DEPARTMENT OF BIOPHYSICS
FACULTY OF SCIENCE
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INTERACTIONS OF SELECTED TRANSITION-METAL BASED COMPLEXES WITH BIOMACROMOLECULES

Doctoral Thesis

Tereza Suchánková

Supervisor: prof. RNDr. Jana Kašpárková, Ph.D.

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Abstract

Cellular DNA is sensitive to chemical modifications caused by various environmental agents, endogenous agents produced by cellular processes and chemoterapeutic agents. Metal-based coordination complexes, especially platinum compounds, represent an important class of anticancer agents that belong currently to the drugs most frequently used in the clinic. There is a large body of experimental evidence indicating that success of platinum complexes in treatment of tumor cells is based on their ability to damage DNA by forming several types of covalent adducts, such as monofunctional adducts, intrastrand or interstrand cross-links.

DNA bases modified by metallodrugs are potentially mutagenic and cytotoxic and so can lead to the cell death. Cells have developed complex repair systems to remove these lesions and thus preserve the integrity of their genomes. However, in cancer cells, repair of the modification caused just by antitumor agents, is an undesired mechanism by which tumors can acquire resistance to anticancer drugs. Mammalian cells remove lesions, produced by platinum drugs, mainly by using the pathway known as nucleotide excision repair (NER).

This thesis consists of two parts. Firstly, the experimental part has been focused on the development of specific conditions for *in vitro* NER assay. After that the level of DNA repair synthesis induced by various novel antitumor metal-based drugs was compared. Although these types of drugs form several types of DNA lesions, one of the most disruptive are interstrand cross-links. Understanding how interstrand cross-links are repaired has been a challenging problem because there is wide variation in chemical structures of these adducts. It is unclear how the physical and chemical properties of a given interstrand cross-link affect ability of the cell to repair such a complex form of DNA damage, so in the second part of this thesis, the molecular structures of these lesions were investigated by means of molecular dynamics simulations. The NER pathway involves damage recognition, unwinding of the DNA around the site of damage, incision on either side of the lesion, removal of a fragment containing lesion, and finally DNA synthesis and ligation to form a repair patch of cca 30 nucleotides. DNA repair synthesis was investigated by incubating circular plasmid DNA damaged by various antitumor compounds, with cell-free extracts containing DNA repair proteins in a reaction mixture which included radiolabeled deoxyribonucleotides. After gel electrophoresis and autoradiography the incorporation of radioactivity was quantified and used as a measure of the level of DNA repair. DNA damage by all studied compounds, including platinum complexes with *cis/trans*-geometry and polynuclear complexes or ruthenium complexes, was less efficiently removed from DNA than damage by conventional cisplatin. Thus, they exhibited enhanced resistivity against repair, which was one of important

factors contributing to their activity in tumor cells.

In the second part, it was shown that computational techniques, provide an useful complement to experiments and are often capable to give further specific information about the interactions of DNA with antitumor compounds. Molecular dynamics simulations, using molecular modeling software package AMBER11 have shown how interstrand crosslinks formed by platinum ligands affect structure of DNA duplex by formation of sterical clashes with DNA backbone and thus leading to change of the bend angle or unwinding of the double helix.

Abstrakt

V buněčné DNA může docházet k chemické modifikaci bází způsobené jak různými vlivy životního prostředí, tak látkami, které vznikají při vnitrobuněčných procesech či chemoterapeutiky. Protinádorově účinné látky s centrálními atomy těžkých kovů, především sloučeniny platiny, patří v současnosti k nejvíce používaným v klinické praxi. Bylo mnohokrát experimentálně prokázáno, že úspěch léčiv na bázi platiny je založen na jejich schopnosti tvořit na DNA několik typů kovalentních aduktů, které zahrnují monofunkční adukty, vnitrořetězcové a mezivláknové můstky. Modifikované báze DNA jsou potenciálně mutagenní a cytotoxické a v důsledku toho tak mohou vést k buněčné smrti. V buňkách se proto vyvinul komplexní systém oprav, který je využíván k odstranění poškození bazí a tím k zajištění integrity genomu. V rakovinných buňkách je ovšem oprava DNA modifikované léčivy nežádoucí a je právě jedním z mechanismů, díky kterému nádory získávají k léčbě rezistenci. V buňkách savců jsou poškození způsobená sloučeninami na bázi platiny odstraňována z DNA především pomocí tzv. nukleotidové excisní opravy (NER). Předkládaná disertační práce sestává ze dvou částí. První, experimentální část, se zaměřila na specifikaci podmínek pro studium nukleotidové excisní opravy v laboratorní praxi. Po optimalizaci byla metoda použita pro porovnání úrovně reparace poškození způsobených nově syntetizovanými protinádorově účinnými komplexy kovů. I když tato činidla tvoří na DNA různé typy poškození, k nejzávažnějším patří adukty spojující oba komplementární řetězce DNA – mezivláknové můstky. Pochopení mechanismu, jakým jsou tyto adukty v buňkách opravovány, je dlouhotrvajícím problémem, především kvůli velké variabilitě jejich strukturně-chemických vlastností. Doposud není jasné jak fyzikální a chemické vlastnosti jednotlivých můstků ovlivňují schopnost buňky odstranit takto komplexní typ poškození. Druhá část této práce se tedy zaměřila na studium strukturních vlastností mezivláknových můstků pomocí počítačových simulací.

Mechanismus nukleotidové excisní opravy zahrnuje rozpoznání místa poškození, rozvinutí DNA v tomto místě, vystřižení a odstranění úseku obsahujícího poškození a následně syntézu nového fragmentu DNA o délce cca 30 nukleotidů a jeho vložení do vzniklé mezery. Kruhová plazmidová DNA byla po inkubaci s různými protinádorově účinnými látkami studována v reakční směsi s buněčným extraktem, obsahujícím proteiny účastnící se opravy DNA, a s radioaktivně značenými deoxyribonukleotidy. Po gelové elektroforéze a autoradiografické kvantifikaci množství zainkorporované radioaktivity byla určena úroveň reparace aduktů jednotlivých látek. Všechny studované sloučeniny, zahrnující komplexy platiny s *cis* či *trans* geometrií a polynukleární komplexy nebo komplexy s centrálním

atomem ruthenia, byly z DNA méně efektivně odstraňovány v porovnání s již dříve používanými léčivy, prokázaly tedy zvýšenou odolnost svých aduktů k reparačním procesům a tím vylepšenou protinádorovou aktivitu.

Cílem druhé části disertační práce bylo prokázat, že techniky počítačových simulací mohou být užitečným doplňkem laboratorních experimentů a poskytnout další detailnější informace o interakci DNA s protinádorově účinnými látkami. Výsledky molekulárně dynamických simulací s použitím modelovacího balíku AMBER11 ukázaly, jak mezivláknové můstky tvořené komplexy platiny stericky ovlivňují strukturu DNA a způsobují tak změny v ohybu a rozvinutí dvojité šroubovice.

Statutory declaration

I hereby declare that this doctoral thesis has been written solely by myself. All the sources quoted in this work are listed in the "References" sections. All published results included in this work are approved by co-authors.

Olomouc, April 2012 …….….....

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1 INTRODUCTION

1.1 Cancer as a challenging problem

Neoplastic transformation (i. e. cancer) is caused when genetic damage in the cells prevents them from being responsible for normal tissue control. The disease spreads when affected cells multiply rapidly, forming tumors of varying degrees. Curing cancer requires eliminating all cancer cells. The major modalities of therapy are surgery and radiation therapy, usually used for local and local-regional disease and chemotherapy for treatment of systemic disease. Often, modalities are combined to create a program that is appropriate for the patient and is based on patient and tumor characteristics.

A chemotherapeutic agent kills the rapidly dividing cells, and thus stops the tumor from spreading. In chemotherapy, the key issue is to kill the tumor cells, with as few as possible consequences for the patient body. The ideal chemotherapeutic drug would target and destroy only cancer cells. Anticancer drugs can originate from a variety of sources, including dyestuffs and chemical warfare agents, and from natural products such as plants, microbes and fungi[1]. Metal-based coordination complexes, especially platinum compounds are considered an important class of antitumor agents. A very effective cancer drug with major clinical impact, particularly for patients with testicular or ovarian cancers, is cisplatin.

1.2 Historical background for metal-based antitumor drugs

The journey of cisplatin to be the most used anticancer drug has started accidentally over 40 years ago in the laboratory of prof. Barnett Rosenberg at Michigan State University. As a biophysicist, Prof. Rosenberg was interested in applying electromagnetic field to investigate its effect on bacterial and mammalian cells. The early experiments used Escherichia coli and a set of platinum electrodes. By the influence of the field the bacteria became very long filaments compared to normal short rods. Consequently, this effect was

shown to be due to chemical compounds originating from the electrolysis on the platinum electrodes instead of the influence of the field itself. Detailed analysis identified chemical compound - *cis*-diamminedichloridoplatinum(II) (cisplatin, Fig. 1) - as the cause of this intriguing biological effect [2,3].

After the regression of animal tumors caused by cisplatin was observed, clinical trials on solid tumors in humans followed rapidly. Phase I clinical trials started in 1971[4], and Food and Drug Administration approval as a chemotherapeutic agent was obtained in 1978 under the name Platinol. Today, over 30 years after its approval, cisplatin is still one of the world's best selling anticancer drugs. It is responsible for the cure of testicular cancer with a cure rate as high as 90% and it plays an important role in some cancer treatments such as ovarian, head and neck cancer, bladder cancer, cervical cancer, melanoma, and lymphomas[5].

The impressive number of patients who have been cured after cisplatin treatment has resulted in great interest in metal-containing antitumor drugs generally and cisplatin chemistry has provided huge field for bioinorganic chemistry research.

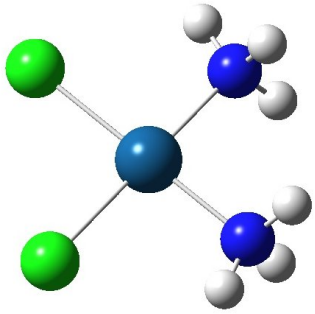

Fig. 1: Formula of cisplatin

1.3 Mechanisms of action of platinum-based drugs

1.3.1 DNA as the critical target

After the discovery of the anticancer properties of cisplatin, work began to elucidate its mechanism of action. One of the first issues was to settle the biological target for the drug because of many cellular components suitable for reaction (including nucleic acids, proteins,

membrane phospholipids or cytoskeleton[4]). Soon, a number of observations pointed to DNA as the critical cellular target of cisplatin and related metal-based antineoplastic agents[7].

Prior to DNA attack, cisplatin became activated intracellulary by aquation. Being able to hydrolyse, cisplatin will be susceptible to aquation as soon as it enters the body. The high concentration of chloride ions in blood plasma (ca. 100 mM) preserves its stability. However, the chloride concentrations under 100 mM (ca. 3-20 mM), as found inside cells, facilitates rapid hydrolysis of the chloride ligands of the molecule, leading to activated cationic mono- or diaqua species, which can subsequently react with DNA. The process of cellular uptake and activation of cisplatin is illustrated by the Fig. 2 [8].

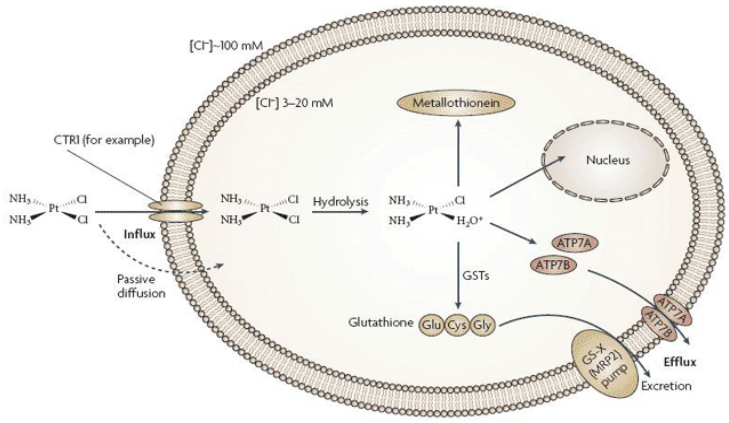

Fig. 2 Cellular uptake and *in vivo* chemistry of cisplatin [8]

1.3.2 DNA adducts of cisplatin and consequences for the duplex

Although activated cisplatin can interact with various biomolecules, its antitumour activity derives from its capability to form bifunctional DNA adducts (crosslinks). These types of lesions involving either intrastrand or interstrand DNA crosslinks or protein-DNA crosslinks were settled to be crucial for biological activity of platinum drugs. Crosslinks cause specific distortion to the duplex, which can be at the beginning of many events that the recognition of platinum adducts can initiate in the cell. This is also supported by the fact that cisplatin-related inactive compounds can only bind as monofunctional adducts[9].

The adducts are predominantly formed as guanine-guanine (GG) crosslinks, where the chlorido ligands have been replaced first by a water molecule through aquation and then by the nitrogen N7 of adjacent guanine (G) residues (Fig. 3, [10]). The coordination of guanines is enhanced by the fact, that their N7 atoms are the most electron-dense and accessible sites in DNA for electrophilic attack by platinum. They are exposed in the major groove of the double helix, and not involved in base-pair hydrogen bonding.

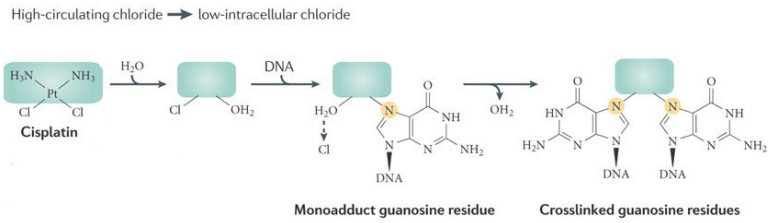

Fig. 3: Formation of the crosslink by cisplatin ([10], modified)

The major cisplatin-DNA adducts (see Fig. 4) – comprising around 90% of all the adducts – both *in vitro* and *in vivo* were found to be: 1,2-intrastrand d(GpG) crosslinks (between adjacent guanines) in ca. 50-65%, and 1,2-intrastrand d(ApG) crosslinks in ca. 25%, (between adenine and adjacent guanine from 5′ to 3′)[11,12]. In addition, 1,3-intrastrand d(GpNpG) crosslinks (intrastrand adducts between purines separated by one or more intervening bases) were present in less than 10%, together with interstrand cross-links, monofunctional adducts to guanine residues, and DNA-Pt-protein cross-links[13,14].

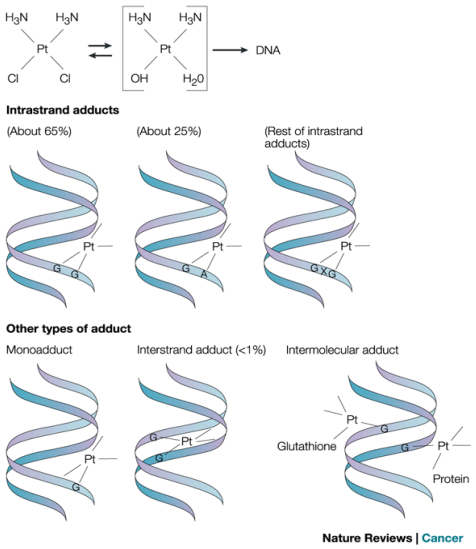

Fig. 4: Types of DNA-cisplatin adducts

1.3.3 Cellular response to cisplatin-DNA adducts

Once cisplatin has reached DNA, the cell tries to remove the damage. This effect is crucial for the anticancer activity of cisplatin. Mammalian cells remove lesions, produced by platinum drugs, mainly by using the pathway known as nucleotide excision repair (NER). In cancer cells repair of the modification caused by antitumor agents is an undesired mechanism. In other words, if the repair is not efficient, the probability of cancer cell to survive decreases.

It was shown, that the failure of repair mechanisms is caused by structural changes in DNA duplex after the cisplatin was bound[15]. This altered DNA conformation could be recognized and suitable for binding of HMG-domain proteins (HMG = high mobility group), which lead to “repair shielding”[14,16,17]. Another consequence of cisplatin-damaged DNA, is attraction of HMG-domain proteins and other nuclear proteins, thus leading to lack in their natural binding sites and eventually resulting in cell death. It is also believed, that these two effect can operate together[18].

HMGB1 and HMGB2 proteins are structure-specific DNA-binding proteins. They belong to architectural chromatin proteins that play some kind of structural role in the formation of functional higher order protein-DNA or protein-protein complexes[18]. The

HMGB1 protein contains two tandem HMG-box domains, A and B (HMGB1a and HMGB1b), and an acidic C-terminal tail[19]. The domains A and B in the HMGB1 protein are linked by a short sequence (A/B linker) containing a short lysine-rich segment of seven amino acids at the N-terminus of the domain B, whereas the domain B and C tail are linked by a longer chain (containing 23 amino acids, B/C linker region) .

The domain HMGB1a binds to the minor groove of the DNA double helix opposite the cisplatin 1,2-intrastrand CL located in the major groove[17,20]. Distortions such as prebending, unwinding at the site of platination as a consequence of the CL formation are important for the recognition and affinity of the proteins that specifically bind DNA damaged by cisplatin[17,20]. Intercalating residues in HMG-domain proteins influence both the affinity and orientation of the protein binding to this CL[21]. Moreover, the lysine-rich region of the A/B linker in the full-length HMGB1 protein attached to the N-terminus of the domain HMGB1b markedly enhances binding of this domain to DNA containing site-specific 1,2-GG intrastrand CL of cisplatin stressing an importance of this domain for specific binding of the HMGB1 protein to this platinum lesion[22]. It has been also shown[23] that binding of the A/B linker region within the major groove of DNA helps the two HMGB1 domains anchor to the minor DNA groove facilitating their DNA binding. The structural representation of the highly specific complex of DNA and HMG-domain protein is shown in the Fig. 5.

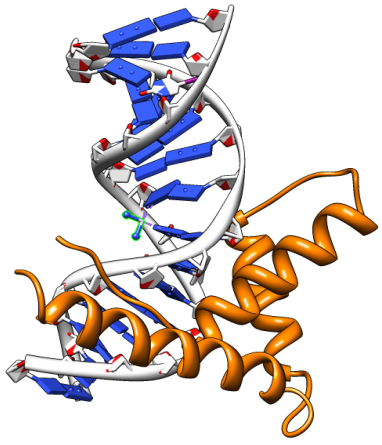

Fig. 5: Adduct formed between the nonsequence-specific domain A of HMGB1 and cisplatin-modified DNA (coordinates taken from the Protein Data Bank, 1CKT)

Another essential functions of the cellular metabolism affected by lesions formed on DNA by platinum compounds are DNA replication and transcription. These are crucial processes in rapidly proliferating tumor cells, so that their inhibition should result in cytostatic effects. There is a large body of experimental evidence[24,25], that inhibition of DNA replication and transcription is an important part of the mechanism underlying antitumor effects of cisplatin.

1.4 The drawbacks of platinum-based cancer chemotherapy

As had been written, cisplatin is a very effective antitumor drug and has had a major clinical impact, particularly for patients with testicular or ovarian cancers. But quite severe side effects of treatment can occur - nausea, ear damage, vomiting and also the nephrotoxicity which has to be reduced by saline (hydration) and diuresis. These side effects limit the dose that can be administered to patients to 100 mg per day for 5 consecutive days[26].

1.4.1 Cellular resistance to cisplatin

Another drawbacks coupled with cisplatin toxicity are also associated with resistance of tumor cells to this drug. Some tumors can have natural resistance to cisplatin, while others develop resistance after the initial treatment. Resistance is multifactorial and in general it may consist of mechanisms either limiting the formation of DNA adducts and/or inducing downstream effects with essential impact on cell survival. The formation of DNA adducts by cisplatin can be limited by reduced accumulation of the drug, enhanced drug efflux, and cisplatin inactivation by coordination to sulfur-containing proteins (including glutathion (GSH) and metallothioneins (MT-2)), whose production also may be increased as a consequence of cisplatin treatment. The second group of mechanisms includes enhanced repair of DNA adducts of cisplatin and increased tolerance of the resulting DNA damage [27].

To prevent the detrimental consequences of DNA damage a complex network of complementary DNA repair mechanisms has arisen (overview of repair processes illustrates Fig. 6 [28]). Briefly, double-strand breaks are repaired by homologous recombination-dependent repair or in an end-joining reaction, and most small base modifications are removed by base excision repair. Nucleotide excision repair (NER) removes primarily bulky,

helix-distorting adducts. However, considerable overlap exists in substrate specificity of repair pathways, and certain proteins are used in more than one pathway[29]. As had been written above, DNA repair has a significant role in modulating cytotoxicity of platinum drugs. Mammalian cells remove lesions, produced by these agents, mainly by using the NER pathway.

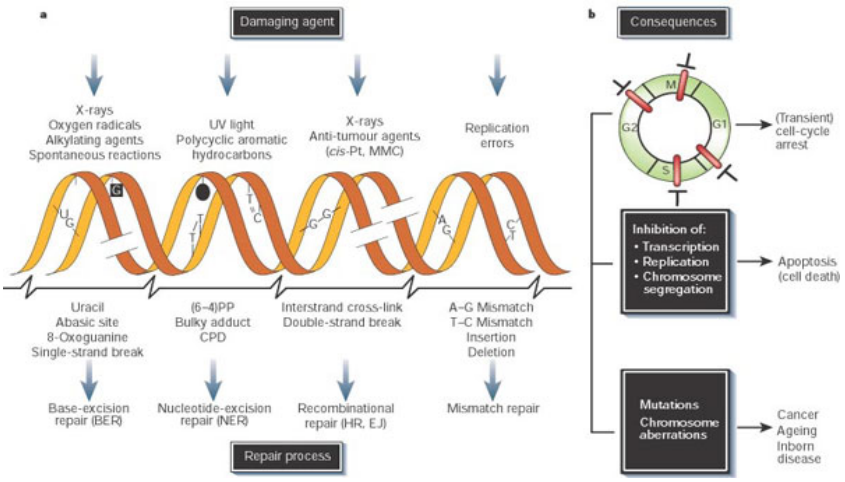

Fig. 6: DNA repair pathways in mammalian cells [28]

1.4.2 Nucleotide excision repair

The NER process involves the action of about 20–30 proteins in successive steps of damage recognition, local opening of the DNA double helix around the injury, and incision of the damaged strand on either side of the lesion. After excision of the damage-containing oligonucleotide the resulting gap is filled by DNA repair synthesis, followed by strand ligation[30].

Initial damage-recognizing step in the NER process is binding of the XPC/HHR23B complex[31], recruiting the entire repair protein apparatus to the lesion. The complex may, in addition to lesion detection, increase single-strandedness at the site of the DNA injury. The next step is the formation of an open complex, requiring a local unwinding of the DNA helix and demarcation of the lesion. XPA has many interactions with other NER components, for instance with the single-strand binding complex replication protein A (RPA)[32], the TFIIH complex[33] and the ERCC1/ XPF endonuclease[34], in addition to affinity for a variety of

lesions particularly when base pairing is disrupted[35]. Hence, XPA may organize the repair machinery around the DNA lesion when XPC/HHR23B with or without the help of TFIIH has locally opened the helix. Full opening of the DNA helix around the lesion is dependent on the presence of ATP [36]. DNA unwinding by TFIIH may be facilitated by RPA, a heterotrimeric complex involved in NER, replication and recombination[37]. The optimal binding patch of RPA is 30 nucleotides[38], which is similar to the size of the fully opened repair complex and the size of the released damage-containing oligonucleotide.

TFIIH is a protein complex of nine subunits, which was originally identified as an essential factor in basal transcription initiation[39]. The p89 and p80 subunits were identified as the previously discovered XPB and XPD NER helicases[40,41], which contain, respectively, ATPase-driven 3' → 5' and 5' →3' directed DNA unwinding activity. TFIIH is required for local unwinding of the DNA helix around the lesion in NER[42] and in the transcription initiation of RNA polymerase II at the promoter[43].

After local unwinding and demarcation of the lesion, an oligonucleotide of 24–32 nucleotides containing the lesion is excised. This requires the structure-specific endonuclease activities of XPG at the 3' side of the open complex[44]. The ERCC1/XPF complex cuts at the single-strand to double-strand transition 5' of the damage[45]. In principle, both XPG and ERCC1/XPF can also incise the undamaged strand, but specificity of ERCC1/XPF seems to be coordinated by RPA, which binds with defined polarity to the undamaged strand. Its 3'-oriented side stimulates ERCC1/XPF whereas the 5'- oriented side inhibits endonuclease activity of ERCC1/XPF in the undamaged strand[46]. RPA and XPG interact directly, but RPA alone is not sufficient to endow strand specificity upon XPG. The strong interaction between XPG and TFIIH[47] suggests that TFIIH is involved in XPG positioning.

The last step in the NER reaction, gap-filling of the excised patch by repair DNA synthesis, is used for assaying NER activity in vitro and in vivo. An in vitro reconstituted repair reaction showed that efficient repair synthesis occurs after addition of the mammalian DNA replication factors RPA, RFC, PCNA and DNA polymerase δ and ε[48]. The NER reaction is completed by ligation of the newly synthesized DNA. DNA ligase I is a likely candidate for this reaction[49].

Two modes of NER can be distinguished which differ only in a step involving recognition of the DNA lesion. The reaction mechanism described above is designated global

genome nucleotide excision repair (GG-NER), which removes DNA damage from any place in the genome. In contrast, lesions in the transcribed strand of actively transcribed genes are preferentially repaired via the NER-subpathway transcription-coupled repair (TC-NER) [34,35] A simplified model of steps in NER is shown in the Fig. 7[50].

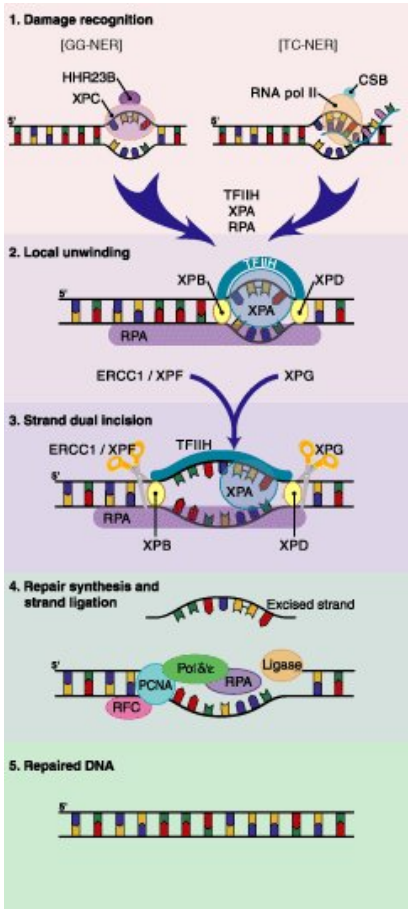

Fig. 7: Model of the nucleotide excision repair

1.5 The development of new platinum drugs

Despite the success of cisplatin and platinum-based drugs, the market is still accessible for new advantageous metal-based drugs that offer better viability, such us oral administration, which might help to diminish severe side-effects and clinic costs[5]. The first wave of drug-development activity was to discover a less toxic analogue that retained anticancer activity. The major breakthrough in this regard was the drug design based on the hypothesis that a more stable leaving group than chloride might lower toxicity without affecting antitumor efficacy[51]. This hypothesis turned out to be correct. Thus, the research is nowadays focused on drugs that interact differently with the target (DNA), consequently can overcome inherent or acquired resistance to cisplatin, and are active towards tumors which are non-responsive to current chemotherapy.

1.5.1 Antitumor analogs of cisplatin

Six platinum-based drugs are in clinical use today, i.e. cisplatin, carboplatin, and oxaliplatin worldwide, and nedaplatin, lobaplatin, and heptaplatin in Japan, China and South Korea, respectively (see Fig. 9)[5]. Carboplatin was the first example of development of the compound in the search for a drug less toxic than cisplatin. In carboplatin, the ligands susceptible to nucleophilic substitution are the oxygen carboxylates of chelated cyclobutanedicarboxylate. By having less labile ligands, this analogue reacts with nucleophiles to a lesser extent than cisplatin, and is less toxic. Thus, it was found that carboplatin is less toxic than cisplatin, with no significant renal or neural toxicity, and at the same time is as effective as, or even more active than cisplatin in some tumors. Due to the presence of the same carrier ligands (NH_3), carboplatin and cisplatin eventually form the same DNA adducts, and the differences between these two drugs is due to the much slower DNA binding kinetics of carboplatin[52,53]. The same scenario is expected for nedaplatin where the carrier ligands are also ammonia groups. Thus the principle is established that not only thermodynamic and structural features are important in the design of metal complexes as drugs, but kinetic factors are too.

There appear to be few published studies on DNA adducts formed by lobaplatin or

heptaplatin[54,55]. The case is different for oxaliplatin.

Oxaliplatin, (trans-1R,2R-diaminocyclohexane)oxalato-platinum(II) (Fig. 9), first synthesized in 1978[56], is the latest worldwide approved platinum-based anticancer agent. It has shown a broad spectrum of antitumor activity[57] with a partial or a non-cross-resistance with cisplatin in a wide range of human tumors *in vitro* and *in vivo*[57–59]. Oxaliplatin also has similar DNA binding properties, leading to the same type of DNA-Pt cross-links, although the amount of the different types of adduct varies in comparison to cisplatin. Several studies show that oxaliplatin is less reactive and forms fewer adducts with cellular DNA than cisplatin. The different response of cellular proteins to these structurally-different DNA adducts may be responsible for the differences in cytotoxicity and spectrum of activity against different tumors for oxaliplatin and cisplatin. Therefore, the reason why oxaliplatin adducts lead to fatal lesions in cisplatin-resistant cell lines, and differences in mutagenicity in comparison to cisplatin and carboplatin, might be explained by the different discrimination of cisplatin and oxaliplatin adducts by cellular proteins[60].

Success of oxaliplatin, which incorporates the 1R,2R-DACH carrier ligand as PtII complex, raised considerable research interest over the past three decades in platinum–DACH complexes. Thus, in a search for more effective platinum antitumor complex that is also capable of overcoming cisplatin resistance *in vivo* a large-ring chelate complex [$PtCl_2$(*cis*-1,4-DACH)] (Fig. 8) was synthesized and tested for cytotoxicity in tumor cell lines and for antitumor activity against the murine leukemias and human solid tumors[61]. Our further experiments, which were concerned on this compound, resulted in Paper II.

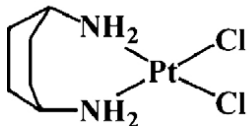

Fig. 8: Structure of chelate complex

The interesting properties of oxaliplatin reported above and also recent results published in our laboratory[62] were also the main motivation for further biophysical studies of interstrand crosslink of oxaliplatin and its *S,S*-enantiomer. Our findings supported by structures obtained by molecular dynamics simulations, are summarized and published in Paper VI.

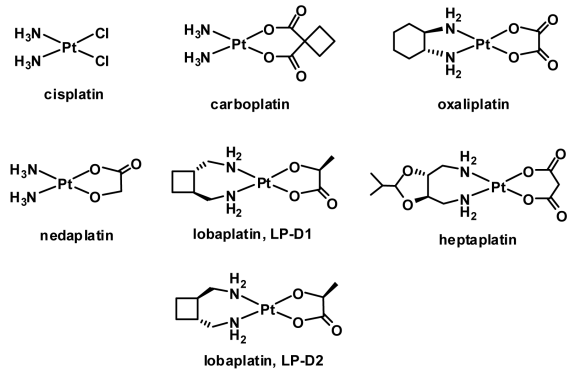

Fig. 9: Analogs of cisplatin

1.5.2 *Trans*-platinum complexes (activation of *trans* geometry)

The original *cis/trans* empirical structure–pharmacological activity relationships for mononuclear bifunctional platinum complexes considered transplatin [*trans*-diamminedichloridoplatinum(II)] (Fig. 11, in comparison with cisplatin) and its analogs to be clinically inactive, whereas cis-oriented complexes, such as cisplatin and its analogs were considered to be antitumor-active[63]. A distinct difference between cisplatin and its trans isomer is that transplatin is kinetically more reactive than cisplatin, and therefore is more susceptible to deactivation. Later, it was shown that some trans compounds are active in vitro and in vivo and show anticancer activity distinct from cisplatin (interestingly some new trans-platinum compounds are even more potent than cisplatin itself)[30,43,64].

An explanation for the clinical inefficiency of transplatin is believed in the different nature of its bifunctional DNA adducts relative to those of cisplatin. While major DNA adducts of cisplatin and its analogs are intrastrand crosslinks between neighboring purine residues, stereochemical limitations prevent transplatin from forming this type of lesion. DNA adducts of transplatin are mainly interstrand crosslinks between complementary guanine and cytosine residues, intrastrand crosslinks between nonadjacent base residues, and a relatively large portion of adducts remain monofunctional. It has also been suggested[65] that in cells transplatin forms only a small number of crosslinks because of the slow closure of the monofunctional adducts coupled to their trapping by intracellular sulfur nucleophiles. Importantly, adducts of ineffective transplatin also affect DNA conformation although less severely than the crosslinks of cisplatin. Examples of new analogs of transplatin which exhibit

a different spectrum of cytostatic activity including activity in tumor cells resistant to cisplatin are analogs containing iminoether groups, an asymmetric aliphatic ligand or a heterocyclic amine ligand[27,32].

Recently, new asymmetric dichloridoplatinum(II) complexes comprising an isopropylamine ligand cis (**1** in Fig. 10) and trans (**2** in Fig. 10) to an azole ligand were synthesized as well[66]]. These complexes were also investigated in this thesis (Paper I).

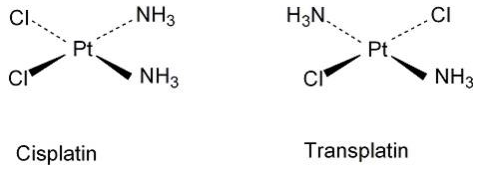

Fig. 11: Formulas of cisplatin and transplatin

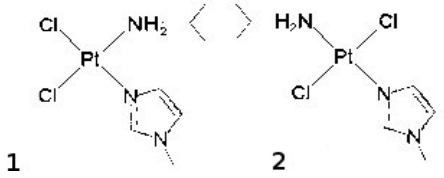

Fig. 10: new asymmetric dichloridoplatinum(II) complexes

1.5.3 Antitumor ruthenium compounds

One approach in the search for the new, metal-based anticancer agent which would exhibit antitumor activity markedly different from that of cisplatin and its direct analogs is to examine complexes that would contain another transition metal. The possible advantages in using transition metal ions other than platinum may involve additional coordination sites, alterations in ligand affinity and substitution kinetics, changes in oxidation state, and photodynamic approaches to therapy[68] .

Today, there are two Ru-based anticancer drugs in clinical trials: NAMI-A and KP1019[69,70]. NAMI-A has a relatively low toxicity towards cancer cells, but is particularly effective against metastases from solid tumors both in experimental mouse tumors and against human tumors engrafted in the nude mouse [56,68,69] being scarcely effective in solid tumours. The drug has now completed phase I clinical trials[74].

Organometallic RuII–arene complexes of the type $[Ru^{II}(\eta^6\text{-arene})(Cl)(en)][PF_6]$ (en = 1,2-diaminoethane) constitute a relatively new group of anticancer compounds[75–77]. These monodentate complexes appear to be novel anticancer agents with a mechanism of action different from those of platinum and other ruthenium complexes that have been tested for antitumor activity. Although the pharmacological target for antitumor ruthenium compounds has not been unequivocally identified, there is a large body of evidence indicating that the

cytotoxicity of several ruthenium complexes correlates with their ability to bind DNA, although several exceptions have been reported[78,79]. Because the RuII–arene complexes bind strongly to DNA[80,81], DNA modifications and the downstream effects of these modifications are of great interest. It has been shown that RuII–arene complexes of the type[Ru^{II}(η^6-arene)(Cl)(en)][PF_6] can form monofunctional adducts with DNA at guanine residues. Some of these RuII complexes, in particular those containing multiring arenes, bind to DNA not only through coordination to the N7 atoms of guanine (G), but also noncovalently through hydrophobic interactions between the arene and DNA[82,83]. These hydrophobic interactions might include intercalation of the noncoordinated arenes between DNA bases and minor-groove binding.

Recently, new complexes of the type [Ru^{II}(η^6-arene)-(Cl)(en)]$^+$ (arene = ortho-, meta-, or para-terphenyl) have been synthesized to investigate the effect on cytotoxicity and DNA binding of structural isomerization of the multring terphenyl arene ligand[83]. Importantly, the complex containing para-terphenyl as the arene ligand (complex 1, Fig. 12) exhibits promising cytotoxic effects in several human tumor cell lines, including those resistant to conventional cisplatin. In contrast, complexes containing meta- or ortho-terphenyl arene ligands (complexes 2 and 3, respectively; Fig. 12) are much less cytotoxic. Complexes 1 and 2 were selected for an initial study focused on global modification of natural, high-molecular-mass DNA[83]. The results of this study have revealed that, concomitant with the relatively high cytotoxicity of 1 in tumor cells, its DNA-binding mode involves combined intercalative and monofunctional (coordination) binding modes. In contrast, complex 2, which is much less cytotoxic, binds to DNA through only a monofunctional coordination to DNA bases. Further examination of these complexes was described in the paper IV.

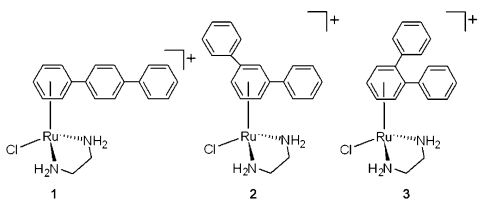

Fig. 12: Formulas of studied ruthenium compounds

1.5.4 Multinuclear platinum complexes

Although some progress had been made in reducing the toxic side effects and overcoming resistance, little improvement in cytotoxicity or spectrum of activity had been observed for the many analogues of cisplatin[84]. Evidence suggests that this is due to the cisplatin analogues forming a similar array of DNA adducts as cisplatin[84].

Consequently, attention turned to the synthesis of 'non-classical' platinum complexes which were capable of forming a different range of DNA adducts - which could therefore display a different spectrum of anti-cancer activity compared to cisplatin. One group of non-classical platinum complexes include trans complexes, of the general formula trans - $[PtCl_2(NH_3)L]$ where L is a planar N donor[85], mono-functional complexes, like cis - $[Pt(NH_3)_2(Am)Cl]^+$ where Am is pyridine, pyrimidine, or purine[86,87] and multinuclear complexes.

Multinuclear platinum complexes contain two or more linked platinum centres that can each covalently bind to DNA, and hence are capable of forming a completely different range of DNA adducts compared to cisplatin and its analogues[85]. These multinuclear complexes represent a completely new paradigm for platinum based anti-cancer complexes[85], and appear to offer great potential as new anticancer agents.

In an effort to design a polynuclear platinum compound that maintains the target (DNA) binding profile of the "parent" compound and is less susceptible to metabolic decomposition, the new, long-chain bifunctional dinuclear platinum complex structurally cognate with BBR3610 – $[\{PtCl(DACH)\}_2\text{-}\mu\text{-}Y]^{4+}$ [DACH = 1,2-diaminocyclohexane, Y = $H_2N(CH_2)_6NH_2(CH_2)_2NH_2$ $(CH2)_6NH_2$ (spermine-like chain)] (Fig. 13) – was synthesized[88]. Importantly, preliminary results showed that there was no labilization of the polyamine spermine-like linker groups of this complex in the presence of sulfur-containing species at physiological pH, which was attributed to the chelate effect of the DACH ring[88]. Complex 1 should, besides enhanced stability to metabolic deactivation, also exhibit other main features of potent di- or trinuclear platinum compounds. This compound was investigated in Paper III.

Also another long-chain bifunctional dinuclear cis platinum complex with enhanced stability to metabolic deactivation was synthesized[89]. Its formula is show in the Fig. 14.

This complex was studied, and compared to the previous one, in Paper IV.

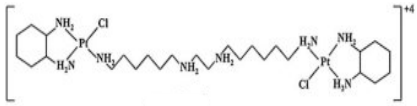

Fig. 13: bifunctional dinuclear platinum complex investigated in Paper III

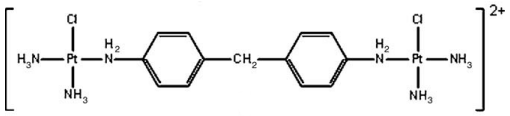

Fig. 14: Dinucelar platinum complex with cis geometry (Paper V)

1.6 DNA (deoxyribonucleic acid)

The discovery of the double-helix structure of deoxyribobnucleic acid (DNA) was the seminal event in the origin of the field of molecular biology. DNA occurs naturally almost exclusively as the genetic material of organisms. The structure and chemical properties of DNA are the basis of its central role in storing and transmitting the genetic information.

1.6.1 Building blocks of DNA

DNA is a long linear polymer, composed of four kinds of deoxyribose nucleotides. Each repeating nucleotide in a DNA consists of the three units linked together – a phosphate group, a sugar, and one of the four bases. The bases are planar aromatic heterocyclic molecules and are divided into two groups – the pyrimidine bases (thymine and cytosine) and the purine bases (adenine and guanine). When the bases are attached covalently to the sugar moiety, they are known as the nucleosides. In each nucleoside, the N9 atom of a purine or N1 of a pyrimidine is bonded in a glycosidic bond to the C1' atom of the sugar (by convention, the numbers of atoms of the sugar are given primes). A nucleotide is a nucleoside joined to one or more phosphate groups by an ester linkage to the deoxyribose sugar. (Fig. 15).

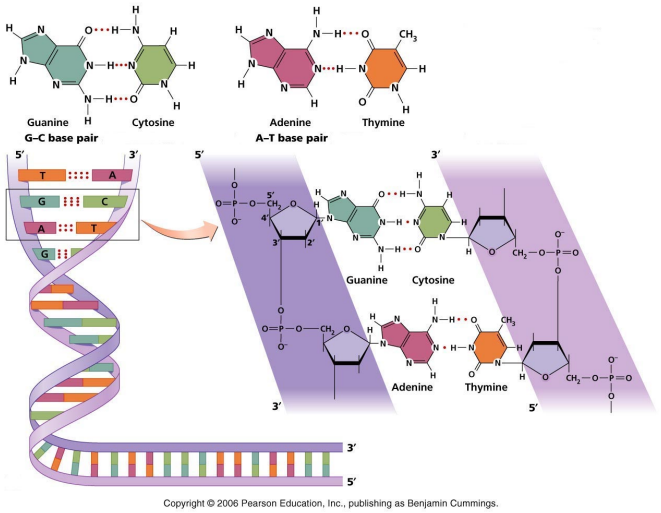

Fig. 15: The organization of repeating units in a polynucleotide chain

Polynucleotide like DNA is produced by polymerizing the nucleotides, linking them by phosphodiester bridges, in which the 3´ - hydroxyl group of the sugar moiety of the nucleotide is esterified to a phosphate group, which is joined to 5' – hydroxyl group of the adjacent sugar. The chain of sugars linked by phosphodiester bridges comprises the backbone of the DNA molecule. A linear polynucleotide has a 5' - end and a 3' – end. By convention, the sequence of a polynucleotide chain is described in the 5'- to 3'- direction.

A single-stranded polynucleotide is very flexible because rotations can take place about six bonds of each nucleoside of the backbone. There are very significant steric restrictions on what combinations of torsion angles can occur. The number of distinct conformations for each of the bond rotations is believed to be about one for torsion angle α, two for δ, ζ and χ, and three for β, γ and ε (Fig. 16, [90]).

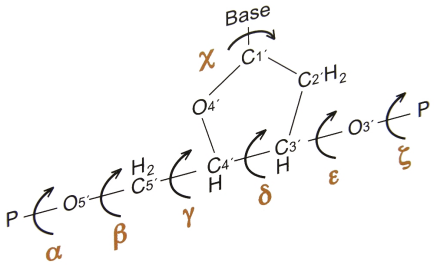

Fig. 16: Definition of the torsion angles of a DNA sugar-phosphate backbone and of the glycosyl angle

Sugars as deoxyribose normally exist in solution as three different tautomeric forms in equilibrium. Replacing the hydrogen atom normally on C1' by a base, to generate a nucleoside, however, locks the sugar into the cyclic form. The stereochemistry of this glycosidic bond is important. In natural nucleic acids the glycosidic bond is always β, that is the base is above the plane of the sugar when viewed onto the plane and therefore on the same face of the plane as the 5′ - hydroxyl substituent.

The deoxyribose ring does not have all five atoms lying in the same plane. The five torsion angles of this ring (describe as υ_0 to υ_4) would be zero in planar conformation, but this would be very strained. Such strain is relieved by having at least one of the five atoms lying out of the plane, introducing the pucker of the ring. The carbons most likely to be out of the plane are the 2' and 3'. If the nonplanar atom is on the same side as the base and the C5' – carbon, the conformation is said to be *endo*; if on the other side, the conformation is *exo*.

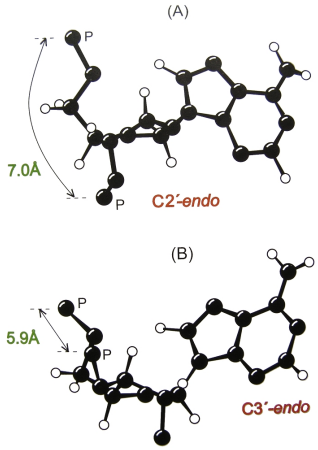

Fig. 17: Effects on the backbone of nucleotides in the (A) *C2′-endo* and (B) *C3′-endo* conformation

The sugar pucker is important for nucleic acids because it governs the relative orientations of the phosphate substituents (see Fig. 17, [90]).

Nucleotides and nucleosides can exist with either of two orientations about the glycosidic bond (as defined by the torsion angle χ), commonly designed as *syn* and *anti* (Fig. 18, [90]). Rotations about this bond are hindered, so these two most stable conformations are not readily interconverted. For pyrimidine nucleosides or nucleotides, only the anti

conformation is stable, because otherwise the sugar residue would sterically interfere. The anti conformation is found in all DNA structures, except of Z-DNA, where the purine residues are syn.

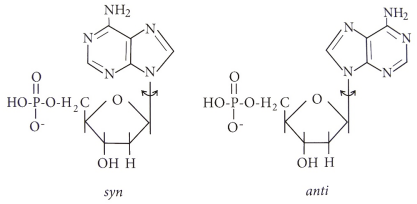

Fig. 18: Orientations about the glycosidic bond in nucleotides

1.6.2 Formation of DNA double helix

DNA consists of two associated polynucleotide strands that form a structure described as a double helix**.** The two sugar-phosphate backbones are on the outside of the double helix, and the bases project into the interior. The adjoining bases in each strand stack on top of one another in parallel planes. The orientation of the two strands is antiparallel (their 5′ → 3′ directions are opposite). The strands are held in precise manner by a regular base pairing between the two strands. The adenine:thymine (A•T) base pair has two hydrogen bonds compared to the three in a guanine:cytosine (G•C). This base-pair complementarity is a consequence of the size, shape, and chemical composition of the bases (Fig. 19, modified from [90]). To maintain the geometry of the double-helical structure, a larger purine (A or G) must pair with a smaller pyrimidine (C or T). In natural DNA, A almost always forms hydrogen bonds with T and G with C, forming A·T and G·C base pairs (called Watson-Crick base pairs). In theory and in synthetic DNA other interactions can occur (forming nonstandard base pairs).

The presence of thousands of such hydrogen bonds in a DNA molecule contributes greatly to the stability of the double helix. Hydrophobic and van der Waals interactions between the stacked adjacent base pairs also contribute to the stability of the DNA structure.

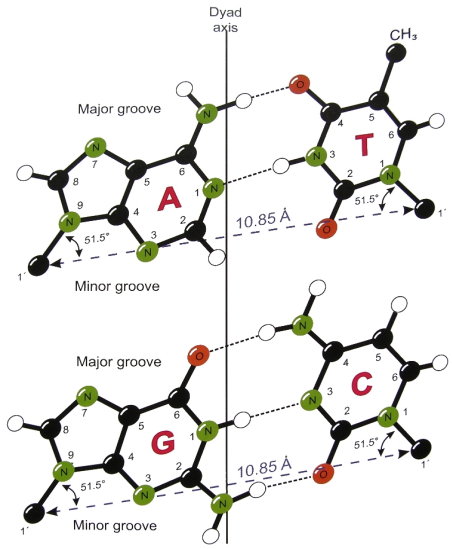

Fig. 19: A·T and G·C Watson-Crick base pairs

1.6.3 Base and base-pair flexibility

The individual bases in a nucleic acid are flat, but base pairs can show considerable flexibility. This flexibility is to some extent dependent on the nature of the bases and base pairs themselves, but is more related to their base-stacking environments. Thus descriptions of base morphology have become important in describing and understanding many sequence-dependent features and deformations of nucleic acids (Fig. 20, adapted from [90]). The former features are often considered primarily at the dinucleoside local level, whereas longer-range effects such as helix bending, can also be analyzed at a more global level.

For individual base pairs[91] :

- *Propeller twist* between bases is the dihedral angle between normals to the bases, when viewed along the long axis of the base pair. The angle has a negative sign under normal circumstances, with a clockwise rotation of the nearer base when viewed down the long axis. The long axis for a purine–pyrimidine base pair is defined as the vector between the C8 atom of the purine and the C6 of a pyrimidine in a Watson–Crick base pair. Analogous definitions can be applied to other nonstandard base pairings in a

duplex including purine–purine and pyrimidine–pyrimidine ones.

- *Buckle* is the dihedral angle between bases, along their short axis, after propeller twist has been set to 0° . The sign of buckle is defined as positive if the distortion is convex in the direction 5′→3′ of strand 1. The change in buckle for succeeding steps, termed cup, has been found to be a useful measure of changes along a sequence. Cup is defined as the difference between the buckle at a given step, and that of the preceding one.
- *Inclination* is the angle between the long axis of a base pair and a plane perpendicular to the helix axis. This angle is defined as positive for right-handed rotation about a vector from the helix axis toward the major groove.
- *X and Y displacements* define translations of a base pair within its mean plane in terms of the distance of the midpoint of the base pair long axis from the helix axis. X displacement is toward the major-groove direction, when it has a positive value. Y displacement is orthogonal to this, and is positive if toward the first nucleic acid strand of the duplex.

For base pair steps[91]:

- *Helical twist* is the angle between successive base pairs, measured as the change in orientation of the C1′–C1′ vectors on going from one base pair to the next, projected down the helix axis (Fig. 2.7). For an exactly repetitious double helix, helical twist is 360°/n, where n is the unit repeat defined above.
- *Roll* is the dihedral angle for rotation of one base pair with respect to its neighbor, about the long axis of the base pair. A positive roll angle opens up a base pair step toward the minor groove.
- *Tilt* is the corresponding dihedral angle along the short (i.e. x-axis) of the base pair.

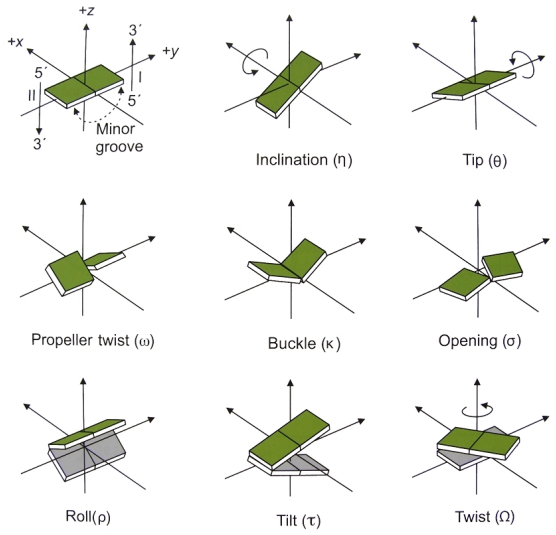

Fig. 20: The conformational parameters associated with DNA base pairs

1.6.4 Polymorphism of DNA structures

Double-stranded DNA almost always adopts a helical structure that is much more rigid and extended than a single strand. The three most common types of DNA double helices are B-, A- and Z-DNA. Their structures are illustrated by Fig. 21 .

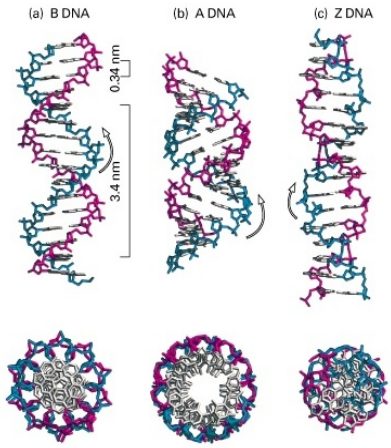

Fig. 21: B-, A-, and Z- form of DNA double helix

B-DNA

B-DNA is the predominant DNA structure under normal physiological conditions. The B-DNA double helix is right-handed, with base pairs perpendicular to the helical axis, which passes through the centers of the base pairs. The pitch of the helix is determined by the stacking between the adjacent base pairs. The major and minor grooves are roughly equivalent. In depth, 8,5 Å and 7,5 Å, respectively, but the width of major groove is about 12 Å, whereas that of the minor groove only about 6 Å. The sugar pucker of the deoxyribose ring favors the *C2'-endo* conformation. The bases that define the sequence are more accessible in the major groove, and this is where proteins tend to bind to specific nucleotide sequences. B-DNA structures are very flexible depending upon the exact sequence and the environment. In general, runs of adenines lead to a very narrow minor groove and a high propeller twist, which introduce a bend in the axis.

A-DNA

B-DNA can be converted to A-DNA under conditions of low hydration and by adding alcohols. The conversion is reversible and occurs on the microsecond time scale. A-DNA was first recognized from X-ray diffraction analysis, where the hydration of the insoluble samples can be varied drastically. The double helix of this DNA form is fat and short, with the base pairs and backbone wrapped further away from the helix axis than in B-DNA. The base pairs are tilted significantly relative to the helix axis, by about 19°, and display a minor propeller twist. The equivalent of the major groove of B-DNA is deep and very narrow, with the width of only 3 Å and a depth 13 Å. In contrast, the minor groove has a breadth of 11 Å and a shallow depth of 3 Å.

Z-DNA

Z-DNA structure was unexpected and is unusual in being left-handed. Its name originates from zig-zag path of the phosphate groups along the DNA backbone. Relative to B- and A-DNA the Z-DNA double helix is tall and thin, with a diameter of about 18 Å and a helical pitch of about 45 Å. Z-DNA is favored by alternating pyrimidine-purine sequences, especially $(dC\text{-}dG)_n$. The repeat unit is CpG dinucleotide with the glycosyl conformation of

cytosine being *anti* and the guanine being *syn*. The sugar pucker of cytosine C2'-endo, while the guanine is predominantly C3'-endo. There are 12 base pairs per helical turn. The base pairs are sligthly inclined by 9°, and the helical twist angle per dinucleotide repeat is -60°, about -8° for the CpG and -52° for the GpC. The rise per dinucleotide repeat is about 7,4 Å. The Z-DNA conformation has been observed in the crystal structures of several other oligonucleotides. The single groove of Z-DNA is narrow and deep, with an opening of only 6-7 Å. The equivalent of the major groove is almost non existent, being very shallow and exposed to the solvent.

DNA is in a dynamic state and forms Z-DNA when circumstances stabilize it, then relaxing right-handed B-DNA. DNA with alternating dCdG sequences, like poly(dCdG), can interconvert especially easily between the B- and Z-DNA conformations. This interconversion requires substantial change of the purines from *anti* to *syn* conformations plus a change in the handness of the helix. Each time the a DNA segment turns into Z-DNA, two B-Z junctions form (Fig. 25). There is continuous stacking of bases across the junction, except that one base pair at the junction is broken and the bases one each side are extruded. The tendency to form the Z conformation is increased by negative supercoiling, increasing the ionic strength, and by the presence of proteins that bind only to Z-DNA.

1.7 Methods for studying nucleic acid structure

The discovery of the double helix, as Watson and Crick realized[92], immediately provided fundamental and new insights into the nature of genetic events. We now have extensive knowledge of the variety of DNA and RNA structures themselves, together with the manner in which they are recognized by regulatory, repair, and other proteins, as well as by small molecules. All this is giving us more possibilities to understand the basis of processes of gene regulation, transcription and translation, carcinogenesis, and drug action at the atomic and molecular levels.

These advances in nucleic acid structural studies have been largely due to the increased power and sophistication of the experimental approach of X-ray crystallography, which have provided most of the highly detailed structural information to date. The dominance of the crystallographic approach still continues. NMR (nuclear magnetic

resonance) spectroscopy, molecular modeling/simulation, and chemical/biochemical probe techniques also play important roles in providing information on structure, dynamics, and flexibility that can approach near-atomic resolution in at least some of its detail[93].

1.7.1 X-ray crystallography and nuclear magnetic resonance

Short oligonucleotides can be crystallized and their detailed structures have been elucidated using X-ray crystallography. The detailed structures obtained are remarkably variable, however, indicating that the canonical double helix is not a rigid structure but relatively flexible. The DNA structures in crystals appear to be very subject to crystal-packing forces. Structure determination of DNA molecules by NMR does not suffer from these crystal constraints, but the traditional NMR approach is inherently more difficult with DNA than with proteins because DNA molecules are long and cylindrical. In addition, DNA molecules have a lower density of hydrogen atoms. Therefore, the number of short distances of less than 5 Å between hydrogen atoms that can be observed by NMR (NOEs - nuclear Overhauser effects) is comparatively small in oligonucleotides. NOEs are typically found only between adjacent base pairs, so there are usually few long-range NOEs to define the overall structure. As a result, the structure tends to be defined only locally by NMR, and not very accuratelly. Fortunately, the orientation information contained in residual dipolar couplings has been very useful in determining accurate DNA structures.

1.7.2 Molecular modeling and simulation of nucleic acids

The complexities of biological macromolecules and also their flexibilities, make it difficult to elucidate and describe the details of theirs structures and functions using only experimental techniques. All the physical and biological properties of a macromolecule should in theory be predictable from a mathematical description of its energy as a function of the atomic coordinates of its atoms. Such computer simulations offer one way of describing the functions of complex macromolecules.

Crystallographic analysis provide a quasi-static view of molecular structure. The

process of X-ray data acquisition from a single crystal, can take typically several minutes. The vast majority of crystal structures provide a time-averaged picture of molecular motions about the low-energy structure in the crystal. By contrast, molecular modeling techniques enable dynamic changes in structure and conformation to be calculated and visualized in terms of their effects on molecular energetics.

The first step in modeling a molecule is to obtain the appropriate potential function or force field, which relates the position of the molecule's atoms to its energy. Potential functions can be derived for small molecules using quantum mechanical calculations, but this is not practical for macromolecules. Hybrid quantum/molecular mechanics (QM/MM) approaches have made some progress, but generally it is necessary to use approximate and empirical potential functions. Potential functions can be derived from “all – atom” molecular models that treat the interactions between all the atoms of the given molecule, or from simplified models, where forces due to groups of several atoms are treated together as a single unit. Using the corresponding potential functions to analyze molecular properties is known as molecular mechanics (MM) and is used in energy minimization and normal mode analysis methods. Solving the equation of motion of atoms of the basis of the potential functions is known as molecular dynamics (MD) or Brownian dynamics which describes the atomic positions as a function of time and provides dynamic and time-averaged properties. Starting conformations for the search for the global energy minimum can be generated by Monte Carlo calculations and other such sampling approaches[94].

The energetics of macromolecules are described by potential functions that include covalent bondindg and van der Waals interactions, which determine the steric properties of the given molecule, plus eletrostatic interactions, which include hydrogen bonds and other dipolar and ionic interactions, including their compensation by polarization of the solvent around the biomolecule. Electrostatic energies are long-range interactions which are difficult to evaluate, but they can be modeled using implicit models where the solvent molecules and biopolymer's dipoles are represented implicitly. The concept of the uniform dielectric constant is a type of implicit model which is extremely useful with homogenous environments.

The validity of the result obtained by computer simulations always depends upon the accuracy of the energy functions used and the assumptions implicit in the computer modeling. Even when started from experimentally determined structure, it is not trivial to find a simulated structure that is identical to the observed one.

It is not feasible at present to compute conformational or energetic properties for significant lengths of nucleic acid sequence by *ab initio* quantum mechanics. Instead empirical force field methods are widely used. These have been derived from experimental data that describe the energetics of a DNA or RNA molecule in terms of the sum of a number of factors

- van der Waals nonbonded interaction (6-12 in the formalism of eq. 1.)
- bond length and angle distortions
- barriers to rotation about single bond
- Columbic electrostatic contributions from full and partial electrostatic-potencial-derived atomic charges
- Hydrogen-bonding (often incorporated as an implicit part of the electrostatic component)

$$V(\mathbf{r}) \triangleq \sum_{bonds} K_b(b-b_0)^2 + \sum_{angles} K_\theta(\theta-\theta_o)^2 + \sum_{dihedrals} (V_n/2)(1+\cos[n\phi-\delta] + \sum_{nonb\,ij} (A_{ij}/r_{ij}^{12}) - (B_{ij}/r_{ij}^{6}) + (q_i q_j/r_{ij})$$

(eq. 1)

The more recently developed nucleic acid force fields can also include the contributions of polarization effects, which can be especially important when examining the interactions of nucleic acids with drug or protein molecules. The major empirical nucleic acid force fields have been incorporated into algorithms that can be used to minimize the conformation of a molecule with respect to its internal energy. This is the method of molecular mechanics, which in effect optimizes local low-energy minima. Much more extensive explorations of conformations can be made by molecular dynamics (MD), which applies Newton's equations of motion to an empirical force field, for all atoms in a molecule, although, this technique is computer-intensive.[91]

1.7.3 Amber package

In this thesis, to achieve goals of molecular simulations, the Amber 10[95] (Assisted Model Building with Energy Refinement), Amber 11[96] and AmberTools, were used. Amber is the collective name for a suite of programs that allow users to carry out molecular dynamics simulations, particularly on biomolecules. This program package provides a powerful framework for many common calculations[97,98]. The term “amber” is also sometimes used to refer to the empirical force fields that are implemented here[99,100]. The force fields are in the public domain, whereas the codes are distributed under a license agreement.
According the manual of the Amber 11 suite, the following procedure is recommended to obtain MD trajectories for further analysis:

- define Cartesian coordinates for each atom in the system (usually from X-ray crystallography, NMR spectroscopy, or model-building data; in Protein Data Bank (PDB) or Tripos "mol2" format)
- set the "topology" - connectivity, atom names, atom types, residue names, and charges
- choose the force field - parameters for the bonds, angles, dihedrals, and atom types in the system (the standard parameters for several force fields are also found in the amber11 directories)
- prepare commands - the user specifies the procedural options and state parameters desired. These are specified in the input files to the sander or pmemd programs.

Preparatory programs of Amber suite package:

- LEaP is the primary program to create a new system in Amber, or to modify old systems
- ANTECHAMBER is the main program from the Antechamber suite used, if the system contains more than just standard nucleic acids or proteins

Simulation programs of Amber suite package:

- SANDER is the basic energy minimizer and molecular dynamics program. This program relaxes the structure by iteratively moving the atoms down the energy

gradient until a sufficiently low average gradient is obtained. The molecular dynamics portion generates configurations of the system by integrating Newtonian equations of motion. MD will sample more configurational space than minimization, and will allow the structure to cross over small potential energy barriers. Configurations may be saved at regular intervals during the simulation for later analysis, and basic free energy calculations using thermodynamic integration may be performed. More elaborated conformational searching and modeling MD studies can also be carried out using the SANDER module. This allows a variety of constraints to be added to the basic force field, and has been designed especially for the types of calculations involved in NMR structure refinement.

- PMEMD is a version of sander that is optimized for speed, parallel scaling. The name stands for "Particle Mesh Ewald Molecular Dynamics," but this code can now also carry out generalized Born simulations. The input and output have only a few changes from sander.

Analysis programs Amber suite package:

- PTRAJ is a general purpose utility for analyzing and processing trajectory or coordinate files created from MD simulations (or from various other sources), carrying out superpositions, extractions of coordinates, calculation of bond/angle/dihedral values, atomic positional fluctuations, correlation functions, clustering, analysis of hydrogen bonds, etc.
- PBSA is an analysis program for solvent-mediated energetics of biomolecules. It can be used to perform both electrostatic and non-electrostatic continuum solvation calculations with input coordinate files from molecular dynamics simulations and other sources. The electrostatic solvation is modeled by the Poisson-Boltzmann equation. Both linear and full nonlinear numerical solvers are implemented. The nonelectrostatic solvation is modeled by two separate terms: dispersion and cavity.

The basic information flow in Amber, briefly described above, is illustrated by Fig. 22 [96].

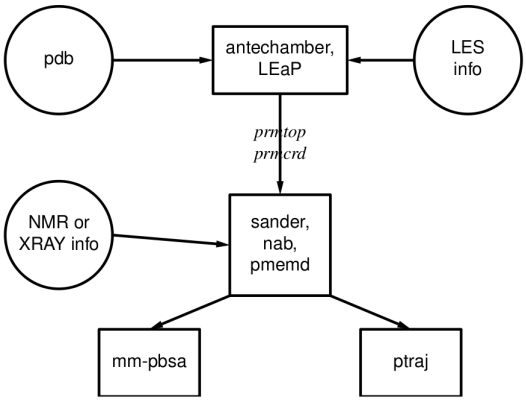

Fig. 22: Basic information flow in Amber

1.7.4 Structural studies of DNA adducts formed by platinum drugs

Understanding the mechanistic basis for the anticancer activity of cisplatin requires knowledge of the structural consequences of the Pt-DNA adducts formed upon drug binding. Due to unprecedented advances of techniques described above, also the exact structures of several DNA duplexes containing different platinum lesions were obtained. Although several structures has been also published for DNA containing adducts of ruthenium[101] or multinucelar complexes[102,103], this section summarizes the most important and well described duplexes bearing cisplatin and oxaliplatin adducts.

Early structural information about the major cisplatin adduct, 1,2-intrastrand d(GpG) crosslink (between adjacent guanines) with a dinucleotide[104], was obtained by X-ray crystallography, validating preliminary NMR solution studies. The nucleobases were in anti conformation, and the two oxygen O6 atoms are on the same side of the platinum coordination plane, i.e. head-to-head orientation. The same group also published the first crystal structure of the cisplatin fragment coordinated to a double-stranded oligonucleotide (a dodecamer), cis-$\{Pt(NH_3)_2\}^{2+}$ -{d(CCTCTG6G7TCTCC)·d(GGAGACCAGAGG)}] (Fig. 23) [105]. They observed rolling of the guanines towards one another by 49°.

Three years after this crystal structure, the NMR structure of the same adduct in solution was resolved[15]. This NMR study showed that the rolling of the guanines towards one another was 61° leading to an overall helix bend angle of 78° and unwinding of the helix at the site of platination of 25°. Common to both structures was the coordination of the *cis*-

$\{Pt(NH_3)_2\}^{2+}$ fragment to G6 and G7 with bending of the duplex significantly towards the major groove without disruption of the Watson-Crick hydrogen bonding. As a result, the minor groove opposite the platinum adduct is widened and flattened, affording a geometry with characteristics of A- and B-type DNA.

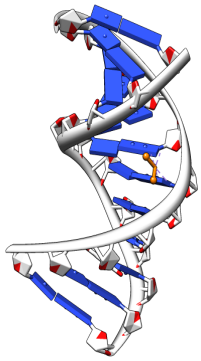

Fig. 23: X-ray structure of the adduct formed by interaction of cisplatin and the DNA duplex (12-mer) (coordinates taken from the Protein Data Bank, 1AIO)

The interstrand crosslink represents only a small fraction of the adducts formed by cisplatin on duplex DNA. Although 1,2-intrastrand d(GpG) cross-link is generally believed to represent the cytotoxic lesion, the ICL may at least also contribute to antitumor effect. Formation of cisplatin ICL occurs best at the GpC step and is expected to cause significant distortion of B-form of DNA due to the long distance between N7 atoms of the guanine bases involved.

These DNA crosslinks have also been a subject of study by NMR and X-ray methods. NMR structures of two different DNA decamers containing interstrand crosslinks, where the platinum is bound to two guanine bases, have been determined[106,107]. One of these structures is illustrated in Fig. 24.

The crosslinked guanine residues are not paired with hydrogen bonds to the complementary cytosines, which are located outside the duplex and not stacked with other aromatic rings. All other base residues are paired, but distortion extends over at least 4 bp at the site of the CL. In addition, the *cis*-diammineplatinum(II) bridge resides in the minor groove and the double helix is locally reversed to a left-handed, Z-DNA-like form (schematically illustrated by Fig. 25, adapted from [90]). This adduct induces not only the helix unwinding by 76-80 ° relative to B-DNA but also the bending of 20-40 ° of the helix axis at the cross-linked site toward the minor groove.

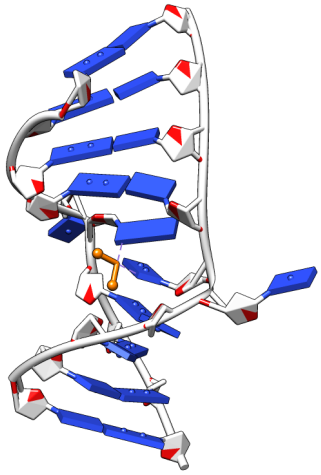

Fig. 24: X-ray structure of the ICL formed by interaction of cisplatin and the DNA duplex (coordinates taken from the Protein Data Bank, 1A2E)

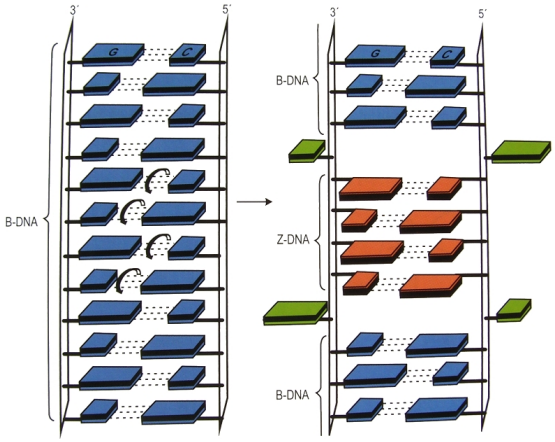

Fig. 25: Schematic illustration of the convertion from B to Z conformation of the DNA duplex

There are also two published structures of oligonucleotide-oxaliplatin adducts for two different dodecamers: a 2.4 Å crystal structure of a 1,2-d(GpG) intrastrand crosslink in a DNA dodecamer duplex formed by reaction with oxaliplatin[108], and a related NMR solution structure[60]. However, the differences in cytotoxic activity of the two platinum complexes are not readily correlated with these structures. Very recently, the solution structures of the DNA dodecamer duplex with and without a cisplatin 1,2-d(GG) intrastrand cross-link, and the detailed comparison with the 1,2-d(GG) intrastrand cross-link from the reaction of the same oligonucleotide with oxaliplatin, had been reported[60,109]. For example, when comparing average solution structures, the platinum-oligonucleotide adduct from reaction of the oligonucleotide with oxaliplatin bends the DNA by 31° (overall bend angle), while the cis-{$Pt(NH_3)_2(GG)$} adduct from cisplatin bends the DNA by 22°[110].

So far, no work has been aimed to study structural properties of DNA duplex containing interstrand crosslink of oxaliplatin. To elucidation of this type of structure contributed this thesis, using molecular dynamics simulations (Paper VI).

2 MOTIVATION AND AIMS OF PHD THESIS

This PhD thesis consists of two parts. Firstly, the experimental part has been focused on the development of specific conditions for *in vitro* NER assay. The second part was concerned on computational techniques as a useful possibility to study DNA structure modified by antitumor compounds.

Thus, the first aim of the presented PhD thesis was to study DNA repair synthesis in vitro. The work outline consisted of developing specific conditions of NER assay, including preparation of several suitable cell free nuclear extracts. The assay was then used to measure the level of DNA repair induced by number of potential antitumor compounds of different nature, which are described in the chapter 1.

Understanding the mechanistic basis for the anticancer activity of cisplatin requires knowledge of the structural consequences of the platinum-DNA adducts formed upon drug binding. So the second aim, was to introduce in our laboratory methods of molecular simulations as a useful complement to experiments. Structural models of several structures of DNA duplexes containing interstrand adducts of different antitumor drugs, were prepared and described.

3 MATERIALS AND METHODS

3.1 Chemicals

3.1.1 Solutions of studied antitumor compounds

The stock solutions of platinum or ruthenium compounds were prepared at the concentration of 5×10^{-4} M in 10 mM $NaClO_4$ and stored at 20°C in the dark. The concentrations of complexes in the stock solutions were determined by flameless atomic absorption spectrometry (FAAS).

- Cisplatin and transplatin were obtained from Sigma (Prague, Czech Republic) (purity was 99.9% based on elemental and ICP trace analysis).
- Complexes *c*is-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and its *trans* isomer (Paper I) were prepared and provided by prof. J. Reedijk, Institute of Chemistry, Leiden University, The Netherlands)[66].
- Complex [$PtCl_2$(*cis*-1,4-DACH)] (paper II) was prepared[111] and provided by prof. G. Natile (Department of Pharmaceutical Chemistry, University of Bari, Italy).
- Complex [{PtCl(DACH)}$_2$-μ-Y]$^{4+}$ (Paper III) was prepared [88] and provided by prof. N. P. Farrell (Virginia Commonwealth University, Richmond, USA).
- Complexes{[*cis*-$Pt(NH_3)_2Cl]_2$(4,4'-methylenedianiline)}$^{2+}$ and {[*cis*-$Pt(NH_3)_2Cl]_2$(α, α'-diamino-p-xylene)}$^{2+}$ (Paper IV) were prepared and provided by prof. Z. Guo[89,112] (Nanjing University, School of Chemistry and Chemical Engineering, China).
- Complexes [Ru^{II}(η^6-arene)-(Cl)(en)]$^+$ (Paper V) were prepared[83] and provided by prof. P. J. Sadler (University of Warwick, UK).

3.1.2 Other chemicals and biological material

- Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 20,000 kDa) was prepared and characterized as described previously[113].
- Plasmids, pUC19 [2686 base pairs (bp)], pSP73KB [2464 bp] and pBR322 [4361 bp] were isolated according to standard procedures.
- Enzymes T4 DNA ligase, Klenow fragment from DNA polymerase I (KF^-), restriction endonucleases EcoRI, HindIII, NdeI, SspI, Circum VentTM Thermal Cycle Sequencing Kit with Vent(exo^-), DNA polymerase and T4 polynucleotide kinase (New England Biolabs; Beverly, MA).
- DMSO, 3-(4,5-dimethylthiazol-2-yl)-2,5-difenyl tetrazolium bromid (MTT), EZBlue gel staining reagent, β-estradiol, progesteron a glutathion (Sigma; Prague, Czech Republic).
- Proteinase K and ATP (Boehringer; Mannheim, Germany)
- Sephadex G-50 coarse (Amersham Biosciences; UK)
- Agarose (FMC BioProducts; Rockland, ME)
- Electrophoresis-grade acrylamide, N,N'-methylenebisacrylamide, ethidium bromide (EtBr), urea, and dithiothreitol (MercK KgaA; Darmstadt, Germany)
- Expression and purification of recombinant rat full-length HMGB1 protein and its domains A (residues 1–84[114]) and B (residues 85–180[114]) (HMGB1a and HMGB1b, respectively) were carried out as described[22,114]
- Nonidet P-40 (Fluka; Prague, Czech Republic)
- Sodium dodecyl sulfate (SDS) (Serva; Heidelberg, Germany)
- MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (Calbiochem;Darmstadt, Germany)
- Radioactive products were from Amersham (Arlington Heights, IL, USA)
- Cell-free extracts (CFEs) were prepared from the repair-proficient HeLa (human cervical carcinoma) or AA8 (chinese hamster ovary) as described below (cell lines provided by Prof. B. Keppler; Univesity of Vienna, Austria)
- Streptavidin-coated magnetic beads (Dynabeads) and biotin-14-dATP (Invitrogen Dynal AS; Oslo, Norway).

3.2 Platination reactions in cell-free media

Calf thymus or plasmid DNAs were incubated with the platinum complexes in 10 mM $NaClO_4$ at 37°C in the dark. After 24 h, the samples were exhaustively dialyzed against the medium required for subsequent biochemical or biophysical analysis. An aliquot of these samples was used to determine the value of r_b (r_b is defined as the number of molecules of the platinum complex bound per nucleotide residue) by flameless atomic absorption spectrophotometry (FAAS), or by differential pulse polarography (DPP). The duplexes containing site-specific adduct of studied platinum or ruthenium complexes were prepared according the procedure described in [7]. The platinated or ruthenated oligonucleotides were purified by ion-exchange high-pressure liquid chromatography (HPLC). It was verified by platinum FAAS and by the measurements of the optical density that the modified oligonucleotides contained one platinum atom. The coordination of platinum compounds to the N7 atoms of the desired guanines was also verified using dimethyl sulfate (DMS) footprinting of platinum on DNA [115][116].

3.3 Sequence preference of DNA adducts

The primer extension footprinting assay was used to evaluate the sequence selectivity of DNA modification by the studied compounds. The fragment of pSP73 DNA linearized by HpaI (2464 bp) was incubated with platinum complexes in 10 mM $NaClO_4$ for 24 h at 37°C to obtain r_b = 0,005 . The excess of drug was removed by ethanol precipitation. Circum VentTM Thermal Cycle Sequencing Kit with Vent(exo^-) DNA polymerase was used along with the protocol for thermal cycle DNA sequencing with 5'-end-labeled 20-mer SP6 primer recommended by the manufacturer with small modifications[117]. The synthesis products were separated by electrophoresis on a denaturing polyacrylamide (PAA) gel (6% PAA/ 8 M urea); sequence ladders were obtained in parallel using untreated control DNA fragment.

3.4 Interstrand crosslink assay

The complexes at varying concentrations were incubated for 24 h with 500 ng of plasmid DNA after it had been linearized by resctriction enzyme and 3'- end labeled by means

of KF^- and [α-^{32}P]dATP. The amount of interstrand crosslinks was analyzed by electrophoresis under denaturing conditions on alkaline agarose gel (1%). After the electrophoresis was completed, the intensities of the bands corresponding to single strands of DNA and interstrand crosslinked duplex were quantified. The frequency of interstrand crosslinks, (% of interstrand crosslinks)/Pt (the number of interstrand crosslinks per adduct), was calculated as %of interstrand crosslinks/Pt = XL/(number of bp for plasmid)·rb[116]. XL is the number of interstrand crosslinks per molecule of the linearized DNA duplex which was calculated assuming Poisson distribution of the interstrand crosslinks as XL = - ln A, where A is the fraction of molecules running as a band corresponding to the non-cross-linked DNA.

3.5 Unwinding of negatively supercoiled DNA

Unwinding of closed circular supercoiled plasmid DNA was assayed by an agarose gel mobility shift assay[118]. The unwinding angle Φ, induced per Pt–DNA adduct was calculated upon the determination of the r_b value at which the complete transformation of the supercoiled to relaxed form of the plasmid was attained. Samples of plasmid DNA were incubated with platinum compounds at 37°C in the dark for 24 h. The samples were subsequently subjected to electrophoresis on 1% agarose gels running at 25°C in the dark with Tris-acetate buffer (TAE) buffer. The gels were then stained with EtBr, followed by photography with transilluminator.

3.6 DNA melting

The melting curves of CT DNAs were recorded by measuring the absorbance at 260 nm. The melting curves were recorded in a medium containing 10 mM or 0.1 M Na^+ with 1 mM Tris–HCl/0.1 mM Na_2H_2EDTA, pH 7.4. The value of the melting temperature (t_m) was determined as the temperature corresponding to a maximum on the first derivative profile of the melting curves.

3.7 Other physical methods

Absorption spectra were measured with a Beckmann DU- 7400 spectrophotometer. The melting curves were recorded using a Varian Cary 4000 UV–VIS spectrophotometer equipped with a thermoelectrically controlled cell holder and quartz cells with a pathlength of 1 cm. FAAS measurements were carried out with a Unicam 939 AA spectrometer with a graphite furnace. DPP curves were recorded with the aid of an EG&G PARC model 384B electrochemical analyzer. Isothermal circular dichroism spectra of CT DNA modified by platinum complexes were recorded at 25 °C in 10 mM NaClO4 busing a JASCO J- 720 spectropolarimeter equipped with a thermoelectrically controlled cell holder. The cell pathlength was 1 cm. Spectra were recorded in the range 230–500 nm in 0.5-nm increments with an averaging time of 1 s. For the interstrand crosslinking assay, the gels were dried and visualized using a FUJIFILM BAS 2500 bioimaging analyzer, and the radioactivities associated with bands were quantitated with AIDA Image Analyzer (Raytest, Germany).

3.8 Reactions with sulfur-containing compounds

Reactions of GSH and mammalian MT-2 with antitumor compounds were investigated by monitoring UV absorption at 260 nm of solutions containing the platinum complex and GSH or MT-2 exactly as described in literature [119][120]. The absorbance at 260 nm reflects the presence of platinum–sulfur and disulfide bonds. The platinum compounds (from aged stock solutions equilibrated in 10 mM $NaClO_4$) were mixed with GSH or MT-2 at 37°C in the medium of 10 mM $NaClO_4$ plus 0.1 mM phosphate buffer, pH 7.0, in the dark. Reactions were initiated by mixing the platinum complex with the buffer followed by immediate addition of GSH or MT-2. The kinetic data were fitted by non-linear regression (GraphPad Prism) to one-phase and two-phase exponential association. The decision as to which fit was more appropriate for each dependence was made by comparing the fits of the two equations by using an F-test (GraphPad Prism).

3.9 DNA repair synthesis by cell extracts

The *in vitro* repair assay relies on the incorporation of radiolabeled deoxyribonucleotide during the repair synthesis assay step. Supercoiled plasmid DNA damaged by various antitumor compound is incubated with cell extract in a reaction mixture including the four dNTPs, one of them radiolabeled, ATP, and an ATP-regenerating system. Undamaged plasmid DNA of a slightly different size is added to the reaction mixture as a inner control. DNA repair synthesis is determined after recovery of plasmid DNA from the mixture, linearization with a restriction enzyme, agarose-gel electrophoresis, autoradiography and the measurement of the radioactivity incorporated into each plasmid. The procedure of the experiment is illustrated in Fig. 26.

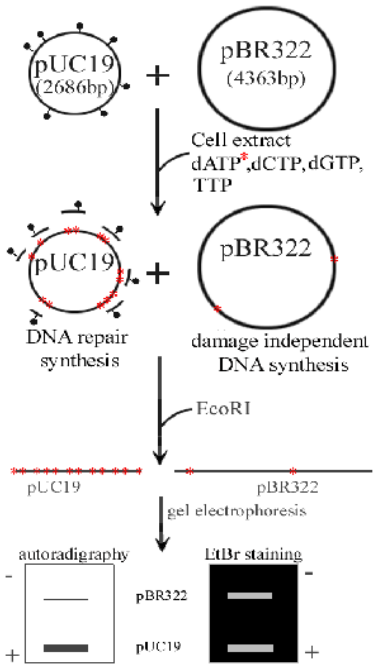

Fig. 26: *In vitro* repair synthesis assay

DNA repair synthesis of CFEs was assayed according to literature[98]. Each reaction of 50 µl contained 600 ng of nonmodified or platinated plasmid pSP73 or PUC19, 600 ng of nonmodified pBR322, 2 mM ATP, 30 mM KCl, 0.05 mg/mL rabbit muscle creatine phosphokinase, 20 mM of each dGTP, dATP and TTP, 8 mM dCTP, 74 kBq of [α-^{32}P]dCTP

(alternatively 20 mM of each dGTP, dCTP and TTP, 8 mM dATP, 74 kBq of [α-^{32}P]dATP) in the buffer composed of 40 mM HEPES–KOH, pH 7.5, 5 mM $MgCl_2$, 0.5 mM dithiothreitol, 22 mM creatine phosphate, 70 mM potassium glutamate, 1.4 mg bovine serum albumin/mL and 20 μg of CFE. Reactions were incubated for 4 h at 30°C and terminated by adding EDTA to a final concentration of 20 mM, SDS to 0.6% and proteinase K to 250 μg/mL and then incubating for 30 min. The products were extracted with 1 volume of 1:1 phenol:chloroform. The DNA was precipitated from the aqueous layer by the addition of 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol. After 30 min of incubation at -20°C and centrifugation at 12,000 g for 30 min at 4°C, the pellet was washed with 0.2 mL 70% ethanol and dried. DNA was finally linearized by SspI before electrophoresis on a 1% agarose gel. The resulting gel was stained by SybrGreen® or EtBr according to the manufacturer's protocol. The amount of DNA in each bend was quantitated by densitometry and the radioactivity associated with the bands was also quantitated, using AIDA Image Analyzer .

3.10 Preparation of cell free extract CFE

The HeLa (human cervical carcinoma) or AA8 (chinese hamster ovary) were used for preparation of CFEs. The cells were collected from confluent monolayer cultures. After the removal of the culture medium, they were washed by pipetting sufficient PBS (phosphate-buffered saline) to cover them, swirled gently, the PBS then poured off. All steps were performed on ice. The cells were scraped into fresh PBS, pooled in centrifuge tubes and pelleted by centrifuging (200 g, 10min, 0°C). The supernatants were discarded and the pellets were rapidly resuspend in 1 ml of lysis buffer I (composition shown bellow) and allowed to swell on ice 10 min. After centrifugation (800 g, 4 minutes, 0°C) the pellet was carefully re-suspended by 1 ml of lysis buffer II and again centrifuged (300 g, 4 minutes, 0°C). The nuclear extraction buffer was added into the pellet and the sample was slowly tilted on a tiltboard for 30 minutes at 4°C. After final centrifugation (12 000 g, 15 minutes, 0°C) the aliquots from the supernatant were prepared and imediately frozen at -80°C.

Lysis buffer I:
Tris base (10 mM), KCl (60 mM), EDTA (1,2 mM)
DTT (1 mM), PMSF (0,1 mM) , NP-40 (0,1 mM) (added immediately before use)

Lysis buffer II:
Tris base (10 mM), KCl (60 mM), EDTA (1,2 mM)
DTT (1mM) (added immediately before use)

Nuclear extraction buffer:
Tris base (20 mM), NaCl (420 mM), $MgCl_2$ (0,7 mM), EDTA (0,25 mM), Glycerol (25% (v/v)

3.11 Molecular modeling

The molecular mechanics calculations and MD simulations used the SANDER module of the program suites AMBER10[95] and AMBER11[96]. The parmbsc0 forcefield[122] was supplemented by parameters describing the platinum coordination sphere as described previously or by parameters calculated using program Mopac2009[123]. After preparation of starting structures, appropriate number of sodium counterions was added using the XLEaP module, to neutralize the system. Structures were then subjected to an energy minimization in vacuo. These energy-minimized structures were solvated in a periodic box of randomly oriented TIP3P water molecules having the form of a rectangular box of ~100.5 × ~71.6 × ~63.4 Å, and subsequently relaxed using MD simulations. The protocol for the MD runs[124], [125] are described in tables 1 and 2. Slight differencies in preparation of starting structures and MD cycles are summarized in next two chapters and tables.

3.11.1 Duplexes bearing two enantiomers of oxaliplatin and cisplatin as a control

Structures were generated from the X-ray based model of the decamer d(C1C2T3C4G*5C6T7C8T9C10)-d(G11A12G13A14G*15C16G17A18G19G20) with the central interstrand cis-Pt(NH3)2-G*G* crosslink (PDB entry 1A2E)[107]. The C4G17 pair was replaced by a T8A33 pair using exchange of residues in the program MolMol[126]. Both

ends were extended with canonical B-DNA duplexes created by the NUCGEN module of AMBER10 to produce the cisplatinated 20mer d(C1T2C3T4C5C6-T7T8G*9C10T11C12T13C14C15T16T17C18T19C20) d(G21A22G23A24A25G26G27-A28G29A30G*31C32A33A34G35G36A37G38A39G40). The Pt(R,R- DACH)$^{2+}$ and Pt(S,S-DACH)$^{2+}$ adducts were constructed by least-squares fitting of the Pt(DACH)$^{2+}$ moiety (derived from Density Functional Theory calculations)[127] into the structure of starting X-ray based structure (using the three-atoms unit cis-PtN_2 as reference in the program MolMol).

3.11.2 Duplexes bearing complex {[*cis*- Pt$(NH_3)_2Cl]_2$(4,4'-methylenedianiline)}$^{2+}$

Canonical B-DNA 22-mers were prepared using module NAB. Their sequences and positions (blue lines) of desired interstrand crosslinks formed by complex are illustrated by Fig. 27 (chemical formula of the complex by the Fig. 28). Structure of the compound and two guanine residues (connected through N7 nitrogens of the bases and Pt centers of the complex) was built in program Avogadro[128] and subsequently minimized. The complex with attached guanines was divided into two parts, which were fitted to corresponding desired guanine residues in canonical duplexes using least-square fitting in program Sirius[129]. The values of charges of atoms, bond lengths, bond angles and dihedral angles were computed by program Mopac2009 (using of the PM6 Hamiltonian with corrections for dispersion and hydrogen-bonding[130]).

Fig. 27: Schematic illustration of DNA duplex sequences with crosslinks at desired positions

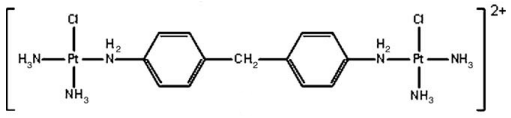

Fig. 28: Dinucelar platinum complex with cis geometry

The particle-mesh Ewald (PME) method using a charge grid spacing of approximately 1 Å with cubic B-spline interpolation and sum tolerance of 10 − 6 Å was used to calculate the electrostatic energy. A 9 Å cutoff was applied to Lennard–Jones nonbonded terms and for Ewald summations. The temperature was kept at (300 ± 2) K using the Berendsen coupling algorithm. Center of motion was removed every 100 steps of calculation. The SHAKE algorithm was applied to constrain all bonds involving hydrogen atoms. Structures were saved every 1 ps during the production period to obtain the trajectory file. The PTRAJ module of AmberTools 1.4 was used to determine average structures over the whole production period or for different conformational families. Before structural analysis, the averaged structures were submitted to an energy minimization using 100 steps of steepest descent energy minimization with a Generalized Born implicit solvent representation. The analysis of trajectories employed the program CURVES+[131] for the calculation of helical parameters and the backbone torsion angles. The programs VMD[132], MolMol[126], Pymol[79], Chimera[134], Sirius[129] were used for superposition and visualizations of molecules. The XmGrace program[94] has been used to plot all graphs.

Step of molecular dynamics/ energy minimization	Time [ps]	Thermodynamics conditions	Constraint - DNA-Pt [kcal·mol^{-1}·Å^{-2}]	Constraint - ions [kcal·mol^{-1}·Å^{-2}]	Torsion /distance constraint [kcal·mol^{-1}·Å^{-2}]
Minimization 1					-
Heating	100	NVT, 100K to 300K	25	25	-
Minimization 2			5	5	-
Equilibration 1	50	NPT, 300K	5	5	-
Minimization 3			4	4	-
Equilibration 2	50	NPT, 300K	4	4	-
Minimization 4			3	3	-
Equilibration 3	50	NPT, 300K	3	3	-
Minimization 5			2	2	-
Equilibration 4	50	NPT, 300K	2	2	-
Minimization 6			1	1	-
Equilibration 5	50	NPT, 300K	1	1	-
Equilibration 6	50	NPT, 300K	0.5	0.5	-
Production 1	50	NPT, 300K	-	-	-
Production 2	20 000	NPT, 300K	-	-	-

Table 1: MD simulation protocol for duplexes bearing complex $\{[cis\text{-}Pt(NH_3)_2Cl]_2(4,4'\text{-methylenedianiline})\}^{2+}$

Step of molecular dynamics/ energy minimization	Time [ps]	Thermodynamics conditions	Constraint - DNA-Pt [kcal·mol^{-1}·Å^{-2}]	Constraint - ions [kcal·mol^{-1}·Å^{-2}]	Torsion /distance constraint [kcal·mol^{-1}·Å^{-2}]
Minimization 1					-
Heating	100	NVT, 100K to 300K	25	25	-
Minimization 2			5	5	-
Equilibration 1	50	NPT, 300K	5	5	-
Minimization 3			4	4	-
Equilibration 2	50	NPT, 300K	4	4	-
Minimization 4			3	3	-
Equilibration 3	50	NPT, 300K	3	3	-
Minimization 5			2	2	-
Equilibration 4	50	NPT, 300K	2	2	-
Minimization 6			1	1	-
Equilibration 5	50	NPT, 300K	1	1	-
Equilibration 6	50	NPT, 300K	0.5	0.5	-
Production 1	50	NPT, 300K	-	-	-
Production 2	260	NPT, 300K	-	-	Torsion - 50*
Production 3	1000	NPT, 300K	-	-	Distance –10/20**

*	Torsion constraints used :	1-PtRRDACH	1-PtSSDACH	1-cisPt
	50 kcal·mol^{-1}·Å^{-2}	C_6: C3'-O3'-P-O5'$_{(n+1)}$ C_6: C4'-C3'-O3'-P T_7: $_{(n-1)}$O3'-P-O5'-C5'	C_6: C3'-O3'-P-O5'$_{(n+1)}$ C_6: C4'-C3'-O3'-P T_7: $_{(n-1)}$O3'-P-O5'-C5'	C_6: C3'-O3'-P-O5'$_{(n+1)}$ C_6: C4'-C3'-O3'-P T_7: $_{(n-1)}$O3'-P-O5'-C5'

**	Distance constraints used :	1-PtRRDACH and 1-PtSSDACH	1-cisPt
	20 kcal·mol^{-1}·Å^{-2}	G_9O4'-T_{11}N1 G_{31}O4'-A_{33}C4 G_9O4'-T_{11}N3 G_{31}O4'-A_{33}C5 G_9O4'-T_{11}C5 G_{31}O4'-A_{33}O4 G_9O4'-T_{11}O4'	G_9O4'-T_{11}N1 G_{31}O4'-A_{33}C4 G_9O4'-T_{11}N3 G_{31}O4'-A_{33}C5 G_9O4'-T_{11}C5 G_{31}O4'-A_{33}O4' G_9O4'-T_{11}O4'
	10 kcal·mol^{-1}·Å^{-2}	-	$A_{34}O_4$' -N (proximate NH_3ligand)

Table 2. MD simulation protocol for duplexes bearing two enantiomers of oxaliplatin

4 SUMMARY OF RESULTS AND DISCUSSION

Here presented PhD thesis is based on 6 papers published in international journals. The originals of these papers with comments on author's contribution are enclosed in the part Appendix. In this sections, the overall results which were obtained, are briefly summarized. The last chapter also contains short summary of new results which are just prepared for publication.

4.1 DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[PtCl2(isopropylamine) (1-methylimidazole)]: relations to their different antitumor effects (Paper I)

The original cis/trans empirical structure–pharmacological activity relationships for mononuclear bifunctional platinum complexes considered transplatin *[trans*-diamminedichlor- idoplatinum(II)] (Fig. 11) and its analogs to be clinically inactive, whereas cis-oriented complexes, such as cisplatin [*cis*-diamminedichloridoplatinum(II)] and its analogs were considered to be antitumor-active[63]. To continue in the design of new, more effective antitumor *trans*-platinum drugs, it is of interest to understand differences in the details of the molecular and biochemical mechanisms of the biological effects of such trans-platinum compounds and their *cis* counterparts.

We compared alterations in DNA induced by *c*is-[$PtCl_2$(isopropylamine)(1-methylimidazole)] (1) and its trans isomer (2) (Fig. 10 in section 1) in cell-free media and recognition and repair of these alterations. In addition, we also examined inactivation of these asymmetric platinum(II) complexes by a sulfur-containing compound. Earlier cytotoxicity studies had revealed that the replacement of the NH_3 groups in cisplatin by the azole and isopropylamine ligands lowers the activity of cisplatin in both sensitive and resistant cell lines. To contribute to understanding why, on one hand, replacement of both ammonia groups in cisplatin by isopropylamine and azole ligands results in a reduced potency, whereas, on the other hand, the same replacement in transplatin markedly enhances cytotoxicity in a number of tumor cell lines, the experiments described in the present work were carried out.

The results show that this replacement does not considerably affect the DNA modifications by this drug, recognition of these modifications by HMGB1 protein, their repair, and reactivity of the platinum complex with GSH. These results were interpreted to mean that the reduced activity of this analog of cisplatin in tumor cell lines is due to factors that do not operate at the level of the target DNA. In contrast, earlier studies had shown that the replacement of the NH_3 groups in the clinically ineffective trans isomer (transplatin) by the azole and isopropylamine ligands results in a radical enhancement of its activity in tumor cell lines. Importantly, this replacement also markedly alters the DNA binding mode of transplatin, which is distinctly different from that of cisplatin, but does not affect reactivity with GSH. Hence, the results of the present work are consistent with the view and support the hypothesis systematically tested by us and others that platinum drugs that bind to DNA in a

fundamentally different manner from that of conventional cisplatin may have altered pharmacological properties.

4.2 Cytotoxicity, cellular uptake, glutathione and DNA interactions of an antitumor large ring Pt chelate complex incorporating the 1, 4-diaminocyclohexane carrier ligand (paper II)

A primary objective of this work was to understand more fully the molecular mechanism of action of [$PtCl_2$(*cis*-1,4-DACH)] (DACH = diaminocyclohexane) (Fig. 8). Earlier studies have described promising antitumor activity of this large-ring chelate complex. Encouraging antitumor activity of this analogue of cisplatin prompted us to perform studies focused on the mechanistic basis of pharmacological effects of this drug. Four early steps in the mechanism of biological activity of cisplatin and its analogues have been delineated, namely: cell entry, reactions with sulfur-containing compounds, platinum– DNA binding and processing of platinated DNA by proteins (enzymes) and DNA repair. The comparative experiments (involving also cisplatin) showed

- improved cytotoxicity of [$PtCl_2$(*cis*-1,4-DACH)] in human tumor ovarian cell lines
- enhanced cellular uptake (~1.5-fold) of [$PtCl_2$(*cis*-1,4-DACH)]
- somewhat enhanced rate of reactions of [$PtCl_2$(*cis*-1,4-DACH)] with GSH (~1.5-fold), but a similar rate of reactions with MT-2
- enhanced rate of DNA binding of [$PtCl_2$(*cis*-1,4-DACH)] in cell-free media (~2-fold)
- similar sequence preference of DNA binding of [$PtCl_2$(cis-1,4-DACH)] in cell-free media
- identical DNA interstrand crosslinking efficiency (6%)
- similar bending (32°) and enhanced local unwinding (~1.5-fold) induced in DNA by the major 1,2-GG-intrastrand cross-link
- markedly enhanced inhibiting effects of DNA adducts of [$PtCl_2$(*cis*-1,4-DACH)] on processivity of DNA polymerase

- a slightly lower efficiency of DNA repair systems to remove the adducts of the complex from DNA.

The cytotoxicity caused by DNA damage is the result of many events. It is not a simple task to reveal all aspects of the mechanism underlying antitumor effects of platinum complexes, and it is apparently incorrect to attribute cytotoxicity to their single property. Further studies are therefore warranted to reveal a relative contribution of all potential factors contributing to the potency of [$PtCl_2$(*cis*-1,4- DACH)] in various types of cancer cells.

4.3 Conformation and recognition of DNA modified by a new antitumor dinuclear PtII complex resistant to decomposition by sulfur nucleophiles (Paper III)

The primary objective of this study was to understand more deeply the molecular mechanism of action of antitumor dinuclear PtII complex [{PtCl(DACH)}$_2$-μ-Y]$^{4+}$ [DACH = 1,2- diaminocyclohexane, Y =$H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2$] (here abbreviated "complex 1", Fig. 13). Experiments, which were performed, studied the DNA binding mode of complex 1 in cell-free media, the effects of DNA adducts of complex 1 on processivity of RNA polymerase II (the enzyme RNA pol II has been a major focus of the experiments designed to investigate cellular responses to DNA damage by platinum drugs), repair of DNA adducts of complex 1 (since the integrity of this process in human cells is a key indicator of the sensitivity of a tumor to platinum-based therapy), reactivity of complex 1 with GSH, which has been chosen for investigation in this study because of its role as a determinant of cellular sensitivity to a wide variety of drugs and cytotoxic agents and cellular accumulation of 1. For comparative purposes most of the experiments performed with complex 1 were also performed with BBR3610 [{*trans*-PtCl$(NH_3)_2$}$_2$-μ-{*trans*-$(H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2)$}]$(NO_3)_4$, to further study its mechanism of action as the complex exhibits a remarkable efficacy against glioma and colon cancer cells in culture and animal models [136,137].

The main results have shown, that DNA adducts of dinuclear complex 1 not only block transcription markedly more efficiently than those of cisplatin, but also repair of

adducts of complex 1 by the mammalian repair systems is markedly more reduced relative to repair of cisplatin (Fig. 29). Since DNA adducts of complex 1 can largely escape repair and yet inhibit very effectively transcription, they should persist longer than those of cisplatin. Hence, they could trigger a number of downstream cellular effects different from those triggered in cancer cells by DNA adducts of cisplatin. This might lead to the therapeutic effects that could radically improve chemotherapy by platinum complexes or could be at least an alternative to chemotherapy by platinum complexes already used in the clinic. Together, the findings of the present work help understand differential pharmacological effects of 1 or BBR3610 and conventional mononuclear cisplatin and thereby make new insights into mechanisms associated with antitumor effects of dinuclear/trinuclear platinum complexes possible.

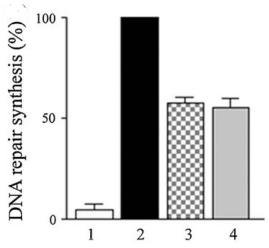

Fig. 29: In vitro DNA repair synthesis assay (for further description see the Paper III)

4.4 Mechanistic insights into antitumor effects of new dinuclear cis Pt(II) complexes containing aromatic linkers (Paper IV)

The polynuclear platinum compounds represent a class of new antitumor metallodrugs that is structurally distinct from conventional cis-diamminedichloridoplatinum(II) (cisplatin) and its mononuclear analogs, and whose clinical profile and mechanism of action are different from these established platinum mononuclear compounds.

The primary objective of this paper was to understand more deeply the molecular mechanism underlying different antitumor effects of dinuclear PtII complexes containing aromatic linkers of different length, {[*cis*- $Pt(NH_3)_2Cl]_2$(4,4'-methylenedianiline)}$^{2+}$ (**1**) and {[*cis*-$Pt(NH_3)_2Cl]_2$(α, α'-diamino-p-xylene)}$^{2+}$ (**2**). (formulas are shown in the Fig. 30).

Results obtained with the aid of methods of molecular biophysics and pharmacology reveal differences and new details of DNA modifications by **1** and **2** and recognition of these modifications by cellular components. The results indicate that the unique properties of DNA interstrand cross-links of this class of polynuclear platinum complexes and recognition of these crosslinks may play a prevalent role in antitumor effects of these metallodrugs. The enhanced resistivity of DNA adducts of **1** and **2** against repair processes appears to be an important factor contributing to ability of **1** and **2** to overcome resistance toward cisplatin. Moreover, the results show for the first time a strong specific recognition and binding of high-mobility-group-domain proteins, which are known to modulate antitumor effects of clinically used platinum drugs, to DNA modified by a polynuclear platinum complex.

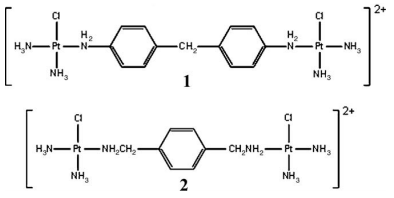

Fig. 30: Dinuclear PtII complexes containing aromatic linkers

4.5 Energetics, Conformation, and Recognition of DNA Duplexes Modified by Monodentate RuII Complexes Containing Terphenyl Arenes (Paper V)

Recently, new complexes of the type $[Ru^{II}(\eta^6\text{-arene})\text{- (Cl)(en)}]^+$ (arene = ortho-, meta-, or para-terphenyl), described in chap. 1, have been synthesized to investigate the effect on cytotoxicity and DNA binding of structural isomerization of the multiring terphenyl arene ligand[74]. Importantly, the complex containing para-terphenyl as the arene ligand (complex **1**, Fig. 12) exhibits promising cytotoxic effects in several human tumor cell lines, including those resistant to conventional cisplatin. In contrast, complexes containing meta- or ortho-terphenyl arene ligands (complexes **2** and **3**, respectively; Fig. 12) are much less cytotoxic.

The results of our initial work have further supported the view that the presence of the arene ligand capable of noncovalent, hydrophobic interaction with DNA considerably

enhances cytotoxicity in tumor cell lines. Herein, we have studied the thermodynamic properties, conformation, and recognition of DNA duplexes uniquely and site-specifically modified by the two monodentate RuII arene complexes **1** and **2** to elucidate in detail the DNA-binding mode of these compounds. We compare previously obtained cytotoxicity data with new data obtained in the present work on conformational distortions induced by single, site-specific monofunctional adducts of the RuII in short oligodeoxyribonucleotide duplexes, associated alterations in the thermodynamic stability of these duplexes, recognition and repair of these adducts by two specific proteins, that is, the important factors that modulate the antitumor effects of antitumor metallodrugs already used in clinic.

An analysis of conformational distortions revealed more extensive and stronger distortion and concomitantly greater thermodynamic destabilization of DNA by the adducts of nonintercalating 2. Moreover, affinity of replication protein A to the DNA duplex containing adduct of **1** was pronouncedly lower than to the adduct of 2. On the other hand, another damaged-DNA binding protein, xeroderma pigmentosum protein A, did not recognize the DNA adduct of **1** or **2**. Importantly, the adducts of **1** induced a considerably lower level of repair synthesis than the adducts of **2**, which suggests enhanced persistence of the adducts of the more potent and intercalating **1** in comparison with the adducts of the less potent and nonintercalating **2**. Also interestingly, the adducts of **1** inhibited DNA polymerization more efficiently than the adducts of **2**, and they could also be bypassed by DNA polymerases with greater difficulty.

Results of the present work along with those previously published support the view that monodentate RuII arene complexes belong to a class of anticancer agents for which structure–pharmacological relationships might be correlated with their DNA-binding modes.

4.6 Platinum–DNA interstrand crosslinks: Molecular determinants of bending and unwinding of the double helix (PAPER VI)

In this work, we have used MD simulations to identify the molecular origin of the different geometrical features observed experimentally[62] for the *cis*-$Pt(NH_3)_2^{2+}$-GG, Pt(R,R-$DACH)^{2+}$-GG, and Pt(S,S- $DACH)^{2+}$-GG interstrand crosslinks formed at a $d(GC)_2$ sequence of the same DNA duplex.

Cisplatin [*cis*-$Pt(NH_3)_2^{2+}$] and oxaliplatin [Pt(R,R-$DACH)^{2+}$] are platinum complexes that belong currently to the most frequently used anticancer drugs. While the molecular structures of the two drugs are relatively similar, the biological activities show some important differences. Cisplatin as well as oxaliplatin have two labile binding sites and are thus capable of forming bifunctional DNA lesions, crosslinking two residues, including both intrastrand and interstrand.

Molecular dynamics simulations of three DNA duplexes with interstrand cross-links of *cis*- $Pt(NH_3)_2^{2+}$, Pt(RR-$DACH)^{2+}$ and Pt(SS-$DACH)^{2+}$, respectively, were carried out with explicit inclusion of water. The simulations started with an X-ray based model[35] and the dynamics were performed for 1 ns for each structure. We have found out that the steric bulk and hydrophobic nature of the cyclohexyl ring induce subtle but significant reorganization of the near environment of the cross-links, which explains why the geometric perturbations of the DNA double helix are different in the different complexes.

The cis-$Pt(NH_3)_2^{2+}$ interstrand adduct is stabilized, as we found out while re-analyzing the published X-ray structure[107] , by lone-pair–π interactions within each DNA-strand. The physical origin of lone-pair-π interactions has not yet been fully evaluated and may include a complex combination of weak σ-bonding with dispersion forces. An adequate description may prove fairly difficult, and will possibly require recourse to the QM/MM strategy. Notwithstanding these difficulties in portraying platinum-DNA interstrand adduct structures, we were able to identify the reasons for which the $Pt(DACH)^{2+}$ adducts cannot adopt the same structure as the *cis*-$Pt(NH_3)_2^{2+}$ adduct.

The replacement of the two NH_3 ligands by a DACH ligand, in the model for the *cis*-$Pt(NH_3)_2^{2+}$ adduct led to clashes between the DACH ligand and the sugar of the cytidine C12 of the crosslinked sequence (Fig. 31). MD simulations of the adducts, although they were accounting for the above mentioned lone-pair–π interactions only in a very improvised

manner, revealed two principal ways of adaptation to the bulky DACH ligand: one consisting mainly in untwisting the T11/A28–C12/G29 step and the other combining an in-plane translation of the T11/A28 and C12/G29 base-pairs against each other along the shift and slide vectors with a local BI→BII transition. The in-plane movements of the base-pair C12/G29 against T11/A28 involved in each mode of adaptation are followed by the whole arm of the kinked helix. Thus, while the first mode results in stronger global unwinding, the second causes stronger bending toward the minor groove. Comparison with the experimentally determined bending and unwinding angles enabled us to assign the first mode to the Pt(R,R-DACH)$^{2+}$, and the second mode to the Pt(S,S- DACH)$^{2+}$ adduct.

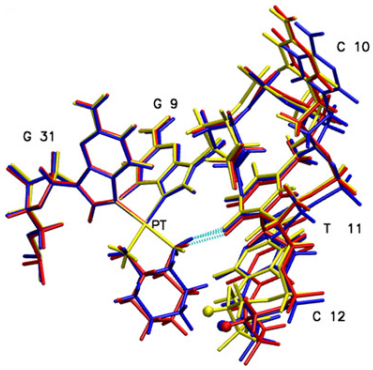

Fig. 31: Clashes between the DACH ligand and the sugar of the cytidine C12 (for further description see paper VI)

4.7 Structural analysis of DNA interstrand crosslinks with unique properties formed by new dinuclear platinum complex (publication in preparation)

The recent results published[138] and described in the Paper IV have shown that the unique properties of DNA interstrand crosslinks of newly synthesized compound from class of polynuclear platinum complexes and recognition of these crosslinks may play a prevalent role in antitumor effects of this metallodrug. Moreover, the results show for the first time a strong specific recognition and binding of high-mobility-group domain proteins, which are known to modulate antitumor effects of clinically used platinum drugs, to DNA modified by a polynuclear platinum complex. Thus, these interesting results, motivated us to further

investigate this dinucelar platinum complex (Fig. 33).

So the goal was to shed light in the structural alterations, which these specific compounds induce in DNA. Firstly, the molecular biology and biochemical work was preformed, to narrow relatively large number of possibilities of binding modes of the studied complex. Due to nature of the compound, especially due to its flexible linker, interstrand crosslinks of different lengths, involving relatively distant bases, had to be assumed. Thus, we designed suitable sequences of DNA oligonucleotides, each allowing only two possibilities of the adduct formation. Sequences of oligonucleotides, which were used, are shown in the Fig. 32, with blue lines symbolizing the molecule of the complex (Fig. 33).

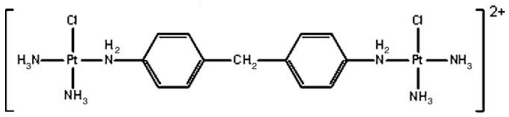

Fig. 33: Dinucelar platinum complex with cis geometry

Fig. 32: Sequences of oligonucleotides used in experiments

Firstly, the site-specific interstrand crosslinks according to Fig. 32 were prepared and kinetic of their formation was observed. The rate of ICL formation was almost the same for all types of duplexes used in this experiment (Fig. 34).

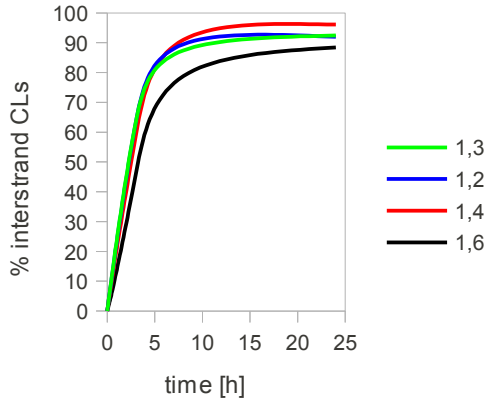

Fig. 34: Kinetics of formation of interstrand crosslinks

Thus, we deiced to further investigate the position of interstrand crosslinks by DMS and hydroxyl radical footprinting. Results from these experiments (Fig. 35 and Fig. 36) have clearly shown the exact location of all lesions. Surprisingly, only one preferential adduct in each sequence was formed on DNA duplex in the 3' → 3' direction. This first observation showed, that these adducts are unusual in comparison with those previously described for drugs of a similar nature.

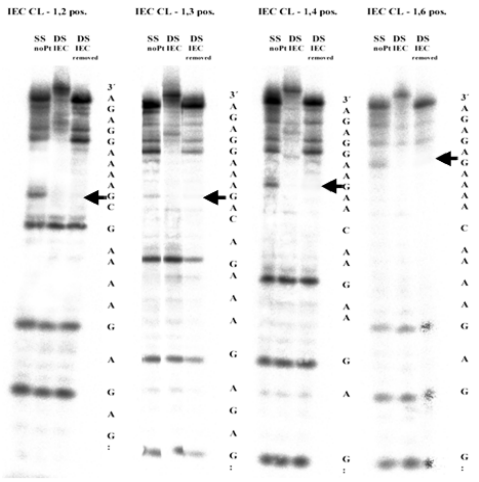

Fig. 35: DMS footprinting

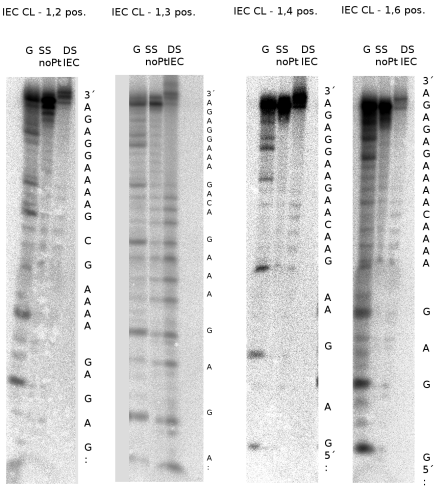

Fig. 36: Hydroxyl radical footprinting

As a further step, we decided to investigate these structures by means of molecular dynamics. The program Mopac2009 was used for computing of quantum-chemical properties of the complex. Using molecular modeling package AMBER11 and linked preparatory programs (NAB, Xleap), the structures of two sequences used in experiments (marked [1,2] and [1,6], Fig. 27), with molecule of complex attached as an single interstrand crosslink in the 3' → 3' direction, were prepared. The exact procedure for energy minimization and consequent 20 ns long runs of molecular dynamic is described in section 2. Even though, the calculations are still in progress, we can deduce from preliminary results marked differences in DNA duplex conformations, especially in their bending (kink of the duplex) (Fig. 37 and Fig. 38). Thus, hopefully the full analysis of trajectories will provide useful structural information, which at least partly, will be able to elucidate the unique properties of interstrand crosslinks formed by the studied dinuclear drug.

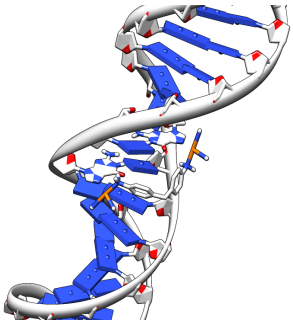

Fig. 37: 1,2 position of the interstrand crosslink

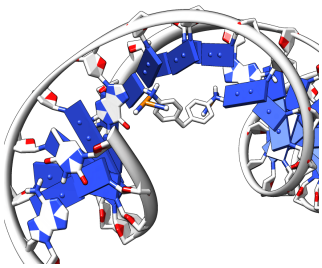

Fig. 38: 1,6 position of the interstrand crosslink

5 CONCLUSION

DNA repair has a significant role in modulating cytotoxicity of platinum drugs as had been reported in chap. 1. Thus, it is of great interest to study repair of damage to DNA induced by platinum antitumor drugs and its downstream effects in tumor cells. Any contribution to elucidation of these repair mechanism can help to understand cellular response to platinum-based antitumor therapy. Consequently, greater knowledge of these mechanisms can lead to rational design and improving the efficacy of new therapies. Thus, effort to employ pharmacological inhibition of DNA repair was one of the aspects which lead to the development of new promising antitumor compounds, studied in this work. It was shown that DNA adducts of the different complexes of platinum or ruthenium examined in this study, were more difficult to recognize and to remove by DNA repair systems. This effect for most of studied drugs markedly contributed to their antitumor effect.

This PhD thesis also focused on structural studies of DNA bearing interstrand crosslinks of antitumor drugs. Interstrand crosslinks are highly toxic DNA lesions that prevent transcription and replication by inhibiting DNA strand separation. Agents that induce interstrand crosslinks were one of the earliest, and are still the most widely used, forms of chemotherapeutic drug. Only recently, we have begun to understand how cells repair these lesions. Also not only DNA repair mechanism, but a large number of another cellular proteins are involved in modulating antitumor effect of metal drugs. Many observations clearly shown that the propensity of the adduct to induce conformational distortions is one of the important factors involved in recognition by celullar protein as the early event in the cascade of processes leading to cancer cells' apoptosis.

In this work, the simulated structures with interstrand crosslinks of oxaliplatin, as recently successfully used drug in clinics, and of new promising dinuclear complex were described. The results of the analysis referred mainly to the structural features of DNA duplex in comparison to previously studied properties of cisplatin adducts, which are known to be responsible for its antitumor effect. Hopefully, the obtained molecular models provide useful information about the nearest environment and nature of the studied lesions and thus can at least partly contribute to explanation of their mechanisms of action in cancer therapy.

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7 LIST OF ABBREVIATIONS

A - adenine

C - cytosine

G - guanine

T - thymin

AA8 - chinese hamster ovary

AMBER - Assisted Model Building with Energy Refinement

Cisplatin - *cis*- diamminedichloroplatinum(II)

CT - calf thymus

DMS - dimetyl sulfate

DNA - deoxyribonucleic acid

DPP - differential pulse polarography

EtBr - ethidium bromid

FAAS - flameless atomic absorption spectrophotometry

HeLa - human cervical carcinoma

HMG protein - high mobility group protein

HPLC - high-pressure liquid chromatography

MD - molecular dynamics

MM - molecular mechanics

MT-2 – metallothionein-2

NER – nucleotide excision repair

NMR - nuclear magnetic resonance

Oxaliplatin – (*trans*-1R,2R-diaminocyclohexane)oxalato-platinum(II)

PBS - phosphate-buffered saline

QM - quantum mechanics

$\mathbf{r_b}$ - the number of molecules of the platinum complex bound per nucleotide residue

TAE - tris-acetate buffer

UV - ultraviolet light

VIS - visible light

8 CURRICULUM VITAE

Personal details:

Name: **Tereza Suchánková**

Address: Záhumenice 28, Brno, 619 00

E-mail: suchankovatereza@centrum.cz

Date of birth: November 23, 1983

Marital status: Married

Education

2008 – present Palacky University in Olomouc, Faculty of Science, Department of Biophysics, doctoral studies in Biophysics

2003 – 2008 Masaryk University in Brno, Faculty of Science, Department of Physics, MSc in Biophysics

Professional experience

9 / 2008 – present research assistant, Institute of Biophysics, AS CR v. v. i

2 / 2009 – present research assistant, Department of Experimental Physics, Palacky University in Olomouc

Research interest

Study of the mechanism of new metallodrugs´ anticancer activity. DNA interactions and distortions caused by binding of these compounds. Molecular modeling.

Research fellowship

1 / 2010 – 4 / 2010 Lab. de Chimie et Biochimie - UMR 8601, CNRS, Universite Rene Descartes, Paris (supervisor: prof. Jiri Kozelka)
research topic: Molecular modeling of interstrand crosslink of oxaliplatin

Computer skills

MS Windows/Office; OS UNIX; software for molecular modeling and visualization

Language skills

English – fluent (FCE - First Certificate in English, 2003)
German – intermediate

9 LIST OF PUBLICATIONS

1. **Suchankova, T.**, Vojtiskova, M., Reedijk, J., Brabec, V., and Kaspárková, J. (2009). DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[PtCl2(isopropylamine)(1-methylimidazole)]: relations to their different antitumor effects. J. Biol. Inorg. Chem. *14*, 75–87. *(IF = 3.287; times cited = 5)*
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Scientific presentations

- **Suchankova T.**, Kubicek K., Monnet J., Kasparkova J., Brabec V., *Oral presentation*: Molecular simulations of oxaliplatin-dna interstrand crosslinks. COST Chemistry, Working group meeting D39/0004/06. Brno, Czech Rebuplic, May 13. – 14., 2011, Book of Abstracts: p. 19
- **Suchankova T**., Kubicek K., Kasparkova J., Brabec V., Kozelka J., *Oral presentation*: Molecular simulations of a DNA duplex bearing an interstrand crosslink formed by cisplatin or its chiral analogues. Struktura a interakce biomolekul, konference mladých vědeckých pracovníků v rámci řešení projektu GAČR, Registrační číslo 301/09/H004. Brno, Czech Republic, April 29., 2010 , Book of Abstracts: p. 18
- **Suchankova T.**, Vojtiskova M., Brabec V., Kasparkova J., *Oral presentation*: DNA binding mode of the cis and trans geometries of new antitumor nonclassical platinum complexes containing isopropylamine and 1-methylimidazole ligands: relation to their activity in cancer cell lines. COST Chemistry D39, Working group meeting D39/0004/06. Palermo, Italy, June 30. – July 1., 2009 , Book of Abstracts: p. 23
- **Suchankova T.**, Vojtiskova M., Brabec V., Kasparkova J., *Poster*: Effects of isopropylamine and 1-methylimidazole ligands on the mechanism of action of antitumor cisplatin and ineffective transplatin. 10th Meeting in Inorganic Chemistry, FIGIPAS. Palermo, Italy, July 1. - 4., 2009 , Book of Abstracts: PO 169

APPENDIX

PAPER I

DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[PtCl2(isopropylamine) (1-methylimidazole)]: relations to their different antitumor effects

T. Suchankova, M. Vojtiskova, J. Reedijk, V. Brabec, and J. Kasparkova

Journal of Biological Inorganic Chemistry **14**, 75–87 (2009)

I declare that my role in preparation of this paper was as following:
Realization of all the experiments except electrophoretic mobility shift assays with HMGB1 protein and replication mapping.
Preparation of the manuscript.

….....

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ORIGINAL PAPER

DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes *cis*- and *trans*-[$PtCl_2$(isopropylamine)(1-methylimidazole)]: relations to their different antitumor effects

Tereza Suchánková · Marie Vojtíšková · Jan Reedijk · Viktor Brabec · Jana Kašpárková

Received: 12 July 2008 / Accepted: 24 August 2008 / Published online: 6 September 2008

Abstract The global modification of mammalian and plasmid DNAs by the novel platinum compounds *cis*-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and *trans*-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and the reactivity of these compounds with reduced glutathione (GSH) were investigated in cell-free media using various biochemical and biophysical methods. Earlier cytotoxicity studies had revealed that the replacement of the NH_3 groups in cisplatin by the azole and isopropylamine ligands lowers the activity of cisplatin in both sensitive and resistant cell lines. The results of the present work show that this replacement does not considerably affect the DNA modifications by this drug, recognition of these modifications by HMGB1 protein, their repair, and reactivity of the platinum complex with GSH. These results were interpreted to mean that the reduced activity of this analog of cisplatin in tumor cell lines is due to factors that do not operate at the level of the target DNA. In contrast, earlier studies had shown that the replacement of the NH_3 groups in the clinically ineffective *trans* isomer (transplatin) by the azole and isopropylamine ligands results in a radical enhancement of its activity in tumor cell lines. Importantly, this replacement also markedly alters the DNA binding mode of transplatin, which is distinctly different from that of cisplatin, but does not affect reactivity with GSH. Hence, the results of the present work are consistent with the view and support the hypothesis systematically tested by us and others that platinum drugs that bind to DNA in a fundamentally different manner from that of conventional cisplatin may have altered pharmacological properties.

T. Suchánková · M. Vojtíšková · V. Brabec · J. Kašpárková (✉)
Institute of Biophysics,
Academy of Sciences of the Czech Republic, v.v.i.,
Kralovopolska 135,
61265 Brno, Czech Republic
e-mail: jana@ibp.cz

J. Reedijk
Leiden Institute of Chemistry,
Gorlaeus Laboratories,
Leiden University,
P.O. Box 9502,
2300 RA Leiden, The Netherlands

V. Brabec
Laboratory of Biophysics,
Department of Experimental Physics,
Faculty of Sciences,
Palacky University,
tr. Svobody 26,
77146 Olomouc, Czech Republic
e-mail: brabec@ibp.cz

Keywords DNA · Conformation · Cisplatin · Transplatin · Antitumor activity

Abbreviations

bp	Base pair
CD	Circular dichroism
CFE	Cell-free extract
CL	Cross-link
CT	Calf thymus
DPP	Differential pulse polarography
EtBr	Ethidium bromide
FAAS	Flameless atomic absorption spectrophotometry
GSH	Glutathione
KF	Klenow fragment from DNA polymerase I (exonuclease minus, mutated to remove the 3′ → 5′ proofreading domain)
Tris	Tris(hydroxymethyl)aminomethane
TU	Thiourea

PAPER II

Cytotoxicity, cellular uptake, glutathione and DNA interactions of an antitumor large-ring Pt chelate complex incorporating the 1, 4-diaminocyclohexane carrier ligand

J. Kasparkova, **T. Suchankova**, A. Halamikova, L. Zerzankova, O. Vrana, N. Margiotta, G. Natile, and V. Brabec

*Biochemical Pharmacology **79**, 552–564 (2010)*

I declare that my role in preparation of this paper was as following:
Realization of the experiments with sulfur-containing compounds, interstrand crosslink assay and DNA repair synthesis.
Preparation of the corresponding part of the manuscript.

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Cytotoxicity, cellular uptake, glutathione and DNA interactions of an antitumor large-ring Pt^{II} chelate complex incorporating the *cis*-1,4-diaminocyclohexane carrier ligand

Jana Kasparkova [a], Tereza Suchankova [a,b], Anna Halamikova [a], Lenka Zerzankova [a], Oldrich Vrana [a], Nicola Margiotta [c], Giovanni Natile [c], Viktor Brabec [a,*]

[a] Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-61265 Brno, Czech Republic
[b] Department of Experimental Physics, Faculty of Sciences, Palacky University, tr. 17. listopadu 12, 77146 Olomouc, Czech Republic
[c] Department of Pharmaceutical Chemistry, University of Bari, I-70125 Bari, Italy

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ABSTRACT

Earlier studies have described promising antitumor activity of a large-ring chelate complex [$PtCl_2$(*cis*-1,4-DACH)] (DACH = diaminocyclohexane). Encouraging antitumor activity of this analogue of cisplatin prompted us to perform studies focused on the mechanistic basis of pharmacological effects of this complex. Four early steps in the mechanism of biological activity of cisplatin have been delineated: cell entry, reactions with sulfur-containing compounds, platinum–DNA binding along with processing platinated DNA by proteins (enzymes) and DNA repair. Here, we describe comparative experiments (involving also cisplatin) revealing: (i) improved cytotoxicity (3.4–5.4-fold) of [$PtCl_2$(*cis*-1,4-DACH)] in human tumor ovarian cell lines; (ii) enhanced cellular uptake (~1.5-fold) of [$PtCl_2$(*cis*-1,4-DACH)]; (iii) somewhat enhanced rate of reactions of [$PtCl_2$(*cis*-1,4-DACH)] with glutathione (~1.5-fold), but a similar rate of reactions with metallothionenin-2; (iv) enhanced rate of DNA binding of [$PtCl_2$(*cis*-1,4-DACH)] in cell-free media (~2-fold); (v) similar sequence preference of DNA binding of [$PtCl_2$(*cis*-1,4-DACH)] in cell-free media; (vi) identical DNA interstrand cross-linking efficiency (6%); (vii) similar bending (32°) and enhanced local unwinding (~1.5-fold) induced in DNA by the major 1,2-GG-intrastrand cross-link; (viii) markedly enhanced inhibiting effects of DNA adducts of [$PtCl_2$(*cis*-1,4-DACH)] on processivity of DNA polymerase; and (ix) a slightly lower efficiency of DNA repair systems to remove the adducts of [$PtCl_2$(*cis*-1,4-DACH)] from DNA.

1. Introduction

Serendipitous discovery of antitumor activity of cisplatin [*cis*-diamminedichloridoplatinum(II)] (Fig. 1B) and ensuing introduction of cisplatin, carboplatin [*cis*-diammine(cyclobutane-1,1-dicarboxylato)platinum(II)], nedaplatin [*cis*-diammineglycolatoplatinum(II)] and oxaliplatin {[(*1R,2R*-diaminocyclohexane)oxalatoplatinum(II)] (diaminocyclohexane = DACH)} into the clinical use evoked a renaissance in inorganic chemistry and led to the synthesis and biological evaluation of many thousands of cisplatin analogues, and a thorough investigation of other nearby elements from the periodic table (for example, gold, osmium, palladium and ruthenium) [1,2]. Much of the early effort in the design of new platinum drugs was aimed at making cisplatin-based therapy safer to patients, in particular, lessening or removing unpredictable and severe nephrotoxicity and/or providing oral bioavailability. A second major, ongoing, initiative is to overcome tumor resistance to cisplatin, either that acquired during cycles of therapy with cisplatin (as

Abbreviations: bp, base pair; cisplatin, *cis*-diamminedichloridoplatinum(II); CFE, cell-free extract; CL, cross-link; CT, calf thymus; DACH, diaminocyclohexane; DEPC, diethyl pyrocarbonate; dNTP, deoxyribonucleoside 5′-triphosphate; DMSO, dimethylsulfoxide; DPP, differential pulse polarography; FAAS, flameless atomic absorption spectrophotometry; GSH, glutathione; HPLC, high-pressure liquid chromatography; IC_{50}, concentration inhibiting cell growth by 50%; KF^-, Klenow fragment from DNA polymerase I (exonuclease minus mutated to remove the 3′→5′ proofreading domain); MT, metallothionein; MT-2, metallothionein isoform MT-2; oxaliplatin, [Pt(oxalate)(*1R,2R*-DACH)]; PAA, polyacrylamide; r_b, the number of molecules of the Pt^{II} complex bound per nucleotide residue; r_i, the molar ratio of free metal complex to nucleotide phosphates at the onset of incubation with DNA; $t_{1/2}$, half time; transplatin, *trans*-diamminedichloridoplatinum(II).

* Corresponding author. Tel.: +420 541517148; fax: +420 541240499.
E-mail address: brabec@ibp.cz (V. Brabec).

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PAPER III

Conformation and recognition of DNA modified by a new antitumor dinuclear PtII complex resistant to decomposition by sulfur nucleophiles

L. Zerzankova, T. Suchankova, O. Vrana, N. P. Farrell, V. Brabec, and J. Kasparkova

Biochemical pharmacology **79**, 112–121 (2010).

I declare that my role in preparation of this paper was as following:
Realization of the measurements of DNA repair synthesis.
Preparation of the corresponding part of the manuscript.

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Conformation and recognition of DNA modified by a new antitumor dinuclear Pt^{II} complex resistant to decomposition by sulfur nucleophiles

Lenka Zerzankova[a], **Tereza Suchankova**[b], **Oldrich Vrana**[a], **Nicholas P. Farrell**[c], **Viktor Brabec**[a], and **Jana Kasparkova**[a,*]

[a]Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-61265 Brno, Czech Republic

[b]Department of Experimental Physics, Faculty of Sciences, Palacky University, tr. Svobody 26, 77146 Olomouc, Czech Republic

[c]Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284-2006, USA

Abstract

Reported herein is a detailed biochemical and molecular biophysics study of the molecular mechanism of action of antitumor dinuclear Pt^{II} complex [{PtCl(DACH)}$_2$-μ-Y]$^{4+}$ [DACH = 1,2-diaminocyclohexane, Y = $H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2$] (complex **1**). This new, long-chain bifunctional dinuclear Pt^{II} complex is resistant to metabolic decomposition by sulfur-containing nucleophiles. The results show that DNA adducts of **1** can largely escape repair and yet inhibit very effectively transcription so that they should persist longer than those of conventional cisplatin. Hence, they could trigger a number of downstream cellular effects different from those triggered in cancer cells by DNA adducts of cisplatin. This might lead to the therapeutic effects that could radically improve chemotherapy by platinum complexes. In addition, the findings of the present work make new insights into mechanisms associated with antitumor effects of dinuclear/trinuclear Pt^{II} complexes possible.

Keywords

Antitumor; Dinuclear platinum complex; DNA damage; DNA repair; RNA polymerase II; Glutathione

1. Introduction

The development of new platinum-centered complexes as antitumor DNA-interactive agents has traditionally been focused on mononuclear bidentate platinum complexes. In order to contribute to the design of new antitumor platinum drugs we and others have been systematically testing the hypothesis that there is a correlation between clinical efficacy of platinum compounds and their ability to induce a certain sort of damage or conformational change in target DNA or, in other words, that platinum drugs that bind to DNA in a manner

*Corresponding author. Tel.: +420 541517174; fax: +420 541240499. jana@ibp.cz (J. Kasparkova).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2009.08.009.

!!! To meet copyright requirements, the full text of this article is available at:

http://www.sciencedirect.com/science/article/pii/S0006295209006893

PAPER IV

Mechanistic insights into antitumor effects of new dinuclear cis Pt(II) complexes containing aromatic linkers

L. Zerzankova, H. Kostrhunova, M. Vojtiskova, O. Novakova, **T. Suchankova**, M. Lin, Z. Guo, J. Kasparkova, and V. Brabec

Biochemical Pharmacology **80**, 344–351 (2010)

I declare that my role in preparation of this paper was as following:
Realization of the measurements of DNA repair synthesis.
Preparation of the corresponding part of the manuscript.

….....

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Mechanistic insights into antitumor effects of new dinuclear *cis* Pt^{II} complexes containing aromatic linkers

Lenka Zerzankova [a], Hana Kostrhunova [a], Marie Vojtiskova [a], Olga Novakova [a], Tereza Suchankova [b], Miaoxin Lin [c], Zijian Guo [c], Jana Kasparkova [a,*], Viktor Brabec [a,*]

[a] Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-61265 Brno, Czech Republic
[b] Department of Experimental Physics, Faculty of Sciences, Palacky University, tr. Svobody 26, 77146 Olomouc, Czech Republic
[c] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China

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ABSTRACT

The primary objective was to understand more deeply the molecular mechanism underlying different antitumor effects of dinuclear Pt^{II} complexes containing aromatic linkers of different length, {[*cis*-$Pt(NH_3)_2Cl]_2$(4,4′-methylenedianiline)}$^{2+}$ (**1**) and {[*cis*-$Pt(NH_3)_2Cl]_2$(α,α′-diamino-p-xylene)}$^{2+}$ (**2**). These complexes belong to a new generation of promising polynuclear platinum drugs resistant to decomposition by sulfur nucleophiles which hampers clinical use of bifunctional polynuclear *trans* Pt^{II} complexes hitherto tested. Results obtained with the aid of methods of molecular biophysics and pharmacology reveal differences and new details of DNA modifications by **1** and **2** and recognition of these modifications by cellular components. The results indicate that the unique properties of DNA interstrand cross-links of this class of polynuclear platinum complexes and recognition of these cross-links may play a prevalent role in antitumor effects of these metallodrugs. Moreover, the results show for the first time a strong specific recognition and binding of high-mobility-group-domain proteins, which are known to modulate antitumor effects of clinically used platinum drugs, to DNA modified by a polynuclear platinum complex.

1. Introduction

The polynuclear platinum compounds represent a class of new antitumor metallodrugs that is structurally distinct from conventional *cis*-diamminedichloridoplatinum(II) (cisplatin) and its mononuclear analogs, and whose clinical profile and mechanism of action are different from these established platinum mononuclear compounds [1–6]. DNA adducts of polynuclear platinum complexes, whose formation is associated with antitumor effects of these agents, differ significantly in structure and type from those of mononuclear platinum complexes. Especially because of markedly more distant leaving groups, long-range intra- and interstrand cross-links (CLs) are formed in DNA which affect DNA conformation, are recognized by specific proteins and repaired differently in comparison with the CLs of cisplatin and its mononuclear analogs [7–11]. Importantly, cells with resistance to cisplatin showed no cross-resistance to polynuclear platinum compounds [12–14]. The polynuclear platinum complexes also exhibit significantly higher levels of cellular uptake very likely because of their lipophilic linker chains and hydrophilic platinum-amine coordination spheres, which may enhance membrane permeability [13,15,16].

The biological activity of polynuclear platinum complexes may be modulated by the geometry and number of leaving groups in the coordination sphere of platinum atoms as well as by the nature of linkers connecting the platinum centers. In contrast with the mononuclear complexes, such as antitumor cisplatin and clinically ineffective transplatin, in the dinuclear case both geometries are antitumor active [2], although DNA adducts (CLs) as well as local conformational distortions on DNA and their recognition by cellular components are affected by geometry [7,10,17]. The dinuclear *cis* isomer [{*cis*-$PtCl(NH_3)_2$}$_2$(H_2N-$(CH_2)_6$-NH_2]$^{2+}$ is kinetically more inert in its reactions with DNA and in double-stranded DNA produces more interstrand cross-links than its *trans* counterpart [10].

Abbreviations: BBR3464, [{*trans*-$PtCl(NH_3)_2$}$_2$-μ-*trans*-$Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2$]$^{4+}$; BBR3610, [{*trans*-$PtCl(NH_3)_2$}$_2$-μ-{*trans*-$H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2$}]$^{4+}$; bp, base pair; Cisplatin, *cis*-diamminedichloridoplatinum(II); CFE, cell-free extract; CL, cross-link; Complex **1**, {[*cis*-$Pt(NH_3)_2Cl]_2$(4,4′-methylenedianiline)}$^{2+}$; Complex 2, {[*cis*-$Pt(NH_3)_2Cl]_2$(α,α′-diamino-p-xylene)}$^{2+}$; CT, calf-thymus; DPP, differential pulse polarography; EtBr, ethidium bromide; FAAS, flameless atomic absorption spectrophotometry; HMG, high-mobility-group; IC_{50}, concentration inhibiting cell growth by 50%; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; PAGE, polyacrylamide gel electrophoresis; r_b, the number of molecules of the metal complex bound per nucleotide residue; r_i, the molar ratio of free metal complex to nucleotide-phosphates at the onset of incubation with DNA.

* Corresponding authors. Tel.: +420 541517148; fax: +420 541240499.
E-mail addresses: jana@ibp.cz (J. Kasparkova), brabec@ibp.cz (V. Brabec).

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PAPER V

Energetics, Conformation, and Recognition of DNA Duplexes Modified by Monodentate RuII Complexes Containing Terphenyl Arenes

O. Novakova, J. Malina, **T. Suchankova**, J. Kasparkova, T. Bugarcic, P. J. Sadler, and V. Brabec

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I declare that my role in preparation of this paper was as following:
Realization of the measurements of DNA repair synthesis.
Preparation of the corresponding part of the manuscript.

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Energetics, Conformation, and Recognition of DNA Duplexes Modified by Monodentate Ru^{II} Complexes Containing Terphenyl Arenes

Olga Novakova,[a] Jaroslav Malina,[a] Tereza Suchankova,[b] Jana Kasparkova,[a] Tijana Bugarcic,[c] Peter J. Sadler,[c] and Viktor Brabec*[a]

Abstract: We studied the thermodynamic properties, conformation, and recognition of DNA duplexes site-specifically modified by monofunctional adducts of Ru^{II} complexes of the type $[Ru^{II}(\eta^6\text{-arene})(Cl)(en)]^+$, in which arene = *para*-, *meta*-, or *ortho*-terphenyl (complexes **1**, **2**, and **3**, respectively) and en = 1,2-diaminoethane. It has been shown (*J. Med. Chem.* **2008**, *51*, 5310) that **1** exhibits promising cytotoxic effects in human tumor cells, whereas **2** and **3** are much less cytotoxic; concomitantly with the high cytotoxicity of **1**, its DNA binding mode involves combined intercalative and monofunctional (coordination) binding modes, whereas less cytotoxic compounds **2** and **3** bind to DNA only through a monofunctional coordination to DNA bases. An analysis of conformational distortions induced in DNA by adducts of **1** and **2** revealed more extensive and stronger distortion and concomitantly greater thermodynamic destabilization of DNA by the adducts of nonintercalating **2**. Moreover, affinity of replication protein A to the DNA duplex containing adduct of **1** was pronouncedly lower than to the adduct of **2**. On the other hand, another damaged-DNA-binding protein, xeroderma pigmentosum protein A, did not recognize the DNA adduct of **1** or **2**. Importantly, the adducts of **1** induced a considerably lower level of repair synthesis than the adducts of **2**, which suggests enhanced persistence of the adducts of the more potent and intercalating **1** in comparison with the adducts of the less potent and nonintercalating **2**. Also interestingly, the adducts of **1** inhibited DNA polymerization more efficiently than the adducts of **2**, and they could also be bypassed by DNA polymerases with greater difficulty. Results of the present work along with those previously published support the view that monodentate Ru^{II} arene complexes belong to a class of anticancer agents for which structure–pharmacological relationships might be correlated with their DNA-binding modes.

Keywords: arenes · calorimetry · DNA · DNA recognition · ruthenium

Introduction

Ruthenium complexes have attracted much interest as alternative drugs to cisplatin (*cis*-diamminedichloridoplatinum(II)) and its analogues in cancer chemotherapy. Organometallic Ru^{II}–arene complexes of the type $[Ru^{II}(\eta^6\text{-arene})(Cl)(en)][PF_6]$ (en = 1,2-diaminoethane) constitute a relatively new group of anticancer compounds.[1–4] These monodentate complexes appear to be novel anticancer agents with a mechanism of action different from those of platinum and other ruthenium complexes that have been tested for antitumor activity. Although the pharmacological target for antitumor ruthenium compounds has not been unequivocally identified, there is a large body of evidence indicating that the cytotoxicity of several ruthenium complexes correlates with their ability to bind DNA,[5] although several exceptions have been reported.[6,7] Because the Ru^{II}–arene complexes bind strongly to DNA,[8–15] DNA modifications

[a] Dr. O. Novakova, Dr. J. Malina, Dr. J. Kasparkova, Prof. Dr. V. Brabec
Institute of Biophysics
Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135
61265 Brno (Czech Republic)
Fax: (+420) 541240499
E-mail: brabec@ibp.cz

[b] T. Suchankova
Laboratory of Biophysics
Department of Experimental Physics, Faculty of Sciences
Palacky University
771 46 Olomouc (Czech Republic)

[c] Dr. T. Bugarcic, Prof. Dr. P. J. Sadler
Department of Chemistry
University of Warwick
Coventry C4V 7AL (UK)

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PAPER VI

Platinum–DNA interstrand crosslinks: Molecular determinants of bending and unwinding of the double helix

T. Suchankova, K. Kubicek, J. Kasparkova, V. Brabec, and J. Kozelka

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I declare that my role in preparation of this paper was as following:
Realization of the whole molecular modeling procedure including processing of the results.
Preparation of the manuscript.

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Platinum–DNA interstrand crosslinks: Molecular determinants of bending and unwinding of the double helix

Tereza Suchánková [a], Karel Kubíček [b], Jana Kašpárková [a], Viktor Brabec [a], Jiří Kozelka [b,c,*]

[a] Department of Biophysics, Faculty of Sciences, Palacky University, tr. 17. listopadu 12, 77146 Olomouc, Czech Republic
[b] Institute of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic
[c] Université Paris Descartes, UMR 8601 CNRS, 45 rue des Saints-Pères, 75270 Paris, France

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ABSTRACT

Platinum diamine complexes are able to crosslink the guanines of $d(GC)_2$ dinucleotides within double-stranded DNA. The interstrand crosslink thus formed causes a bend of the double helix toward the minor groove and the helical sense changes locally to left-handed, resulting in a considerable unwinding. The bend and unwinding angles have been shown to depend on the platinum ligands. Here, we have used molecular dynamics simulations to investigate the DNA 20-mer $d(C_1T_2C_3T_4C_5C_6T_7T_8G^*_9C_{10}T_{11}C_{12}T_{13}C_{14}C_{15}T_{16}T_{17}C_{18}T_{19}C_{20})$-$d(G_{21}A_{22}G_{23}A_{24}A_{25}G_{26}G_{27}A_{28}G_{29}A_{30}G^*_{31}C_{32}A_{33}A_{34}G_{35}G_{36}A_{37}G_{38}A_{39}G_{40})$ with the G* guanines crosslinked by *cis*-$Pt(NH_3)_2^{2+}$, $Pt(R,R\text{-DACH})^{2+}$, or $Pt(S,S\text{-DACH})^{2+}$. Previous investigations on cisplatin interstrand adducts indicated that the structure is similar in solid state and in solution; thus, we used the reported X-ray structure of a cisplatin adduct as a starting model. Replacing in the MD-relaxed model for the DNA duplex crosslinked with *cis*-$Pt(NH_3)_2^{2+}$ the two NH_3 platinum ligands by *R,R*-DACH or *S,S*-DACH led to clashes between the DACH residue and the deoxyribose of C_{12}. Confrontation of MD-derived models with gel shift measurements suggested that these clashes are avoided differently in the adducts of $Pt(R,R\text{-DACH})^{2+}$ *versus* $Pt(S,S\text{-DACH})^{2+}$. The *R,R*-isomer avoids the clash by untwisting the T_{11}/A_{30}–C_{12}/G_{29} step, thus increasing the global unwinding. In contrast, the *S,S*-isomer modifies the shift and slide parameters of this step, which dislocates the helical axis and enhances the bend angle. The clash that leads to the differentiation of the structures as a function of the diamine ligand is related to a hydrogen bond between the platinum complex and the T_{11} base and could be characteristic of interstrand crosslinks at d(pyG*Cpy)-d(puG*Cpu) sequences.

1. Introduction

Cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) and oxaliplatin ($[Pt(oxalate)(R,R\text{-DACH})]$; DACH = 1,2-diaminocyclohexane) are platinum complexes that belong currently to the most frequently used anticancer drugs [1]. Although the molecular structures of the two drugs (see formulas below) are relatively similar and both drugs react with DNA to form similar adducts, their biological activities show some important differences. Albeit oxaliplatin forms fewer DNA lesions than cisplatin, it is more cytotoxic against some cell lines [2]. The nuclear protein HMGB1 and the mismatch repair complex protein MutS exhibit higher affinity for cisplatin adducts on DNA than the corresponding oxaliplatin lesions [3]. In addition, eukaryotic DNA polymerases β, ζ, γ, and η bypass oxaliplatin adducts more readily than cisplatin adducts and the misincorporation frequency of DNA polymerase β is slightly greater with oxaliplatin adducts than with those of cisplatin [3–5]. For cisplatin, a number of observations point to DNA as the critical cellular target: filamentous growth of bacteria following cisplatin treatment [6–8], induction of lambda prophage in lysogenic bacteria [9], inactivation of hemophilus-transforming DNA [10], and particularly lethal effects on *Escherichia coli* mutants having deficient DNA repair systems [11, 12]. For oxaliplatin, a tumor-killing mechanism triggered by an initial DNA attack is assumed as well, although the evidence is less elaborated in this case.

Both cisplatin and oxaliplatin have two labile binding sites and are thus capable of forming bifunctional DNA lesions, crosslinking two residues. Guanine N7 atoms are the most reactive nucleophilic centers in DNA and therefore guanine–guanine crosslinks are the most abundant lesions. By far the most frequent are intrastrand crosslinks between adjacent guanines [13, 14]. Cisplatin and oxaliplatin form also interstrand crosslinks. The latter are formed almost exclusively at $(GC)_2$ sequences, and are more than one order of magnitude less frequent than intrastrand crosslinks at GG sequences [15]. This is due to intrinsic reactivity of individual guanines: An adjacent guanine increases the relative reactivity of a neighboring guanine, whereas a 3′-cytosine reduces the reactivity considerably [16, 17]. Although the interstrand crosslinks are formed less frequently than intrastrand crosslinks, they are more inhibitory to DNA replication and transcription because

* Corresponding author at: Université Paris Descartes, UMR 8601 CNRS, 45 rue des Saints-Pères, 75270 Paris, France. Tel.: +33 1 42 86 20 86; fax: +33 1 42 86 83 87.
E-mail address: kozelka.jiri@gmail.com (J. Kozelka).

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