Mechanism and energy diagram for O–O bond formation in the oxygen-evolving complex in photosystem II

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The recent finding of a transition state with a significantly lower barrier than previously found, has made the mechanism for O–O bond formation in photosystem II much clearer. The full mechanism can be described in the following way. Electrons and protons are ejected from the oxygen-evolving complex (OEC) in an alternating fashion, avoiding unnecessary build-up of charge. The S_0 – S_1 and S_1 – S_2 transitions are quite exergonic, while the S_2 – S_3 transition is only weakly exergonic. The strong endergonic S_3 – S_4 transition is a key step in the mechanism in which an oxygen radical is produced, held by the dangling manganese outside the Mn_3Ca cube. The O–O bond formation in the S_4 -state occurs by an attack of the oxygen radical on a bridging oxo ligand in the cube. The mechanism explains the presence of both a cube with bridging oxo ligands and a dangling manganese. Optimal orbital overlap puts further constraints on the structure of the OEC. An alternating spin alignment is necessary for a low barrier. The computed rate-limiting barrier of 14.7 kcal mol^{−1} is in good agreement with experiments.

Keywords: photosystem II; transition state; density functional theory; spins

1. INTRODUCTION

After years of intensive calculations based on the recent X-ray structures (Ferreira *et al.* 2004; Loll *et al.* 2005), significant progress was finally made when a new type of transition state for the O–O bond formation with a very low barrier was found (Siegbahn 2006). In the present paper, new insights concerning this mechanism will be described. Full mechanisms and energy diagrams will be discussed for most of the recent structural suggestions, including a few of the new structures suggested based on extended X-ray absorption fine-structure (EXAFS; Yano *et al.* 2006). The results for the most recent X-ray structure are surprisingly accurate when compared to experiments.

2. MATERIAL AND METHODS

The calculations discussed here were made using the density functional theory (DFT) hybrid functional B3LYP (Becke 1993), with procedures very similar to those used in previous studies (Lundberg & Siegbahn 2004; Siegbahn & Lundberg 2005, 2006; Siegbahn 2006). Small basis sets for the geometries (lacvp), large basis sets for energies (cc-pvtz(-f)) and a surrounding dielectric medium with dielectric constant equal to 4.0 were used (basis lacvp*). The calculations were performed with the programs JAGUAR (Schrödinger 1991–2003) and GAUSSIAN03 (Frisch *et al.* 2003). An alternative computational approach to the mechanism for dioxygen evolution has recently been presented (Sproviero *et al.* 2006, 2007). In that approach, the focus has been on the geometries

rather than on the energies, and the entire protein has been included in the model using a quantum mechanics/molecular mechanics (QM/MM) procedure.

3. RESULTS

In this section a few topics will be discussed. The first topic is the possibility to use the Tyr_Z radical as a hydrogen atom abstractor in the water oxidation process. The second one is the reliability of using IR frequency shifts for determining which manganese atom is oxidized. The third topic is the main one and concerns a comparison of energy diagrams which have been computed for most of the recently suggested structures. Finally, the effect of having a chloride ligand has been investigated, as well as substituting calcium with cadmium and strontium.

In the discussion below, use is made of the driving force of the full catalytic cycle. This value can be obtained from known redox potentials. In the case of water oxidation in photosystem II (PSII), the redox potential of the donor O₂/H₂O is 0.8 V and the acceptor P₆₈₀⁺ is 1.3 V (Diner 2001; Rappaport & Lavergne 2001). The driving force therefore becomes 4 (1.3–0.5) eV = 46 kcal mol^{−1}. It is interesting to note that the driving force for the reverse process of respiration in cytochrome *c* oxidase is approximately the same. However, in that case, practically all this energy is used up by moving charges across the membrane, not only for the chemistry but also for pumping protons across the membrane. For some reason this is not done in PSII, and even at full gradient (pH 4) as much as 29.6 kcal mol^{−1} is lost as heat. This value is obtained by subtracting the additional cost

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due to the gradient of four proton donations ($4.1 \text{ kcal mol}^{-1}$ each), from the driving force without the membrane gradient of 46 kcal mol^{-1} .

(a) *Hydrogen atom abstraction by Tyr_Z*

A quite different idea of a mechanism for proton and electron abstraction from water in the dioxygen formation process was suggested approximately a decade ago by Babcock and co-workers (Hoganson *et al.* 1995). In this mechanism, the Tyr_Z radical plays a key role as a hydrogen atom abstractor from water molecules bound to manganese in the OEC. From the driving force of the catalytic cycle, together with the known bond strengths of water and tyrosine, this hypothesis can be tested.

The first reaction of interest is the gas phase reaction,

With an atomization energy of H₂O of $219.3 \text{ kcal mol}^{-1}$, and a binding energy of O₂ of $118.0 \text{ kcal mol}^{-1}$ (Curtiss *et al.* 1991), this reaction is endothermic by $320.6 \text{ kcal mol}^{-1}$. Since H₂O is taken from the lumen in PSII, where the binding energy of a H₂O molecule can be estimated to be 14 kcal mol^{-1} , and O₂ is released with an entropy gain of 10 kcal mol^{-1} , the endergonicity of the actual reaction becomes $338.6 \text{ kcal mol}^{-1}$. For a hydrogen abstractor X to give the required exergonicity of the full cycle of 46 kcal mol^{-1} , the X–H bond strength needs to be $(338.6 + 46)/4 = 96.2 \text{ kcal mol}^{-1}$. However, the O–H bond strength of tyrosine is only $85.8 \text{ kcal mol}^{-1}$ (Kerr 1996), indicating that it would not suffice energetically to be a hydrogen abstractor in PSII.

A more general way to realize that it is not optimal to have Tyr_ZO(rad) as a hydrogen atom abstractor is to divide the process into an electron and a proton transfer. P₆₈₀⁺ is a very strong oxidant with a redox potential of 1.3 V. The electron transfer step from Tyr_Z to P₆₈₀⁺ should ideally be almost isoergonic in order not to lose energy unnecessarily. This means that the redox potential of Tyr_Z should be close to 1.3 V. This is a much higher redox potential than tyrosine normally has, which means that Tyr_Z should be in a positively charged enzyme surrounding, which would increase the redox potential. With Tyr_Z in a positive enzyme surrounding it should be a very poor proton acceptor. In fact, since any covalent bond strength, like the one in Tyr_ZO–H, is almost unaffected by the enzyme surrounding, an increase of the redox potential due to the enzyme surrounding would automatically lead to a corresponding decrease of the pK_a value to a good approximation. It is therefore much more optimal to send the proton to another acceptor than Tyr_Z, in this case directly to the lumen. Hydrogen atom abstraction is thus not a powerful enough mechanism, at least in the higher S-state transitions. It could be used in the lower S-state transitions, but there appears to be no possibility, or even reason, for this.

(b) *Comment on IR frequencies*

FTIR difference spectroscopy has yielded a wealth of information about the steps before dioxygen is formed

(Chu *et al.* 2004; Debus *et al.* 2005). By comparing the spectra for wild-type and different mutants, it has been possible to assign some of the frequencies in the spectra. In this way, the carboxylate stretching frequencies of some amino acids have been observed to shift, or not to shift, in the different S-transitions. By the assumption that a significant shift of a carboxylate group of a certain amino acid is connected with an oxidation of the particular manganese centre on which that amino acid is attached, conclusions concerning which manganese centres are being oxidized in the different S-transitions have been drawn.

The first conclusion drawn from this type of experiment was that the carboxylate of the C-terminus of the D1 polypeptide (Ala344) is bound to the manganese centre that is being oxidized in the S₁–S₂ transition (Chu *et al.* 2004). In the model shown in figure 1, built on the most recent X-ray structure (Loll *et al.* 2005), this would be Mn1. The second conclusion was that Asp170 is not attached to a manganese centre that is being oxidized in any of the S₀–S₃ transitions (Debus *et al.* 2005). Since this amino acid is assigned as ligating to the dangling manganese (Mn4) in the crystallographic analysis, this means that this manganese should not be oxidized in the lower S-transitions. This would be quite remarkable since most mechanisms suggested assign a key role to this manganese. For example, in the hybrid DFT studies performed so far, it has been energetically quite impossible to keep the same oxidation state of the dangling manganese in all these S-transitions, whether it is Mn(III) or Mn(IV) (Lundberg & Siegbahn 2004; Siegbahn & Lundberg, 2005, 2006; Siegbahn 2006).

Since the analysis of the FTIR spectra is built on an assumption, it was considered important to get more information by calculating the carboxylate CO stretching frequencies by hybrid DFT calculations. The results are given in table 1 for structures corresponding to the most recent X-ray structure (Loll *et al.* 2005). The model used for the computational studies is shown in figure 1, where chlorine has been added to Mn4. The labelling of the S-states is the same as in previous studies, with the lower index denoting the S-state and the upper index denoting the charge of the model. The assignment of a frequency to a particular amino acid was straightforward in almost all cases. The only exception was the 1319 cm^{-1} frequency in S₂⁰, which is the average of two interacting modes of 1311 and 1327 cm^{-1} for Glu333 and Glu354. The absolute values of the computed frequencies are not very accurate, but this is substantially improved using a large basis set. Since the large basis set calculations are quite time consuming and the computed shifts do not change, the small basis set was kept.

Some qualitative conclusions can be drawn from the computed frequencies, even though the assignment of the ligand positions based on the X-ray analysis is probably not entirely correct at the present stage. The first conclusion is that the relation between the shift and the manganese centre being oxidized is not at all simple. Although there is, in general, a downshift of the frequencies going from S_n^{–1} to S_{n+1}⁰ as the complex becomes less negatively charged, in agreement with the assumptions in the experiment, the relation to the

Figure 1. The transition state for the O–O bond formation using a model based on the most recent X-ray structure (Loll *et al.* 2005) and where chlorine has been added to **Mn4**. Most important spin populations are indicated.

Table 1. Computed symmetric C–O stretching frequencies (cm^{-1}) for different S-states of the OEC model described in the text. **Mn3** is oxidized in S_0 – S_1 , **Mn4** in S_1 – S_2 , and **Mn2** in S_2 – S_3 . The centre (see figure 1) to which the amino acid is coordinated is given below its label.

state	Asp170 Mn4	Ala344 Mn1	Glu333 Mn3, Mn4	Glu354 Mn1, Mn3	Glu189 Mn2, Ca	Asp342 Mn1, Mn2
S_0^{-1}	1324	1265	1345	1322	1308	1296
S_1^0	1317	1216	1336	1312	1296	1300
S_1^{-1}	1319	1257	1298	1321	1305	1309
S_2^0	1282	1224	1319	1319	1299	1302
S_2^{-1}	1310	1268	1311	1323	1305	1313
S_3^0	1260	1233	1323	1314	1273	1317
S_3^{-1}	1330	1253	1295	1313	1291	1319
S_4^0	1316	1231	1332	1314	1278	1320

oxidized centre is much less clear. For example, even though there is a shift of approximately -30 cm^{-1} in the S_1^{-1} – S_2^0 transition on the C–O stretch of the Ala344 terminus, as observed experimentally, there is a similar shift in the same transition on Asp170 located at the other end of the complex. A general result appears to be that the frequencies which are most stable, with only small shifts, are the ones for the bidentately bound amino acids. The observation that there is no shift of the C–O stretch belonging to Asp170 in any of the lower S-states, may therefore be related to an actual bidentate bonding of this ligand, rather than to its location on a centre that is never oxidized, as suggested in the experimental study. For example, even though Asp342 is coordinated to a manganese centre that is oxidized in S_2 – S_3 , its C–O frequency hardly changes. Furthermore, the frequency associated with Glu333 does not shift more than the other ones, even though it is bound to two manganese centres that are being

oxidized in two different transitions. In summary, there is, in principle, a large amount of useful information in the measured frequencies, but a deeper analysis would probably have to start with a comparison of experimental and computed frequencies if correct conclusions should be drawn. However, in order to make a comparison of this type, a better assignment of the positions and binding modes of the ligands than at present has to be made. For example, with the present assignment it is clear that the computed results do not qualitatively match the experimental result that there is no shift of the frequency for Asp170.

(c) Requirements for a low O–O bond formation barrier

The main result from the DFT studies of the mechanism for the O–O bond formation came after years of calculations, following a slightly different strategy than before (Siegbahn 2006). After numerous

attempts to find a low barrier for the O–O bond formation starting with the original X-ray structure (Ferreira *et al.* 2004), it was realized that a lower energy S_4 structure was obtained when the dangling manganese (**Mn4**) was placed further out from the Mn_3Ca cube. The original position was a direct coordination to a bridging oxo ligand in the cube. Instead, **Mn4** was now placed connected to a manganese in the cube (**Mn3**) via a single terminal oxo bridge (see figure 1), just as in the most recent X-ray structure (Loll *et al.* 2005). The best S_4 -state obtained with this structure has the oxygen radical bound to the dangling manganese. From this structure, essentially all possible ways to form an O–O bond were investigated with full transition-state optimizations. The O–O bond formation was studied for an attack of the oxygen radical on: second-sphere water molecules, calcium-bound water, manganese-bound hydroxide, the oxo ligand connecting the dangling manganese with manganese in the cube and finally the oxo ligand in the Mn_3Ca cube. Ferromagnetic and antiferromagnetic coupling between the manganese centres were compared for all pathways. This investigation gave a very clear answer, where one pathway stood out compared with the other ones with a much lower barrier. The type of mechanism found has since been tested for the other structures suggested and remains by far the best one also for these other structures.

The optimized transition state for the most recent X-ray structure is shown in figure 1. The character of this transition state is essentially identical to the one originally found (Siegbahn 2006) using the assignment of the ligand positions from the first X-ray structure (Ferreira *et al.* 2004). However, the barrier for the one in figure 1 is only $5.1 \text{ kcal mol}^{-1}$, compared to the previous one of $11.4 \text{ kcal mol}^{-1}$. The O–O bond formation occurs by an attack of the oxygen radical on a bridging oxo ligand in the Mn_3Ca cube, which was extremely surprising when it was originally found.

The main features of the O–O bond formation found are illustrated in figure 2. The first requirement for a low barrier is that the spins on the atoms involved are alternating. The reason for this is partly obvious. If the oxygen radical has β -spin, as in the figure, it is clear that it should form a bond with an oxygen with α -spin. Therefore, the electron transferred from the oxo ligand to **Mn2** has to have β -spin, with the α -spin on the electron remaining on the oxo ligand. Since a high-spin configuration is the groundstate of all Mn-states, **Mn2** has to have all its d-electrons with β -spins. This explains the sequence of spins on the oxygen radical, the oxo ligand and **Mn2**. The least obvious spin requirement for a low barrier is that **Mn4** should have α -spin. One reason for this is that an antiferromagnetic coupling between **Mn4** and the oxygen radical is a few kcal mol^{-1} lower than the ferromagnetic coupling for the reactant, which follows from an introduction of some double-bond character. In fact, the alternating alignment of the spins in the figure is a requirement if the groundstate of the reactant should go smoothly over to the groundstate of the product, without crossing to another surface, but the energetic effect on the barrier is surprisingly large.

Figure 2. Requirements for a low O–O bond formation barrier. α and β denote spin directions.

The second most important requirement for a low barrier is that **Mn2** must be easily reduced to Mn(III) in the step where the O–O bond is formed. This leads to a few requirements for the ligands on **Mn2**. First, since Mn(III) is Jahn–Teller (JT) active, a good JT axis has to be available. Also, since the bond between **Mn2** and the oxo ligand becomes broken in the O–O bond formation process, the JT axis has to point along this Mn–O bond. With the first X-ray assignment of the ligands, His332 was placed opposite to the **Mn2**–O bond, which is good since histidine is a neutral ligand and can therefore easily extend its bond length to manganese. A negative monodentate carboxylate would have a much stronger bond to manganese and would therefore be a significantly less optimal ligand along the JT axis. However, a bidentate ligand, like Asp342 in the most recent X-ray structure, is even better since its loss of binding to **Mn2** can be compensated by a strengthening of its bond to **Mn1** (see figure 2). An easy reduction of **Mn2** also puts important demands on the charges of the ligands on this centre. In this context, it is noteworthy that **Mn2** has no place for a water-derived ligand (except the bridging oxo ligands) and also has a predetermined neutral ligand, His332. This means that at the end of the oxidation process, its ligand surrounding leads to the best conditions for forming Mn(III). In contrast, **Mn3** has two water-derived ligands that become deprotonated in the lower S-transitions, reducing its ability to go to Mn(III) in the final S-state transition. **Mn1** has three anionic carboxylates, making its Mn(IV) oxidation state favoured already in S_0 . **Mn2** is therefore both easily reduced to Mn(III) and has an ideal JT axis at the stage when the O–O bond is formed.

The final requirement for a low barrier for formation of the O–O bond is illustrated by the orbitals in the

Figure 3. Energy diagram for dioxygen evolution in photosystem II. The full line corresponds to the ligand assignment from the first X-ray structure ($17.1 \text{ kcal mol}^{-1}$; Ferreira *et al.* 2004) while the dashed line corresponds to the latest one ($14.7 \text{ kcal mol}^{-1}$; Loll *et al.* 2005).

figure. In the bond forming process there are mainly four molecular orbitals involved. These orbitals are formed from the oxygen p-orbitals and a d-orbital on **Mn2**, which can form bonding (σ) and anti-bonding (σ^*) orbitals between the two oxygens and between manganese and the oxo ligand. For a smooth bond forming process, these orbitals should have good overlap, requiring that they are in a reasonably linear arrangement. For this reason the **Mn2**–O–O angle is 146° at the transition state in figure 1.

(d) Comparison between structures

The picture of the O–O bond formation in PSII is not complete without an energy diagram showing all S-state transitions. With calculated proton and electron affinities for each S-state, an energy diagram can be set up by fitting the computed driving force to the experimental value (46 kcal mol^{-1} , see above). This diagram will contain only every other step, where both a proton and an electron have been removed from the OEC between the different S-states. This diagram is essentially independent of the enzyme surrounding, including the choice of dielectric constant. Still, it

contains the main features of the full diagram as described below. To draw a full diagram, one parameter has to be used, here determined as the one that minimizes the barriers and at the same time makes every S-state transition exergonic. The diagrams for the ligand assignment of both the first and the latest X-ray structures are shown in figure 3. Unlike the case in §3b on the frequencies, the chloride is no longer present in the complex. It should also be noted in this context that in the case of the first X-ray structure, the bicarbonate has been removed and the dangling manganese has been placed further out than originally suggested. Otherwise, the barrier for the O–O bond formation would have been much higher.

The main features of the two curves in the figure are quite similar. The two first S-state transitions are strongly exergonic, the one for the first X-ray structure more so than for the latest one, however. The S_2 – S_3 transition is only weakly exergonic. The most interesting development starts after the S_3 – S_4 transition has been reached. For both structures there is a strongly endergonic transition leading to the S_4 – S_0 state, involving both an endergonic electron transfer and an

exergonic proton transfer. The overall rate-limiting barrier should be counted from the resting state at S_3^{-1} to the TS for the O–O bond formation in the S_4^{-1} -state. This barrier for the latest X-ray structure is then $(32.6 - 17.9) = 14.7 \text{ kcal mol}^{-1}$, while the one for the first structure is $(24.6 - 41.7) = 17.1 \text{ kcal mol}^{-1}$, indicating that the latest X-ray structure is probably a slightly better representation of the actual OEC. The experimental turnaround time in milliseconds indicates a rate-limiting barrier of $13\text{--}14 \text{ kcal mol}^{-1}$. The result for the latest X-ray structure is thus remarkably accurate considering the remaining uncertainties in the ligand assignments.

An important result demonstrated by the diagrams in figure 3 is that the rate-limiting barrier for the O–O bond formation is not only determined by the local O–O bond formation, but also by the endergonicity of the S_3 – S_4 transition. This means that even though the difference between the local O–O barriers for the two structures is $(11.4 - 5.1) = 6.3 \text{ kcal mol}^{-1}$, the difference between the rate-limiting barriers is only $2.6 \text{ kcal mol}^{-1}$. Another interesting conclusion is that a proton transfer is included in the rate-limiting step, which means that the barrier will increase by at least $4.1 \text{ kcal mol}^{-1}$ (3 pH units) for the full membrane gradient. The exergonicity of the O_2 release in the S_3 – S_4 transition will also be affected by the membrane gradient to the same extent, which is of importance for analysing the consequences of the results of the experiment on the pressure dependence of the O_2 release (Clausen & Junge 2004). With this background, it is here suggested that the intermediate observed in those experiments could be the bound peroxide formed directly after the O–O bond formation. It is much less likely that the intermediate would be a state prior to the O–O bond formation, since this would imply that the stability of the intermediate would be sensitive to the membrane gradient.

The experimental observation that the S_1 – S_2 transition probably only consists of an electron transfer leading to charging up of the OEC, has often been emphasized as an important factor in the O–O bond formation process. From the curves in the figure, it can be seen that for the first X-ray structure this transition is actually computed to involve only an electron transfer, since S_2^{-1} is higher in energy than S_2^0 . However, the energy difference is only $0.6 \text{ kcal mol}^{-1}$ and can hardly be termed significant in this context. It is also important to note that the S_1 – S_2 transition is anyway very exergonic. Furthermore, for the latest X-ray structure, the S_2^{-1} -state is actually lower than the S_2^0 -state by $0.8 \text{ kcal mol}^{-1}$, but still has a lower rate-limiting barrier for the O–O bond formation.

The most recently suggested structures based on a combination of EXAFS and X-ray crystallography (Yano *et al.* 2006) have also been used in a construction of complete energy diagrams. It is important to note in this context that the models discussed above based on the two most recent X-ray structures do not match the changes of the distances as observed by EXAFS. The main point raised in the EXAFS analysis is that there should be three rather than two short Mn–Mn distances. Only two of the four types of structures suggested in detail could be carried all the way to full

diagrams. For the structure labelled I in the EXAFS paper, it was too difficult at present to assign reliable positions of the ligands, and the optimizations of structure II converged to the same geometries as those based on structure IIa. For IIa, a similar investigation to the one as described above, led to structures with protonations of two of the bridging oxo ligands, which meant that the optimizations failed to reproduce three short Mn–Mn distances for this type of structure. The computed rate-limiting barrier for IIa is $29.4 \text{ kcal mol}^{-1}$, much higher than for the X-ray structures. The most interesting structural results were obtained for structure III, where it was actually possible to retain three short Mn–Mn distances from S_0 through S_3 . However, the energetics obtained are again substantially poorer than those obtained for the X-ray structures in figure 3. The calculations for structure III led to a rate-limiting barrier of $22.2 \text{ kcal mol}^{-1}$, as compared to only $14.7 \text{ kcal mol}^{-1}$ for the most recent X-ray structure and $13\text{--}14 \text{ kcal mol}^{-1}$ indicated from experiments. A major reason for this is that the manganese corresponding to Mn2 in figure 1 is very difficult to reduce in the O–O bond formation step since it is surrounded by three negative oxo ligands and two carboxylates. The driving force in this critical step therefore becomes too low. It should be emphasized that it may still be possible to find another ligand assignment, which is different from the ones suggested so far, giving agreement with the EXAFS interpretations and a good picture of the energetics. However, given the quite strict requirements on the OEC structure for an optimal transition state for the O–O bond formation, as described above, it is hard to see how the OEC could be very different from the one in the most recent X-ray structure and still give a low barrier.

(e) *The effects of cadmium, strontium and chloride*

The effects of substituting calcium with cadmium or strontium, and the effect of adding chloride will finally be briefly mentioned. Cadmium is usually considered to be the metal that most strongly resembles calcium, and it is therefore somewhat surprising that replacement of calcium by cadmium prevents dioxygen formation (Vrettos *et al.* 2001). The calculations on cadmium confirm the picture that it is very similar to calcium, with energy diagrams that do not differ very much. Still, the rate-limiting barrier increases from 14.7 to $16.9 \text{ kcal mol}^{-1}$, which may be enough to explain the experiments. For strontium, the individual steps differ much more, but the rate-limiting barrier of $15.1 \text{ kcal mol}^{-1}$ is only $0.4 \text{ kcal mol}^{-1}$ higher than the one with calcium of $14.7 \text{ kcal mol}^{-1}$. This result is consistent with the observation that strontium is still active, but with a lower overall rate. However, it should be remembered that the accuracy of the calculations is hardly enough to draw definite conclusions about rates with so similar barriers.

The effects of chloride are also found to be very small. The optimal position for chloride is on the dangling manganese, as shown in figure 1. A comparison to the case without chloride becomes slightly parameter dependent. As discussed above, a parameter

has to be chosen to set up the diagrams, and this parameter is chosen to minimize the barriers involved. It can be considered as a parameter for the optimal enzyme surrounding of the OEC. If different parameters are chosen for the cases with and without chloride, the rate-limiting barriers become very similar at 14.8 and 14.7 kcal mol^{−1}, respectively. However, if chloride is present in the actual cluster it can be argued that the environment should be optimized for chloride. Therefore, if the optimal parameter for chloride is used also for the case without chloride, the barriers become 14.8 and 15.5 kcal mol^{−1}, showing a slight preference for the case with chloride. Again, the main conclusion is that the results are very similar, the differences being too small for drawing firm conclusions.

4. CONCLUSIONS

The main steps in the formation of the O–O bond in PSII, as obtained from hybrid DFT calculations, have been described. The first key step is an endergonic formation of an oxygen radical in the S₃–S₄ transition. The O–O bond is then formed by an attack of the oxygen radical on a bridging oxo ligand in the Mn₃Ca cube. There are several requirements on the OEC complex, schematically illustrated in figure 2, for making the barrier for the O–O bond formation as low as possible. An alternating spin alignment is one of the most important of these. An easily reduced Mn²⁺ centre with a good JT axis is another one. Optimal orbital overlaps lead to other requirements on the transition-state structure. In fact, these requirements can be used to define most structural features of the oxygen-evolving complex, with a dangling manganese that can hold an oxygen radical in an optimal approach towards an oxo ligand with a reasonably linear Mn–O–O arrangement. In turn, a highly coordinated oxo ligand is optimal, since one of its bonds (to Mn²⁺) can be broken without much cost of energy, by a compensating strengthening of its remaining coordinations. Finally, the coordination environment around Mn²⁺ makes it optimal for reduction to the JT-active Mn(III)-state in the O–O bond forming process.

As a final remark it is interesting to note that so much energy is wasted in the O–O bond formation process, even with full membrane gradient. In contrast, mitochondrial respiration with a very similar driving force loses almost no energy. In that case energy is saved by an additional pumping of protons across the membrane. PSII has apparently not been able to save energy in this way. One reason for this could be that the very strong demand on the OEC structure for forming the O–O bond with a low barrier has been impossible to combine with the equally strong demand on a complex for allowing translocation of protons as in cytochrome *c* oxidase.

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Discussion

J. Barber (*Imperial College London*). I have two questions. What are the main factors which give rise to the differences in the barrier for the (Ferreira *et al.* 2004; Loll *et al.* 2005; Yano *et al.* 2006) structures and when does the Ca^{2+} –O–Mn bond reform in your scheme?

P. E. M. Siegbahn. First, it should be emphasized that the differences are not very large, only a few kcal mol^{-1} on the barrier. With such a small difference, it is difficult to say exactly where the effects are. One difference is the *trans* ligand along the JT axis, which in the Ferreira *et al.* structure is a histidine and in the Loll *et al.* structure a carboxylate which binds bidentately to two metals. The latter is probably slightly favoured. Also, the metal in the cube that turns from Mn(IV) to Mn(III) as the O–O bond is formed is in a slightly less negative surrounding in the Berlin structure, thereby making a transition to Mn(III) more favourable, leading to a larger driving force for the O–O bond formation step. Regarding your second question, the Ca–O–Mn bond reforms as dioxygen is released and two waters become bound in the S_4 – S_0 transition.

D. Nocera (*MIT, Boston, USA*). You propose to effectively couple oxygen based radicals within the Mn_4Ca cluster of OEC. Do you know any details for the O_2 release presumably after ‘peroxide’ formation?

P. E. M. Siegbahn. I have looked a little bit at the mechanism for dioxygen release after peroxide has been formed. It is a very complicated part of the entire mechanism where also two water molecules become bound to the complex. In my model calculations there is no thermodynamic problem in releasing dioxygen, but there are, of course, some barriers. Probably the best information on this part of the mechanism can be found in the work by Clausen & Junge (2004), who estimate a rather high barrier in the last step after they detected an intermediate, still unidentified. From my model results I think that their intermediate is likely to be the bound peroxide directly after the O–O bond formation.

H. Dau (*Freie University, Berlin, Germany*). You stated that in the S-state cycle of photosynthetic oxygen-evolution transitions 30 kcal mol^{-1} is ‘wasted’ in the form of heat release. The redox potentials of the donor-side redox factors are most likely more positive than previously assumed (E_m of P680/P680^+ of $\sim 1.25 \text{ V}$; see Rappaport *et al.* (2002) and Grabolle & Dau (2005)). Nonetheless, I feel that the figure of 30 kcal mol^{-1} is clearly too high and in conflict with common assumptions on the PSII energetics. Can you comment on this?

P. E. M. Siegbahn. The driving force for dioxygen formation in PSII can be calculated from the redox potential of P_{680} of 1.3 V and the one for $\text{H}_2\text{O/O}_2$ of 0.8 V , to be 2 V , or approximately 45 kcal mol^{-1} . Without any membrane potential all of this energy will be wasted as heat. However, with the maximum membrane potential, only approximately 30 kcal mol^{-1} is wasted. This is different from respiration in cytochrome oxidase where much less energy is wasted as heat since it can be saved by additional proton pumping. The reason for this difference is, no doubt, the much more difficult chemistry performed by PSII in the O–O bond formation step.

F. Armstrong (*University of Oxford, UK*). What is your prediction of the individual Mn valencies (oxidation states) immediately upon release of O_2 ? Do you expect relaxation (redistribution) of valences to achieve a stable S_0 ?

P. E. M. Siegbahn. The lowest energy S_0 -state from the calculations is one with three Mn(III) and one Mn(IV). The detailed pathway from the bound peroxide, formed after the O–O bond formation in S_4 , has not been investigated. For the peroxide state there are three Mn(IV) and one Mn(III). It is quite possible that in between the peroxide and the S_0 -state there are several other states, may be even involving Mn(II).

P. Rich (*University College London, UK*). What is the expected level of confidence and accuracy in your carboxylate frequency predictions? Would not the calculated values be different if the model resembled the amino acids more accurately by replacing the H-COO^- in your model by $\text{R-CH}_2\text{-COO}^-$, for example?

P. E. M. Siegbahn. The absolute accuracy of the calculated frequencies is not very high and they would, for example, be improved by extending the models of the amino acids, as you suggest. However, the main point of doing the calculations was to test the hypothesis that the presence of a shift in a frequency of an amino acid means that the Mn centre to where the amino acid is bound must have been oxidized. The calculations show that a large shift can appear even if the amino acid is not bound to an oxidized centre. In fact, the oxidation might even have occurred in the other end of the complex and still give rise to a substantial shift. It is unlikely that this type of qualitative behaviour would change in a more accurate treatment of the frequencies.

Additional references

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