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Mechanism of color tuning in retinal protein: SAC-CI and QM/MM study

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Abstract

The SAC-CI and QM/MM methods were applied to calculations of the absorption peaks of the five retinal proteins, bovine rhodopsin (Rh), bacteriorhodopsin (bR) in BR, K, and KL states, and sensory rhodopsin II (sRII). The results nicely agree with the experimental excitation energies and provided an insight into the mechanism of the large blue shifts in Rh and sRII from BR: geometric distortion in Rh and protein electrostatic effect in sRII. These results indicate that the present approach is useful for studying the excitation spectra and the mechanism of the color tuning in the retinal proteins.

Photo-absorption is the initial event of vision, photosensing, and ion-pumps in retinal proteins [1,2]. The absorption maxima of the retinal proteins 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 excitation energy of retinal is, therefore, controlled by interaction with the apoprotein, opsin [1]. In order to physically characterize the electronic excitation of retinal, several theoretical studies have been carried out using modern quantum chemistry methodologies [3–8]. These studies, however, have not yet succeeded in definitive elucidation of the color tuning mechanism due to a lack of sufficient methodological accuracy for evaluation of the excitation energy. In a very recent report, the CASPT2 method was applied to one of the retinal proteins, and the result has been significantly improved [9]. Theoretical approaches that make it possible to accurately predict the excitation energy would open the door toward the rational protein design for the color tuning.

In this study, we applied the symmetry-adapted cluster-configuration interaction (SAC-CI) method [10–13] to the ground and excited states of retinal proteins: bovine rhodopsin (Rh), bacteriorhodopsin (bR) and its early intermediate states, K and KL, and sensory rhodopsin II (sRII), which possess varied absorption maxima and protein structures. The SAC-CI method treats sufficiently and consistently electron correlations for both the ground and excited states, and thus has been established as a reliable tool for calculating the excitation energy and excited state properties [13]. The computational program of the SAC-CI method is now available in GAUSSIAN03 package [14]. In order to obtain chemically reliable protein structures, we refined the X-ray crystallographic structures [15–17] through geometry optimization with an ab initio quantum mechanical/molecular mechanical (QM/MM) method [6–8] at the Hartree–Fock/AMBER94 [18] level of theory (see, [6–8]). Fig. 1 shows the optimized structure of the

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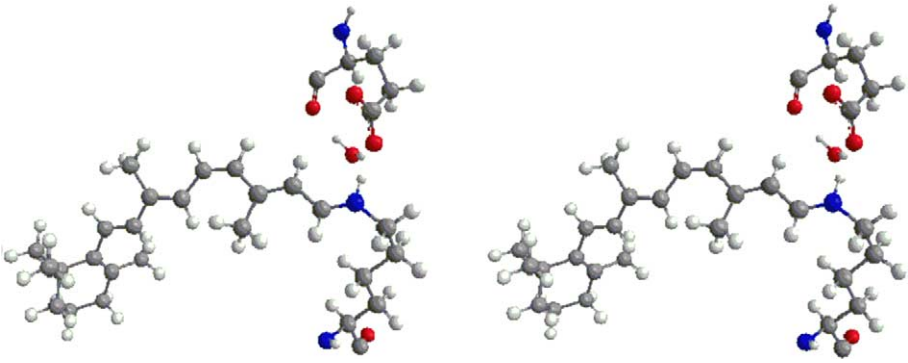

Fig. 1. The QM/MM optimized structure of retinal, Glul13, and Wat2b in Rh (stereo view).

chromophore-Lys296 moiety with the counter-ion group Glul13 and one water molecule, (Wat2b) [15,19], in Rh. In the SAC-CI calculations, the excitation energies were computed for the entire protonated Schiff base retinal chromophore with the side chain of lysine shown in Fig. 2 under the electrostatic (ES) field of protein represented by the point charges of AMBER94 [18]. The double-ζ plus polarization basis sets (D95(d)) were used for the C and N atoms in the π-system, and the double-ζ sets (D95) for the other atoms.

Table 1 summarizes the SAC-CI theoretical excitation energies and compares them with experimental values [1,2,20–25] and theoretical ones reported previously [3,6,9], The SAC-CI calculations assign the first absorption peaks of all the proteins to the first excited states which exhibit large oscillator strengths (0.53–1.18). The main character of the first excited states is π–π* excitation from HOMO to LUMO. The computed *absolute* values nicely agree with the experimentally observed absorption energies for *all* the systems studied, and are significantly improved compared to the previous theoretical ones [3,6]. The root mean square error is around 0.07 eV, and the maximum error is 0.14 eV for the K-state of bR. Recently, CASPT2 excitation energies for bovine rhodopsin have been reported and the result reproduced the experimental value with the deviation of 0.1 eV [9]. However, our results would be the first report that the absorption energies of the retinal proteins are systematically reproduced. The results also support the reliability of the QM/MM optimized structures [6–8].

The highly accurate SAC-CI method now allows us to identify the molecular factors determining the spectral shifts between the proteins. The spectral shifts are analyzed in terms of geometric distortion of the chromophore itself due to protein confinement and ES interaction of the chromophore with the surrounding protein. Excitation energy difference for the isolated chromophores in the absence of the protein ES interaction (in vacuo) determines contribution of the geometric distorsion and the rest of the overall shift is defined as the ES contribution.

Excitation energies of Rh (2.55 eV) and sRII (2.53 eV) exhibit similar large shifts from that of BR (2.22 eV), but the underlying mechanisms are completely different. In Rh, the geometric distortion gives rise to a blue shift by 0.21 eV, about two third of the overall blue shift, 0.33 eV. In fact, the geometries of chromophore are largely different from each other. The chromophore is in almost planer all-*trans* conformation in bR, whereas it is in strongly distorted 11-*cis* one in Rh. Strong torsion around a single bond C_6–C_7 appearing in Rh breaking a conjuga-

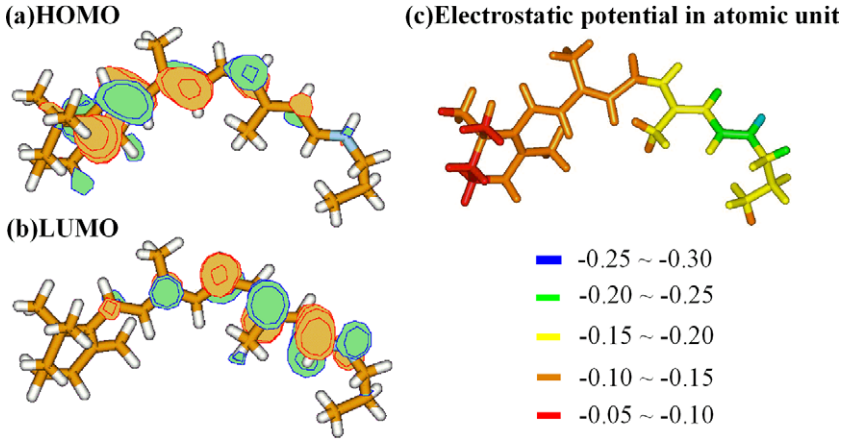

Fig. 2. (a) HOMO and (b) LUMO of retinal in Rh, and (c) the electrostatic potential by the protein environment of Rh.

Table 1
Excitation energies (eV) of retinal proteins

	Bacteriorhodopsin (bR)				Rhodopsin (Rh)	Sensory rhodopsin II (sRII)	
	BR	BR (R82A)	K	KL		sRII	sRII (R72A)
SAC-CI							
In vacuo	1.51	–	1.41	1.44	1.72	1.49	–
In opsin	2.22	2.26	1.89	2.08	2.55	2.53	2.48
Other theoretical							
In vacuo	2.05[a]				2.72[b], 2.72[c]		
In opsin	2.75[a]				2.78[b], 2.59[c]		
Experimental	2.18[d]	2.23[e]	2.03[f]	2.10[g]	2.49[h]	2.49[i]	2.48[j]

[a] MRMP result described in [6].
[b] CASPT2 result described in [3].
[c] CASPT2 result described in [9].
[d] Ref. [20].
[e] Ref. [21].
[f] Ref. [22].
[g] Ref. [23].
[h] Refs. [1,2].
[i] Ref. [24].
[j] Ref. [25].

tion of the polyene chain is mainly responsible for the shift. The other one third of the overall spectral shift is attributed to the ES interaction (see below). On the other hand, in the spectral shift between bR and sRII, only minor contribution of the geometric distortion (−0.02 eV) to the overall spectral shift (0.31 eV) is found, and most of the shift is due to the ES interaction (0.33 eV), as previously suggested [7,26].

The red shifts upon the formations of early intermediates of bR, K (0.33 eV) and KL (0.14 eV), are also analyzed. As contrasted to Rh, strong torsions of the chromophore around double bonds, especially around C_{13}=C_{14}, in K [8] leads to a red shift of 0.10 eV from BR. Reduction of ES interaction of the Schiff base with polar groups upon the K formation [8] also gives a red shift of 0.23 eV. Relaxation from K to KL decreases the torsions and increases the ES interaction [8], resulting in the smaller red shift.

Comparison of the excitation energies in the protein environments with those in vacuum clearly shows that the protein electrostatic effect leads to very large blue shifts for all the proteins (0.4–1.0 eV), The LUMO energy levels which become higher upon introducing the protein electrostatic potentials are the direct causes of the blue shifts. Figs. 2a,b depict HOMO and LUMO of the chromophore in Rh, respectively. The LUMO is localized in the Schiff base half of the π-chain, while the HOMO is in the other β-ionone ring side. In Fig. 2c, the protein ES potential at the nuclei is also shown. The figure clearly illustrates that the electrostatic potential is negative around the protonated Schiff base linkage. Therefore, the LUMO is more destabilized than the HOMO in the opsin. This orbital analysis is consistent with a conventional classical view that the blue shift is due to destabilization of the protonated Schiff base upon the excitation by migration of the positive charge along the polyene chain from the Schiff base toward the β-ionone ring [6–8,26].

The main sources of the negative potential are anionic carboxylates of Glu113 in Rh, of Asp85 and Asp212 in bR, and of Asp75 and Asp201 in sRII, which are the counter ions of the Schiff base. The magnitude of the shift depends on positions of the charged residues, i.e. the strength of the ES potential field. A remarkable difference in distance between the Schiff base and a counter ion has been suggested to account for the large spectral shift between bR and sRII [7].

The large spectral shift between bR and sRII has also been suggested to be dominantly determined by difference in chromophore–protein interaction due to opposite orientations of the side chains of positively charged residues Arg82 in bR and Arg72 in sRII [26,27]. In order to test this mechanism, we carried out *theoretical mutation*, in which Arg82 of bR and Arg72 of sRII were replaced by a neutral residue alanine, respectively, to remove positive ES potential field produced by those arginines. The SAC-CI excitation energies of the mutated bR and sRII are 2.26 and 2.48 eV, respectively, and undergo only small changes. The spectral shift between bR and sRII is also only moderately altered by 0.09 eV upon the mutations, indicating the ES interaction with the arginines plays a minor role in the spectral shift. The reason is that those arginines create positive ES potential almost equally over the entire retinal molecule, and thus the orbital energies of HOMO and LUMO are equally stabilized. The analysis explains the experimental results [21,25] that have shown minor effects of those mutations on the absorption maxima.

The present results indicate that the SAC-CI calculation with the QM/MM optimized structure is a promising approach to study the excitation spectrum of the retinal protein. This approach would be useful for studying the color

tuning of the retinal proteins led by the theoretical prediction.

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