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Modelling a binuclear metal binding site in the photosynthetic reaction centre II

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Based on the experimental data and homologous sites in Protein Data Bank (PDB) a model for metal binding sites in D1/D2 heterodimer has been proposed. On searching for tetranuclear and binuclear Mn binding sites in the PDB, a suitable sequence homology in thermolysin and D1 could be observed. From the homology and site-directed mutagenesis data, a model for binuclear Mn-Ca or Mn-Mn has been built and it is extended to a tetranuclear Mn centre.

The photosynthetic oxidation of water to molecular oxygen has been suggested to take place at a tetranuclear Mn-cluster, located in the reaction centre (RCII) of photosystem II (PSII)[1-5]. In addition, one Ca^{2+} ion[6] and undetermined number of Cl^- ions are also observed to be associated with the evolution of oxygen[7,8]. Mn-cluster is proposed to be located in the hetrodimer of the D1 and D2 polypeptides, which form the RCII, where primary photochemistry and charge separation takes place[4,9]. Various experimental approaches have been made to study the organization of metal cluster associated with oxygen evolution. Reconstitution of inactivated PSII, extended X-ray absorption fine structure (EXAFS), electron paramagnetic resonance (EPR), site-directed mutagenesis and chemical modification of amino acids are some of the tools employed for the study. Site-directed mutagenesis studies have indicated that the potential ligands for Mn^{2+} are from the D1/D2 heterodimer[10-13].

Photo activation of PSII to restore molecular oxygen evolution in leaves require both Mn^{2+} and Ca^{2+} ions[14]. The differential pool of Mn^{2+} ions in the binding site can also be observed during the release of Mn^{2+} ions upon Tris-washing, heat shock, alkaline *p*H or NH_2OH treatment[15,16].

EXAFS studies indicate that either O or N atoms form the co-ordination sphere of the Mn-cluster[17,18]. It also provides evidence for the Mn-Mn distances of 2.7 Å and 3.3 Å in the S1-state. 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 a mono-μ-oxo and mono- or di-carboxylato bridges yielding a Mn-Mn distance of 3.3 Å (ref.19,20).

Ca^{2+} ions are proposed to be closely associated with Mn-cluster and the bond distance is 3.7 Å (ref.18). Such a close distance between Ca^{2+} ions and Mn-cluster implies an intimate role of these ions in the oxidation of water. Besides these data, the chemical cross linking analysis has shown that the carboxy-terminal domains of the D1-subunit (D308-A344) and those of the D2-subunit (Y297-L353) are in close proximity to Mn-cluster[21]. The LF^1 mutant of *Scenedesmus obliquus* has no post-translational cleavage of the carboxy-terminal extension of the D1-polypeptide and thus, it cannot assemble a functional Mn-complex[22].

We have searched the Protein Data Bank (PDB) for tetranuclear Mn-cluster containing proteins. An inorganic phosphatase (1WGJ) contains four Mn per unit. Thermolysin (1LNC) contains three Mn and one Ca per unit. In 1LNC Mn-Mn distances are between 17-28 Å. In 1WGJ only two Mn-Mn distances are 3.7 Å and 3.6 Å but none at 2.7 Å. It has μ-carboxylato, μ-phosphato and μ-oxo bridges. However, there is a Mn-Ca distance of 3.7 Å in 1LNC with tri-μ-carboxylato bridge. We also searched for binuclear Mn-containing proteins. The proteins, namely fructose 1,6 bis phosphatase (1FBD), ribonucleotide reductase substituted with Mn (1MRR) and arginase (1RLA), have Mn-Mn distances 3.7 Å, 3.7 Å and 3.3 Å respectively. Models for Mn dimers can be built based on the structures of these sites. Semin and Parak have built a model for Mn-cluster based on the Mn-cluster of 1MRR[22]. In most of these cases, the ligating amino acids are from different distant locations in the sequence except in 1MRR, 1RLA and 1LNC. In 1MRR, two pairs of amino acids in the sequence DXXH (D and H represent aspartic acid and histidine respectively as per the single letter symbols

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for the amino acid residues whereas X denotes any amino acid) participate in metal binding. Such a tetrapeptide sequence is found both in D1 and D2 polypeptides. In 1RLA, DXHXXXDXDXD sequence is involved in metal binding. But such a sequence is neither found in D1 nor in D2 polypeptides. Interestingly, 1LNC shows a continuous segment providing most of the ligands to Mn-Ca pair and homologous sequence is found in D1 C-terminal loop, E333-A344, which may serve as a binuclear metal binding site (Fig. 1). Further, E189, E333, D342 and H337 of D1 are suggested by site-directed mutagenesis studies to be ligands to Mn-cluster and are essential for molecular oxygen evolution[10,11]. Information from site-directed mutagenesis and homology in PDB suggest that E333-A344 segment is suitable for modelling a binuclear metal binding site in RCII. Therefore, in the present paper, we have modelled the metal binding site based on Mn-Ca binding site in 1LNC.

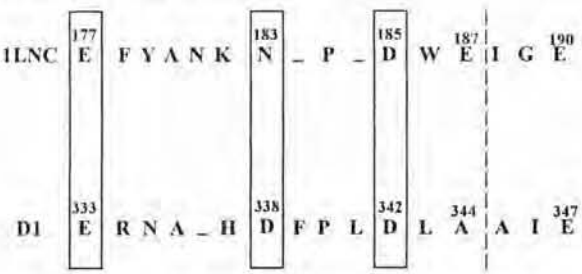

Fig. 1—Sequence alignment of metal binding sites in 1LNC and proposed site in D1 protein of PSII. [Boxes indicate ligand forming conserved residues. Dotted vertical line indicates cleavage site in post-translational modification of D1.]

The Ca^{2+} ions in 1LNC ligate to the carboxylate group of glutamate residues E177 and E190, aspartate residues D138 and D185, water (OW346) and backbone carbonyl of glutamate residue E187. The Mn^{2+} ions in 1LNC ligate to carboxylate group of glutamate residues E177 and E190, aspartate residue D185, carbonyl of the amide group of asparagine N183 and two molecules of water, OW353 and OW475. E177, E190 and D185 form tri-μ-carboxylato bridges between Ca^{2+} and Mn^{2+} ions.

During the post-translational modification, peptide sequence A345-G353 of the D1 polypeptide is cleaved. It renders A344 as the terminal residue. Therefore, the terminal carboxylate group of A344 of the D1 polypeptide may be one of the ligands in homology with the main chain carbonyl group of E187 in 1LNC. E333 and D342 may form di-μ-carboxylato bridge in homology with E177 and D185 of 1LNC (Fig 2a). The rest of the secondary valencies of the metal ion may be satisfied either by side chain O atom of D, E, N, Q, or N atom of H in the vicinity either from D1 or D2 polypeptides, or by Cl^- ions or water molecules. In the absence of E347 homologous with E190, an oxo-bridge may be incorporated in the model. E69 in cyanobacteria (and E70 in higher plants) of D2 is also suggested to be a ligand to Mn-cluster[23]. Therefore, E70 may be one of the ligands to Mn-cluster.

The Ca^{2+} ions are known to be substituted by Mn^{2+} ions in proteins without significant change in the structural parameters. Mn^{2+} ion is substituted in place of Ca^{2+} ion in troponin C (1NCY) and intestinal

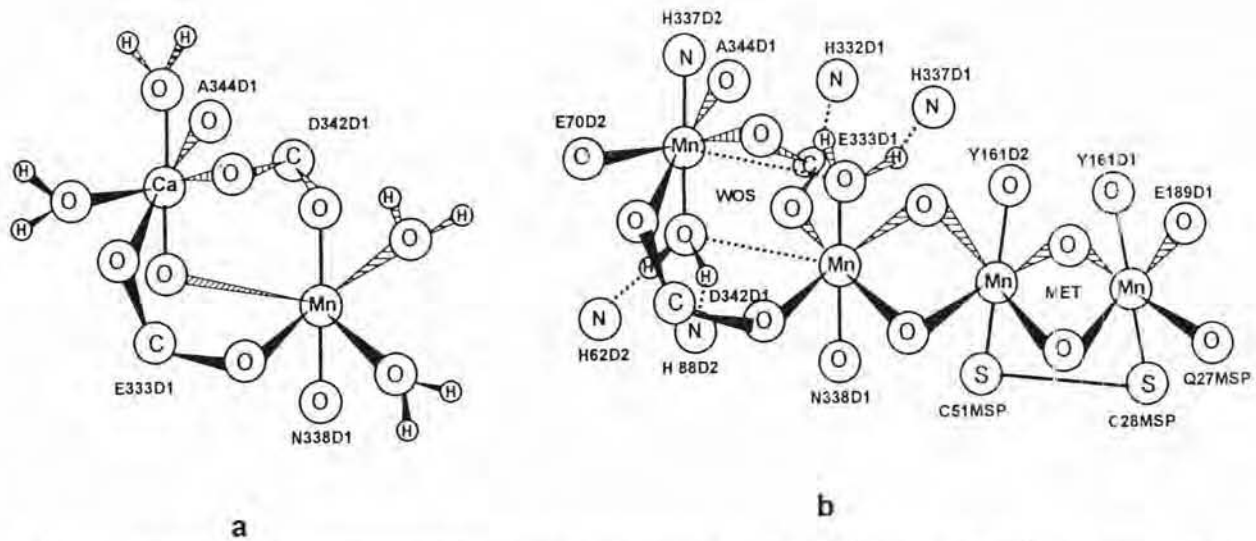

Fig. 2—(a): Mn-Ca binuclear model constructed based on the structure of 1LNC. (b): Mn tetranuclear model by extension of substitution of Mn in place of Ca and modification of the model of Raval and Biswal[24]. [MSP, 33kDa extrinsic manganese stabilizing protein; MET, manganese electron trap; WOS, water oxidizing site.]

calcium binding protein (6ICB). Hence, a Ca^{2+} ion may be replaced by a Mn^{2+} ion in the model and it can be a part of the model tetranuclear Mn-cluster[24] (Fig. 2b).

Further information from site-directed mutagenesis in terms of ligands to Mn^{2+} or Ca^{2+} ions would be needed to build a model for Mn-Ca or Mn-tetranuclear centre of RCII.

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