Mechanism of Proton Transfer in Bacteriorhodopsin

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Abstract

Photoinduced proton pumping in bacteriorhodopsin (bR) was extensively studied using multiple experimental methods and utilizing various theoretical modeling approaches. These studies usually refer to well-resolved structural data of bacteriorhodopsin. However, despite obtained results, the origin of the proton pumping force initiated by the electronic excitation of retinal remains questionable. Here we discuss the challenges and approaches to modeling the proton transfer in bR and demonstrate that the process, which starts from the electronic excitation of the retinal molecule, is mainly due to the detailed arrangement of the protein environment. Using quantum chemical calculations, we have revealed that the retinal molecule after its excitation is fixed in the ground state of 13-*cis*,15-*syn* configuration, as a result of interaction with specific protein residuals. Thus, reaching this fixed configuration, the proton is first transferred to the Asp-85 residue from the water molecule, which is subsequently restored by the proton initially located in the Schiff base.

1. Introduction

Bacteriorhodopsin (bR) is the light-transducing protein in the purple membrane of *Halobacterium salinarum* [1, 2]. This hydrophobic protein contains seven transmembrane helices (A through G), which span the membrane at small angles to the perpendicular and surround the centrally located retinal linked to helix G by a protonated Schiff base (SB) with Lys-216 [3-6]. As a result of the light absorption, the retinal yields the transition into the 13-*cis*,15-*anti* form, which triggers a cyclic reaction that comprises the series of spectral intermediates usually named J, K, L, M, N, and O (see Figure 1), reflecting the proton pumping across the membrane [7-13]. It should be noted that proton transport starts from the middle of the protein structure, with the proton transfer from SB to Asp-85 residue. Subsequently, the proton is transferred to the extracellular side of the membrane and the SB is reprotonated from the cytoplasmic side completing the cycle. In addition to the ability of the proton pumping of bR, it was also experimentally demonstrated that a Cl^-/H^+ exchanger could be converted into a passive channel for Cl^- by substitution of only two key residues [14]. Site-directed mutagenesis combined with various spectroscopic methods, crystallographic studies, quantum chemical, and molecular dynamics simulations revealed many details of the structure and its changes in the course of the bR photocycle [8]. However, the main questions concerning the exact pathway of the energy conversion from the electronic excitation of retinal into the proton pumping force in this photoactive protein, as well as the unidirectionality of the proton transfer processes, are still unanswered.

In the present paper, we discuss the challenges for the modeling of bR related to its intermediate size, as it is too large for the fully quantum-chemistry-based description, but on the other side, the system of helices and residues attached to them cannot be described statistically, as the uniform dielectric environment. Moreover, the energy exchange between various parts of the whole system, such as the excited electronic states, transferred protons, twisted retinal, and bent helices, is not unidirectional, as none of them serves as the energy sink. Correspondingly, the calculations should be carried out keeping in mind the energetic states of the entire system, even if it is impossible to take into account all the

specific interactions. As an example, we address the proton transfer event associated with the transition between L and M₁ intermediates, the deprotonation of the SB and the protonation of the Asp 85 residue. We show that the irreversibility of the proton transfer can be achieved within the quantum chemistry calculations, if the initial retinal configuration was 13-*cis*,15-*syn*. This isomerization does not correspond to the minimum of the energy for the retinal separated from the rest of the complex. However, we argue that it *might be* defined as the state corresponding to the proper energy redistribution in the full system at this configuration when all the interactions with the residues at the bent helices are taken into account. We also would like to stress that according to our analysis, proton transport starts from the middle of the protein, with one of the water molecules donating its proton to Asp-85 with subsequent reprotonation from the SB.

The rest of the paper is structured as follows. In Section 2 we address the challenges of the modeling and outline the approach to qualitative description of the energy redistributions during the photocycle, which is discussed in Section 3. The molecular dynamics reviews and quantum chemistry calculations of the photoisomerization and the initial proton transfer step are presented in Section 4. The obtained results are discussed in Section 5 and Section 6 contains the conclusions of our work.

2. Challenges In The Bacteriorhodopsin Modeling

The modeling of charge transport and energy transfer processes in bR is extremely challenging because of the intermediate size of this protein complex. It is too large to be included in quantum chemistry calculations as a whole, but at the same time, it is too small to be effectively separated into the dynamical part and the environment. Such separation is at the heart of the Marcus theory of electron transfer [15, 16] with the transfer rate given by

$$k_{ET} = \frac{2\pi}{\hbar} |H_{12}|^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left(-\frac{(\lambda + \Delta G^0)^2}{4\lambda k_B T}\right), \quad (1)$$

where $|H_{12}|$ is the coupling between the initial and final states, λ is the total reorganization energy of the environment, k_B is the Boltzmann constant, T is the absolute temperature, and ΔG^0 is the total Gibbs free energy change for the transfer reaction. It was shown that such an approach can be successfully used to describe proton transport as well [17, 18]. However, there are two caveats for specific bR case, with both of them related to the smallness of the system and corresponding impossibility of the separation of the dynamical subsystem and environment. First, it is very difficult to define the reorganization energy, with all the bR residues reacting to the proton transfer events in a rather dynamical, not statistical manner. Second, in the calculation of the differences of free energies between the initial and final states, the direct electrostatic interaction between the transferring proton and charges on the residues should be taken into

account. In previous works based on the energetic conceptions [19, 20], it is missing. For the calculations of the free energies, they used only the energy of the proton sites (based on the corresponding pK_a factors) and the electrostatic interaction of these sites. The rest of the system is included via the reorganization energy and non-vacuum dielectric permittivity, with the electrostatic interaction of the protons with electrons being completely ignored, in particular. Water molecules, which are shown to be crucial for the proton transfer in bR [21-23], are considered electrically neutral, non-dissolved, and dipoleless. While this approach can be appropriate for large protein systems [24], its applicability for small systems, such as bR, is questionable.

A proper description of the full bR dynamics could be based on the inclusion of all the helices and residues into the Gibbs free energy and consideration of various vibrational degrees of freedom as the environment. In this case, the reorganization energy would be small, and the functional form of Eq. (1) becomes close to a delta-function. With small dissipation, the transfer between the states would be almost resonant, but slightly downhill with respect to the energy of the *full system*. As was mentioned above, the bR complex is too large for such quantitative treatment, but the qualitative discussion is possible. In particular, if the exact dynamics of some fragment of the full complex is being calculated, the initial and final energies *of this fragment alone* do not have to be minimal for given configurations, as the requirement of the energy minimum for the relaxed system applies only to the whole complex. Moreover, when two final states are available, the system can have a higher probability to be transferred to the more energetic of the two, because it is impossible to unload a large amount of energy to the vibrational environment in order to proceed to the minimal energy state, i.e, if the system is in the inverted Marcus regime.

3. Energy Redistribution Processes During The Photocycle

Let us consider now how the energy supplied by the photon is redistributed over various degrees of freedom in the transitions between the intermediates keeping in mind that these transitions are almost resonant with small dissipation. Immediately after the photon absorption, the energy is entirely in the electronic degrees of freedom of the excited state of retinal. The excited electronic state cannot relax back to the all-*trans* ground state because the internal degrees of freedom are not able to accept all the energy of the electronic excitation. Instead, the conformation to the 13-*cis* form (J to K transition) allows the excitation energy to be transferred to the strain of retinal. It should be noted that the part of the energy is still contained in the electron system, as the ground state energy of the 13-*cis* isomerization is larger than that of all-*trans*. In the consequent transition between K and L intermediates, the retinal is more relaxed and the energy is redistributed to the bending of the helix C resulting in modification of the energies of the Coulomb interaction between residues [25, 26].

These modified interactions compensate for the huge pK_a differences between the SB and Asp-85 (more than 11 and 2.5, respectively, for bR state [8]) and allow the initial proton transfer between these residues (L to M_1 transition). It should be emphasized that the pK_a factors of both protonable sites are modified bringing them to the pK_a of about 6.5 [13]. Accordingly, the energy transferred to the proton system is

much less than half of the electron-volt related to the initial pKa difference. Protonation of Asp-85 modifies the electrostatic interactions in the proton release group, so the corresponding proton energy becomes larger than the chemical potential of the extracellular space and the proton can be released (M_1 to M_2 transition). Deprotonation of the SB leads to conformational changes (relaxation of the helix C and bending of helices B and F), which, in turn, allow both the increase of the pKa of the SB to 8.3 and decrease of the pKa of Asp-96 to 7 [8]. Moreover, it leads to the penetration of water molecules in the space between Asp-96 residue and the SB. As a result, the reprotonation of the SB from Asp-96 via the water bridge becomes energetically favorable (M_2 to N_1 transition). Consequently, the proton energy level of Asp-96 again becomes smaller than the chemical potential of the cytoplasmic side and the Asp-96 residue is reprotonated (N_1 to N_2 transition). Accordingly, in the series of events the bR complex releases the high-energetic proton to the extracellular side and takes the low-energetic proton from the cytoplasmic side, i.e. the proton is effectively pumped. The energy gain can be estimated from the difference between the pKa of the Asp-96 residue at the initial state (about 11 [9]) and the pH factor of the extracellular space, which is about 0.3 eV for pH = 5. The proton translocations from and to Asp-96 give rise to a new sequence of conformational changes, such as relaxation of the helices B and F, and modifications of the electrostatic interactions, so the retinal reisomerization to the all-*trans* configuration is now possible (N_2 to O transition). The reprotonation of the SB leads to the decrease of the pKa of Asp-85 and its proton is slowly transferred downhill to the proton release group (O to bR transition) completing the photocycle.

4. Molecular Dynamics And Quantum Chemistry Calculations

4.1. Methods

Mimicking photoinduced processes in bR is not a trivial task since the resulting outcome is sensitive to the amount of the protein environment, which has to be taken into account for calculations. For this purpose, Molecular Dynamics (MD) simulations have been intensively used previously [4, 27-37]. Indeed, the rotation of the Schiff base as a result of the retinal isomerization can be obtained by modeling the retinal with its protein environment [34, 35]. However, in order to achieve it, many strong approximations should be used due to the lack of computational resources. One very crucial approximation suggests that the retinal structure can be subdivided into Quantum Mechanics (QM) and Molecular Mechanics (MM) segments, and the division between them is set across covalent bonds. The different QM/MM simulations have different approximations used for the description of MM and its connections to the QM. The QM-MM boundary major problem is the treatment of covalent bonding and adaptative schemes [4, 33, 37] Hayashi, et al., [34, 35] exploited only a part of retinal structure using the QM approach, while the other part of the molecule was parametrized. The full retinal in QM simulations is complicated by not demonstrating rotations as expected from experimental data [36, 37]. The later studies showed that the SB bound to retinal does not rotate in the vacuum models but instead, only the β -ring is rotating in the excited state [38, 39]. Starting from the excited state, structure optimization of the ground state headed back to the initially optimized ground state structure, which implies that the minima of the ground- and excited-state potential energy surfaces are not separated by any significant energy barrier [40].

In the vicinity of the local minimum of the S_1 state, the retinal coupled to the SB remains almost planar, but the single and double bonds turn to become inverted according to the MD simulations and *ab initio* modeling [29, 30, 32, 37, 40]. As follows from these data, the existence of a small barrier between *trans* and *cis* structures in the excited states could partially explain the fluorescence effects related to the structural evolution of retinal bound to the protonated SB in bR [29]. Our studies of the retinal-type conjugated systems indicate similar results: the S_1 excited state demonstrates an inversion between single and double bonds, which should be caused by the presence of the S_2 (also called $1^1B_u^+$) excited state properties, known for polyene and carotenoid molecules [41]. According to our EOM-CCSD data, the lowest excited state of the retinal bound to the protonated SB is of a similar origin to that of the polyene type $1^1B_u^+$ state, while the excited state of the retinal bound to the non-protonated SB of the same origin (as in the polyene type $1^1B_u^+$ state) was above the excited state of the optically forbidden state $1^1A_g^-$, which can be predicted only by taking into account the electron correlations. The EOM-CCSD is computationally expensive and the excited state energies are largely overestimated. Thus, the lowest S_1 state of the deprotonated SB should be of the $1^1A_g^-$ symmetry for the polyene while the lowest S_1 state of the protonated SB should be of the $1^1B_u^+$ symmetry. On the other hand, the retinal energy surfaces should be more complicated if we join our studies of the retinal isomerization and possible flexibility of helices. There we would expect some additional energy transfer mechanisms which fix the retinal 13-*cis*,15-*syn* and 13-*cis*,15-*anti* configurations, and finally pass the absorbed light energy from the retinal into the helices.

Deprotonation of the SB initiates the first proton transfer step in bR [10]. Protonation of Asp-85 upon deprotonation of the SB together with the *all-trans* to 13-*cis* photoisomerized retinal is followed by the release of a proton from a location within a network of protein residues and bound water [43-48] to the extracellular membrane surface. As follows from studies based on theoretical calculations, there should be various possible pathways for retinal deprotonation in the ground state what at moderate approximations agrees with the experimental data [40, 49]. The possibility to understand the detailed mechanism of proton pumping across the bR membrane as a result of the photoexcitation of the retinal chromophore is the aim of our consideration. The retinal deprotonation in the excited state would be the simplest model for explaining the irreversible mechanism without the requirement for the retinal 13-*cis*,15-*syn* isomer to exist in the ground state (Figure 2). Here we show possible energetic surfaces of the proton transfer from SB to ASP85 group which could open the proton transfer pathway, if the retinal 13-*cis*,15-*syn* isomer would be stable in the ground state.

4.2. Modeling of retinal isomerization

According to crystallographic data (PDB ID: 2NTW [50]), the photoisomerized 13-*cis* retinal in L is twisted at the C13 = C14 and C15 = N16 double-bonds resulting in the formation of retinal 13-*cis*,15-*syn* isomer. The protonated SB is connected to the proton acceptor Asp-85 via the water (W402) molecule. In the vicinity of the active center involved in the photoinduced proton transfer, additional water molecules,

W401 and W406, are also present. The retinal is surrounded by the protein residuals TRP86, TRP182, and TYR185. In modeling, the all-*trans* retinal was taken from crystallographic data (PDB ID:1C3W[5]). For QM calculations, the DFT modelling approach was performed in terms of Gaussian [51] by using HSEH1PBE and B3LYP functionals with cc-pVDZ basis set. The used HSEH1PBE and CAM-B3LYP functionals involve the long-range corrections. According to our study, the HSEH1PBE functional did not affect the results comparing with that of B3LYP functional. The CAM-B3LYP functional resulted in the β -ring rotation when all-*trans* retinal was optimized in the excited state what is out of the scope of this paper study.

It follows that the *trans*-retinal (corresponding to the ground state) had only one global minimum in the rotational pathway while optimization of the structure in the excited state showed two possible minima (Figure 3A). One of these minima is at about -92° , which correlates with the structure defined by the crystallographic data 2NTW. Moreover, the ground state in this configuration was also reorganized what suggested a conical intersection between these two states. The retinal 13-*cis*,15-*syn* isomer existed in the excited state only when TRP86, TRP182, and TYR185 structures defined according to crystallographic data were taken into account (Figure 3B). However, the optimization procedure in the ground state always resulted in the configuration of the *trans*-retinal. The same results were also obtained from QM/MM or QM/QM (ONIOM) studies by adding TRP86, TRP182, and TYR185 structures or even more structural data into the calculation scheme using molecular mechanics UFF or semiempirical PM6. Thus, minima corresponding to the excited states of the retinal 13-*cis*,15-*syn* isomer are available by using the supermolecular approach containing retinal and TRP86, TRP182, and TYR185 residuals in accord to the earlier suggestion [12]. However, this was obtained for the case of the ground state, while our calculations support this conclusion for the excited state minima (Figure 3). Evidently, additional important effects should be taken into account to obtain minima of retinal 13-*cis*,15-*syn* isomer in the ground state. Thus, we fixed retinal 13-*cis*,15-*syn* isomer (Figure 4) in order to mimic the active center caused by the protein.

4.3. Modelling of the Initial Step of Proton Transfer

Part of the structures taken from the crystallographic data as shown in Figure 2 and Figure 4 was analyzed in terms of the QM calculations. The first step of the proton transfer is caused by the photoexcitation of the SB with its subsequent rotation towards the active center where the water molecule, which can mediate the Asp-85 structure, is positioned as shown in Figure 4. We did the QM studies of the part of this structure; the results are presented in Figure 5. Initial *trans*-retinal (0°) should change into the 13-*cis* structure (-92.4°) after the light-induced transition into optically allowed excited S_1 state.

To model the initial stage of the proton transfer, let us keep the key atoms taken from the crystallography data (with retinal in the 13-*cis*-configuration, PDB ID: 2NTW [50]) fixed. In order to mimic the protein environment, the ASP85 and water molecules (except W402) were also kept in fixed positions (Figure 4). The modeling data demonstrate the proton displacement from W402 to the Asp-85 group (Figure 5A) what opens the pathway of the proton transfer from the SB to W402 (Figure 5B).

Thus, the presence of the water molecules of W401 and W406 is crucial for the proton transfer from W402 to Asp-85 group (Figure 5A). These molecules make an essential influence on the energy surfaces meaning that they open the protonation pathways which could be controlled by the protein of bR. This could explain the subsequent pathway of the protonation of Asp-85 by the SB in the L→M stage of the photoreaction of bR.

5. Discussion

Here we want to combine energy redistribution processes during the photocycle with the initial step of proton transfer. Proton transfer is driven by a series of proton locations in the different sites at various bR stages which correlate with protein changes, especially C and F helices (Figure 1). The proton transfer out to the extracellular side is correlated with C-helix; proton uptake from cytoplasmic side is correlated with F-helix. The estimated energy gain from the difference between the pKa of the Asp-96 residue at the initial state was about 0.3 eV, and the proton translocations from and to Asp-96 give rise to a new sequence of conformational changes in helices.

As follows from our quantum chemistry study, the rotation energy surface cannot explain the initial energy transfer without the interaction with the protein. Indeed, the protonated 13-*cis*,15-*syn* retinal cannot occur without the support of the protein: this configuration is absent in the ground state structure (Figure 3A), while the relevant minima might be present in the first excited state, if three structural parts from the bR are taken into account (Figure 3 B). Thus, the energy transfer from the retinal to protein should be expected by taking into account the relevant parts of the protein during the retinal excitation (Figure 2). This type of consideration redetermines the ground state of the system for the relaxed and still protonated retinal (Figure 4). The energy should be evidently translocated to the C-helix for rearrangement positions of the three water molecules (Figure 5A), which are present in the vicinity of the protonated Schiff base. This complex dynamics of the molecules and protein residuals in the vicinity of the excited retinal could result in irreversible proton transfer by (i) temporary shifting the OH- group from W402 and subsequently protonating Asp85 (Figure 5B) and (ii) translocating the proton from the SB group to W402 (Figure 5A). Thus, the initial energy should be transferred to the protein in the initial step.

As a result of such configuration changes, the energy of the electronic states of the retinal should be modified as schematically shown in Figure 6. According to our calculations, we conclude that the proton should be transferred to Asp 85 (with the help of the water molecule) from the 13-*cis*,15-*syn* configuration, which is not of the lowest energy state of the retinal. To be fixed in this configuration, we have to postulate that this state is minimized as a result of the interaction with the protein. The ground state of retinal at the 13-*cis*,15-*syn* conformation should be almost at the same energy level as it is for *cis*/*trans* minima. Thus, we should have two similar centers which differ by water translocation but with similar structural parts causing different irreversible proton transfer (Figure 6).

The energy translocation from retinal to protein in the primary step allows us to qualitatively determine the energy evolution in the protein, as shown in Figure 6. The proton transport is initiated from the middle

of the structure by step-by-step pushing and uptaking protons over all the bR proteins resulting in the pKa changes.

6. Conclusions

In conclusions, we have discussed the challenges and approaches to modeling of the proton transfer in bacteriorhodopsin (bR) and suggested that the retinal molecule responsible for the light absorption cannot be completely separated from the protein environment. In the spirit of the Marcus transport theory, all the energies of the initial and final states of the transfer event should be taken from the whole system, including the energies of the bent helices, stretched bonds, and modified electrostatic couplings to the charged and electric-dipole-possessed residues and water molecules. If the part of the whole system is involved in specific exact or high-level numerical calculations, it does not have to be at its ground state, as one of the excited states can correspond to the energy minimum for the whole system.

For the illustration, we have discussed molecular dynamics and performed quantum chemistry calculations of the initial proton transfer event in bR, the deprotonation of the Schiff base (SB) and the protonation of the Asp-85 residue, with the retinal isomerization and the energy of the retinal electron state taken into account. We have shown that the proton transport becomes irreversible, if the retinal is initially in the 13-*cis*,15-*syn* configuration, which is not the ground state. However, this state *might* correspond to the energy minimum of the whole system including the energy of the modified electrostatic interaction with the residues of the *bent* helix C. We have revealed actual mechanism of the proton transfer, as initially the Asp-85 residue is protonated by the water molecule which is restored, thereafter, by the SB proton. In some sense, it is comparable to the proton transfer through the whole complex, as it is not the situation when the single proton being transferred from the cytoplasmic side to the extracellular space, but instead proton transport is initiated from the middle of the structure.

We believe that the outlined approach will offer additional prospects for future research, because the restrictions on the initial and final states can be lifted. Correspondingly, molecular dynamics and quantum chemistry calculations can be performed in much wider range of parameters and allow detailed descriptions of individual transfer events. We also believe that eventually all these individual events will be combined in the unified self-consistent picture of the bR machinery.

Declarations

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Author contributions

L. M. and L. V. analyzed and discussed energy redistribution processes during the photocycle. M. M. performed quantum chemistry calculations. All authors contributed to manuscript text and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

The photocycle of bacteriorhodopsin showing the main proton transfer events and the main mechanical transformations of the protein environment. The details are discussed in Section 3.

Figure 2

Primary proton transfer schematic representation: initial state (PDB ID: 1C3W [5]) structure; structure after excitation and isomerization process (PDB ID: 2NTW [40]); the structure after the proton release. “rotation” presents rotation part which actually result in structural changes from the all-trans retinal (RET) to the 13-cis,15-syn RET.

Figure 3

Energy surface of protonated Schiff-base rotation: 0 deg represents all-trans structure (A); -92.4 deg represents 13-cis structure from 2NTW (B).

Figure 4

Primary proton transfers active center (PDB ID: 2NTW): RET301, LYS216, W401, W402, W406, and Asp-85. The retinal Schiff base with its polyene chain has -92.4 deg dihedral angle.

Figure 5

The proton pathway from protonated Schiff base (SBH) of retinal to Asp85 group by mediating water (W402): A - energetic surface between a water molecule and ASP85 group; B – energetic surface between Schiff base (SB) and a water molecule (W402).

Figure 6

Energy model for the Schiff base electron interaction with protein. The red marks represent the rotation energy surface of retinal, while the green ones correspond to the energy changes of protein.