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Color Tuning in Rhodopsins: The Origin of the Spectral Shift between the Chloride-bound and Anion-free Forms of Halorhodopsin

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Abstract

Detailed knowledge of the molecular mechanisms that control the spectral properties in the rhodopsin protein family is important for understanding the functions of these photoreceptors and for the rational design of artificial photosensitive proteins. Here, we used a high-level *ab initio* QM/MM method to investigate the mechanism of spectral tuning in the chloride-bound and anion-free forms of Halorhodopsin from *Natronobacterium pharaonis* (phR) and the interprotein spectral shift between them. We demonstrate that the chloride ion tunes the spectral properties of phR via two distinct mechanisms: (i) the electrostatic interaction with the chromophore that results in the 95 nm difference between the absorption maxima of the two forms and (ii) the induction of the structural reorganization in the protein that changes the positions of charged and polar residues and reduces this difference to 29 nm. The present study expands our knowledge on the role of the reorganization of the internal H-bond network for the color tuning in general and provides a detailed investigation of the tuning mechanism in phR in particular.

Spectral and photochemical properties of rhodopsins, light-responsive seven-helix transmembrane proteins, have been a subject of intense studies for decades. This interest is motivated by the important role that these photoreceptors play in biophysical processes such as vision, ion transport and photo-taxis and by their use in nanotechnology and optogenetic applications.^{1,2} Here, we report the first *ab initio* multireference ONIOM (QM:MM (SORCI + Q//B3LYP/6-31g(d):Amber96)) investigation of the spectral properties of the Halorhodopsin from *Natronomonas pharaonis* (phR). The results of the investigation not only revealed specific properties of this rhodopsin but also elucidated the mechanisms that control color tuning in rhodopsins in general.

While microbial rhodopsins (type 1 rhodopsins, bR) and the visual pigments (type 2 rhodopsins, Rh) have different primary structures, their overall topologies are similar. Both types of rhodopsins consist of the retinal chromophore (PSB) and seven transmembrane helices that form an interior pocket for the chromophore. The chromophore – all-trans retinal (PSBT) in microbial rhodopsins or 11-cis retinal (PSB11) in the visual pigments – is

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ASSOCIATED CONTENT

Supporting Information. Cartesian coordinates of all the models discussed in this study, contributions of all the polar residues of the binding pocket into σ_{max} . This material is available free of charge via the Internet at <http://pubs.acs.org>.

covalently bound via a protonated Schiff base linkage to the rest of the protein (opsin). Photoisomerization of the retinal is the first step that triggers conformational reorganization in a surrounding protein environment and leads to signal transduction or ion transport. The electronic transition responsible for this photoisomerization is an optically active transition from the ground state to the first excited singlet state ($S_0 \rightarrow S_1$). Experimental studies show that the absorption maxima (λ_{max}) corresponding to this transition vary over a wide range of wavelengths. This variation is possible due to the high sensitivity of the $S_0 \rightarrow S_1$ transition in PSB to the protein microenvironment. The origin of the opsin shift – the blue spectral shift between λ_{max} for the chromophore in the gas phase and in rhodopsins – has been the central question of color tuning for many years. The electrostatic effect of a protein environment was found to be the dominant factor that is responsible for the opsin shift.^{3–10} The steric interaction of the chromophore with an opsin was shown to modify the spectral properties of visual rhodopsins by deforming the planar structure of the chromophore.^{7,8,10} In contrast, in bR and sRII, the deviation from the planar structure was found to be negligibly small.³ To understand color tuning in rhodopsins, the electrostatic potential in the region of the PSB generated by an opsin can be decomposed into individual contributions of each amino acid. The most prominent contribution to the potential originates from the negatively charged residues (counterions) situated close to ^+N-H part of the chromophore – one Glu in visual rhodopsins and two Asp's in bR and sRII. A number of theoretical^{3–10} and experimental¹¹ studies demonstrate that the counterions cause a prominent blue shift of the spectral band going from the gas phase to the protein environment. In archaeal rhodopsins such as bR and sRII, the rest of an opsin (excluding the counterions) was shown to produce a red shift that partially counterbalances the effect of the counterions.³ The analogous red shift in Rh is still matter of debate.^{5–7,12}

Another important issue in the field is to elucidate the origin of the spectral shift between different rhodopsins (interprotein shifts). A recent QM/MM study of bR and sRII³ shows that two factors are essential to evaluate the 70 nm blue shift between these two rhodopsins: the composition of polar amino acids in the binding pocket (up to 5 Å from the chromophore) and the reorganization of the internal H-bond network that changes the positions of the charged residues, the most important being the counterions. However, SAC-CI QM/MM studies of the human red, green and blue visual pigments reveals that the contribution of Glu113, the counterion, to the interprotein shifts between these rhodopsins is small.¹² However, in this case, a reorganization of the internal H-bond network leads to a change in the position of several polar residues located in the binding pocket. This reorganization was shown to contribute to the tuning (along with the amino acid composition and the deformation of the retinal). Recently we have shown that H-bond network also regulates optical properties of short wavelength sensitive visual rhodopsins.¹³ Therefore, in all cases, the reorganization of the internal H-bond network was found to be an important mechanism to control spectral properties.

The recently published X-ray structures of the chloride-bound (phR-Cl)¹⁴ and the anion-free forms (phR-af)¹⁵ of phR enable a computational investigation that complements the present knowledge of the molecular mechanisms of color tuning in rhodopsins. Experimental studies show that titration of the bound anion-free form of phR by a sodium chloride solution moves the λ_{max} from 600 nm to 578 nm.¹⁶ Shortly after the discovery of phR, this 22 nm blue shift was attributed to the electrostatic effect of the chloride ion that supposedly moves from the solution to the ^+N-H region of the protein. Indeed, the X-ray structure¹⁴ reveals that the chloride ion is situated in this region and thus should induce a blue shift. However, our investigation shows that the electrostatic effect of the chloride ion is only one of the contributing factors. Similar to bR and sRII or the visual pigments, the reorganization of the protein structure is also crucial to understand the tuning mechanism. Moreover, since the two forms of phR differ in only one component (Cl^-), the impact due to the electrostatic

effect of the Cl^- can be clearly separated from the impact due to the structural reorganization of the protein. From a theoretical perspective, these two proteins provide representative models for an investigation of the effect of the protein reorganization on spectral properties of rhodopsins – an important factor that is still not completely understood. In the rest of the communication we will give a detailed description of the tuning mechanisms in phR and discuss how this knowledge can be used to understand the molecular mechanisms that control spectral properties of rhodopsins in general.

To construct the models we started from the 2.0 Å X-ray structure (PDB code 3A7K)¹⁴ for phR-Cl and the 1.80 Å structure (3QBG) for phR-af.¹⁵ PROPKA¹⁷ and PDB2PQR¹⁸ programs in conjunction with visual inspection are used to assign protonation states of the titratable residues (pH=7) and add hydrogen atoms. The resulting structure is then optimized using two-layer ONIOM (QM:MM-EE) scheme. (QM=B3LYP/6-31G*; MM=AMBER96 for aminoacids and Cl^- and TIP3P for water, EE=electronic embedding)¹⁹ implemented in the Gaussian09 package. To calculate the spectral properties of the chromophore in the presence and absence of the protein environment (described as AMBER96 point charges), we employ SORCI+Q/6-31G* level of the theory implemented in ORCA6.0 package.²⁰ This approach has been successfully used to study the structure and spectroscopic properties of visual pigments in our previous studies.^{5,6} More details can be found in SI.

phR-Cl is composed of seven transmembrane α -helices and the all-*trans* 15-*anti* retinal chromophore. Negatively charged Asp252, Cl401 and three water molecules (wat502, wat503, wat504) form a pentagonal H-bond cluster that is situated in the vicinity of $^+\text{N-H}$ part of PSBT (Fig. 1). Similar clusters have been found in other bacterial rhodopsins such as bR and sRII with the only difference being that Cl401 is replaced by a second Asp. Remarkably, the very recent X-ray structure of phR-af¹⁵ reveals the pronounced difference compared to phRCl (Fig. 1A–D). The main change can be seen in the middle of the helix C (in the region of the binding pocket, Fig. 1A, E, D) and in the chloride uptake pathway. In phR-af, the side chain of Thr126, the residue in the middle of the helix C that forms an H-bond with Cl401 in phR-Cl, occupies the place of this chloride ion. The water molecule (Wat502) that is H-bonded to Cl401 in phR-Cl moves out from the cavity and the remaining two water molecules change their positions (Fig 1D). Cl^- binding reorganizes the internal H-bond network of the protein leading to a significant change in the position of the polar OH groups of Thr126, Ser78 and Ser130 and a smaller reorganization of other polar residues in the binding pocket. The five charged residues that fall into the region up to 15 Å from the chromophore – Asp252, Asp156, Arg176, Arg123 and Glu234 – undergo a substantial rearrangement as well. As we will show, the polar residues in the binding pocket (up to 5 Å) and the charged residues up to 15 Å from the chromophore are essential for the color tuning, and the change in their positions after the Cl^- binding contributes to the interproteine shift.

Another important structural effect that should be investigated is the change in the geometry of the chromophore going from the gas phase to phR-af and then to phR-Cl. The geometry of π -conjugated compound is sensitive to external electrostatic field that can change the length of single and double bonds by contracting the former and elongating the latter. This geometrical distortion can be quantified by the bond length alteration (BLA), defined as the average difference in length between single and double bonds in the conjugated chain. The effect of protein electrostatic field on BLA in PSB was previously reported for the bovine rhodopsin^{5,6} and archaeal rhodopsins, such as bRh and sRII.³ These studies demonstrated that BLA increases going from PSB in the gas phase to the protein environment. Our results corroborate these findings. The calculated BLA in the gas phase PSBT (0.027 Å) increases to 0.045 Å in phR-af and to 0.064 Å in phR-Cl. To confirm that the origin of the BLA change is the polarization of the chromophore electron density by the protein environment, we reoptimized the models at the ONIOM (QM:MM-ME) (ME = mechanical embedding

with the RESP charges calculated for the gas phase chromophore) level. In contrast to the EE scheme, the ME scheme neglects the reorganization of the electronic density in the QM part caused by the MM part and treats the electrostatic interaction between them at the MM level. BLA in PSBT at this level of the theory is 0.024 Å for phR-Cl and 0.026 Å for phR-af, which is close to the gas phase value (see SI). In addition, we found that the deviation of dihedral angles from the gas phase planar structure both in phR-af and in phR-Cl is very small (see SI). Therefore, similar to bR and sRII, the main change in the geometry of the chromophore going from the gas phase to the proteins is the change in BLA that originates from the electrostatic field of the opsin but not from the steric interactions.

The calculated absorption maxima of the retinal in the protein environment are in close agreement (within 0.18 eV) with experimental data (Table 1). The blue shift between phR-af and phR-Cl observed experimentally (600 nm – 578 nm) is reproduced by our calculations (561 nm – 532 nm). To evaluate the mechanisms that are responsible for the opsin and the interprotein shifts in phR, we carried out a series of model calculations. We created models that take into account the electrostatic effects of only part of the residues that are included in a model. We kept AMBER charges of the residues included in the corresponding model, set the charges of the rest of the opsin residues to zero, and performed a SORCI+Q calculation for each model. Thus, the model phR-Cl(PSBT) includes only the bare chromophore without any external charges; phR-Cl(PSBT+count) includes the charges of the counterions (Asp252 and Cl401); phR-Cl(PSBT+5.0Å) includes charges of all the residues that fall into the region within 5 Å of the chromophore (counterions and 22 neutral and polar residues, see SI for a complete list); and phR-Cl(PSBT+5.0Å+chr) includes all charges from phR-Cl(PSBT+5.0Å) plus the charges of the four charged residues that are situated in the distance up to 15 Å from the chromophore (Arg123, Asp156, Arg176 and Glu234). The models for phR-af were constructed in the same way. Thus, phR-af(PSBT+count) includes the charges of Asp252, etc. The results of these calculations are shown in Fig. 2 A and B. The residues included in the models phR-Cl(PSBT+5.0Å+chr) and phR-af(PSBT+5.0Å+chr) are sufficient to obtain λ_{max} that are within 12 nm of the number calculated when charges of all residues are included (models phR-Cl and phR-af). The counterions – Asp252 for phR-af and Asp252 + Cl401 for phR-Cl – cause a prominent blue shift (models phR-Cl(PSBT+count) and phR-af(PSBT+count)). This shift is larger in phR-Cl (638 - 431 = 207 nm) than in phR-af (625 - 527 = 98 nm). In both cases, the electrostatic field of the rest of the protein reduces the effect of the counterions. This leads to a back red shift in both proteins, as it was also found for bR and sRII.³ However, the magnitude of the shift in these two forms is different: 101 nm and 34 nm for phR-Cl and phR-af, respectively. This difference in the back red shift explains the relatively small 29 nm net interprotein shift between phR-Cl and phR-af. Specifically, in phR-Cl, the electrostatic effect of Asp252 (model phR-Cl(PSBT+Asp252)) accounts for the 112 nm blue shift, which is slightly larger than that 98 nm calculated for phR-af (model phR-af(PSBT+count)). The negative charge located on the chloride ion induces the additional 95 nm blue shift resulting in 96 nm interprotein shift between phR-af and phR-Cl. On the other hand, the residues up to 5 Å from the chromophore, excluding the counterions, reduce this shift to 49 nm. Finally, the presence of additional four charged residues in the models phR-Cl(PSBT+5.0Å+chr) and phRaf(PSBT+5.0Å+chr) reduces the shift to 29 nm. As the next step, we estimated the influence of each polar residue in the binding pocket and the charged residues within 15 Å of the chromophore on the spectral properties by setting the charges of these residues to zero. We found that the impact of some residues on the spectral shift differs considerably for phR-Cl and phR-af. The contribution of the three most significant polar residues of the binding pocket and the four charged residues to λ_{max} are shown in Fig. 1B and C. Contributions of all the polar residues of the binding pocket can be found in SI.

The spectral shift caused by the electrostatic field of a residue depends on the distance from this residue to the chromophore. A number of quantum chemical calculations demonstrated that shortening or elongation of the distance from a counterion to the ^+N-H region leads to a larger or smaller blue shift, respectively.²¹ In phR-Cl, the reorganization of H-bond network caused by Cl^- leads to a change in the position of Asp252 compared to that in phR-af. However, as shown in Fig. 1D and E, the reorientation of the carboxyl group of Asp252 results in the shortening of the distance from the ^+N-H group to one of the carboxyl oxygens and the elongation of the distance from ^+N-H group to the other carboxyl oxygen. The effects of this reorganization partially cancel each other out and contribute 14 nm to the interprotein shift. Besides, the change in the orientation of the OH groups (dipole moments) of Ser78, Thr126, Ser130 and the change in the position of Asp156 and Arg123 (charges) are found to significantly affect the color tuning. Fig. 1C shows the positions of these residues, the directions of the dipole moments of the OH groups and the contribution of these residues into the spectral shifts. For the polar residues (Thr126, Ser78, Ser130), the change in the orientation and position of the OH groups leads to a significant difference in these contributions. Analogously, the changes in the distances from Asp156 and Arg123 to PSBT when comparing phR-af with phR-Cl affect the contributions of these residues on λ_{max} (Fig. 1B). The elongation of the distance from a negatively charged Asp156 to the ^+N-H part of the chromophore leads to a smaller negative impact of this residue on λ_{max} in phR-Cl (−4 nm) compared to phR-af (−14 nm). Accordingly, the shortening of the distance from the positively charged Arg123 results in a larger positive contribution of this residue for phR-Cl (+15 nm) compared to phR-af (+1 nm). The two remaining residues (Arg176 and Glu234) contribute to the λ_{max} significantly. However, their effects are similar to each other in the two forms of phR and they do not significantly affect the interprotein shift.

In conclusion, our theoretical investigation shows that the magnitude of the interprotein shift between phR-af and phR-Cl is controlled in two ways: directly – via the electrostatic interaction of the chloride ion with the electron density of the chromophore – and indirectly – via a change in the position of the polar and charged residues of the protein induced by this chloride ion. Therefore, the composition and three-dimensional structure of this protein determine not only the opsin shift in phR-af and phR-Cl but also the interprotein shift between these two forms. These results can be utilized for the rational design of phR derivatives with different optical properties. Beyond phR, our study clearly demonstrates that a change in the position of charged and polar residues caused by a reorganization of H-bond network leads to a significant interprotein shift. Remarkably, a reorganization of the charged residues that are situated even as far as 6–10 Å from the chromophore is shown to affect the tuning. For the short wavelength-sensitive visual pigments, past experiments and our recent QM/MM study show that mutations of the residues situated far from the chromophore tune λ_{max} by modifying the H-bond network around the retinal.¹³ The present study suggests that a distant mutation affecting the internal H-bond network in a protein can modify positions of not only counterions but also other charged residues leading to a detectable spectral shift. This kind of long-range color tuning mechanisms may explain the recent experimental study that demonstrates that a single amino acid replacement in a distant cytoplasmic loop (A178R) of the proteorhodopsin causes the 20 nm red shift of λ_{max} .^{22,23} Combined theoretical and experimental studies will help to elucidate this mechanism of the color tuning in retinal proteins further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) The superposition of phR-Cl (tan) and phR-af (gray) QM/MM structures; (B) The superposition of QM/MM geometries and the contributions to $V_{\max}$ of the charged residues situated within the 15 Å region from the chromophore, excluding counterions. The geometries are shown in color for phR-Cl and in gray for phR-af. The contributions to $V_{\max}$ are shown in tan for phR-Cl and in gray for phR-af; (C) The QM/MM geometries, the contributions to $V_{\max}$, and the directions of the OH-dipoles for three polar residue in the binding pocket that are found to significantly contribute to the tuning. The color code is the same as in (B); (D and E) H-bond network in the ^+N -H region of the PSBT in the presence of a chloride ion (phR-Cl, D) and in phR-af (E)

Figure 2.

(A) $\lambda_{\max}$ for several models of phR-Cl (green) and phR-af (blue); (B) The interprotein shift for the models.

Table 1

The first vertical excitation energies of phR-Cl and phR-af (in eV) and corresponding absorption wavelengths [in nm]

	phR-Cl	phR-af
Experiment ¹⁹	2.15 [578]	2.07 [600]
Calculations	2.33 [532]	2.21 [561]