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REVIEW

Roles of manganese in photosystem II dynamics to irradiations and temperatures

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Abstract **The most amazing chemistry is the light-driven water splitting reaction occurred in the oxygen-evolving complex of phototsystem II in higher plants, green algae, and cyanobacteria. Mn, in the form of Mn_4CaO_5 cluster in photosystem II, is responsible for the catalytic water splitting reaction as well as plays roles in photosystem II dynamics to irradiation and temperatures. Manganese hypothesis of UV-initiated photoinhibition as a direct target is established, and thermal inactivation of photosystem II involves the valence and structural changes of manganese. Recent progresses in understanding the roles of manganese in photoinhibition especially under UV light and in thermal inactivation including elevated temperatures using synthetic models and native PS II complexes are summarized and evaluated. Potential problems and possible solutions are discussed and presented.**

Keywords photosynthesis, manganese, photosystem II, irradiation, temperature, stress

Introduction

Photosynthesis, which harvests, transfers, converts and stores the solar energy in the form of chemical bonding energy and supports all the living things on earth (Blankenship, 2002), is one of the most important chemical reactions in science. In photosynthetic process, the most amazing chemistry is water splitting reaction, which occurs in the phototsystem II (PS II) protein complex embedded in the thylakoid membranes of higher plants, green algae, and cyanobacteria (Rutherford and Boussac, 2004; Brudvig, 2008). However the molecular basis of photosynthetic water oxidation has remained one of the major mysteries in bioenergetics research.

PS II functions as a water-plastoquinone oxidareductase and is able to catalyze the light-driven transfer of electron and proton from water at the luminal side to a pool of plastoquione molecules at the stromal side of the thylakoid membranes (Diner and Rappaport, 2002). The kinetics of the electron transfer steps in PS II has been thoroughly investigated over the complete time scale of femtosecond to many seconds (van Grondelle et al., 1994; Rappaport et al., 2002; Govindjee and Seibert, 2010). The early process occurring in PS II revealed by ultrafast spectroscopy can be divided into several steps: (1) absorption of light quanta by antenna to form excited states of pigments; (2) trapping of excitation energy by the primary electron donor P_{680} in the reaction center on the picosecond time scale; (3) primary charge separation from the singlet excited state of P_{680} to the primary acceptor pheophytin (Pheo) in about 3–20 ps; (4) stabilization of the separated charges from the radical pair P_{680}^+ $Pheo^-$ on the acceptor side by electron transfer to Q_A in about 200 ps and to Q_B on the hundreds of millisecond time scale; and (5) on the donor side, an electron is supplied to reduce P_{680}^+ from a tyrosine residue (Y_Z) on the nanosecond time scale to microsecond time scale.

In contrast to the knowledge of the kinetics, that of the thermodynamics of electron transfer step in PS II is relatively limited (van Gorkom, 1985; Delosme et al., 1994; Delosme, 2003; Hou and Mauzerall, 2011). The free energy of the electron transfer process is obtained from midpoint redox potentials of the respective components or from the analysis of kinetic data (van Gorkom, 1985; Sakuragi et al., 2002). Using photoacoustics, quantum yield, volume changes, and reaction enthalpy of electron transfer from P_{680} to Q_A in PS II of *Synechocystis* 6803 are determined (Hou et al., 2001a). The apparent entropy change of the reaction in PS II is negative,

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which is different from large positive entropy of PS I of *Synechocystis* 6803 and purple bacteria of *Rb. Sphaeroides* (Edens et al., 2000; Hou et al., 2001b; Hou and Mauzerall, 2006; Mauzerall, 2006; Hou et al., 2009). The negative entropy may be due to a mixture of electron transfer from anionic tyrosine and fast proton transfer to a polar region in PS II (Hou et al., 2001a; Hou and Mauzerall, 2011). The preliminary data using photothermal beam deflection sensor (Krivanek et al., 2008) and an air microphone photoacoustic detector (Mauzerall, 2010) suggests that the S-state cycle in PS II oxygen-evolving complex is likely entropy-driven as are steps in PS I (Mauzerall, 2006).

PS II is very sensitive to changes in environment. Under unfavorable or stressful environmental conditions, such as strong light, high concentration of salt, and low and high temperatures, the activity of PS II declines more rapidly (Pearcy et al., 1977; Aro et al., 1993; Niyogi, 1999; Barbara Demmig-Adams and Autar, 2007; Murata et al., 2007; Takahashi and Murata, 2008). The effects of environmental stress on damage and repair were explored and multiple hypothetical models were proposed.

In this review, we will summarize the recent advances in literature associated with the roles of manganese in photoinhibition and thermal inactivation. Other aspects of manganese structure and function in photosystem II were briefly discussed to provide a background of manganese in photosynthesis. The focus is placed on the response mechanisms of Mn_4CaO_5 cluster in PS II to strong light and elevated temperature. We also discuss the potential problems and possible solutions.

Mn_4CaO_5 cluster in PS II

Mn_4CaO_5 cluster in PS II plays a vital role in oxygenic photosynthesis and is responsible for the catalytic water splitting chemistry (Diner and Babcock, 1996; Diner and Rappaport, 2002; Vrettos and Brudvig, 2002). In PS II, which is integral membrane protein complex of more than 20 subunits with a molecular mass of about 350 kD, Mn_4CaO_5 cluster is the site of water oxidation to oxygen (Brudvig, 2008). When four electrons and four protons are extracted from two molecules of water, one molecule of dioxygen is produced. The mechanism of photosynthetic water oxidation in PS II is believed to occur through four distinct oxidation step as the S-state cycle by Kok et al. (1970) and Joliot et al. (1969).

The structure of PS II has been determined at resolution of 2.9–3.8 Å from *Thermosynechococcus elongates* and *Thermsynechocccus vulcanus* (Kamiya and Shen, 2003; Ferreira et al., 2004; Loll et al., 2005). However due to the sensitivity of Mn cluster to X-ray exposure and relative low resolution crystallographic data, the structure of Mn cluster and substrate water molecules in PS II are revealed. A recent study reported the high-resolution structure of PS II from *Thermosynechococcus vulcanus* at a resolution of 1.9 Å (Umena et al., 2011). The complete geometry arrangement of the Mn cluster including its oxo bridges and ligands were determined. Three manganese, one calcium and four oxygen atoms form a cubane-like structure. The fourth manganese is located outside the cubane. In addition, five water molecules are also identified in the structure of PS II. These molecular details reveal important function for the mechanism of water splitting and O-O bond formation.

Mn in photoinhibition of PS II

PS II plays an important role in response to the environmental conditions such as excess light, which is known as photoinhibition and is observed either under high intensity light conditions when the repair mechanisms have reached maximum capacity or at lower light intensities when an additional external factor inhibits the repair of PS II (Kok et al., 1966; Powles, 1984). The molecular mechanisms of photoinhibition were extensively investigated with great progress made during last decades (Ohad et al., 1990; Aro et al., 1993; Chow, 1994; Stewart and Brudvig, 1998; Niyogi, 1999; Tracewell et al., 2001; Adir et al., 2003; Allakhverdiev and Murata, 2004; Frank and Brudvig, 2004; Carpentier, 2005; Nishiyama et al., 2005; Szabó et al., 2005; Telfer, 2005; Nishiyama et al., 2006; Zsiros et al., 2006; Murata et al., 2007; Tyystjarvi, 2008; Kramer, 2010; Sarvikas et al., 2010a; Sarvikas et al., 2010b). Light-induced damage is targeted mainly to PS II (Powles, 1984; Barber and Andersson, 1992). The action spectra of photoinhibition of spinach chloroplast revealed that ultraviolet light is highly photoinhibitory to PS II (Jones and Kok, 1966). It was proposed that plants have a recovery mechanism that continuously repairs the photoinhibitory damages. The visible light part of the action spectra was found to have a peak in the red region, suggesting that chlorophylls act as photoreceptors of photoinhibition. Photoinhibition is accompanied by selective loss of the D1 protein in the PS II reaction center (Kyle et al., 1984). The environmental stress factors, such as salt, cold, and moderate heat, do not affect photodamage of PS II but inhibit the repair of PS II. In this way, environmental stress enhances the extent of photoinhibition (Allakhverdiev and Murata, 2004, 2008; Takahashi and Murata, 2008).

Photoinactivation of the PS II reaction center is hypothesized to occur by two independent mechanisms associated with the acceptor and donor sides of PS II, respectively, were published in the several comprehensive reviews (Barber and Andersson, 1992; Aro et al., 1993; Tyystjarvi, 2008). As summarized by Hakala et al. (Hakala et al., 2005), both models result in the inhibition of electron transfer in PS II and the subsequent degradation of the D1 protein (Fig. 1). The acceptor side inhibition occurs under high light conditions when the plastoquinone pool is fully reduced, and consequently there is a lack of oxidized plastoquinone to bind to the

Q_B site on the D1 protein (Fig. 1C). In this case, Q_A will become doubly reduced on a second turnover of the reaction center to form Q_A^{2-}, because Q_A^- cannot transfer an electron to Q_B. Q_A^{2-} then becomes protonated to form Q_AH_2 and is released from the Q_A^- binding site on the D1 protein. It is noted that the accumulation of double reduced Q_A in photoinhibition may not occur in aerobic conditions (Setlik et al., 1990; Vass et al., 1993). With the Q_A site unoccupied, the excitation of P_{680} will result in the formation of the radical pair-P_{680}^+ $Pheo^-$. Recombination of charge separation will generate a triplet state of P_{680}, which will react with oxygen to form singlet oxygen. The singlet oxygen as one of the reactive oxygen species (ROS) is highly toxic to proteins and cofactors and can react with the D1 protein, triggering the degradation of the D1 protein (Shipton and Barber, 1991, 1994).

The donor-side photoinhibition of PS II occurs as a result of the formation of the highly reactive P_{680}^+ species under light (Fig. 1B). P_{680}^+ is capable of oxidizing neighboring proteins and chromophores. Oxidation of accessory chlorophylls and β-carotene and degradation of D1 have been found to occur under conditions of P_{680}^+ formation using the isolated PS II reaction center D1/D2/cyt b_{559} complex (Telfer et al., 1990; Peng et al., 1999). Loss of donor-side electron transport and consequent photodamage to the D1 protein have been linked to the generation of high H^+ electrochemical potential across the thylakoid membrane and the possible associated release of Ca^{2+} from the water-oxidizing complex (Ohad et al., 1994). It was also found that the process does not require the presence of oxygen (Jegerschöld and Styring, 1991; Shipton and Barber, 1992) and is not accompanied by singlet oxygen production in Tris-pretreated thylakoids (Hideg et al., 1994).

The first study on UV-B effect on photoinhibition of PS II showed that UV-B primarily attacks the water oxidizing complex in 1989 (Renger et al., 1989). Recently, a Mn-targeted hypothesis for the mechanism of UV photoinhibition of PS II was proposed (Hakala et al., 2005; Ohnishi et al., 2005), as shown in Fig. 1A. The photoinactivation of the thylakoid membranes from *Thermodynechococcus elongatus* by UV and blue light was suggested to be the first step of photoinhibition (Ohnishi et al., 2005). The experimental evidence seems to support that the earliest step in photoinhibition is the release of a manganese ion from PS II (Hakala et al., 2005). A photon absorbed by the manganese ions of the oxygen-evolving complex triggers inactivation of the oxygen-evolving complex, which is supported by measuring the action spectra of photoinhibition. The inhibition occurs like in the donor-side model. Furthermore, it is reported that not only the Mn ions in PS II oxygen-evolving complex (OEC) (Hakala et al., 2005), but also the PS II protein subunits, 33 kD, 24 kD and 18 kD, are released form the spinach PS II membranes under photoinhibitory illumination. The released proteins, 33 kD and 24 kD, were photodamaged and lost the ability to rebind to PS II. In addition, Mn catalase and Mn superoxide dismutase are also photosensitive to both visible and UV light (Hakala et al., 2006). The oxidation state of the water-splitting complex seems play an role in PS II sensitivity by UV-B radiation (Szilárd et al., 2007). The photodamage of the Mn_4CaO_5 complex in single crystals of PS II was also reported by exposure to X-rays during crystallographic data collection (Yano et al., 2005). Their X-ray absorption spectra show that the damage site is the reduction of high-valent manganese ions and associated with structure change.

The mixed-valence Mn(III/IV)-oxo dimer compound, $[Mn(III)(O)_2Mn(IV)(H_2O)_2(Terpy)_2](NO_3)_3$, is the first func-

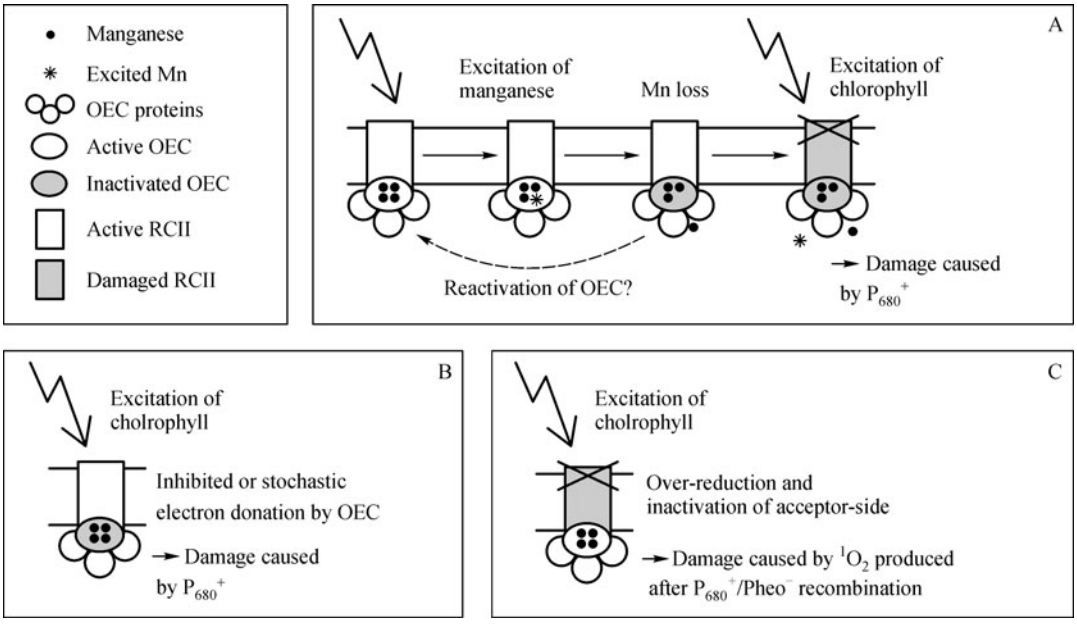

Figure 1 Possible mechanistic models of PS II dynamics to strong light (Hakala et al., 2005) (Reproduction permission from Elsevier).

tional model oxygen-evolution complex of PS II (Limburg et al., 1999). The structure of the Mn-oxo mix-valence dimer is shown in Fig. 2. The Mn-oxo mix-valence dimer is able to catalyze oxygen evolution in the presence of several chemical oxidants such as hypochlorite (Limburg et al., 1999; Cady and Brudvig, 2008), Ce^{4+} ion (Tagore et al., 2007a) and oxone (Chen et al., 2007). The mechanism of its catalytic reaction in water oxidation was well studied (Limburg et al., 2001; Tagore et al., 2007b; Usov et al., 2007; Cady and Brudvig, 2008).

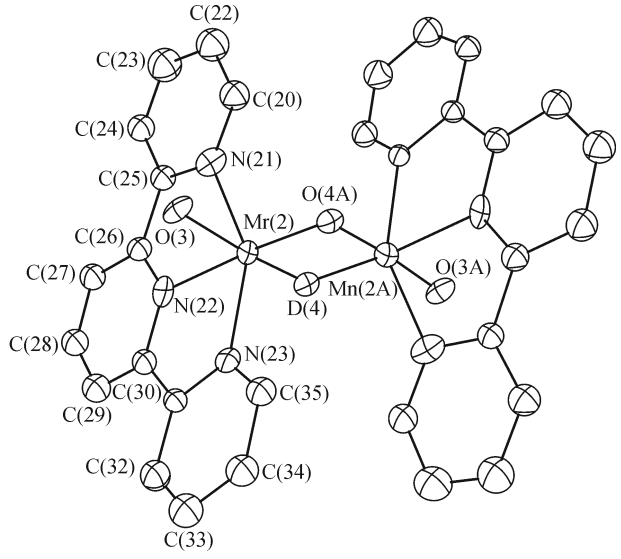

Figure 2 Three-dimensional structure of a synthetic functional model.

When the Mn-oxo dimer was exposed to strong illumination, photodamage of the compound was confirmed by the changes in the UV-visible spectra shown in Fig. 3. The UV illumination at 254 nm and 312 nm caused the appearance of two novel absorption peaks at 440 nm and 400 nm (Wei et al., 2011). The increased absorption at 400–450 nm is a characteristic of formation of a Mn(IV/IV)-oxo species (Limburg et al., 2001; Wei et al., 2011). In contrast, the exposure to light at 365, 453, 555, and 655 nm had no such effects. As the energy of UV light is higher than that of visible light, the formation of a Mn(IV/IV) species during the photoreaction is likely the consequence yield by UV radiation.

In addition, fluorescence spectrometric data on the synthetic Mn-oxo dimer compound showed that the a novel fluorescence peak at 514 nm was observed under UV illumination (Wei et al., 2011). The UV-induced formation of the Mn(IV/IV) species is correlated with the formation of a species that exhibits a fluorescence emission in aqueous solution. This is the first observation of a fluorescent high-valent Mn-oxo complex in the literature. The observed fluorescence peak at 514 nm is most likely emitted by the Terpy (2,2′:6′,6′-terpyridine) ligand and not by the central manganese ions. It was reported that 2,2′-bipyridine and 1, 10-phenanthroline are emissive depending the pH values of the solutions (Henry and Hoffman, 1979). The fluorescent Mn species might involve the hydration or protonation of the Terpy ligand.

Figure 4 is the absorption action spectrum, oxygen-evolution action spectrum of photodamage of the Mn(III/IV) dimer, and oxygen-evolution action spectrum of PS complexes from spinach. The action spectra of the synthetic manganese compound and PS II complexes are similar throughout the UV and visible wavelength range, suggesting that the mechanism of the photodamage reaction in the Mn model compound might be share the similar model with PS II. The UV light may cause a valence change of the Mn ion in the OEC of PS II in photoinhibition and that visible light cannot produce the same effect. This observation support that the high energy UV light is directly absorbed by the Mn ions in the PS II OEC and cause a valence change of Mn. The high-valent Mn(IV) species may oxidize another redox component such the protein or cofactors in PS II. Similar conclusion was reached by use of a tetra nuclear oxo-Mn(IV) complex, $[Mn_4O_6(bpea)_4]_4Br_4$ (Antal et al., 2009). The light-induced changes of the Mn (IV) tetramer are consistent with a one-electron reduction in the compound. The possible photoxidation of bromide by the Mn(IV) tetramer is possible and implies that the oxidation of chloride is involved in photoinhibition (Antal et al., 2009). This Mn-initiated photoinhibition is different from those in visible light-induced photodamage, which involves the photodamage of chlorophylls and carotenoid molecules.

In contrast to the chlorophyll *a*-containing species, a new unique strain chlorophyll *d*-containing cyanobacterium *Acaryochloris marina* is able to use chlorophyll *d* to store solar energy at longer wavelengths of light (690 and 750 nm) (Miyashita et al., 1996; Murakami et al., 2004; Kashiyama et al., 2008). The energetic data of electron transfer in this species has been reported (Allakhverdiev et al., 2010; Allakhverdiev et al., 2011; Tomo et al., 2011). Its energy-storage efficiency is comparable to or higher than that in typical, chlorophyll *a*-utilizing oxygenic species, determined by photoacoustic measurement on whole cells (Mielke et al., 2011). *Acaryochloris* is able to adapt to the low light environment. However under excess light illumination, it is extremely sensitive and vulnerable. HPLC analysis showed that the content of photosynthetic pigments including zeaxanthin, chlorophyll *d*, and *a*-carotene are decreased by about 80% upon strong light treatment. Interestingly, the cells of *Acaryochloris marina* were affected differently upon the illumination of visible light at 700 nm and UV radiation at 312 nm. The data suggested that the UV light at 312 nm targets on Mn cluster and 700 nm on chlorophyll d light-harvesting system.

In the manganese-induced photoinhibition mechanism as shown in Fig. 1, the role of manganese of PS II in photoinhibition may be summarized in Fig. 5. In high plant

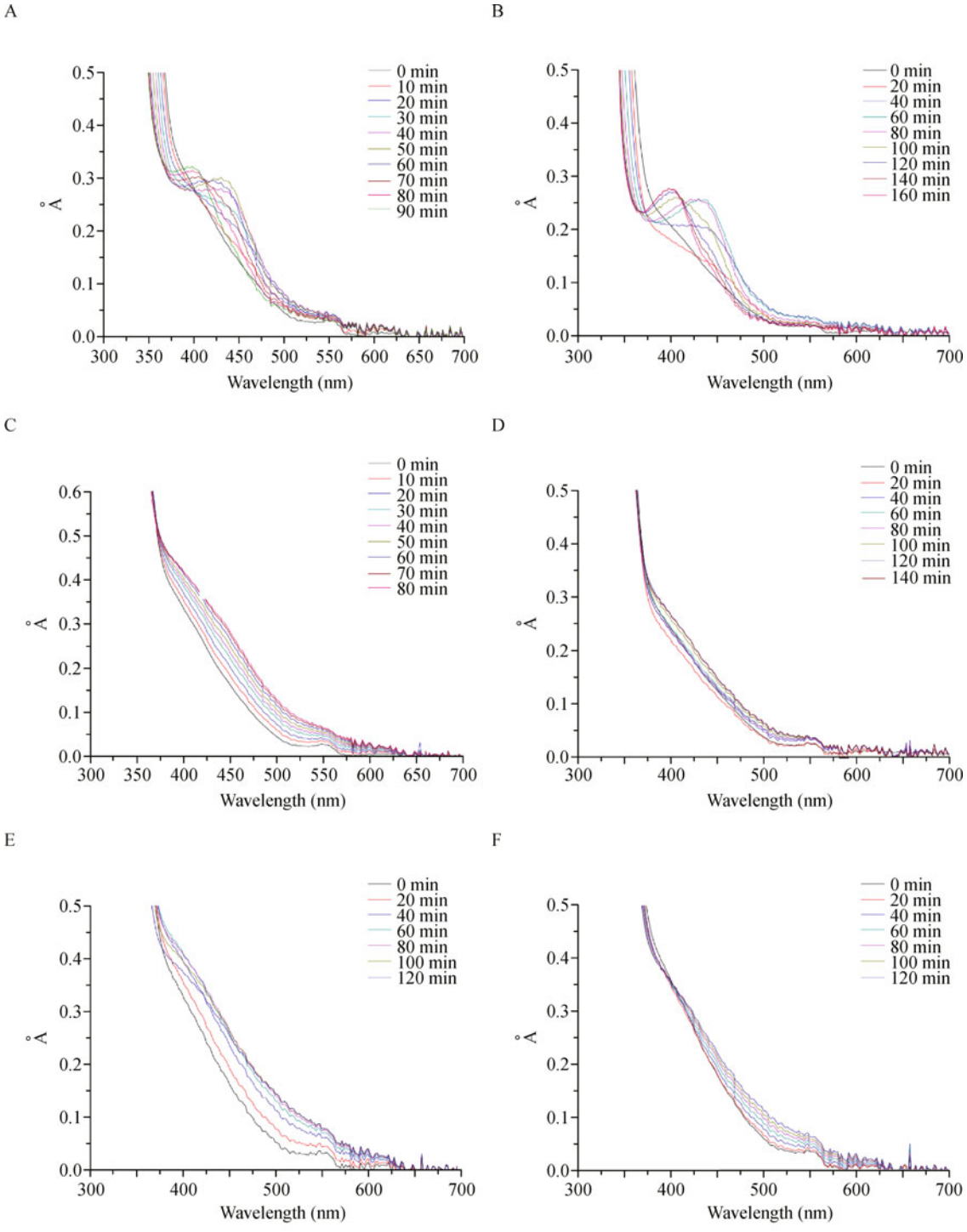

Figure 3 Effects of the wavelength of strong illumination on the absorption spectra of synthetic PS II functional model (Wei et al., 2011).

PS II and cyanobacterium *Acaryochloris marina*, the UV light targets mainly on the manganese cluster and causes subsequently damage of proteins and pigments. In contrast, the visible light aims at different sites: 1) in high plant PSII, the target is the chlorophyll a molecule in the reaction center of PS II, such as primary electron donor P_{680} species; 2) in *Acaryochloris marina*, the objective of light is on the chlorophyll d molecules in the light harvesting system.

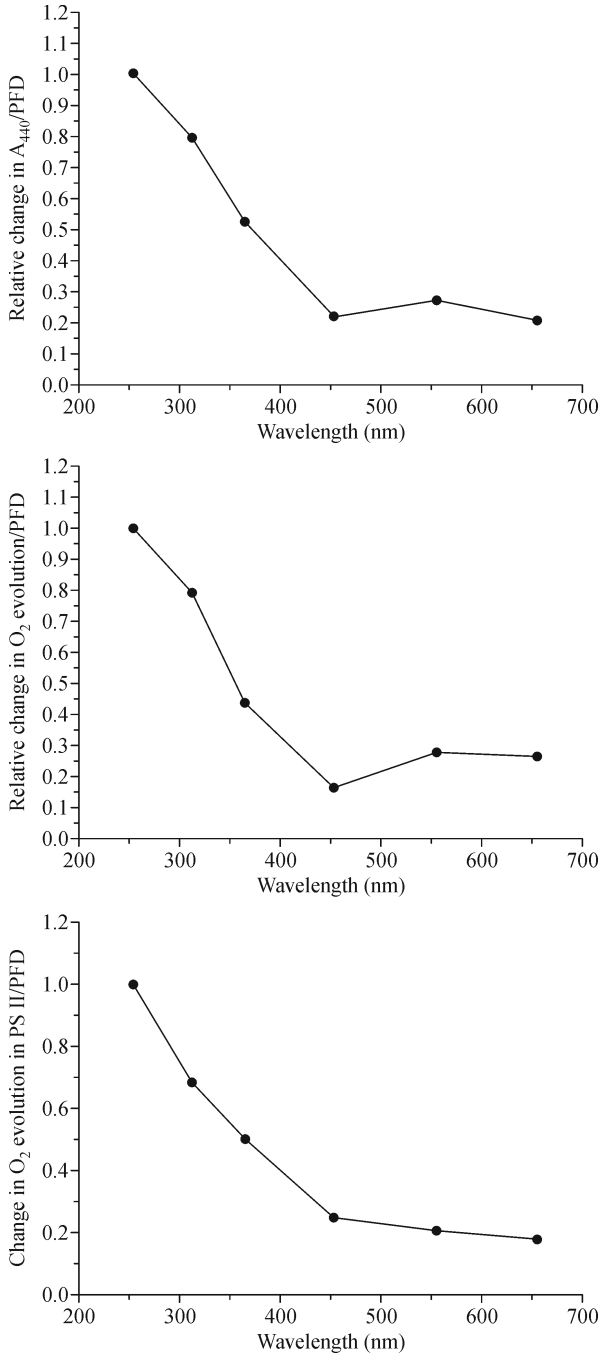

Figure 4 Action spectra of photoinhibition of synthetic functional model and of PS II particles from spinach. Upper panel: Absorption action spectrum of the Mn-oxo mixed-valence dimer compound. (Middle panel) Oxygen-evolution action spectrum of the Mn dimer compound. Lower panel: Oxygen-evolution action spectrum of PS II complexes (Wei et al., 2011).

Mn in thermal inactivation of PS II

The thermal inactivation of the photosynthetic reaction center from *Rb. Sphaeroides* involves at least one intermediate degradation of subunits at elevated temperature, possibly by a reversible transformation between the native and an off-pathway intermediate and followed by a irreversible transformation to the denatured state (Hughes et al., 2006). PS II, like other membrane protein complexes, is unstable upon elevated temperature and undergoes a protein denature reaction. Molecular responses in photosynthesis has been investigated and revealed that the primary targets of thermal damage in plants are the oxygen evolving complexes in PS II (Allakhverdiev et al., 2008). Thermal denaturation of PS II complex was investigated by differential scanning calorimetry (DSC) (Thompson et al., 1986; Thompson et al., 1989). The thermal reaction may involve a reaction of the Mn complex with hydroxide ions, which are oxidized to peroxide or superoxide, and results in the reduction and release of Mn.

Heat inactivation of oxygen evolution by isolated PS II particles is enhanced by depleting chloride ion and exogenous Mn(II) ion (Nash et al., 1985). Weak red light also accelerated heat inactivation. Heat treatment released the 33, 24 and 18 kD proteins and Mn from the Photosystem II particles. Cl^- depletion and exogenous Mn^{2+} stimulated the protein release, and the Mn release was also stimulated by Cl^- depletion. A 50% loss of Mn corresponded to full inactivation of oxygen evolution, whereas no direct correlation seemed to exist between the loss of any one protein and inactivation of oxygen evolution. Removal of the 24 and 18 kD proteins from photosystem II particles only slightly decreased the heat stability of oxygen evolution.

Due to the complexity of PSII, which contains more 20 polypeptides and more than 250 cofactors, the details of thermal disassembly process in PS II is difficult and challenging. To provide novel insights into the structure and mechanisms of the water-oxidation chemistry of the functional PSII model complex, the effect of elevated temperature on the functional PSII model complex, a mixed-valence Mn(III/IV)-oxo dimer compound, $[Mn(III)(O)_2Mn(IV)(H_2O)_2(Terpy)_2](NO_3)_3$, was investigated over the range of 25 to 85°C by UV-visible absorption spectrometry, atomic absorption spectrometry, oxygen evolution measurements, FTIR, and EPR methodologies (Zhang et al., 2011). The UV-visible spectra of the Mn(III/IV)-oxo dimer at different temperature in the range of 25 °C to 85 °C indicated a decomposition of the compound in two transformation steps. The first step started at 65 °C. The increased absorbance at 400 nm may be attributed to the formation of Mn(IV/IV) species from Mn(III/IV) precursor. The significant increase at 300 nm is likely due to the dissociation of the Terpy ligand from the Mn-oxo dime compound.

Figure 6 shows the EPR spectra of frozen solutions of the Mn(III/IV)-oxo dimer collected over time during the heating process (Zhang et al., 2011). The native Mn compound samples in acetate buffer shows a 16-line signal in the range of 2800–4100 G, which is characteristic for the Mn mix-valence species. When the solution was heated at 75 °C, the 16-line EPR signal was decreased by a factor of 90% within 10 min. This indicates that the Mn(III/IV)-oxo dimer is

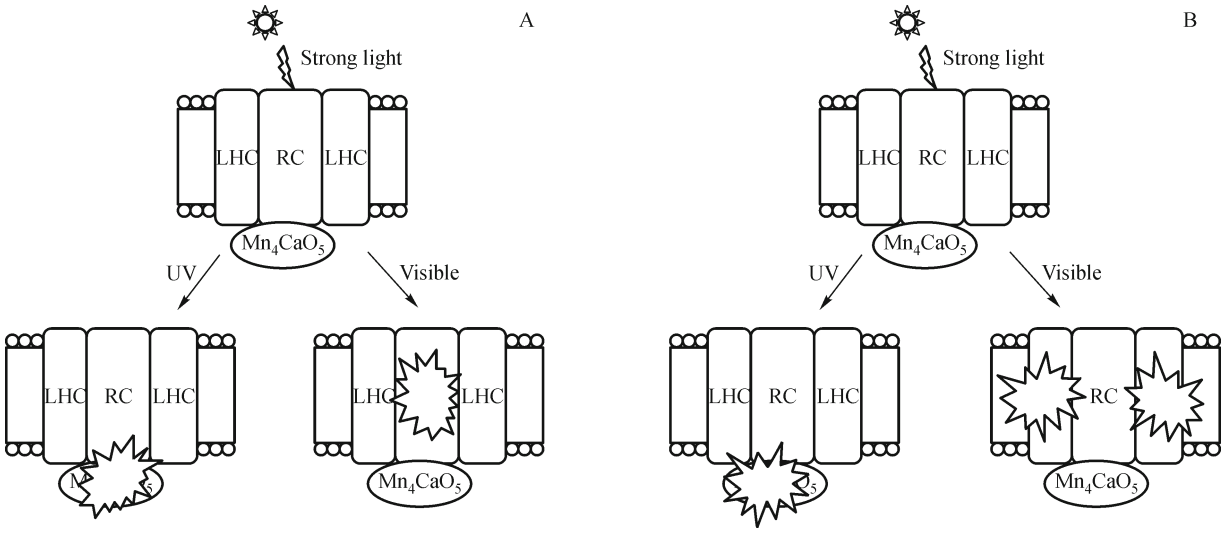

Figure 5 Possible models of PS II in higher plant spinach. (A) and cyanobacterium *Acaryochloris marina;* (B) responding to UV and visible light irradiations.

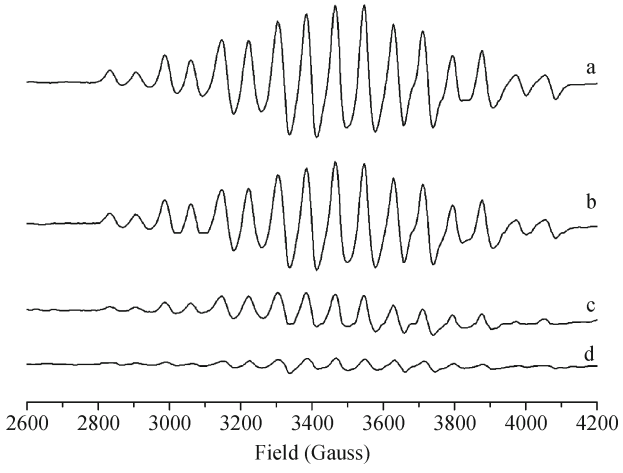

Figure 6 EPR spectra of frozen solutions of the Mn(III/IV)-oxo dimer were measured at 7 K (Zhang et al., 2011) (Reproduction permission from Elsevier).

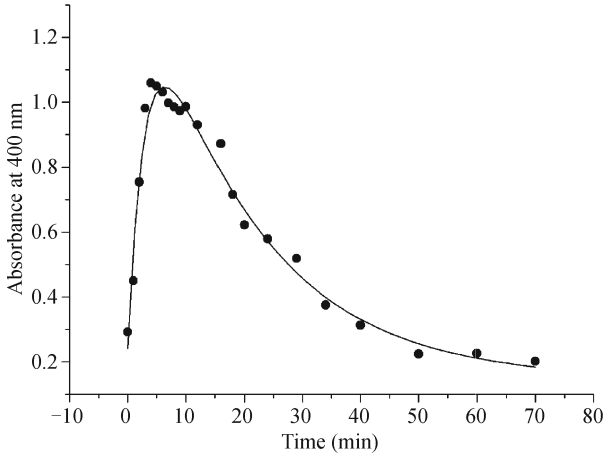

Figure 7 Kinetic curve of the thermal decomposition of the Mn(III/IV)-oxo dimer was monitored at 400 nm during heating at 60 °C. The data were fitted with a two-exponential equation, 3.0±0.5 min and 19±3.6 min (Zhang et al., 2011) (Reproduction permission from Elsevier).

converted by heating into an EPR silent species, such as Mn(IV/IV)-oxo dimer suggested by the UV-visible spectrophotometric data. The kinetics of the decomposition reaction of Mn(III/IV)-oxo dimer was fit and revealed that the half time of transformation of the Mn(III/IV) dimer was 3.5 min in the first step and 19 min in the following slow step (Fig. 7) (Zhang et al., 2011). Using the Arrhenius plots the activation energies for the two steps were determined to be 68 kJ/mol and 82 kJ/mol, respectively (Zhang et al., 2011). Although the Terpy ligand in the synthetic PS II model is far different from the natural amino acids, the Mn valence and geometry are somewhat similar to the Mn_4CaO_5 cluster in PS II OEC. It is speculated that the thermal denaturation of PS II may be couple with a Mn valence change in the Mn_4CaO_5 center.

X-ray absorption spectroscopy provides structural information on the Mn_4CaO_5 cluster in the oxygen-evolving complex (Dau et al., 2003; Pospísil et al., 2003; Yano and Yachandra, 2008). The effect of temperature on PSII revealed that the target of the temperature jump from 25°C to 47°C is the Mn_4CaO_5 cluster (Pospísil et al., 2003, 2007). One interesting intermediate formed within 10 min of hearing is binuclear Mn unit with Mn(III) ions separated by 2.7 Å and connected by one d-μ-oxo bridge (Pospísil et al., 2003). The disassembly of the Mn complex of PSII by a temperature jump from 25°C to 47°C experienced three distinct phases (Fig. 8). First, the oxygen-evolution activity was lost. This phase also involved the release of Ca but the overall structure

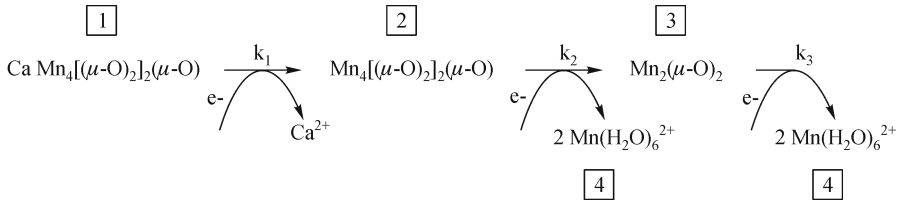

Figure 8 The possible mechanism of thermal inactivation of PS II (Pospísil et al., 2003) (Reproduction permission from the Biophysical Society).

of Mn complex remained largely intact. Subsequently, two Mn(III) or Mn(IV) ions in the native complex were reduced to Mn(II) and released. The two unreleased Mn ions formed a di-μ-oxo bridged Mn(III/III) dimer complex. Finally, the tightly bound Mn(III/III) unit was slowly reduced and released. The rate constants for each step in the model were determined (Pospísil et al., 2003). These data provide insightful information on the response mechanisms of Manganese in PS II thermal inactivation.

Conclusions

Mn, in the form of Mn_4CaO_5 center, plays a central role in oxygenic photosynthesis as the inorganic core of PS II OEC. In sensing and responding the diverse environmental stress factors, Mn plays an important role in regulating and protecting of photosynthetic machineries. In particular Mn is the primary target of photoinhibition under strong UV light and elevated temperature conditions as documented in this review. The manganese hypothesis may be the dominant mechanism in photoinhibition. However the several details of the mechanism including the nature of the intermediates remain to be elucidated. The first observed fluorescent Mn(IV) species may provide novel insights into the mechanism of the UV-based photoinhibition of photosynthesis. The detailed measurements on synthetic manganese compounds, native PS II complexes, and site-directed mutants of PS II using sophisticate biophysical methodologies including X-ray absorption spectroscopy may provide strong evidence to support or disapproval of the current hypotheses and models in photo-induced and heat-initiated inactivation of PS II OEC *in vivo*. Such measurements may also offer novel opportunities and open new routes for understanding the mechanisms of assembly of PS II OEC and shed light on solar energy production using artificial photosynthesis.

Abbreviations

Cyt b_{559}, cytochrome b-559, a 6–9 kD subunit in photosystem II; D1, 32 kD peptide, a subunit of reaction center in photosystem II; D2, 34 kD peptide, a subunit of reaction center in photosystem II; DSC, the differential scanning calorimetry; EPR, the electron paramagnetic spectroscopy; FTIR, the Fourier transform infrared spectroscopy; Mn(IV/IV), manganese-oxo dimer with manganese oxidation states of + 4 and + 4; Mn(III/IV), manganese-oxo dimer with manganese oxidation states of + 3 and + 4; OEC, the oxygen evolving complex of photosystem II; P_{680}, the primary electron donor of photosystem II; Pheo, pheophytin, the primary electron acceptor of photosystem II; PS II, photosystem II; Q_A, the primary quinone electron acceptor of photosystem II; Q_B, the secondary quionone electron acceptor of photosystem II; ROS, reactive oxygen species; UV, ultraviolet light; UV-A, ultraviolet light (315–400 nm); UV-B, ultraviolet light (280–315 nm); UV-C, ultraviolet light (100–280 nm); Y_Z, tyrosine, the secondary electron donor of photosystem II.

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References

Adir N, Zer H, Shochat S, Ohad I (2003). Photoinhibition——a historical perspective. Photosynth Res, 76(1/3): 343–370

Allakhverdiev S I, Kreslavski V D, Klimov V V, Los D A, Carpentier R, Mohanty P (2008). Heat stress: an overview of molecular responses in photosynthesis. Photosynth Res, 98(1–3): 541–550

Allakhverdiev S I, Murata N (2004). Environmental stress inhibits the synthesis *de novo* of proteins involved in the photodamage-repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803. Biochim Biophys Acta, 1657(1): 23–32

Allakhverdiev S I, Murata N (2008). Salt stress inhibits photosystems II and I in cyanobacteria. Photosynth Res, 98(1–3): 529–539

Allakhverdiev S I, Tomo T, Shimada Y, Kindo H, Nagao R, Klimov V V, Mimuro M (2010). Redox potential of pheophytin *a* in photosystem II of two cyanobacteria having the different special pair chlorophylls. Proc Natl Acad Sci USA, 107(8): 3924–3929

Allakhverdiev S I, Tsuchiya T, Watabe K, Kojima A, Los D A, Tomo T, Klimov V V, Mimuro M (2011). Redox potentials of primary electron acceptor quinone molecule $(Q_A)^-$ and conserved energetics of photosystem II in cyanobacteria with chlorophyll *a* and chlorophyll *d*. Proc Natl Acad Sci USA, 108(19): 8054–8058

Antal T K, Lo W, Armstrong W H, Tyystjärvi E (2009). Illumination with ultraviolet or visible light induces chemical changes in the water-soluble manganese complex, $[Mn_4O_6(bpea)_4]Br_4$. Photochem Photobiol, 85(3): 663–668

Aro E M, Virgin I, Andersson B (1993). Photoinhibition of photosystem

II. inactivation, protein damage and turnover. Biochim Biophys Acta, 1143(2): 113–134

Barbara Demmig-Adams W W A, Autar K M (2007). Photoprotection, Photoinhibition, Gene regulation, and Environment. The Netherlands: Springer

Barber J, Andersson B (1992). Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci, 17(2): 61–66

Blankenship R E (2002). Molecular Mechanisms of Photosynthesis. Blackwell Science

Brudvig G W (2008). Water oxidation chemistry of photosystem II. Philos Trans R Soc Lond B Biol Sci, 363(1494): 1211–1219, discussion 1218–1219

Cady C W, Brudvig G W (2008). Functional manganese model chemistry relevant to the oxygen-evolving complex of photosystem II: oxidation of a Mn(III,IV) complex coupled to deprotonation of a terminal water ligand. In: Allen J P, Osmond B, Golbeck J H, and Gantt E Eds, Photosynthesis: Energy from the Sun. Springer, 377–382

Carpentier R (2005). Influence of high light intensity on photosynthesis: Photoinhibition and energy dissipation. In: Mohammad P. Ed., Handbook of Photosynthesis, 2nd Ed., Taylor & Francis, 327–342

Chen H, Tagore R, Olack G, Vrettos J S, Weng T C, Penner-Hahn J, Crabtree R H, Brudvig G W (2007). Speciation of the catalytic oxygen evolution system: $[Mn^{III/IV}{}_2(\mu\text{-}O)_2(terpy)_2(H_2O)_2](NO_3)_3 + HSO_5^-]$. Inorg Chem, 46(1): 34–43

Chow W S (1994). Photoprotection and photoinhibitory damage. Adv Mol Cell Biol, 10: 151–196

Dau H, Liebisch P, Haumann M (2003). X-ray absorption spectroscopy to analyze nuclear geometry and electronic structure of biological metal centers—potential and questions examined with special focus on the tetra-nuclear manganese complex of oxygenic photosynthesis. Anal Bioanal Chem, 376(5): 562–583

Delosme R (2003). On some aspects of photosynthesis revealed by photoacoustic studies: a critical evaluation. Photosynth Res, 76(1/3): 289–301

Delosme R, Beal D, Joliot P (1994). Photoacoustic detection of flash-induced charge separation in photosynthetic systems. Spectral dependence of the quantum yield. Biochim Biophys Acta, 1185(1): 56–64

Diner B A, Babcock G T (1996). Structure, dynamics, and energy conversion efficiency in photosystem II. In: Ort D R, Yocum C F Eds, Oxygenic Photosynthesis: The Light Reactions, Kluwer Academic Publishers, 213–247

Diner B A, Rappaport F (2002). Structure, dynamics, and energetics of the primary photochemistry of photosystem II of oxygenic photosynthesis. Annu Rev Plant Biol, 53(1): 551–580

Edens G J, Gunner M R, Xu Q, Mauzerall D (2000). The enthalpy and entropy of reaction for formation of $P^+Q_A^-$ from excited reaction centers of *Rhodobacter sphaeroides*. J Am Chem Soc, 122(7): 1479–1485

Ferreira K N, Iverson T M, Maghlaoui K, Barber J, Iwata S (2004). Architecture of the photosynthetic oxygen-evolving center. Science, 303(5665): 1831–1838

Frank H A, Brudvig G W (2004). Redox functions of carotenoids in photosynthesis. Biochemistry, 43(27): 8607–8615

Govindjee S M, Seibert M (2010). Picosecond spectroscopy of the isolated reaction centers from the photosystems of oxygenic photosynthesis—ten years (1987–1997) of fun : a tribute to Michael R. Wasielewski on his 60th birthday. Photosynth Res, 103(1): 1–6

Hakala M, Rantamäki S, Puputti E M, Tyystjärvi T, Tyystjärvi E (2006). Photoinhibition of manganese enzymes: insights into the mechanism of photosystem II photoinhibition. J Exp Bot, 57(8): 1809–1816

Hakala M, Tuominen I, Keränen M, Tyystjärvi T, Tyystjärvi E (2005). Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II. Biochim Biophys Acta, 1706(1–2): 68–80

Henry M, Hoffman M (1979). Photophysics and photochemistry of aromatic nitrogen heterocycles. Fluorescence from 2,2'-bipyridine and 1,10-phenanthroline. J Phys Chem, 83(5): 618–625

Hideg E, Spetea C, Vass I (1994). Singlet oxygen and free radical production during acceptor- and donor-side-induced photoinhibition. Studies with spin trapping EPR spectroscopy. Biochim Biophys Acta, 1186(3): 143–152

Hou H J M, Mauzerall D (2006). The A^-F_x to $F_{A/B}$ step in *synechocystis* 6803 photosystem I is entropy driven. J Am Chem Soc, 128(5): 1580–1586

Hou H J M, Mauzerall D (2011). Listening to PS II: enthalpy, entropy, and volume changes. J Photochem Photobiol B, 104(1-2): 357–365

Hou H J M, Shen G, Boichenko V A, Golbeck J H, Mauzerall D (2009). Thermodynamics of charge separation of photosystem I in the *menA* and *menB* null mutants of *Synechocystis* sp. PCC 6803 determined by pulsed photoacoustics. Biochemistry, 48(8): 1829–1837

Hou J M, Boichenko V A, Diner B A, Mauzerall D (2001a). Thermodynamics of electron transfer in oxygenic photosynthetic reaction centers: volume change, enthalpy, and entropy of electron-transfer reactions in manganese-depleted photosystem II core complexes. Biochemistry, 40(24): 7117–7125

Hou J M, Boichenko V A, Wang Y C, Chitnis P R, Mauzerall D (2001b). Thermodynamics of electron transfer in oxygenic photosynthetic reaction centers: a pulsed photoacoustic study of electron transfer in photosystem I reveals a similarity to bacterial reaction centers in both volume change and entropy. Biochemistry, 40(24): 7109–7116

Hughes A V, Rees P, Heathcote P, Jones M R (2006). Kinetic analysis of the thermal stability of the photosynthetic reaction center from *Rhodobacter sphaeroides*. Biophys J, 90(11): 4155–4166

Jegerschöld C, Styring S (1991). Fast oxygen-independent degradation of the D1 reaction center protein in photosystem II. FEBS Lett, 280 (1): 87–90

Joliot P, Barbieri G, Chabaud R (1969). Model of the System II photochemical centers. Photochem Photobiol, 10: 309–329

Jones L W, Kok B (1966). Photoinhibition of chloroplast reactions. II. Multiple effects. Plant Physiol, 41(6): 1044–1049

Kamiya N, Shen J R (2003). Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-Å resolution. Proc Natl Acad Sci USA, 100(1): 98–103

Kashiyama Y, Miyashita H, Ohkubo S, Ogawa N O, Chikaraishi Y, Takano Y, Suga H, Toyofuku T, Nomaki H, Kitazato H, Nagata T, Ohkouchi N (2008). Evidence of global chlorophyll *d*. Science, 321 (5889): 658

Kok B, Forbush B, McGloin M (1970). Cooperation of charges in photosynthetic O_2 evolution-I. A linear four step mechanism. Photochem Photobiol, 11(6): 457–475

Kok B, Gassner E B, Rurainski H J (1966). Photoinhibition of chloroplast reactions. Photochem Photobiol, 4(2): 215–227

Kramer D M (2010). The photonic "smart grid" of the chloroplast in action. Proc Natl Acad Sci USA, 107(7): 2729–2730

Krivanek R, Dau H, Haumann M (2008). Enthalpy changes during photosynthetic water oxidation tracked by time-resolved calorimetry using a photothermal beam deflection technique. Biophys J, 94(5): 1890–1903

Kyle D J, Ohad I, Arntzen C J (1984). Membrane protein damage and repair: Selective loss of a quinone-protein function in chloroplast membranes. Proc Natl Acad Sci USA, 81(13): 4070–4074

Limburg J, Vrettos J S, Chen H, de Paula J C, Crabtree R H, Brudvig G W (2001). Characterization of the O_2-evolving reaction catalyzed by $[(terpy)(H_2O)Mn(III)(O)_2Mn(IV)(OH_2)(terpy)](NO_3)_3$ (terpy = 2,2′:6,2″-terpyridine). J Am Chem Soc, 123(3): 423–430

Limburg J, Vrettos J S, Liable-Sands L M, Rheingold A L, Crabtree R H, Brudvig G W (1999). A functional model for O-O bond formation by the O_2-evolving complex in photosystem II. Science, 283(5407): 1524–1527

Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005). Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature, 438(7070): 1040–1044

Mauzerall D (2006). Thermodynamics in photosystem I. In J. Golbeck (ed): Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase, Dordrecht: Springer, 571–581

Mauzerall D (2010). Changes in enthalpy of the Joliot-Kok four step cycle to produce oxygen in photosynthesis. Biophys J, 98: 173a

Melis A (1999). Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? Trends Plant Sci, 4: 130–135

Mielke S P, Kiang N Y, Blankenship R E, Gunner M R, Mauzerall D (2011). Efficiency of photosynthesis in a Chl *d*-utilizing cyanobacterium is comparable to or higher than that in Chl *a*-utilizing oxygenic species. Biochim Biophys Acta, 1807(9): 1231–1236

Miyashita H, Ikemoto H, Kurano N, Adachi K, Chihara M, Miyachi S (1996). Chlorophyll *d* as a major pigment. Nature, 383(6599): 402

Murakami A, Miyashita H, Iseki M, Adachi K, Mimuro M (2004). Chlorophyll *d* in an epiphytic cyanobacterium of red algae. Science, 303(5664): 1633

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev S I (2007). Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta, 1767(6): 414–421

Nash D, Miyao M, Murata N (1985). Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese. Biochim Biophys Acta, 807(2): 127–133

Nishiyama Y, Allakhverdiev S I, Murata N (2005). Inhibition of the repair of photosystem II by oxidative stress in cyanobacteria. Photosynth Res, 84(1–3): 1–7

Nishiyama Y, Allakhverdiev S I, Murata N (2006). A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. Biochim Biophys Acta, 1757(7): 742–749

Niyogi K K (1999). Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol, 50(1): 333–359

Ohad I, Adir N, Koike H, Kyle D J, Inoue Y (1990). Mechanism of photoinhibition in vivo. A reversible light-induced conformational change of reaction center II is related to an irreversible modification of the D1 protein. J Biol Chem, 265(4): 1972–1979

Ohad I, Keren N, Zer H, Gong H, Mor T S, Gal A, Tal S, Eisenberg-Domovich Y (1994). Light induced degradation of the photochemical reaction center II-D1 protein *in vivo*: An intergrative approach. In: Baker NR, Bowyer JR, Eds, Photoinhibition of Photosynthesis. Oxford: Bios Scientific Publishers, 161–177

Ohnishi N, Allakhverdiev S I, Takahashi S, Higashi S, Watanabe M, Nishiyama Y, Murata N (2005). Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. Biochemistry, 44(23): 8494–8499

Pearcy R W, Berry J A, Fork D C (1977). Effects of growth temperature on the thermal stability of the photosynthetic apparatus of Atriplex lentiformis (Torr.) Wats. Plant Physiol, 59(5): 873–878

Peng D C, Hou J M, Kuang T Y, Tang C Q, Tang P S (1999). Light-induced damage of photosystem II primary electron donor P_{680}: A high performance liquid chromatographic analysis of pigment content in D1/D2/cytochrome b_{559} complex under photoinhibitory conditions. J Integr Plant Biol, 41: 1307–1311

Pospísil P, Michael H, Dittmer J, Solé V A, Dau H (2003). Stepwise transition of the tetra-manganese complex of photosystem II to a binuclear $Mn_2(\mu\text{-}O)_2$ complex in response to a temperature jump: a time-resolved structural investigation employing x-ray absorption spectroscopy. Biophys J, 84(2 Pt 1): 1370–1386

Pospísil P, Snyrychová I, Naus J (2007). Dark production of reactive oxygen species in photosystem II membrane particles at elevated temperature: EPR spin-trapping study. Biochim Biophys Acta, 1767 (6): 854–859

Powles S B (1984). Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol, 35(1): 15–44

Rappaport F, Guergova-Kuras M, Nixon P J, Diner B A, Lavergne J (2002). Kinetics and pathways of charge recombination in photosystem II. Biochemistry, 41(26): 8518–8527

Renger G, Volker M, Eckert H J, Fromme P, Hohm-Veit S, Graber P (1989). On the mechanism of photosystem II deterioration by UV-B irradiation. Photochem Photobiol, 49(1): 97–105

Rutherford A W, Boussac A (2004). Biochemistry. Water photolysis in biology. Science, 303(5665): 1782–1784

Sakuragi Y, Zybailov B, Shen G, Jones A D, Chitnis P R, van der Est A, Bittl R, Zech S, Stehlik D, Golbeck J H, Bryant D A (2002). Insertional inactivation of the *menG* gene, encoding 2-phytyl-1,4-naphthoquinone methyltransferase of *Synechocystis* sp. PCC 6803, results in the incorporation of 2-phytyl-1,4-naphthoquinone into the $A(_1)$ site and alteration of the equilibrium constant between $A(_1)$ and $F(_X)$ in photosystem I. Biochemistry, 41(1): 394–405

Sarvikas P, Hakala-Yatkin M, Dönmez S, Tyystjärvi E (2010a). Short flashes and continuous light have similar photoinhibitory efficiency in intact leaves. J Exp Bot, 61(15): 4239–4247

Sarvikas P, Tyystjärvi T, Tyystjärvi E (2010b). Kinetics of prolonged photoinhibition revisited: photoinhibited Photosystem II centres do not protect the active ones against loss of oxygen evolution. Photosynth Res, 103(1): 7–17

Setlik I, Allakhverdiev S I, Nedbal L, Setlikova E, Klimov V V (1990). Three types of photosystem II photoinactivation. 1. Damaging processes on the acceptor side. Photosynth Res, 23: 39–48

Shipton C A, Barber J (1991). Photoinduced degradation of the D1 polypeptide in isolated reaction centers of photosystem II: evidence for an autoproteolytic process triggered by the oxidizing side of the photosystem. Proc Natl Acad Sci USA, 88(15): 6691–6695

Shipton C A, Barber J (1992). Characterisation of photoinduced breakdown of the D1-polypeptide in isolated reaction centres of Photosystem II. Biochim Biophys Acta, 1099(1): 85–90

Shipton C A, Barber J (1994). *In vivo* and *in vitro* photoinhibition reactions generate similar degradation fragments of D1 and D2 photosystem-II reaction-centre proteins. Eur J Biochem, 220(3): 801–808

Stewart D H, Brudvig G W (1998). Cytochrome b_{559} of photosystem II. Biochim Biophys Acta, 1367(1–3): 63–87

Szabó I, Bergantino E, Giacometti G M (2005). Light and oxygenic photosynthesis: energy dissipation as a protection mechanism against photo-oxidation. EMBO Rep, 6(7): 629–634

Szilárd A, Sass L, Deák Z, Vass I (2007). The sensitivity of Photosystem II to damage by UV-B radiation depends on the oxidation state of the water-splitting complex. Biochim Biophys Acta, 1767(6): 876–882

Tagore R, Chen H, Zhang H, Crabtree R H, Brudvig G W (2007a). Homogeneous water oxidation by a di-μ-oxo dimanganese complex in the presence of Ce^{4+}. Inorg Chim Acta, 360(9): 2983–2989

Tagore R, Crabtree R H, Brudvig G W (2007b). Distinct mechanisms of bridging-oxo exchange in di-μ-O dimanganese complexes with and without water-binding sites: implications for water binding in the O_2-evolving complex of photosystem II. Inorg Chem, 46(6): 2193–2203

Takahashi S, Murata N (2008). How do environmental stresses accelerate photoinhibition? Trends Plant Sci, 13(4): 178–182

Telfer A (2005). Too much light? How β-carotene protects the photosystem II reaction centre. Photochem Photobiol Sci, 4(12): 950–956

Telfer A, He W Z, Barber J (1990). Spectral resolution of more than one chlorophyll electron donor in the isolated photosystem II reaction center complex. Biochim Biophys Acta, 1017(2): 143–151

Thompson L K, Blaylock R, Sturtevant J M, Brudvig G W (1989). Molecular basis of the heat denaturation of photosystem II. Biochemistry, 28(16): 6686–6695

Thompson L K, Sturtevant J M, Brudvig G W (1986). Differential scanning calorimetric studies of photosystem II: evidence for a structural role for cytochrome b_{559} in the oxygen-evolving complex. Biochemistry, 25(20): 6161–6169

Tomo T, Allakhverdiev S I, Mimuro M (2011). Constitution and energetics of photosystem I and photosystem II in the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina*. J Photochem Photobiol B, 104(1–2): 333–340

Tracewell C A, Vrettos J S, Bautista J A, Frank H A, Brudvig G W (2001). Carotenoid photooxidation in photosystem II. Arch Biochem Biophys, 385(1): 61–69

Tyystjarvi E (2008). Photoinhibition of Photosystem II and photodamage of the oxygen evolving manganese cluster. Coord Chem Rev, 252(3–4): 361–376

Umena Y, Kawakami K, Shen J R, Kamiya N (2011). Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature, 473(7345): 55–60

Usov O M, Grigoryants V M, Tagore R, Brudvig G W, Scholes C P (2007). Hyperfine coupling to the bridging ^{17}O in the di-μ-oxo core of a Mn(III)-Mn(IV) model significant to the core electronic structure of the O_2-evolving complex in photosystem II. J Am Chem Soc, 129 (39): 11886–11887

van Gorkom H J (1985). Electron transfer in photosystem II. Photosynth Res, 6(2): 97–112

van Grondelle R, Dekker J P, Gillbro T, Sundstrom V (1994). Energy transfer and trapping in photosynthesis. Biochim Biophys Acta, 1187 (1): 1–65

Vass I, Gatzen G, Holzwarth A R (1993). Picosecond time-resolved fluorescence studies on photoinhibition and double reduction of Q_A in photosystem II. Biochim Biophys Acta, 1183(2): 388–396

Vrettos J S, Brudvig G W (2002). Water oxidation chemistry of photosystem II. Philos Trans R Soc Lond B Biol Sci, 357(1426): 1395–1404, discussion 1404–1405, 1419–1420

Wei Z, Cady C W, Brudvig G W, Hou H J M (2011). Photodamage of a Mn(III/IV)-oxo mixed-valence compound and photosystem II: evidence that a high-valent manganese species is responsible for UV-induced photodamage of the oxygen-evolving complex in photosystem II. J Photochem Photobiol B, 104(1–2): 118–125

Yano J, Kern J, Irrgang K D, Latimer M J, Bergmann U, Glatzel P, Pushkar Y, Biesiadka J, Loll B, Sauer K, Messinger J, Zouni A, Yachandra V K (2005). X-ray damage to the Mn_4Ca complex in single crystals of photosystem II: a case study for metalloprotein crystallography. Proc Natl Acad Sci USA, 102(34): 12047–12052

Yano J, Yachandra V K (2008). Where water is oxidized to dioxygen: structure of the photosynthetic Mn_4Ca cluster from X-ray spectroscopy. Inorg Chem, 47(6): 1711–1726

Zhang F, Cady C W, Brudvig Gary W, Hou H J M (2011). Thermal Stability of $[Mn(III)(O)_2Mn(IV)(H_2O)_2(Terpy)_2](NO_3)_3$ (Terpy = 2,2':6',2'-terpyridine) in aqueous solution. Inorg Chim Acta, 366(1): 128–133

Zsiros O, Allakhverdiev S I, Higashi S, Watanabe M, Nishiyama Y, Murata N (2006). Very strong UV-A light temporally separates the photoinhibition of photosystem II into light-induced inactivation and repair. Biochim Biophys Acta, 1757(2): 123–129