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An Energetic Comparison of Different Models for the Oxygen Evolving Complex of Photosystem II

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Photosystem II is the only system in nature capable of forming dioxygen from water and sunlight. The catalyst for the step where the O−O bond is formed is the oxygen evolving complex (OEC) located close to the lumenal side in the membrane. The OEC complex contains four manganese and one calcium atom. X-ray diffraction studies during the past few years have considerably clarified the detailed structure of the OEC.[1−3] In the first of these studies,[1] it was shown that three of the manganese and the calcium atom form a cuboidal structure, with the fourth manganese situated outside the cube; see Figure 1. The amino acids most likely to be ligated to the complex were also assigned. Waters were assumed to fill up the remaining coordination sites. Since the resolution was rather low (3.5 Å), the ligation pattern could only be suggested. In the more recent X-ray structures,[2,3] the resolution was slightly higher (2.9−3.0 Å), and a different ligation pattern was suggested with most of the carboxylate amino acid ligands assumed to bind bidentately between two different metal atoms. This means that hardly any water derived ligands had to be added to saturate the metal coordinations. The positions of the metal atoms were similar to the ones in the earlier X-ray structure, with the exception that the outside manganese was placed farther out from the Mn_3Ca-cube. These two X-ray structures will in the following be termed the London and the Berlin structures. One problem with the X-ray structures is that they do not agree with EXAFS experiments.[4,5] It has been suggested that this is due to radiation damage[6] but could also come from the low resolution.

Parallel to the experimental structural work, significant progress has been made on the mechanism for O−O bond formation by using density functional theory (DFT). Three different approaches were used for trying to obtain both mechanisms and more detailed structures than are available from experiments. In the first approach,[7] a cluster model of the OEC was used with up to 200 atoms. Backbone atoms were fixed to the positions obtained in the London X-ray structure. In the second approach,[8] the QM/MM (Quantum Mechanics/Molecular Mechanics) methodology was employed. A small QM part was surrounded by a large MM part, together making up the entire protein. In the third approach,[9] 10 different models for the S_2-state were constructed based on the core topology derived by polarized EXAFS spectra,[10] and with a ligand structure chosen to fit reasonably well into the Berlin X-ray structure. Based on the agreement with experiments for the computed spin spectrum, the best candidates for the actual structure of the OEC were selected. These three models will in the following be termed the cluster model, the QM/MM model, and the spin model.

In the present communication, an attempt is made to compare the structures obtained in the different DFT approaches as to their probability of mimicking the actual OEC complex. The structure with the lowest energy will be taken as the best structure. An additional requirement is, of course, that the structure fits into the enzyme backbone structure. This is the case for both the cluster model, where all backbone atoms were taken directly from the X-ray structure, and the QM/MM model, which was obtained from an optimization including the entire protein. It is less clear for the structures assumed in the spin model.

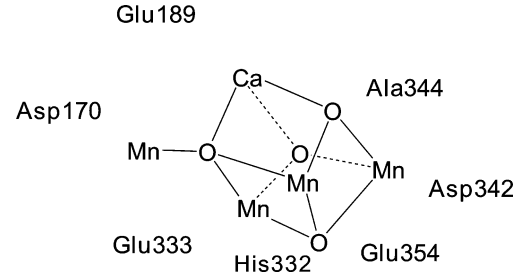

Figure 1. Simplified picture of the structure of the oxygen evolving complex, suggested by X-ray crystallography.

To make the comparison, models have to be constructed of the resting S_1-state, for which it is possible to directly compare the energies. A model was first chosen for the QM/MM structure with 142 atoms with positions taken from that structure. In this model, the backbone atoms were fixed from the optimized QM/MM structure. A charge of +2 is optimal for this model according to the QM/MM calculations. With the backbone atoms fixed, the rest of the atoms were fully optimized at the B3LYP level,[11] using the lacvp* basis set. A single-point energy for the optimized structure was then obtained using the cc-pvtz(-f) basis set with lacv3p+ for the metal atoms. Dielectric effects were added using lacvp* with $\varepsilon = 6$ and a probe radius of $R = 1.40$ Å. The calculations were performed using the Jaguar program.[12] For the structure obtained using the cluster approach, a similar model was constructed with 142 atoms and a charge of +2 (see Figure 2), starting from the most recently optimized structure.[13,14] The optimal charge for the cluster model should actually be −1, but this would make the energetic comparison much harder, and three protons were therefore added. A chloride bound to calcium was included since it is present in the QM/MM structure. The backbone atoms were fixed from the London X-ray structure, and the geometry was optimized with the same basis set (lacvp*) as in the case for the QM/MM model. The oxidation states are two Mn(IV) and two Mn(III), and the total spin is 15. The single-point B3LYP energy (with cc-pvtz(-f), lacv3p+) of this cluster model could then be directly compared to the one obtained for the QM/MM model. The B3LYP energy difference including dielectric effects of −4.9 kcal/mol is +72.8 kcal/mol in favor of the cluster model. At the B3LYP* level (with 15% exact exchange),[15] the energy difference becomes +71.2 kcal/mol. Since, by experience, an energy difference of 5−10 kcal/mol should be enough to discriminate between two DFT structures, which do not differ in the oxidation states, it seems clear that the QM/MM structure can be ruled out as a candidate for the actual OEC.

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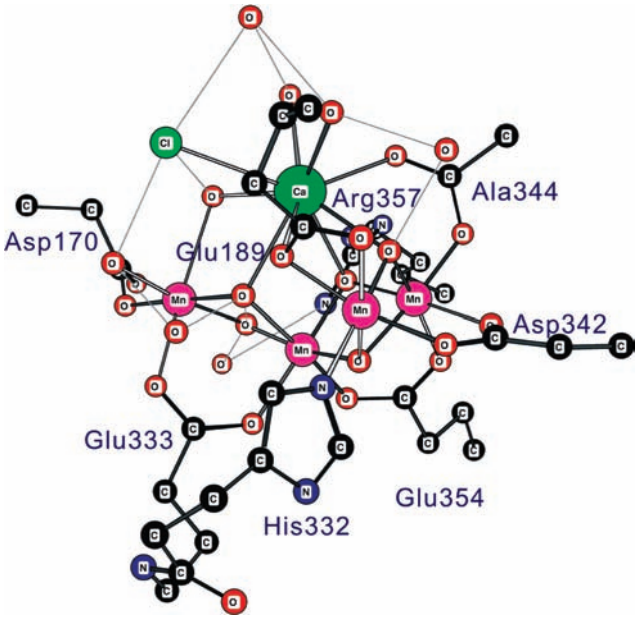

Figure 2. 142-atom model of the S_1-state, constructed from the optimal structure using the cluster approach. The hydrogen atoms are omitted. Hydrogen bonds are shown as thin lines.

It might be argued that in principle it could be possible for an enzyme to adopt a structure which is not the lowest one in energy. However, in that case there must be barriers larger than 20−25 kcal/mol to prevent decay of the structure during the lifetime of the protein. To make sure that this is not the case for the QM/MM structure, a beginning of a pathway for the decay of the 142 atom model of the QM/MM structure was also located. For this pathway the barriers are less than 5 kcal/mol, and the QM/MM structure would therefore decay on the order of a nanosecond to another structure, which is 20 kcal/mol lower in energy. From this point it will most probably decay further, but this was not investigated. The QM/MM structure therefore appears to have been trapped in a local minimum in the QM/MM geometry optimization. It is worth noting that even though the QM/MM structure can be ruled out as a candidate for the OEC by the calculations, it still matches many experimental spectral features, such as those from polarized EXAFS.[16] The present cluster model is found to reproduce experimental solution EXAFS but has not yet been tested against polarized EXAFS.

A similar procedure was used to compare the energies of the 10 spin models for the S_2-state from ref 9 to corresponding cluster models. The oxidation states are three Mn(IV) and one Mn(III), and the total spin is 14. These 10 spin models have different numbers of atoms, and the corresponding cluster models were adapted accordingly. Since the spin models had fixed metal distances (from EXAFS), the cluster models also had fixed distances (from a large model). The same basis sets and methods as described above were used also in this case. The numbers given in Table 1 are B3LYP* results including dielectric effects. It is again clear that the 10 different spin models constructed based on polarized

Table 1. Energy Differences (kcal/mol) between the Cluster Type Models and the Ten Different Spin-Models[a]

structure	1	2	3	4	5
	60.2	63.3	34.4	45.7	50.2
structure	6	7	8	9	10
	43.7	63.4	59.6	53.2	34.8

[a] Positive values mean that the cluster type model is lower in energy.

EXAFS and with assumed ligand structures can be ruled out as possible candidates for the OEC. Most probably, the main part of the energy differences comes from the ligand part of the structures. The energy differences coming from the metal core parts are probably much smaller. Therefore, this type of spectroscopic approach would probably be more successful if the investigation was restricted to ligand structures which are low in energy.

In summary, the computed total energy is a useful property for discriminating between different structural models of the oxygen evolving complex in photosystem II. Using the total energy, it has conclusively been shown that many suggested models for the OEC can be ruled out. The only remaining model obtained by calculations is the one found using the cluster approach.[13,14] However, it should be emphasized that still better structures cannot be ruled out in future investigations.

Supporting Information Available: Coordinates for all structures discussed here. This material is available free of charge via the Internet at http://pubs.acs.org.

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