Tyrosine Deprotonation and Associated Hydrogen Bond Rearrangements in a Photosynthetic Reaction Center

Hiroshi Ishikita

Introduction

In biological systems, tyrosine residues often play an important role in functioning as a redox active group and mediating electron transfer. In photosystem II (PSII), electronic excitation of the chlorophyll (P) and the heme (heme3). While mutation of the tyrosine does not affect the kinetics of electron transfer from heme3 to P, recent time-resolved Laue diffraction studies reported displacement of Tyr-L162 in response to the formation of the photo-oxidized P**+, implying a possible tyrosine deprotonation event. pK_a values for Tyr-L162 were calculated using the corresponding crystal structures. Movement of deprotonated Tyr-L162 toward Thr-M185 was observed in P**+ formation. It was associated with rearrangement of the H-bond network that proceeds to P via Thr-M185 and His-L168.

Abstract

Photosynthetic reaction centers from Blastochloris viridis possess Tyr-L162 located mid-way between the special pair chlorophyll (P) and the heme (heme3). While mutation of the tyrosine does not affect the kinetics of electron transfer from heme3 to P, recent time-resolved Laue diffraction studies reported displacement of Tyr-L162 in response to the formation of the photo-oxidized P**+, implying a possible tyrosine deprotonation event. pK_a values for Tyr-L162 were calculated using the corresponding crystal structures. Movement of deprotonated Tyr-L162 toward Thr-M185 was observed in P**+ formation. It was associated with rearrangement of the H-bond network that proceeds to P via Thr-M185 and His-L168.

Citation: Ishikita H (2011) Tyrosine Deprotonation and Associated Hydrogen Bond Rearrangements in a Photosynthetic Reaction Center. PLoS ONE 6(10): e26808. doi:10.1371/journal.pone.0026808

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Funding: This work was supported by the JST PRESTO program, Grant-in-Aid for Science Research from the Ministry of Education, Science, Sport and Culture of Japan (21770163), Special Coordination Fund for Promoting Science and Technology of MEXT, and Takeda Science Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The author has declared that no competing interests exist.

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Boltzmann equation with consideration of the protonation states of all titratable sites in the entire bRC protein. Using this approach, one will be able to sufficiently consider the equilibrium in protonation states of all titratable groups in bRC [9,11] and clarify the factors (e.g., residues, cofactors, atomic charges, or hydrophobicity of the protein environment) that shift pKs(Tyr-L162) in the protein environment.

Results and Discussion

Movement of deprotonated tyrosine

To investigate the possible presence of deprotonated tyrosine, Tyr-L162 was treated in its deprotonated form, and its geometry was energetically optimized with CHARMM in the P+ state. As a consequence, deprotonated Tyr-L162 moved further toward Thr-M185 (Figure 2); the H-bond distance between Tyr-L162 and Thr-M185 (OThr-M185–OTyr-L162 distance) was 2.7 Å in the resulting geometry with deprotonated Tyr-L162 (Ydeprot position), which was 0.6 Å shorter than that in the light structure (Ylight position). Although the resulting Ydeprot position was not exactly identical to the Ylight position, this result implies that Tyr-L162 deprotonation leads to tyrosine movement from the one in the dark structure (Ydark position) to the Ylight position. Interestingly, the OThr-M185–OTyr-L162 distance obtained with deprotonated Tyr-L162 is 2.7 Å in the Ydeprot structure.

Tyr-L162 deprotonation induces H-bond network rearrangements

In addition to Tyr-L162 movement, a striking rearrangement in the H-bond network containing P and Tyr-L162 was observed in the transition from the initial uncharged P0 and protonated Tyr-L162 state (P0 Y) to the photo-oxidized P+ and deprotonated Tyr-L162 state (P+ Y–). In the P0 Ydark state (Figure 3, left), the hydroxyl H atom of Tyr-L162 can be oriented toward the hydroxyl O atom of Thr-M185 (OThr-M185–OTyr-L162 distance = 4.4 Å). The hydroxyl H atom of Thr-M185, in turn, is oriented to the Nδ site of His-L168 (OThr-M185–NHδ+His-L168 distance = 4.3 Å), forming the OH–H-Tyr-L162 H-bond network that stabilizes Tyr-L162 in the dark structure. The significantly high pKs(Tyr-L162) value shift from the P0 Ydark to the P+ Ydeprot state, since the hydroxyl OH group of Thr-M185 is subject to forming an H-bond with the deprotonated Tyr-L162 (OThr-M185–OTyr-L162 distance = 2.7 Å) to stabilize the negative charge (Figure 3, right). The absence of the hydroxyl H atom near His-L168 promotes protonation of the His-L168 Nδ site (Table 1). In accordance with reorientation of the Thr-M185 hydroxyl group, H atoms of a water molecule at an H-bonding distance with Tyr-L162 were also reoriented toward the deprotonated Tyr-L162. As a consequence, the OH dipole orientations were altered, forming the OThr-M185–H–OH–His-L168 network (Figure 3, right).

pKs(Tyr-L162) value shift from the P0 Ydark to the P+ Ydeprot state

pKs(Tyr-L162) was calculated to be 22 in the P0 Ydark state (Table 1), indicating that this residue will never be deprotonated in the dark structure. The significantly high pKs(Tyr-L162) value of 22, which is even higher than that in aqueous solution (~10), is mainly due to the presence of acidic residues in the bRC that upshift pKs(Tyr-L162), e.g., Asp-M182, Glu-C254, Asp-L155, and Glu-M171 (Table 2). The presence of these negatively charged acidic residues upshifts pKs(Tyr-L162) and thus does not energetically allow deprotonated Tyr-L162 formation.

In contrast to the P0 Ydark state, orientation of the H-bond network is completely different in the P+ Ydeprot state, since the hydroxyl OH group of Thr-M185 is subject to forming an H-bond with the deprotonated Tyr-L162 (OThr-M185–OTyr-L162 distance = 2.7 Å) to stabilize the negative charge (Figure 3, right). The absence of the hydroxyl H atom near His-L168 promotes protonation of the His-L168 Nδ site (Table 1). In accordance with reorientation of the Thr-M185 hydroxyl group, H atoms of a water molecule at an H-bonding distance with Tyr-L162 were also reoriented toward the deprotonated Tyr-L162. As a consequence, the OH dipole orientations were altered, forming the OThr-M185–H–OH–His-L168 network (Figure 3, right).
contributes to stabilization of the deprotonated Tyr-L162 form, downshifting $pK_a$ (Tyr-L162) by 4.2 (2.7 from $P_A$ and 1.5 from $P_B$) in the $P^{+}N_{deprot}$ state (Table 3). The influence of $P^{+}$ on $pK_a$(Tyr-L162) did not essentially differ in the $Y_{dark}, Y_{light}$, and $Y_{deprot}$ positions (Table 3).

Concluding Remarks

Deprotonation of Tyr-L162 resulted in the displacement of the side chain, lowering the $pK_a$ value to 6.7. Movement of deprotonated Tyr-L162 toward Thr-M185 was observed in $P^{+}$ formation. It was associated with rearrangement of the H-bond network that proceeds to P via Thr-M185 and His-L168.

Materials and Methods

Atomic coordinates and charges

For performing computations of bRC from Blastochloris viridis, crystal structures in the photoactivated form (protein data bank (PDB); 2X5V) [7] were used. A crystal structure corresponding to the dark state is available (PDB 2X5U), but this crystal structure does not contain water molecules that can be seen in the photoactivated crystal structure. Furthermore, the conformer labeled with A in the photoactivated crystal structure is essentially identical to the dark state crystal structure in terms of the Tyr-L162 position while the conformer labeled with B in the photoactivated crystal structure is considered to correspond to the photoactivated state. Thus, in the present study, atomic coordinates for the A and B conformers (PDB 2X5V) were used as the dark and light structures, respectively.

The atomic coordinates were obtained using the same procedures used in previous studies (e.g., Refs. [11,13,14]). The positions of H atoms were energetically optimized with CHARMM [15] by using the CHARMM22 force field. While carrying out this procedure, the positions of all non-H atoms were fixed, and the standard charge states of all the titratable groups were maintained, i.e., basic and acidic groups were considered to be protonated and deprotonated, respectively. All of the other atoms whose coordinates were available in the crystal structure were not geometrically optimized. To investigate a possible movement of deprotonated Tyr-L162 (i.e., to yield the $Y_{deprot}$ position, see the later part), atomic coordinates for the minimum set of relevant residues, i.e., Tyr-L162, Thr-M185, and a water molecule (HOH M 2001 in PDB: 2X5V) were released and geometrically optimized (Table S1 for atomic coordinates). As a general and uniform strategy, other crystal waters are removed in our computations [16] because of the lack of experimental information for hydrogen atom positions. Cavities resulting after removal of crystal water are uniformly filled with solvent dielectric of $\varepsilon = 80$.

**Table 1.** Calculated $pK_a$ (Tyr-L162, His-L168, and Glu-C254) and redox potential (Tyr-L162) values in mV and $pK_a$ units, respectively.

<table>
<thead>
<tr>
<th></th>
<th>$P^0$ $Y_{dark}$H</th>
<th>$P^{+}$ $Y_{dark}$</th>
<th>$P^{+}$ $Y_{light}$</th>
<th>$P^{+}$ $Y_{deprot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr-L162</td>
<td></td>
<td>22.2</td>
<td>13.7</td>
<td>10.8</td>
</tr>
<tr>
<td>His-L168</td>
<td></td>
<td>9.7</td>
<td>6.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Glu-C254</td>
<td></td>
<td>4.3</td>
<td>7.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

$^{a}$Side chain. $^{b}$Backbone.
doi:10.1371/journal.pone.0026808.t001

**Table 2.** Main residues that contribute to increase of $pK_a$(Tyr-L162) in $pK_a$ units (i.e., residues that stabilize the Tyr-L162 protonation state).

<table>
<thead>
<tr>
<th></th>
<th>$P^0$ $Y_{dark}$H</th>
<th>$P^{+}$ $Y_{deprot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>side.</td>
<td>b.b.</td>
<td>total</td>
</tr>
<tr>
<td>Asp-M182</td>
<td>2.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Glu-C254</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Asp-L155</td>
<td>2.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Asn-L158</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Glu-M171</td>
<td>1.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

$^{a}$Side chain. $^{b}$Backbone.
doi:10.1371/journal.pone.0026808.t002

Figure 3. Hydrogen bonding pattern in the dark $P^0$ $Y_{dark}$H state (left) and the photooxidized $P^{+}$ $Y_{deprot}$ state (right). $pK_a$ values are indicated in the bracket. Key hydrogen bonds are shown as dotted lines. For clarity, only one of the pair chlorophyll, $P_A$ is shown in the figure. doi:10.1371/journal.pone.0026808.g003
Atomic partial charges of the amino acids were adopted from the all-atom CHARMM22 [15] parameter set. The charges of protonated acidic oxygen atoms in Asp and Glu were both increased symmetrically by +0.5 unit charges to account implicitly for the presence of a proton. Similarly, instead of removing a proton in the deprotonated state, the charges of all protons of the basic groups of Arg and Lys were diminished symmetrically by a total unit charge. For residues whose protonation states are not available in the CHARMM22 parameter set, appropriate charges were computed [17]. The atomic charges for the redox-active tyrosine (Tyr-L162) were adopted from the previous applications [16,18] (deprotonated with negative charge $Y^-$), and protonated with neutral charge ($Y^0$). The atomic charges of BChlb and bacteriopheophytin $b$ (BPheob) were determined from the electronic wave functions obtained with the density functional (DFT) module (B3LYP) in Gaussian03 [19] with 6-31G** basis set by fitting the resulting electrostatic potential in the neighborhood of these molecules by the RESP procedure [20] (Tables S2 and S3). To represent the charge states of the light-induced oxidized special pair $P^+$, a unit positive charge was distributed with a ratio of $P^+ / P^0 = 2/1$ derived from ENDOR studies [21] as done in the previous application [22].

$pK_a$ and protonation pattern

The present computation is based on the electrostatic continuum model by solving the linear Poisson-Boltzmann (LPB) equation with the MEAD program [23]. To facilitate a direct comparison with previous computational results, identical computational conditions and parameters were used (e.g., Refs. [11,13,14]) such as atomic partial charges and dielectric constants. The redox states of all other cofactors (i.e., accessory BChlb, BPheob, and quinones) were kept in their neutral charge state. Hemes in the cytochrome $c$ subunit were kept in the reduced state. The ensemble of the protonation patterns was sampled by the Monte Carlo method with Karlsberg [24] (Rabenstein, B. Karlsberg online manual, http://agknapp.chemie.fu-berlin.de/karlsberg/). The dielectric constants were set to $\varepsilon_w = 80$ for water. All computations were performed at 300 K, pH 7.0, and an ionic strength of 100 mM. The LPB equation was solved using a 3-step grid-focusing procedure at the resolutions 2.5 Å, 1.0 Å, and 0.3 Å. The Monte Carlo sampling for a redox active group yielded the probabilities $[A_{ox}]$ and $[A_{red}]$ of the two redox states of the molecule $A$.

To obtain absolute $pK_a$ values of a target site (e.g. $pK_a$(Tyr-L162)), the electrostatic energy difference was calculated between the two protonation states, protonated and deprotonated, in a reference model system using a known experimentally measured $pK_a$ value. The difference in the $pK_a$ value of the protein relative to the reference system was added to the known reference $pK_a$ value. Experimentally measured $pK_a$ values employed as references are 12.0 for Arg, 4.0 for Asp, 9.5 for Cys, 4.4 for Glu, 10.4 for Lys, 9.6 for Tyr [25], and 7.0 and 6.6 for deprotonation/protonation at $N_e$ and $N_o$ atoms of His, respectively [26,27,28]. All of the other titratable sites were fully equilibrated to the protonation state of the target site during the titration. The Monte Carlo sampling for a titratable residue yielded the probabilities $[\text{[protonated]}]$ and $[\text{[deprotonated]}]$ of the two protonation states of the molecule. The $pK_a$ value was evaluated using the Henderson-Hasselbalch equation. A bias potential was applied to obtain an equal amount of both protonation states $([\text{[protonated]}] = [\text{[deprotonated]}])$, yielding the $pK_a$ value as the resulting bias potential.

Error estimation

The procedures to compute $pK_a$ of titratable residues are equivalent to those of the redox potential for redox-active groups, although in the latter case, the Nerst equation is applied instead of the Henderson-Hasselbalch equation [29]. Therefore, the accuracy of the present $pK_a$ computations is directly comparable to that obtained for recent computations [16]. From the analogy, the numerical error of the $pK_a$ computations can be estimated to be about 0.2 pH units. Systematic errors typically relate to specific conformations that may differ from the given crystal structures.

Supporting Information

Table S1 Energetically minimized atomic coordinates of Tyr-L162 ($Y_{\text{depred}}$), Thr-M185, and a water molecule. (DOC)
Table S2 Atomic partial charge of BChlb. (DOC)
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Table S3  Atomic partial charge of BPheo.

References


Author Contributions

Conceived and designed the experiments: HI. Performed the experiments: HI. Analyzed the data: HI. Contributed reagents/materials/analysis tools: HI. Wrote the paper: HI.