Hypothesis

Coordination sphere and structure of the Mn cluster of the oxygen-evolving complex in photosynthetic organisms

Boris K. Semin[a], Fritz Parak[b,*]

[a]*Biophysics Department, Biological Faculty, Moscow State University, Moscow 119899, Russian Federation*
[b]*Fakultät für Physik E17, Technische Universität München, 85747 Garching, Germany*

Received 8 November 1996

Abstract The great similarity between the binding of Fe(II) and the high-affinity Mn-binding site in the Mn-depleted PSII membranes (Semin et al. (1996) FEBS Lett. 375, 223–226) suggests that the coordination sphere of Mn in PSII is also suitable for iron. A comparison is performed between the primary amino acid sequences of D1 and D2 and diiron-oxo enzymes with the function of oxygen activation. All conservative motifs (EXXH) and residues binding and stabilizing the diiron cluster in diiron-oxo enzymes have been found in the C-terminal domains of D1 and D2 polypeptides. On the basis of these sequence similarities we suggest a structural model for the manganese cluster in the oxygen-evolving complex.

Key words: Photosystem II; Oxygen-evolving complex; Manganese complex; Iron; Binuclear iron protein

1. Introduction

The photosynthetic water cleavage takes place within a multicomponent pigment-protein complex called photosystem II (PSII). The active centre for the charge accumulation and the water oxidation is a tetranuclear Mn cluster located on the lumenal side of PSII (for reviews of PSII, see [1–3]). The structure of the Mn complex is still the subject of numerous investigations. Recently, EXAFS studies on the Mn cluster have provided evidence for two Mn-Mn distances of 2.7 Å and 3.3 Å in the S1 state [4,5]. These data have been explained by a model in which two di-μ-oxo bridged Mn binuclear structures with a Mn-Mn separation of 2.7 Å are linked by mono-μ-oxo and mono- or dicarboxylato bridges yielding a Mn distance of 3.3 Å [6,7].

However, the nature of the terminal amino acid ligands involved in the coordination of the Mn cations and the location in the subunit(s) of PSII are still essentially unknown. EXAFS studies indicate that either O or N atoms form the coordination sphere of the Mn cluster [4]. The participation of histidine as a potential terminal ligand has been revealed in experiments with electron spin echo envelope modulation [8]. The involvement of carboxylate residues and histidines in the coordination of Mn has also been shown using chemical modification of the amino acids [9–12]. Several glutamate, aspartate and histidine residues of D1 and D2 have been identified as potential ligands of Mn by site-directed mutagenesis of PSII (for reviews see [1–3]).

In a preceding work [13] we used Fe(II) and Fe(III) as probes for the high-affinity Mn-binding site of PSII. Using the diphenylcarbazide/2,6-dichlorophenolindophenol assay we studied the interaction of these cations with the high-affinity Mn-binding site in Mn-depleted PSII membranes. Fe(II) binds to the Mn site in the same way as Mn(II). The following common peculiarities of the interaction of these cations with the Mn-binding site were found: (i) the effective concentration for binding is smaller than 1 μM; (ii) the binding is accompanied by the oxidation of the cations; (iii) the affinity of Mn(II) and Fe(II) to this site changes in the presence of different anions in the same manner; (iv) reductants inhibit the binding of Mn(II) and Fe(II) [13]; (v) the ligation of Fe(II) is a light-dependent process leading to the formation of a binuclear 2 Fe(III) cluster, as in the case of Mn(II) [14,15].

The great similarity between the interaction of Fe(II) and Mn(II) with the Mn-binding site in Mn-depleted PSII membranes suggests that the coordination sphere of Mn in PSII is also suitable for iron. This could be very important for the identification of Mn-binding ligands. There exist iron-containing proteins (superoxide dismutase, ribonucleotide reductase, dioxygenase, acid phosphatase [16]) which can perform the same biological function with Mn instead of Fe. For some of them the structure of the metal-binding centre has been determined and can be used to obtain information on the ligation of Mn in PSII. Taking into account the formation of a diiron cluster during the binding of Fe(II) to the Mn-binding site of the oxygen-evolving complex (OEC) we used in our study the enzymes with a binuclear iron centre such as ribonucleotide reductase. We compare the iron-binding domains in the sequence of these polypeptides with sequences of the polypeptides D1, D2, which are thought to bind preferably the Mn cluster (for review see [1]).

2. Results and discussion

Examples of diiron-oxo enzymes are ribonucleotide reductase (R2 component), methane monooxygenase (MMOH), and stearoyl-ACP desaturase (for a review see [17]). These enzymes contain two main conserved motifs, EXXH, participating in the binding of the two irons [18]. These motifs can also be found in the carboxy-terminal domains of the D1 and D2 polypeptides of PSII (Fig. 1). The D1 polypeptide contains the region $E^{329}VMH^{332}$, and the D2 polypeptide contains a similar region $D^{334}QPH^{337}$. These motifs are conserved in 38 sequences of the D1 polypeptide and in 15 sequences of the D2 polypeptide [19]. In D2 the motif contains aspartate instead of glutamate but this is an insignificant difference because both contain carboxyl groups. Thus, these two motifs exactly correspond to those providing the coordination of the iron cluster in R2, MMOH and desaturase [18]. Two copies of

*Corresponding author.

PII S0014-5793(96)01345-2

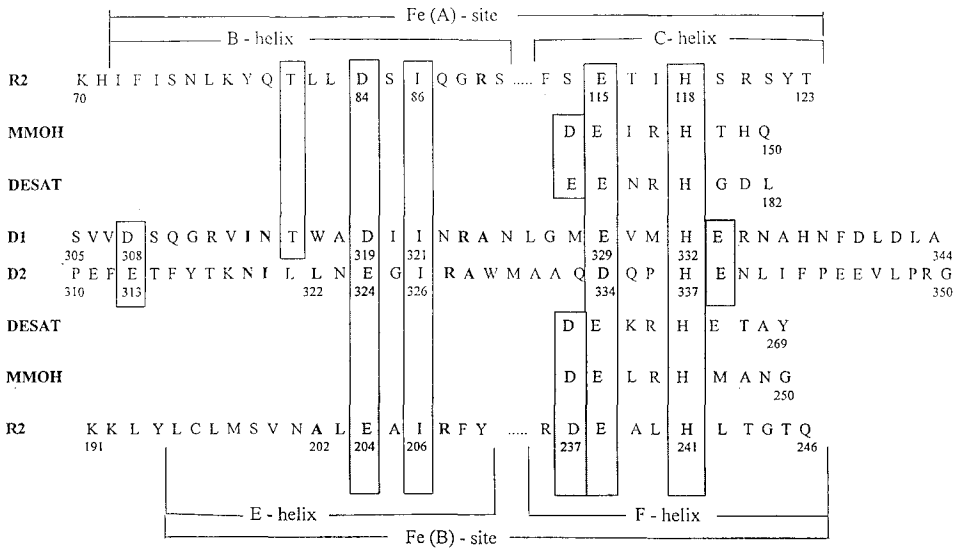

Fig. 1. Comparison of the amino acid sequences of D1, D2 with the Fe_A and Fe_B sites of diiron-oxo enzymes. The sequences of the D1 and D2 polypeptides are aligned relative to the conserved EXXH motif in the C domains of the D1/D2 heterodimer of *Spinacia oleracea* [19]. We also show the amino acid sequences of the iron-binding motifs of diiron-oxo proteins: *E. coli* ribonucleotide reductase R2 protein (R2) [20], *M. capsulatus* methane monooxygenase (MMOH) [21] and castor bean stearoyl-ACP desaturase (Desat) [18]. Iron ligands and hydrogen-bonding partners to the cluster in diiron-oxo proteins and the corresponding residues in the D1, D2 polypeptides are enclosed in shaded boxes. Residues that are identical in D1/D2 subunits and D1/R2 or D2/R2 are boxed. Bold type residues represent amino acids that have a reversible position (a pair IN in D1/D2) or dismissed in one position relative to each other.

the primary sequence motif EXXH provide four of the cluster coordination in R2 [20], MMOH [21] and desaturase [18].

Two other ligands in the class II diiron-oxo proteins are provided by aspartate or glutamate residues preceding the EXXH sequence. In R2 these are D^{84} and E^{204}. A comparison of the primary sequences of the C-terminal domains D1 and D2 polypeptides which were aligned relative to the conserved motifs EXXH reveals two symmetrically located residue pairs preceding the EXXH by 9 and 20 residues respectively (Fig. 1). These are D^{319}/E^{324} and D^{308}/E^{313}. A comparison with domains in the B and E helix respectively which contain the fifth and sixth coordination for the iron cluster in R2 implies that D^{319} of D1 and E^{324} of D2 coordinate the dimanganese cluster. This suggestion is strengthened by the following observations: (i) in R2 after D^{84} and E^{204} there are the residues I^{86} and I^{206} [20]. Likewise, in the same position relative to D^{319} (D1) and E^{324} (D2) one finds I^{321} and I^{326}; (ii) in the E helix of R2, A^{202} or L^{202} is located before E^{204}. The L^{322} is found in the same position relative to E^{324} of D2. The residues I^{321} and I^{326} are conserved in all sequences of the D1 polypeptide besides blue-green alga and L^{322} is conserved in all 15 sequences of D2 polypeptide published in [19].

The comparison shows that the C-terminal domains of D1 and D2 contain all key residues for the coordination of the iron in the diiron-oxo proteins such as R2, MMOH and desaturase. It is interesting that the similarity of the amino acid sequence around the key ligands is closer to R2 which function is connected with the generation of a stable tyrosyl radical [22]. Moreover, an analogy has been made between the function of OEC and oxygen-consuming proteins including ribonucleotide reductase [23,24].

Besides the residues which serve as the iron coordinations diiron-oxo proteins also contain partners performing hydrogen bonding to the cluster. The hydroxylase of MMOH, desaturase and helix F of R2 (Fig. 1) have D/E residues yielding the motifs DEXXH and EEXXH respectively as conserved sequences in these proteins. These aspartate/glutamate stabilise the coordinated histidines by hydrogen bonding. The conserved D^{237} from helix F of R2 (*E. coli*) forms a hydrogen bond to the iron ligand H^{118} in the C-helix. MMOH and desaturase contain identically conserved D^{242} and D^{261} residues respectively and also additional symmetrical identically conserved D^{143} and E^{175} from the helix C which are hydrogen-bonded to the iron ligands H^{246} and H^{265} respectively. The EXXH and the DXXH motifs from D1 and D2 do not contain these residues before E and D respectively, but have E^{333} and E^{338} after the histidines. This opposite location of the glutamates can be understood in the following way. The helices C and F of R2 containing the motifs DEXXH have opposite directions. The antiparallel arrangement determines the location of the hydrogen acceptors D^{143} and D^{242} with respect to the hydrogen donors H^{246} and H^{147}. For the C-terminal domains of the D1 and D2 chains an antiparallel direction is less probable. The membrane-spanning α-helices E of D1 and D2 are bounded by the non-haem iron through H^{272} and H^{269} [1]. The close neighbourhood of these transmembrane helices makes an identical direction of C-terminal domains rather probable. According to the model of the iron centre of MMOH the residues participating in the formation of hydrogen bonds should be disposed opposite to each other. If we consider such an arrangement, the E/D residues accepting the hydrogen can be located only on the side of the histidines. The displacement of E^{333} and E^{338} in a way to allow hydrogen bonds with histidine in a parallel arrangement of the E/DXXH motifs accentuates once more the astonishing resemblance between domains forming the coordination sphere of the diiron centre in diiron-oxo proteins and the primary sequence of the C-terminus of D1/D2 polypeptides.

As discussed, the C-terminal domains of the D1 and D2 polypeptides of PSII contain all key motifs for the coordination of the iron centre in the diiron-oxo enzymes. This explains why we were able to bind two irons to the high-affinity

Mn-binding site of Mn-depleted PSII membranes [13]. There are additional arguments which suggest that these motifs in the D1/D2 heterodimer participate in the coordination of the dinuclear centre of the tetranuclear manganese cluster: (i) mutants which terminate the D2 translation prematurely and mutants which have deletions near the C-domain of D2 have proved the involvement of this region in the formation of the OEC [3]; (ii) the replacement of H^{332} in D1 results in the loss of oxygen-evolving activity [25]; (iii) the chemical cross-linking analysis has shown that the carboxyl-terminal domains of the D1 subunit (D^{308}–A^{334}) and those of the D2 subunit (Y^{297}–L^{353}) are in close proximity [26]; (iv) the C-terminal regions of D1 and D2 are highly conserved among the oxygen-evolving species [19]; (v) the L and M subunits of the reaction centres from purple bacteria which do not evolve oxygen have significant homology in the sequence with the D1 and D2 polypeptides but a shorter carboxy-terminal extensions [27]; (vi) the LF^1 mutant of *Scenedesmus obliquus* has no post-translational cleavage of the carboxy-terminal extension of the D1 polypeptide and thus a failure to assemble a functional Mn complex [28].

In accordance with the known structures of the iron centre in the diiron-oxo proteins R2 and MMOH we suggest the following structure for the dinuclear manganese in the water-splitting system of PSII (Fig. 2). According to this model the dimanganese centre is coordinated by the two histidines H^{332} (D1) and H^{337} (D2). The carboxylate group of E^{329} (D1) builds a bridge between the Mn atoms. One glutamate, E^{324} (D2), and two aspartates, D^{319} (D1) and D^{334} (D2), occupy three coordinations. The residues E^{338} (D2) and E^{333} (D1) form a hydrogen bridge to the histidines H^{332} (D1) and H^{337} (D2) respectively. Since all diiron proteins have a mono-μ-oxo or hydroxo bridge and such a bridge has been suggested on the basis of EXAFS data [7], we include a mono-μ-oxo bridge in our model. A monocarboxylato bridge can be also formed by D^{334} (D2). A slightly greater similarity between the D1 primary sequence and the B-C helices in which the E^{115} forms the carboxylato bridge (see Fig. 1) stimulates us to take the E^{329} of D1 as the bridging ligand. An involvement of histidine in the ligation of manganese in the OEC has been shown [8]. There it is suggested that the histidine ligand could play a role in the proton conduction from oxidised water to the luminal phase. Thus, the pairs H^{332} (D1)–E^{33} (D2) and H^{337} (D2)–E^{333} (D1) in our model could be part of a 'proton channel' for this proton transfer.

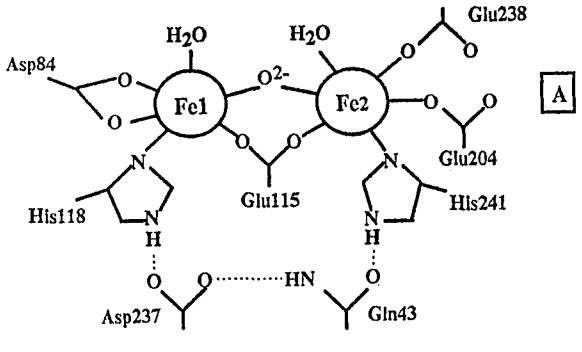

Fig. 2. A schematic representation of the coordination of oxo bridged diferric clusters and the proposed dimanganese centre of the water splitting system. A: R2 component of ribonucleotide reductase from *E. coli* [20]; B: D1/D2 reaction centre of PSII from spinach.

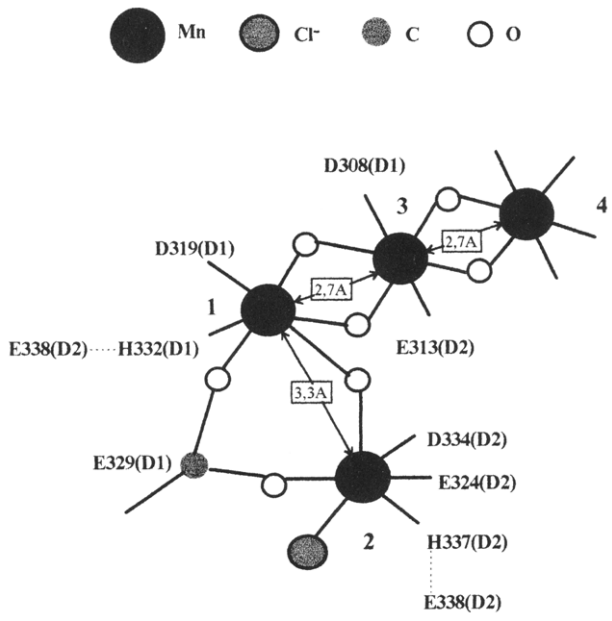

Fig. 3. Proposed model for the Mn complex in PSII. In this figure we place the halide ligand in the position of sixth ligand to Mn2 according to the model constructed by Yachandra et al. from EXAFS measurements [7].

According to the Mn distances obtained by EXAFS the Mn cluster consists of a pair of di-μ-oxo bridged binuclear manganese clusters linked by a mono-μ-oxo, mono- or dicarboxylato bridge [7]. This means that the binuclear cluster proposed by us can be a part of the structure suggested by Yachandra et al. [7]. The question is then the arrangement of the other two manganese atoms. EXAFS implies the existence of two di-μ_2-oxo bridged Mn binuclear structures and this fact has to be taken into account. The Mn atoms in our model are unsymmetrical: one of them has only one free coordination (Mn2), the other has two coordinations (Mn1 in Fig. 2). This implies that the sequence of distances between the Mn atoms cannot be 2.7 Å–3.3 Å–2.7 Å. Instead, the Mn sequence has to be 3.3 Å–2.7 Å–2.7 Å (compare Fig. 3). Such an arrangement of Mn atoms was not ruled out on the basis of EXAFS [7]. Our arrangement shows that the Mn3 atom having a distance of 2.7 Å to Mn1 and Mn4 can accept only two additional ligands.

At present there exists no X-ray structure of the OEC. Only such a structure determination could finally prove or disprove our model.

In our comparison with the reaction centre of the PSII we used enzymes performing O_2 activation [18]. The similarity between oxygen-evolving and oxygen-consuming structures can be helpful for the understanding of the mechanism of oxygen abstraction from water and the development of such a mechanism during evolution.

Acknowledgements: This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

References

[1] Debus, R.J. (1992) Biochim. Biophys. Acta 1102, 269–352.
[2] Renger, G. (1993) Photosynth. Res. 38, 229–247.
[3] Vermaas, W. (1993) Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 457–481.
[4] Yachandra, V.K., Guiles, R.D., McDermott, A., Britt, R.D., Dexheimer, S.L., Sauer, K. and Klein, M.P. (1986) Biochim. Biophys. Acta 850, 324–332.
[5] MacLachlan, D.J., Halahan, B.J., Ruffle, S.V., Nugent, J.H.A., Evans, M.C.W., Strange, R.W. and Hasnain, S.S. (1992) Biochem. J. 285, 569–576.
[6] Klein, M.P., Sauer, K. and Yachandra, V.K. (1993) Photosynth. Res. 38, 265–277.
[7] Yachandra, V.K., DeRose, V.J., Latimer, M.J., Mukerji, M.J., Sauer, K. and Klein, M.P. (1993) Science 260, 675–679.
[8] Tang, X.-S., Diner, B.A., Larsen, B.S., Gilchrist, M.L. Jr., Lorigan, G.A. and Britt, R.D. (1994) Proc. Natl. Acad. Sci. USA 91, 704–708.
[9] Preston, C. and Seibert, M. (1991) Biochemistry 30, 9615–9624.
[10] Preston, C. and Seibert, M. (1991) Biochemistry 30, 9625–9633.
[11] Blubaugh, D.J. and Cheniae, G.M. (1992) Photosynth. Res. 34, 147.
[12] Hsu, B.-D., Lee, J.-I. and Pan, R.-L. (1987) Biochim. Biophys. Acta 890, 89–96.
[13] Semin, B.K., Ivanov, I.I., Rubin, A.B. and Parak, F. (1995) FEBS Lett. 375, 223–226.
[14] Tamura, N. and Cheniae, J.M. (1987) Biochim. Biophys. Acta 890, 179–194.
[15] Miller, A.-F. and Brudvig, G.W. (1990) Biochemistry 29, 1385–1392.
[16] Ochiai, E.-I. (1990) in: General Principles of Biochemistry of the Elements (Frieden, E., Ed.) pp. 60–63, Plenum Press, New York.
[17] Vincent, J.B., Olivier-Lilley, G.L. and Averill, B.A. (1990) Chem. Rev. 90, 1447–1467.
[18] Fox, B.G., Shanklin, J., Ai, J., Loehr, T.M. and Sanders-Loehr, J. (1994) Biochemistry 33, 12776–12786.
[19] Svensson, B., Vass, J. and Styring, S. (1991) Z. Naturforsch. 46c, 765–776.
[20] Nordlund, P., Sjoberg, B.-M. and Eklund, H. (1990) Nature 345, 593–598.
[21] Rosenzweig, A.C., Frederick, C.A., Lippard, C.J. and Nordlund, P. (1993) Nature 366, 537–543.
[22] Sjoberg, B.-M. and Graslund, A. (1983) Adv. Inorg. Biochem. 5, 87–110.
[23] Barry, B.A. and Babcock, G.T. (1988) Chem. Scr. 28A, 117–122.
[24] Babcock, G.T. (1995) in: Photosynthesis from Light to Biosphere (Mathis, P., Ed.) Vol. II, pp. 209–215, Kluwer Academic, Dordrecht.
[25] Diner, B.A., Nixon, P.J. and Farchaus, J.W. (1992) Curr. Opin. Struct. Biol. 1, 546–554.
[26] Tomo, T. and Satoh, K. (1994) FEBS Lett. 351, 27–30.
[27] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[28] Seibert, M., Tamura, N. and Inoue, Y. (1989) Biochim. Biophys. Acta 974, 185–191.