Minireview

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

Armen Y. Mulkidjianian*

Division of Biophysics, Faculty of Biology/Chemistry, University of Osnabrück, D-49069 Osnabrück, Germany

Received 6 October 1999

Edited by Vladimir Skulachev

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), carboxylic 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 pH-switching. Particularly, the pH-switching concept could help to reconcile the contradictory views on the functional protonation state of the redox-active tyrosine Z in the oxygen-evolving photosystem II. It is conceivable that 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.

Key words: Proton transfer; Electron transfer; Photosynthetic reaction center; Bacteriorhodopsin; Photosystem II

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, Δϕ [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 pH 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. pH-switching in the photosynthetic reaction center and in bacteriorhodopsin

2.1. Photosynthetic reaction center (RC)

The photosynthetic reaction center (RC) of purple photosynthetic bacteria is a membrane enzyme that utilizes the energy of light to catalyze the reduction of ubiquinone Q to ubiquinol QH2 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, Qb, to a tightly bound semiquinone anion Qb-. The second reduction of Qb- (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 QbH2 that readily exchanges against a ubiquinone from the membrane pool.

Fig. 1 shows the mechanistic model of the Qb 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 Rh. sphaeroides [15–18]. The reaction cycle starts from the neutral ubiquinone, Qb. The Qb-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]. Qb 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, Qb', is remote from the glutamate 212 in the RC L-subunit (L-Glu-
212). The respective structure A in Fig. 1 shows several water molecules between Qd 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, pK212, has been estimated as 9.6 for the Qd state (see [16] and references therein). In the alternative proximal position Qp, the quinone ring is 5 Å closer to L-Glu-212 and is rotated by 180° compared to Qd (see [12] and structure B in Fig. 1). The presence of Qp 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 Qp keep L-Glu-212 protonated at neutral pH. The pK212 value decreases, supposedly, to a same value of 9.6 as in the Qd-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 QBH to yield QBH2 (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 hR state has been recently estimated as \( \approx 12.0 \) [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 re-protonation 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 \( \text{M}^{\text{closed}} \rightarrow \text{M}^{\text{open}} \) [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 re-protonation of the Schiff base [23] yielding the \( \text{N}^{\text{open}} \) state [32]. The following \( \text{N}^{\text{open}} \rightarrow \text{N}^{\text{closed}} \rightarrow \text{O} \rightarrow \text{bR} \) 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 re-protonation 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 \( \alpha \) of < 1, on the free energy of the reaction, and, accordingly, on \( \Delta pK \) 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 carboxylic groups retain the proton up to pH \( \approx 12.0 \), 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 can 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 competitive 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} and RC-Q_{B} complexes are similar, although the respective pK_{Q} values differ by \( \approx 4 \) pH units i.e. by \( \approx 25 \) 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 inheritance 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 \( \text{F}_{2} \text{F}_{1}-\text{ATPase} \) [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 YZ 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 \( \text{P}_{680} \), a chlorophyll m g moiety with an extremely high redox potential of \( \approx 1.15 \) V, extracts an electron from the redox-active tyrosine \( \text{YZ} \) (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 \( \text{S}_{0} \rightarrow \text{S}_{1} \rightarrow \text{S}_{2} \rightarrow \text{S}_{3} \rightarrow \text{S}_{4} \). Dioxygen release is associated with the spontaneous \( \text{S}_{4} \rightarrow \text{S}_{0} \) 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 \( \text{YZ} \) upon the \( \text{S}_{1} \rightarrow \text{S}_{2} \) transition (\( \tau \approx 50 \) ns) is not steered by proton release at pH \( \approx 5.0 \) [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 \( \text{YZ} / \text{YZ} \) couple [46,47]. (1) According to the first mechanism, D1-Tyr-161 is a tyrosine anion \( \text{YZ}^{-} \) (tyrosinate) with an unusually low pK_{YZ} of \( \approx 4.5 \) in the ground state. Such a low pK_{YZ} could be caused by a hydrogen bonding with a nearby protonated amino acid A \((\text{YZ}^{-}+\text{H}^{+})\text{A}\) in a low-polar environment containing a metal cation. (2) Alternatively, \( \text{YZ}^{-} \) could be a hydrogen-bonded neutral tyrosine
YZH with its phenolic proton pre-shifted towards a nearby strong base \( B \) (YZH-\[H\]\[^{1}B\]).

Recently D1-His-190 has been identified as the hydrogen bond partner of YZ [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 YZ being a tyrosinate anion with a ‘abnormal’ pK\(_{YZ}\) value of \( \approx 4.5 \). An anionic YZ, however, does not fit into the widely discussed hypothesis of hydrogen abstraction in PSII [49,50,57]. According to this hypothesis, a neutral tyrosine YZH releases, on each its oxidation step, a hydrogen-bonded tyrosinate with pK\(_{\approx 4.5} \) (YZ\[^{-}\]) to a neutral tyrosine radical Yc\[^{+}\] (A\(\rightarrow\)B transition in Fig. 2). The absence of proton involvement permits the oxidation of YZ in nanoseconds. Each time, Yc\[^{+}\] is then reduced back by the OEC to yield YZH (B\(\rightarrow\)A transition in Fig. 2). On the final S4 oxidation step, the YZ 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 YZ may lead to the separation of Yc\[^{+}\] 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 provoked by the inevitable enzyme reorganization in response to the accumulation of electron vacancies (positive charges) in the OEC. The interaction of Yc\[^{+}\] with water would increase pK\(_{YZ}\) of the conjugate reduced form of the tyrosine to a value of \( \approx 10.0 \). The latter figure is compatible with the observation that in the D1-His-190\(\rightarrow\)Ala mutant, where no hydrogen

---

**Fig. 2. Tentative scheme of the pK-switching upon water oxidation by PSII.** The mutual arrangement of YZ, three key residues of the D1-subunit, and of the Ca atom, as well as the changes in the mode of the Ca binding upon the S4\(\rightarrow\)S0 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 \( S_{0}^{\text{open}} \rightarrow S_{0}^{\text{closed}} \) transition (thin red arrow). Another proton (thick red arrow) stays with YZH. This proton is released into the bulk later, on \( S_{0}^{\text{closed}} \rightarrow S_{0}^{\text{open}} \) transition. The color code is as in Fig. 1. See text for the further details.

---

YZH with its phenolic proton pre-shifted towards a nearby strong base B (YZH-\[H\]\[^{1}B\]).
bond with histidine can be formed, the functional pKc 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 (YZ → YZH), helping, thus, to drive the OEC components to restore the hydrogen bond to D1-His-190 and reset the low pKc value (D → A transition in Fig. 2). The following reduction of the OEC components would restore the hydrogen bond to D1-His-190 and reset the low pKc value (D → 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 YZ, crucial for the energetics of the rate-limiting S1 → S0 transition [36]. In the hydrogen abstraction model, however, this free energy gain is likely to be surpassed by the unavoidable energy losses (Born solvation penalty model, however, this free energy gain is likely to be surpassed by the unavoidable energy losses (Born solvation penalty model). 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 YZ into the bulk water, across the membrane/water solvation barrier. In the light of these observations, it is conceivable that YZ, the redox-active tyrosine, but with the reset of its low pK state (YZH, see C → D transition in Fig. 2) 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 water, thus, to drive the dioxygen formation (S4 → S0, see C → D transition in Fig. 2). This reduction is indeed coupled with a remarkable net proton release into the bulk [41,54].

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 water. 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 YZ, the redox-active tyrosine of PSII, switches its pKc value from ~4.5 to ~11.0 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.

Acknowledgements: Thanks are due to Drs. Dmitry A. Cherepanov, Michael Y. Galperin, Andrey D. Kaulen and Lev I. Krishtalik for the critical reading of the manuscript. Thanks are also due to Prof. Wolfgang Jung for his stimulating interest in this work and generous support. The helpful discussions with Drs. Gerald T. Babcock, Boris A. Feniouk, Michael Haumann, Monika Hundelt, C. Roy D. Lancaster, Janos K. Lanyi, Andrey D. Vinogradov and Lev S. Yaguzhinsky are appreciated. Prof. Dieter Oesterhelt is thanked for the access to ref. [29] prior to publication. This work was supported in part by grants from the Deutsche Forschungsgemeinschaft (Mu-1285/1) and from the European Commission (INTAS-93-2852-Ex).

References