Minireview

Conformationally controlled pK-switching in membrane proteins: One more mechanism specific to the enzyme catalysis?

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Abstract Internal proton displacements in several membrane photosynthetic enzymes are analyzed in relation to general mechanisms of enzymatic catalysis. In the bacterial photosynthetic reaction center (RC) and in bacteriorhodopsin (BR), carboxy residues (Glu-212 in the RC L-subunit and Asp-96 in BR) serve as indispensable intrinsic proton donors. Both carboxyls are protonated prior to the proton-donation step, because their pK values are shifted to ≥ 12.0 by the interaction with the protein and/or substrate. In both cases, the proton transfer reactions are preceded by conformational changes that, supposedly, let water interact with the carboxyls. These changes switch over the pK values of the carboxyls to ≤ 6.0 and 7.1 in the RC and BR, respectively. The sharp increase in the proton-donating ability of the carboxyls drives the reaction cycles. This kind of catalytic mechanism, where a strong general acid or base emerges, when needed, as a result of a conformational change can be denoted as a *conformationally controlled pK-switching*. Generally, the ability of enzymes to go between isoenergetic conformations that differ widely in the reactivity of the catalytic group(s) may be of crucial importance to the understanding of enzymatic catalysis. Particularly, the pK-switching concept could help to reconcile the contradictory views on the functional protonation state of the redox-active tyrosine Y_Z in the oxygen-evolving photosystem II. It is conceivable that Y_Z switches its pK from ~ 4.5 to ≥ 10.0 upon the last, rate-limiting step of water oxidation. By turning into a strong base, tyrosine assists then in abstracting a proton from the bound substrate water and helps to drive the dioxygen formation.

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1. Introduction

The nature of the enormous catalytic power of enzymes stays unclear in many aspects. The existing hypotheses on the mechanisms of enzymatic catalysis emphasize as a rule the crucial importance of the proton-involving reactions (see [1–4] and references therein). Hence, the kinetic tracing of proton displacements during a catalytic transition may provide insight into its mechanism. Measurements of this kind are possible with the membrane photosynthetic enzymes.

Their reaction cycles can be triggered by flashes of light, and proton transfer events can be experimentally traced via the accompanying changes of pH and of the transmembrane electrical potential difference, $\Delta\psi$ [5–7]. In this review, the mechanisms of proton transfer in the bacterial photosynthetic reaction center, bacteriorhodopsin, and photosystem II are analyzed. Proton displacements turned out to be coupled to the conformational transitions that cause dramatic changes in the acidic strength of the catalytic ionizable groups. Due to these pK shifts, strong proton donors or acceptors arise, when needed, to drive the reaction cycles. It is suggested that the ability of enzymes to pass through series of isoenergetic conformations that differ widely in the pK values of the catalytic, reactive groups may be of crucial importance to the understanding of enzymatic catalysis.

2. pK-Switching in the photosynthetic reaction center and in bacteriorhodopsin

2.1. Photosynthetic reaction center (RC)

The photosynthetic reaction center (RC) of purple phototrophic bacteria is a membrane enzyme that utilizes the energy of light to catalyze the reduction of ubiquinone Q to ubiquinol QH_2 in the non-polar membrane phase (see [8,9] for recent reviews and Fig. 1 for the reaction cycle). The absorption of a light quantum leads to a charge separation in the RC resulting in the reduction of the secondary ubiquinone, Q_B, to a tightly bound semiquinone anion Q_B^-. The second reduction of Q_B^- (e.g. as a result of a next flash of light) is accompanied by the sequential binding of two protons from the negatively charged n-side of the membrane. The reaction yields ubiquinol Q_BH_2 that readily exchanges against a ubiquinone from the membrane pool.

Fig. 1 shows the mechanistic model of the Q_B turnover in the RC of *Rhodobacter sphaeroides*. The model is based on the comparative analyses of the X-ray structures that were obtained under different crystallization conditions [10–13], diverse functional observations (reviewed in [9,14]), and the data on the flash-induced proton displacements in the membrane preparations from *Rb. sphaeroides* [15–18]. The reaction cycle starts from the neutral ubiquinone, Q_B. The Q_B-binding pocket is about 15 Å away from the water boundary and is connected with the surface by several water channels that could serve as proton inlets [11,12]. Q_B is distributed between two binding sites, as it is apparent from the low-temperature X-ray structures of the RC [12], and from functional studies (see [16] and references therein). The *distal* ubiquinone, Q_B^d, is remote from the glutamate 212 in the RC L-subunit (L-Glu-

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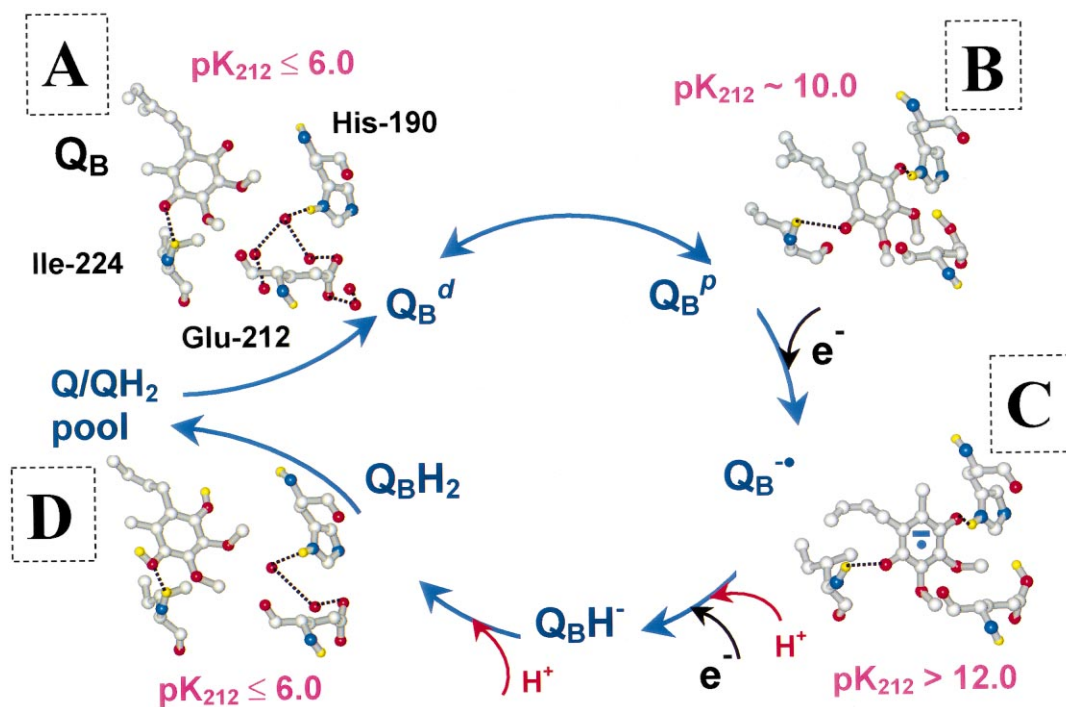E-mail: mulkidjanian@biologie.uni-osnabrueck.de

Fig. 1. Mechanistic scheme of the Q_B turnover in the RC of *Rb. sphaeroides*. A: The distal position of Q_B (Q_B^d) as seen in the low-temperature dark-adapted RC structure [12] (PDB entry 1AIJ). B: The proximal position of Q_B (Q_B^p) according to [10,12] (PDB entry 4RCR). C: The 'semiquinone' position of Q_B^- after [12] (PDB entry 1AIG). (D) $Q_B H_2$ position as seen in the X-ray structure of the RC crystallized in the presence of ascorbate [11,13] (PDB entry 1PCR). The color code: oxygen, red; nitrogen, blue; hydrogen, where shown, yellow. Water molecules are shown as red balls. The electron transfer events are shown by black arrows, whereas the proton transfer reactions are depicted by the red ones. The established hydrogen bonds are shown by dashed lines.

212). The respective structure A in Fig. 1 shows several water molecules between Q_B^d and L-Glu-212; two of them form a bridge between L-Glu-212 and L-His-190 [12,13]. L-Glu-212 seems to serve as a hydrogen bond acceptor in such a bridge at neutral pH [13]. Based on diverse functional data, the pK value of L-Glu-212, pK_{212}, has been estimated as ≤ 6.0 for the Q_B^d state (see [16] and references therein). In the alternative *proximal* position Q_B^p, the quinone ring is ~ 5 Å closer to L-Glu-212 and is rotated by 180° compared to Q_B^d (see [12] and structure B in Fig. 1). The presence of Q_B^p prevents the formation of the water bridge between L-Glu-212 and L-His-190. The absence of the water bridge and the proximity of oxygen atoms of Q_B^p keep L-Glu-212 protonated at neutral pH. The pK_{212} of this state corresponds to the experimentally estimated apparent pK_{212} of ~ 10.0 (see [16,19–21] for more details).

After the first electron transfer, the negative charge of the semiquinone anion Q_B^- shifts pK_{212} to > 12.0 [14]. Q_B^- is seen *proximally* located in the respective X-ray structure ([12], see structure C in Fig. 1). The same is true, supposedly, for the ubiquinone anion $Q_B H^-$ that is formed after the joint transfer of the second electron and the first proton (see [13] for the structural model of the $Q_B H^-$ binding). The second proton is donated to $Q_B H^-$ by L-Glu-212, as it has been concluded from the drastic slowing of the respective reaction in the L-Glu-212 → Gln mutant [19,20]. The transfer of the second proton seems to proceed with a higher activation energy, E_a, than those of the first one (60 kJ/mol versus ~ 10 kJ/mol [17]), that points to a kinetic limitation by a conformational change. Most likely, the second protonation is coupled with the detachment of $Q_B H^-$ from the *proximal* binding site and its movement towards the *distal* one, where ubiquinol $Q_B H_2$ is

seen in the respective crystal structure (see [11,13] and structure D in Fig. 1). On this movement, water molecules wedge in between the withdrawing quinone ring and L-Glu-212 and restore the water bridge between L-Glu-212 and L-His-190 [13]. The pK_{212} value decreases, supposedly, to a same value of ≤ 6.0 as in the Q_B^d-containing RC, inasmuch as the relative positions of the quinone ring, L-His-190 and L-Glu-212 are similar in two states (compare structures A and D in Fig. 1; the smaller number of identified water molecules in the latter structure is due to its lower resolution). L-Glu-212, now an effective proton donor, delivers its proton to $Q_B H^-$ to yield $Q_B H_2$ (see [17] for more details).

2.2. Bacteriorhodopsin (BR)

The extent and range of pK changes in the RC strikingly resemble those in bacteriorhodopsin (BR), a quite different enzyme that serves as a light-driven proton pump in the archaebacterium *Halobacterium salinarium* (see [22,23] for recent reviews and [24–26] for X-ray structures). BR is formed by seven transmembrane α-helices. They surround a molecule of retinal that is covalently linked to Lys-216 via a protonated Schiff base. On absorption of a light quantum, the retinal undergoes an *all trans* to 13-*cis* isomerization which is coupled with the deprotonation of the Schiff base and with the proton release to the positively charged p-side of the membrane (bR → K → L → M transitions, using the notation from [27]). The Schiff base is reprotonated from the opposite n-side of the membrane. It receives a proton from the aspartate 96 (Asp-96) that is located on the half-way between the Schiff base and the membrane surface. The substitution of Asn for Asp-96 slows the reprotonation dramatically [28]. The pK value of

Asp-96 (hereafter pK_{96}) in the initial bR state has been recently estimated as ≥ 12.0 [29]. This extremely high pK value is attributed to the influence of a hydrophobic environment and to the electrostatic interaction with Tre-46 [23]. The reprotonation of the Schiff base is preceded by a conformational change which, supposedly, allows water to form a proton-conducting chain between Asp-96 and the Schiff base (see [22,23,30,31] and references therein). The transition has been denoted as $M^{closed} \rightarrow M^{open}$ [31]. The conformational change and/or the appearance of water switches over the pK_{96} to 7.1 [29]. Turning into an effective proton donor, Asp-96 *drives* the reprotonation of the Schiff base [23] yielding the N^{open} state [32]. The following $N^{open} \rightarrow N^{closed} \rightarrow O \rightarrow bR$ transitions are believed to reflect the closing of the water channel, the re-isomerization of the retinal, the reset of the high pK_{96}, and the reprotonation of Asp-96 from the surface [22,23,32]. Thus, the turnover of BR seems to be driven by a conformationally controlled pK-switching that, even quantitatively, resembles those in the RC.

3. Is pK-switching inherent to enzymatic catalysis?

According to the Brønsted Catalysis Law, the stronger the acid, the better the general acid catalysis [33]. This empirically derived rule reflects a more general relation, according to which the rate of proton transfer depends, with a transmission coefficient α of < 1, on the free energy of the reaction, and, accordingly, on ΔpK between the donor and the acceptor of proton [34]. In a homogenous solution, the maximal rate of proton transfer is, however, limited by the ambient pH: a catalytic acid becomes deprotonated at pH above its pK, and the reaction rate slows down, following the drop in the concentration of the protonated form.

Both the RC and BR have found the same way to overcome this fundamental limitation. Their catalytic, reactive carboxyls retain the proton up to pH ~ 12.0, owing to their interaction with the substrate and/or protein. Due to a properly timed conformational change leading to a drastic pK decrease, the protonated carboxyls turn into effective proton donors, when needed, that drives the reaction cycles. The rate of such a proton delivery does not depend on the external pH in the whole physiological pH range. The mechanism could be utilized for the general base catalysis as well, provided that the unprotonated, low pK form of a catalytic residue is preserved by the enzyme, so that a conformationally controlled pK increase yields a strong base, when needed (see the next section). Importantly, the pK values of the general acids (bases) that arise from conformational changes may be well below (above) the ambient pH. The catalytic power of such acids and bases does not have precedents in the non-enzymatic chemistry. Accordingly, the *conformationally controlled* pK-*switching* could be classified as one more mechanism that is specific to the enzymatic catalysis.

The pK-switching could be kinetically competent only if the energies of the low and high pK states of enzyme are close to each other. Relevantly, the almost even distribution of Q_B between two binding sites in the dark-adapted, ground state RC (see the previous section, Fig. 1, and [12,16]) indicates that the total energies of the $RC-Q_B^p$ and $RC-Q_B^d$ complexes are similar, although the respective pK_{212} values differ by ≥ 4 pH units i.e. by ≥ 25 kJ/mol. This example shows that the whole enzyme-substrate complex could serve to balance the energy difference between the different protonation states of a catalytic residue. Not just the active site, but the whole bulk of the conformationally mobile enzyme seems to be crucial for balancing the enzyme conformations that differ in the reactivity of the catalytic group(s).

The pattern of a typical enzymatic reaction is compatible with the inherence of the conformationally controlled pK-switching to enzymatic catalysis. The substrate binding is usually accompanied by the expulsion of water from the active site and by the formation of new salt bridges. Both processes are known to cause changes in the pK values of catalytic residues (see e.g. [2]). Then the substrate binding could be accompanied by the 'charging', via protonation and/or deprotonation, of those acids and/or bases that would be needed as catalysts on the subsequent steps of reaction. A conformationally controlled and properly timed later 'discharge' (e.g. on the rate-limiting stage) would decrease the reaction activation barrier(s). The free energy contributions from pK shifts of some ionizable residues have been hypothesized to be essential for the catalytic mechanisms in the protonic F_0F_1-ATP-synthase [35] and in photosystem II [36]. Conformationally controlled pK-switching can be suspected, from the data on pK shifts an/or unusual pK values of catalytic residues, in glutathione S-transferase [37], xylanase [38] and lactose permease [39], to name just some examples. Still, the definitive identification of a pK-switch implies the necessity to track down the proton displacements during the catalytic transition. Such a tracing is currently possible only with a limited group of photosynthetic membrane proteins. Thus, a search for some other, more widely applicable way to identify the catalytic pK-switches might be a formidable challenge.

4. Is the redox-active tyrosine Y_Z of photosystem II another pK-switch?

The pK-switching concept could be applied to reconcile the contradictory views on the mechanism of water oxidation to oxygen by photosystem II of green plants (PSII), one more membrane photosynthetic enzyme for which the proton transfer reactions have been traced [40,41]. Here, the flash-generated P_{680}^+, a chlorophyll $\underline{a}$ moiety with an extremely high redox potential of ~ 1.15 V, extracts an electron from the redox-active tyrosine Y_Z (Tyr-161 in the D1-subunit) that, in its turn, oxidizes the oxygen-evolving complex (OEC). The four Mn atoms and one Ca atom-containing OEC accumulates electron vacancies, four of which are needed to oxidize water, by going through the increasingly oxidized states $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4$. Dioxygen release is associated with the spontaneous $S_4 \Rightarrow S_0$ transition (see [40,42–44] for recent reviews, and Fig. 2 for the scheme of the reaction cycle).

It has been found that the fast oxidation of Y_Z upon the $S_1 \rightarrow S_2$ transition ($\tau \sim 50$ ns) is not steered by proton release at pH ≥ 5.0 [45]. Two possible mechanisms have been suggested in refs. [45,46] to be equally compatible both with the latter finding and with the UV-Vis and FTIR difference spectra of the $Y_Z^{\bullet}/Y_Z$ couple [46,47]. (1) According to the first mechanism, D1-Tyr-161 is a tyrosine anion Y_Z^- (tyrosinate) with an unusually low pK_{Y_Z} of ~ 4.5 in the ground state. Such a low pK_{Y_Z} could be caused by a hydrogen bonding with a nearby protonated amino acid A ($Y_Z^- \bullet\bullet\bullet H^+ A$) in a low-polar environment containing a metal cation. (2) Alternatively, Y_Z could be a hydrogen-bonded neutral tyrosine
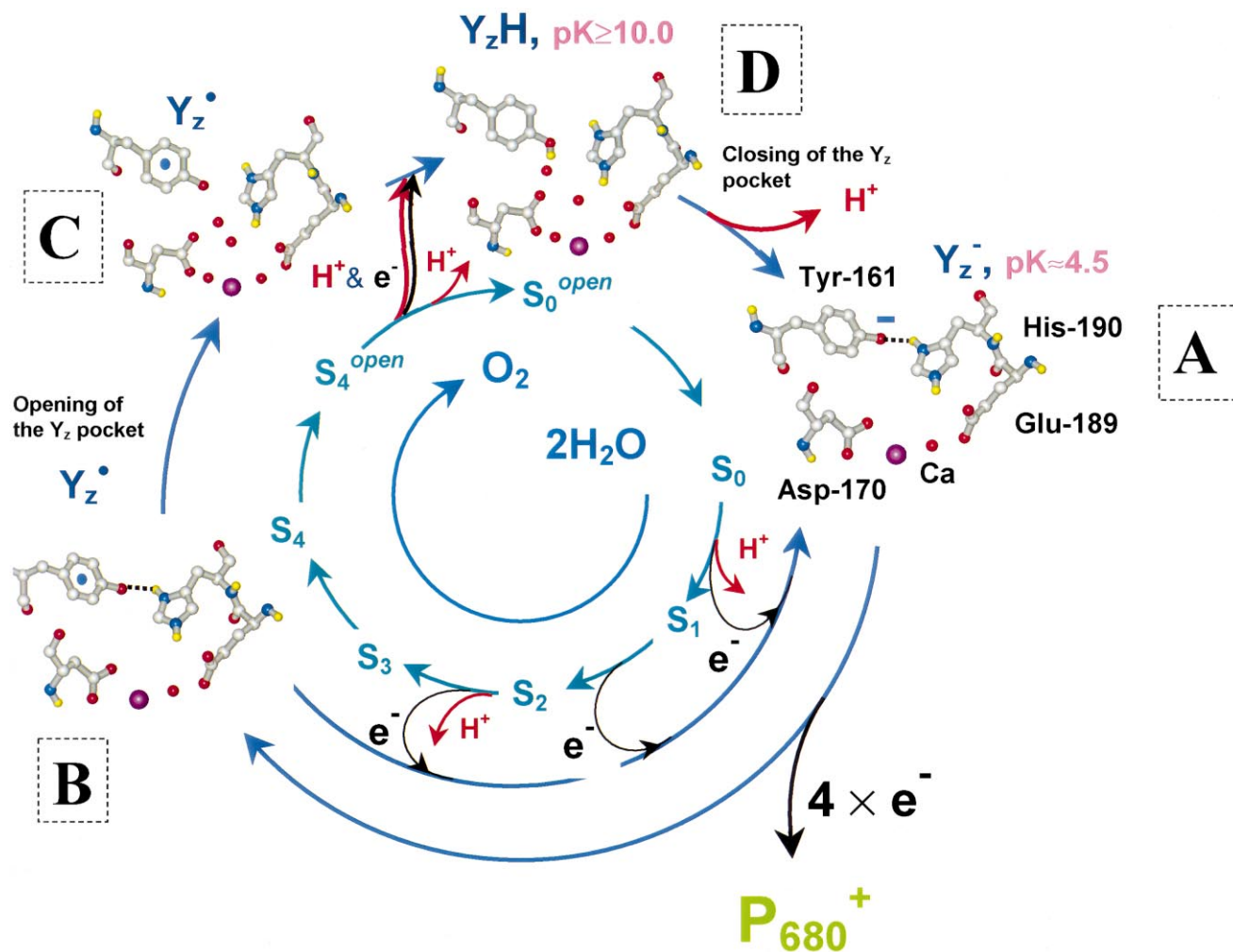
Fig. 2. Tentative scheme of the pK-switching upon water oxidation by PSII. The mutual arrangement of Y_Z, three key residues of the D1-sub-unit, and of the Ca atom, as well as the changes in the mode of the Ca binding upon the $S_3 \rightarrow S_4 \Rightarrow S_0$ transition are hypothetical. The scheme, however, is based on the molecular modeling and on the functional studies of oxygen evolution (see [45,46,48,55–58] and references therein). Only one protein is released into the bulk on $S_4^{open} \rightarrow S_0^{open}$ transition (thin red arrow). Another proton (thick red arrow) stays with Y_ZH. This proton is released into the bulk later, on $S_0^{open} \rightarrow S_0$ transition. The color code is as in Fig. 1. See text for the further details.

Y_ZH with its phenolic proton pre-shifted towards a nearby strong *base* B ($Y_Z\cdots H\cdots B$).

Recently D1-His-190 has been identified as the hydrogen bond partner of Y_Z [48]. Although histidine may serve both as an acceptor and as a donor of a hydrogen bond, the site-specific mutagenesis has revealed that D1-His-190 may be functionally substituted only by hydrogen bond donors, namely by arginine or lysine [48]. These data rather identify D1-His-190 as a functional donor of a hydrogen bond and, correspondingly, favor the case (1) of Y_Z being a tyrosinate anion with an 'abnormal' pK_{Y_Z} value of ~ 4.5. An anionic Y_Z^-, however, does not fit into the widely discussed hypothesis of hydrogen abstraction in PSII [49,50,57]. According to this hypothesis, a neutral tyrosine Y_ZH releases, on each its oxidation by P_{680}^+, a proton into the bulk p-phase and abstracts instead a hydrogen atom from the bound substrate water in the OEC. It is noteworthy that the hypothesis has been pro-voked by the Krishtalik's analysis of the energetics of water oxidation [36]. Krishtalik concluded that a strong proton ac-ceptor is indispensable upon the rate-limiting $S_3 \rightarrow S_4$ step of water oxidation. Fig. 2 shows a hypothetical scheme that uti-lizes the pK-switching concept to reconcile the experimental

evidence of the anionic properties of Y_Z [45–47] with the requirements following from the Krishtalik's analysis [36]. It is assumed that during each of the initial $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3$ steps, a hydrogen-bonded tyrosinate with p$K \leq 4.5$ (Y_Z^-) is oxidized by P_{680}^+ to a neutral tyrosine radical $Y_Z^\bullet$ (A $\rightarrow$ B tran-sition in Fig. 2). The absence of proton involvement permits the oxidation of Y_Z^- in nanoseconds. Each time, $Y_Z^\bullet$ is then reduced back by the OEC to yield Y_Z^- (B $\rightarrow$ A transition in Fig. 2). On the final $S_3 \rightarrow S_4$ oxidation step, the $Y_Z^\bullet$ state has been shown to dwell until dioxygen is finally released [51–53]. It is a viable hypothesis that the long dwell time (over 1 ms) and the weakening of the hydrogen bond with D1-His-190 in the absence of a negative charge of Y_Z^- may lead to the sep-aration of $Y_Z^\bullet$ and D1-His-190 and to the wedging of water in between them (B $\rightarrow$ C transition in Fig. 2). It is conceivable that the conformational change could be additionally pro-voked by the inevitable enzyme reorganization in response to the accumulation of electron vacancies (positive charges) in the OEC. The interaction of $Y_Z^\bullet$ with water would increase pK_{Y_Z} of the conjugate reduced form of the tyrosine to a value of ≥ 10.0. The latter figure is compatible with the observation that in the D1-His-190 $\rightarrow$ Ala mutant, where no hydrogen

bond with histidine can be formed, the functional pK_{Y_Z} value has been estimated as ~ 10.3 [48]. After turning into a strong base, tyrosine would abstract a proton together with an electron from the bound substrate water ($Y_Z^\bullet \to Y_Z H$), helping, thus, to drive the dioxygen formation ($S_4 \Rightarrow S_0$, see $C \to D$ transition in Fig. 2). The following reduction of the OEC components would restore the hydrogen bond to D1-His-190 and reset the low pK_{Y_Z} value ($D \to A$ transition in Fig. 2).

The main difference between the pK-switching scheme in Fig. 2 and the hypothesis of hydrogen abstraction [49,50] is in the number of proton release events and in their timing. Both models capitalize on the free energy gain from the simultaneous abstraction of an electron and a proton from the substrate water by $Y_Z^\bullet$, crucial for the energetics of the rate-limiting $S_3 \to S_4 \Rightarrow S_0$ transition [36]. In the hydrogen abstraction model, however, this free energy gain is likely to be surpassed by the unavoidable energy losses (Born solvation penalties) coupled with *four* proton expulsions from $Y_Z H$ into the bulk water, across the membrane/water solvation barrier. In the case of the pK-switch in Fig. 2, the Born penalty is paid only *once*, and only after the free energy gain has been already utilized for dioxygen formation. Here, the single event of proton release from YH into the bulk is coupled not with the fast oxidation of tyrosine, but with the reset of its low pK state ($Y_Z H \to Y_Z^\bullet$) that follows the slow reduction of $Y_Z^\bullet$ upon the $S_4 \Rightarrow S_0$ transition. This reduction is indeed coupled with a remarkable net proton release into the bulk [41,54].

5. Concluding remarks

The catalytic performance of general acids is known to improve with the decrease of their pK values, but to deteriorate as pK becomes lower than the ambient pH. The RC and BR have found the same way to overcome this fundamental limitation. Their catalytic carboxyls stay protonated up to pH 12.0 owing to their interaction with the protein and/or substrate. Due to the properly timed and energetically tuned conformational changes leading to drastic drops in their pK values, the protonated carboxyls turn into strong proton donors, when needed, to drive the reaction cycles. In the light of these observations, it is conceivable that Y_Z, the redox-active tyrosine of PSII, switches its pK_{Y_Z} from ~ 4.5 to ≥ 10.0 on the last, rate-limiting step of water oxidation. After turning into a strong base, tyrosine could help to abstract a proton from the bound substrate water.

The conformationally controlled pK-switching could yield general acids (bases) with pK values that are much lower (higher) than the ambient pH. Their catalytic power does not have analogies in the non-enzymatic chemistry. The described ability of enzymes to go between isoenergetic conformations, that differ widely in the reactivity of the catalytic group(s), may be of crucial importance to the understanding of enzymatic catalysis.

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