Role of reactive oxygen species in oxidative damage and dynamic nature of PSII-LHCII supercomplexes

Ph.D. Thesis

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Klíčová slova: reaktivní formy kyslíku; fotosystém II; PSII-LHCII superkomplex; oxidativní stres; fotoinhibice; elektronová paramagnetická rezonanční spektroskopie; konfokální laserová skenovací mikroskopie; hmotnostní spektroskopie; CN-PAGE; elektronová mikroskopie.

Annotation

This thesis characterizes the role of reactive oxygen species on the structural and functional changes occurring within the photosystem II - light-harvesting complex II (PSII-LHCII) suprecomplex under high light stress. Goal of the study is focused on 1) the role of free oxygen radicals in the oxidative damage of PSII proteins, 2) characterization of a high resolution 3D structure of the PSII-LHCII supercomplex from Arabidopsis, and 3) singlet oxygen formation under leaf wounding. The oxidation of specific amino acid residues of D1 and D2 proteins was shown to nearby to the site of hydroxyl radical formation at both the Mn_4O_5Ca cluster and the non-heme iron. Using cryo-electron microscopy and single particle analysis, evidence was provided on the coordination of chlorophylls to the protein subunits of PSII-LHCII supercomplex. Based on the structural data, energy transfer pathway from the Lhcb proteins to the PSII core complex was proposed. Apart to well described singlet oxygen formation by triplet-triplet energy transfer from triplet excited chlorophyll to molecular oxygen under high light, singlet oxygen was shown to be formed by triplet-triplet energy transfer from triplet excited carbonyl to molecular oxygen formed by lipid peroxidation.

Anotace

Disertační práce popisuje vliv reaktivních forem kyslíku na strukturální a funkční změny v PSII-LHCII (fotosystém II s navázanými světlosběrnými komplexy fotosystému II) superkomplexu za působení vysoké ozářenosti. Cílem studie bylo studovat 1) roli volných kyslíkových radikálů na oxidativní poškození PSII, 2) 3D strukturu PSII-LHCII superkomplexu u huseníčku a 3) tvorbu singletního kyslíku během poškození listu. K oxidaci aminokyselin D1 a D2 proteinů dochází v místě tvorby hydroxylového radikálu v blízkosti Mn_4O_5Ca komplexu a nehemového železa. Charakterizace aminokyselin oxidovaných hydroxylovým radikálem umožní pochopit mechanismus poškození centrálních proteiůn reakčního centra PSII. Pomocí elektronové mikroskopie a jednočásticová analýzy byla charakterizována koordinace chloropfylů na proteiny jednotlivých proteinových komplexů LHCII-PSII superkomplexu. Pomocí těchto strukturálních dat byl navržen možný přenos excitační energie z Lhcb do jádra PSII. Dále je popsána nový mechanismus tvorby singletního kyslíku triplet-tripletním přenosem excitační energie z tripletního karbonylu na molekulární kyslík, který vzniká během lipidové peroxidace při poškození listu.

Contents

Declaration

I, Ravindra Kale, hereby declare I have written and submitted this thesis independently without any additional authorized/unauthorized support and counselling with inclusive of my original work and all necessary literature sited here within.

In Olomouc, date:…… Signature:……

List of enclosed publications

This thesis is based on 3 publications and 1 manuscript in preparation

Publications:

1. **Kale R.**, Hebert A.E., Frankel L.K., Sallans L., Bricker T.M. and Pospíšil P. (2017) Amino acid oxidation of D1 and D2 protein by free oxygen radicals in photosystem II. *Proceedings of the National Academy of Sciences of the United States of America*: 114 (11); 2988-2993.
2. van Bezouwen L.S., Caffarri S., **Kale R**, Kouřil R., Thunnissen A-M. W. H., Oostergetel G.T. and Boekema E. J. (2017) Subunit and chlorophyll organization of the plant photosystem II supercomplex. *Nature Plants* 3; 17080.
3. Prasad A., Sedlářová M., **Kale R.** and Pospíšil P. (2017) Singlet oxygen formation in *Arabidopsis thaliana* in response to wounding: in vivo imaging study. *Scientific Reports* 7 (1); 9831.

Other publication obtained during Ph.D. study

1. Datta K.J., Gawande M.B., Datta K.K.R., Ranc V., Pechousek J., Krizek M., Tucek J., **Kale R**., Pospíšil P., Varma R.S., Asefa T., Zoppellaro G., Zbořil R. Micro-Mesoporous ,Iron Oxides with Record Efficiency for Decomposition of Hydrogen Peroxide: Morphology Driven Catalysis for Degradation of Organic Contaminants (2016) *Journal of Materials Chemistry A*: 4 (2); 596-604.

Declaration

Ravindra Kale's contribution to publications was following:

Paper I) Main author – contributed in designing the research, performing the experiments and analysis of the obtained data, participated in manuscript preparation. He performed isolation of PSII membranes, separation of protein via SDS-PAGE, detection of protein radicals, carbonyl group formation and protein immunoblot analysis and detection of superoxide anion and hydroxyl radicals using EPR spin-trapping spectroscopy. Whilst his research stays in laboratory of Prof. Bricker he also prepared the samples for mass-spectrometric analysis, which were isolated from the non-oxidizing LDS-PAGE.

Paper II) Co-author – contributed in isolation of thylakoid membranes and performed the separation of PSII-LHCII supercomplex via CN-PAGE for EM negative stain specimens.

Paper III) Co-author – contributed in performing experiments of imaging of the ultra-weak photon emission and sample preparation for SOSG fluorescence imaging by confocal laser scanning microscopy.

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Doc. RNDr. Pavel Pospíšil, Ph.D.

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Abbreviations

ATP	adenosine triphosphate
CCD	charge coupled device
Chl	chlorophyll
CN-PAGE	clear native polyacrylamide gel electrophoresis
Cyt b_{559}	cytochrome b_{559}
D1, D2	D1 and D2 proteins of photosystem II
EPR	electron paramagnetic resonance
H_2O_2	hydrogen peroxide
$HO^{\bullet}$	hydroxyl radical
kDa	kilo Dalton
$L^{\bullet}$	lipid alkyl radical
LDS-PAGE	lithium dodecyl sulfate polyacrylamide gel electrophoresis
LHCI	Light harvesting complex of Photosystem I
LHCII	Light harvesting complex of Photosystem II
LLM	low molecular mass
$LOO^{\bullet}$	peroxyl radical
LOOH	lipid hydroperoxide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
$^1O_2$	singlet oxygen
$O_2^{\bullet -}$	superoxide anion radical
$HOO^{\bullet}$	hydroperoxyl
P_{680}	chlorophyll in the reaction centre of PSII
PC	plastochromanol
PQH_2	plastoquinol
Pheo	pheophytin
PQ	plastoquinone
PQH_2	plastoquinol
PSI	photosystem I
PSII	photosystem II
Q_A, Q_B	primary and secondary quinone in PSII
ROS	reactive oxygen species
SOSG	Singlet oxygen sensor green
Y_Z	redox active tyrosine-161 of the D1 protein in PSII

Introduction

Photosynthesis is photobiochemical process involving the conversion of light energy for the synthesis of organic molecules occurring in cyanobacteria, algae and plants. In higher plants, photosynthetsis occurs in chloroplast containing stacked (grana) and unstacked (stroma lamellae) regions of thylakoid membrane (Fig. 1A). Photosynthesis process is divided into the light reaction which involves energy transfer and electron transport in the thylakoid membrane and the dark reaction which maintains carbon fixation in the stroma (Fig. 1B). During the light reaction, light absorbed by chlorophylls is utilized for water oxidation to molecular oxygen and reduction of $NADP^+$ to NADPH. A proton gradient across the membrane is formed during electron transport, which is in turn used for ATP synthesis. The light reactions occurs in the membrane bound proteins photosystem II (PSII), cytochrome b_6f complex ($cytb_6/f$), photosystem I (PSI), and ATP synthase linked by two mobile electron carriers, plastoquinone (PQ) and plastocyanine (PC). Both ATP and NADPH as a product of light reaction are utilized in the dark reaction in which triose phosphates and consequently carbohydrates are formed in Calvin-Bassham cycle.

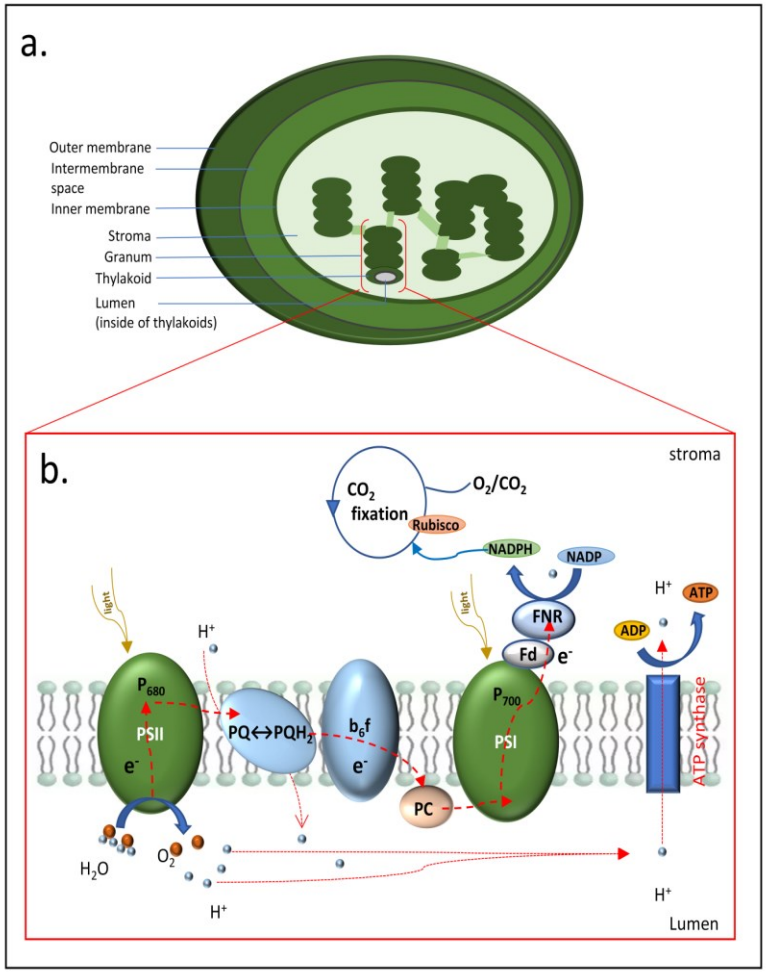

Figure 1: A) Thylakoid membrane of the chloroplast with stacked (grana) and unstacked (stroma lamellae) regions. B) Electron transport (light reaction) and CO_2 fixation (dark reaction).

1. PSII-LHCII supercomplex

1.1. PSII-LHCII supercomplex structure

PSII-LHCII supercomplex is a large homodimeric multi-subunit pigment protein with molecular weight of 350 kDa per monomer implanted in the grana region of thylakoid membranes. In thylakoid membranes, PSII-LHCII supercomplex is situated across the lipid membrane from the lumen to stroma (Fig. 2A). PSII-LHCII supercomplex is consists of dimeric PSII core complex and light harvesting complexes (LHCII). Several high-resolution crystal structures of PSII core complex have been described in thermophilic cyanobacterial (Ferreira et al., 2004, Loll et al., 2006, Guskov et al., 2009, Umena et al., 2011) and red algal (*Cyanidium caldarium*) (Ago et al., 2016). Crystal structure of spinach LHCII was resolved at 2.72 Å resolution (Liu et al., 2004). Using a single-particles cryo-electron microscopy, the structure of spinach PSII-LHCII supercomplex at 3.2 Å resolution have recently reported (Wei et al., 2016).

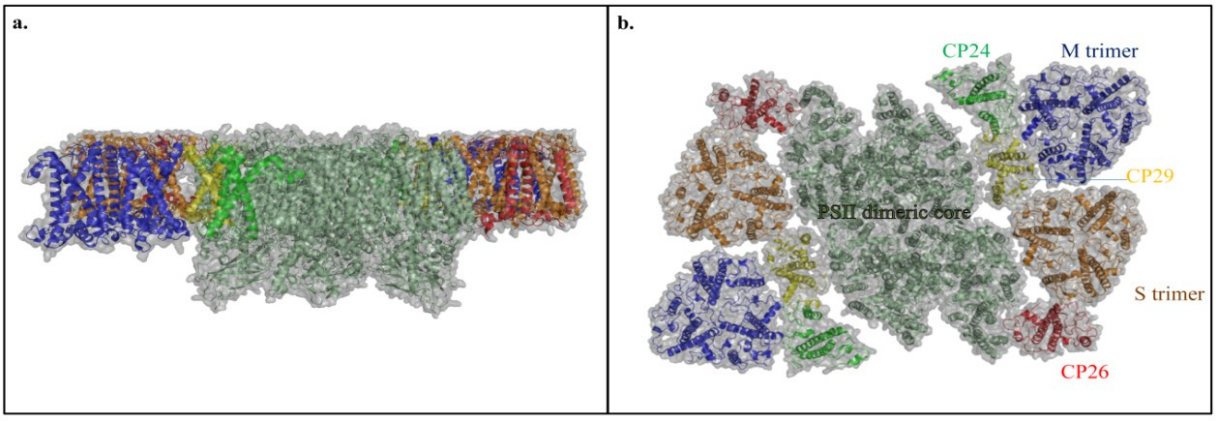

Figure 2: Cryo-EM structure of PSII-LHCII supercomplex at 5.3 Å. A) Side view along membrane plane. B) Top view from the lumen side (adopted from PDB ID: 5MDX).

1.1.1. Light Harvesting Antenna Proteins

Light harvesting complexes are present in thylakoid membrane in trimeric (major) and monomeric (minor) forms. Trimeric LHCII is composed of Lhcb1, Lhcb2 and Lhcb3 proteins (Dekker and Boekema, 2005) arranged as either homo-trimer of 3Lhcb1 and 3Lhcb2 or mixed Lhcb1-Lhcb2-Lhcb3 hetero-trimers (Fig. 3). Monomeric LHCII are Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24) proteins (Albanese et al., 2016). Each Lhcb1-3 monomer binds 14 chlorophylls (Chl) (8 Chl *a* and 6 Chl *b*) and 4 xanthophylls (1 neoxanthin, 2 lutein and 1 violaxanthin) (Fig. 4).

A variable number of LHCII proteins can associate with dimeric PSII core complexes to form various PSII–LHCII supercomplexes with a different composition. The C_2S_2 supercomplex

is composed of a dimeric PSII core complex (C_2) that binds to two strongly bound LHCII trimers (S trimers). It is well know that binding of S trimer to C_2 occurs via Lhcb4 and Lhcb5. Larger PSII-LHCII supercomplexes $C_2S_2M_{1\text{-}2}$ consist of one or two moderately bound LHCII trimers (M trimers). It is considered that binding of M-trimer to C_2 occurs via Lhcb4 and Lhcb6. Moreover, loosely bound LHCII trimers can be detected near the PSII-LHCII supercomplex (L trimer). The dynamic and rearranging variation of LHCs and their associated proteins to the supercomplex may assist in forming PSII-LHCII megacomplex or possibly arrays of an even larger order.

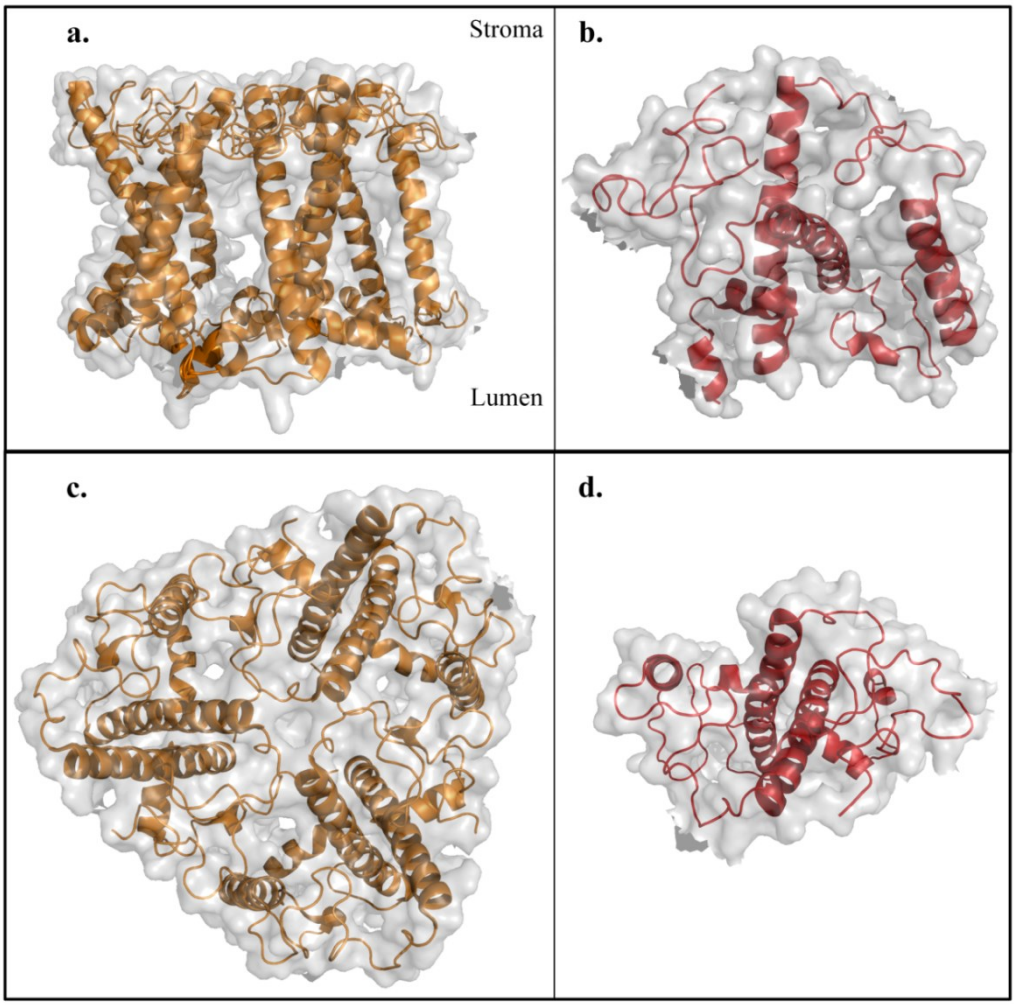

Figure 3. Structure of trimeric and monomeric LHCII proteins. LHCII side (A) and top (C) view; Lhcb6 side (b) and top (d) view.

1.1.2. PSII core complex

PSII core complex is composed of PSII reaction center and PSII core antennae. PSII reaction center is consists of two homologous proteins called D1 (PsbA)/D2 (PsbD) and α and β subunits (PsbE and PsbF) of cytochrome b_{559} (cyt b_{559}) (Table 1). PSII core antennae is formed by intrinsic proteins CP47 (PsbB) and CP43 (PsbC). The D1 and D2 are equipped with total six

chlorophylls which are utilized in the charge separation and electron transfer, whilst CP47 and CP43 are associated with 16 and 13 chlorophylls, respectively, which transfer excitation energy from LHCII to PSII reaction center. The extrinsic PsbO, PsbP and PsbQ proteins appear to interact with the inorganic cofactors manganese, calcium and chloride; all of which are required for optimal water splitting. It becomes increasingly clear that these proteins are also required for the assembly and stability of the PS II complex *in vivo*. In addition, PSII core complex is also equipped with several other smaller intrinsic protein subunits PsbH, PsbI-M, PsbR, PsbTc, PsbTn, PsbW, PsbX, PsbY and PsbZ.

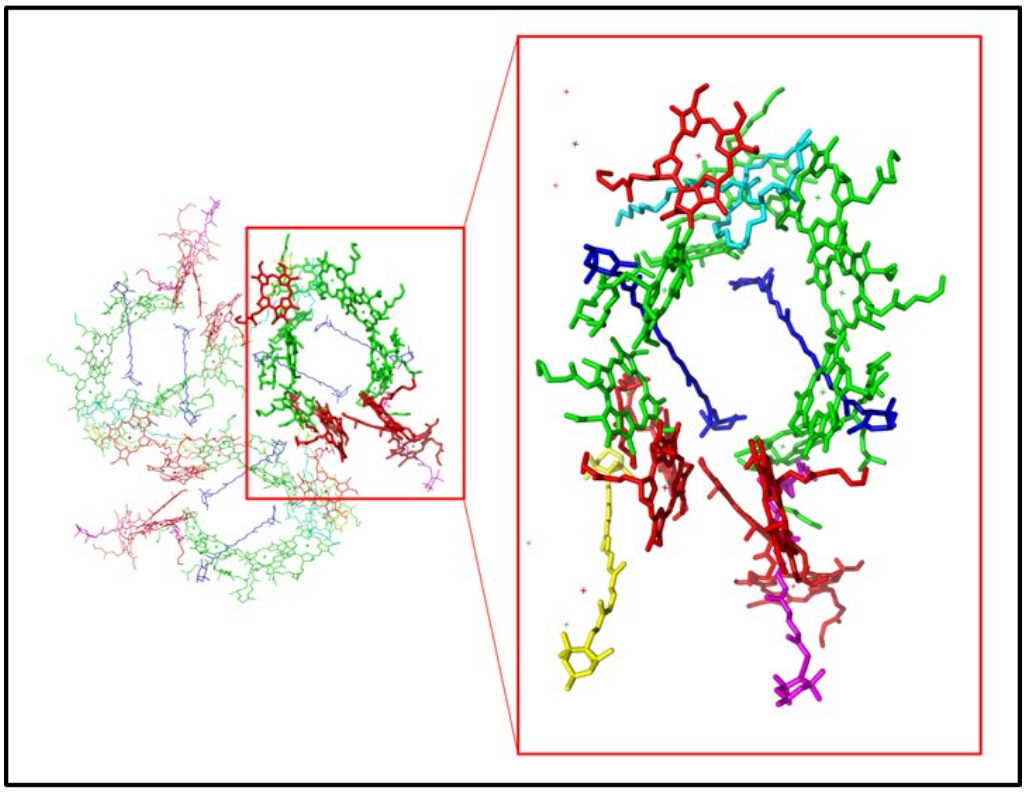

Figure 4. Pigment molecules within major LHCII trimer. Green, Chl a; red, Chl b; blue, lutein (Lut); yellow, violaxanthin (Vio); maganta, neoxanthin (Neo); cyan, phosphatidyl glycerol (PG).

Protein names(s)	Gene	Mature protein size (kDa)	Location/ function(s) (c-chloroplast; n-nuclear)	
D1/PsbA	psbA	37.978	c	RC protein/charge separation and ligands for Mn4CaO5 cluster
CP47/PsbB	psbB	56.037	c	Inner antenna protein for RC
CP43/PsbC	pshC	50.043	c	Inner antenna protein for RC and ligand for Mn4CaO5 cluster
D2/PsbD	psbD	39.416	c	RC protein/charge separation
PsbE/α-Cyt b_{559}	psbE	9.265	c	RC proteins/early steps of PSII assembly; photoprotection
PsbF/β-Cyt b_{559}	psbF	4.424	c	RC proteins/early steps of PSII assembly; photoprotection
PsbH	psbH	7.571	c	LMM protein of PSII core/PSII assembly and stabilization; electron transport al acceptor side of PSII; photoprotection
PsbI	psbl	4.168	c	LMM protein of PSII core/Early steps of PSII assembly Stabilization of PSII dimers and supercomplexes
PsbJ	psbJ	4.117	c	LMM protein of PSII core/forward electron transfer within PSII; necessary for assembling PsbP to PSII core
PsbK	psbK	4.237	c	LMM peripheral protein of PSII core/necessary for assembling PsbZ and Psb30 to PSII core; PSII dimer stabilization
PsbL	psbL	4.47	c	LMM protein at monomer–monomer interface of PSII core/ PSII assembly;PSII dimer stabilization donor side and acceptor electron transfer
PsbM	psbM	3.783	c	LMM protein at monomer–monomer interface of PSII core/ PSII dimer stabilization; Electron transfer at QB site
PsbO/33 kDa	psbO	26.566	n	Extrinsic protein-lumenal side, close to OEC/Mn4CaO5 cluster stabilization; Environment optimization for water oxidation; D1 turnover
PsbP/ 23–24 kDa	psbP	20.212	n	Extrinsic protein-lumenal side, close to OEC/binding of CI- and Ca2+; light-induced assembly of Mn4CaO5 cluster; structural integrity of thylakoids architecture; stabilization of PSII-LHCII supercomplexes
PsbQ/ 16–18 kDa	psbQ	16.194	n	Extrinsic protein-lumenal side, close to OEC/binding of CI- and Ca2+; photoaoutotrophy PSII assembly and stability under low light
PsbR	psbR	10.342	n	Protein anchored to lumenal side, close to OEC/docking protein for PSII extrinsic proteins in plants
PsbS	psbS	21.527	n	Peripheral protein/dissipation of excess light energy; interaction between LHCll and PSII core
PsbTc	psbTc	3.822	c	LMM protein at the monomer–monomer interface of PSII core/early steps of PSII assembly; PSII dimerization and stabilization; recovery of photodamage
PsbTn	psbTn	3.172	n	Extrinsic protein-lumenal side/unknown function
PsbW	psbW	6.036	n	LMM protein of PSII core with uncertain localization/ photoprotection; stabilization of PSII–LHCII supercomplexes
PsbX	psbX	4.186	n	LMM protein of PSII core/electron transfer function of QA and QB
PsbY	psbY	4.202	c.n	LMM peripheral protein of PSII core/unknown function
PsbZ	psbZ	6.569	c	LMM peripheral protein of PSII core/linker between LHCll and PSII core
Psb30/ Ycf12	psb30	4.143	c	LMM peripheral protein of PSII core/stabilization of PSII dimers; prevention of Cyt b559 from converting to low potential form under high light
Psb31	psb3l	13.256	n	Extrinsic protein-lumenal side/supporting oxygen evolution

Tabel 1. Structural subunits of PSII core complex. RC: reaction center; LLM: low molecular mass proteins; chloroplast (c) or in the nuclear (n) genome. Adopted from (Pagliano et al., 2013).

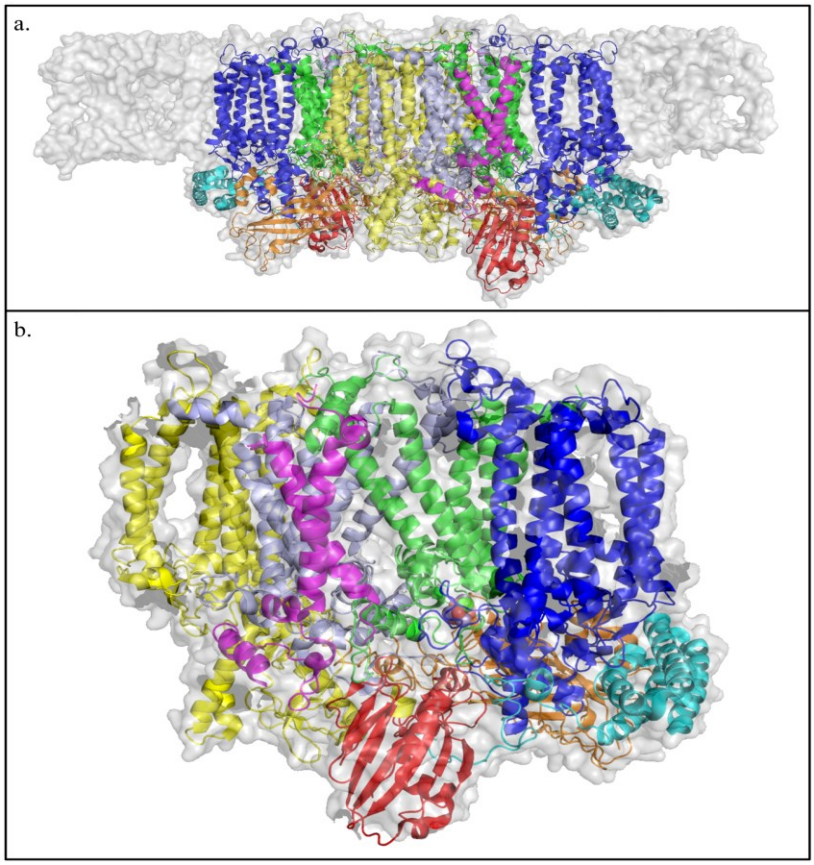

Figure 5. Structure of PSII core complex. A) Dimeric PSII core complex (side view). B) Monomeric PSII core complex (top view). Green, D1; light blue, D2; yellow, CP47; blue, CP43; magenta, cyt b_{559}; orange, PsbO; red, PsbP; cyan, PsbQ.

1.1.2.1 Mn_4O_5Ca cluster

A recent study oriented towards the structure of Mn_4O_5Ca cluster by X-ray absorption spectroscopy depicts that Mn_4O_5Ca cluster is formed by di-μ-oxo and one mono-μ-oxo-bridged Mn-Mn (Zein et al., 2008, Yano and Yachandra, 2008, Yano and Yachandra, 2014). The differences in the distances (from 2.4 to 3.3Å) between the Mn and associated oxygen atoms are too long to withstand and to be bridged by a di-μ-oxo which is stabilized by the presence of the oxygen molecules in the complex, thus giving rise to the cubane structure (Umena et al., 2011, Robblee et al., 2001). Ca is connected to the cluster via three of the bridging oxygens which are associated with more isolated Mn_4. There are several ligands directly ligated to Mn_4CaO_5 cluster: six carboxyl ligands (aspartate and glutamate), one imidazole ligand (histidine), the D1 subunit is associated with the cluster via CD luminal loop (^{170}Asp and ^{189}Glu), and the C-terminal region (^{332}His and ^{344}Ala) of CP43on EF luminal loop.

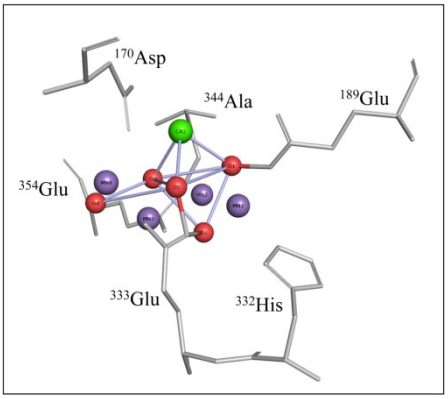

Figure 6. Structure of Mn_4O_5Ca cluster with ligands of Mn (purple) and Ca (green) and four oxygen atoms (red).

1.2. PSII-LHCII supercomplex function

LHCII and PSII core complex (CP43 and CP47) maintain absorption of light and energy transfer to PSII reaction center. PSII reaction center is the site of primary charge separation, where electron transfer is initiated that oxidizes water and reduces plastoquinone.

1.2.1. Excitation energy transfer

The capturing of light energy is achieved by LHCII and PSII core antennae CP43 and CP47. After the absorption of a photon by chlorophylls, excitation energy is transferred to PSII reaction center, converted into heat or emitted as fluorescence. LHCII and PSII core antennae are arranged to be very efficient in excitation energy transfer to PSII reaction center. However, under various types of abiotic and biotic stresses, limitation in electron transport enhances conversion of excitation energy to heat or fluorescence emission.

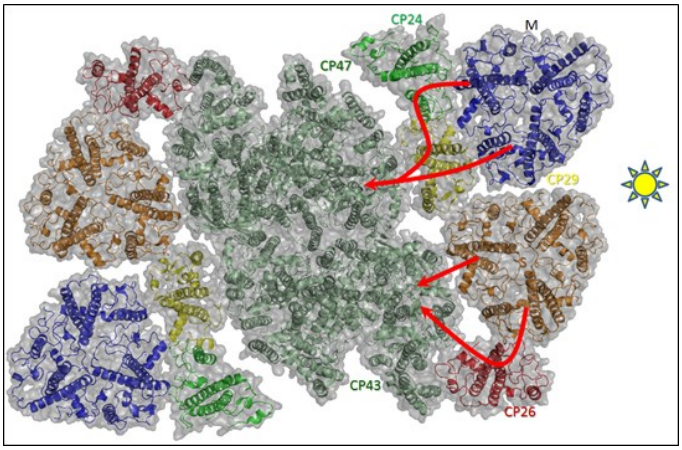

Figure 7: Excitation energy transfer in PSII-LHCII

$C_2S_2M_2$ does not follow strict pathways for excitation energy transfer, which affect the energy transfer efficiency, transfer pathways and transfer limiting obstacles. This flexibility in the appropriate transfer route improves and optimizes the delivery of the energy transfer. The excitation energy transfer occurs from Lhcb to the PSII core complex on faster time scale, whereas the energy transfer to PSII reaction center is rather slow. The absorbed light energy in outer LHCII antenna complexes is transferred *via* the minor complexes CP24, CP26, and CP29 to CP47 and CP43 of PSII (Bennett et al., 2013, Kreisbeck and Aspuru-Guzik, 2016).

1.2.2. Electron transport

Primary charge separation between the chlorophyll monomer (Chl_{D1}) and pheophytin ($Pheo_{D1}$) of D1 protein forms $^1[Chl_{D1}^{\bullet+}Pheo^{\bullet-}]$ radical pair. Oxidation of weakly-coupled chlorophyll dimer P_{D1} and P_{D2} (P680) by $Chl_{D1}^{\bullet+}$ forms $^1[P680^{\bullet+}Pheo_{D1}^{\bullet-}]$ radical pair. The charge separation induces electron transport which results in the oxidation of water and reduction of plastoquinone on the luminal and stromal side of PSII, respectively (Fig. 8). Electron from $Pheo_{D1}^{\bullet-}$ is transferred to the tightly bound plastoquinone Q_A forming $Q_A^{\bullet-}$ which subsequently transfers electron to the loosely bound plastoquinone Q_B. When two reducing equivalents are formed at Q_B, protonated forms PQH_2 is released to PQ pool. Electron transfer from the redox active tyrosine residue D1:161Y (Y_Z) to $P680^{\bullet+}$ forms $Y_Z^{\bullet}$ which successively oxidizes manganese in the Mn_4O_5Ca cluster. Formation of four oxidizing equivalents in the Mn_4O_5Ca cluster causes four-electron oxidation of two H_2O to O_2 which is released via channels into the lumen.

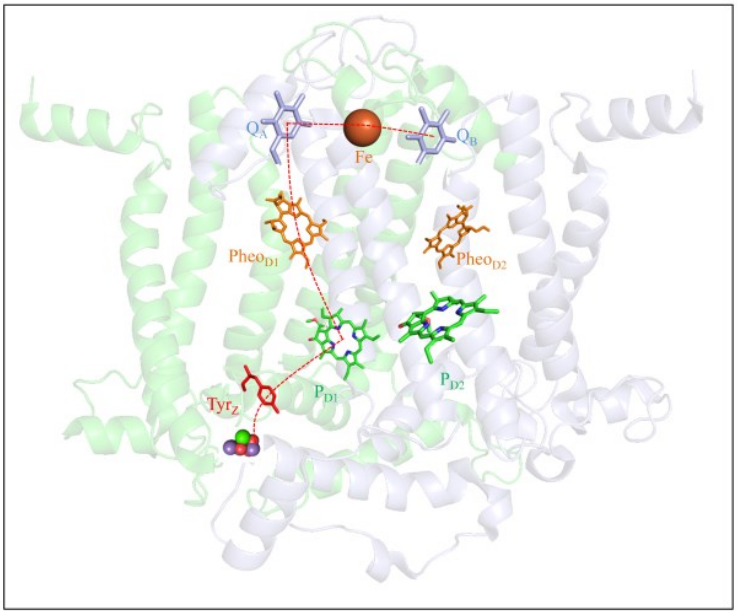

Figure 8. Electron transfer in PSII reaction center

To control water oxidation, the delivery of water and molecular oxygen has to be maintained. It has been proposed that Mn_4O_5Ca cluster is equipped with a number of networks and channels which help transfer substrate and products to and from the arena (Fig. 9). Several molecular dynamically and computationally based methods have been advanced in order to study the nature and characteristics of the diffusion of water and molecular oxygen in and out of PSII. In total, nine channels are associated with Mn_4O_5Ca cluster that are most likely influenced by the presence of the Ca^{2+} and Cl^- ions (Barber, 2008, Guskov et al., 2010, Kern and Guskov, 2011, Najafpour et al., 2016). These studies have opened the understandings of the roles of individual oxygen and water molecules, as well as proton channels and migration patterns of the substrate and products of the photosynthetic reaction.

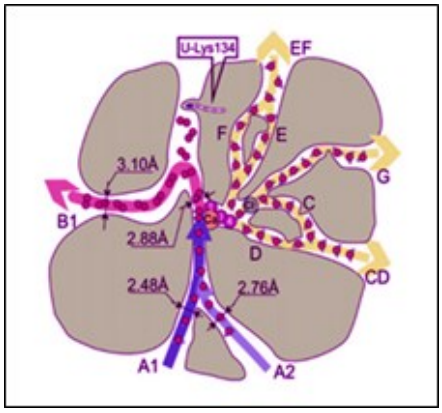

Figure 9. Possible trajectories of the substrate/product through the channels to and from the Mn_4O_5Ca cluster and to the lumen. Thick colored arrows indicate the suggested paths for water supply (blue), release of molecular oxygen (pink) and protons (yellow). Adopted from (Najafpour et al., 2016).

2. ROS formation

The simultaneous occurrence of molecular oxygen with excited chlorophylls and highly reducing/oxidizing species is potentially harmful to plants as reactive oxygen species (ROS) might be formed. The mechanistic aspects of ROS formation can be grouped as 1) ROS formation by energy transfer and 2) ROS formation by electron transfer. Energy transfer is associated with the formation of singlet oxygen ($^1O_2$), whereas electron transport is accompanied by the formation of superoxide anion radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) (Pospíšil, 2016). Under moderate levels of ROS, when scavenging systems effectively eliminate ROS, ROS serves as a signaling molecule which can further initiate protective mechanisms. Under high level of ROS, if the scavenging system fails to eliminate ROS, PSII proteins and lipids are oxidized (Aro et al., 1993, Komenda J, 2006, Triantaphylides et al., 2008).

2.1. ROS by excitation energy transfer

Under high light conditions, if the energy of singlet excited chlorophyll ($^1Chl^*$) in the PSII antenna complexes is not transferred properly to the PSII reaction center, the lifetime of $^1Chl^*$ is increased and the probability of the formation of long-lived triplet excited chlorophyll ($^3Chl^*$) occurs (Fig. 10). When molecular oxygen is in the proximity of $^3Chl^*$, triplet-triplet energy transfer from $^3Chl^*$ to molecular oxygen results in $^1O_2$ formation (Triantaphylides and Havaux, 2009, Pospíšil, 2012, Fischer et al., 2013). PSII is equipped with a number of protective processes to avoid $^1O_2$ formation and thus plausible damage of PSII protein and lipid by $^1O_2$. In the first line, $^1Chl^*$ formation is avoided by the dissipation of excitation energy in the form of heat, known as non-photochemical quenching. It involves either 1) quenching of $^1Chl^*$ by xanthophylls or 2) structural changes of Lhcb protein subunits maintained by PsbS to minimize chlorophyll over-excitation (Li et al., 2000). However, any hindrance in quenching may results in $^3Chl^*$ formation and transfer of treiplet energy to nearby molecular oxygen forming $^1O_2$. In the second line, $^1O_2$ is eliminated by physical (quenching) or chemical (scavenging) processes. In the $^1O_2$ quenching, triplet-triplet energy transfer from $^1O_2$ is transferred to quencher (carotene) which converts excitation energy to heat (Ruban et al., 2012). In $^1O_2$ scavenging, oxidation of scavenger (tocochromanol and plastoquinone) by $^1O_2$ forms scavenger radical which recombines with another scavenger radical to terminate radical reaction.

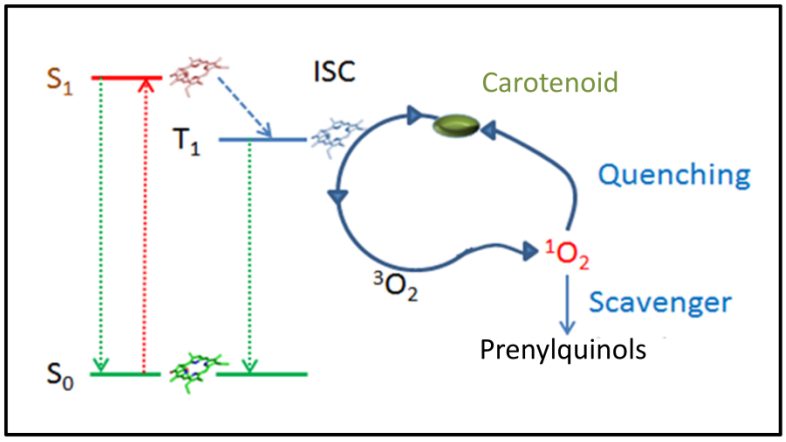

Figure 10: Singlet oxygen formation by triplet-triplet energy transfer from triplet chlorophyll to molecular oxygen and its elimination by quenching maintained by carotenoids and scavenging mediated by prenylquinols.

2.1.1. PSII antennae complexes

In PSII antennae complex, $^3Chl^*$ is formed by intersystem crossing comprising a change in spin state of the excited electron (Scheme 1) (Pospíšil, 2016). Comparison of the monomeric and the trimeric Lhcb showed that the monomeric antenna proteins produced more $^1O_2$ as

compared to trimeric antenna proteins (Ballottari et al., 2013). It has been demonstrated that monomeric Lhcb contributes to $^1O_2$ formation in following order: Lhcb6 > Lhcb5 > Lhcb4. The authors demonstrated that lutein bound in the L1 binding site and zeaxanthin bound in the L2 binding site in monomeric Lhcb4-6 proteins can efficiently quench the neighboring $^3Chl^*$. Evidence has been provided that xanthophylls in L1 and L2 binding sites are coupled with chlorophylls Chl610-Chl614 and Chl602-Chl604, respectively. Whereas quenching of $^3Chl^*$ by xanthophyll in L2 is highly efficient, xanthophyll in L1 binding site have limited effect on $^3Chl^*$ quenching. Due to the lack of coupling between chlorophyll and xanthophyll in site L1, chlorophyll is not protected and it possibly contributes significantly to the overall $^3Chl^*$ formation in Lhcb.

$$^1Chl^* \longrightarrow {}^3Chl^* + O_2 \longrightarrow Chl + {}^1O_2 \qquad (1)$$

2.1.2. PSII reaction center

In PSII reaction center, $^3P680^*$ is formed by the charge recombination of triplet radical pair $^3[P680^{\bullet+}Pheo^{\bullet-}]$ (Scheme 2). It was reported that $[P680^{\bullet+}Q_A^{\bullet-}]$ radical pair recombines to the ground state P680, whereas primary radical pair $^1[P680^{\bullet+}Pheo^{\bullet-}]$ formed by the reverse electron transport from $Q_A^{\bullet-}$ to Pheo either recombines to the ground state P680 or is convert to the triplet radical pair $^3[P680^{\bullet+}Pheo^{\bullet-}]$ by change in the spin orientation. Subsequently, triplet radical pair $^3[P680^{\bullet+}Pheo^{\bullet-}]$ recombines to the triplet chlorophyll $^3P680^*$. It has been reported that triplet excitation energy is delocalized on the weakly-coupled chlorophyll dimer P_{D1} and P_{D2} (Fischer et al., 2013; Telfer, 2014). Due to the fact that β-carotenes (Car_{D1} and Car_{D2}) are localized far away from chlorophyll dimer P_{D1} and P_{D2}, it was evidenced that β-carotenes are not able to quench triplet chlorophyll $^3P680^*$.

$$^1[P680^{\bullet+}Pheo^{\bullet-}] \longrightarrow {}^3[P680^{\bullet+}Pheo^{\bullet-}] \longrightarrow {}^3P680^* + O_2 \longrightarrow P680 + {}^1O_2 \qquad (2)$$

2.1.3. Triplet excited carbonyls

Apart to $^3Chl^*$, $^1O_2$ might be formed by triplet-triplet energy transfer from triplet excited carbonyl ($^3L{=}O^*$) to molecular oxygen formed during lipid peroxidation (Scheme 3) (Pospíšil and Prasad, 2014). In this process, fatty acids are oxidized by non-enzymatic and enzymatic reaction pathways. In the non-enzymatic reactions, hydrogen abstraction from fatty acids by $HO^\bullet$ forms lipid alkyl radical ($L^\bullet$). The reaction of $L^\bullet$ with molecular oxygen results in the formation of peroxyl radical ($LOO^\bullet$). Another hydrogen abstraction form nearby fatty acid by $LOO^\bullet$ causes formation of hydroperoxy polyunsaturated fatty acids (lipid hydroperoxide, LOOH). In the enzymatic reaction, LOOH is formed by lipoxygenase. Under reducing conditions, LOOH is reduced by transition metals to lipid alkoxyl radical ($LO^\bullet$); however, under the oxidizing

condition, LOOH is oxidized to $LOO^{\bullet}$ by oxidized transition metals, ferric heme iron of cytochrome c, peroxynitrite, chloroperoxide, and hypochlorous acid. The cyclization of $LOO^{\bullet}$ is known to form a cyclic endoperoxide (dioxetane) LOOH, whereas recombination of the two LOO• forms a linear tetroxide LOOOOH. These high-energy intermediates decompose to $^3L{=}O^*$ which might transfer triplet energy either to Chl forming $^1Chl^*$ or molecular oxygen forming $^1O_2$. In addition, tetroxide might decompose directly to $^1O_2$ by Russell mechanism. It has to be noted that amount of $^1O_2$ formed by the triplet-triplet energy transfer from $^3Chl^*$ is considerably higher than from $^3L{=}O^*$ (Pospíšil, 2016). It has been demonstrated that exposure of Chlamydomonas cells to heat stress results in $^1O_2$ formation by triplet-triplet energy transfer from $^3L{=}O^*$ to molecular oxygen (Prasad et al., 2016).

$$L \longrightarrow L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \longrightarrow LOOH \longrightarrow LO^{\bullet} \qquad (3)$$

$$LOO^{\bullet} \longrightarrow LOOH \text{ or } LOOOOH \longrightarrow {}^3L{=}O^* + O_2 \longrightarrow {}^1O_2$$

2.2. ROS by electron transport

In electron transfer mechanisms, ROS can be formed by reduction of molecular oxygen on PSII electron acceptor side and oxidation of water on PSII electron donor side (Fig. 11).

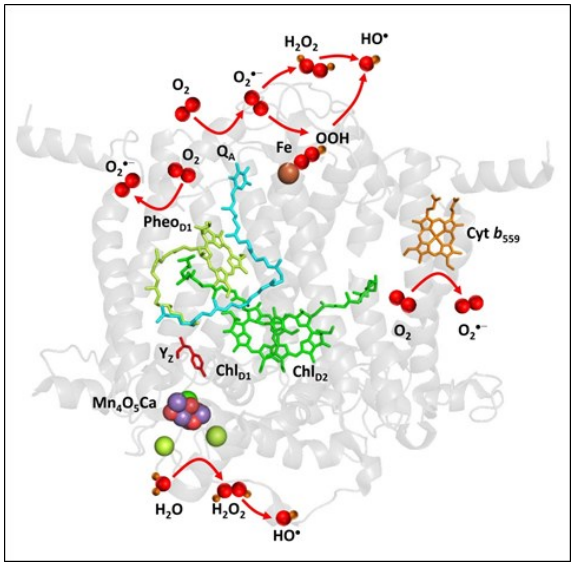

Figure 11: Light-induced formation of $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$ by PSII. Adopted from (Pospíšil, 2016).

2.2.1. PSII electron acceptor side

Limitation in electron transport on the PSII electron acceptor side is associated with reduction of molecular oxygen (Pospíšil, 2014). One-electron reduction of molecular oxygen results in $O_2^{\bullet-}$ formation. It has been evidenced that PSII cofactors such as $Pheo_{D1}^{\bullet-}$, $Q_A^{\bullet-}$, $Q_B^{\bullet-}$ and possibly reduced low potential cyt b_{559} have redox potentials and lifetimes sufficient to reduce molecular oxygen. Superoxide anion radical either spontaneously dismutates to free H_2O_2 or form bound peroxide by the interaction with non-heme iron. In the dismutation, two $O_2^{\bullet-}$ are instantaneously reduced and oxidized to H_2O_2 and O_2, respectively. It has been proposed that bound peroxide is formed by the interaction of $O_2^{\bullet-}$ with the non-heme iron forming ferric-peroxo and ferric-hydroperoxo species (Pospíšil et al., 2004). Both free H_2O_2 and bound peroxide are reduced by free iron and non-heme iron to $HO^{\bullet}$. The reduction of free H_2O_2 occurs via Fenton reaction, whereas the reduction of bound peroxide (ferric iron-hydroperoxo intermediate) was proposed to take place via ferric iron-oxo intermediate (Pospíšil et al., 2004).

2.2.1. PSII electron donor side

Limitation in electron transport on the PSII electron donor side is associated with incomplete H_2O oxidation catalyzed by the Mn_4O_5Ca cluster. An incomplete H_2O oxidation results in the formation of H_2O_2 which is either reduced to $HO^{\bullet}$ or oxidize to $O_2^{\bullet-}$. It has been proposed that H_2O_2 is reduced to $HO^{\bullet}$ by manganese ion released from the damaged Mn_4O_5Ca cluster, whereas H_2O_2 is oxidized to $O_2^{\bullet-}$ by redox active tyrosine residue Y_Z.

AIMS OF THE STUDY

This study mainly aims at the efficiency of PSII under high light with respect to structural and functional characterization of PSII-LHCII supercomplex and current understanding of how PSII behaves with respect to highly oxidizing ROS. This study also depicts the major pathways and protective mechanisms which occur whilst the damage caused by ROS responsible for the photoinactivation of PSII and the oxidative modification which occurs in protein subunits of PSII. While we are aware that modification occurred due to ROS and the structural damages they cause, this process remains a topic of greater interest, which has been addressed in this study to larger extent.

The much anticipated and most crucial evets which occur within higher plants under high light stress were addressed in this study. The primary aim of the study was to:

1. address the formation of $O_2^{\bullet -}$ and $HO^{\bullet}$ under high light in PSII electron donor and acceptor sides and identify specific amino acid residues of the D1 and D2 proteins oxidized by these ROS.
2. provide high resolution 3D structure of the $C_2S_2M_2$ supercomplex from *Arabidopsis* and describe structural change associated with shift in the position of M trimer a lateral movement of the two halves of the dimeric core complex
3. describe mechanism of $^1O_2$ by triplet-triplet energy transfer form $^3L{=}O^*$ to molecular oxygen under leave wounding.

SUMMARY

Despite the prevailing mechanisms against excess light energy, PSII reaction center is vulnerable to oxidative modifications. The D1 and D2 proteins, lying at PSII reaction center, are susceptible to oxidative modification by ROS that are formed by PSII during high light illumination. With the obtained data, it was evident that high light induces the formation of ROS which oxidizes the specific amino acids at the electron donor as well as acceptor sides of the PSII. With high light, $O_2^{\bullet-}$ and $HO^{\bullet}$ formation was detected which is associated with the oxidative damage to D1 and D2 proteins. With the photoinhibitory time course, we have identified several specific amino acid residues of the D1 and D2 proteins that are oxidized by $HO^{\bullet}$, and possibly $O_2^{\bullet-}$.

The dynamic nature of PSII-LCHII suprecomplex which gives the insights of the flexibility of trimeric antennas of supercomplex with respect to energy transfer and also structural changes in dark and light adapted state. We analyzed a set of 400,000 negatively stained projections in which M trimer of the $C_2S_2M_2$ supercomplex shows the flexibility within the peripheral antenna. With the obtained data, it was evident that M trimer has two different positions compared to S trimer. With shift in peripheral antenna, there is a displacement of the CP24 creating a gap in PSII core complex. In fewer particles, lateral movement of the dimeric core complex was observed with the displacement of both M trimers. The relative orientation of M trimer and CP24 obtained from the PSII model indicates a contact of CP24 with M trimer in the region between helix B and helix E which have the greater implications on the expected energy flow.

Molecular mechanism on the formation of $^1O_2$ in wounded Arabidopsis plants was studied in WT and lox2 mutant lacking chloroplast lipoxygenase (LOX2). Our data revealed that $^1O_2$ is formed by triplet-triplet energy transfer from $^3L{=}O^*$ to molecular oxygen formed during lipid peroxidation. As $^3L{=}O^*$ and $^1O_2$ formation was prevented in lox2 mutant, it was proposed that the chloroplast lipoxygenase plays a key role in the formation of $^3L{=}O^*$ and $^1O_2$ in wounded Arabidopsis plants. The plant response to wounding by $^1O_2$ formation can lead to hypothesis on existence of wound induced signaling pathway mediated by $^1O_2$.

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