Available online at www.sciencedirect.com
ScienceDirect
Coordination Chemistry Reviews 252 (2008) 296–305
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

The calcium and chloride requirements of the O_2 evolving complex

Charles F. Yocum [a,b,*]

[a] *Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA*
[b] *Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1048, USA*

Received 12 June 2007; accepted 10 August 2007
Available online 22 August 2007

Contents

Abstract

Manganese oxidation, catalyzed by chlorophyll photochemistry in photosystem II, is the key step in the pathway of redox reactions leading formation of O_2 from H_2O. A unique feature of Mn redox chemistry is its dependence on the presence of both Ca^{2+} and Cl^-; the active site of H_2O oxidation consists of four Mn atoms, and one atom each of Ca^{2+} and Cl^-. The best current structural model of the inorganic ion cluster, based on polarized EXAFS experiments on crystals of the photosystem, shows Ca^{2+} ligated by carboxyl groups from amino acid residues (alanine, glutamate) that bridge to Mn atoms. Chloride is not resolved in the current structures. Calcium is an essential structural element of the cluster during its assembly, and is required for efficient Mn oxidation. There is also compelling evidence that Ca^{2+} is involved in catalysis of H_2O oxidation.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EPR, electron paramagnetic resonance; ESEEM, electron spin echo envelope modulation; EXAFS, extended X-ray absorption fine structure; FTIR, Fourier transform infrared; H_2Q, hydroquinone; Ln^{3+}, lanthanide; OEC, O_2 evolving complex; PS, photosystem; PsbO, the 33 kDa extrinsic protein of photosystem II; PsbP, the 23 kDa extrinsic polypeptide of spinach photosystem II; PsbQ, the 17 kDa extrinsic polypeptide of spinach photosystem II; PsbU, the 12 kDa polypeptide of cyanobacterial photosystem II; PsbV, cytochrome C550, an extrinsic subunit of cyanobacterial photosystem II; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine; XANES, X-ray absorption near edge structure; XAS, X-ray absorption spectroscopy; Y_Z, $Y_Z^\bullet$the reduced and oxidized forms of the tyrosine residue that catalyzes electron transfer between the Mn cluster in photosystem II and the redox active chlorophyll a molecule(s) that transfer electrons to the reducing side of the photosystem

* Correspondence address: Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA.
Tel.: +1 734 647 0897; fax: +1 734 647 0884.
E-mail address: cyocum@umich.edu.

doi:10.1016/j.ccr.2007.08.010

Chloride is also required for Mn oxidation by photosystem II, and it has been demonstrated that the anion is required for the Mn redox reactions immediately preceding oxidation of H_2O to O_2. Although there are no structural data on the site of Cl^- binding in photosystem II, spectroscopic probing and ligand competition experiments position it in the vicinity of the Mn cluster.

Keywords: Calcium; Chloride; Manganese; Oxygen evolution; S-states; Photosystem II

1. Introduction

Other articles in this volume describe the components as well as the many interesting properties of electron transfer in the O_2 evolving complex (OEC) of photosystem II (PSII). Here, an unusual property of the H_2O oxidizing reaction itself is discussed, namely the requirement for both Ca^{2+} and Cl^-. These inorganic ions act as essential activators of the Mn redox chemistry that culminates in the release of O_2 from PSII. A survey of the various redox reactions catalyzed by transition metals fails to turn up other cases where oxidation reduction reactions require these cofactors, but this is perhaps not unexpected given the fact that Mn-catalyzed H_2O oxidation is unique; no comparable analogs exist among other biological redox reactions. In addition to this paper, readers are referred to other reviews [1–7]. It is useful to begin with a brief survey of the other functions of Ca^{2+} and Cl^- in biological systems before addressing their roles in the OEC.

1.1. Ca^{2+} in biological systems

Of all of the metals, Ca^{2+} may exert the widest range of effects in biological systems of all of the metals [8]. A cursory overview would include functions related to structure, intercellular and intracellular signaling, regulation of enzyme activity, muscle contraction and conduction of nerve impulses. The common coordination numbers for Ca^{2+} are 6 or 7, and the solubility of the metal is turned to good advantage in its many roles in biology. For example, the insoluble phosphates of Ca^{2+} (hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and octacalcium phosphate ($Ca_8H_2(PO_4)_6$) constitute the central building blocks for bones and teeth. The carbonates are prominent in exoskeletons and are stored in plants [9]. In its soluble form, Ca^{2+} plays a wide range of roles in biology that depend on its interaction with a number of proteins. The most thoroughly characterized of these is probably the Ca^{2+}-calmodulin interaction [10–12]. Calmodulins possess four Ca^{2+} binding sites of very high affinity ($K_d = \sim 1\ \mu M$). Upon binding of Ca^{2+}, the metallated calmodulin binds specifically to a target enzyme that is activated by the protein–protein interaction. The calmodulin Ca^{2+} binding site, called an EF-hand motif on account of its tertiary structure, is comprised of a loop of 12 amino acids. The most highly conserved amino acids in this loop are an aspartate residue at position 1, a glycine at position 6 and a glutamate at position 12, while aspartate or asparagine residues are found at positions 3 and 5. Ligation of Ca^{2+} involves the side chains of amino acid residues 1, 3, 5 and 12 and the peptide backbone carbonyl at residue 7. The oxygens on Glutamate 12 in this arrangement form a bidentate ligand, and a water molecule completes the shell of ligands. The other amino acids in this sequence (numbers 2, 4, 8–11) are "spacers" in the Ca^{2+} binding sequence. Proteins bearing the EF hand motif can function either as regulators of enzyme activity (calmodulins, troponin C) or as buffers of intracellular Ca^{2+} concentrations (calbindin D_{9K}, parvalbumin).

Other Ca^{2+} binding proteins are more flexible in the amino acid sequences of their sites [2]. These proteins, known as Ca^{2+} cofactor proteins, include proteases (trypsin and thermolysin, for example) as well as enzymes that degrade nucleic acids (nucleases) and lipids (lipases). As a general rule, these proteins are stabilized by Ca^{2+} binding to sites that include oxoanions associated with glutamate and aspartate. The carbohydrate binding protein concanavalin A is of interest in that it contains a binuclear site comprised on an atom of Mn^{2+} and an atom of Ca^{2+} [8]. Binding of either metal is contingent of the presence of the other.

The metal ion specificity of Ca^{2+} binding proteins has been the subject of a number of investigations. One approach has been to use Ln^{3+} ions as probes of Ca^{2+} binding sites [13,14]; the natively bound Ca^{2+} atom can be displaced by a Ln^{3+} such as Tb^{3+}. Irradiation of the modified protein with UV light excites aromatic amino acid residues (phenylalanine, tyrosine) in the vicinity of the metal, and energy transfer to the Tb^{3+} ion produces light emission from ff orbital transitions [14]. The technique has found broad applications in the characterization of Ca^{2+} binding sites (binding constants, hydration number, and total number of coordinating atoms in a site, for example).

The effects of Ca^{2+} displacement by Ln^{3+} can have differing consequences depending on the role of the native metal in the protein. A large number of proteins retain their function upon Ca^{2+} (or Mg^{2+}) displacement by a Ln^{3+}. A brief list would include hydrolytic enzymes (thermolysin, subtilisin, elastase, chymotrypsin, trypsin, thermolysin, phospholipase A_2 and α-amylase) along with other proteins such as concanavalin A and galactosyl transferase. In each of these cases, binding of Ca^{2+} confers structural stability on a particular protein. A second, smaller category of enzymes, including staphylococcal nuclease and Ca^{2+} ATPase, along with some Mg^{2+}-dependent enzymes such as phosphoglycerate and pyruvate kinases, are inhibited by Ln^{3+} substitution [13]. The difference in the sensitivity of the activity of these proteins resides in the fact that in the former examples, Ca^{2+} is a structural component, whereas in the latter case, the displaced metal is a functional component of the active site of the enzyme. This observation will be relevant to the discussion of the role of Ca^{2+} in PSII activity.

In addition to Ln^{3+}, other metals will also occupy protein-associated Ca^{2+} binding sites. Replacement of Ca^{2+} by Cd^{2+}

has enabled the use of ^{113}Cd-NMR as a probe of Ca^{2+} sites in the EF-hand proteins [15,16]. It is less widely appreciated that Na^+ and K^+ will also bind to sites in parvalbumin [17] and the Ca^{2+} ATPase [18].

1.2. Cl^- in biology

There is much less to be said about the function of this anion in biological systems. Some living systems can accumulate more Cl^- than any other inorganic ion. For example, human plasma contains 100 mM Cl^-, but concentrations as high as 0.5 M are found in some marine algae. This anion contributes negative charge in the formation of membrane potentials, for example, and is responsible for the regulation of osmotic pressures in cells. A number of proteins function as Cl^- channels in biological membranes [19]. A few enzymes (for example, α amylase [20]) require Cl^- for activity, and the haloperoxidases use Cl^-, Br^- and I^- as substrates along with H_2O_2 to halogenate aromatic amino acids or to produce reactive species that are bactericidal [21]. Chloride has also been found to associate as a bridging ligand between heme a and Cu_B in the oxidized form of cytochrome oxidase [22]. In this case, the binding kinetics of the anion are too slow to play a role in the catalytic activity of the enzyme.

2. The roles of Ca^{2+} and Cl^- in PSII-catalyzed H_2O oxidation

2.1. Photosystem II structure: proteins

Most of the biochemical and spectroscopic (EPR, ESEEM, XAS, FTIR and time-resolved UV–vis absorption measurements) characterizations of PSII have employed the enzyme isolated from spinach [23]. This includes investigations of the structure of the enzyme using electron microscopy [24,25]. Crystals of the enzyme [26–29] that diffract to 3.0 Å [30] have been obtained using PSII isolated from thermophilic cyanobacteria. The properties of the enzyme from both sources include the presence of a number of amphipathic proteins that reside in a lipid bilayer and ligate the inorganic and organic cofactors of the enzyme (see the articles by Barber and Debus in this issue). Also present in the isolated enzyme are a group of water soluble proteins that bind to the membrane proteins with high affinity (K_d = 2–10 nM). In plant and algal preparations, these proteins are known as PsbO (also called the 33 kDa manganese stabilizing protein), PsbP and PsbQ [31–33]. The cyanobacterial enzyme also contains PsbO, but PsbP and PsbQ are replaced by PsbU and PsbV (a cytochrome, c550) [33]. Extraction of PsbP and PsbQ from spinach PSII exposes the Mn cluster in the OEC to rapid reduction by exogenously added reagents (hydroquinone, hydroxylamines, *p*-phenylenediamines) [34,35]. The Mn^{2+} produced by this reaction is released from the enzyme, with a corresponding loss of O_2 evolution activity. As will be seen, dissociation of the extrinsic proteins from PSII also has profound consequences for its activity and its ability to retain the Ca^{2+} and Cl^- cofactors needed for activity.

2.2. Photosystem II activity, Mn redox states and the S-state cycle

Isolated PSII is capable of high rates of water oxidation in the presence of a synthetic electron acceptor such as 2,6-dichloro-*p*-benzoquinone or *p*-phenyl-*p*-benzoquinone. This activity requires four Mn atoms per PSII [36]. In dark adapted PSII, the oxidation states of these Mn atoms have been established to be 2 Mn^{4+}/2 Mn^{3+} [37–39]. Light-catalyzed Mn oxidation initiates a cycling of PSII between the dark adapted, resting state and its higher oxidation states, which reveals a pattern of O_2 release that peaks on the 3rd, 7th, and 11th exposures to a train of very short (<10 μs) flashes of light. This response was modeled by Kok et al. [40] as a linear sequence of one-electron oxidation reactions, where each oxidation state of the enzyme was called an "S" state:

$$S_1 \xrightarrow{h\nu} S_2 \xrightarrow{h\nu} S_3 + 2H_2O \xrightarrow{h\nu} S_4 \rightarrow O_2 + 4H^+ + S_0 \xrightarrow{h\nu} S_1 \xrightarrow{h\nu} S_2 \ldots \quad (1)$$

The advancement in the S-states is accompanied by Mn oxidation (see Dau and Britt, this issue); other contributors to this volume review the current status of models for the $S_3 \rightarrow S_0$ transition, and Hillier and Wydrzynski review the evidence for H_2O exchange with, and binding to, the various S-states. As will be seen, S-state cycling is profoundly affected by the Ca^{2+} and Cl^- status of the OEC.

2.3. The role of Ca^{2+} in the OEC

Extraction of the PsbP and PsbQ subunits from PSII using high ionic strength (≥1 M NaCl) produces an inhibition of steady state O_2 evolution activity. This defect is repaired by addition of Ca^{2+} and Cl^- to the assay reaction mixture [41,42]; addition of Sr^{2+} is also capable of restoring activity, but at a level less than about 50% that of the rate obtained with added Ca^{2+} [41,43]. Subsequent investigations [41] correlated Ca^{2+} retention by the OEC with the presence of PsbP and PsbQ, and it was also shown that exposure of PSII preparations to acidic conditions (pH 3) released Ca^{2+} without perturbing binding of the polypeptides [44]. In this PSII preparation, restoration of activity required incubation of the enzyme with Ca^{2+} for up to 50 min prior to assay in order to allow the metal to permeate the barrier imposed by the extrinsic proteins. The two preparations (minus or plus the PsbP and PsbQ subunits) present contrasting behaviors with respect to the sensitivity of activity to the divalent metal chelators EDTA or EGTA. In its native state, OEC activity is unaffected by addition of a chelator. In the case of the polypeptide-depleted enzyme, the activity restored by addition of Ca^{2+} is sensitive to the chelator, which indicates that rebinding of the metal must occur with low affinity, or that under steady state illumination one or more S-states bind it with lowered affinity. For polypeptide reconstituted samples equilibrated with Ca^{2+}, activity is chelator insensitive [44]. This behavior is consistent with current structural models of PSII that place the Ca^{2+} binding site in a position that is shielded from the external medium by the extrinsic polypeptides of the enzyme [28–30].

2.3.1. Ca^{2+} stoichiometry and binding affinity

Several groups have reported widely varying Ca^{2+} contents of PSII preparations before and after extraction treatments to release the metal. The first experiments detected large amounts of Ca^{2+} in intact PSII preparations. Only minimal depletion of the metal was observed after extraction of the extrinsic polypeptides [41]. Other experiments indicated that as many as three Ca^{2+} atoms were bound to the enzyme, two of which could be removed by extraction of the PsbP and PsbQ subunits, which resulted in the loss of O_2 evolution activity as well [45]. Other investigations reached a similar conclusion, namely that PSII contained two populations of Ca^{2+}, one of which was extractable and one that was more tightly bound [46,47]. Some of these results were also interpreted to indicate that there was only a limited correlation between loss of the metal and inhibition of activity. Other experiments monitoring Ca^{2+} binding to PSII with a sensitive ion selective electrode detected up to four separate binding sites associated with the enzyme ([48], see below). The issue of the stoichiometry of Ca^{2+} in the active site of the OEC was settled by experiments using $^{45}Ca^{2+}$ [49]. Results of these studies showed that about one extractable Ca^{2+} atom is associated with the OEC; other high and low affinity binding sites for additional Ca^{2+} atoms reside outside the OEC. The 4 Mn:1 Ca^{2+} stoichiometry is consistent with models for the Mn_4Ca cluster derived from crystallographic studies of the enzyme [28–30]; see Barber, this issue.

Initiatives to determine the binding affinity of the OEC for Ca^{2+} have produced a substantial literature that will be reviewed very briefly here. Two methods have been employed. In the first, the dissociation constant of the metal has been estimated from plots of steady state activity versus Ca^{2+} concentration, which are then transformed to double reciprocal Lineweaver–Burk plots to obtain the K_M or K_d value for the binding site. Values obtained by this method range from about 50 μM to >1 mM [5,7]. The disparity has been attributed to the existence of heterogeneity in the binding site, induced by the methods used to extract Ca^{2+} from the OEC [50]. The alternate methods determined the K_d value from assays of rebinding of $^{45}Ca^{2+}$ to the OEC [49], or by an analysis of Ca^{2+} binding using an ion-selective electrode [48]. Some heterogeneity in binding was observed in the $^{45}Ca^{2+}$ experiments; K_d's of 26 μM and 0.5 mM were determined for preparations depleted of Ca^{2+} by extraction of the extrinsic polypeptides. For samples that had been Ca^{2+} depleted by low pH treatment, K_d's of 60 μM and 1.7 mM were reported. In the experiments employing a Ca^{2+} selective electrode, four sites external to the OEC with K_d values of 1.8 μM were detected [48]. Exposure of the OEC by extraction of PsbP and Q revealed >2 binding sites with K_d values of 0.15 μM, and other sites of high affinity binding were uncovered upon removal of PsbO and the Mn cluster. The origins of the very substantial heterogeneity in Ca^{2+} binding evident in the literature will be difficult to resolve, given the almost universal occurrence of the metal as a contaminant in H_2O and biochemical reagents, the presence of several nonspecific binding sites for the metal in PSII, and the S-state dependence of Ca^{2+} binding, which is discussed in Section 2.3.4 below. However, one useful observation emerges from the data that are currently available. The lower limit of Ca^{2+} affinities measured in those instances where activity was also monitored is higher that one would expect for the EF-hand type sites found in regulatory proteins, but the values are well within the range expected for protein ligation by oxoanion and carbonyl groups.

2.3.2. Competition by alternate metals for the OEC Ca^{2+} site

A number of metals have been tested for their ability to displace Ca^{2+} from PSII, or to interfere with its rebinding to the OEC of Ca^{2+}-depleted PSII preparations. The results are summarized in Table 1, which is adapted from [5]. While there is general agreement that ions such as Ln^{3+} and Cd^{2+} are capable of competing for the Ca^{2+} ligand environment, the situation with respect to monovalent metals is less clear. Assays of steady state activity in PSII preparations depleted of Ca^{2+} show that K^+, Cs^+ and Rb^+ are able to block Ca^{2+} activation of the OEC [51,52] as long as the inhibitory metal is added to the reaction mixture along with Ca^{2+}. In addition, it has been shown that K^+ will extract the extrinsic polypeptides and displace Ca^{2+} from the OEC [53]. An alternate method of examining PSII-Ca^{2+} interactions employs preincubation of the Ca^{2+} depleted sample with an alternate metal, and under these conditions, the subsequent assay of activity fails to detect any interference with Ca^{2+} activation of the OEC by either Na^+ or K^+ [54]. The overall picture that emerges from metal substitution studies is that the OEC Ca^{2+} site exhibits a preference for di- and trivalent metals whose ionic radii are close to that of Ca^{2+} (∼1 Å).

Table 1
Metal ion activators and inhibitors of the OEC

Metal	Ionic radius (Å)	Coordination number	Effect on O_2 evolution activity	K_d (estimated) (mM)
Ca^{2+}	0.99	6–8	Activator	0.01–1.0
Sr^{2+}	1.13	6–8	Activator	–
Ba^{2+}	1.35	6–8	Weak inhibitor	–
Mg^{2+}	0.65	6	No effect	2.84
Mn^{2+}	0.80	6	Weak inhibitor	–
Cd^{2+}	1.03	6–8	Competitive inhibitor	0.14–0.30
La^{3+}	1.04	7–9	Competitive inhibitor	0.05
K^+	1.33	6–8	Competitive inhibitor	3–8
Cs^+	1.65	6–8	Competitive inhibitor	8–10

2.3.3. The Ca^{2+}–Mn interaction in the OEC

Prior to the appearance of crystal structures of the OEC, spectroscopic and biochemical probes established a close interaction between Ca^{2+} and the Mn cluster. The first indication of a Ca^{2+}–Mn interaction came from experiments to define the conditions for assembly of the OEC metal cluster [55,56]. In these experiments, Mn-depleted PSII is incubated with Mn^{2+} under weak illumination; only if Ca^{2+} is also included in the reaction mixture is a functional OEC reconstituted. It has also been shown that the OEC metal cluster will assemble in vivo in a cyanobacterium if Sr^{2+} replaces Ca^{2+} in the growth medium [57]. An exploration of the Ca^{2+} binding capacity of Mn-depleted PSII found a corresponding result, namely that in the absence of a functional Mn cluster, Ca^{2+} binding could not be observed (see [49] and the paper of Dismukes and Dasgupta in this issue). The mutual dependency of Ca^{2+} and Mn in assembly of the OEC is reminiscent of the Ca^{2+}–Mn^{2+} interaction in the lectin concanavalin A (Section 1.1), where the two metals share bidentate bridging carboxyl ligands from aspartyl and glutamyl residues [8].

Results of Mn EXAFS experiments on Ca^{2+} depleted/Sr^{2+} reconstituted PSII samples, and of Sr^{2+} and Ca^{2+} EXAFS experiments as well, were interpreted to indicate that Ca^{2+} could be a scatterer at a distance of ca. 3.4 Å from the Mn cluster [58,59]. Solid state magic angle spinning NMR characterization of PSII samples containing ^{113}Cd concluded that Ca^{2+} binding occurred in a site that was probably octahedral; the behavior of the ^{113}Cd signal with respect to temperature was rationalized in terms of proximity of the metal to a nearby paramagnet, presumably a Mn atom [60]. The advent of crystals of cyanobacterial PSII has provided the potential for an improved view of the Mn–Ca^{2+} arrangement in the OEC. The first models for the cluster [26–28] were compromised by damage to the metal cluster caused by exposure to the high X-ray fluxes used to obtain diffraction data. On the basis of XANES and EXAFS data from these crystals [61], it can be concluded that little, if any, of the native structure is retained in after exposure to X-rays at the intensities needed for structure determination. For example, the Mn XANES edges are shifted to lower energies, consistent with the presence of large amounts of Mn^{2+}, and the EXAFS spectra show that the Mn cluster is completely disrupted, as evidenced by the presence of a lone first shell scatterer at ∼1.9 Å [61]. Polarized EXAFS using undamaged PSII crystals has provided structural models of the metal cluster that represent the most reliable picture of this site that is currently available [62]. A representative structure is shown in Fig. 1. A notable feature of this model is the presence of amino acid side chain ligands to the Ca^{2+} atom, which were not included in the first detailed structural model of the cluster [28].

Structural models of the OEC derived from X-ray data may eventually provide useful insights into the active site of water oxidation, if radiation damage can be contained. In the meantime, another approach to obtaining information on the OEC has been to employ FTIR to probe the identity of amino acids that might function as ligands to the redox active subset of Mn atoms that bind substrate and oxidize to O_2. This method exploits the ability to use cyanobacteria to label amino acid residues with

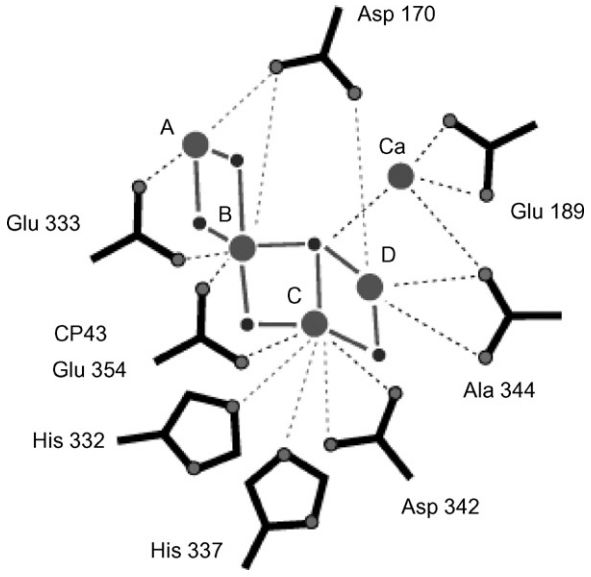

Fig. 1. A model of the OEC Mn_4Ca cluster derived from polarized EXAFS measurements on PSII crystals from *Thermosynechococcus elongatus* [63]. The experimental conditions used to obtain the data minimize the radiation damage to the Mn cluster that occurs in crystallographic studies [62]. The four Mn atoms are indicated by A–D, with μ-oxo bridges between the metals. The predicted ligating amino acids are alanyl (Ala), aspartyl (Asp) and glutamyl (Glu) residues (oxoanion ligand donors) along with N ligands contributed by the imidazole side chains of histidine (His).

^{13}C to provide a marker for the FTIR experiments. One such investigation [63] concluded that the prediction that Ala344 at the C-terminus of the PSII D1 polypeptide was a Ca^{2+} ligand was incorrect; effects of Sr^{2+} substitution and light induced changes in carboxylate stretching frequencies support this residue's function as a Mn ligand instead. Other results (see R.J. Debus in this issue) have made use of the ability to selectively alter amino acid residues that are putative Mn or Ca^{2+} ligands, using the technique of site-directed mutagenesis. Results obtained from PSII preparations carrying such mutations have been interpreted to suggest that Asp 170 and Glu 189 function as Mn ligands in the OEC, but the results of FTIR experiments comparing the differences between native and mutated preparations in the various S-states are interpreted to suggest that the side chains of these amino acids do not ligate Mn atoms that undergo oxidation on the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3$ transitions [64,65]. Another FTIR study using Ca^{2+}/Sr^{2+} exchanged samples has identified carboxylate stretching modes that are assigned to ligation of Mn atoms that are strongly coupled to the Ca^{2+} atom [66], and yet other FTIR characterizations of Sr^{2+}-exchanged PSII samples are interpreted to indicate that this metal in the Ca^{2+} site produces structural changes and perturbations to a H-bonding network containing H_2O and peptide carbonyl groups [67]. These data and those from future characterizations of undamaged crystals will no doubt provide a clearer picture of the nature of Ca^{2+} ligation by PSII.

An alternate method for probing the Mn–Ca^{2+} interaction has employed Mn reductants (hydroxylamines, H_2Q, TMPD). Addition of NH_2OH to untreated or polypeptide depleted PSII causes a loss of enzymatic activity coincident with the appearance of

$Mn^{2+}(H_2O)_6$ that is detectable by EPR at room temperature [36], or by NMR (measurements of H_2O proton relaxation rate enhancements [68]). In the presence of Ca^{2+}, release of Mn^{2+} is delayed [69]. Supplementation of PSII samples with Ca^{2+} prior to reductant addition produces one of two effects: stabilization of the reduced Mn cluster (H_2Q), or a blockage of Mn reduction (CH_3NHOH; $(CH_3)_2NOH$; TMPD) [70,35]. Characterization of these samples by XAS and EPR showed that H_2Q reduced, Ca^{2+}-supplemented samples, which possessed high rates of O_2 evolution activity, contained a reduced Mn cluster (2 Mn^{4+}/2 Mn^{2+}) containing $Mn^{2+}(H_2O)_6$ that was EPR detectable at room temperature [70]. In these samples, the scatterer at 3.3 Å was lost, as was one of the 2.7 Å scatterers assigned to a Mn–Mn interaction. These features were restored upon illumination of the reduced sample [70,71]. In the case of methylated reductants, steric factors appear to impede access to the Mn cluster in the presence of Ca^{2+}, and the reductant with the highest redox potential ($(CH_3)_2NOH$; $E_{m,7} = +0.550$ V) is unable to attack the Mn cluster when Ca^{2+} is present [35]. A couple of tentative conclusions from these results are that (1) Ca^{2+} is situated in an access site (or channel) such that it can block access of bulky methylated reductants to the Mn cluster and (2) the ability of Ca^{2+} to interfere with the reaction between $(CH_3)_2NOH$ and the Mn cluster could indicate that the target of this reductant is high potential Mn atoms that might be sites of H_2O oxidation. Taken together, these results would be consistent with a model of the active site of H_2O oxidation in S_1 composed of a $2Mn^{3+}$–Ca^{2+} cluster. One interpretation of these observations, in terms of current structural models, would be that the "C" and "D" atoms in the model shown in Fig. 1 are the sites of H_2O oxidation by PSII.

2.3.4. The role of Ca^{2+} in S-state advancement and H_2O oxidation

Calcium release from the OEC is accelerated by illumination. Photosystem II samples exposed to high ionic strength to release the polypeptides retain the ability to cycle through the S-states, and this is lost by exposure of the samples to an extended (>100) series of short single turnover flashes [72]. Inactivation of steady state activity is also accelerated by illuminating PSII samples during the polypeptide extraction reaction at high ionic strength [73]. Experiments to further characterize the relationship between illumination and Ca^{2+} release from the OEC showed that the S_3 state, and to a lesser extent S_2, bind the metal with a much lower affinity than either S_1 or S_0 [74]. Taken together, these results indicate that polypeptide depleted PSII samples that are inactive under steady state assay conditions probably lose functional Ca^{2+} from OEC sites during the initial period of illumination. This process is reversed if adequate Ca^{2+} is added to the assay buffer system. The same results also suggest that for single turnover experiments, Ca^{2+} is probably retained in the OEC even after treatments that release the PsbP and PsbQ extrinsic subunits.

Substantial attention has focused on the question of whether Ca^{2+} is necessary for the S_1–S_2 transition. Long term illumination of samples in the presence of high ionic strength and EGTA are conditions which should release the PsbP and PsbQ subunits along with Ca^{2+}. Subsequent dialysis to lower the ionic strength and rebind the polypeptides generates samples containing an abnormally stable S_2 state [75]; the modified form of the $g = 2$, $S = 1/2$ multiline EPR signal in these samples has a lifetime of about 40 min. after addition of Ca^{2+} or Sr^{2+}. Illumination of the stable S_2 state created by Ca^{2+} depletion in the light results in photoaccumulation of a tyrosine radical called $Y_Z^\bullet$ [76], an electron carrier between the Mn cluster and the PSII reaction center chlorophyll(s) termed P680. From this result, it is apparent that S-state advancement beyond S_2 is blocked when Ca^{2+} is removed. The same is probably true for the $S_1 \rightarrow S_2$ transition after extraction of Ca^{2+}; the diminished ability to form the S_2 multiline signal under continuous illumination at 200 K correlates with the increase in the detection of a slow-decaying form of $Y_Z^\bullet$ [77], and the OEC depleted of Ca^{2+} by pH 3 treatment also exhibits a slow decaying $Y_Z^\bullet$ signal, and no evidence of S_2 formation [78].

A pair of models for the behavior of the Ca^{2+} binding site in the presence of a Ca^{2+} chelator (EGTA) to facilitate removal of the metal would be as follows:

$$Y_ZS_1(Ca^{2+}) + EGTA \rightarrow Y_ZS_1(\,)EGTA(Ca^{2+}) \xrightarrow{h\nu} Y_Z^\bullet S_1(\,) + EGTA(Ca^{2+}) \rightarrow Y_ZS_2(\,) + EGTA(Ca^{2+}) \quad (1)$$

$$Y_ZS_1(Ca^{2+}) + EGTA \xrightarrow{h\nu} Y_Z^\bullet S_1(Ca^{2+})EGTA \rightarrow Y_ZS_2(Ca^{2+}) + EGTA \rightarrow S_2(\,) + EGTA(Ca^{2+}) \quad (2)$$

The data currently available in the literature are consistent with the reaction sequence given in (2) above, where Ca^{2+} is required for advancement from S_1 to S_2, although some recent data support the model in Eq. (1). Samples in which Ca^{2+} has been exchanged for either Cd^{2+} or Dy^{3+} in the dark, in the absence of the PsbP and PsbQ subunits, will produce a multiline signal of lower amplitude than that detected in a native sample [79]. The same substitution protocols applied to samples incubated with Cu^{2+} inhibit steady state activity, but these samples fail to form S_2. This is a somewhat unusual result in that it has previously been shown that Cu^{2+} does not compete effectively with Ca^{2+} for the OEC binding site [54], and its ionic radius (0.72 Å) should exclude it from ligation in the Ca^{2+} site. It is possible that Cd^{2+} and Cu^{2+} are capable of inhibiting PSII electron transfer by binding to sites outside the OEC; this is the conclusion already reached for Cd^{2+}, where inhibition of PSII electron transfer at several sites has been detected [80]. It is also possible that $S_1 \rightarrow S_2$ transitions can occur in Dy^{3+} or Cd^{2+} substituted samples, but with a low quantum yield that necessitates long term illumination protocols, such as those used to advance EPR samples from S_1 to the S_2 state.

The data that currently available support two proposals for the function of Ca^{2+} in the OEC. One of these is structural. As noted above, a number of experiments demonstrate a requirement for Ca^{2+} for the functional assembly and stability of the OEC Mn cluster. This is not surprising in view of the large number of biological systems in which the metal plays a role as a structural element. That Ca^{2+} plays more than a structural role

in the OEC is demonstrated by the inhibitory effects of depletion of the metal, but also by the inhibitory effects of Ln^{3+} substitutions on activity. As noted above, this phenomenon is associated with those Ca^{2+} binding proteins in which the metal has a function in the active site of the enzyme [13]. There are numerous models for water oxidation, and a number of these depict Ca^{2+} as a binding site for substrate H_2O. The first such model [81] proposed that a H_2O ligated to Ca^{2+} carried out a nucleophilic attack on a Mn^{5+}=O species in S_4 to form the initial O–O bond, which leads to release of O_2 and reduction of the cluster to S_0. Later models have elaborated on this proposal [82], replacing the H_2O with a Ca^{2+}-bound hydroxyl group [54], based on the observation that the strongest Lewis acids (Ca^{2+}, Sr^{2+}) are the only metals effective in catalysis of H_2O oxidation. Later versions have been altered in light of one of the early structures of the OEC derived from PSII crystals [83], and further alterations will be necessary in light of the compelling evidence that the cluster has been disrupted by radiation damage. Essential features and predictions from these models about the role of Ca^{2+} as a substrate binding site and as a site for Cl^- binding (bridging to a Mn [82] or ligated only to Ca^{2+} [4]) need to be explored in more detail, hopefully with structural data from undamaged OEC preparations.

2.4. The role of Cl^- in the OEC

Early research on the role of Cl^- in the OEC produced equivocal results, owing to a lack of high purity distilled H_2O. Plants grown on the purest water available at the time appeared to be normal, but when isolated photosynthetic membranes were examined, they required addition of Cl^-, Br^-, NO_3^- or I^- to the assay medium in order to produce O_2 [83]. Later research identified Cl^- as the native anion that was required for electron donor reactions in the OEC [84,85].

2.4.1. Chloride stoichiometry and binding affinity

The OEC-Cl^- stoichiometry has been determined using PSII preparations isolated from plants grown on $^{36}Cl^-$. Analyses of this material gave an estimate of about one atom of the anion per OEC [86]. As in the case of Ca^{2+}, Cl^- binding is regulated by the presence of the PsbP and PsbQ subunits. The intact OEC exhibits a high affinity for Cl^- in the light (K_d = 20 μM [87]). Binding of $^{36}Cl^-$ cannot be detected in dark adapted samples lacking PsbP and PsbQ [86] and steady state assays of these preparations give K_d estimates of 1–2 mM [88]. These results emphasize again the importance extrinsic subunits of PSII in facilitating cofactor retention in the OEC. Similar results have been reported in cyanobacteria. Site directed mutations of PSII membrane proteins in domains that are involved in the binding of the PsbO subunit [89] alter the growth of the bacteria; increased Cl^- concentrations are required for the mutants to grow at near normal rates. These data, and a number of similar observations in other mutants, would seem to contradict the claim that biochemical manipulations of PSII in vitro create a Cl^- requirement for O_2 evolution activity [90]. An alternate strategy for removing Cl^- from the OEC, dialysis [87], removes Cl^- but the PsbP and PsbQ subunits remain bound to PSII. Examination of Cl^- binding affinity after dialysis gave a K_d of ~500 μM. By incubation of dialyzed samples with added Cl^- in darkness, stable high-affinity binding (K_d = 20 μM) was restored. A model has been proposed, based on these results, for a single Cl^- binding site in PSII that exists in both low and high affinity states. The low affinity site (K_d = 500 μM) binds Cl^- somewhat more avidly than do preparations lacking the PsbP and PsbQ subunits, but the differences are not dramatic (added Cl^- is rapidly lost from dialyzed samples), and this suggests that dialysis may have produced some structural changes, perhaps to extrinsic subunits, in the vicinity of the OEC Cl^- binding site.

Table 2
Effects of Lewis bases on OEC activity

Activators	$Cl^- > Br^- > NO_3^- > I^-$
Inhibitors	
Anions	^-OH, F^-, CH_3COO^-, N_3^-
1° amines	$(CH_2OH)_3$-C-NH_2, NH_3, CH_3NH_2, $CH_3CH_2CH_2NH_2$
No effect	PO_4^{2-}, SO_4^{2-}

2.4.2. Alternate ligands and the Cl^-–Mn interaction

As noted above, a small group of anions (Br^-, NO_3^-, I^-) will substitute for Cl^- as activators of the OEC, although the activity produced by these anions is lower than that observed with Cl^- [91–94]. The mechanism of action of inhibitors at the Cl^- site is linked to the observation that NH_3 inhibits the $S_2 \rightarrow S_3$ transition [95]. A characterization of this phenomenon led to the discovery that in the steady state, Lewis bases interfered with Cl^- binding to a site in the OEC [96,97]; primary amines compete with Cl^- for its site of action. For NH_3, a second Cl^- insensitive site was also detected [97]. Results of a number of investigations have produced a list of Lewis bases that affect the activity of the OEC by interference with Cl^- [96–99]. Representative results are summarized in Table 2. For primary amines, a linear relationship between their apparent binding constants for the OEC site, and their pK_a's was discovered [96,100]. This is the typical behavior of a Lewis acid–Lewis base interaction, and it was proposed that Cl^-, functioning as a Lewis base, was a ligand to Mn [96].

Extensive spectroscopic probing of PSII has produced a body of data that places Cl^- in the vicinity of the OEC Mn cluster, but evidence for direct ligation of the anion to Mn is lacking. None of the crystal structures include Cl^-, but this is not surprising given the extent of radiation damage to the OEC in these samples. Results of EXAFS experiments on cyanobacterial PSII taken from cells grown on Br^- [101] can be interpreted to indicate the presence of Cl^- near the Mn cluster, but these results are not conclusive. In PSII samples where Br^- has partially replaced Cl^-, Br^- EXAFS data are interpreted to indicate that the Cl^- site can be no closer than about 5 Å from a metal (Ca^{2+} or Mn) [102]. Other experiments have utilized CW [103] and pulsed EPR (ESEEM) [104] to probe interactions between acetate and the OEC in the S_2 state (the EPR multiline signal). In the EPR experiments, two acetate binding sites were detected, only one of which is associated with the OEC (the second site is a non-heme iron atom located on the reducing side of the photosystem).

The CW EPR experiments were interpreted to indicate a close spatial relationship between Cl^- and $Y_Z^\bullet$, but no conclusions concerning Cl^-–Mn interactions were presented. In the case of the ESEEM experiments that detected binding of deuterated acetate in the OEC, analyses of the pulsed data were interpreted to indicate that the deuterons could be at a distance ranging from about 3 to 5 Å from Mn, and models were presented for acetate binding as a bridging or monodentate ligand [104]. Probing of $^{15}N_3^-$ inhibited PSII by ESEEM also gave results that were interpreted to indicate binding of this anion in the vicinity of, or directly to the Mn cluster [105]. A series of FTIR experiments have also been carried out using $^{15}NO_3^-$-substituted PSII samples [106,107]. The authors of these studies conclude that although the substituting anion may bind in the vicinity of the Mn cluster, they find no evidence for direct ligation to Mn. Lastly, the Cl^- binding affinity for the OEC has been examined as a function of the S-states; binding affinity decreases with increasing Mn oxidation [108].

2.4.3. The role of Cl^- in S-state advancement

Depletion of Cl^- from PSII followed by illumination to generate the S_2 state prevents formation of the EPR multiline signal that is the spectroscopic signature for Mn oxidation on advancement from S_1; instead, a species centered at about $g = 4.1$ is detected [109]. Upon addition of Cl^-, this state is converted to the multiline signal. The ca. $g = 4.1$ species formed in Cl^- depleted samples decays very slowly back to S_1, with a halftime of about 10 min, as opposed to the multiline signal decay under the same conditions with Cl^- (<1 min). In the presence of adequate Cl^- to permit normal S-state advancement, illumination of PSII with added NH_3 produces an altered multiline signal (narrowed linewidths) that was proposed to arise from direct binding of the amine to a site normally occupied by H_2O [110]. Application of ESEEM spectroscopy to PSII samples illuminated with added Cl^- and NH_3 provided direct evidence for binding of the amine to the Mn cluster [111]. For technical reasons, it has been difficult or impossible to carry out experiments to characterize the consequences of amine binding to the site normally occupied by Cl^-. Other EPR experiments to characterize the effects of Cl^- on S-state advancement have made use of the inhibitory anion F^- [112,113]. In this case, formation of the S_2 multiline signal is blocked, and instead the broad lower-field $g = 4.1$ signal is obtained upon illumination. Variations on this treatment, including Cl^- depletion at high pH (10) induce modifications to the S_2 multiline signals [114,115].

An alternate approach to characterization of the role of Cl^- in the S-state cycle employed kinetic studies of UV–vis absorption changes in the OEC Mn cluster in the region around 295 nm [72]. These changes are proposed to arise from ligand-to-metal charge transfer bands [116] and display positive absorbance changes on the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ oxidation state advances, and a decrease on the $S_4 \rightarrow S_0$ transition; plots of absorbance change versus flash number generate the period-four oscillation expected for a component of the S-state cycle [72,116]. In the Cl^--depleted OEC, the first flash produced an absorbance change consistent with the $Mn^{3+} \rightarrow Mn^{4+}$ transition that occurs on $S_1 \rightarrow S_2$ [94]. Subsequent flashes produced signals consistent with formation of $Y_Z^\bullet$ and its decay by recombination with an electron from the reducing side of the photoreaction. By rapid depletion/reconstitution of Cl^-, it was shown that the anion was required only for the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4 \rightarrow S_0$ transitions of the OEC. Other absorbance experiments revealed that the affinity of Cl^- binding to the OEC decreased with increasing oxidation states, and that alternate ions (Br^-, NO_3^-, I^-) decreased the stability of the advanced S-states [117]. These data are, like other spectroscopic results, consistent with a close interaction between Cl^- and the Mn cluster.

A variety of proposals have been advanced to explain the Cl^- requirement for OEC redox activity. An early idea was that the anion functioned as a bridging ligand between Mn atoms [96]. It has also been proposed that the role of Cl^- is to regulate the redox properties of the Mn cluster [118]. One model proposed to account for the block in electron transfer hypothesized that Cl^- ligation to a Mn^{3+} atom proximal to Y_Z altered its redox properties so that it could function as the oxidant of a second Mn^{3+} distal to Y_Z, i.e., $Mn^{3+} \rightarrow Mn^{3+}(Cl^-) \rightarrow Yz$ [94]. Other models for the function of Cl^-, noted earlier, suggest that it is either a bridging ligand between Ca^{2+} and one of the Mn atoms involved in H_2O oxidation [96] or, more recently, that Cl^- is ligated solely to Ca^{2+} [4]. It has also been suggested that Cl^- is a component of a proton transfer network from the site of H_2O oxidation to the external medium, and it has been proposed that Cl^- is not an essential cofactor for H_2O oxidation [93], but this claim has not been investigated further. If this were to be so, then an alternate role for Cl^- in the OEC might be to exclude the binding of small, hard inhibitory ligands (most notably ^-OH) to the Mn cluster [119].

3. Concluding remarks

The ambiguities associated with the functions of Ca^{2+} and Cl^- in their roles as cofactors that promote Mn redox chemistry in PSII arise from a number of factors. One of these is that the redox chemistry catalyzed by the OEC has no analog among other metalloenzymes: this is the only enzyme-catalyzed reaction in the biosphere that oxidizes water. A second is the fairly strict correlation between structural integrity of the OEC and the affinity of cofactor binding, and a third is the sensitivity of cofactor binding affinity to changes in Mn oxidation states. On account of the lack of other well-characterized biological systems that can serve as points of comparison, PSII researchers have had to develop techniques and models in a vacuum. The second and third complications listed above have added a layer of difficulty to the efforts to unravel the mechanisms of action of the cofactors by complicating efforts to assess the extent to which a given ion has or has not been released from the OEC. In spite of these complexities, there has been substantial progress in understanding why Ca^{2+} and Cl^- are essential cofactors for the O_2 evolving reaction.

In the case of Ca^{2+}, the combined biochemical and biophysical characterizations of the effects of depletion of the ion on the OEC have yielded tentative, yet reasonable models for its location in the OEC and its role in H_2O oxidation. The model for the Ca^{2+}–Mn cluster structure, shown in Fig. 1, will certainly be

subjected to modifications in the future, as more refined structural data become available. For the present time, however, it is consistent with the observation that Ca^{2+} extraction, or substitutions of either Sr^{2+} or Ln^{3+} after extraction of Ca^{2+}, have no effect on the XANES and EXAFS features of the OEC that arise from Mn–Mn interactions [120]. The model also positions Ca^{2+} so that it can function as site for ligation of H_2O or ^{-}OH, consistent with its proposed role as a direct participant in the mechanism of H_2O oxidation [54,4]. What must be kept in mind is that any model for PSII in the S_1 state provides at a best a fragmentary view of the OEC. Enzymes are not immobile structures, and ligand exchange probably occurs in the course of the S-state cycle. It will be interesting to see whether the results now being obtained with FTIR spectroscopy may be due in part to shifts of carboxyl ligands and substrate H_2O to and from Ca^{2+}, and perhaps Mn atoms as well, in the course of substrate binding and O–O bond formation in the OEC.

Chloride's role in H_2O oxidation remains elusive. The fact that it is not resolved in the current crystal structures is not particularly worrisome given the radiation damage issue and the long times required for crystal formation, which would allow for loss of the anion. The weight of experimental evidence favors a role for Cl^- as an essential cofactor in the S-state cycle, at least for S-state advancements from $S_2 \rightarrow S_4$. The physical probes to locate the anion in the OEC have produced data that at first glance seem to be contradictory. This is not necessarily the case. The XAS experiments with Br^- [102] provide data on the S_1 state, where Cl^- is not required for the transition to S_2 [108,109], and is predicted to be at least 5 Å from the nearest metal atom. The results of EPR and ESEEM experiments rely on the paramagnetic S_2 state to generate a point of reference for determining distances, and in this case, the probes (CH_3COO^-, N_3^-) seem to be located in closer proximity to the Mn cluster than would be predicted from the XAS results. This may simply be a reflection of a ligand shift by Cl^- that occurs on the $S_1 \rightarrow S_2$ transition. The functional significance of Cl^- binding to the OEC remains open to speculation. Neutralization of charge on the Mn cluster is a possibility. However, any number of anions could function in this role, but the OEC has a very selective preference for Cl^- and a very small number of other ions. The correlation of the presence of Cl^- in the OEC with formation of the S_2 multiline signal, and the dependence on Cl^- for S-state advancement points to a role in modulation of Mn redox activity or of metal–metal couplings within the Mn cluster.

Acknowledgement

The author is grateful for research support from the National Science Foundation (MCB 0110455 and 0716541).

References

[1] R.J. Debus, Biochim. Biophys. Acta 1102 (1992) 269.
[2] C.F. Yocum, Biochim. Biophys. Acta 1059 (1991) 1.
[3] Vrettos, Limburg, Brudvig, Biochim. Biophys. Acta 1503 (2001) 229.
[4] J.P. McEvoy, G.W. Brudvig, Chem. Rev. 106 (2006) 4455.
[5] H.J. van Gorkom, C.F. Yocum, in: T.J. Wydrzynski, K. Satoh (Eds.), Photosystem II: the light-driven water: plastoquinone oxidoreductase, Springer, Dordrecht, 2005, p. 307.
[6] H. Popelkova, C.F. Yocum, Photosynth. Res. 93 (2007) 111.
[7] M. Miqyass, H.J. van Gorkom, C.F. Yocum, Photosynth. Res. 92 (2007) 275.
[8] R.H. Kretsinger, D.J. Nelson, Coord. Chem. Rev. 18 (1976) 29.
[9] T.J. Lyons, D.J. Eide, in: I. Bertini, H.B. Gray, E. Stiefel, J.S. Valentine (Eds.), Biological Inorganic Chemistry: Structure and Reactivity, University Science Books, Sausalito, CA, 2007, p. 57.
[10] T. Drakenberg, B. Finn, S. Forsen, in: I. Bertini, H.B. Gray, E. Stiefel, J.S. Valentine (Eds.), Biological Inorganic Chemistry: Structure and Reactivity, University Science Books, Sausalito, CA, 2007, p. 635.
[11] N.C.J. Strynadka, M.N.G. James, Ann. Rev. Biochem. 58 (1989) 951.
[12] A. Lewit-Bentley, S. Rety, Curr. Opin. Struct. Biol. (2000) 637.
[13] R.B. Martin, in: T. Spiro (Ed.), Metal Ions in Biology. Calcium in Biology, vol. 6, John Wiley & Sons, New York, NY, 1983, p. 237.
[14] C. Turro, P.K.-L. Fu, P.M. Bradley, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 40, Marcel Dekker, New York, NY, 2003, p. 323.
[15] L.T. Kakalis, M. Kennedy, R. Sikkink, F. Rusnak, I.M. Armitage, FEBS Lett. 352 (1995) 55.
[16] R.D. Brokx, H.J. Vogel, Prot. Sci. 9 (2000) 964.
[17] J. Grandjean, P. Laszlo, FEBS Lett. 81 (1977) 376.
[18] M.-J. Moutin, Y. Dupont, J. Biol. Chem. 266 (1991) 5580.
[19] X. Li, S.A. Weinman, Ann. Rev. Physiol. 64 (2002) 609.
[20] G. Feller, O. LeBussy, C. Houssier, C. Gerday, J. Biol. Chem. 271 (1996) 23836.
[21] M. Morrison, G.R. Schonbaum, Ann. Rev. Biochem. 45 (1976) 861.
[22] M. Fabian, L. Skultety, C. Brunel, G. Palmer, Biochemistry 40 (2001) 6061.
[23] D.A. Berthold, G.T. Babcock, C.F. Yocum, FEBS Lett. 134 (1983) 231.
[24] E.J. Boekema, J.F. van Breemen, H. van Roon, J.P. Dekker, J. Mol. Biol. 301 (2000) 1123.
[25] J. Nield, E.V. Orlova, E.P. Morris, B. Gowen, M. van Heel, J. Barber, Nat. Struct. Biol. 7 (2000) 44.
[26] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, Nature 409 (2001) 739.
[27] N. Kamiya, J.R. Shen, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 98.
[28] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Science 303 (2004) 1831.
[29] J. Biesiadka, B. Loll, J. Kern, K.D. Irrgang, A. Zouni, Phys. Chem. Chem. Phys. 6 (2004) 4733.
[30] B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Nature 438 (2005) 1040.
[31] D.F. Ghanotakis, T.A. Bricker, in: D.R. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer, Dordrecht, 1996, p. 113.
[32] A. Seidler, Biochim. Biophys. Acta 1277 (1996) 35.
[33] T.A. Bricker, R.L. Burnap, in: T.J. Wydrzynski, K. Satoh (Eds.), Photosystem II: the light-driven water: plastoquinone oxidoreductase, Springer, Dordrecht, 2005, p. 95.
[34] D.F. Ghanotakis, J.N. Topper, C.F. Yocum, Biochim. Biophys. Acta 767 (1984) 524.
[35] T. Kuntzleman, R. McCarrick, J.E. Penner-Hahn, C.F. Yocum, Chem. Phys. Phys. Chem. 6 (2004) 4897.
[36] C.F. Yocum, C.T. Yerkes, R.R. Sharp, R.E. Blankenship, G.T. Babcock, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 7507.
[37] P.J. Riggs, R. Mei, C.F. Yocum, J.E. Penner-Hahn, J. Am. Chem. Soc. 114 (1992) 10650.
[38] V.K. Yachandra, V.J. DeRose, M.J. Latimer, I. Mukerji, K. Sauer, M.P. Klein, Science 260 (1993) 675.
[39] T. Kuntzleman, C.F. Yocum, Biochemistry 44 (2005) 2129.
[40] B. Kok, B. Forbush, M. McGloin, Photochem. Photobiol. 11 (1970) 457.
[41] D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, FEBS Lett. 167 (1984) 127.
[42] M. Miyao, N. Murata, FEBS Lett. 168 (1984) 118.
[43] A. Boussac, A.W. Rutherford, Biochemistry 27 (1988) 3476.
[44] T.-A. Ono, Y. Inoue, FEBS Lett. 277 (1988) 147.
[45] K.V. Cammarata, G.M. Cheniae, Pl. Physiol. 84 (1987) 587.

[46] J.R. Shen, K. Satoh, S. Katoh, Biochim. Biophys. Acta 936 (1988) 386.
[47] J.R. Shen, S. Katoh, Plant Cell Physiol. 32 (1991) 439.
[48] G.N. Grove, G.W. Brudvig, Biochemistry 37 (1998) 1532.
[49] P. Adelroth, K. Lindberg, L.-E. Andreasson, Biochemistry 34 (1995) 9021.
[50] K.C. Han, S. Katoh, Biochim. Biophys. Acta 1232 (1993) 230.
[51] C.M. Waggoner, V. Pecoraro, C.F. Yocum, FEBS Lett. 244 (1989) 237.
[52] T.-A. Ono, A. Rompel, H. Mino, N. Chiba, Biophys. J. 81 (2001) 1831.
[53] Z. Nagel, C.F. Yocum, in: A. van der Est, D. Bruce (Eds.), Photosynthesis: Fundamental Aspects to Global Perspectives, Alliance Communications Group, Lawrence, KS, 2005, p. 372.
[54] J.S. Vrettos, D.A. Stone, G.W. Brudvig, Biochemistry 40 (2001) 7937.
[55] C.G. Chen, J. Kazimir, G.M. Cheniae, Biochemistry 34 (1995) 13511.
[56] G.C. Dismukes, G.M. Ananyev, R. Watt, in: T.J. Wydrzynski, K. Satoh (Eds.), Photosystem II: The Light-Driven Water: Plastoquinone Oxidoreductase, Springer, Dordrecht, 2005, p. 609.
[57] A. Boussac, F. Rappaport, p. Carrier, J.-M. Verbavatz, R. Goin, D. Kirilovsky, A.W. Rutherford, M. Sugiura, J. Biol. Chem. 279 (2004) 22809.
[58] R.M. Cinco, J.H. Robblee, A. Rompel, C. Fernandez, V.K. Yanchandra, K. Sauer, M.P. Klein, J. Phys. Chem. B 102 (1998) 8248.
[59] R.M. Cinco, L.L.M. Holman, J.H. Robblee, J. Yano, S.A. Pizarro, E. Bellacchio, K. Sauer, V.K. Yanchandra, Biochemistry 41 (2002) 12928.
[60] J. Maysik, Alia, G. Nachtegaal, H.J. van Gorkom, A.J. Hoff, H.J.M. de Groot, Biochemistry 39 (2000) 6751.
[61] J. Yano, J. Kern, K.-D. Irrgang, M.J. Latimer, U. Bergmann, P. Glatzel, Y. Pushkar, J. Biesiadka, B. Loll, K. Sauer, J. Messinger, A. Zouni, V.K. Yachnadra, Proc. Natl. Acad. Sci. U.S.A. 102 (2006) 12047.
[62] J. Yano, J. Kern, K. Sauer, M.J. Latimer, J. Pushkar, J. Biesiadka, B. Loss, W. Saenger, J. Messinger, A. Zouni, V.K. Yachandra, Science 1314 (2006) 821.
[63] M.A. Strickler, L.M. Walker, W. Hillier, R.J. Debus, Biochemistry 44 (2005) 8571.
[64] R.J. Debus, M.A. Strickler, L.M. Walker, W. Hillier, Biochemistry 44 (2005) 1367.
[65] M.A. Strickler, W. Hillier, R.J. Debus, Biochemistry 45 (2006) 8801.
[66] H. Suzuki, Y. Taguchi, M. Sugiura, A. Boussac, T. Noguchi, Biochemistry 45 (2006) 13454.
[67] A. De Riso, D.L. Johnson, B.A. Barry, Biophys. J. 91 (2006) 1999.
[68] R.R. Sharp, C.F. Yocum, Biochim. Biophys. Acta 635 (1981) 90.
[69] R. Mei, C.F. Yocum, Biochemistry 30 (1991) 7836.
[70] R. Mei, C.F. Yocum, Biochemistry 31 (1992) 8449.
[71] P. Riggs-Gelasco, R. Mei, C.F. Yocum, J.E. Penner-Hahn, J. Am. Chem. Soc. 118 (1996) 2387.
[72] J.P. Dekker, D.F. Ghanotakis, J.J. Plijter, H.J. van Gorkom, G.T. Babcock, Biochim. Biophys. Acta 767 (1984) 515.
[73] M. Miyao, N. Murata, Photosynth. Res. 10 (1986) 489.
[74] A. Boussac, A.W. Rutherford, FEBS Lett. 236 (1988) 432.
[75] A. Boussac, J.L. Zimmermann, A.W. Rutherford, Biochemistry 28 (1989) 8984.
[76] X.S. Tang, D.W. Randall, D.A. Force, B.A. Diner, R.D. Britt, J. Am. Chem. Soc. 118 (1996) 7638.
[77] J.C. DePaula, P.M. Li, A.F. Miller, B.W. Wu, G.W. Brudvig, Biochemistry 25 (1986) 6487.
[78] M. Haumann, W. Junge, Biochim. Biophys. Acta 1411 (1999) 121.
[79] C.-I. Lee, K.V. Lakshmi, G.W. Brudvig, Biochemistry 46 (2007) 3211.
[80] K.G.V. Sigfridsson, G. Bernat, F. Mamedov, S. Styring, Biochim. Biophys. Acta 1659 (2004) 19.
[81] V.L. Pecoraro, M.J. Baldwin, M.T. Caudle, W.Y. Hsieh, N.A. Law, Pure Appl. Chem. 70 (1998) 925.
[82] J.S. Vrettos, J. Limburg, G.W. Brudvig, Biochim. Biophys. Acta 1503 (2001) 229.
[83] D.I. Arnon, F.R. Whatley, Science 110 (1949) 554.
[84] J. Bove, C.M. Bove, F.R. Whatley, Z. Naturforsch. 18 (1963) 683.
[85] S. Izawa, R.L. Heath, G. Hind, Biochim. Biophys. Acta 180 (1969) 388.
[86] K. Lindberg, T. Vänngård, L.-E. Andréasson, Photosynth. Res. (1993) 401.
[87] K. Lindberg, L.E. Andréasson, Biochemistry 35 (1996) 14259.
[88] P. Homann, Photosynth. Res. 15 (1988) 205.
[89] T.M. Bricker, J. Lowrance, H. Sutton, L.K. Frankel, Biochemistry 40 (2001) 11483.
[90] T. Wydrzynski, F. Baumgart, F. Macmillan, G. Renger, Photosynth. Res. 25 (1990) 59.
[91] P.M. Kelly, S. Izawa, Biochim. Biophys. Acta 502 (1978) 198.
[92] D.I. Bryson, N. Doctor, R. Johnson, S. Baranov, A. Haddy, Biochemistry 44 (2005) 7354.
[93] K. Oleson, L.-E. Andréasson, Biochemistry 42 (2003) 2025.
[94] H. Wincencjusz, C.F. Yocum, H.J. van Gorkom, Biochemistry 38 (1999) 3719.
[95] B.R. Velthuys, Biochim. Biophys. Acta 396 (1975) 392.
[96] P.O. Sandusky, C.F. Yocum, Biochim. Biophys. Acta 766 (1984) 601.
[97] P.O. Sandusky, C.F. Yocum, Biochim. Biophys. Acta 849 (1986) 85.
[98] P. Homann, Biochim. Biophys. Acta 934 (1988) 1.
[99] A. Haddy, J.A. Hatchell, R.A. Kimel, R. Thomas, Biochemistry 38 (1999) 6104.
[100] D.F. Ghanotakis, P.J. O'Malley, G.T. Babcock, C.F. Yocum, in: Y. Inoue, A.R. Crofts, Govindjee, N. Murata, G. Renger, K. Satoh (Eds.), The Oxygen Evolving System of Photosynthesis, Academic Press, Tokyo, 1983, p. 91.
[101] M.P. Klein, K. Sauer, V.K. Yachandra, Photosynth. Res. 38 (1993) 265.
[102] M. Haumann, M. Barra, P. Loha, S. Losher, R. Krivanek, A. Grundmeier, L.-E. Andréasson, H. Dau, Biochemistry 45 (2006) 13101.
[103] H. Kuhne, V.A. Szalai, G.W. Brudvig, Biochemistry 38 (1999) 6604.
[104] K.L. Clemens, D.A. Force, R.D. Britt, J. Am. Chem. Soc. 124 (2002) 10921.
[105] H. Yu, C.P. Aznar, X. Xu, R.D. Britt, Biochemistry 44 (2005) 12022.
[106] K. Hasegawa, Y. Kimura, T.-A. Ono, Biochemistry 41 (2002) 13839.
[107] K. Hasegawa, Y. Kimura, T.-A. Ono, Biophys. J. 86 (2004) 1042.
[108] H. Wincencjusz, H.J. van Gorkom, C.F. Yocum, Biochemistry 36 (1997) 3663.
[109] T.-A. Ono, J.L. Zimmermann, Y. Inoue, A.W. Rutherford, Biochim. Biophys. Acta 851 (1986) 193.
[110] W. Beck, J.C. DePaula, G.W. Brudvig, J. Am. Chem. Soc. 108 (1986) 4018.
[111] R.D. Britt, J.L. Zimmermann, K. Sauer, M.P. Klein, J. Am. Chem. Soc. 111 (1989) 3522.
[112] A. Haddy, W.R. Dunham, R.H. Sands, R. Aasa, Biochim. Biophys. Acta 1099 (1992) 25.
[113] V. DeRose, M.J. Latimer, J.-L. Zimmermann, I. Mukerji, V. Yachandra, K. Sauer, M.P. Klein, Chem. Phys. 194 (1995) 443.
[114] P. van Vliet, A. Boussac, A.W. Rutherford, Biochemistry 33 (1994) 12998.
[115] P. van Vliet, A.W. Rutherford, Biochemistry 35 (1996) 1829.
[116] J.P. Dekker, In: V.L. Pecoraro (Ed.), Manganese Redox Enzymes, VCH Press, New York, NY, p. 85.
[117] H. Wincencjusz, C.F. Yocum, H.J. van Gorkom, Biochemistry 37 (1998) 8595.
[118] A.W. Rutherford, Trends Biochem. Sci. 14 (1989) 227.
[119] H.J. van Gorkom, C.F. Yocum, in: A. van der Est, D. Bruce (Eds.), Photosynthesis: Fundamental Aspects to Global Perspectives. Alliance Communications Group, Lawrence, KS, 2005, p. 378.
[120] P.J. Riggs-Gelasco, R. Mei, D.F. Ghanotakis, C.F. Yocum, J.E. Penner-Hahn, J. Am Chem. Soc. 118 (1996) 2400.