ONLINE SUPPORTING MATERIAL FOR:

Structural/Functional Role of Chloride in Photosystem II

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- Molecular dynamics (MD) simulations (Figure S0)
- Muli-Conformer Continuum Electrostatics (MCCE) method
- QMMM model of the S₁ state (Figure S1)
- Chloride binding sites (BS) (Figure S2)
- Chloride relocation into the BS2 binding site (Figure S3)
- MCCE (D1-D61) conformers (Figure S4)
- References

Molecular dynamics (MD) simulations

The computational structural models of PSII are based on the X-ray crystal model at 1.9 Å resolution (*I*). A sphere containing all amino acid residues within 20 Å of the Mn_4Ca cluster is extracted from the protein complex (see Figure S0). All crystal waters belonging to this portion of the protein complex were kept and 6 extra water molecules have been added for substituting (6 hydroxyl groups of) two glycerol molecules. Hydrogen atoms were added using AmberTools (version 1.4). In order to preserve the ligation scheme of the X-ray structural model of the Mn_4Ca cluster, amino acid residue H332 has been protonated at the δ position and bound to the Mn cluster with its N_{ϵ} .

Molecular dynamics simulations of the above described computational models (~5600 atoms) are based on the AMBER-ff99SB (2) force field for protein residues and TIP3 model (3) for explicit water molecules, as implemented in the NAMD2 software package (4). Only nonbonding interactions between atoms in the Mn_4O_5Ca cluster were included and harmonic constrains with force constants set to 5 kcal mol⁻¹Å² were applied to all the cluster atoms and to the atoms of the protinaceous and water ligands directly bound to the cluster. The atom partial charges for the OEC cluster were derived by using the our new QM/MM model described below (5). Amino acid alpha-C atoms lying between 15-20 Å from the Mn_4Ca cluster have been harmonically constrained with force constants set to 5 kcal mol⁻¹Å⁻² in order to avoid unfolding of protein secondary structures at the periphery of the sphere, defining the system boundary conditions. Terminal residues at the sphere periphery were capped using C- and N-terminus groups.

MD simulations succeed the following pre-equilibration procedure: hydrogen atoms and capping groups added to the crystal structure model are optimized, constraining the rest of the atoms at the crystal structure positions. The system was then slowly heated to 300 K, performing MD simulations (150 ps) at 100, 200 and 300 K using Langevin dynamics, with a damping coefficient of 0.1 ps⁻¹. During this heating procedure different parts of the system were gradually unconstrained. Equilibration of the system at 300 K was then evaluated monitoring potential and kinetic energies of the system.

Electrostatics and van der Walls interactions were calculated using a switching distance of 10 Å and a cutoff of 12 Å. A multiple time-stepping algorithm (6) was used, where bonded and short-range nonbonded interactions were evaluated at every time step while long-range electrostatic interactions were evaluated at every two time steps, using a timestep of integration set to 0.5 fs.



Figure S0. Computational structural model used for MD simulations. A spherical portion of the protein complex (right) extracted from the X-ray crystal model of PSII at 1.9 Å resolution (left). The sphere of 20 Å radius is cenetered at the Mn_4O_5Ca cluster (green, red and cyan spheres).

Multi-Conformer Continuum Electrostatics (MCCE) method

The Monte Carlo sampling for amino acid and terminal OEC water protonation states, rotamer positions and chloride binding sites was carried out at pH 7 using MCCE (Muli-Conformation Continuum Electrostatics) (7). The 1.9 Å crystal structure was initially relaxed by MD simulation. During minimization OEC, its ligands and other residues within 5 Å of OEC (in total 17 aminoacids: chain A ASN87, GLN165, SER167, SER169, ASP170, VAL185, GLU189, GLU329, GLU333, ARG334, HIS337, ASP342, LEU343, ALA344; chain C GLU354, ARG357, LEU352) were harmonically constrained with the force constant of 100 kcal mol⁻¹ Å⁻². In addition all protein backbone atoms were constrained with the same force constant. The AMBER-99 force field (8) was used for protein and the GLYCAM-2000a force field (9) was used for the headgroups of galactolipids. The force field for cofactors was based on the parameters described previously. The PMEMD program from AMBER package (version 10) was used to relax the structure. Relaxation utilized the generalized Born implicit solvent model with external dielectric constant of 80 and salt concentration 0.15 M. Steepest descent/conjugate gradient minimization was terminated when RMS gradient of energy became less than 0.1 kcal/mol-Å.

The four manganese ions of the OEC were assigned the S₁ valance charges (IV, III, IV, III) according to our new QM/MM model (5). All μ -oxo oxygens are deprotonated and there are 4 terminal waters, with a bridging hydroxyl between Mn(4) and Mn(3). The amino acid ligands of the Mn₄Ca cluster have the same ionization states as the MD simulations. All water molecules except those that are direct ligands to the OEC were removed and replaced with implicit water with a dielectric constant of 80. The protein has a dielectric constant of 4. Poisson-Boltzmann electrostatic interactions and the solvation energies were calculated using Delphi, (10) while the Lennard–Jones and torsion energies were obtained from AMBER (8).

The IPECE5 subroutine was used to place the chloride in the pocket between D2-K317 and D1-D61. Each conformer has a charge of -1 and a solvent accessible radius of 1.937 Å. Each intra-protein CI^{-} is paired with one in water with a solvation energy of -20.3 kcal/mol. Some of this energy is lost when the CI^{-} is buried in the proteins. MCCE determines if this is replaced by sufficient favorable interactions with the protein for it to be bound in the simulation.

QMMM model of the S₁ state

Both MD simulations and MCCE calculations are based on our new OMMM model (5), as described below. The QM/MM model of the S₁ state (IV,III,IV,III) was built using the coordinates from the new crystal structure at the resolution of 1.9 Å (1). All water molecules and chloride atoms within 15 Å distance as well as all amino-acid residues with the α -carbon within 15 Å distance from the OEC of chain A were included and hydrogen atoms were added where appropriate. The amino acid residues were protonated in accordance with physiological pH. The minimum QM layer that we have considered includes the inorganic core of the OEC, the carboxylate groups and corresponding α -carbons of the ligands D170, A344, E189, E354, E333, and D342, the imidazole rings of H332 and H337, the guadinium group of R357 and several water molecules. The structure optimization was performed using the ONIOM (our own N-layered integrated molecular orbital plus molecular mechanics) method (11) with the link-hydrogen atom scheme as implemented in Gaussian 09 (12). Unrestricted density functional theory (DFT) with the BP86 (13, 14) or B3LYP (15, 16) density functionals and the LACVP* basis set as implemented in the Jaguar 7.7 program were employed for the QM part. The initial-guess wavefunction for the QM part was generated with the Jaguar 7.7 program (17) using the same density functional and basis set as in the QM/MM calculation. The MM part was calculated using the Amber force field (8). The α -carbons (except of the QM layer) were fixed during the optimization. Two isoenergetic tautomers of the S_1 state have been localazied by using a large QM/MM model that includes amino acid residue D1-D61. In one tautomer the protonated D1-D61 is hydrogen-bonded (as donor) to a µ-hydroxo bridge of Mn(3)-Mn(4) and (as acceptor) to an hydrooxo ligand of Mn(4), see Figure S1 (left panel). In the second tautomer D1-D61 is hydrogen-bonded (as donor) to a µ-oxo bridge of the Mn(3)-Mn(4) unit and (as acceptor) to a water ligand of Mn(4), see Figure S1 (right panel).



Figure S1. Two isoenergetic tautomers of the S₁ state.



Figure S2. Chloride binding sites and OEC cluster coordination sphere as resolved in the recent crystal structure (*1*). a) Mn_4CaO_5 cluster (ball and sticks) and first coordination sphere of the metal atoms (bound ligand atoms in shaded red balls). Substrate waters (w_1^* and w_2^*) are indicated with dashed red circles. The two Cl⁻ (magenta large balls) binding sites (BS1 and BS2) are highlighted with blue squares. b) Cl⁻ (2) binding site: a water molecule bridges unprotonated D61 and K317 sidechains via hydrogen bonds. c) Cl⁻ (1) binding site: chloride is interacting with NH groups of surrounding amino acids backbones and a water molecule.



Figure S3. MD simulation of chloride ($C\Gamma_{(2)}$) relocation into the binding site (BS2) proposed by high-resolution Xray structure (in magenta). a) $C\Gamma_{(2)}$ is initially located between K317 and protonated D1-D61. Simulation time is set to 0 after 15 ns pre-equilibrium simulation constraining chloride in this position. b) After almost 3 ns of simulation chloride moves to the BS2 binding site position. As a consequence, D1-K317 sidechain rearranges in the "hooking" configuration and protonated D1-D61 makes and hydrogen bond with D1-E333. c,d) $C\Gamma_{(2)}$ maintains its location around the Xray position for the rest of the simulation. The other two hydrogen bond interactions observed in a different MD simulation (see Figure 3 in the text) are restored and similar configurations having D61 hydrogen bonded to O(5) (panel c) and to a close water molecule (w5 in panel d) are observed.



Figure S4. The selected conformers through the MC sampling. Conformer 1 (in red) and 2 (in orange) of D61 are almost equally occupied, with 55% and 45% occupancy, respectively. The terminal water hydrogen bonded to the D61 is shown in sphere scheme and cyan color. The OEC amino acids ligands are shown in black and line scheme and chloride is shown in green. Conformer 1 has more favorable interactions with the positive potential created by the backbone and less desolvation and torsion penalty than conformer 2, which balances these energy terms by more favorable electrostatic interactions with the CP43-R357 and the H337.

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