The Role of Metals in Production and Scavenging of Reactive Oxygen Species in Photosystem II

Pavel Pospíšil*

Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

*Corresponding author: E-mail, pavel.pospisil@upol.cz; Fax, +420-58-5225737

(Received January 13, 2014; Accepted April 7, 2014)

Metal ions play a crucial role in enzymatic reactions in all photosynthetic organisms such as cyanobacteria, algae and plants. It is well known that metal ions maintain the binding of substrate in the active site of the metalloenzymes and control the redox activity of the metalloenzyme in the enzymatic reaction. A large pigment-protein complex, PSII, known to serve as a water-plastoquinone oxidoreductase, contains three metal centers comprising non-heme iron, heme iron of Cyt b559 and the water-splitting manganese complex. Metal ions bound to PSII proteins maintain the electron transport from water to plastoquinone and regulate the pro-oxidant and antioxidant activity in PSII. In this review, attention is focused on the role of PSII metal centers in (i) the formation of superoxide anion and hydroxyl radicals by sequential one-electron reduction of molecular oxygen and the formation of hydrogen peroxide by incomplete two-electron oxidation of water; and (ii) the elimination of superoxide anion radical by one-electron oxidation and reduction (superoxide dismutase activity) and of hydrogen peroxide by two-electron oxidation and reduction (catalase activity). The balance between the formation and elimination of reactive oxygen species by PSII metal centers is discussed as an important aspect in the prevention of photo-oxidative damage of PSII proteins and lipids.

Keywords: Cytochrome b559 • Non-heme iron • Photosystem II • Photo-oxidative damage • Reactive oxygen species • Water-splitting manganese complex.

Abbreviations: Chl, chlorophyll; CP43, the core antenna complex of PSII; Cyt b559, midpoint redox potential; EPR, electron paramagnetic spectroscopy; Fe^{3+}–OO, ferric-peroxo intermediate; Fe^{3+}–OOH, ferric-hydroperoxo intermediate; HP, high potential; IP, intermediate potential; LP, low potential; PSI, Photosystem I; PSII, Photosystem II; QA, primary quinone electron acceptor of PSII; QB, secondary quinone electron acceptor of PSII; ROS, reactive oxygen species; SOD, superoxide dismutase; SOO, superoxide oxidase; SOR, superoxide reductase.

Introduction

As a by-product of photosynthetic water splitting in chloroplasts, molecular oxygen is released, which is essential for cellular respiration in mitochondria, a process required for all aerobic organisms. The splitting of two water molecules into protons, electrons and molecular oxygen proceeds in the water-splitting manganese complex in PSII embedded in the thylakoid membrane of cyanobacteria, algae and higher plants (Dau et al. 2012). The process is driven by solar energy absorbed by Chls in the PSII antenna complex followed by the charge separation in the PSII reaction center. The charge separation initiates electron transport in PSII comprising the oxidation of water and the reduction of plastoquinone on the electron donor and electron acceptor side of PSII, respectively (Cardona et al. 2012, Vinyard et. al. 2013).

When solar energy absorbed by Chls exceeds its utilization for electron transport reactions, reactive oxygen species (ROS) are formed by consecutive one-electron reduction of molecular oxygen and by concerted two-electron oxidation of water (Pospíšil 2009, Pospíšil 2012). The consecutive one-electron reduction of molecular oxygen on the electron acceptor side of PSII leads to the formation of the superoxide anion radical (O_2^-) known to dismutate spontaneously or enzymatically to hydrogen peroxide (H_2O_2) and subsequently be reduced to the hydroxyl radical (HO^•) via the Fenton reaction. The concerted two-electron oxidation of water on the electron donor side of PSI forms H_2O, known to be oxidized and reduced to O_2 and HO^•, respectively. Apart from PSII, PSI serves as a source of ROS in the thylakoid membrane. Several lines of evidence have been provided that due to the fact that the midpoint redox potential of reductant on the electron acceptor side of PSI is more negative as compared with PSII, one-electron reduction of molecular oxygen in PSI (Mehler reaction) occurs with high efficiency. It has been shown previously that the rate of O_2^- production in PSI is 15 μmol (mg Chl)^-1 h^-1 (Asada and Nakano 1978). In spite of the fact that the rate of O_2^- production in PSII has not been determined yet, it is generally considered that it is less significant compared with PSI (Asada 2000, Badger et al. 2000).
Whereas the non-radical form of ROS (H₂O₂) are rather inert to biomolecules such as proteins and lipids, the radical forms of ROS (O₂•− and HO•) have the ability to oxidize proteins and lipids. While experimental evidence on the oxidation of proteins and lipids by O₂•− is rather limited (Tiwari et al. 2013), several lines of evidence have been provided on the oxidation of proteins and lipids by HO•. As the standard redox potential of the HO•/H₂O redox couple (E° = 2.3 V, pH 7) is highly positive, HO• has a high capability to oxidize proteins and lipids. Due to its high reactivity toward proteins and lipids, the lifetime of HO• is in the nanosecond time range, indicating that the diffusion of HO• is rather restricted. Based on these considerations, it is generally accepted that the oxidation of proteins and lipids occurs at the site of HO• formation. To eliminate the deleterious effect of ROS on proteins and lipids, an antioxidant defense system has been developed in PSII. When ROS are efficiently eliminated by the antioxidant defense system, the oxidative effect of ROS on proteins and lipids is fully prevented. However, when ROS are formed in excess, the oxidation of proteins and lipids by ROS initiates the oxidative damage of proteins and lipids (Yamamoto 2001). To prevent the oxidative effect of ROS on proteins and lipids efficiently, ROS have to diffuse reliably from the site of ROS formation to the antioxidant catalytic center through specific channels (Frankel et al. 2012, Frankel et al. 2013). The channels are proposed to direct dangerous ROS towards the antioxidant catalytic center located either within or outside of the thylakoid membrane. The elimination of ROS by antioxidant catalytic centers within the thylakoid membrane plays an important role in the first-line defense, whereas the antioxidant catalytic centers outside the thylakoid membrane maintain the second-line defense against the oxidative effect of ROS on proteins and lipids.

Metal ions bound to PSII proteins play a crucial role in the maintenance of electron transport from the electron donor to electron acceptor side of PSII (Liu and Wang 2012). Recent crystal structures of PSII from the cyanobacteria Thermosynechococcus elongatus and Thermosynechococcus vulcanus showed that three types of metal centers, i.e. non-heme iron, heme iron of Cyt b₅₅₉ and the water-splitting manganese complex (M₅₅₉Ca) are coordinated to amino acids in PSII (Ferreira et al. 2004, Guskov et al. 2009, Umena et al. 2011). Whereas non-heme and heme iron are located on the PSII electron acceptor side, manganese ions in the M₅₅₉Ca complex are positioned on the PSII electron donor side. Several structural forms of metal centers coordinated to PSII proteins are known to be involved in electron transport: (i) a metal ion directly coordinated to amino acids (non-heme iron); (ii) a metal ion incorporated in the heme structure (cyt b₅₅₉); and (iii) metal ions arranged into a specific cluster coordinated to amino acids directly or via inorganic ions (M₅₅₉Ca complex).

Apart from the participation of PSII metal centers in electron transport from water to plastoquinone, several lines of evidence have been provided on the role of PSII metal centers in the formation and elimination of ROS (Kruk and Strzalka 1999, Nugent 2001, Kruk and Strzalka 2001, Pospíšil et al. 2006, Tiwari and Pospíšil 2009, Pospíšil and Tiwari 2010, Pospíšil 2011). Similarly to the mitochondrial membrane, where the participation of metal ions in electron transport is closely associated with pro-oxidant (free iron) and antioxidant [Mn-superoxide dismutase (SOD) and Cu-Zn-SOD] activity of metal ions (Kirkiniezos and Moraes 2001), the coupling of electron transport with pro-oxidant and antioxidant activity of metal ions in the thylakoid membrane is of significant importance as PSII is a source of molecular oxygen. The pro-oxidant and antioxidant activity of metal ions is driven by the redox state and redox potential of PSII metal centers. It is well known that metal ions are involved in electron transport directly by changes in the metal oxidation state during electron transport (Fe²⁺/Fe³⁺ in Cyt b₅₅₉ and Mn²⁺/Mn³⁺/Mn⁴⁺ in the M₅₅₉Ca complex). The advantageous property of metal ions bound to PSII proteins is modulation of their redox potential properties. The redox potential of metal ions is finely tuned by the protein environment surrounding the metal ions. The fine tuning of redox potential properties of metal ions in PSII plays an important role predominantly under high light stress conditions. The non-heme iron undergoes an increase in its redox potential accompanied by minor modification of the bicarbonate-binding site during high light stress (Vass et al. 1995). The redox potential of heme iron of Cyt b₅₅₉ decreases under high light stress (Ortega et al. 1999). This review attempts to focus on the pro-oxidant and antioxidant properties of non-heme iron, heme iron of Cyt b₅₅₉ and the M₅₅₉Ca complex and their role in the photo-oxidative damage of PSII proteins and lipids.

Non-heme iron

Non-heme iron is hexacoordinate ferrous iron located in close proximity to PsBA (D1 protein) and PsBD (D2 protein). X-ray crystallography data from the cyanobacterium T. elongatus showed that non-heme iron is at a distance of about 7.5 Å from the primary (QA) and secondary (QB) quinone electron acceptors on the electron acceptor side of PSII. Non-heme iron is coordinated by four histidine residues at the D1 protein (D1-His215, D1-His272) and D2 protein (D2-His214, D2-His268) and a bidentate bicarbonate ligand. D2-His214 and D1-His215 are coordinated to QA and QB and donate an H-bond to the carbonate O atoms of QA and QB, respectively. Bicarbonate is coordinated to D1-Tyr246 and D2-Tyr244. It has been demonstrated that H-bonding from D1-Tyr246 and D2-Tyr244 to the carbonate ligand of the non-heme iron contributes to the stability of the semiquinones (Saito et al. 2013). It is widely accepted that non-heme iron regulates electron transport from QA to QB even if the molecular mechanism is not fully understood (Diner et al. 1991, Müh et al. 2012, Müh and Zouni 2013).
Pro-oxidant activity of non-heme iron

One-electron reduction of H$_2$O$_2$ by ferrous iron leads to the formation of HO$^*$ (reaction 1). One-electron reduction of O$_2^{2-}$ by ferrous non-heme iron to the non-heme ferric-hydroperoxo species (Fe$^3+$–OO) (superoxide reductase) (reaction 2). One-electron oxidation of O$_2^{2-}$ by ferric non-heme iron to molecular oxygen (superoxide oxidase) (reaction 3). One-electron reduction of molecular oxygen by the ferric iron of the LP form of Cyt $b_{559}$ to O$_2^{2-}$ (oxygen reductase) (reaction 4). One-electron oxidation of O$_2^{2-}$ by the ferric iron of the LP form of Cyt $b_{559}$ to molecular oxygen (superoxide oxidase) (reaction 5). One-electron oxidation of O$_2^{2-}$ by the ferric iron of the HP form of Cyt $b_{559}$ to H$_2$O$_2$ (superoxide reductase) (reaction 6). Two-electron oxidation of water to H$_2$O$_2$ (water oxidase) (reaction 7). One-electron reduction of H$_2$O$_2$ to HO$^*$ by a manganese ion (Fenton reaction) (reaction 8). Two-electron reduction of H$_2$O$_2$ to water (reaction 9) and two-electron oxidation of H$_2$O$_2$ to molecular oxygen (reaction 10) (catalase activity).

![Diagram](https://example.com/diagram.png)

**Fig. 1** The arrangement of the metal center in PSII involved in the production (red arrow) and scavenging (green arrow) of ROS. One-electron reduction of the non-heme ferric-hydroperoxo species to HO$^*$ (reaction 1). One-electron reduction of O$_2^{2-}$ by ferrous non-heme iron to the non-heme ferric-hydroperoxo species (Fe$^3+$–OO) (superoxide reductase) (reaction 2). One-electron oxidation of O$_2^{2-}$ by ferric non-heme iron to molecular oxygen (superoxide oxidase) (reaction 3). One-electron reduction of molecular oxygen by the ferric iron of the LP form of Cyt $b_{559}$ to O$_2^{2-}$ (oxygen reductase) (reaction 4). One-electron oxidation of O$_2^{2-}$ by the ferric iron of the LP form of Cyt $b_{559}$ to molecular oxygen (superoxide oxidase) (reaction 5). One-electron reduction of O$_2^{2-}$ by the ferric iron of the HP form of Cyt $b_{559}$ to H$_2$O$_2$ (superoxide reductase) (reaction 6). Two-electron oxidation of water to H$_2$O$_2$ (water oxidase) (reaction 7). One-electron reduction of H$_2$O$_2$ to HO$^*$ by a manganese ion (Fenton reaction) (reaction 8). Two-electron reduction of H$_2$O$_2$ to water (reaction 9) and two-electron oxidation of H$_2$O$_2$ to molecular oxygen (reaction 10) (catalase activity).
formation of HO• revealed that the ligation of bicarbonate to non-heme iron is crucial for HO• formation (Pospíšil et al. 2004). Alternatively, it has to be considered that tyrosine peroxide might be formed by radiation damage caused by X-ray radiation. In spite of the fact that tyrosine peroxide might be due to X-ray radiation damage, the physiological formation of tyrosine peroxide cannot be fully excluded mainly due to the fact that the formation of tyrosine peroxide was D1 protein specific with no formation of tyrosine peroxide on its counterpart D2-Tyr244. The site of damage in the D1 protein was shown to be the stroma-exposed DE-loop of the D1 protein. The D1 DE-loop (D1-238–249 including D1-Tyr246) was proposed to be the first target for cleavage during photodegradation of D1 protein (Greenberg et al. 1987). Further, 23 kDa N-terminal and 9 kDa C-terminal fragments were shown to be formed after cleavage of the D1 protein at the DE-loop (De Las Rivas et al. 1992). The observation that the addition of H2O2 to PSII core complexes that contained the non-heme iron caused significant oxidative damage to D1 protein, where no oxidative damage was observed in isolated PSII reaction centers depleted of the non-heme iron, indicated that non-heme iron is involved in the oxidative damage of D1 protein (Miyao et al. 1995). It seems to be likely that HO• formed at the site of non-heme iron caused hydrogen abstraction from amino acids in the close vicinity of the non-heme iron. Recently, it has been proposed that non-heme iron might serve as a lipoxigenase known to initiate lipid peroxidation (Chan et al. 2012). The authors proposed here that a secondary product of lipid peroxidation, malondialdehyde, formed by cyclization of peroxyl radicals, and singlet oxygen formed by recombination of lipid peroxyl radicals via the Russell mechanism might cause modification of light-harvesting complex II (LHCII) and D1 protein damage, respectively.

Antioxidant activity of non-heme iron

Superoxide anion radicals formed on the electron acceptor side of PSII are eliminated in a reaction catalyzed by SOD. SOD catalyzes one-electron reduction of O2•− to H2O2 (superoxide reductase, SOR) and one-electron oxidation of O2•− to molecular oxygen (superoxide oxidase, SOO). The midpoint redox potential of the Fe3+/Fe2+ redox couple in SOD has to be between the standard redox potential of the O2•−/H2O2 redox couple (E0’ = +890 mV, pH 7) (Wood 1988) and the standard redox potential of the O2/O2•− redox couple (E0’ = −160 mV, pH 7) (Ilan et al. 1976). Evidence has been provided that non-heme iron exhibits SOD activity (Nugent 2001, Pospíšil et al. 2004). Whereas ferrous non-heme iron has been proposed to exhibit SOR activity (Fig. 1, reaction 2) (Pospíšil et al. 2004), the ferric non-heme iron has been assigned SOD activity (Fig. 1, reaction 3) (Mcevoy and Brudvig 2008). In SOR activity, one-electron reduction of O2•− by the the ferrous non-heme iron forms the ferric peroxy intermediate (Fe3+-OO), the protonation of which brings about formation of the ferric-hydroperoxo species (Fe3+-OOH) (Fig. 1, reaction 2) (Pospíšil et al. 2004). As the midpoint redox potential of the Fe3+/Fe2+ redox couple of the non-heme iron is +400 mV (pH 7), the reduction of bound peroxide by the non-heme iron is feasible. In SOD activity, it has been suggested that ferric non-heme iron formed by the recombination of ferrous non-heme iron and highly oxidized species on the electron donor side of PSII, such as Chl•−, Car•− and YD, might be re-reduced to ferrous non-heme iron by O2•− forming molecular oxygen (Fig. 1, reaction 3) (Mcevoy and Brudvig 2008). These authors proposed that the reduction of ferric non-heme iron to ferrous non-heme iron by O2•− occurs on the millisecond time scale. As the standard redox potential of the O2/O2•− redox couple (E0’ = −160 mV, pH 7) is lower than the midpoint redox potential of the Fe3+/Fe2+ redox couple in non-heme iron (E h = +400 mV, pH 7), the reduction of the ferric heme iron by O2•− is favorable from the thermodynamic point of view. Due to the fact that non-heme iron is at a distance of about 7.5Å from QA and QB, it seems to be likely that non-heme iron eliminates O2•− formed at QA and QB sites. The effective elimination of O2•− by non-heme iron requires the direct accessibility of O2•− from the QA and QB sites to non-heme iron. As no channels were identified in the X-ray crystallography data in the vicinity of QA, it is proposed that O2•− diffuses in a random direction from QA. Indeed, using Fourier transform ion cyclotron resonance mass spectrometry, several oxidatively modified residues were determined in the proximiy of QA on both D1 (Phe239, Glu241 and Gin242) and D2 (Pro238, Thr239, Glu242 and Met247) proteins (Frankel et al. 2013). In contrast to the QA site, plastoquinone transfer channels have been identified in the vicinity of the QB site. Based on the fact that no modified residues were observed in the proximity of QB (Frankel et al. 2013), it seems to be likely that O2•− formed at the QB site is directed to the heme iron of Cyt b559 via plastoquinone transfer channels.

Heme iron

Cyt b559 is heme-bridged heterodimer formed by subunits PsbE (α) and PsbF (β) and located in close proximity to PsbD (D2 protein). X-ray crystallography data from the cyanobacterium T. elongatus showed that the heme iron of Cyt b559 is located about 25Å from QA. Several different redox potential forms of Cyt b559 spread over the redox potential range from 400 to 0 mV have been described in the literature (Cramer and Whitmarsh 1977, Ortega et al. 1988, Thompson et al. 1989). The midpoint redox potential of the high potential (HP) form of Cyt b559 is in the range from +310 to +400 mV (pH 7) (Roncel et al. 2001), whereas the intermediate potential (IP) and the low potential (LP) forms exhibit a midpoint redox potential of +125 to +240 mV (pH 7) and from −40 to +80 mV (pH 7), respectively (Kaminskaya et al. 2009, Pospíšil and Tiwari 2010). In spite of extensive interest in the past three decades, the
physiological role of Cyt b559 is still unknown. Based on the fact that Cyt b559 is required for PSII structure, it was proposed that Cyt b559 plays an essential role in the early steps of assembly of the PSII complex (Morais et al. 1998). It is well accepted that Cyt b559 is not involved in the linear electron transport chain from water to plastoquinone; however, it participates in the cyclic electron transport around PSII as a protective mechanism during the process of photoinhibition (Shinopoulos and Brudvig 2012). It is generally believed that the HP form of Cyt b559 avoids oxidation of the PSII electron donor side by donation of an electron to highly oxidizing P680** from CarO2 and ChlZD2 (Hanley et al. 1999, Tracewell et al. 2011), whereas the LP form of Cyt b559 prevents overreduction of the PSII electron acceptor side by acceptance of an electron from plastoquinone (Barber and De Las Rivas 1993) or plastoquinol (Buser et al. 1992). In addition to the involvement of Cyt b559 in the cyclic electron transport around PSII, several lines of evidence have been provided on the enzymatic function of Cyt b559 (for a review, see Pospíšil 2011). It has been proposed that Cyt b559 exhibits oxygen reductase (Kruk and Strafatka 2001, Pospíšil et al. 2006), SOD (Ananyev et al. 1994, Kruk and Strafatka 1999, Tiwari and Pospíšil 2009) and plastoquinol oxidase (Bondarava et al. 2003, Bondarava et al. 2009) activities.

**Pro-oxidant activity of heme iron of cyt b559**

One-electron reduction of molecular oxygen by the ferrous heme iron of b-type cytochromes has been shown to result in the formation of O2**. As the standard redox potential of the O2/O2** redox couple is negative (Em′ = −160 mV, pH 7), only a ferrous heme iron with a negative midpoint redox potential exhibits the capability to reduce molecular oxygen. It is well established that one-electron reduction of molecular oxygen is catalyzed by b-type cytochromes in the cytoplasmic and mitochondrial membranes (Isogai et al. 1995, Quinlan et al. 2011). In the cytoplasmic membrane, the reduction of molecular oxygen by ferrous heme iron in Cyt b558 in NADPH oxidase during the phagocyte respiratory burst is feasible as the midpoint redox potential of the Fe3+/Fe2+ redox couple is highly negative (Em = −245 mV, pH 7). In the mitochondrial membrane, Cyt b562 [Em(Fe3+/Fe2+) = −90 mV, pH 7] and Cyt b566 [Em(Fe3+/ Fe2+) = +50 mV, pH 7] of complex III are involved in the formation of O2** within the respiratory electron transport chain. Similarly, evidence has been provided regarding the reduction of molecular oxygen by ferrous heme iron of LP form of Cyt b559 in PSII (Fig. 1, reaction 4) (Pospíšil et al. 2006). As the standard redox potential of O2/O2** redox couple is −160 mV (pH 7), the reduction of molecular oxygen by ferrous heme iron of LP form of Cyt b559 (Em = −40 to +80 mV, pH 7) seems to be thermodynamically infeasible. However, in the thylakoid membrane, the concentration of the electron donor to molecular oxygen is typically several orders lower compared with the concentration of molecular oxygen. Under these circumstances, the operational redox potential of the O2/O2** redox couple is shifted according to the Nernst equation E = −0.16 + 0.06 log [O2/O2**] to a value close to 0 mV. Based on these considerations it is likely that the ferrous heme iron of the LP form of Cyt b559 acts as oxygen reductase known to catalyze the one-electron reduction of molecular oxygen to O2**.

**Antioxidant activity of heme iron of Cyt b559**

Experimental evidence has been provided showing that Cyt b559 exhibits both SOO (Fig. 1, reaction 5) and SOR (Fig. 1, reaction 6) activity (Tiwari and Pospíšil 2009, Pospíšil and Tiwari 2010). One-electron oxidation of O2** to molecular oxygen is maintained by the ferric heme iron of the IP form of Cyt b559. In this reaction, the ferric heme iron of the IP form of Cyt b559 is reduced to the ferrous heme iron. As the standard redox potential of the O2/O2** redox couple (Em′ = −160 mV, pH 7) is lower than the midpoint redox potential of the Fe3+/Fe2+ redox couple in the IP form of Cyt b559 (Em = +125 mV, pH 7), the oxidation of O2** by the ferric heme iron of IP form of Cyt b559 is feasible. One-electron reduction of O2** to H2O2 is sustained by the ferrous heme iron of the HP form of Cyt b559. In this reaction, the ferrous heme iron of the HP form of Cyt b559 is oxidized to the ferric heme iron. As the standard redox potential of the O2**/H2O2 redox couple (Em′ = +890 mV, pH 7) is lower than the midpoint redox potential of the Fe3+/Fe2+ redox couple in the HP form of Cyt b559 (Em = +310 mV, pH 7), the reduction of O2** by the ferric heme iron is thermodynamically favorable. To maintain effective elimination of O2** and full prevention of oxidative damage of proteins and lipids on the electron acceptor side of PSII, the accessibility of O2** from the site of its formation to the heme iron of Cyt b559 has to be guaranteed. X-ray crystal structural analysis of PSII complexes showed that the heme iron of Cyt b559 is located in the vicinity of two plastoquinone transfer channels (Guskov et al. 2009). It has been demonstrated that photoreduction and photo-oxidation of the heme iron in PSII deprived of the Mn4O5Ca complex were completely diminished by exogenous SOD (Sinha et al. 2010). Based on these observations, it has recently been proposed that these channels facilitate diffusion of O2** toward the heme iron and thus maintain reduction and oxidation of the heme iron by O2**. Using EPR spin-trapping spectroscopy, it has recently been demonstrated that the oxidation of proteins and lipids by the protonated form of superoxide, known as the perhydroxyl radical (HO2.), results in the formation of a carbon-centered radical (Tiwari et al. 2013). Interestingly, when the QA site was occupied by DCMU, the formation of the carbon-centered radical was enhanced. These observations confirmed that diffusion of O2** to the heme iron of Cyt b559 via channel I and II is crucial for effective elimination of O2**.
**Mn₄O₅Ca complex**

It is well established that the Mn₄O₅Ca complex consists of four manganese, Mn(1), Mn(2), Mn(3) and Mn(4), and one calcium ion bound to specific amino acid residues. Five oxygen atoms serve as oxo bridges linking manganese and calcium ions. Two water molecules are coordinated to Mn(4), whereas two other water molecules are bound to calcium. In addition, two chloride ions have been shown to be coordinated to manganese via specific amino acid residues at a distance of 6.7 Å from Mn(4) and 7.4 Å from Mn(2) (Kawakami et al. 2009, Kawakami et al. 2011). It has recently been pointed out that the redox potential of manganese is modulated by binding and re-binding of calcium without any change in the structural arrangement of manganese (Tsui and Agapie 2013). Electrochemical studies of heterometallic manganese–oxido compounds showed that the redox potential of manganese is highly dependent on the Lewis acidity of the redox-inactive metal (Tsui et al. 2013). The tuning of the redox potential in both directions allows thermodynamic control of the pro-oxidant and antioxidant properties of the Mn₄O₅Ca complex. The formation of molecular oxygen by the Mn₄O₅Ca complex as a by-product of water splitting is accompanied by the inevitable probability of ROS formation and consequently oxidative modification of proteins and lipids within PS II.

**Pro-oxidant activity of the Mn₄O₅Ca complex**

Apart from the formation of H₂O₂ by two consecutive one-electron reductions of molecular oxygen on the electron acceptor side of PSII, H₂O₂ is formed by two-electron oxidation of water on the electron donor side of PSII. Light-driven splitting of two water molecules by the Mn₄O₅Ca complex is considered to occur as a four-electron oxidation reaction, in which either the manganese or the water sub-strate is oxidized (Grundmeier and Dau 2012). Several lines of evidence have been provided that a two-electron oxidation of water comprising the transition from the S₂ to the S₀ state or from the S₁ to the S₋₁ state results in the formation of H₂O₂ (Fig. 1, reaction 7) (Schröder and Åkerlund 1986, Fine and Frasch 1992, Taoka et al. 1993). The release of a chloride ion coordinated to manganese was shown to play a crucial role in the formation of H₂O₂ (Wydrynski et al. 1989, Bradley et al. 1991, Fine and Frasch 1992). Recently, it has been demonstrated that the two-electron oxidation of water coordinated to Mn(4) results in the formation of H₂O₂ under heat stress (Yadav and Pospíšil 2012). It has been proposed that the coordination of the chloride ion to Mn(4) prevents the formation of H₂O₂ (Fig. 2). It has been proposed that the coordination of the chloride ion to Mn(4) prevents the formation of H₂O₂ (Fig. 2). However, it has never been shown that chloride depletion does occur under physiologically relevant conditions. H₂O₂ was proposed to be reduced to HO* by a manganese ion released from the damaged Mn₄O₅Ca complex (Fig. 1, reaction 8) (Pospíšil et al. 2007, Yamashita et al. 2008, Yadav and Pospíšil 2012). From a thermodynamic point of view, the reduction of H₂O₂ to HO* by free manganese ions is not feasible as the standard redox potential of manganese is highly positive \( E_{\text{red}}^\circ (\text{Mn}^{3+}/\text{Mn}^{2+}) = +1,200 \text{ mV, pH } 7 \). However, the reduction of H₂O₂ by manganese is thermodynamically possible, when the redox potential is decreased by a temporal coordination of manganese to the extrinsic protein bound on the lumenal side of PSII (Pospíšil 2012). Based on the observation that HO* caused more pronounced damage to PsbO (33 kDa), it has been proposed that it coordinates more metal compared with PsbP (23 kDa) and PsbQ (17 kDa) (Henmi et al. 2004). In contrast, Krieger-Liszkay and co-workers demonstrated that PsbP coordinates manganese during the turnover of the D1 protein (Bondarava et al. 2005, Bondarava et al. 2007). It has been suggested that manganese is coordinated at two binding sites comprising either Glu50, Asp51, Asp54 and Glu39 or Glu50, Asp51 and Asp54 and Glu39 located close to Trp34, or Glu177, Asp16S and His144 located in the vicinity of Trp168 (Bondarava et al. 2007, Bricker and Frankel 2011). It was previously demonstrated that high light caused a partial release of extrinsic PsbO, PsbP and PsbQ proteins from PSII in parallel to a significant degradation of D1 protein (Henmi et al. 2004). The authors proposed that release of the PsbO protein from PSII and the subsequent damage of PsbO protein were caused by HO* formed by the Fenton reaction mediated by transition metals bound to the PsbO protein. It has been shown that the CP43 residues (Glu354, Thr355, Met356 and Arg357) in close proximity to the Mn₄O₅Ca complex are oxidized (Frankel et al. 2012). It seems to be likely that the oxidation of amino acids at the C-terminal domain of the D1 protein and PsbO, PsbP and PsbQ proteins is caused by HO* formed on the electron donor side of PSII.
Antioxidant activity of the Mn$_4$O$_5$Ca complex

H$_2$O$_2$ formed by two-electron oxidation of water is eliminated by the Mn$_4$O$_5$Ca complex known to catalyze two-electron reduction (Fig. 1, reaction 9) and two-electron oxidation (Fig. 1, reaction 10) of H$_2$O$_2$ to water and molecular oxygen, respectively. In the dark, the two-electron oxidation of H$_2$O$_2$ to molecular oxygen involves either $S_0$ to $S_0$ or $S_1$ to $S_{-1}$ state transitions, whereas two-electron reduction of H$_2$O$_2$ to water comprises either $S_0$ to $S_2$ or $S_{-1}$ to $S_1$ state transitions (Fine and Frasch 1992, Mano et al. 1997). In the light, the two-electron oxidation of H$_2$O$_2$ to molecular oxygen is mediated by manganese released from the damaged Mn$_4$O$_5$Ca complex (Sandusky and Yocum 1988). Later, Sheptovitsky and Brudvig (1998) provided evidence that the Mn$_4$O$_5$Ca complex is less likely to be involved in the enzymatic disproportion of H$_2$O$_2$ to water and molecular oxygen. The author demonstrated that the heme catalase attached to the luminal side of the thylakoid membrane close to PSII is involved in the elimination of H$_2$O$_2$ formed by two-electron oxidation of water. The latest crystal structural data of PSII have enabled detailed analyses of water channels in the vicinity of Mn$_4$O$_5$Ca. It is generally accepted that these channels permit the flow of water substrate to the Mn$_4$O$_5$Ca complex and in turn the flow of molecular oxygen from the Mn$_4$O$_5$Ca complex (Linke and Ho 2014). Furthermore, when incomplete oxidation of water substrate brings about the formation of H$_2$O$_2$, it is crucial to direct H$_2$O$_2$ away from the Mn$_4$O$_5$Ca complex toward the surface and thus prevent formation of HO$^\cdot$. Four CP43 residues (Glu354, Thr355, Met356 and Arg357) in the vicinity of the Mn$_4$O$_5$Ca complex have been proposed to be associated with the channel leading from the Mn$_4$O$_5$Ca complex to the surface of the thylakoid membrane (Frankel et al. 2012).

Funding

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic grants No. LO1204 (National Program of Sustainability I).

Acknowledgments

I am grateful to Dr. Ankush Prasad for his support with figure preparation.

Disclosures

The author has no conflicts of interest to declare.

References


Manno, J., Ushimaru, T. and Asada, K. (1997) Ascorbate in thylakoid lumen as an endogenous electron donor to Photosystem II: protection of thylakoids from photoinhibition and regeneration of...


Results of the study indicate that heterometallic manganese-oxido clusters modulate the reduction potential in multimetallic manganese–oxidino clusters. Nat. Chem. 5: 44–46.


