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Chemical Physics Letters 432 (2006) 252–256
CHEMICAL PHYSICS LETTERS
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On the color-tuning mechanism of Human-Blue visual pigment: SAC-CI and QM/MM study

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Received 8 August 2006
Available online 12 October 2006

Abstract

Color-tuning mechanism of Human-Blue pigment, visual color-receptor in the cone cells of the eye, has been investigated. Based on a previous Homology-modeling structure and experimental evidences, a working model was constructed, and the structure has been optimized by QM(B3LYP)/MM(Amber) method. SAC-CI calculation was performed to obtain photo-absorption energy. The calculated absorption energy reasonably agrees with the experiment. A decomposition analysis was performed and compared with the case of Bovine rhodopsin. The electrostatic effect from the opsin is primarily important for the color-tuning. The electronic interaction (quantum effect) of the counter-residue is indispensable for quantitative calculation of the absorption energy.

Photo-absorption is the initial event of vision, photo-sensing, and ion-pumps in retinal proteins [1,2]. The visual pigments are classified into two types: rod pigment for dim-light vision and three cone pigments for color vision [1,3]. The absorption peak positions of these visual pigments are well regulated in wide spectral range to furnish the photo-receptors with color sensitivity, whereas the proteins include a common identical chromophore, retinal. The electronic absorption energy of retinal is therefore controlled by interaction with the apoprotein, opsin [2]. Many studies have been reported on the excited states of bovine rhodopsin by using modern quantum chemistry methodologies [4–11]. However, since no X-ray structure is available so far, there is no report on the excited states of the visual cone pigments. Therefore, molecular mechanism of the color-tuning is still veiled in secrecy.

In the present circumstances, there is one possibility to investigate the molecular mechanism of color-tuning. It is based on the homology modeling (HM) structure (PDB code: 1KPN) reported by Stenkamp et al. [12]. The G-protein coupled receptors (GPCR) family including retinal proteins have highly conserved seven *trans*-membrane helices [2,12], and overall homology in its amino acid sequence is 41% between the visual Human-Blue (HB) and Rhodopsin (Rh) [12]. HM would be a reasonable starting point to construct a working model. However, this model has two problems. First, the protonation states of the charged residues were ignored. Second, no water molecule was included in this structure. Therefore, based on the similarity to Rh and also on chemical intuition, we improved the model as follows: Glu178 was treated as a neutral form, because the corresponding residue in Rh, Glu181, is neutral [13]. Mutation E181Q (Glu181 → Gln) does not affect the absorption peak position, and thus Glu181 is considered to be neutral (protonated). Seven water molecules are attached in the positions similar to Rh. In the next step, to obtain numerically stable structure, we optimized the structure by an ab initio quantum mechanical/molecular mechanical (QM/MM) method [4,5,14,15]. Density functional theory (DFT) with B3LYP functional and Amber99 force-field [16] were used for the QM and MM methods, respectively. In our recent study [17], DFT (B3LYP) could obtain reasonable structure for the retinal chromophore

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doi:10.1016/j.cplett.2006.10.011

[17]. The detail of the QM/MM program and calculation were described in the previous article [4,5]. We have tested several protonation states and hydrogen-bonding patterns, and the present structure was turned out to be the most stable of all the others. In the last step, we performed SAC-CI (Symmetry-adapted cluster-configuration interaction) [18–21] calculation to determine the absorption energy of the model.

The SAC-CI method treats the electron correlations of the ground and excited states, and has been established as a reliable tool for calculating the excitation energy and excited state properties [21]. In our recent study, SAC-CI calculation with the QM/MM geometry gave rather precise absorption energy for the retinal proteins, Rh, bacteriorhodopsin (bR), and sensory rhodopsin II (sRII) [15,17].

In this communication, we report the color-tuning mechanism of visual HB pigment. Since HB shows significant blue-shift in absorption energy, we focus on the comparison with bovine rhodopsin (Rh) and analyzed the mechanism in terms of three contributions: molecular structures of the chromophore in the binding pockets, electrostatic (ES) interaction of the chromophore with the opsin environment, and electronic interaction (quantum effect) between the chromophore and the counter-ion groups.

Fig. 1 shows the optimized structure of the chromophore-lysine (HB: Lys293, Rh: Lys296) moiety with the counter-ion groups (HB [12]: Glu110 and Wat2b, Rh [22]: Glu113 and Wat2b). They are treated as QM region in the single point SAC-CI calculations for the absorption energies. The ES effect of the other residues was included by the point charges of AMBER99 [16]. Hereafter, we call this class of the computational models as 'active site (AS)' model. To analyze quantum effect of the neighboring residue and water, we also used a model 'RET' which includes only the chromophore in the QM segment. The basis functions used were Dunning's double-ζ plus polarization basis sets (D95(d)) [23] for C atoms of the retinal π-system, N- and H-atoms of Schiff base (SB), and O- and C-atoms of the carboxylate of the counter residue and the water molecule. For the other atoms, Dunning's double-ζ sets (D95) [23] were employed. In addition, single p-type anion functions ($\alpha = 0.059$) [23] were augmented on the anionic O-atoms of the counter-ion group to properly describe the charge transfer interaction between the counter-ion groups and the chromophore. In order to check the quality of the valence basis sets, we tested Dunning's correlation consistent basis sets (cc-pVDZ) [24] instead of D95(d) sets. This change causes only 0.02 eV of deviation as shown in Table 1.

Table 1 shows the SAC-CI theoretical absorption energies. We also compared them with the experimental values (2.99 eV for HB [25] and 2.49 eV for Rh [1,2]). Based on the SAC-CI result, the observed absorption peaks of HB and Rh are assigned to their first excited states (2.85 eV for HB and 2.45 eV for Rh) which exhibit large oscillator strength. The main configuration of the first excited states is $\pi - \pi^*$ excitation from the highest occupied molecular orbital (HOMO) to the lowest unoccupied MO (LUMO). The SAC-CI theoretical result reproduced the experimental value with the maximum deviation of 0.14 eV. The difference between HB and Rh was calculated to be 0.4 eV, which agrees with the experimental value of 0.5 eV. Therefore, we could discuss the molecular mechanism of color-tuning with adequate reliability.

As shown in Table 1, the calculated absorption energy for the RET model considerably underestimates that of the experiment and the AS system. Such point-charge treatment (RET model) explains only 76% (1.45 eV) of the overall spectral blue-shift obtained by the AS model. The interactions of the chromophore with the nearest charged group are not fully described only by the ES interactions using the point-charge model. The electronic interaction (quantum effect) together with the ES interaction provides important contributions to the quantitative description for that absorption energy [4,5] and hence to the spectral blue-shift in HB.

In order to identify the molecular factors determining the color tuning in HB, we analyzed the reason of the spectral blue-shifts compared to Rh (0.4 eV by SAC-CI and 0.5 eV in the experiment). This spectral shift can be decomposed into three contributions: geometric distortion of the chromophore due to the protein confinement, ES interaction of the chromophore with the surrounding proteins,

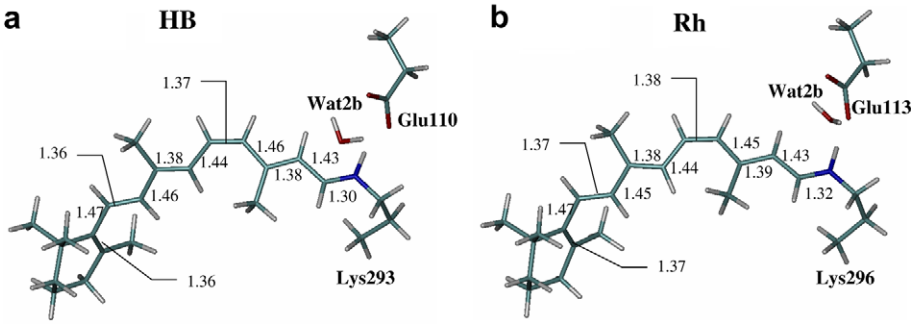

Fig. 1. Molecular structure of the retinal chromophore, counter ion group, and proximal water molecule taken from the QM/MM refined structures. (a) HB and (b) Rh. The C–C bond lengths along the retinal π-chain are also shown.

Table 1
SAC-CI absorption energies in the human-blue (HB) and bovine rhodopshin (Rh) [a]

Protein	Environment	QM size[b]	Calc[c]	SAC-CI			Exptl. (ev)
				Main configuration($\|C\| > 0.3$)	F[d](a.u.)	E_{ex}[e](ev)	
HB	*SAC-CI results*						
	In opsin	AS	a	0.94 (H → L)	1.16	2.85	2.99[i]
		RET	b	0.93 (H → L)	0.97	2.50 (2.52)	
	Bare	RET	c	0.91 (H → L)	0.58	1.40 (1.42)	
	Decomposition analysis using the SAC-CI results						
	Electrostatic[f]		b − c			1.10 (1.09)	
	Electronic[g]		a − b			0.35	
Rh	*SAC-CI results*						
	In opsin	AS	d	0.94 (H → L)	1.03	2.45	2.49[j]
		RET	e	0.93 (H → L)	0.88	2.06 (2.06)	
	Bare	RET	f	0.91 (H → L)	0.63	1.36 (1.37)	
	Decomposition analysis using the SAC-CI results						
	Electrostatic[f]		e − f			0.70 (0.68)	
	Electronic[g]		d − e			0.39	
HB–Rh	*Mechanism of the color tuning*						
	Total		a − d			0.40	0.50
	Structural[h]		c − f			0.04	
	Electrostatic[f]		(b − c) − (e − f)			0.40	
	Electronic[g]		(a − b) − (d − e)			−0.04	

The result of the decomposition analysis is also shown.
[a] Ref. [17]
[b] 'AS' and 'RET' denote 'Active site' and 'RETinal chromophore' models, respectively.
[c] Index of the calculations.
[d] Oscillator strength.
[e] Excitation (absorption) energy. The numbers in parenthesis are results with cc-pVDZ basis sets.
[f] Electrostatic effect from opsin environment.
[g] Electronic (quantum) effect from the counter-ion groups.
[h] Chromophore structural effect.
[i] Ref. [25].
[j] Ref. [1,2].

and electronic interaction with the counter-ion groups in the vicinity of the retinal chromophore. Those contributions can be deduced from the absorption energies listed in Table 1. The geometric distortion effect corresponds to the difference in the absorption energies of the 'bare' chromophores (result 'c' minus result 'f' in Table1). The ES effect of the protein surroundings is introduced by the point-charge model 'RET', 'b' − 'c' in HB and 'e' − 'f' in Rh. The ES effect to the blue-shift is thus defined as ('b' − 'c') − ('e' − 'f'). The electronic effect of the counter-ion group and water is defined as the difference between the 'AS' and 'RET' systems, 'a' − 'b' in HB and 'd' − 'e' in Rh. The electronic effect to the spectral shift is defined as ('a' − 'b') − ('d' − 'e'). The result of the analysis is also summarized in Table 1.

As seen in Table 1, most of the shift is due to the ES interaction (0.40 eV). The geometric distortion gives rise to a blue-shift of 0.04 eV (about 10% of the overall blue-shift). This smallness is related to the similarity in the chromophore geometries as shown in Fig. 1. HB has slightly stronger bond-length alternation (~0.02 Å) which would be mainly responsible for the blue-shift [17]. On the other hand, the electronic interaction (quantum effect) between chromophore and counter-ion groups slightly contributes to the spectral red-shift of 0.04 eV from Rh.

As explained above, the ES interaction is the main source of the spectral blue-shift from Rh to HB. In order to analyze the ES interaction in some more detail, we compared the electrostatic potential (ESP) caused by the opsin environment. Fig. 2a shows the ESP of HB and Rh acting on carbon atoms of the retinal π-chain. The ESP gradually increases toward the β-ionone ring side, creating a gradient of the ESP along the chain. As described in our previous study [15,17], the first excited state, HOMO–LUMO transition, has the intramolecular charge transfer (CT) character. The LUMO of retinal chromophore is localized in the SB half of the π chain, while the HOMO is in the other β-ionone ring side. Therefore, larger decrease in ESP toward the SB side causes larger blue-shift in the absorption wave length. We note that this ESP difference is larger in HB than that in Rh. Therefore, HB has the HOMO–LUMO gap larger than that of Rh. The differences of ESP between the N_ζ side (the SB side) and the C5 side (the β-ionone ring side) are 0.15 and 0.11 a.u. for HB and Rh, respectively, which increases HOMO–LUMO gap of HB (7.89 eV) by 0.35 eV larger than that of Rh (7.54 eV).

In Fig. 2a, we also show the ESP created by counterions, glutamate and water (see Fig. 1). The ESP distributions for HB and Rh show the same trend as those from total protein environment. These proximal groups contrib-

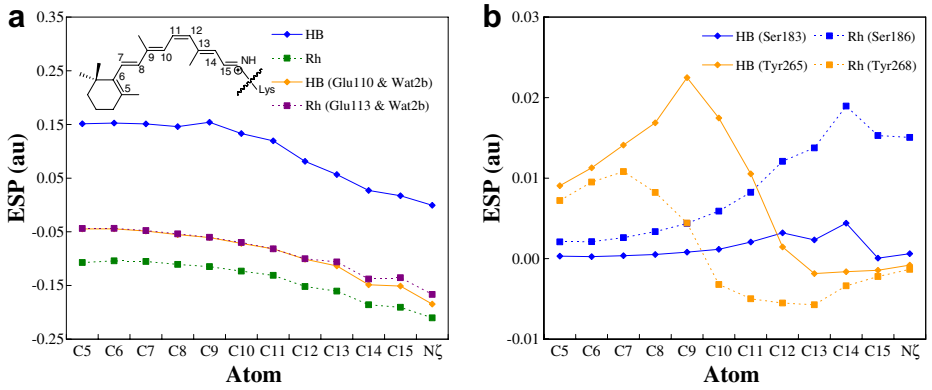

Fig. 2. The electrostatic potential (ESP) from the opsin environment at the carbon atoms in the π-chain. (a) Blue: human blue (HB), Rh: bovine rhodopsin (Rh), Yellow: contribution only from Glu110 and Wat2b in HB, Purple: contribution only from Glu113 and Wat2b in Rh; (b) Blue solid line: contribution only from Ser183 in HB, Blue broken line: contribution only from Ser186 in Rh, Yellow solid line: contribution only from Tyr265 in HB, Yellow broken line: contribution only from Tyr268 in Rh.

ute to the total ESP distribution. However, the deviation between HB and Rh around $C_{13} \sim N_\zeta$ region is not so large in the ESP from the proximal groups (at most 0.01 a.u.). It would be thus difficult to explain the spectral blue-shift of HB only by the ESP of the proximal residues. This result indicates that the absorption peak of HB is controlled not only by the counter-ion groups.

To understand how HB controls the absorption energy, we analyzed the ES interaction between the chromophore and the residues. We found that many residues are contributing to the blue-shift. This result agrees to the previous experimental results [26,27]. Among them, Ser183 and Tyr265 give leading contributions. In Fig. 2b, the ESP distribution along the π-chain is compared. Ser186 in Rh, which is the counter part of Ser183 in HB, increases the ESP around the SB region. This ESP distribution contributes to red-shift in the absorption energy. Therefore, Ser183 in HB contributes to the blue-shift, because it does not contribute to red-shift as in Ser186 in Rh. Tyr265 works as the blue-shift contributor in the same way as Glu110. We further performed Hartree–Fock calculations of the retinal chromophore including only these residues as point charges. Compared with Rh, Ser183 and Tyr265 increase HOMO–LUMO gap of the chromophore by 0.10 and 0.05 eV, respectively.

Fig. 3a,b shows the protein environment in the vicinity of the retinal SB region of HB and Rh, respectively. Significant differences are found in the hydrogen-bonding patterns near Ser183 in HB (Ser186 in Rh) and Tyr265 in HB (Tyr268 in Rh) between the two proteins. As shown in Fig. 2b, the ESPs created by Ser183 and Tyr265 in HB are different from those in Rh (Ser186 and Tyr268). This is due to the directions of polarized O–H bonds in these residues. The O–H bond of Ser183 in HB is vertical to the retinal π-chain. On the other hand, the O–H bond of Ser186 in Rh which is parallel to the π-chain creates positive ESP around the SB region as seen in Fig. 2b. This ESP decreases the absorption energy compared with HB.

What controls the hydrogen-bonding pattern in HB and Rh? As seen in Fig. 3a,b it is caused by the residue Ser289 in HB and Ala292 in Rh. Ser289 in HB acts as proton donor, while hydrophobic Ala292 cannot mediate hydrogen-bonding network. Consequently, Ser289 controls position of Wat2a and creates stable hydrogen-bonding network including Ser183. In order to check the stability, we artificially changed the hydrogen-bonding patterns of HB to be similar to those of Rh. However, these hydrogen-bondings returned back to the original ones after the QM/MM optimization. Therefore, Ser289 in HB (Ala292 in Rh) would regulate the hydrogen-bonding patterns

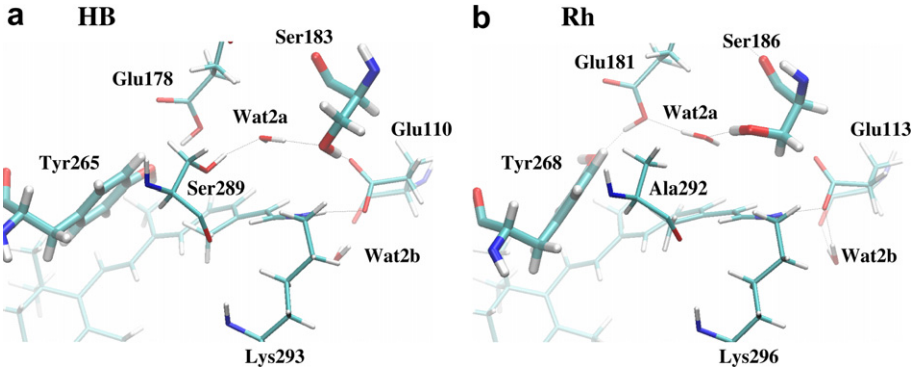

Fig. 3. Protein environment in the vicinity of the retinal SB region. (a) HB and (b) Rh.

around the SB region and contributes to the spectral blue-shift. Although contribution from single O–H bond is not as significant as the counter residue dose, there are many other hydrogen-bonding residues contribute in the similar way. Such effects to the spectral shift have been clarified by Resonance Raman experiments in red-sensitive pigments [26,27].

We note that ES effect also contributes to the blue-shift indirectly by through the structure of the retinal chromophore. The bond alternation of HB is slightly larger than that of Rh as shown in Fig. 1. In our previous study, we showed that stronger bond-alternation gives more blue-shift [17]. Therefore, the environmental effect controls the bond alternation and hence the color of the retinal protein.

Before closing, we note that the HM is used for the initial guess of our working model. If the HM is totally different in the structure around the hydrogen-bonding network, the present analysis would be worthless. However, considering the difficulty in experiment for HB, we hope that theoretical approach could provide useful information.

Acknowledgements

This study was supported by a Grant-in-Aid for Creative Scientific Research and for Young Researcher from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan, and also by Japan Science and Technology Agency. One of the authors (K.F.) gratefully acknowledges the Research Fellowship for Young Scientists from the Japan Society for the Promotion of Science.

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