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Review

A cluster of carboxylic groups in PsbO protein is involved in proton transfer from the water oxidizing complex of Photosystem II

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Abstract

The hypothesis presented here for proton transfer away from the water oxidation complex of Photosystem II (PSII) is supported by biochemical experiments on the isolated PsbO protein in solution, theoretical analyses of better understood proton transfer systems like bacteriorhodopsin and cytochrome oxidase, and the recently published 3D structure of PS II (Pdb entry 1S5L). We propose that a cluster of conserved glutamic and aspartic acid residues in the PsbO protein acts as a buffering network providing efficient acceptors of protons derived from substrate water molecules. The charge delocalization of the cluster ensures readiness to promptly accept the protons liberated from substrate water. Therefore protons generated at the catalytic centre of PSII need not be released into the thylakoid lumen as generally thought. The cluster is the beginning of a localized, fast proton transfer conduit on the lumenal side of the thylakoid membrane. Proton-dependent conformational changes of PsbO may play a role in the regulation of both supply of substrate water to the water oxidizing complex and the resultant proton transfer.

Keywords: Photosystem II; PsbO protein; Proton transfer; Cluster of carboxylic group

1. Introduction

Photosynthetic water oxidation is the primary process responsible for the production of oxygen present in the biosphere. This biological process takes place at a manganese containing centre, Mn_4CaO_X, located in the water oxidizing complex (WOC) on the lumenal side of Photosystem II (PS II) [1,2]. The oxidation of water by PS II and the subsequent electron transfer in the photosynthetic electron transport chain are accompanied by trans-thylakoid pumping of protons from the stroma to the lumen resulting in conversion of light energy to an electrochemical gradient. This gradient is energetically coupled with ATP synthesis that mainly occurs in stroma thylakoids. The light induced lowering of pH in the lumen [3,4] activates lumen localized enzymes and regulates non-photochemical quenching [5]. When the light intensity increases or the Calvin cycle slows down, the acidification of the lumen can increase relative to the demand. This may also occur when ATP synthesis is inhibited. Under such potentially destructive conditions, the WOC may become damaged resulting in a subsequent release of manganese [6,7]. Therefore a fine tuning of proton reactions in the lumen is desirable.

In higher plants and cyanobacteria at least six intrinsic proteins (D1, D2, CP47, CP43 and the two subunits of cytochrome b559) are required for oxygen evolving activity [8]. The extrinsic 33 kDa protein, encoded by the PsbO gene, is the only extrinsic subunit present in all oxygenic organisms [9]. Substituting PsbO from different photosynthetic organisms, including cyanobacteria, red algae and higher plants, did not significantly affect the reconstitution ability [10], which suggests that the PsbO structure and function are highly conserved among various oxytrophs. Somewhat in contrast, multiple sequence alignment of all known PsbO proteins revealed that only 7.7% of the sequence found in *Thermosynechococcus elongatus* is fully conserved [11].

The possible roles PsbO may play in the structure and function of PSII have received much research attention since

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the discovery of PsbO 20 years ago (see [11,12] for a review). Removal of PsbO from PSII membrane fragments by treatment with high concentration of salt or urea suppresses oxygen evolution. Such PSII, lacking the PsbO protein, requires much greater than physiological levels of calcium and chloride ions for oxygen evolution [8,13,14]. At low chloride concentrations, two of the four manganese atoms are released from PS II [15], while all four remain bound if the chloride concentration is sufficiently high [16]. Removal of the PsbO affects the turnover and stability of the higher redox states of the WOC [17]. PSII devoid of PsbO is susceptible to donor side photoinhibition [8]. DNA insertion into the PsbO gene resulted in the complete absence of mRNA and PsbO protein in *Chlamydomonas reinhardtii*. This prevented the assembly of PSII and photoautotrophic growth of the algae [18]. Although the precise role of PsbO remains uncertain, some recent suggestions have emerged from the intense research conducted on the protein. One such recent hypothesis suggests that the PsbO protein plays a key role in the GTP turnover in the lumen [19,20] but further work is needed to fully prove this hypothesis. Hiller and Wydrzynski [21] proposed an alternative function based on the fact that the rate of exchange of substrate water at the WOC is slowed by removal of the PsbO suggesting a possible role in the supply of substrate to WOC.

The PsbO protein has an elongated shape [1,2], which explains its unusual hydrodynamic properties [22]. The eight anti-parallel β-strands form a cylinder filled with hydrophobic amino acid residues [1]. It should be cautioned, however, that the hydrophobic core was proposed based on spectral properties of PsbO in solution [23–25]. The long axis of PsbO is tilted about 40° relative to the plane of the membrane with the C- and N-termini in close proximity on the lumenal side. The small distance between the C- and N-termini of the PsbO protein was proposed by cross-linking studies [26,27] and by analysis of PsbO fluorescence [24,25]. Two fully conserved cysteine residues form a disulfide bridge between the N-terminal loop and the β1 strand [1]. The reduction of the S–S bond leads to unfolding of PsbO and loss of ability to restore oxygen evolution [15]. A *Synechocystis* mutant lacking the disulfide

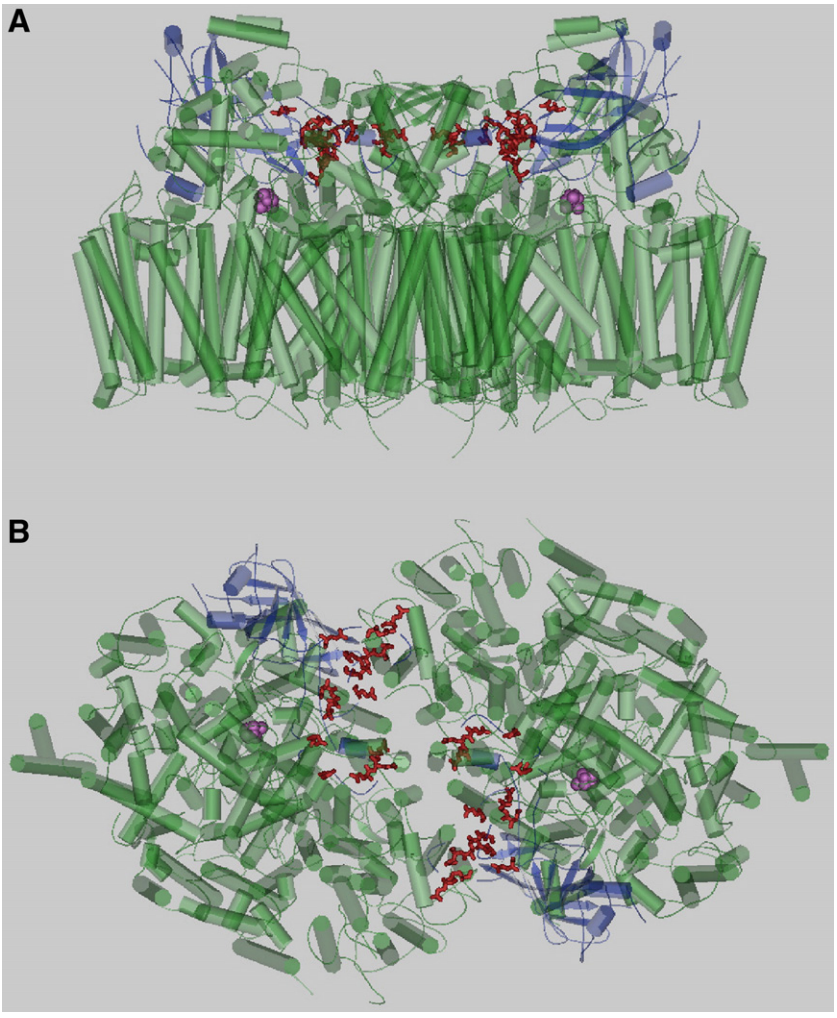

Fig. 1. Overview of the structure and location of PsbO (blue) on the lumenal side of PSII of *T. elongatus* (Pdb entry 1S5L). For the detailed structure see [1]. The proposed negatively charged cluster of carboxylic groups (CCG) in the PsbO 3D structure is shown in red whereas the Mn_4CaO_X centre as magenta spheres. This figure was generated with PyMOL Viewer. (A) Side view; (B) viewed from the lumen.

bridge in the PsbO protein (C20S substitution) accumulated the normal level of mRNA, but it failed to accumulate the protein [28]. The authors suggested that the mutated PsbO is subjected to rapid protein degradation because it does not fold and bind properly. Interestingly, findings on redox-regulated proteins from the lumen identified PsbO as a potential target for the redox regulation [29].

An NMR study on overexpressed, isolated PsbO [30] demonstrated that the PsbO protein consists of two parts: a well folded part that probably corresponds to a rigid β-barrel and another part consisting of up to 40% of the protein with a high degree of flexibility. The apparent contradiction between extraordinary stability [22] and flexibility of the PsbO protein may be explained by the existence of these two parts having very different dynamics. The β-barrel is a very stable structure that is unlikely to differ whether the protein is free in solution or associated with PSII. In contrast, the hydrophilic loops are stabilized by interaction with other PS II proteins [11] and therefore are more flexible in the absence of those interactions, i.e. when the protein is not associated with PS II. Proton dependent structural dynamics of the PsbO were analyzed with respect to lumenal pH [23,31–33]. The observed proton induced conformational changes displaying a characteristic hysteresis effect are particularly interesting and have not been observed for the other extrinsic proteins. These conformational changes are likely to be of functional relevance for photosynthetic water oxidation since they occur within the physiological pH range.

2. The cluster of carboxylic groups in the PsbO

The hysteresis was explained by protonation/deprotonation of 12 carboxylic acid residues of side chains with a pK_a as high as 5.7 (pK_a in water around 4.0). Other experiments have also indicated that side chains of PS II proteins with pK_a values around 5.7 are important for water oxidation [34,35]. The pattern of proton release from the PS II core complexes can be satisfactory simulated by deprotonation of acidic groups with a pK_a of 5.7 [34], supporting a direct participation of these residues in proton transfer. Further, the flash induced FTIR difference spectrum gives direct evidence for deprotonation of glutamic and aspartic acid residues of the PsbO with high pK_a during the S1 to S2 transition [35].

One way to explain the high pK_a values of the carboxylic groups of glutamic and aspartic acid (there is no histidine in the spinach PsbO) is to assume a small spatial distance between these groups in the protein structure. This has also been shown for carboxylic groups in side chains of other proteins. For example, the estimated pK_a of 6.36 for the E41 residue (pK_a in water 4.2) at the ligand-binding site of the rat CDd2 protein was attributed to the reciprocal interaction with a neighbouring E29 [36]. The pK_a of E41 decreased significantly in the E29Q mutant due to the mutant's lack of the negative charge present in the wild type. A similar pK_a and coupled titration behaviour of neighbouring carboxylic groups have also been observed for D10 (pK_a=6.1) and D70 in ribonuclease H1 [37] and E172 (pK_a=6.7) and E78 in xylanase [38].

We suggest that carboxylic groups with high pK_a that are responsible for the hysteresis in the spinach PsbO are in close proximity to each other and form a cluster, referred hereafter as a cluster of carboxylic groups (CCG). Based on the recently published 3D structure of PSII [1] (Pdb entry 1S5L), we identified 16 carboxylic groups of glutamic and aspartic acid in side chains of the cyanobacteria *Thermosynechococcus elongates* that are the most likely candidates for the CCG (Fig. 1).

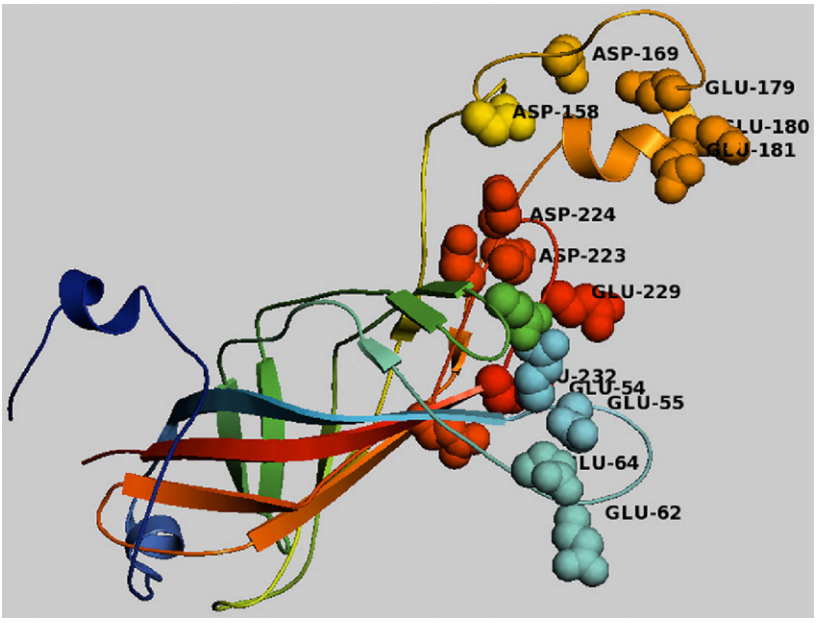

Fig. 2. Stereo ribbon diagram of the PsbO protein from *T. elongatus* (Pdb entry 1S5L) [1]. The carboxylic groups of those side chains involved in the proposed negatively charged cluster (CCG) in the PsbO 3D structure are shown as spheres.

Figs. 1A and B gives an overview of the structure and location of CCG (red) of the PsbO (blue) in PSII on the luminal side of the thylakoid membrane. The average distance between the neighbouring residues in the cluster was estimated to be 7.5± 2 Å. A more precise determination of distances was not possible considering the structure resolution of abut 3 Å [1,2]. The assumption that the hysteresis effect is caused by a cluster of neighbouring carboxylic residues in side chains is consistent with these structural data.

Fig. 2 shows the location of the proposed CCG in the PsbO protein from *T. elongatus*. The number of carboxyl groups in the PsbO from spinach was estimated to be about 12 per PsbO subunit [23] suggesting that the number of residues in the CCG may differ somewhat between cyanobacteria and higher plants (16 vs. 12 per PsbO subunit, respectively) [23]. The side chains of the CCG contain the fully conserved residues E55, E114, D158, D169, E179, D223 and D224, residues E54, E64, E229, E180 and E181 conserved among higher plants and cyanobacteria only, and also E62, E218, D222 and E232.

Most of the CCG is located in a region of the PsbO sequence with maximal hydrophilicity (see Fig. 3). Nevertheless the K159–K186 region that forms part of the CCG was found to be shielded from cross-linking in experiments where the PsbO protein was associated with PSII [39]. These results together with the 3D X-ray structure of PS II, suggest that the CCG is located in a hydrophilic pocket on the lumenal side of PSII forming a pool of bound substrate water. This would fit the data from *in situ* experiments by Hiller and Wydrzynski [21] where they clearly demonstrate that in the absence of PsbO the rate of substrate water exchange with the WOC is slowed down. Functioning of WOC requires a rather hydrophobic environment, so that shortage in substrate water might become a problem. A small cavity that is lined by CCG might provide a continuous water supply and, in the same time, affect the overall electrostatics of the WOC only marginally. As electrostatic interactions are long-range ones, they are determined by the "average" polarity that would remain low, being dominated by the large hydrophobic moiety of the β-barrel domain of PsbO.

3. PsbO as a localized buffer

A short distance between two or more acidic groups creates a common negative field [40] and ensures high efficiency of proton transfer as was shown in bacteriorhodopsin, where the average distance is approximately 7.8 Å [44–46]. In a chemical model system, 7 Å between acidic groups was the optimal distance for the arrangement of a hydrogen bonded water/proton network that could facilitate proton transfer [41]. Furthermore, a 7 Å distance was calculated to be the most likely distribution of negative surface charges of six proton transporters with known 3D structure [42]. Acidic residues and histidine are often found in the proton gateways of membrane enzymes where patches with high density of acidic groups form a conduit for lateral proton transfer [42]. We suggest that the negative charges distributed on average 7.5±2 Å apart in the CCG domain of PsbO effectively attract the protons released during water oxidation.

The proton transfer reactions at the membrane–water interface have been widely discussed in the literature [42–45]. It was for example shown, that the flux of protons across the gramicidin channel was limited by the diffusion near the entrance of the channel, and that different buffers enhanced the conductance of the channel to different extents [46]. A similar buffer effect was shown to be inherent to HCO^- dehydration catalyzed by carbonic anhydrase [47] and in chemical model systems [40,48]. It was further found that the rate of proton release from bacteriorhodopsin increased with an increase in buffer concentration [49]. The reason that increased buffer capacity increases the rate of proton transfer is that removal of protons is often the limiting step in catalytic reactions.

The proton acceptor problem in the photosynthetic water oxidation mechanism was discussed recently in detail [44,50]. The pK_a of an efficient proton acceptor in the water oxidizing cycle should be sufficiently high to abstract protons from the donor (a substrate water molecule) yet not so high that it attracts protons also from other potential proton donors. McEvoy and Brudvig [51] proposed that the Arg357 residue of the CP43 protein is such a proton acceptor, and that it is

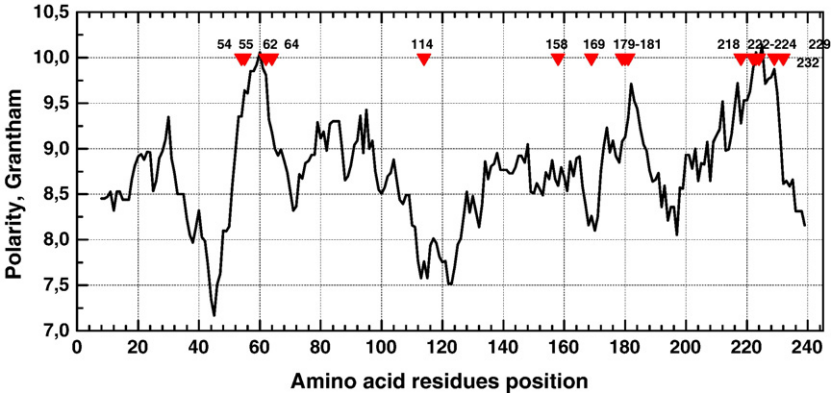

Fig. 3. The polarity of side chains of the PsbO sequence from *T. elongatus* according to [56] was calculated according to procedures available at http://www.expasy.org/tools/protparam.html. The locations of glutamic and aspartic acid residues that we predict are part of the CCG are indicated with red triangles.

able to abstract protons from a substrate water molecule and pass them through a proton/water channel to the membrane surface.

It is thought to be important for the energy requirement of water oxidation that the protons that are generated at the WOC are efficiently removed from there. Dilution of protons can provide an entropy gain that contributes about 200 meV to the driving force of the water oxidation reaction [52]. Such a dilution effect must not necessarily result from dilution of protons by diffusion into the bulk of the lumen, but can be achieved if protons are accepted by a flexible "antenna" network located in the thylakoid itself (compare with the light-harvesting antenna). The charge delocalization of the CCG will ensure readiness to accept the protons that are released by PSII as was shown using molecular dynamic simulations for nearby carboxylates in cytochrome *c* oxidase [53].

It has been proposed that a proton/water channel leading from the Mn_4CaO_X site starts at D1D61 and then proceeds along a hydrophilic pathway including D1E65, D2E312 and D2K317 and further via the six PsbO side chains including the D158, D222, D223, D224, H228 and E229 residues finally to exit into the 267 lumen [1,11]. A recent paper by Ishikita and co-workers considered the proposed proton channel in greater detail [54]. Based on pK_a values, they defined more precisely the putative proton exit pathway. The computations indicate a monotonous increase in pK_a yielding an energetically downhill proton transfer pathway leading toward the membrane surface. However, the final residue in the proton transfer chain, PsbO D224, was calculated to have a pK_a of 9.0–9.1, which means that it would always be protonated at normal lumen pH and therefore not favour proton transfer directly into the bulk of the lumen. Based on these findings and the concept of lateral proton transport suggested by Williams [55], we propose, that protons derived from substrate water in PSII do not need to be released from the WOC into the lumen. Instead the CCG located close to the Mn_4CaO_X centre can serve as an efficient antenna removing protons released by the catalysis of water. We further propose that this cluster is the beginning of a localized, fast proton transfer conduit on the lumenal side of the thylakoid membrane spanning the distance to the ATPase.

Long distance proton transport via hydrogen-bonded water molecules on protein surfaces according to the Grotthuss mechanism has been reviewed in detail [42,45]. It involves a cascade of proton jumps, so the proton arriving at the final acceptor is not the same as the one that initiated the cascade at the source (in our case ATPase and WOC, respectively). Such rapid proton transfer requires precise distance and orientation of the chain of hydrogen-bonded water molecules and can conceivably be regulated by protein dynamics. NMR measurements of proton transfer at the surface of bacteriorhodopsin supports the notion of water-bridged hydrogen bonded networks [42].

Light induced lowering of pH in the lumen (pH < 6.0 [4]) might lead to a conformational changes of PsbO [33], so that its function, whatever it is, becomes impaired. This suggestion is reasonable as both the water supply and proton transfer require a constellation of negatively charge CCG, so the protonation of some of them should affect both these putative functions that do not exclude each other.

4. Conclusion

We propose that the CCG, located in a hydrophilic pocket close to the Mn_4CaO_X centre, acts as a proton antenna and facilitates the removal of the protons from the vicinity of the WOC catalytic site. The most interesting features of the CCG hypothesis are: (i) the charge delocalization in the CCG will ensure readiness to accept the protons released in WOC; (ii) the pK_a of CCG is optimal considering the lumenal pH in the light; and (iii) it offers protein dynamics-regulated fine tuning of proton transfer over the protein surface in PSII.

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