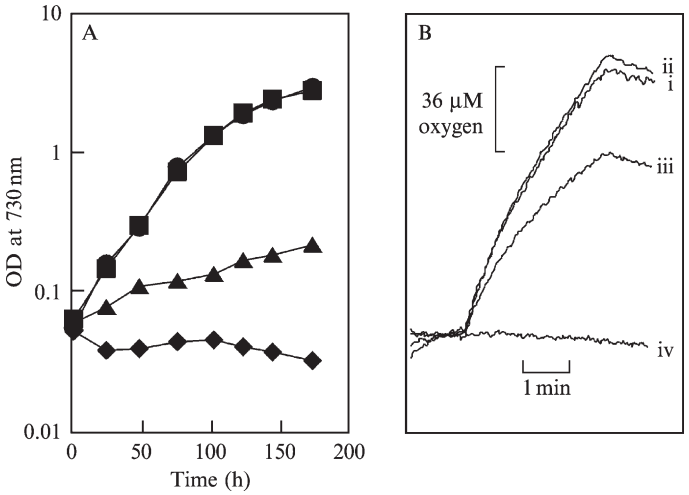

Figure 2: Characterization of D1:Trp-317 mutants. (A) Photoautotrophic growth of control (squares); W317F (circles); W317A (triangles), and W317Y (diamonds). (B) Oxygen evolution: (i) control; (ii) W317F; (iii) W317A, and (iv) W317Y. The control rate of oxygen evolution was 330 μmol O_2 (mg chlorophyll)$^{-1}$ h^{-1}.

W317F strains were both 16 h. In contrast the W317A strain exhibited a doubling time of 62 h while W317Y cells were not photoautotrophic (Fig. 2A). Oxygen evolution for the W317F strain was found to exhibit a similar rate to the control while this was reduced to 67% in the W317A mutant (Fig. 2B). However, the W317Y mutant did not evolve oxygen even though it assembled PSII centers at ~47% of the control strain. The level of assembled centers detected by herbicide-binding assays in the W317F and W317A mutants were 100% and 60% of the control, respectively (data not shown).

DISCUSSION

PsbO Mutants. The substitution of Glu at Lys-60 and Lys-70 in *Synechocystis* sp. PCC 6803 lowered oxygen evolution rates and therefore these residues may be involved in electrostatic interactions that contribute to PsbO binding. In the PSII 3.5 Å model from *T. elongatus* (Ferreira et al 2004) Lys-59 may interact with Glu-405 on loop E of CP47 from the other PSII monomer. Likewise Lys-69 is located <6 Å from Asp-87 on loop A of this CP47 as well as Asp-103 from D1 on its own monomer. Thus although these putative interactions require further experimental investigation they do raise the possibility that protein-protein interactions between hydrophilic domains of the neighboring reaction center monomers contribute to the stability of the functional dimeric complex. Our results also suggest that an intra-molecular electrostatic interaction between Lys-53 and Glu-218 (*T. elongatus* numbering) may play a role in stabilizing the binding of PsbO to the PSII complex. The phenotype of the K38E:Δ(S42-M46):K54E mutant resembled that of the ΔPsbO strain suggesting that binding of PsbO may be affected as a result of a structural perturbation.

Mutations at Phe-430 in CP47 and Phe-302 in D1. Our results indicate that Phe-430 participates in a hydrophobic pocket formed by loop E of CP47 that is important for PSII stability under conditions where the extrinsic proteins dissociate from the complex. The interaction of Phe-302 in D1 with surrounding residues on D2, CP43 and PsbJ also appears to be important for PSII stability. In both of these examples the removal of PsbV resulted in a more severe phenotype than the removal of PsbO. Examples where removing PsbV has resulted in perturbing PSII to a greater extent than the removal of PsbO have also been seen in other CP47 mutants (Morgan et al 1998, Clarke & Eaton-Rye 2000).

Mutations at Trp-317 of D1. Interactions between Trp-317 of D1 and surrounding residues on D2 appear to be important since perturbing these interactions abolishes water-splitting activity in assembled PSII centers. This is the first report of a putative D1-D2 protein-protein interaction that is required for the assembly of a functional PSII complex.

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COMPUTATIONAL STRUCTURAL MODEL OF THE OXYGEN EVOLVING COMPLEX IN PHOTOSYSTEM II: COMPLETE LIGATION BY PROTEIN, WATER AND CHLORIDE

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Keywords: oxygen-evolving complex, structure, molecular mechanics, photosystem II, manganese

INTRODUCTION

The recently published 3.5 Å resolution X-ray crystal structure of a cyanobacterial photosystem II (PSII) (Ferreira et al 2004) provides the first atomic image of the oxygen-evolving complex (OEC), structurally constraining mechanistic models of photosynthetic water oxidation. The OEC is shown to comprise a Mn_3CaO_4 cuboidal

cluster, with the metal ions at four of its corners. In addition, a fourth manganese ion is attached to the cuboid *via* a μ_4-O atom, forming the so-called "3 + 1 Mn" tetramer predicted by spectroscopists in recent years (Peloquin & Britt 2001). The moderate crystallographic resolution, however, has hindered the identification both of exogenous species (including vital substrate water molecules) and of the detailed proteinaceous coordination of the complex, leaving the metals of the OEC incompletely ligated.

Our recently proposed mechanistic model (Vrettos et al 2001) is largely consonant with the new structure (McEvoy & Brudvig 2004), but the rigorous formulation of a mechanistic cycle requires the development of a more complete structural model. In this paper, we use computational methods to add to (and marginally modify) the recently published X-ray model in order to obtain a chemically sensible structure of the hydrated OEC, maximally consistent with the available structural and mechanistic data. We do this by adding a few small, non-proteinaceous molecules as ligands to the OEC metal ions, further hydration of the structure, and subsequent geometry optimization at the molecular mechanics level of theory. We find a plausible binding site for chloride, strongly suspected to be present in the OEC but not revealed in the X-ray structure, and for acetate, an inhibitor that is thought to bind in place of chloride (Kühne et al 1999). The positions of these ligands in our calculated structures are consistent with pulsed EPR measurements.

MODEL AND METHOD

A reduced model of the protein cavity surrounding the OEC cluster was constructed according to the recently reported X-ray structure of photosystem II (Ferreira et al 2004). The model included all residues whose α-carbons are within 15 Å of any OEC cluster atom. We completed the coordination of the Mn atoms mostly by hydration, assuming a *minimum displacement of the ligating residues* from their crystallographic positions, as well as manganese's preference for octahedral coordination in its higher oxidation states. Calcium's more variable coordination (typically 6–8 coordinate) meant that its ligands were supplied by general hydration of the structure (see below). Computational models were also constructed according to the principle of *minimum incorporation of ligand water molecules*. However, these alternative models were dismissed because they required significant displacements of the ligating amino-acids from their crystallographic positions.

The geometry of the resulting model was optimized using the Amber force field. The positions of the OEC cluster ions were fixed according to their crystallographic coordinates, and it was assumed that protein-cluster interactions are dominated by electrostatic terms. Electrostatic charges on the OEC cluster ions were assigned according to their formal charges as previously hypothesized in the S_0 state (McEvoy & Brudvig 2004, Vrettos et al 2001): Mn(1) = +4, Mn(2) = +4, Mn(3) = +3, Mn(4) = +2 (Ca^{2+} = +2, bridging oxides = −2).

Figure 1 shows the resulting computational structure. Note that the coordinations of OEC protein ligands remain mostly unchanged relative to the X-ray structure. For example, D1-Asp342 binds monodentally to Mn(1) and CP43-Glu354 chelates bidentally to Mn(3); the coordination spheres of the two metals are completed by the addition of two waters to Mn(1) and one water to Mn(3).

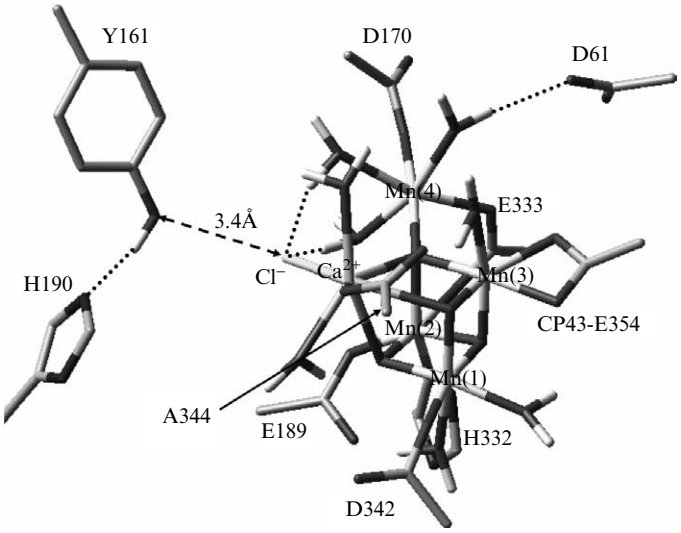

Figure 1: Hydrogen bonds are shown by dotted lines. Note the hydrogen bond connecting one of the water molecules bound to Mn(4) and Asp61, proposed as the start of the proton exit channel.

Three water molecules are added to complete the coordination of Mn(4), ligated monodentally by D1-Asp170 and D1-Glu333. To complete the coordination of Mn(2), D1-Glu333 was moved to form an η^2 carboxylate bridge between Mn(4) and Mn(2). The resulting root mean square displacement of the residue is ∼0.15 Å, with a ∼1.3 Å displacement of its carboxylate oxygens from their original X-ray positions.

The resulting molecular mechanics model has been compared to an *ab initio* model system optimized at the B3LYP/lacvp level of theory, in which ligating carboxylate groups were modeled as formates and D1-His332 as imidazole. The resulting minimized *ab initio* model is entirely consistent with the molecular mechanics model. In particular, the validity of the carboxylate bridge between Mn(2) and Mn(4) is confirmed, as well as the bidentate coordination of CP43-Glu354 to Mn(3) and the monodentate coordination of D1-Asp342 to Mn(1). Furthermore, all *ab initio* Mn—Mn distances are within 0.25 Å of those reported by EXAFS experiments (Robblee et al 2001), which is the expected error of *ab initio* calculations at this moderate level of theory (Lundberg & Siegbahn 2004).

Hydration. Additional water molecules not directly bound to Mn ions were added to the computational model by embedding the molecular structure in a box of water molecules at thermal equilibrium. Water molecules within 7 Å of the cluster's center of mass were added if they did not sterically conflict with the protein or with coordinating water molecules. The completed structure was then relaxed by geometry optimization. Because such a relaxation creates room for more water molecules, the procedure was repeated until the number of water molecules converged to a constant value. Hydration added eight water molecules to the model. These waters either completed the coordination of calcium or were hydrogen-bonded to polar species.

Addition of chloride. There is experimental evidence suggesting that one Cl^- ion is bound to the OEC (Lindberg & Andréasson 1996) near to D1-Tyr161 (Force et al 1997) and participates in the mechanism of oxygen evolution, perhaps by affecting proton

movement in the later S-states (Olesen & Andréasson 2003). In order to determine the most likely position of Cl^-, we analyzed the relative stabilities of molecular structures obtained by replacing one of the non-Mn-bound water molecules by Cl^-. After geometry optimization, the most stable structure was selected. The resulting model became electrostatically neutral around the OEC cluster after the addition of Cl^-, and contained 1872 atoms.

RESULTS

Chloride Ligation. The most stable structure was found to have Cl^- bound to Ca^{2+} as shown in Fig. 1. The Cl^- ion is 2.48 Å from Ca^{2+} and 3.4 Å from the phenoxy oxygen of D1-Tyr161. The Ca^{2+} ion has seven ligands: Cl^-, two water molecules, the monodentate carboxylate terminus of D1-Ala344 and the three bridging oxides of the cuboidal structure. Note that one of the two water molecules ligated to Ca^{2+} is potentially a substrate water molecule, which is proposed to react in the S_4-state with the closest Mn(4)-bound water, only 2.8 Å away (McEvoy & Brudvig 2004). Furthermore, D1-Asp61 is found to hydrogen-bond to one of the non-substrate water ligands of Mn(4). This finding is consistent with our proposed mechanism of water-splitting, which involves proton transfer from the coordination sphere of Mn(4) to the protein lumen exterior *via* D1-Asp61 (Ferreira et al 2004, McEvoy & Brudvig 2004).

Acetate Ligation. Acetate has been shown to bind competitively with Cl^- (Kühne et al 1999) and to block catalysis at the S_2 OEC state (Wincencjusz et al 1997). Pulsed EPR experiments have revealed a distance of 3.1 Å from the methyl hydrogens of the bound acetate to the phenoxy oxygen of D1-Tyr161 (Force et al 1997). In order to determine the possible nature of acetate ligation to the OEC and to validate the proposed Cl^- binding site in the computational model, the Cl^- ion was replaced by acetate. Its carboxylate group was initially placed in the position of Cl^-, and its C–C bond was collinear with the previously modeled Cl^-–Ca^{2+} bond. There is evidence that adding acetate to the OEC displaces several water molecules from the protein cavity (Clemens et al 2002). To be consistent with this observation, and assuming that the displaced waters are near Cl^-, the two waters originally coordinated to Ca^{2+} were removed. After geometry optimization, the phenoxy oxygen of D1-Tyr161 was found to be 3.2 Å from the averaged position of the acetate methyl hydrogens (3.1 Å from the methyl carbon), in excellent agreement with experimental observations. Figure 2 shows the relaxed configuration of the acetate-bound OEC model. It was found that removing a third water molecule, initially bound to Mn(4), caused acetate to relax by forming a bridge between Mn(4) and Ca^{2+}. This produced a methyl—phenoxy oxygen distance of 3.35 Å, only 0.25 Å longer than the experimentally determined distance.

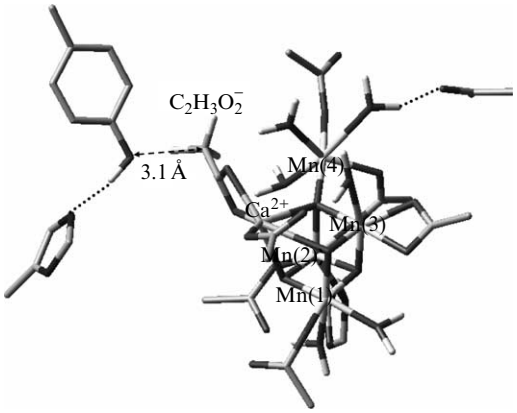

Figure 2: Optimized OEC model after replacing Cl^- by acetate.

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TARGETING OF PCB LIGHT HARVESTING PROTEINS TO PSII AND PSI IN *PROCHLOROCOCCUS MARINUS*

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