The Mechanism of Water Delivery to the Active Site of Photosystem II along the S_2 to S_3 Transition

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Abstract: The two water molecules serving as substrate for the oxygen evolution in Photosystem II are already bound in the S_2 state of the Kok-Joliot's cycle. Nevertheless, an additional water molecule is supposed to bind the cluster during the transition between the S_2 and S_3 states, which has been recently revealed to have the Mn_4CaO_5 catalytic cluster arranged in an open cubane fashion. In this letter by means of *ab initio* calculations we investigated the possible pathways for the binding of the upcoming water molecule. Upon the four different possibilities checked in our calculations, the binding of the crystallographic water molecule, originally located nearby the Cl^- binding site, showed the lowest activation energy barrier. Our findings therefore support the view in which the W2 hydroxyl group and the O5 oxygen act as substrates for the oxygen evolution. Within this framework the role of the open and closed Mn_4CaO_5 conformers is clarified as well as the exact mechanistic events occurring along the S_2 to S_3 transition.

Photosynthetic water splitting takes place in the Mn_4CaO_5 active site of the unique complex Photosystem II. The catalytic cycle, known as Kok-Joliot's cycle [1,2] proceeds through five intermediate states S_0-S_4, towards the subsequent photo-activated abstraction of four electrons from the active site, eventually leading to oxygen evolution. Structural properties of the reaction center of PSII have been revealed in the past decades at higher level of resolution by means of X-ray crystallography and extended X-ray absorption fine structure (EXAFS) experiments. [3–7] Additionally, an important contribution to the understanding of the catalytic mechanism occurring in the reaction center of Photosystem II (PSII) came from several theoretical studies carried out in the last years. [8–13] A crucial step for the detailed understanding of the reaction mechanism resides in the transition between the S_2 and S_3 states. In this respect different conformers have been identified by EPR experiments [14–17] and calculations: [18,19] an open cubane and a closed cubane structure in the S_2 state (namely, S_2^A and S_2^B, respectively, see also Fig.1), and an open cubane conformer in the S_3 state. Although thermodynamically slightly less stable than the corresponding open cubane conformer, the closed cubane S_2^B conformer has been indicated as a key intermediate in the passage towards the S_3 state. [17] Recent calculations [20,21] have also demonstrated that the passage between the closed cubane S_2^B conformer and the open cubane S_3 conformer is possible via a reorganization between the W2 hydroxyl group and the μ-oxo O5. The

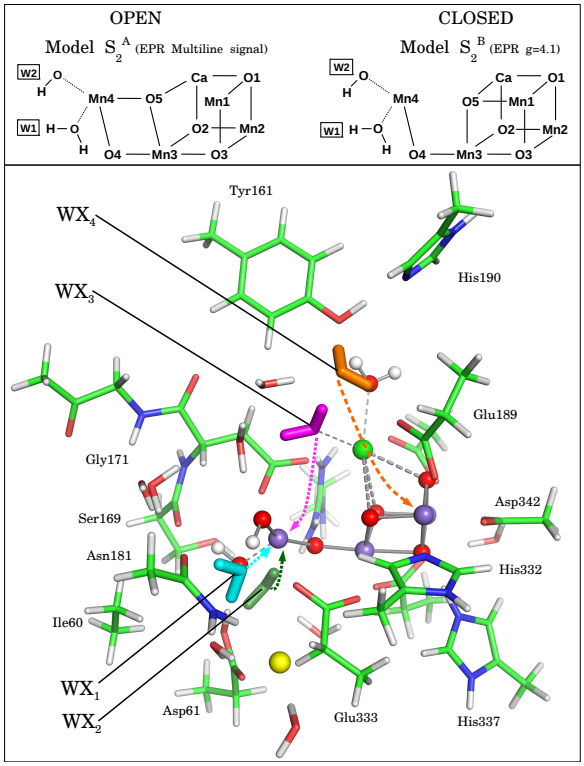

Figure 1. Upper panel: the two different conformers in the S_2 state: on the left the open cubane conformer and on the right the closed cubane conformer. Lower panel: The Quantum Mechanical model of the Mn_4CaO_5 catalytic cluster of Photosystem II considered in the present calculations at DFT+U level of theory. The model represents the S_2^{B+} state prior the upcoming water binding. Four possible binding water molecules are highlighted in different colors. The WX_{1-3} paths bind in the closed cubane model, while WX_4 in the open cubane.

exchange of substrate waters to the Mn_4CaO_5 cluster has been investigated by means of isotopic labeling [22] suggesting that both substrate water molecules are already bound in the S_2 state. [23] Whereas different experimental and theoretical studies have been indicated the oxygen O5 as one of the two substrate oxygen, [24,25] the identification of the second substrate water is still matter of debate. Moreover, an additional water molecule WX was suggested to bind a manganese ion of the cluster between S_2 and S_3 states, [26,27] this hypothesis was recently confirmed by a study based on high field EPR. [28] In this regard, several hypothesis have been proposed on the identity of such water molecule, including the water molecule bound to Ca^{2+} ion, [18] two water molecules coming from the channel ending to the Asp61 and a water molecule belonging to the water channel eventually leading to the coordination of the Mn1 ion [29] (see Fig.1). It

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has to be pointed out that this last possibility suggested by Siegbahn refers to a water molecule not present in the crystal structure, whereas in the current work we have evaluated the energetics of the water binding reaction to Mn1 considering the closest water molecule to the Mn1 ion identified in the X-ray structure. The energetic of the water permeation along the above mentioned water channel was also explored by classical molecular dynamic simulations.[30]

As mention before, based on isotopic labeling experiments,[23] the possibility that the WX water molecule acts as substrate is considered highly unlikely. Therefore the identification of WX automatically limits the possibilities and may lead to the definitive recognition of the two substrate molecules.

It is evident that understanding the water binding mechanism occurring between the S_2 and the S_3 state is mandatory for the sake of a full comprehension of the molecular details behind the Kok-Joliot cycle. In this regard, important insight for the identification of the WX water molecule and the surrounding H-bond network recently arise from theoretical studies.[31–33] In particular Yamaguchi and coworkers elucidated two favorable reaction pathways for the transition from S_2 to S_3. Both pathways involve the binding of a originally calcium-bound water to the pentacoordinate Mn(III) ion.[31] Conversely, Retegan *et al.* suggested a water binding mechanism between the S_2 and S_3 state involving the water molecule originally located close to the Asp61.[33]

In the present work a systematic computational investigation of different water binding pathways was carried out in order to find the energetically most favorable path among those proposed in literature. We performed Minimum Energy Path (MEP) calculations in a Density Functional Theory (DFT) framework, taking into account four different water binding pathways, each involving a different water molecule. The coordinates of the gas phase models used in our calculations have been taken from the last solved crystallographic structure,[7] and the hydrogens have been added using a protocol similar to that used in previous works.[20,21,34] Overall, the gas phase models used here consist of 236 atoms (see Fig.1).

The DFT+U based calculations[35] were performed following the same setup employed in Ref.[34] The structures taken into consideration as initial point are the open and closed cubane conformers of the oxidized S_2 state, namely S_2^{A+} and S_2^{B+}. In order to characterize from the kinetic and thermodynamic point of view the water binding pathway, we carried out Minimum Energy Path calculations based on the nudged elastic band method as implemented in the CP2K package.[36,37] Additional details concerning the models setup and composition, the parameters employed in the quantum calculations, and the procedure adopted for the MEP calculations are reported in Supplementary Materials.

Below we will describe the results obtained in the thermodynamic and structural characterization of the four investigated water binding pathways.

-WX_1: this water molecule in its crystallographic position is supposed to be bound by H-bond to the W2 water molecule (formally a hydroxyl group coordinated to the Mn4 ion in the S_2^+ state), with the residue Asn181 and with an additional X-ray water molecule. The binding of WX_1 to the Mn4 ion occurs in a position between the two W1 and W2 hydroxides, turning out an hexacoordination of the Mn4 and a consequent rearrangement of the two previously bound hydroxyl ions. Interestingly, the spin populations of the WX_1

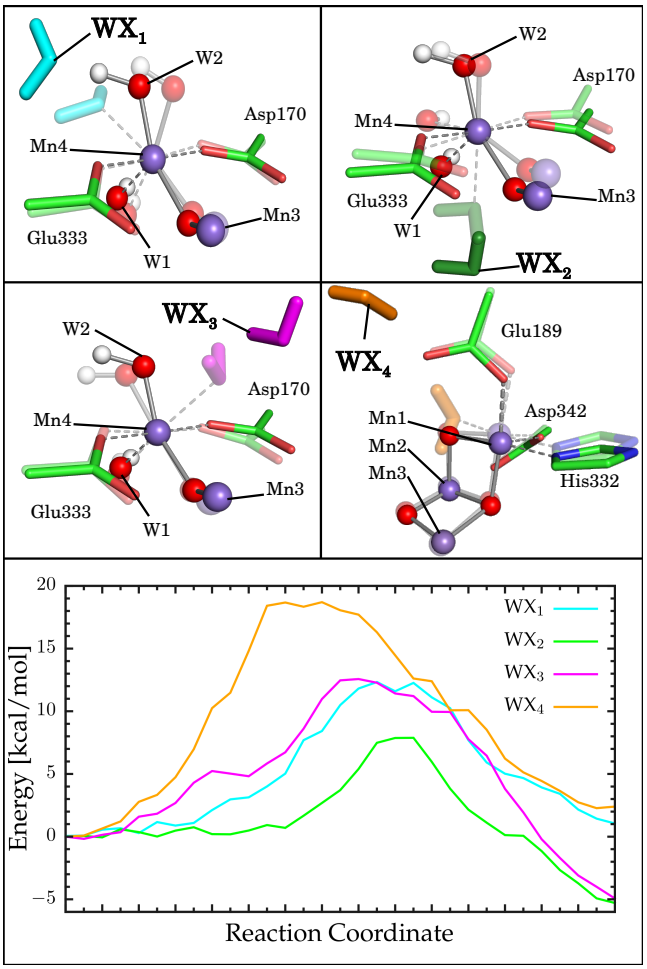

Figure 2. Upper panel: sketches of the paths considered for the WX_1, WX_2, WX_3 and WX_4 water molecules binding to the cluster in the S_2^+ state. The different water molecules are represented as solid sticks. The final conformation of the Mn ligands along the minimum energy pathways is represented as semi-transparent sticks. Lower Panel: the minimum energy path along the binding reaction coordinates of WX_{1-4} water molecules.

oxygen and the Mn4 ion are strongly coupled, thus the manganese ion reaching a maximum positive charge alongside the transition state (data not shown). The MEP predicted an activation energy barrier for the binding pathway involving the WX_1 water molecule of $\sim$ 12 kcal/mol. Based on the Eyring-Polanyi equation, this energy barrier corresponds at room temperature to an half-life time $\tau \sim$ 1-10 μs, thus allowing the water binding process to occur within the experimental time scale. Nevertheless, our results show a positive ΔE $\sim$ 1 kcal/mol that leads to discard this path in favor of other exergonic pathways with either similar or smaller activation energy barriers.

-WX_2: based on the position found in the X-ray structure this water molecule, similarly to the case of WX_1, is characterized by the presence of three hydrogen bonds with Asp61, with the μ-oxo O4 and with an additional X-ray water molecule. The oxygen of WX_2 points to the opposite direction of the Mn4 ion, so such water binding process has to involve the rotation of the binding water molecule. This movement is responsible for the lost of the hydrogen bond with the μ-oxo O4, which is in turn replaced by a different hydrogen bond with an adjacent H_2O. Among the four investigated pathways, this one shows the lowest energy bar-

rier ∼ 8 kcal/mol and is characterized by a clearly exergonic energy landscape (ΔE = ∼ -5 kcal/mol). The energetic of this pathway clearly indicates the WX_2 as a good candidate for the binding between the S_2 and S_3 state. Moreover, consistently with isotopic labeling experiments,[23] the upcoming water would not act as a substrate molecule, leaving this role to the previously bound hydroxyl ion W2.

-WX_3: it is the W3 water molecule directly bound to the Ca ion in the X-ray structure. A water binding pathway involving the WX_3 molecule was suggested by Bovi *et al.* based on QM/MM Molecular Dynamics simulations with a constraint applied on the Mn1-Mn4 distance.[18] The mechanism involving the binding of WX_3 requires a drastic rearrangement of the hydrogen bond network and of the closest water molecules. This mechanism is supposed to be a chain process, in which the movement of WX_3 towards the Mn4 ion occurs while an external water molecule replaces the WX_3 in its original position coordinating the Ca ion. The calculated activation energy barrier for this pathway is around 12 kcal/mol, which would represent a rate limiting step of the S_2-S_3 transition. This path would represent a good candidate for the water binding mechanism also due to the stabilization of ∼ 5 kcal/mol found for the bound state when compared to the starting state. On the other side, it is worth to mention that a similar mechanism implies that the binding water molecule could act as a substrate in apparent disagreement with isotopic labeling experiments. Albeit from the energetic point of view we cannot exclude this pathway, the WX_2 water molecule remains although a better candidate for the water binding mechanism here investigated.

Another possibility to complete the coordination sphere of the Mn4 ion might consists in the binding of the deprotonated form of WX_3. However, such option was not explored in our calculations due to the unlikely of such scenario, as already pinpointed by previous pK_a calculations.[12,33]

-WX_4: unlike the other three investigated cases, an eventual binding mechanism between the WX_4 molecule and the Mn1 ion entails the involvement of the open cubane form of the Mn_4CaO_5 cluster. Additionally, the nearest water molecule to Mn1 is located ∼ 6 Å faraway form such ion. Therefore the water molecule requires a movement of ∼ 4 Å to reach the final binding site against a maximum displacement of ∼ 2 Å required by the WX_1, WX_2 and WX_3 water molecules to eventually bind the Mn4 ion. The approach of WX_4 to Mn1 is partially impeded by the presence of the Glu189 residue. Indeed, the transition state for this pathway was found to coincide with the overcoming of the carboxylate group of Glu189. Consistently with such evidence, an activation energy barrier of ∼ 19 kcal/mol was calculated for the concerned pathway, making together the endergonic nature of this path (ΔE ∼ 2.3 kcal/mol), the mechanism highly disfavored.

Based on our results, the binding of the WX_1 and WX_4 water molecules can be excluded due to the endergonic character of the process and, in the case of WX_4, also to the energy barrier of ∼ 19 kcal/mol. Conversely, from a thermodynamic point of view, both WX_2 and WX_3 could bind to the Mn4 ion during the S_2 to S_3 transition, with the former molecule characterized by a considerably lower activation energy barrier when compared to the second (∼ 8 vs ∼ 12 kcal/mol). Nevertheless, assuming that one of the two substrate water molecules is represented by the μ-oxo binding the Mn4 and coordinated to the Ca^{2+} ion (namely O5 in

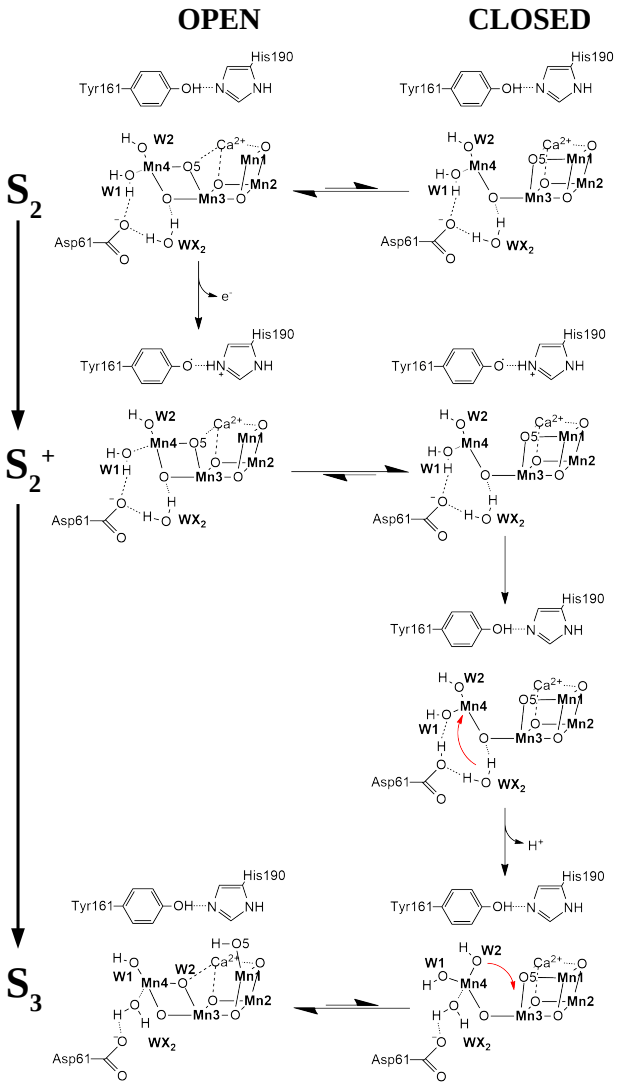

Figure 3. Final complete scheme of the S_2 to S_3 transition with all the studied intermediates

the X-ray structure), the WX_3 water molecule after binding to Mn4 would act as substrate. This is in disagreement with isotope labeling experiments suggesting that the substrate water molecules has to be already bound in the S_2 state,[23] thus excluding the possibility that the upcoming WX_3 water molecule could directly participate to the formation of the O-O bond in the S_4 state. In contrast, WX_2 after binding to the Mn4 ion would not be directly involved in the O-O bond formation. The binding of WX_2 completes the hexacoordination of the Mn4 in the closed cubane structure, leading the W2 hydroxide to assume the most convenient conformation in order to transit from the closed cubane to the open cubane conformation as suggested in Ref.[21] (see also Fig. 3).

In the final open-cubane S_3 state, the W2 oxygen already bound in the S_2 state as a hydroxide, takes the place of the μ-oxo O5, while the O5 atom turns out to be bound to the Mn1 ion as a hydroxide. It is worth noting that in the present scenario, the two possible substrates (the W2 oxygen and the O5 atom, already suggested to act as substrates by Messinger and coworkers[38,39]) interchange their chemical nature during the S_2 to S_3 transition (from hydroxide

to μ-oxo and *vice versa*). This means that, in both S_2 and S_3 states, the substrate moieties consist of a μ-oxo and a hydroxide, independently of their specific identity. This potentially explains the similar fast and slow water exchange velocities measured in such states by isotopic labeling experiments.[23]

Based on our calculations and the available experimental data, herein we suggest the WX_2 as the additional water molecule binding to the manganese cluster during the transition from the S_2 to the S_3 state.

Our results confirm the conclusions achieved by Retegan *et al.*,[33] albeit the values of the activation energy calculated in the present work for the binding of WX_2 water molecule, is ~ 3 kcal/mol higher with respect to the activation energy reported in Ref.[33] This discrepancy could arise from the different number of atoms considered by the two calculations. In particular the two models differ by the number of crystallographic water molecules taken into account (13 in our model and 8 in their model *et al.*).

In Fig.3 we report the suggested mechanism leading from the open cubane S_2 conformer to the open cubane S_3 state. In the S_2 state two interconvertible conformers were suggested to exist,[18,19] the more stable S_2^A state characterized by an open cubane structure and a second less stable closed cubane state (S_2^B). Upon the removal of an electron from the S_2 state the relative stability of the two conformers is reversed and the Tyr-Z assumes a radical character.[20] While in the S_2^A state Tyr-Z was found to keep such radical character, in the closed cubane S_2^B state an oxidation of the Mn4 by the Tyr-Z radical spontaneously occurs in few hundreds of fs, thus leading the cluster to an electronic oxidation pattern consistent with the S_3 state.[20] The next step consists in the binding of an additional water molecule, addressed in the present work. The water molecule, originally close to the Asp61, can efficiently bind th e Mn4 ion in the closed cubane S_2^{B+} state. After the water binding a transition from the closed cubane to the open cubane structure can occur in the μs time range as recently shown by Guidoni and coworkers,[21] finally leading to the open cubane S_3 state suggested by Cox *et al.*[28]

Overall, this work adds one more piece to the puzzle represented by the catalytic mechanism of the photosynthetic water splitting, identifying the water molecule which binds between the S_2 and the S_3 states and reconciling previous apparently contrasting experimental and theoretical studies.

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Supporting Information Available: Four movies graphically showing the results of the minimum energy path obtained for WX_1, WX_4, WX_3 and WX_4 are available as supplementary information. Additional details of computational method and the coordinates (XYZ) of the principal Replica are also available as supplementary information. This material is available free of charge via the Internet at `http://pubs.acs.org/`.

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