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Review

Reconstitution of the photosystem II Ca^{2+} binding site

Kirk A. Vander Meulen, Amanda Hobson, Charles F. Yocum*

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA

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Abstract

The roles of Ca^{2+} in H_2O oxidation may be as a site of substrate binding, and as a structural component of the photosystem II O_2-evolving complex. One indication of this dual role of the metal is revealed by probing the Mn cluster in the Ca^{2+} depleted O_2 evolving complex that retains extrinsic 23- and 17-kDa polypeptides with reductants (NH_2OH and hydroquinone) [*Biochemistry* 41 (2002) 958]. Calcium appears to bind to photosystem II at a site where it could bind substrate H_2O. Equilibration of Ca^{2+} with this binding site is facilitated by increased ionic strength, and incubation of Ca^{2+} reconstitution mixtures at 22 °C accelerates equilibration of Ca^{2+} with the site. The Ca^{2+} reconstituted enzyme system regains properties of unperturbed photosystem II: Sensitivity to NH_2OH inhibition is decreased, and Cl^- binding with increased affinity can be detected. The ability of ionic strength and temperature to facilitate rebinding of Ca^{2+} to the intact O_2 evolving complex suggests that the structural environment of the oxidizing side of photosystem II may be flexible, rather than rigid.

Keywords: Photosystem II; Water oxidation; Calcium; Manganese; Chloride

1. Introduction

The discovery that Ca^{2+} is an essential activator of the OEC [1,2] was surprising in light of the fact that it is not commonly associated with redox-active metal clusters. In fact, the role of the single atom of Ca^{2+} in PSII is not yet resolved. Although a considerable number of metals can compete with Ca^{2+} for its binding site in the enzyme [3–7], only Sr^{2+} can replace Ca^{2+} to restore some, but not all, of the activity that is observed with the native metal [1,8]. The observation that Sr^{2+} and Ca^{2+} are the strongest Lewis acids in the series of competing metals [6] has supported proposals that one role of Ca^{2+} in the OEC is to function as a binding site for substrate H_2O or OH^- [5,9,10]. This is hypothesized to increase the nucleophilicity of the bound substrate molecule, which would enhance its attack on a putative $Mn^V=O$ intermediate in the $S_3 \rightarrow S_0$ transition [5]. It has also been suggested that limitations in electron transfer within the OEC that are observed upon Sr^{2+} substitution for Ca^{2+} arise from effects on H-atom transfer in the course of sequential oxidation of H_2O [11]. When Ca^{2+} is present, the PSII Mn cluster is stabilized against dissociation under conditions where some Mn atoms have been reduced to Mn^{2+} [12]. This is interpreted to indicate that Ca^{2+} has a role in maintaining the integrity of the structure that ligates Mn atoms in the OEC. On the basis of these observations, it is likely that Ca^{2+} fulfills a dual role in PSII, as a part of the structure of the active site of H_2O oxidation, and also as an active participant in the mechanism of the reaction itself.

The exact location of Ca^{2+} within the OEC is not resolved. EXAFS studies [13–17] place Ca^{2+} at distances from 3.3–3.5 Å to >4 Å from the Mn cluster. The recent X-ray structures of PSII at 3.7–3.8-Å resolution [18,19] have not, as yet, provided any information on the location of Ca^{2+} within the OEC. Biochemical experiments have identified the 23- and 17-kDa polypeptides as part of a structure that can affect the rate of equilibration of Ca^{2+} with its binding site [20,21], and the 33-kDa manganese stabilizing protein is also an important component of the Ca^{2+} binding site in the OEC [21]. To probe the relationship between the extrinsic polypeptides of PSII, and the location of the Ca^{2+} binding site relative to the Mn cluster, Ca^{2+} can be removed from PSII by a brief pH 3 exposure in citrate [22] with minimal perturbation of OEC proteins. This opens a path-

Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; EXAFS, extended X-ray absorption fine structure; OEC, O_2-evolving complex; PS, photosystem; SMN, buffer composed of sucrose (0.4 M), MES (50 mM, pH 6), and NaCl (10 mM)

* Corresponding author. Tel.: +1-734-647-0897; fax: +1-734-647-0884.

E-mail address: cyocum@umich.edu (C.F. Yocum).

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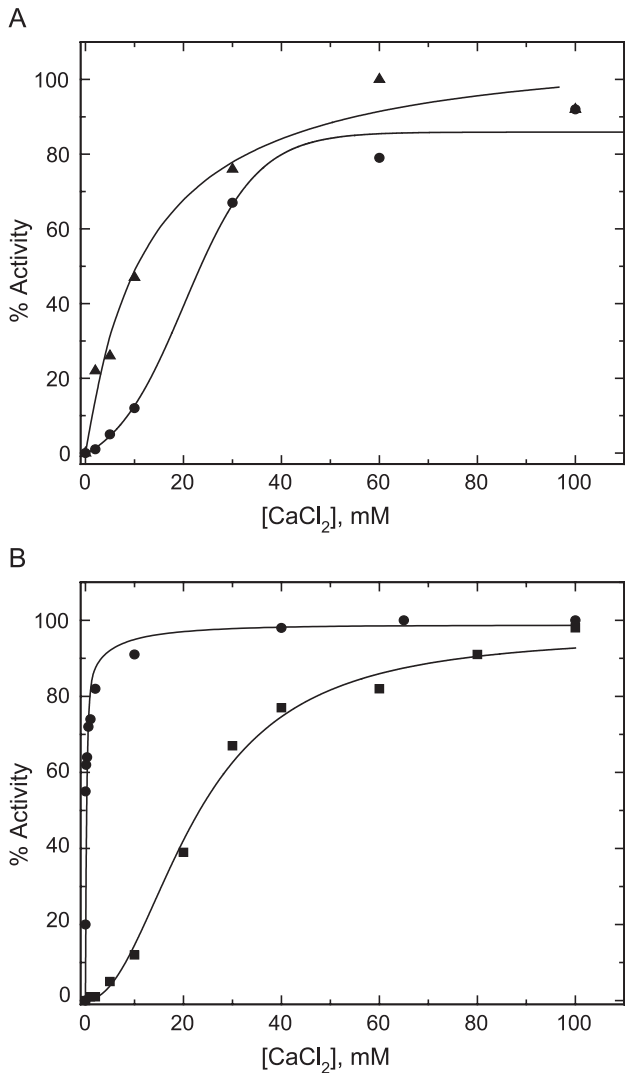

Fig. 1. (A) Reactivation of pH 3-treated PSII: Effect of incubation time with Ca^{2+}. The Ca^{2+}-depleted PSII samples were incubated with the indicated concentrations of Ca^{2+} (as $CaCl_2$) in SMN buffer for 1 or 12 h prior to O_2 activity assays with DCBQ as the acceptor. Symbols: (●) 1-h incubation before assay; (▲) 12-h incubation before assay. The 100% activities were 325 (1 h) and 350 (12 h) μmol O_2/h/mg Chl, respectively. The data shown in this and subsequent figures are the average of at least three separate assays. The error in the measurements was ± 5%. (B) A nonactivating divalent ion, Mg^{2+}, decreases the concentration of Ca^{2+} required to activate pH 3-treated PSII. Samples were incubated with the concentrations of Ca^{2+} shown in the figure for 1 h, plus (●), or minus (■) 60 mM Mg^{2+} as $MgCl_2$. All samples were assayed in SMN, as described in Ref. [23]; the acceptor was 300 μM DCBQ. The 100% activities are 275 (+Mg^{2+}) and 330 (−Mg^{2+}) μmol/h/mg Chl.

way to the Mn cluster that is accessible to a small reductant (NH_2OH), but not to a larger probe, hydroquinone [23]. Reconstitution of activity in this preparation requires high (>10 mM) Ca^{2+} concentrations and long incubation times. Increases in ionic strength or temperature increase the effectiveness of Ca^{2+} rebinding. These observations indicate that the structure of the OEC may be flexible, rather than rigid.

2. Discussion

2.1. Factors affecting Ca^{2+} binding to the OEC

Fig. 1A shows that the unusual sigmoidal behavior observed upon Ca^{2+} rebinding to a pH 3-treated PSII preparation [23] is abolished by extending the incubation time to 12 h. A simple explanation for cooperative behavior is that Ca^{2+} facilitates its equilibration with the binding site by increasing the ionic strength, which would weaken binding of the extrinsic polypeptides. A test of this hypothesis, shown in Fig. 1B, used a nonactivating divalent ion (Mg^{2+} [1,5]) to increase ionic strength. The resulting K_d (+Mg^{2+}) is about 120 μM, as compared to 24 or 11 mM (1 or 12 h incubations, Fig. 1A). Correcting for the Ca^{2+}–

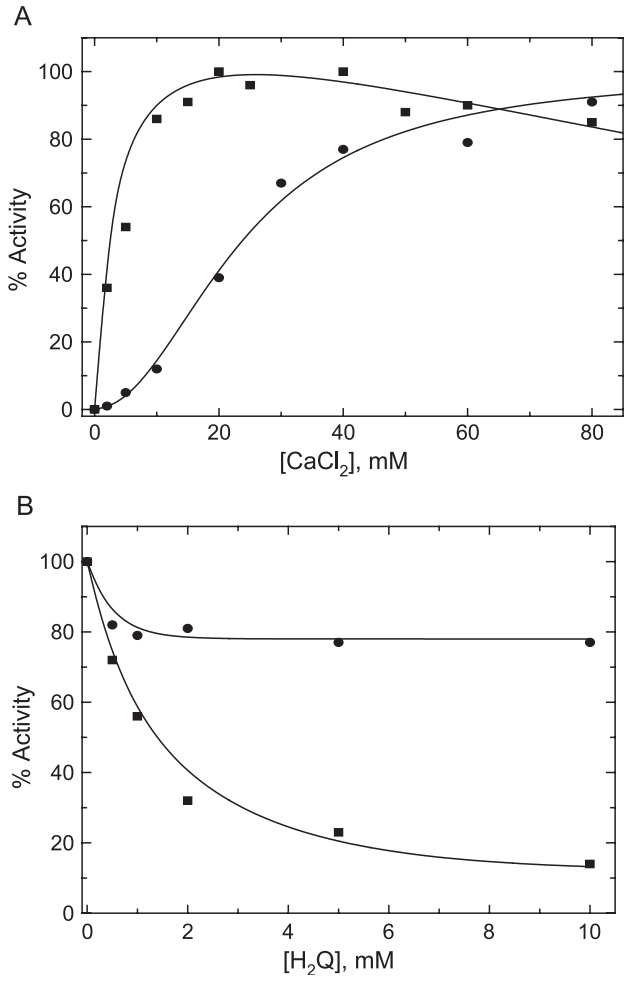

Fig. 2. (A) Increased temperature lowers the concentration Ca^{2+} required for reconstitution of pH 3-treated PSII. Samples were incubated in the $CaCl_2$ concentrations shown for 1 h at 4 (●) or 22 (■) °C, and assayed as described in the legend to Fig. 1. The 100% activities are 310 (22 °C) and 280 (4 °C) μmol O_2/h/mg Chl. (B) Effect of temperature on the sensitivity of the OEC in pH 3-treated PSII to inactivation by a bulky reductant, hydroquinone (H_2Q). Unreconstituted samples were incubated for 1 h at 4 (●) or 22 (■) °C in the presence of the concentrations of H_2Q shown in the figure. Activities were assayed as described in the legend for Fig. 1, but 10 mM $CaCl_2$ was added to the assay buffer.

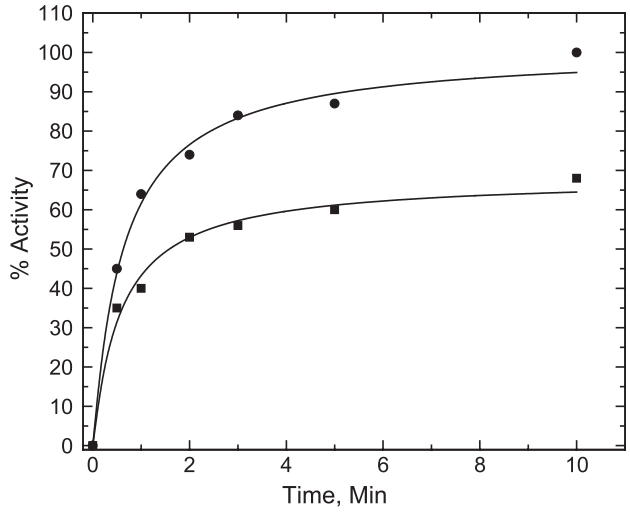

Fig. 3. Effect of temperature on the rate of Ca^{2+} reconstitution of pH 3-treated PSII. Calcium depleted samples were incubated with 60 mM $CaCl_2$ at 4 (■) or 22 (●) °C and assayed for activity at the times shown. The 100% activity is 240 μmol O_2/h/mg Chl.

Mg^{2+} competition (K_i = 2.84 mM [5]) using the equation $K_{dapp} = K_d(1+[I]/K_I)$ [24] yields an estimated Ca^{2+} K_d of about 6 μM. This is close to a value (11 μM) reported by Chen and Cheniae [25], also for a low pH-treated sample, and is lower by a factor of 5 to 10 from those reported for PSII preparations lacking the 23- and 17-kDa polypeptides [5,20,21]. The origin of this discrepancy in Ca^{2+} affinities is not readily apparent. However, Chen and Cheniae also report a Ca^{2+} K_d value of 3 μM for a low-pH treated sample lacking the 23- and 17-kDa polypeptides. It is therefore possible that the Ca^{2+} affinities detected upon reconstitution of the OEC are a function of the prior treatment of PSII used to release Ca^{2+}. Samples exposed to low pH appear to exhibit higher affinities for the metal, regardless of whether the 23- and 17-kDa polypeptides are present, than do samples from which these proteins have been removed by exposure of PSII to high ionic strength.

Under identical conditions of incubation time and Ca^{2+} concentration, raising the temperature from 4 to 22 °C during reconstitution induces a 10-fold decrease in the Ca^{2+} K_d of pH 3-treated PSII, from 24 to 2.4 mM (Fig. 2A). Higher Ca^{2+} concentrations are inhibitory at 22 °C, perhaps as result of weakened binding of extrinsic polypeptides. In Fig. 2B, the OEC structure was probed with hydroquinone to determine whether incubation of the enzyme at 22 °C induces a structural change that exposes the Mn cluster to the external medium. Activity loss increased substantially when the sample was incubated with reductant at 22 °C, whereas incubation of control PSII preparations at 22 °C for periods of several hours produces at most a 5% loss of activity (data not shown). Fig. 3 shows results of experiments to examine the kinetics of reactivation of pH 3-treated PSII by Ca^{2+} at 4 and 22 °C. Incubation of samples at 22 °C yields more rapid reconstitution of activity with Ca^{2+} than at 4 °C, although the latter sample will recover about the same amount of activity as the 22 °C sample after incubation for periods up to an hour (data not shown). These results are consistent with the report that at 20 °C Sm^{3+} binds more rapidly to the PSII Ca^{2+} site than it does at 0 °C [26]. However, although increased temperature accelerates the rate of Ca^{2+} rebinding to PSII, it does not lower the apparent K_d to the extent that is observed for samples incubated with Mg^{2+}. This suggests that ionic strength and temperature may facilitate Ca^{2+} equilibration with its binding site by different mechanisms.

Fig. 4 presents data on restoration of structural integrity to the OEC by Ca^{2+} rebinding. Resistance to NH_2OH inactivation of the OEC is recovered, to levels that approach that of the control. Assays have also been done to assess the effect of Ca^{2+} rebinding on Cl^- affinity of the OEC. Polypeptide-depleted and pH 3-treated PSII bind Cl^- with relatively high K_M values (1900 and 1800 μM, respectively [23]). Steady-state assays of Ca^{2+}-reconstituted pH 3 preparations yield a Cl^- K_M of 620 μM, and rebinding of Cl^- by incubation in the dark, followed by assays in Cl^--free medium gives a K_d of 350 μM (data not shown).

2.2. Implications for the nature of the Ca^{2+} binding site and its role in H2O oxidation

The extrinsic polypeptides that affect access to the binding sites of the inorganic ions comprising the OEC are an impediment to obtaining data on the Ca^{2+} binding properties of the native enzyme system. Although the extrinsic proteins slow the equilibration of Ca^{2+} with its binding site [5,20–

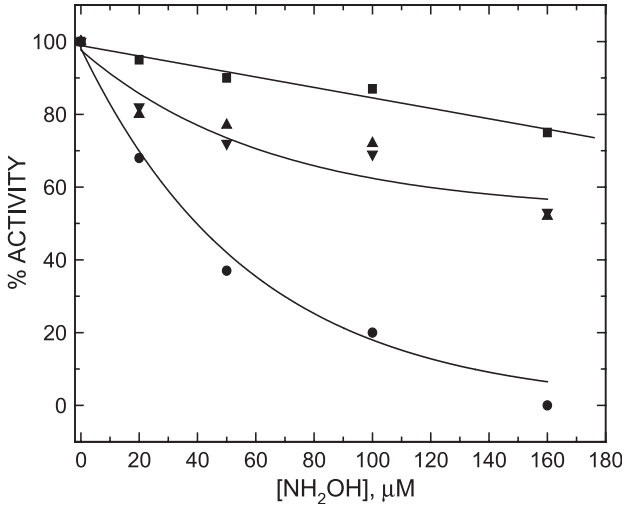

Fig. 4. Reconstitution of Ca^{2+} to the OEC restores resistance to NH_2OH inhibition. Samples of pH 3-treated, Ca^{2+} reconstituted (at 4 or 22 °C) samples were exposed to for 1 h to the concentrations of NH_2OH shown. Assays were carried out as described in Fig. 1. Control (100%) rates (μmol O_2/h/mg Chl) are: intact PSII (■), 560; pH 3-treated PSII (●), 275; pH 3-treated PSII reconstituted with Ca^{2+} at 4 °C (▲), 310; reconstituted at 22 °C (▼), 325.

23], appropriate conditions of ionic strength permit Ca^{2+} rebinding to occur with a high affinity ($K_d = \sim 6$ μM, Fig. 1B) that may approximate the actual Ca^{2+} K_d of the OEC site in vivo. Temperature accelerates Ca^{2+} rebinding, but the affinity ($K_d = 2.4$ mM) is not lowered to the extent observed with increased ionic strength. Reductant probing of the structure of PSII suggests that at 22 °C, the OEC exists in a more open conformation than at 4 °C, which would explain the accelerated binding of Ca^{2+} and Sm^{3+} [26]. The failure to obtain the same high affinity binding that is seen with added Mg^{2+} (Fig. 1B) would indicate that negatively charged amino acid residues may interfere with facile Ca^{2+} incorporation into its binding site. The structural consequences of restoration of Ca^{2+} to its site in intact PSII include resistance to NH_2OH attack on the Mn cluster and enhanced binding of Cl^-.

These aspects of the Ca^{2+} interaction with PSII are relevant to current models for the role of the metal in H_2O oxidation and to its participation in the structure of the OEC. Models for the roles of Ca^{2+}, Cl^- and the Mn cluster in the OEC have included proposals that Ca^{2+} may be directly involved in Cl^- binding. One model proposes transient binding of the anion to either Ca^{2+} or a Mn atom [27], while the second [28] envisions a structure in which Cl^- is bound to both Ca^{2+} and Mn. Either proposal might be invoked to explain the effect of Ca^{2+} in enhancing Cl^- retention by PSII under steady-state illumination, but alternate explanations cannot be eliminated at this point. For example, Ca^{2+} binding might shift the OEC structure to a form that retains Cl^- more avidly at a site that does not involve direct binding of the anion to Ca^{2+}. Further experimentation is needed to clarify this issue.

The enhancement of Ca^{2+} binding at room temperature, coupled with the demonstration that the Mn cluster is open to attack by a bulky reductant at this temperature, suggests that the OEC structure may be dynamic, rather than fixed. If so, then some aspects of the relationship between the structure revealed by analyses of PSII crystals [18,19] and the actual structure of the enzyme in solution may need to be reconsidered. The estimated distance between the Mn cluster of PSII and Y_Z is about 7 Å, which could present a barrier to H-atom transfer in the mechanism of H_2O oxidation. If amino acid side chains of the OEC are mobile, it is possible that the actual distance between Y_Z and a Mn-bound OH^- or H_2O may be shorter than the distance estimated from the crystal structure.

3. Conclusion

The discovery that Ca^{2+} is required for H_2O oxidation presented a number of interesting questions. No accessible model redox systems existed to provide a basis of comparison with PSII, and the structural complexities of the enzyme system have made it difficult to probe the Ca^{2+} binding site without first inducing changes to PSII structure and activity. Nevertheless, there has been real, steady progress in defining the roles of Ca^{2+} in H_2O oxidation. In this regard, a significant portion of the recent activity can be attributed to the impact of the work of Jerry Babcock and his associates to reevaluate mechanisms of oxygen evolution in terms of binding and sequential oxidation of substrate H_2O. While the final impact of this work is yet to be known, it is clear at this time that Jerry's insights and research will heavily influence our thinking, and our planning and interpretation of experiments, for some time to come.

Acknowledgements

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References

[1] D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, Calcium reconstitutes high-rates of oxygen evolution in polypeptide depleted photosystem II preparations, FEBS Lett. 167 (1984) 127–130.
[2] M. Miyao, N. Murata, Calcium ions can be substituted for the 24-kDa polypeptide in photosynthetic oxygen evolution, FEBS Lett. 168 (1984) 118–120.
[3] D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, Structure of the oxygen-evolving complex of photosystem II: calcium and lanthanum compete for sites on the oxidizing side of photosystem II which control the binding of water-soluble polypeptides and regulate the activity of the manganese complex, Biochim. Biophys. Acta 809 (1985) 173–180.
[4] C.M. Waggoner, V. Pecoraro, C.F. Yocum, Monovalent cations (Na^+, K^+, Cs^+) inhibit calcium activation of photosynthetic oxygen evolution, FEBS Lett. 244 (1989) 237–240.
[5] J.S. Vrettos, D.A. Stone, G.W. Brudvig, Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: evidence for direct involvement of Ca^{2+} in O_2 formation, Biochemistry 40 (2001) 7937–7945.
[6] T.-A. Ono, A. Rompel, H. Mino, N. Chiba, Ca^{2+} function in photosynthetic oxygen evolution studied by alkali metal cation substitution, Biophys. J. 81 (2001) 1831–1840.
[7] A. Boussac, A.W. Rutherford, Nature of the inhibition of the oxygen-evolving enzyme of photosystem II induced by NaCl washing and reversed by the addition of Ca^{2+} or Sr^{2+}, Biochemistry 27 (1988) 3476–3483.
[8] V.L. Pecoraro, M.J. Baldwin, M.T. Caudle, W.Y. Hsieh, N.A. Law, A proposal for water oxidation in photosystem II, Pure Appl. Chem. 70 (1998) 925–929.
[9] A.W. Rutherford, Photosystem II, the water-splitting enzyme, Trends Biochem. Sci. 14 (1989) 227–232.
[10] C.F. Yocum, Calcium activation of photosynthetic water oxidation, Biochim. Biophys. Acta 1059 (1991) 1–15.
[11] K.L. Westphal, N. Lydakis-Simantiris, R.I. Cukier, G.T. Babcock, Effects of Sr^{2+} substitution on the reduction rates of Y_Z in PSII membranes-evidence for concerted hydrogen-atom transfer in oxygen evolution, Biochemistry 39 (2000) 16220–16229.
[12] R. Mei, C.F. Yocum, Comparative properties of hydroquinone and hydroxylamine reduction of the Ca^{2+}-stabilized O_2-evolving complex of photosystem II—reductant-dependent Mn^{2+} formation and activity inhibition, Biochemistry 31 (1992) 8449–8454.
[13] M.J. Latimer, V.J. Derose, I. Mukerji, V.K. Yachandra, K. Sauer, M.P. Klein, Evidence for the proximity of calcium to the manganese cluster

of photosystem II-determination by X-ray absorption spectroscopy, Biochemistry 34 (1995) 10898–10909.
[14] R.M. Cinco, J.H. Robblee, A. Rompel, C. Fernandez, V.K. Yachandra, K. Sauer, M.P. Klein, Strontium EXAFS reveals the proximity of calcium to the manganese cluster of oxygen-evolving photosystem II, J. Phys. Chem., B 102 (1998) 8248–8256.
[15] P.J. Riggs-Gelasco, R. Mei, D.F. Ghanotakis, C.F. Yocum, J.E. Penner-Hahn, X-ray absorption spectroscopy of calcium-substituted derivatives of the oxygen-evolving complex of photosystem II, J. Am. Chem. Soc. 118 A (1996) 2400–2410.
[16] R.M. Cinco, K.L.M. Holman, J.H. Robblee, J. Yano, S.A. Pizarro, E. Sauer, K. Sauer, V.K. Yachandra, Calcium EXAFS establishes the Mn-Ca cluster in the oxygen-evolving complex of photosystem II, Biochemistry 41 (2002) 12928–12933.
[17] W.J. Chen, Y. Ta, The EXAFS study on the local structure of lanthanum in spinach PSII, Biol. Trace Elem. Res. 82 (2001) 231–237.
[18] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, Crystal structure of photosystem II from Synechococcus elongatus at 3.8 angstrom resolution, Nature 409 (2001) 739–743.
[19] N. Kamiya, J.-R. Shen, Crystal structure of oxygen-evolving photosystem II from Thermosynechococcus vulcanus at 3.7-Å resolution, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 98–103.
[20] D.F. Ghanotakis, J.N. Topper, G.T. Babcock, C.F. Yocum, Water-soluble 17-kDa and 23-kDa polypeptides restore oxygen evolution activity by creating a high-affinity binding site for Ca^{2+} on the oxidizing side of photosystem II, FEBS Lett. 170 (1984) 169–173.
[21] P. Adelroth, K. Lindberg, L.E. Andreasson, Studies of Ca^{2+} binding in spinach photosystem II using $^{45}Ca^{2+}$, Biochemistry 34 (1995) 9021–9027.
[22] T.A. Ono, Y. Inoue, Discrete extraction of the Ca atom functional for O_2 evolution in higher plant photosystem II by a simple low pH treatment, FEBS Lett. 227 (1988) 147–152.
[23] K.A. Vander Meulen, A. Hobson, C.F. Yocum, Calcium depletion modifies the structure of the photosystem II O_2-evolving complex, Biochemistry 41 (2002) 958–966.
[24] I.H. Segal, Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems, Wiley, New York, NY, 1993. 977 pp.
[25] G.C. Chen, G.M. Cheniae, High affinity binding of the Ca^{2+} essential for O_2 evolution is dependent on the existence of the Mn-cluster but it independent of the extrinsic proteins, in: P. Mathis, P. Ed (Eds.), Photosynthesis: From Light to Biosphere, vol. II, Kluwer Academic Publishing, Drodrecht, 1995, pp. 329–332.
[26] T. Ono, Effects of lanthanide substitution at Ca^{2+}-site on the properties of the oxygen evolving center of photosystem II, J. Inorg. Biochem. 82 (2000) 85–91.
[27] C.W. Hoganson, G.T. Babcock, A metalloradical mechanism for the generation of oxygen from water in photosynthesis, Science 277 (1997) 1953–1956.
[28] J.S. Vrettos, J. Limburg, G.W. Brudvig, Mechanism of photosynthetic water oxidation: combining biophysical studies of photosystem ii with inorganic model chemistry, Biochim. Biophys. Acta 1503 (2001) 229–245.