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Review

Important roles of tyrosines in Photosystem II and cytochrome oxidase

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Abstract

Theoretical studies (B3LYP) on models of the active sites in Photosystem II (PSII) and cytochrome oxidase are discussed. The role of a tyrosyl radical in the O—O bond formation in PSII is investigated, as well as the tyrosyl radical formation. In cytochrome oxidase, mechanisms for O—O bond cleavage involving tyrosyl radical formation are investigated, together with possible roles for the tyrosine in the proton translocation.

Keywords: Theory; B3LYP; Mechanism; Photosystem II; Cytochrome oxidase; Tyrosyl radical

1. Introduction

Photosystem II (PSII) and cytochrome oxidase (CcO) are responsible for essentially all of the creation of dioxygen and for most of its consumption by living organisms. There are many striking and fascinating parallels between the mechanisms of these two enzymes. For example, both of them have catalytic cycles with four distinct and well-defined intermediate states. In each transition, one electron leaves (PSII) or enters (CcO), and this is coupled to proton translocation across the membrane for both systems. The active sites of the enzymes are designed to control this electron and proton flow so that as little energy as possible becomes wasted. When the first structural information appeared for these enzymes, other notable similarities became evident. For example, in cytochrome oxidase there is an interesting tyrosine covalently linked with a histidine ligand of Cu_B at the active site [1,2]. In the low-resolution structure of PSII [3,4] it is still not possible to precisely define the positions of individual amino acids, but it had long been known that a tyrosine in the neighborhood of the active site for dioxygen formation is oxidized in every S-state [5]. This tyrosine, Tyr_Z, had been identified as D1-Y161 [6]. Gerald Babcock, to whom this volume is dedicated, was perhaps the one who most strongly emphasized the interesting parallels between these two enzymes and, in particular, the roles of the tyrosines in the mechanisms. He has inspired much of the work described in the present mini-review.

2. Results

During the past years a series of theoretical studies on the mechanisms of PSII and cytochrome oxidase have been performed. Hybrid density functional (B3LYP [7]) theory was used on model systems, with up to 120 atoms in the most recent models, and some of the results obtained are briefly summarized below.

2.1. PSII

The mechanism for water oxidation in PSII, occurring at a manganese cluster, has traditionally been assumed to involve a simple electron transfer to the nearby tyrosyl radical, Tyr_Z, see scheme (b) in Fig. 1. Gerald Babcock and coworkers suggested that Tyr_Z acts as a hydrogen abstractor, mediating electron transfer between the manganese cluster and the photo-oxidized chlorophyll [8], see scheme (a) in Fig. 1. A requirement for the viability of this mechanism is that the O—H bond strength of a water molecule coordinated to manganese decreases by about 30 kcal/mol as compared to a free water molecule to match the tyrosine O—H bond strength. In an early quantum chemical investigation it was shown that this is actually the case [9],

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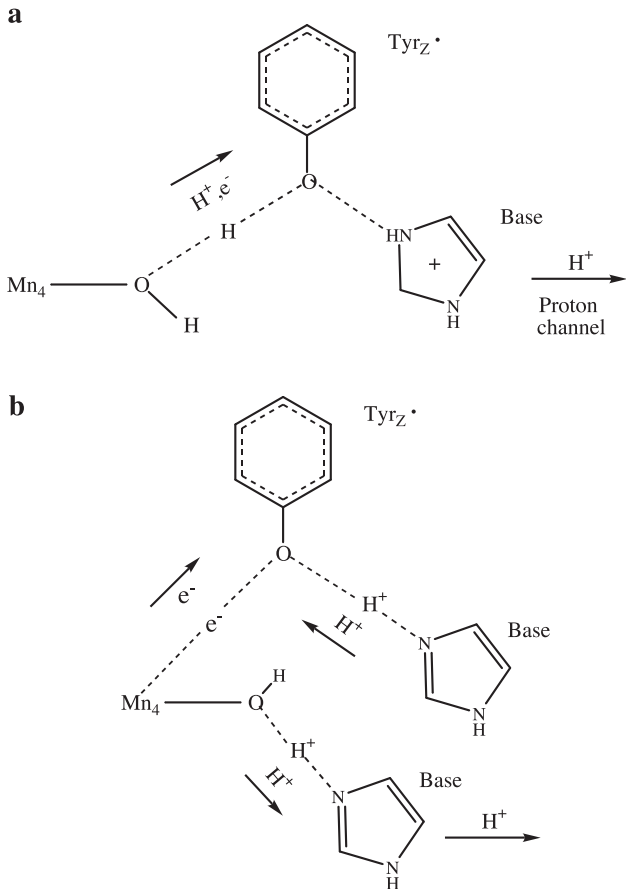

Fig. 1. Schematic picture of the hydrogen abstraction scheme (a) and the electron transfer scheme (b) for tyrosyl reduction in PSII.

thus giving thermodynamical support for the suggested hydrogen abstraction mechanism. An important insight gained from that and other studies is that the thermodynamics of a step in the water oxidation reaction is essentially the same regardless of the mechanism. For example, both the hydrogen abstraction and the simple electron transfer mechanisms, as depicted in Fig. 1, can be described in terms of differences in bond strengths. To approach the difference between these mechanisms, information about the kinetics therefore has to be obtained. In a preliminary study of the kinetics, transition states of the two mechanisms were obtained [10]. However, the results showed that more precise information about the structures has to be obtained in order to safely discriminate between the different mechanisms.

In another quantum chemical study the energetics of the formation of the tyrosyl radical was investigated by comparing calculated reduction potentials for P680 and Tyr$_Z$ [11], see Table 1. Absolute reduction potentials are difficult to calculate accurately, but relative values should be more accurate. For P680 a value of 0.54 V was obtained from the calculations, in reasonably good agreement with the experimental value of 1.1 V, considering that there was no X-ray structure available for PSII, which obviously affected the possibility to construct good models. One conclusion which could be drawn was that for a tyrosine to be able to form a radical at all, it has to be connected to a reasonably strong base. At the time it was thought that a histidine was hydrogen bonding to Tyr$_Z$, and using a phenol-imidazole model, a value of 1.34 V was obtained for the reduction potential, also in reasonable agreement with the experimental value of 0.97 V for Tyr$_Z$. However, the relative error between P680 and Tyr$_Z$ is as large as 0.9 V, indicating that the models used are not describing the true systems, which in turn is an effect of the lack of a crystal structure. Since the potential of Tyr$_Z$ is most sensitive to the surrounding protein, different models were tried for this system. The only way to obtain a reduction potential reasonably close to the one of P680 was to put a negatively charged carboxylate (corresponding to a Glu or an Asp) on the other side of the histidine, yielding a potential of 0.24 V, leading to a slightly exergonic electron transfer from Tyr$_Z$ to P680. It is interesting to note that the low-resolution structure recently obtained for PSII indicates that the nearest neighbor to Tyr$_Z$ may not be a histidine but a glutamate [3]. New calculations on a negatively charged phenol-carboxylate pair, see Fig. 2, gave a reduction potential of 0.34 V for Tyr$_Z$. A more accurate value can only be obtained when a better structure is available, making it possible to construct more realistic models. The P680 potential was also recalculated, using a better chlorophyll model than was possible in the previous study, giving a new value of 0.79 V, in better agreement with the experimental value. It can be noted that in a recent study of cytochrome oxidase [12], the reduction potential for heme a was computed to be 0.3 V, which is actually within the experimental range of values, 0.2–0.4 V. In that case the structure is well known, and the most important hydrogen bonding residues were included in the model.

2.2. O—O bond cleavage in cytochrome oxidase

In 1998, Proshlyakov et al. [13] suggested a hydrogen abstraction mechanism for the O—O bond cleavage step in cytochrome oxidase, where one of the main features was the formation of a tyrosyl radical at the binuclear center (at Tyr244). The suggestion was built on results from resonance Raman spectroscopy on the mixed valence form of the enzyme, indicating that the O—O bond is cleaved already at that level of reduction. At that time it was not generally

Table 1
Calculated and experimental reduction potentials for P680 and Tyr$_Z$

P680 small model[a]	0.54 V
P680 large model[b]	0.79 V
P680 exp	1.1 V
Tyr–His[a]	1.34 V
Tyr–His–Glu$^-$,[a]	0.24 V
Tyr–Glu$^-$,[b]	0.34 V
Tyr$_Z$ exp	0.97 V

[a] From Ref. [11].
[b] Present study.

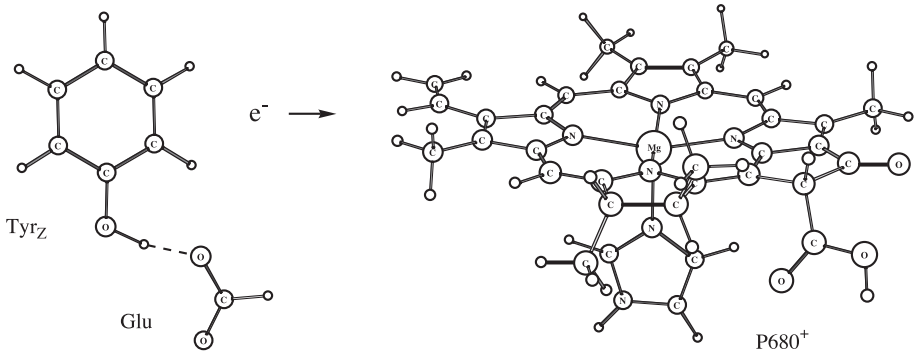

Fig. 2. The creation of the tyrosyl radical Tyr_Z in PSII.

accepted that the O—O bond actually could be cleaved without another electron delivered to the binuclear center from heme a, a possibility not available in the mixed valence form of the enzyme. To further investigate the conditions for the O—O bond cleavage, quantum chemical calculations were performed on models of the binuclear center. Already at an early stage using rather small models, these calculations showed that the O—O bond cleavage in the mixed valence form of the enzyme fits the required thermodynamics when the missing electron is taken from tyrosine, resulting in the formation of a neutral tyrosyl radical in the P_M product [14,15]. More recent calculations on a larger model system confirm the early results, giving an exergonicity of 4 kcal/mol for this reaction step, see Fig. 3 [16]. These results for the thermodynamics thus support the suggested mechanism. However, the barrier for a mechanism where both the electron and proton are simultaneously delivered from tyrosine to O_2 does not appear to be low enough to comply with the short measured life time of the compound A precursor. In fact, the barrier calculated for the suggested mechanism is more than 10 kcal/mol too high.

Modifications of the suggested hydrogen abstraction mechanism therefore have to be introduced. One possible modification is to introduce an extra proton at the binuclear center. Calculations on different versions of the O—O bond cleavage mechanism, with the dioxygen molecule bridging or non-bridging between the metal atoms, have shown that with an extra proton available in compound A, the O—O bond can be cleaved with a barrier in good agreement with experimental observations, and with a tyrosyl radical in the product [15–17]. Another possible modification of the mechanism, not yet investigated, would be that another residue in the vicinity of the binuclear center could deliver an electron at an early stage of the O—O bond cleavage reaction. This may make it possible to cleave the O—O bond with a low barrier without an extra proton at the binuclear center. In that case the initial product of the O—O bond cleavage process would be a tyrosinate and a radical on some other residue. In the final stage of the O—O bond cleavage, the radical should then migrate to the tyrosine residue, giving a tyrosyl radical in compound P_M. A possible electron donor could be the tryptophan (Trp236) which is π-stacking with one of the histidine ligands of Cu_B. This tryptophan, which is as close to the metal centers as the tyrosine, is conserved in all heme-copper oxidases, and is found to be essential for the functioning of the enzyme [18].

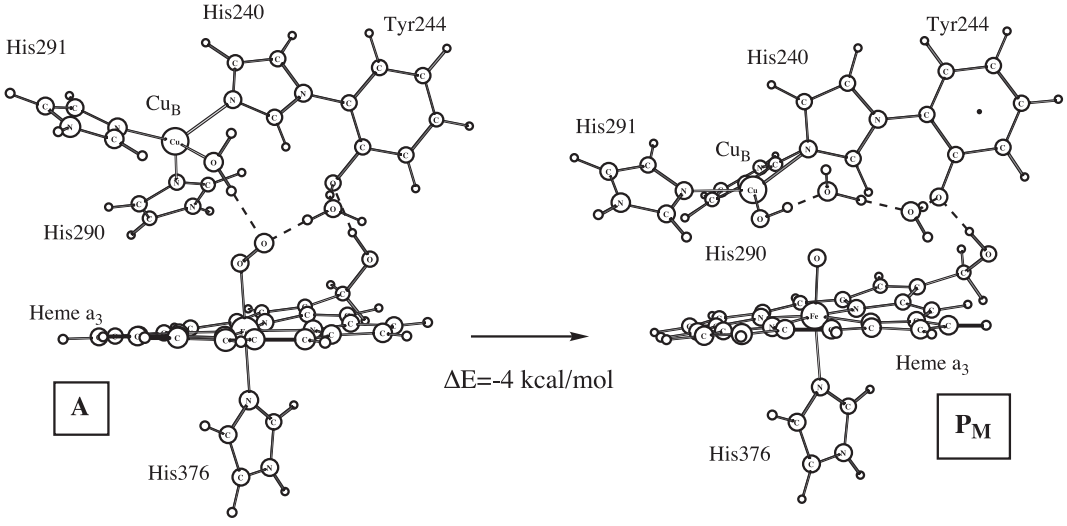

Fig. 3. Reactants and products for O—O bond cleavage in cytochrome oxidase.

A similar situation is actually found in ribonucleotide reductase (RNR), where a tryptophan radical is initially formed during the O—O bond cleavage and a stable tyrosyl radical is formed only at a later stage [19,20].

2.3. Proton and electron flow in cytochrome oxidase

One of the major questions remaining to be solved in biochemistry is how protons are pumped across the membrane in cytochrome oxidase. Several mechanisms have been suggested [21,22]. Recently this problem has been attacked by quantum chemical calculations [12], where pK_a values and reduction potentials were calculated for a model of the active site comprising more than 100 atoms [12]. This model is similar to the one in Fig. 3 but also contains the heme propionates which are important for proton translocation. The calculated values were used to set up a new mechanism for how oxygen reduction is coupled to proton pumping. This mechanism will not be described in its entirety here but only one step, the transition between the two intermediates F and O, to illustrate the main points. As described above, the covalently linked Tyr244 is likely to play an important role in the cleavage of the O—O bond of dioxygen. This tyrosine continues to play a role in the

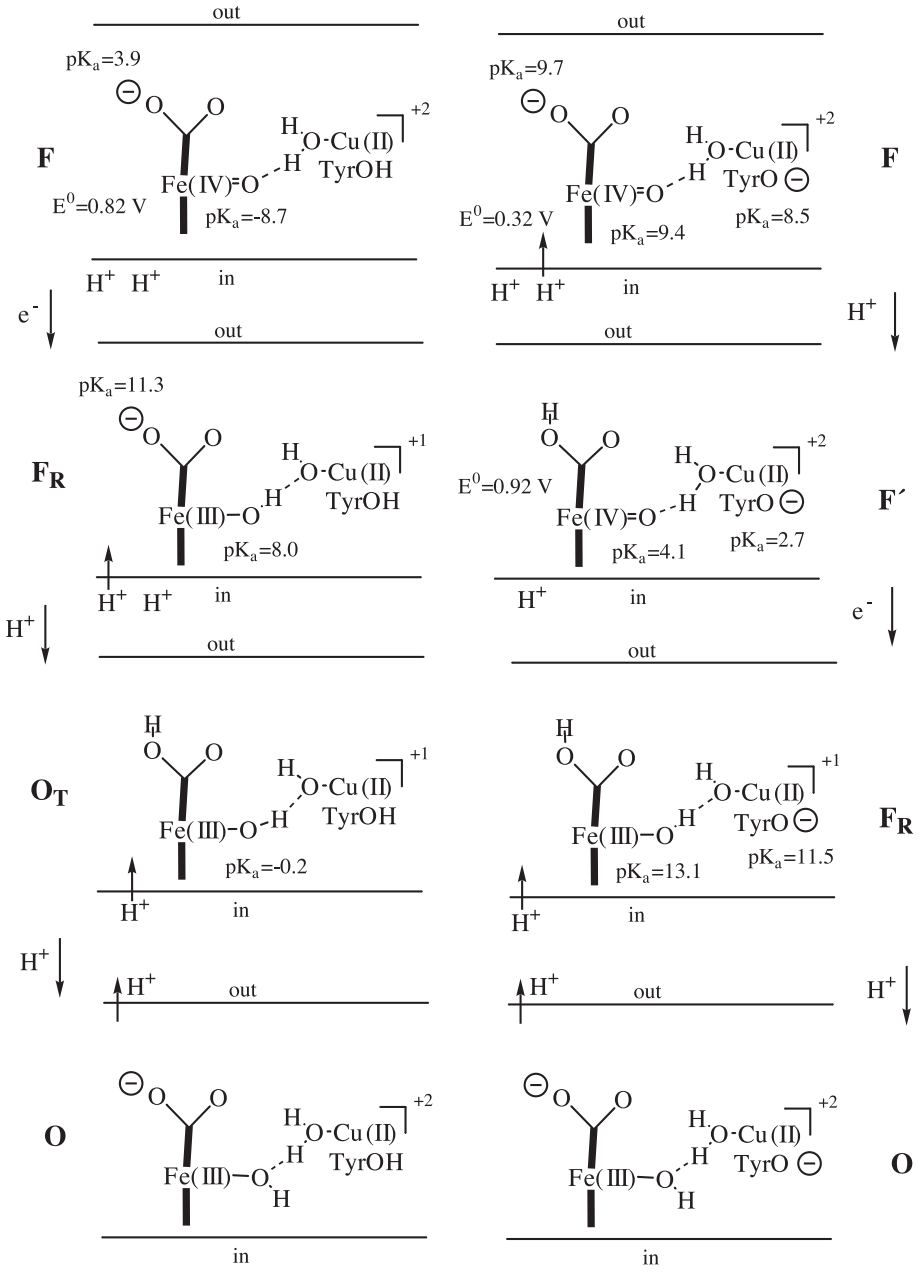

Fig. 4. Proton- and electron-flow in the F to O transition for two different models. Note that the reduction potentials are calibrated to give a value of 0.4 V for heme a.

proton translocation process that follows the O—O bond cleavage. Two alternative possibilities for the effect of the tyrosine is discussed below.

Before the pumping mechanism is discussed a few important points need to be described. After O—O bond cleavage, the oxygens are reduced to water in four transitions. In each transition an electron is transferred to the binuclear center from heme a. In this context the calibration is made such that the calculated reduction potential of heme a becomes 0.4 V. After the electron transfer, a proton should be transferred from the inside of the membrane to the binuclear center to complete the oxygen chemistry. At the same time, a proton should be pumped across the membrane, from the inside to the outside via the propionates of heme a_3. Almost all the protons involved in these transitions go through the so-called D-channel. A major question concerns how the protons to be pumped can be directed towards the propionates rather than to the binuclear center.

The recently suggested scheme for the F to O transition [12] is shown to the left in Fig. 4. In this scheme Tyr244 is suggested to be protonated. The oxidation states in F are Fe(IV) and Cu(II). An electron enters from heme a and reaches Fe(IV) which becomes reduced to Fe(III). This electron transfer is calculated to be exergonic by 9.7 kcal/mol and state F_R is reached. As this happens the pK_a values of both the propionate and the iron-oxo group are strongly increased, for the propionate by 7.4 units and for the oxo-group by 16.7 units. Even though the increase is largest for the oxo-group, the surprising result emerges that the pK_a value is actually larger for the propionate, 11.3 compared to 8.0 for the oxo-group. This has the interesting consequence that a proton taken up from the inside will actually prefer to go to the propionate rather than to the binuclear center. This step is exergonic by 5.9 kcal/mol reaching state O_T. With the proton on the propionate, the pK_a value of the iron-oxo group is strongly reduced by electrostatic repulsion down to −0.2 units. However, to continue the catalytic cycle a proton must go to the binuclear center, since otherwise the next electron from heme a cannot enter. As this proton goes through the D-channel towards the binuclear center, it will repel the proton on the propionate to the outside and state O will be reached. The final step from O_T to O is endergonic by 4.5 kcal/mol but will be driven by the steps following this one. The net result of the F to O transition is that one proton will be translocated across the membrane. A key point is that the high pK_a value after the electron transfer helps the proton to be gated towards translocation rather than consumption at the binuclear center where the energy would just be wasted.

More recently it has been realized that with only minor modifications, the calculated pK_a's and reduction potentials can actually be used in a slightly different way than described above. It is important to take slight modifications of the values into consideration since the accuracy cannot be expected to be higher than 3–5 kcal/mol. In one possible alternative to the mechanism described above, another F state is reached where Tyr244 is a tyrosinate rather than a neutral protonated tyrosine. With a tyrosinate the scheme to the right in Fig. 4 is obtained. This suggestion should not be regarded as more likely than the one to the left but rather as an alternative possibility. A main difference between the schemes is seen already for intermediate F. Due to the electrostatic effect of the tyrosinate, the pK_a value of the propionate is increased from 3.9 to 9.7. This means that a proton will move from the inside (pH = 7) to the propionate already before the electron comes in to the binuclear center. This leads to the formation of intermediate F′ . The electrostatic effect of this proton will in turn increase the reduction potential of Fe(IV) from 0.82 to 0.92 V, which makes the electron transfer from heme a exergonic by 11.9 kcal/mol. In F_R the pK_a value for the hydroxyl group on Fe(III) is 13.1 compared to 11.5 for the tyrosinate, which means that the tyrosine will also remain unprotonated for the next transition. The transfer of a proton from the inside to the binuclear center is, as in the scheme to the left, accompanied by a repulsion of the propionate proton to the outside. This step is exergonic by 4.2 kcal/mol. The same net result is thus reached with the two schemes in the figure, but the transition energies between the individual steps are different. To decide upon which scheme is likely to be the one actually used by the enzyme, detailed comparisons to experiments for all transitions have to be made.

3. Conclusions

The present mini-review has described some of the model calculations on PSII and CcO that were initiated by Gerald Babcock. For PSII, recent calculations of the reduction potentials of P680 and Tyr_Z have improved the results compared to those previously obtained for electron transfer between these groups. However, more accurate structural information than presently available is needed to reach quantitative agreement with experiments and to draw more firm conclusions about the mechanism. For O—O bond cleavage in cytochrome oxidase the model calculations have demonstrated the thermodynamic feasibility of a mechanism where a radical is formed on Tyr244 at the end of this process. However, the calculations also show that the electron and proton needed during the bond cleavage cannot come simultaneously from Tyr244. The most recent model studies suggest that the electron instead might come from the nearby Trp236. The role of Tyr244 during the proton translocation process has also been investigated and two possibilities were described. In the first one Tyr244 is a neutral protonated species and in the second one it is a tyrosinate. Similar mechanisms were obtained in these cases where the most striking feature is that a proton coming from the inside of the membrane will actually prefer to go to the propionates for proton pumping rather than to go to the binuclear center and be consumed. However, the detailed energetics of the two mechanisms are slightly different. It is

at present too early to decide which possibility is most plausible.

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