Identification of a Calcium-Binding Site in the PsbO Protein of Photosystem II[†]

James W. Murray and James Barber*

Wolfson Laboratories, Division of Molecular Biosciences, Faculty of Life Sciences, South Kensington Campus, Imperial College, London SW7 2AZ, U.K.

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ABSTRACT: Analysis of the anomalous X-diffraction data reported by Ferreira et al. (PDB entry 1S5L) for crystals of photosystem II isolated from *Thermosynechococcus elongatus* indicates that a calcium ion is bound to the PsbO protein. The Ca^{2+}-binding site is located close to the lumenal exit of a putative proton channel leading from the water splitting site.

The PsbO protein, often called the 33 kDa protein, forms a part of the oxygen-evolving complex (OEC) of photosystem II (PSII). It is ubiquitous to all known forms of PSII whether from eukaryotic or prokaryotic photosynthetic organisms (*1*). The main function of this protein is to stabilize the cluster of four manganese ions that form the catalytic center of the OEC where water splitting occurs, and it is often termed the "manganese stabilizing protein" (*2*). Recently, the complete structure of the PsbO protein of *Thermosynechococcus elongatus* was obtained by X-ray crystallography as part of the intact PSII complex (*3*). Previous X-ray structures of PSII had not elucidated the full structure of the PsbO (*4*, *5*), although the most recent (*6*) confirms the model presented in ref *3*. The X-ray diffraction studies have shown that the PsbO protein consists of a β-barrel composed of eight antiparallel β-strands with a large loop joining β-strands 5 and 6 (β5−6) and a smaller loop linking strands β1 and β2 (β1−2). The β-barrel is the body of the protein and is ∼40−45 Å long and 15−20 Å wide, while the β5−6 loop forms an extended "head" domain 25−30 Å in length. This head domain is involved in binding of PsbO to the lumenal surface of the PSII complex via the hydrophilic surface of the intrinsic PSII reaction center subunit proteins D1 and D2 and chlorophyll-binding proteins CP43 and CP47. Also involved in this binding are the β1−2 loop and the N-terminal domain of PsbO (*7*).

Several of the strands of the β-barrel are not continuous, and its central part is full of bulky hydrophobic residues, including seven phenylalanines (F50, F95, F120, F142, F146, F215, and F239), typical for the core of a "folded" protein. The head domain consists mainly of nonregular loops and turns with a well-defined α-helix and some β-structures. The long axis of the protein is oriented ∼40° to the membrane plane with its C- and N-termini at its lumenal end and separated by ∼20 Å.

It has been suggested many times that the PsbO protein not only stabilizes the Mn ions that make up the catalytic center of the OEC but also optimizes the available Ca^{2+} and Cl^- which are required for the oxygen-evolving reaction (reviewed in refs *8* and *9*). Indeed, X-ray crystallography has clearly shown that Ca^{2+} forms a part of the catalytic center of the OEC (*3*), as anticipated from a range of other studies (*10*−*12*). It has also been suggested that the PsbO itself is a Ca^{2+}-binding protein, and a number of studies support this notion, ranging from isotope binding experiments (*13*, *14*) to those that monitor the effects of Ca^{2+} on the conformational state of the isolated protein assayed by UV−CD and FTIR (*15*).

RESULTS

Here we have investigated the possibility that PsbO binds Ca^{2+} using the anomalous diffraction data of Ferreira et al. (*3*) available in the protein database. Ferreira et al. (*3*) collected anomalous diffraction data at 5.5 keV and used this to identify a Ca ion closely associated with the Mn cluster of the OEC. It was proposed that this Ca^{2+} formed a cubane-like structure with three Mn ions via oxo bridges. The fourth Mn was suggested to be linked to the $Mn_3Ca^{2+}O_4$ cubane by μ-oxo bridging. This same data set also reveals a strong Ca^{2+} signal within the PsbO, not previously discussed by Ferreira et al. (*3*) (see Figure 1a). The mean σ value over the two NCS-related sites in PsbO is 13.4. This value contrasts with the weaker average value of 5σ for the Ca^{2+} in the OECs. The ligands for this Ca^{2+}-binding site are Glu54, Glu114, and His231 (Figure 1b).

The X-ray structure of intact PSII elucidated by Zouni et al. (*4*) identified a Cd^{2+}-binding site very close to the Ca^{2+}-binding site reported in this work. However, because of the limited resolution of the earlier work, the model for the PsbO protein was incomplete and contained only the α-carbon atoms. Our work therefore shows the coordination of the metal-binding site within the PsbO protein. It is also consistent with speculation by Rutherford and Faller (*16*) that the Cd^{2+}-binding site identified by Zouni et al. (*4*) could be a Ca^{2+}-binding site under physiological conditions. In the same paper, Rutherford and Faller (*16*) also proposed that the β-barrel of the PsbO protein might provide a water/proton channel to and from the catalytic site of the OEC. However,

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* To whom correspondence should be addressed: Wolfson Laboratories, Division of Molecular Biosciences, Faculty of Life Sciences, South Kensington Campus, Imperial College, London SW7 2AZ, U.K. Phone: 44 207 594 5266. Fax: 44 207 594 5267. E-mail: j.barber@imperial.ac.uk.

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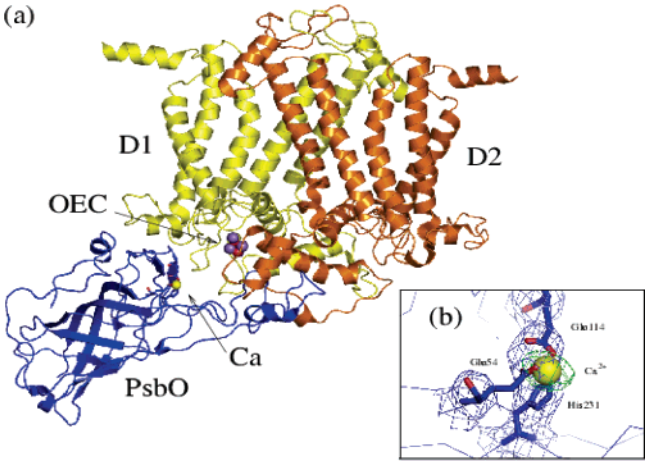

FIGURE 1: Identification of a Ca^{2+}-binding site in the PsbO protein. (a) Structure and special relationship of the PsbO (blue), D1 (yellow), and D2 (orange) proteins of PSII shown as cartoon views. The position of the metal cluster of the oxygen-evolving center (OEC) containing four Mn ions and a Ca^{2+} is shown, as is the location of a Ca^{2+} bound to a specific site in the PsbO protein. (b) Details of the Ca^{2+}-binding site in the PsbO protein showing the ligating residues: Glu54, Glu114, and His231 (numbering as in PDB entry 1S5L). Based on the X-ray structure of Ferreira et al. (*3*) and coordinates available for PDB entry 1S5L. The blue mesh shows a σ_A weighted $2F_o - F_c$ map contoured at 1σ, and the green mesh shows the anomalous difference map at 5.5 keV contoured at 5σ.

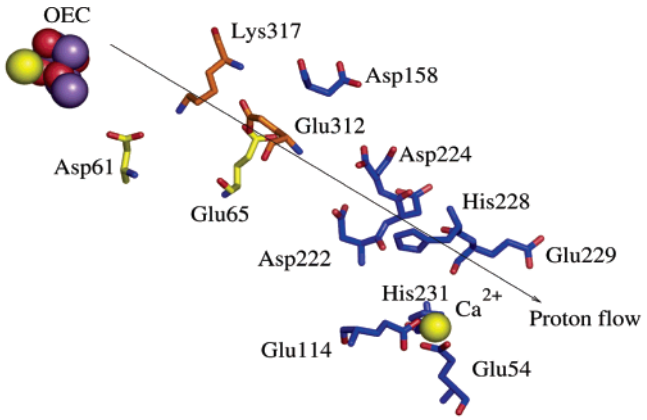

FIGURE 2: Position of the Ca^{2+} bound to the PsbO protein of PSII in relation to a putative proton channel suggested by Ferreira et al. (*3*) and emphasized in the detailed structural analysis of PsbO (*7*). The suggested proton channel leads from the catalytic site of the OEC, indicated as a cluster of four Mn ions (magenta), one Ca^{2+} ion (yellow), and four bridging oxygen atoms (red), to the lumenal surface where the PsbO-bound Ca^{2+} is located as shown.

the X-ray structure of Ferreira et al. (*3*) identified a polar channel starting with D1 Asp61 close to the water-splitting/oxygen-evolving site (Figure 2). The channel proceeds over a distance of ~15 Å toward the docking site of the PsbO protein involving D1 Glu65, D2 Glu312, and D2 Lys317. The channel then continues an additional 20 Å along a hydrophilic pathway which traverses the "neck" region of the PsbO protein, consisting of Asp158, Asp222, Asp223, Asp224, His228, and Glu229, at which point it exits into the lumen. As shown in Figure 2, the Ca^{2+}-binding site identified here is located very close to the lumenal exit site of this channel. Whether it binds Ca^{2+} or another metal ion under physiological conditions has yet to be clarified. Trimethyllead acetate was present in the crystallization experiment, but the site reported here is chemically incompatible with trimethyllead. At this energy, calcium has an anomalous signal (2.45 e^-) similar to those of potassium (2.1 e^-) and zinc (1.3 e^-). However, no potassium or zinc was present in the crystallization buffers or during the purification of the complex, whereas calcium ions were present at a concentration of 10 mM.

DISCUSSION

Studies have shown that the binding of Ca^{2+} to the isolated PsbO protein is weak (*14*), although other studies involving the intact OEC suggest that the protein may have a high-affinity Ca^{2+}-binding site (*17*). Others suggest that perhaps the Mn is the binding cation and in so doing imposes carbonic anhydrase activity (*18*). The positioning of the metal site at the lumenal end of the water/proton channel is of particular note. This feature suggests that it may be involved in regulating the conformation of the lumenal domain of the channel or perhaps in helping to order water molecules for entry into the channel or aiding proton release to the lumen. Interestingly, a metal binding site is located at the exit of the proton channel in cytochrome oxidase involving conserved Glu, Asp, and His residues (*19*). However, of the three residues involved in the binding of Ca^{2+} to the PsbO of *T. elongatus*, only Glu114 is highly conserved; Glu54 is less conserved, while His231 is specific to *T. elongatus*, being a lysine in most other sequences. The existence of only one highly conserved Glu at this site may account for the finding that Ca^{2+} is only weakly bound to PsbO.

MATERIALS AND METHODS

The PSII structure (*3*) and structure factors were taken from the Protein Data Bank (entry 1S5L). Phases and the figure of merit (FOM) were calculated from the structure in REFMAC. Anomalous difference maps (coefficients DANO and ϕ-90) were calculated using the 1.89340 Å (6.548 keV) and 2.25430 Å (5.4997 keV) datasets previously collected corresponding to the Mn-detection and Ca-detection data sets, respectively. Maps were calculated and analyzed using programs of the CCP4 suite. The top sites in the anomalous difference map correspond to four chemical sites related by noncrystallographic symmetry to the other PSII monomer in the asymmetric unit. None of these sites correspond to peaks in the manganese map and so cannot be attributed to manganese atoms. The only significant divalent cation likely to be present was calcium. At 5.5 keV, calcium and sulfur have anomalous signals of 2.5 and 1.1 e^-, respectively (*20*). Therefore, sulfur atoms visible in the anomalous difference map served as positive controls for the accuracy of the map. Indeed, the presence of peaks at known sulfur positions and the replication of peaks at crystallographically independent NCS positions served as a positive control for the location of peaks due to metal ions.

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