



Current analysis of cations substitution in the oxygen-evolving complex of photosystem II

Boris Semin¹ · Aleksey Loktyushkin¹ · Elena Lovyagina¹

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Abstract

Water oxidation in photosystem II (PSII) is performed by the oxygen-evolving complex Mn_4CaO_5 which can be extracted from PSII and then reconstructed using exogenous cations Mn(II) and Ca^{2+} . The binding efficiency of other cations to the Mn-binding sites in Mn-depleted PSII was investigated without any positive results. At the same time, a study of the Fe cations interaction with Mn-binding sites showed that it binds at a level comparable with the binding of Mn cations. Binding of Fe(II) cations first requires its light-dependent oxidation. In general, the interaction of Fe(II) with Mn-depleted PSII has a number of features similar to the two-quantum model of photoactivation of the complex with the release of oxygen. Interestingly, incubation of Ca-depleted PSII with Fe(II) cations under certain conditions is accompanied by the formation of a chimeric cluster Mn/Fe in the oxygen-evolving complex. PSII with the cluster $2Mn2Fe$ was found to be capable of water oxidation, but only to the H_2O_2 intermediate. However, the cluster $3Mn1Fe$ can oxidize water to O_2 with an efficiency about 25% of the original in the absence of extrinsic proteins PsbQ and PsbP. In the presence of these proteins, the efficiency of O_2 evolution can reach 80% of the original when adding exogenous Ca^{2+} . In this review, we summarized information on the formation of chimeric Mn–Fe clusters in the oxygen-evolving complex. The data cited may be useful for detailing the mechanism of water oxidation.

Keywords Photosystem II · Oxygen-evolving complex · Iron · Manganese · Manganese replacement · Calcium

Abbreviations

Chl	Chlorophyll
D1	Integral protein of the photosystem II reaction center
DPC	Diphenylcarbazide
EP	Extrinsic proteins
HAS	High-affinity Mn-binding site
H_2Q	Hydroquinone
LAS	Low-affinity Mn-binding site
OEC	Oxygen-evolving complex
PSII	Photosystem II
PSII(-Ca)	Ca^{2+} -depleted PSII membranes
PSII(-Mn)	Mn-depleted PSII membranes
RC	Reaction center
Tris	Tris(hydroxymethyl)amino methane

SOD	Superoxide dismutase
Y_Z	Redox-active tyrosine D1-Tyr161, the first electron donor to $P680^+$ in PSII

Introduction

Oxygen-producing organisms (which include the higher plants, algae, and cyanobacteria) carry out photosynthesis, the most important light-dependent biological process that is the basis of life on Earth. The main components of the photosynthetic biochemical apparatus associated with the light-dependent part of photosynthesis are photosystem II (PSII) and photosystem I (PSI). PSII contains a unique catalytic center facilitating the oxidation of water molecules which are the donors of protons and electrons required for $NADP^+$ reduction and ATP synthesis with the resulting molecular oxygen released into the atmosphere as a by-product. This reaction is essentially the only source of O_2 on our planet. The catalytic center of water oxidation consists of four manganese cations and one calcium cation interconnected by oxygen bridges— Mn_4CaO_5 . The structural organization of

✉ Boris Semin
semin@biophys.msu.ru

¹ Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia 119234

the manganese/calcium cluster has been solved by X-ray diffraction at 1.9 Å resolution (Umena et al. 2011). The atomic structure of the oxygen-evolving complex (OEC) within PSII is shown in Fig. 1. It is an irregular cube formed by three manganese cations, a calcium cation and four oxygen atoms. The fourth manganese cation (Mn^4 according to the numbering scheme adopted by Umena et al. (2011),

also called the “dangler” cation) is located at some distance from the cube but is connected to it. This “dangler” manganese cation is connected to the Mn_3Ca cube by two oxygen bridges O4 and O5. The Mn^4 cation and the calcium cation each have two water molecules as ligands. Whether these water molecules are substrate water remains to be seen. The catalytic center is connected to the protein structure PSII by

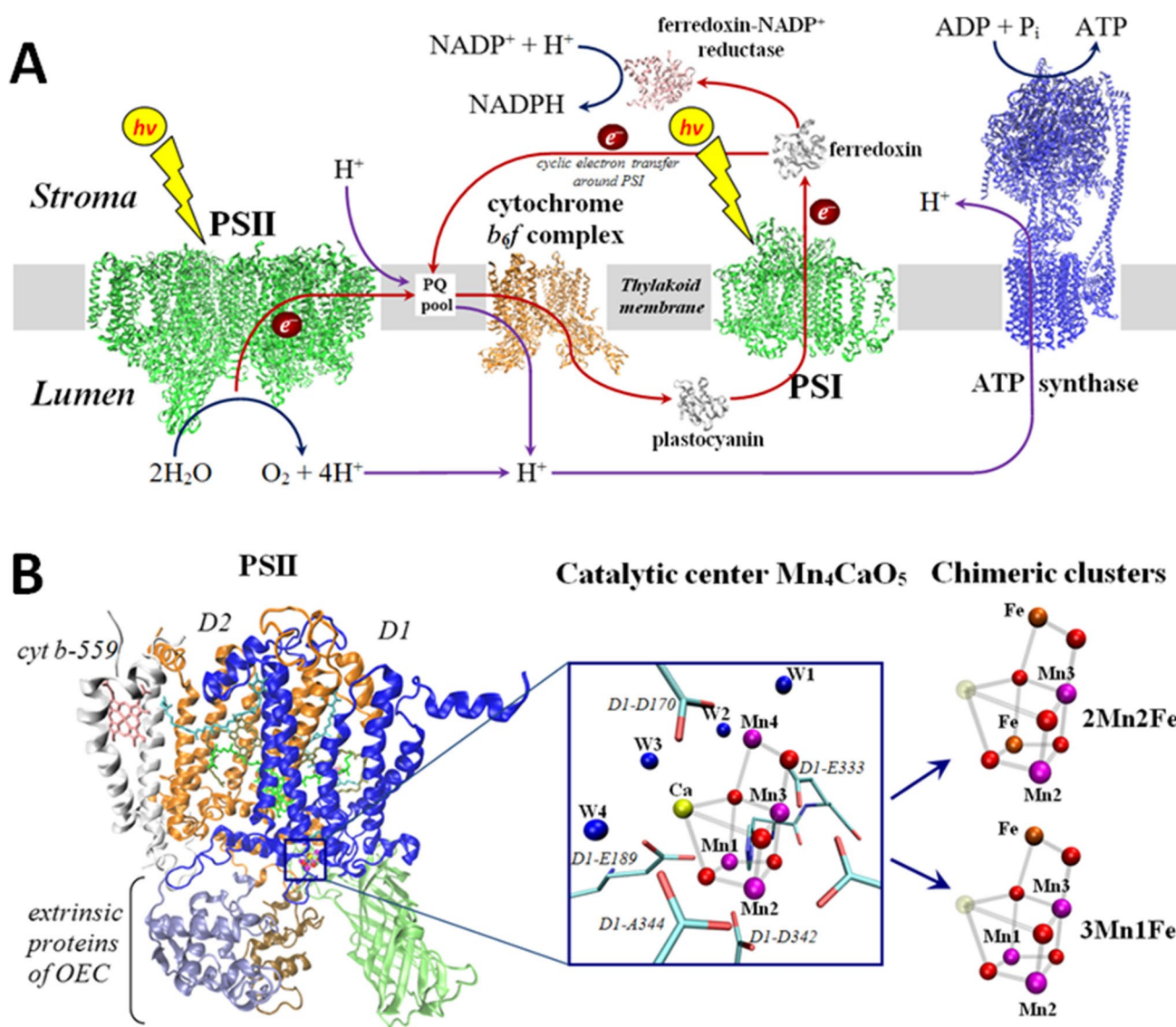


Fig. 1 **A** Photosynthetic apparatus and electron transport chain in plants. Four protein macrocomplexes participate in the production of ATP and NADPH: photosystems PSII and PSI, the cytochrome b_6/f complex, and the ATP synthase. PSII functions as a water/PQ oxidoreductase, which has a manganese complex [Mn_4O_5Ca], located on D1 protein on the PSII donor side, as well as pheophytin, plastoquinones Q_A and Q_B , and a non-heme iron binding a bicarbonate ion on the PSII acceptor side. The structural data on membrane-protein complexes and soluble proteins were obtained from Protein Data Bank (PDB): PSII–6JLJ, PSI–6JBO, *cyt b₆/f* complex–7QRM, ATP synthase–6VOF, plastocyanin–2CJ3, ferredoxin–4ZHP, ferredoxin-

NADP⁺ reductase–1FNB. **B** Structure of PSII and catalytic center of oxygen-evolving complex: Ca, Mn(1–4), and O (red circles) are ions of calcium and manganese and oxygen atoms; W(1–4) are oxygen atoms of water molecules acting as ligands of manganese and calcium. PDB ID: 6jlj. Mn1 ligands: D1-Glu189, His332 and Asp342, D1-Asp342; Mn2 ligands: Ala344 and CP43-Glu354; Mn3 ligands: D1-Glu333 and CP43-Glu354; Mn⁴ (HAS) ligands: D1-Asp170 and D1-Glu333; Ca²⁺ ligands: D1-Asp170 and D1-Ala344 (Umena et al. 2011). The right panel shows the structures of chimeric Mn-Fe clusters in the oxygen-evolving complex, obtained after removing the calcium cation

amino acid residues. The cation Mn^{4+} is bound to the high-affinity Mn-binding site (HAS). A negative criticism of the work of Umena et al. (2011) was raised in regard to the possibility of X-ray-induced reduction of manganese cations during the experiment, which could affect the accuracy of distance determination between cluster elements. This possible artifact was later eliminated using serial femtosecond X-ray free-electron laser crystallography (Suga et al. 2015) for investigation of the Mn/Ca cluster structure in the S1 state. In subsequent works, this method was used to study the structural changes occurring in the cluster during the transition from S1 state to S2 and S3 states (Suga et al. 2017, 2019; Kern et al. 2018). Another method of structural investigation, cryo-electron microscopy, was recently used to study monomeric PSII from *Synechocystis* sp. PCC 6803 lacking the OEC (Gisriel et al. 2020). The authors found significant differences in the structure of the Mn-binding HAS obtained by the cryo-electron microscopy and X-ray diffraction methods earlier (Zhang et al. 2017). Quite recently, a number of advances have been made in the study of the OEC structure, with regard to changes in the catalytic cycle process and understanding the details of the water oxidation mechanism and molecular oxygen synthesis (Shen 2015; Lubitz et al. 2019; Oliver et al. 2022; Shevela et al. 2023). The most discussed hypotheses of which oxygen atoms are involved in the synthesis of O_2 , the oxygen bridge O5 is the most likely candidate. However, the nature of the second oxygen atom is less clear. It could be a newly inserted water molecule W_x (Shen 2015) or W_f (Lubitz et al. 2019) or water molecule W3 bound to Ca^{2+} ion (Shen 2015).

When studying the role and mechanism of metal cations in enzymes, the method of replacing a native cation with other metal cations is often used in order to clarify the question—what features of a native metal cation are important for its special function? In the case of PSII, the possibilities of binding and replacing Ca^{2+} and Mn cations in Mn_4CaO_5 catalytic center of the OEC with different cations have been extensively previously investigated. The Ca^{2+} cation can be substituted while retaining oxygen-evolving function only by Sr^{2+} (Ghanotakis et al. 1984a). Other cations can effectively bind to the Ca site of the OEC but without recovery of OEC activity, with these including Cd^{2+} cations (Waggoner and Yocum 1990) and various lanthanides (Ghanotakis et al. 1985; Ono 2000; Lovyagina et al. 2021). The efficiency of binding of the metal cation to the Ca site and its ability to restore the functional activity of OEC depends on the ion radius and the value of the aqua ion pK_a (Vrettos et al. 2001). The possibility of binding other metal cations to the Mn-binding sites of the PSII has also been investigated, but less extensively. Ghirardi et al. (1996), using the method of flash-probe fluorescence, showed that the cations Zn^{2+} and Co^{2+} bind to the Mn-binding site in PSII(-Mn) membranes less efficiently than Mn cations. However, at the same time,

it was found that the Mn-binding site(s) bind(s) Fe(II) cations with an efficiency and specificity comparable to the Mn cations (Semin et al. 1995). In subsequent works, this effect was studied in detail and a method was developed for replacing 1 or 2 manganese cations in a catalytic cluster with an Fe cation. In this paper, we provide a brief review of published studies concerned with the Mn cation(s) substitution process in the OEC with iron cations and the effects that have been observed from doing so.

Binding of Fe(II) cations to Mn-binding sites in PSII lacking oxygen-evolving complex

Manganese cations can be extracted from the OEC of PSII by using hydroxylamine or Tris at alkaline pH. Treatment with these compounds results in the reduction of Mn cations and these reduced cations then exit from the binding site. The difference in the effect of these compounds is that Tris extracts all extrinsic proteins, whereas hydroxylamine does not extract PsbO extrinsic protein (Tamura and Chennia 1987). PSII(-Mn) membranes are capable of oxidizing exogenous electron donors under light and have two oxidation sites located on the donor side. One of these sites is the high-affinity Mn-binding site (HAS); the second site is the low-affinity site (LAS) (Hsu et al. 1987; Kurashov et al. 2009). HAS is the oxidation site for Mn(II) cations, donor pair ($Mn(II) + H_2O_2$), and diphenylcarbazide (DPC) (Hsu et al. 1987; Semin et al. 2004; Kurashov et al. 2009), while Mn(II) and DPC are oxidized also at the LAS (Hsu et al. 1987; Kurashov et al. 2009). At the HAS, the oxidant is Y_Z' (Hoganson et al. 1989), while at the LAS, the His190 residue of the integral protein of the PSII reaction center D1 may participate in oxidation (Kurashov et al. 2009).

Hsu et al. (1987) showed that binding of the Mn(II) cation to the HAS resulted in blocking of the oxidation of the DPC at that site. This is reflected in a 50% decrease in the rate of oxidation of DPC (DPC is oxidized only through the LAS in this case). The use of this test in the study of Fe(II) binding parameters showed that Fe(II) cations bind to a high-affinity Mn-binding site with a high efficiency (Semin et al. 1995) comparable to that of binding manganese cations (Hsu et al. 1987). This is not unique in biological important molecules. There exist a number of enzymes where the catalytic center can contain iron or manganese although the function is the same. To such proteins belong the family of superoxide dismutases (SOD) utilizing either Fe or Mn to catalyze the dismutation of $O_2^{\cdot-}$. In these enzymes, the coordination of ligands at the Fe site of Fe-SOD closely corresponds to the Mn coordination found in Mn-SOD (Lah et al. 1995). Another example would be binuclear iron-containing enzymes such as ribonucleotide reductase in which the iron cofactor can be replaced with manganese (Atta et al. 1992).

Additional evidence of highly effective binding of Fe(II) cations to the Mn-binding site are experiments of Allen with coworkers (Kálmán et al. 2010; Tang et al. 2009). Using the EPR method, they showed the binding of Fe(II) cations to an artificially created Mn-binding site in bacterial centers and its oxidation by primary electron donor. Binding of Fe(II) cations requires light, as no effect of Fe(II) cations was observed in the dark. This indicates that only oxidized Fe cations bind to the Mn-binding HAS. However, it should be noted that incubation of the PSII(-Mn) membranes with the Fe(III) is not accompanied by binding of the oxidized iron cation to the HAS and blocking it. The reason for this effect may be the following. Salts of Fe(III) do not dissolve in the buffer with a neutral pH due to the fast formation of insoluble $\text{Fe}(\text{OH})_3$. The use of Fe(III) solution stabilized with sucrose (Charley et al. 1963) also did not result in HAS binding and blocking (Semin et al. 1995). However, it has been found relatively recently that such a preparation is a suspension of nanoparticles (Kudasheva et al. 2004), which may be the reason for the lack of binding of iron cations.

These results were confirmed using the method of recording the kinetics of fluorescence decay in response to a flash of light (Semin et al. 2002). In the same work, it was shown that oxidized iron cations, by binding to a HAS with great efficiency, prevent the binding of manganese cations to this site—i.e., this site is blocked by bound Fe(III) cation. Blocking iron cations are bound to the HAS very firmly and cannot be removed by centrifugation. However, they can be extracted with citrate buffer at pH 3.0 (citrate is an efficient chelator of ferric cations having first stability constant ($\log K_1$) 11.8 (Sillen and Marten 1964)) (Semin and Seibert 2006a).

Kinetic studies have shown that not one iron cation is involved in blocking, but two or more (Semin et al. 1995, 2002). When measuring the concentration of iron cations in the buffer during blocking, it was found that oxidation of five iron cations was necessary to provide blocking effect (Semin and Seibert 2004). Carboxyl amino acid group, possibly amino acid D1-Asp170, is involved in binding the iron cations blocking the HAS (Semin and Seibert 2006a).

The need for light-dependent charge separation in PSII(-Mn) membranes, accompanied by the generation of oxidant Y_Z , and, accordingly, electron transport for oxidation of Fe(II) cations is quite clear from the above facts. However, in the experiment, blocking takes place during the short incubation of the sample (several min) under room light and without the addition of an exogenous electron acceptor. The exogenous acceptor increases the rate of Fe(II) oxidation, and the diuron inhibits this process (Lovvyagina et al. 2005). These data suggest that the PSII(-Mn) membrane preparation contains an electron acceptor that ensures the transport of electrons from Fe(II) and that molecular oxygen can be such an acceptor. Indeed, under anaerobic conditions, the

rate of Fe(II) oxidation is significantly slowed down (Lovvyagina et al. 2005).

The catalytic center of OEC contains in addition to 4 redox-active Mn cations one redox-inactive Ca^{2+} cation which is essential for the oxygen-evolving activity of the catalytic Mn_4CaO_5 complex. Without calcium cation, molecular oxygen is not synthesized, indicating its important role in the water oxidation process. The mechanism of Ca^{2+} participation in this process is unclear. Possibly, it binds the substrate water molecule (W3 or W4 (Kawakami et al. 2011)) or participate in controlling the redox potential of Mn cluster (Tsui et al. 2013; Saito et al. 2021). E_m (MnIII/IV) depends on the Lewis acidity of redox-inactive metals (Ca^{2+} in the OEC) (i.e., the $\text{p}K_a$ of aqua complexes of redox-inactive metals) (Tsui et al. 2013). Quantum chemical calculations showed that the valence of metals predominantly affects E_m (MnIII/IV), whereas the ionic radius of metals affects E_m (MnIII/IV) only slightly (Saito et al. 2021). Ca^{2+} is a necessary component for the reconstruction of Mn/Ca catalytic center during photoactivation (self-assembly of Mn cluster). In this regard, the effect of calcium cations on the binding of iron cations to the Mn-binding sites, in particular, on the reduction of Y_Z by the Fe(II) cation in response to a flash of light and on the blocking of HAS by the Fe cation, was studied (Semin et al. 2021). It was shown that the efficiency of Fe(II) oxidation and the efficiency of HAS blocking, depending on the Ca^{2+} concentration, do not coincide (Semin et al. 2021). The maximum level of inhibition of the Fe(II) oxidation process was 20%, while the inhibition of the blocking process was twice as high at the same Ca^{2+} concentration. These results indicate that the blocking process is a more complex process than the oxidation of Fe(II) cations at HAS.

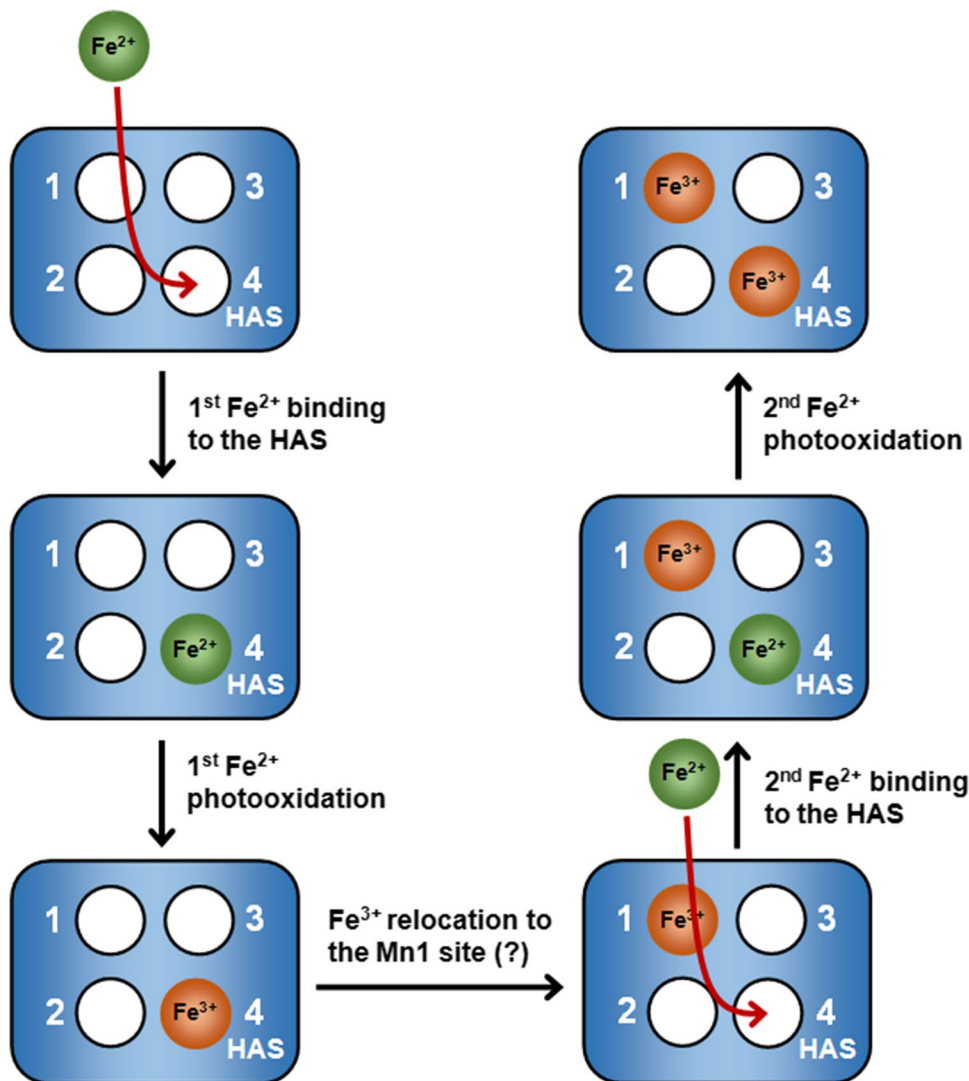
The process of iron cations binding to HAS in PSII(-Mn) membranes is also of significant interest because it reduces a number of electron transport chain properties altered by Mn removal. Extraction of Mn from OEC leads to a significant change in the characteristics of the electron transporter Y_Z such as kinetics and temperature characteristics of Y_Z oxidation and reduction (see for a review Semin et al. 2005). This may be a consequence of the hydrogen bond break between Y_Z and D1-His190. The use of the incorporation of iron cations into PSII(-Mn) membranes made it possible to find that the hydrogen bond between these two amino acid residues is not regular, but is a low barrier hydrogen bond (Semin et al. 2005). This bond is broken by extraction of Mn and restored by binding of Fe cation. The data obtained with X-ray diffraction method confirmed our results and conclusions (Saito et al. 2011).

The process of self-assembly of the manganese cluster in apo-PSII is described by a two-quantum model according to the following sequence of events: **A** (Mn(II)) \Rightarrow **B** (Mn(III), unstable) \Rightarrow dark rearrangement \Rightarrow **C** (Mn(III) + Mn(II), unstable) \Rightarrow **D** (Mn(III)-Mn(III), stable). At the first stage

(A), Mn(II) cation binds to HAS, then it is oxidized by tyrosine Y_Z (B), followed by a mandatory dark period (the 100 ms half-time). Dark period according to one hypothesis represents the relocation of a Mn(III) ion from the HAS to its final location in the assembled cluster which is a thermodynamically more stable site (Avramov et al. 2020). A free HAS binds the second Mn(II) cation (C). Mn(II) cation is oxidized by tyrosine and a stable dimer (D) is formed. Thus, the most important step in the process of photoactivation (in fact, the reaction of Mn(II) oxidation and binding on the donor side of PSII(-Mn)) is the presence of a mandatory dark period. Taking into account the high similarity between the oxidation processes of Mn(II) and Fe(II) in the PSII(-Mn) membranes, the oxidation of Fe(II) cations in the pulsed illumination mode was studied by adjusting the flash duration and the dark interval between flashes (Semin and Seibert 2006b). The flash-probe fluorescence method was used to estimate the blocking efficiency. It has been shown that the yield of blocking increases with flash number

and reaches 50% after seven flashes. When the dark interval between the flashes (Δt) was varied, it was found that the percentage of blocking decreases at $\Delta t < 100$ ms ($t_{1/2}$, 4–10 ms). This result shows the necessity of a dark rearrangement during the blocking process and indicates the formation of a binuclear iron center. Schematically, the process of interaction of Fe(II) cations with Mn-depleted PSII membranes is shown in Fig. 2. An important circumstance should be noted. Concentration binding curves of Mn(II) and Fe(II) cations to the HAS are practically the same (concentration of 50% inhibition of DPC oxidation are equal 0.6 and 0.9 μM (Semin et al. 1995)), which suggests the identity of kinetic parameters. For Mn(II), such parameters (dissociation constant K_D and second-order rate constant for Y_Z reduction k_2) are respectively 0.15 μM (Hsu et al. 1987) and $6 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (Hoganson et al. 1989). These parameters represent the binding process of cations Mn(II) or Fe(II) to HAS and their oxidation by tyrosine. The process of HAS blocking with an iron cation requires binding of at least two

Fig. 2 Hypothetical mechanism of Fe(II) cations binding to the Mn-binding sites of OEC in the Mn-depleted PSII membranes and blocking of the high-affinity Mn-binding site. 1st Fe(II) cation binding to the HAS: $[\text{Fe(II)} (\text{solution}) \rightarrow \text{Fe(II)} (\text{HAS})]$; 1st Fe²⁺ cation photooxidation: $[\text{Fe(II)} (\text{HAS}) + h\nu \rightarrow \text{Fe(III)} + e^-]$; relocation of Fe(III) cation to another Mn-binding site: $[\text{Fe(III)} (\text{HAS}) \rightarrow \text{Fe(III)} (\text{Mn1 site})]$; 2nd Fe(II) cation binding to the HAS: $[\text{Fe(II)} (\text{solution}) \rightarrow \text{Fe(II)} (\text{HAS})]$; 2nd Fe(II) cation photooxidation: $[\text{Fe(II)} (\text{HAS}) + h\nu \rightarrow \text{Fe(III)} + e^-]$



Fe cations (Semin et al. 1995; Semin and Seibert 2004) and oxidation of 4–5 Fe cations (Semin and Seibert 2004) and 50% blocking takes about 5 s (Semin and Seibert 2004).

PSII(-Mn) membranes with iron cations bound to the Mn-binding sites do not have any activity: they do not evolve oxygen under illumination (Semin et al. 2002), and do not oxidize water to hydrogen peroxide (Semin et al. 2013); i.e., there is no electronic transport in such membranes. Thus, the iron cluster replacing the entire catalytic center Mn_4CaO_5 in the OEC is inactive. In this regard, the question arises—will the tetranuclear manganese cluster in which 1 or 2 Mn cations are replaced by an iron cation show activity? Investigation of this problem has yielded very interesting results.

Interaction of Fe(II) cations with PSII containing manganese cluster: substitution of manganese cations in OEC with iron cations

PSII(-Ca) with 2Mn2Fe chimeric cluster

In 1984 year, Ghanotakis with colleagues investigated the protective function of extrinsic proteins (EP: PsoO, PsoQ, PsoP) (Ghanotakis et al. 1984b). The authors found that EP cover the Mn cluster, providing protection against exogenic reductants reducing Mn cations, thereby destroying the Mn cluster. EPs provide protection mainly against the action of bulky reductants such as hydroquinone (not hydroxylamine). The authors also mentioned a protector function of EP against charged reductants such as iron cations (Ghanotakis et al. 1984b). In PSII preparations without EP, for example, in the Ca-depleted PSII, the Mn cluster is available for all reducing agents. This feature made it possible to study the effect of Fe(II) cations on the Mn cluster in PSII(-Ca) membranes. The experiments were carried out on the so-called Ca-depleted PSII membranes that do not contain the Ca cation in OEC and EP PsoQ and PsoP. Such membranes are obtained by washing with a buffer with high ionic strength (Ono and Inoue 1990). Experiments showed that long-term (more than 2 h) incubation of PSII(-Ca) membranes with $FeSO_4$ in the dark was accompanied by extraction of two Mn cations (Semin and Seibert 2016). Two Mn cations per reaction center (RC) is the maximum level of extraction. Other reductants such as H_2O_2 and hydroxylamine are more effective by extracting 3 Mn/RC (Table 1). Taking into account the results obtained (see above), the authors assumed that 2 Mn cations are replaced by Fe cations.

The substitution process may proceed as follows. Bivalent iron cations are quite strong reductants in a medium with a neutral pH. The mid-point reduction potential of Fe(II)/Fe(OH)₃ is about 0 V at neutral pH (Widdel et al. 1993), which is significantly less than the redox potential of bound

Table 1 Efficiency of manganese cations extraction from the oxygen-evolving complex by various reductants at different pH values and the effect of Ca^{2+} cations on this process

Reductant	Number of Mn cations per reaction center remaining in PSII after treatment with a reducing agent		References
	pH 6.5	pH 5.7	
PSII(-Ca), untreated	4.0 ± 0.2	4.0 ± 0.2	Semin et al. (2015, 2021)
PSII(-Ca) + hydroxylamine	0.4 ± 0.1	0.4 ± 0.1	Unpublished data
PSII(-Ca) + H_2O_2	1.0 ± 0.2	2.0 ± 0.1	Semin et al. (2015)
PSII(-Ca) + H_2Q	1.0 ± 0.1	2.0 ± 0.2	Semin et al. (2015)
PSII(-Ca) + Fe(II)	2.0 ± 0.1	2.9 ± 0.2	Semin et al. (2021)
PSII (-Ca) + H_2Q + Ca^{2+}	1.8 ± 0.1	2.1 ± 0.1	Semin et al. (2015)
PSII (-Ca) + Fe(II) + Ca^{2+}	3.1 ± 0.2	3.9 ± 0.2	Semin et al. (2021)

Mn cations (close to 0.9 V (Pace et al. 2012)). Fe(II) cations reduce 1 or 2 Mn cations, which are in the trivalent state (valence of at least 2 Mn cations in S1 state according to the high-valent and low-valent schemes is ≥ 3 . (Najafpour et al. 2017)). This enables the monovalent reduction reaction of the Mn(III) cations by the Fe(II). Mn(II) cations leave the binding sites, and Fe(III) cations bind to free Mn-binding sites with high affinity and specificity (see above) (Fig. 1B, right panel). The following evidence indicate that iron cation is bound to at least one Mn-binding site. Selective extraction of Mn or Fe cations from the 2Mn/2Fe Fe heteronuclear cluster demonstrates that the high-affinity Mn-binding site is occupied by Fe cation (Semin and Seibert 2016). In this work, a citrate was used to extract Fe from the chimeric cluster, which has previously been shown to extract Fe cations but not Mn cations (Semin and Seibert 2006a). In favor of the possibility of the HAS in binding of the Fe(III) cation after substitution is the fact that a carboxyl-containing amino acid (possibly D1-Asp170) is involved in binding of the Mn cation in the native PSII membranes and the Fe(III) cation in the apo-PSII membranes (Semin and Seibert 2006a). Another evidence in favor of Fe binding to Mn-binding sites is a significant increase in resistance of Mn cations in the chimeric cluster to the action of hydroquinone (H_2Q) (Semin et al. 2018). In PSII(-Ca) membranes, H_2Q extracts 3 Mn cations from four, in PSII membranes containing 2 Mn/RC extracts 1, but does not extract any Mn cations from chimeric cluster in PSII(2Mn,2Fe) (Semin et al. 2018). The resulting chimeric cluster 2Mn2Fe is unable to synthesize molecular oxygen, but capable of oxidizing water, but at a slower rate (53%) than native preparations, and not to molecular oxygen, but to hydrogen peroxide (Semin et al. 2018).

Effect of pH on the functional activity of PSII

The rate of O₂ generation by the oxygen-evolving complex in photosynthetic organisms is significantly dependent on the pH of the environment. The dependence of the oxygen-evolving activity of PSII preparations has a bell-shaped form with a maximum in the pH region of 6.0–7.0 and falling areas with 50% inhibition in the pH region of 4.8–5.5 and 7.4–8.0 (Damoder and Dismukes 1984; Schlodder and Meyer 1987; Vass and Styring 1991; Haddy et al. 1999; Schiller and Dau 2000; Semin et al. 2004). In the acidic pH region, the inactivation of OEC is largely determined by the loss of extrinsic proteins PsbP, PsbQ, and PsbO. Extrinsic proteins PsbP, PsbQ, and PsbO have corresponding pK 5.0, 4.1, and 3.6 (Shen and Inoue 1991). An increase in the concentration of protons in the medium also affects the manganese cluster of OEC, including the S transitions of the catalytic cycle. In Bernát et al., and Suzuki et al., almost identical results were obtained in PSII preparations isolated from spinach (Bernát et al. 2002) and thermophilic cyanobacteria *Thermosynechococcus elongatus* (Suzuki et al. 2005). In the experiments performed, it was found that in the OEC of spinach S1 → S2, the transition is independent of pH in the region 4.1–8.4, while in S2 → S3, S3 → [S4] → S0, and S0 → S1 the transitions have pK of 4.0, 4.5, and 4.7, respectively (Bernát et al. 2002).

Resistance of Mn cations in the oxygen-evolving complex of PSII to the action of reductants at different pH

Investigating the pH influence on the effect of reductants on the manganese cluster found that in the pH region 5.7, there is an increase in the resistance of one of the Mn cations to its reduction by reductant. If three out of four manganese cations were extracted at pH 6.5 by H₂Q and H₂O₂, only two Mn cations were extracted at pH 5.7 (Table 1). The same is the case with Fe(II). Fe(II) cations extract two Mn cations at pH 6.5 and only one at pH 5.7 (Table 1). Thus, pH-dependent resistance to exogenous reducing agents exists only for one manganese cation that is part of the manganese OEC cluster. Since the extraction of manganese cations occurs as a result of their reduction, the results obtained indicate the important role of the ratio of redox potentials of the reducing agent and manganese cations in this process (Kuntzleman et al. 2004). In this regard, it can be assumed that an increase in resistance to the action of reducing agents of one of the manganese cations in the cluster is associated with a decrease in its redox potential with a decrease in pH.

Ca²⁺ effect on redox property of Mn cluster

The catalytic center of OEC contains Ca²⁺ cation, the function of which, despite intensive research, remains largely unclear. Recently, a new hypothesis relative to Ca²⁺ function has appeared based on model experiments. Tsui and Agapie (2013) found a linear relationship between the redox potential of the heterometallic metal-oxide cluster and the Lewis acidity of the redox inactive metal cation. The authors hypothesized that this correlation is evidence of the involvement of calcium cation in modulating the redox potential of the manganese cluster. Thus, the calcium cation in OEC can affect the redox potential of one or more manganese cations and regulate the efficiency of reducing manganese cations with exogenous redox agents. In this regard, the effect of Ca²⁺ cations on the efficiency of Mn extraction by hydroquinone and Fe(II) cations was investigated. The results are presented in Table 1 and show the effect of Ca²⁺ cation on the reduction of manganese cations in OEC.

Incubation of PSII(-Ca) membranes in the presence of Ca²⁺ with hydroquinone prevents the extraction of one manganese cation at pH 6.5 but does not affect the extraction process at pH 5.7 (Table 1). The inhibitory effect of Ca²⁺ on the extraction of manganese cations from OEC by hydroquinone and iron cations confirms the possibility of the influence of the Ca²⁺ cation on the redox potential of one or more manganese cations in OEC.

The above results suggest that at pH 5.7, either the oxygen bridge/bridges or one or more amino acid groups of the manganese cluster or its immediate environment is protonated. This process is accompanied by a decrease of the redox potential of one the manganese cations, making it inaccessible to the reducing agent. It should be noted that the pH dependence of the oxygen-evolving activity of the membrane preparations, measured in sufficient detail in 0.1 pH increments, has a small arm at pH 5.7 (Schiller and Dau 2000). A more prominent arm in pH dependence of PSII functional activity at pH 5.3 appears in D1-S169A and D2-K317A mutants (Ghosh et al. 2019).

PSII(-Ca) with 3Mn1Fe chimeric cluster without extrinsic proteins PsbP and PsbQ

Taking into account that the extraction of Mn cations from OEC by iron cations is accompanied in fact by the substitution of Mn cations by Fe cations (see above) (Fig. 1B, right panel), the process of interaction of Fe(II) cations with PSII(-Ca) membranes was investigated in more detail. It was found only 1Mn/RC extraction, that is, the formation of a chimeric cluster in OEC (Semin et al. 2018). It was also shown that the presence of Fe cations in the 3Mn1Fe heteronuclear cluster increases the resistance of the remaining Mn cations to H₂Q action, since this reducing agent is

able to extract Mn cations from homonuclear Mn clusters of PSII(-Ca,4Mn) and PSII(-Ca,2Mn) membranes but not from the heteronuclear cluster in PSII(-Ca,3Mn,1Fe) membranes. This fact indicates that the cluster contains an iron cation stabilizing the Mn cations, as in the case of the chimeric cluster 2Mn2Fe. Interestingly, extraction of the Fe cation from the 3Mn1Fe cluster is accompanied by a loss of 1 Mn cation, which confirms the stabilizing role of Fe in the chimeric cluster. Functional activity of PSII with a 3Mn1Fe cluster was investigated. PSII preparations with a 3Mn1Fe cluster in the OEC are able to photoreduce the exogenous electron acceptor 2,6-dichlorophenolindophenol, possibly due to incomplete oxidation of water molecules as is the case with PSII(-Ca,2Mn,2Fe) samples. However, in the contrast to PSII(-Ca,2Mn,2Fe) samples, PSII(-Ca,3Mn,1Fe) membranes can evolve O₂ at a low rate in the presence of exogenous Ca²⁺ (at about 27% of the rate of O₂ evolution in native PSII membranes). The possibility of water oxidation by a chimeric cluster with oxygen release is of interest for studying the mechanism of photosynthetic oxidation of water (identification of manganese cations that play a general role in water photolysis, etc.), as well as for the development of artificial water photolysis systems as generators of molecular oxygen and hydrogen.

PSII(-Ca) with 3Mn1Fe chimeric cluster with extrinsic proteins

To replace Mn cations in OEC, it is necessary to ensure their availability for Fe cations. To this end, in previous studies (Semin and Seibert 2016; Semin et al. 2018), PSII membranes were treated with a high-molar solution of NaCl, which provided extraction of extrinsic proteins PsbP and PsbQ and Ca²⁺ cation from membranes. To preserve all extrinsic proteins in the PSII preparation, another method of Ca²⁺ extracting from OEC was used—the method developed by Ono and Inoue (1988). This method (treatment of the PSII membranes with citrate buffer with acidic pH) allows to obtain the PSII membranes without Ca²⁺ cation in OEC but with EP. But, crucially, unlike the native PSII membranes in citrate-treated PSII (-Ca) membranes, the Mn cluster is available for small-sized reducing agents (Vander Meulen et al. 2002, 2004). Indeed, one Mn cation in OEC found to be replaced by an Fe cation after incubation of Ca-depleted PSII membranes with Fe(II) cations after removal of citrate; i.e., PSII membranes with a chimeric cluster 3Mn1Fe in OEC were obtained in the presence of all extrinsic proteins. It is important to note that there are 2 significant differences between the characteristics of PSII preparations with a chimeric cluster 3Mn1Fe with (Semin and Davletshina 2023) and without EP (Semin et al. 2018). In preparations prepared by replacing Mn cation in the presence of EP (Semin and Davletshina 2023), only 1 Mn cation

is substituted at pH 6.5, while without EP at the same pH 2 Mn cations are replaced (substitution of one cation occurs only at pH 5.7) (Semin et al. 2018). But the most significant difference in preparations is their evolving activity in the presence of exogenous Ca²⁺. PSII preparations with a chimeric cluster without EP have an activity of about 25% (Semin et al. 2018), while in the presence of EP their activity increases to 80% (Semin and Davletshina 2023). What could be the reason for such differences? Taking into account the fact that EP increases the redox potential of one of OEC Mn cations (Fe(II) extracts 2Mn at pH 6.5 in the absence of EP, but 1Mn in the presence of EP), we can assume that the influence of EP is carried out in the step before the substitution of Mn cation with Fe(II) cation, and not after.

Conclusion

This review summarized the recent progress and understanding associated with the replacement of manganese cations in OEC with Fe cations. Semin with colleagues discovered highly efficient and highly specific binding of iron cations to Mn-binding sites in Mn-depleted PSII (Semin et al. 1995; Semin et al. 2002, 2005; Semin and Seibert 2004, 2006a,b). Using this feature, Semin and co-authors developed a method for producing chimeric clusters in OEC consisting of iron and manganese cations in different ratios (Semin and Seibert 2016; Semin et al. 2018). It should be noted that recently there has been increasing interest in the possible use of metal cations located in the row with Mn, either in the replacement of Mn cations in the OEC, or in the creation of artificial water oxidation catalysts (Saito et al. 2023; Gates et al. 2022; Kondo et al. 2021). Saito et al. (2023) investigated the Fe₄CaO₅ cluster in the PSII protein environment using a quantum mechanical/molecular mechanical approach as the first step in resolving the issue why Mn and not Fe is employed by nature in the water oxidation catalyst. The results obtained showed significant differences between the protonation states and oxidation states of the Fe₄CaO₅ cluster and Mn₄CaO₅ cluster. Gates with coworkers obtained photoactivation of OEC using Co²⁺ ions instead of Mn²⁺ in combination with Ca²⁺ and water (Gates et al. 2022), although the activity of the reconstituted preparations was significantly less (25%) than in the case of Mn(II). Mn, Fe, Co, Ni, and Cu cations are being intensively investigated in the works on the creation of artificial water-splitting catalysts (see review Kondo et al. 2021).

Studies of the possibility of replacing Mn cations in OEC with cations of other metals may be of interest to clarify the mechanism of water oxidation and molecular oxygen synthesis. In this regard, we want to draw attention to the following interesting fact obtained during the study of the chimeric cluster 3Mn1Fe (Semin and Davletshina 2023).

Currently, there are a number of hypotheses for the mechanism of water oxidation (Shen 2015; Lubitz et al. 2019; Oliver et al. 2022; Shevela et al. 2023). All of them assume participation in the oxidation of water and the synthesis of O₂ of all 4 Mn cations, including the cation (Mn⁴) associated with HAS, which implies its oxidation during the Kok cycle. In this regard, it should be noted that according to Semin and Davletshina (2023), the chimeric cluster containing not 4, but 3 Mn has a very high oxygen-evolving activity (about 80%) in the presence of exogenous Ca²⁺ and EP. This fact suggests that the oxidation of the H₂O occurs with the participation of three Mn cations, since the Fe cation in the cluster is in the 3-valence state, and further oxidation of it requires very strong oxidizing agents. This data suggests that the Mn cation bound to HAS and HAS itself are not the main participants of O₂ synthesis. More likely, HAS is mostly involved in resynthesis of Mn cluster, destroyed by photoinhibition process. In fact, this scheme is discussed now in literature (see review Oliver et al. 2022).

It should be noted that iron cations embedded in the chimeric cluster increase the resistance of Mn cations to the action of exogenic reductants (Semin et al. 2018). Combined with the high catalytic activity of the chimeric cluster 3Mn1Fe in the presence of extrinsic proteins (Semin and Davletshina 2023), this object is of interest for more detailed studies in order to create artificial energy systems for water decomposition. In this regard, studies aimed at increasing the oxygen-evolving activity of PSII preparations with a chimeric cluster are of interest, namely the development of new methods for the reconstruction of extrinsic proteins and the preparation of chimeric clusters in the presence of Ca²⁺, which, as it was found not so long ago, is very important for synthesis of Mn cluster during the photoactivation process (Avramov et al. 2020). In addition, a study of the possibility of synthesis on the donor side of PSII(-Mn) an iron-sulfur cluster is of interest, taking into account the high affinity of Fe cations binding to Mn-binding sites. Such preparations would be interesting in terms of the possibility of catalytic activity of such clusters and evolutionary origin PSII. Computational approaches can be used in parallel with experimental studies. They are able to provide a number of interesting conclusions regarding the structure and functioning of OEC with chimeric clusters.

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Declarations

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