Nucleophilic water attack is not a possible mechanism for O–O bond formation in photosystem II

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Two different types of mechanisms are at present suggested for the O–O bond-formation step in photosystem II. The first one is a coupling between an oxyl radical and a bridging oxo. The second one is a nucleophilic water attack on a terminal oxo (or oxyl) group. In the present short paper, the six most reasonable versions of the latter mechanism have been studied and compared with the oxo-oxyl mechanism. The barriers are found to be much too high for the water attack, and that mechanism can therefore safely be ruled out. The reason is that the protonated peroxide product is always very high in energy.

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he leading mechanism for O–O bond formation has for a long time been an attack by an oxyl radical on a bridging oxo-bond (1–7). This mechanism, first suggested in 2006 (1), was reached after trying all possibilities for a small, but reasonable, model of the oxygen-evolving complex (OEC), including an additional oxo-ligand compared with the low-resolution structures. The number of possibilities is not large and can therefore all be tested (also see below for the nucleophilic attack mechanism). This oxo-oxyl mechanism fulfills all requirements so far set by spectroscopy. Detailed structures for the S3−S1 states were suggested in 2008 (2) and refined in 2011 (3). After a set of remarkable spectroscopic experiments in 2011–2014, the structures for S2−S1 were confirmed in detail (8–10). Likewise, the involvement of a bridging oxo in the O–O bond formation step was confirmed in 2012 based on a combination of EPR and water-exchange experiments (8, 9). It can be added that, already in 2004, it was speculated that the O–O bond should be formed between a terminal water ligand to Mn and an oxo bridge between Mn and Ca (11). The involvement of an oxo bridge was a very controversial suggestion at the time because it was known that an oxo group in general would not exchange with water as fast as observed by water-exchange experiments on photosystem II (PSII).

Despite the present consensus between theory and experiments, there are still arguments put forward for another mechanism (12, 13). In that mechanism, a nucleophilic water, bound to calcium, attacks an oxyl radical (or oxo group) on the dangling manganese. In the present work, calculations have been performed to reinvestigate the viability of this water attack mechanism. For completeness, it should be mentioned that very different mechanisms have also been suggested. In one of these, a key aspect is the absence of substrate–water deprotonations before S4 (14). In another one, the oxyl radical has a very low energy already in S4, implying a possible start of the formation of the O–O bond already at that stage (15). Neither of these two mechanisms will be discussed further here. Mechanisms found for heterogeneous systems (16) will not be discussed either, because it is not clear at the present stage how to translate those mechanisms to the homogeneous case.

It is true, as is often claimed, that a suggested mechanism can never be proven to be correct. Conversely, a suggested mechanism can be proven to be incorrect. This conclusion can be made both based on experiments and on model calculations. In theoretical model studies, there are simple rules based on the past decade of experience. If two mechanisms have barriers within 3 kcal/mol, density functional theory (DFT) calculations cannot safely distinguish which is the right one. Already with a difference of 5 kcal/mol, the mechanism with the highest barrier is quite unlikely. In addition, when the difference is 10 kcal/mol or more, the safe conclusion can be made that the mechanism with the higher barrier is not viable. These conclusions can be made given that the chemical model used is sufficiently large and that the methods are well tested. A scientific argument against these conclusions requires a demonstration that the rules do not hold in some cases for a similar system. In the present calculations, models and methods are used that have been very carefully tested over at least a decade (5). For the mechanism of water oxidation, a large number of predictions using these methods and models for the S1−S3 states have obtained very strong support from experiments (see above). A problem in this context is that the critical S4 state, where the O–O bond is formed, is so short-lived that it will probably never be possible to study this state directly by experiments.

Approximately 10 years ago, a number of mechanisms for O–O bond formation were compared (1), but for a small model with ~60 atoms, and with only low-resolution X-ray structures available (17–19). In that case, one mechanism stood out as having a barrier much lower than any other one. This was the oxyl-oxo mechanism, found to have a barrier of 12.5 kcal/mol with respect to an S4 state with an oxyl radical. In comparison, the lowest water attack mechanism had a barrier of 18.0 kcal/mol, which was therefore considered unlikely to be the correct mechanism. The difference was rather clear, but still not completely decisive. Because the model of the OEC was small (of the closed form) and only derived from preliminary calculations, a larger and more correct model might decrease this energy difference to make the mechanisms more comparable. A reinvestigation of the nucleophilic attack mechanism is therefore urgent, now that much better structures are available (2, 3, 20, 21) and the methods have been refined (5).

Results

The model used here is the same one as used in earlier studies with 200 atoms (4). The O–O bond was found to be formed between O3 and O4 (Fig. 1) in the open form of the OEC. The

Significance

In this short paper, a detailed investigation is made of one of the leading suggestions for O–O bond formation in photosystem II. The mechanism studied is a nucleophilic attack by a water molecule on an oxyl group in the oxygen-evolving complex. The calculations show quite clearly that this type of mechanism is not possible in this case.

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barrier obtained for O–O bond formation using the oxyl-oxo mechanism is 6.2 kcal/mol, with respect to an oxyl S4 reference state. In the present study, DFT calculations were performed at the B3LYP level by using a Poisson–Boltzmann continuum solvent (dielectric constant 6) and including the Grimme D2 empirical London Dispersion correction (see Methods and Models for more details). Compared with the methods used in the original study, full geometry optimization was performed of the transition states (TSs), and entropy effects were included. In the present case, the entropy effects are quite small and could in fact be neglected. Other cases—for example, when O2 is released—need another treatment, which has been described elsewhere (6). The old result using the same 200-atom model was 7.7 kcal/mol (4). For all mechanisms discussed here, there is an estimated cost of 3.6 kcal/mol to reach the oxyl S3 state, which should be added to the barrier obtained for the O–O bond-formation step. The oxyl-oxo mechanism is also possible in the closed form of the OEC, with a bond between O5 and an oxyl radical bound to Mn4, and this variant has a similar barrier (6, 11).

For the water attack mechanism, six possible variants can be identified. The oxyl radical could be either on O3 or on O8 (Fig. 1). Attempts to converge oxyl radicals at other positions led to substantially higher energies, 18–19 kcal/mol above the O3 radical. Furthermore, the OEC could be either in the open form with O3 bound to Mn4 or in the closed form with O8 bound to Mn4. Two types of spin states are relevant, with either a high-spin coupling between the oxygen radical and the manganese to which it is bound or in a low-spin coupling. In the high-spin coupling case, all manganese spins were chosen parallel, whereas in the low-spin case, one manganese spin was chosen antiparallel to the rest, following the mechanism used previously for the oxyl-oxo mechanism. For the water attack mechanism, only one manganese atom is involved, and the spin directions of the other manganese atoms hardly affect the results. The results for all six possibilities are given in Table 1. It has been shown (7) that the low-spin state is the ground state for the oxyl-reactant S3 state, whereas the high-spin state is the ground state for the peroxy product of the water attack mechanism. This means that for this mechanism there has to be a spin-crossing during the O–O bond formation, which is avoided in the oxyl-oxo mechanism (1).

In mechanisms 1 and 2, a water attack is done on O8 bound to Mn4. The open form of the OEC was used for this attack. As seen in Table 1, the barriers are very high, both if they are in the low-spin or in the high-spin states, 48.6 and 43.2 kcal/mol, respectively. The reason for this is that the product peroxy formed directly after O–O bond formation is in the O–OH2 form, which is very unstable. All reasonable bases are too far away to be used to deprotonate the water before O–O bond formation. If a proton is any way moved from the incoming water to a base far away (in an unknown pathway), a high-energy OH-radical is formed. For mechanism 1, a lower barrier of 34.6 kcal/mol is then obtained for O–O bond cleavage, whereas for mechanism II, the barrier is approximately the same as the one in Table 1. If the proton is moved only after the O–OH2 product has been formed, to form an OOH peroxy, the energy of the product goes down to +29.4 kcal/mol for mechanism 1 and +19.2 kcal/mol for mechanism 2. The pathways to these OOH products have low barriers.

In mechanisms 3 and 4, the oxyl radical is on Mn4 but in another position than O8, therefore termed O9. The closed form of the OEC is used. This is the mechanism that most closely resembles the originally suggested water attack mechanism (12, 13). One difference from that suggestion is that the attacking water is no longer bound to Ca at the TS. This loss of Ca bond occurs automatically because water on Ca is too far away from the oxyl radical. In addition, it is actually more positive than a free water, and therefore not better suited as a nucleophile. To restrict the water to be bound to calcium makes the barrier significantly higher, as shown before (1). Instead, the water is replaced by another water on calcium. Equivalently, an outside water makes the attack directly, rather than one bound to Ca. Again, the barriers are high, 32.3 kcal/mol for low spin and 29.8 kcal/mol for high spin, but still the lowest barrier of the six mechanisms investigated. These barriers are lower than the other ones because the OH group on Mn4 can serve as a reasonably good base and deprotonate the water before the TS. In mechanisms 5 and 6, the oxyl radical is on Mn1, and the barriers are still high, with 33.4 and 39.8 kcal/mol, respectively. The open form of the OEC is used. Here, O5 deprotonates the water before, or at, the TS for mechanism 5, whereas the base in mechanism 6 is the OH group on Mn4.

As an aside, the pKa of Arg-357 was also calculated because it has been suggested to be unprotonated in S3 (12, 13). The calculated pKa in S3 is 12.4, indicating that it is not favorable to

![Fig. 1. Schematic picture of the OEC. O8 and O9 denotes the two possible positions for the oxyl radical. Two different positions of O9 exists. If it is bound to Mn4 as in the figure, it is termed the open structure; if it is bound to Mn1, it is termed closed.](image-url)
have it unprotonated. The value was obtained by comparing the proton affinity of Arg-357 to the one of the waters bound to Mn₆, which has been suggested to be deprotonated only when Tyr₂ becomes oxidized. Its pKₐ was therefore set to 6 at that stage. Before Tyr₂ is oxidized, the pKₐ for Arg-357 is significantly higher.

Conclusions

In summary, the nucleophilic water attack mechanism has very high barriers in all cases, with the lowest one being 29.8 kcal/mol. This barrier should be compared with the best oxyl-oxo mechanism, which has a barrier of 6.2 kcal/mol using the same model and methods. According to the general rules developed in the past decade, the water attack mechanism is therefore concluded to not be a viable mechanism. The reason this mechanism has such high barriers is clearly connected to the very poor energies for the product where the O–O bond has been formed. There are only two alternatives for the product—either being in an OOH form or in an OOH₂ form. The latter one is, not surprisingly, very high in energy, but also the former one is high. In contrast, the oxyl-oxo mechanism forms an unprotonated peroxide directly, which is strongly bound to the Mn cluster. To afterward protonate this peroxide is very unfavorable. The lowest form of the protonated peroxide has been calculated to be 17.3 kcal/mol above the unprotonated peroxide, by moving a proton from the water on Mn₆ and placing it on the peroxide. It is obviously an advantage that the peroxide does not want to be protonated before it is released from the OEC, because this will avoid unwanted hydrogen peroxide formation.

Another reason for the high barrier for the water attack mechanism is that a transition between two potential surfaces is required, unlike the case of the oxyl-oxyl mechanism, where this problem is avoided (1).

The conclusion from the present calculations is that the nucleophilic water attack can be ruled out for PSII. A very large error in the method and model is extremely unlikely, in particular considering the fact that the predictions made for the S₁–S₃ states have been confirmed in detail by experiments.

Finally, it should also be mentioned that there are recent strong experimental indications that the nucleophilic water attack is an unlikely mechanism for PSII. By using arguments based on mass-spectrometric and spectroscopic results, it was concluded that the nucleophilic attack is strongly disfavored compared with the oxyl-oxo mechanism (22). In a comparison between the two mechanisms, it was recently argued that current experimental data favor a coupling-type mechanism of the oxyl-oxyl type (23).

Methods and Models

The hybrid functional B3LYP* (24, 25) was used with polarized basis sets for the geometries (lcaup*), large basis sets for energies [cc-pvtz(f)], and a surrounding dielectric medium with dielectric constant equal to 6.0 (basis lcaup*). Dispersion effects were added by using the empirical G2 formula of Grimme (26). Zero-point and entropy effects were obtained from Hessians calculated by using the lcaup* basis set. The entropy effects were obtained by projecting out the frequencies connected with the fixed coordinates. Fully optimized transition states were located at the same computational level. The calculations were performed with the Jaguar (27) and Gaussian (28) programs.

The model is the same as the one used in refs. 3 and 4. It includes the following amino acids: the directly binding amino acids, Asp-170, Glu-189, His-332, Glu-333, His-334, Glu-342, Ala-344, and Glu-354; the second shell residues, Asp-61, His-337, and Arg-357, and the region around the chlorides are also included. This region also contains, besides chloride, LyS-317 and three water molecules, forming a hydrogen-bonding network, as in the X-ray structure (5).

All atoms in the model along the backbone, carbons, and hydrogens have been fixed from the X-ray structure. Exceptions are backbone atoms between His-332 and Glu-333 and between Asp-342 and Ala-344, where only two carbon atoms were fixed. Coordinates for all structures can be obtained from the author upon request.

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