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Origin of color tuning in human red, green, and blue cone pigments: SAC-CI and QM/MM study

Kazuhiro Fujimoto [a,b], Jun-ya Hasegawa [a,c], Hiroshi Nakatsuji [a,c,*]

[a] Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Nishikyo-ku, Kyoto 615-8510, Japan
[b] Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[c] Quantum Chemistry Research Institute (QCRI) and JST-CREST, Kyodai Katsura Venture Plaza, Goryou Oohara 1-36, Nishikyo-ku, Kyoto 615-8245, Japan

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ABSTRACT

Human color vision is controlled by the red, green, and blue cone pigments. Their photo-absorption wavelengths spread uniquely over the three primary colors, although these pigments include common chromophore, retinal. In this study, molecular mechanism of color tuning in the cone pigments was clarified. The protein effect represented by the electrostatic potential is primarily important for the spectral tuning among the pigments. The structural distortion effect of the retinal chromophore is important in the human blue pigment. The result of the analysis indicates that amino acids at specific positions in the opsins regulate the color tuning.

1. Introduction

Origin of human color vision lies in the photo-electronic processes in visual cone pigments in retina [1–3]. The absorption wave lengths of the human blue (HB), green (HG), and red (HR) cone pigments spread uniquely over the three primary colors, 414 nm (2.99 eV) [4], 532 nm (2.33 eV) [5], and 563 nm (2.20 eV) [5], respectively. These cone pigments include only one common chromophore, retinal, so that the origin of the color tuning known as 'opsin shift' [6] was thought to lie in the different interactions of this chromophore with different apoproteins (opsins). Pioneering mutagenesis studies (for review, see Ref. [1]) were summarized for clarifying the biological origin of the color tuning. It is now possible to clarify the basic physics and chemistry behind the color tuning in visual cone pigments by theoretical means. This would help to understand, at the same time, the origin of the color vision deficiency.

Regarding theoretical studies on the absorption spectrum of retinal, starting with the quantum-mechanical/molecular-mechanical (QM/MM) studies in solutions [7,8], many studies on the spectral tuning of the retinal proteins were reported (for example, see [9–17] and references therein). In our knowledge, there is, however, only a CI-single level of study [14] on the human cone pigments.

In this study, we determined theoretically the structures of the human visual pigments and clarified the molecular mechanisms behind the color tuning in the three cone pigments. We clarified the electronic structure of the retinal protonated Schiff base (PSB) and the interactions between PSB and the opsin environments. The structures and the excited states were calculated using hybrid QM/MM method [18] implemented in the development version of GAUSSIAN 03 [19]. The geometry optimizations were done with the DFT with B3LYP functional. The excitation energies of the chromophores in the different pigments were calculated with the symmetry-adapted-cluster configuration-interaction (SAC-CI) method [20–22] which has already been established as a reliable method for calculating the electronic excited states from small molecules to photo-biological systems [23]. In particular, our successful applications to photosynthetic reaction centers [24–26] and retinal proteins such as HB [15], rhodopsin (Rh) [16,17] and proton-pump system [16,17] were impressive.

In the geometry optimizations, we started from the homology modeling structures (PDB: 1KPX, 1KPW, and 1KPN for HR, HG and HB, respectively) [27] as initial guesses. We improved the protonation states of the charged residues by Poisson–Boltzmann calculations [28], the positions of the water molecules according to the X-ray structures of Rh (PDB: 1L9H) [29], and the Cl^- binding sites in the HG and HR structures [30].

In the QM/MM calculations, we used two computational models, 'active site (AS)' model and 'RET' model shown in Fig. 1. In the AS model, the retinal PSB-lysine and the counter-ion group were treated as a QM segment. The RET model was used to analyze the QM effect of the counter-ion group. In both B3LYP and SAC-CI calculations, double-ζ polarization basis [31] were used for the carbons of the retinal π-system, the N and H atoms of the PSB, and the O and C atoms of the carboxylate group in the counter residue and water. The simpler double-ζ sets [31] were used for the rest. Further, a single p-type anion function ($\alpha = 0.059$) [31] was

* Corresponding author. Address: Quantum Chemistry Research Institute (QCRI), Kyodai Katsura Venture Plaza, Goryou Oohara 1-36, Nishikyo-ku, Kyoto 615-8245, Japan. Fax: +81 75 634 3211.
E-mail address: h.nakatsuji@qcri.or.jp (H. Nakatsuji).

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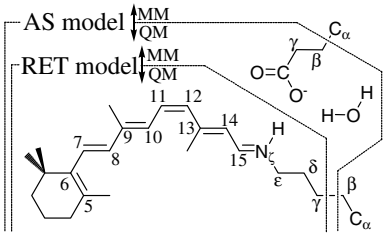

Fig. 1. Retinal PSB in the active site. The dotted lines show the QM–MM borders in the RET and AS models used for the QM/MM calculations. The H atoms were placed for terminating the QM boundary.

augmented on the O atoms of the counter-ion group. For selecting double excitation operators, the perturbation selection with energy thresholds of 5.0×10^{-6} a.u. and 5.0×10^{-7} a.u. ('LevelTwo') were performed for the SAC and SAC-CI wave functions, respectively. This level of calculation was sufficient for reproducing the absorption energies of the systems [15–17]. We also examined the applicability of the present method by calculating the excitation energy of 11-cis protonated Schiff base (11PSB) in hexane solution [32]. The 11PSB and its counter ion, trichloroacetate [32], were described quantum-mechanically. The effect of solution was calculated with the polarizable continuum model ($\varepsilon = 1.92$, heptane). The atomic coordinates were optimized using DFT (B3LYP). The excited state calculation was performed using the SAC-CI method. The excitation energy was calculated to be 2.59 eV (at LevelTwo) and 2.61 eV (at LevelThree), which reasonably agrees to the peak position observed in the experiment (2.71 eV [32]). The LevelThree energy threshold set defined in GAUSSIAN 03 is 1.0×10^{-6} a.u. and 1.0×10^{-7} a.u. for the SAC and SAC-CI wave functions, respectively.

We note that the present study used the energy minimum structures. MD calculations were performed in constructing the homology modeling structures [27] which were used as the reference in this study. The statistical average by the MD sampling study should be done in the next step [33]. Regarding the line shape of the spectra, we refer previous studies on the method [8] and its application to the spectrum of retinal chromophore in protein [10].

The SAC-CI method successfully reproduced the experimental excitation energies of the six pigments as shown in Table 1. The SAC-CI values for the wild-type HB, HG, and HR are 2.94, 2.32, and 2.08 eV, respectively, while the experimental absorption energies are 2.99, 2.33, and 2.20 eV, respectively. For the triple (3MUT) [5] and double (2MUT) [20] mutants of HG, the SAC-CI values were 2.20 and 2.40 eV, respectively, and the experimental values were 2.23 and 2.48 eV, respectively. The observed absorption peaks were assigned to the first excited states that have the largest oscillator strength in this energy region. They are one-electron homo(π)-lumo(π^*) transitions. The present calculations do not include electronic polarization effect of proteins. In previous studies of bacteriorhodopsin, polarizable force field models gave a red shift of 0.19 eV [10] to 0.34 eV [34]. A recent DFTB study gave smaller amount of red shift (0.07 eV) [35].

Table 1
Absorption energies (eV) of HB, HG, HR, Rh, and mutants

Visual pigments		SAC-CI AS[a] (model)	Experimental
HB	Wild type (WT)	2.94	2.99[e]
Rh[b]	WT	2.45[b]	2.49[h]
HG	WT	2.32	2.33[f]
	Triple mutant (3MUT)[c]	2.20	2.23[f]
	Double mutant (2MUT)[d]	2.40	2.48[g]
HR	WT	2.08	2.20[f]
	2MUT[d]	2.28	

[a] For 'AS' model, see Fig. 1.
[b] Ref. [17].
[c] 'A180, F277Y, A285T' triple mutant.
[d] 'H197A, K200A' double mutant.
[e] Ref. [4].
[f] Ref. [5].
[g] Ref. [30].
[h] Refs. [2,3].

The calculated excitation energies in the opsin environment of the AS model may be analyzed as,

$$E_{ex}^{X}(\mathrm{AS,Opsin}) = E_{ex}^{X}(\mathrm{RET,Bare}) + \mathrm{ES}^{X} + \mathrm{QM}^{X} \quad (1)$$

where X = HB, Rh, HG, and HR. $E_{ex}^{X}(\mathrm{RET,Bare})$ denotes the excitation energy of the 'bare' RET model. ES^{X} is defined by $E_{ex}^{X}(\mathrm{RET}, Opsin) - E_{ex}^{X}(\mathrm{RET,Bare})$ and represents the opsin electrostatic (ES) effect on the excitation energy. QM^{X} represents the counter-residue QM effect and evaluated by $E_{ex}^{X}(\mathrm{AS,Opsin}) - E_{ex}^{X}(\mathrm{RET,Opsin})$. The excitation energies of the additional two models, $\{E_{ex}^{X}(\mathrm{RET}, Opsin), E_{ex}^{X}(\mathrm{RET,Bare})\}$, are {2.63 eV, 1.61 eV}, {1.99 eV, 1.36 eV}, {1.71 eV, 1.33 eV}, and {2.06 eV, 1.36 eV} for HB, HG, HR, and Rh, respectively. Fig. 2 compares the three components of Eq. (1) for the HB, HG, and HR pigments using Rh as a reference.

Fig. 2 clearly shows the physical origin of the color tuning: the main origin is the protein ES interaction between PSB and the opsin environment. It amounts to 0.32 eV of the spectral blue shift of 0.49 eV of HB. The spectral red shifts of HG (0.13 eV) and HR (0.37 eV) are also dominated by the protein ES effect (0.08 eV for HG and 0.33 eV for HR). The structural effect of the chromophore (0.24 eV) is a second important factor in the spectral blue shift of HB.

The protein ES effect on the excitation energy originates from the following. The homo–lumo transition is actually an intramolecular electron transfer from the ring to the N_ζ region (from the left-half to right-half of the chromophore, see Fig. 1), so that the excitation is affected by the external ESP [14–16]. In all the retinal proteins, the ESP gradually decreases toward the N_ζ atom [14–16], which increases the homo–lumo gap. The N_ζ–C_5 difference in the ESP is −0.15, −0.11, −0.10, and −0.07 a.u. for HB, Rh, HG, and HR, respectively. We clearly see from Table 1 that the excitation energy of the pigment correlates well with the N_ζ–C_5 ESP difference.

The protein ES effect is expressed further as a sum of the contribution from each amino acid I as

$$\mathrm{ES}^{X}(I) = \sum_{A \in I} \int \frac{\Delta\rho(r) \cdot Q(r_A)}{|r - r_A|} dr \quad (X = \mathrm{HB, Rh, HG, and\ HR}) \quad (2)$$

where $\Delta\rho(r)$ is the electron-density difference upon transition and $Q(r_A)$ is the atomic charge of atom A of the amino acid I. For searching important positions I, the relative numbers $\Delta\mathrm{ES}^{X-Y}(I) = \mathrm{ES}^{X}(I) - \mathrm{ES}^{Y}(I)$ were calculated for all I and for all pairs of pigments X and Y (X, Y = HB, Rh, HG, and HR) and the important amino acids were picked up in Fig. 3.

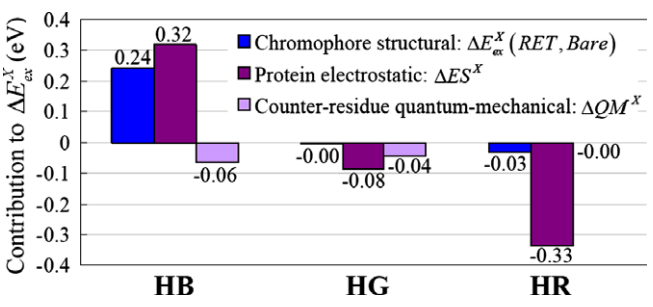

Fig. 2. Physical origin of the color tuning. Decomposition analysis on the absorption-energy shifts. Rh was used as a reference.

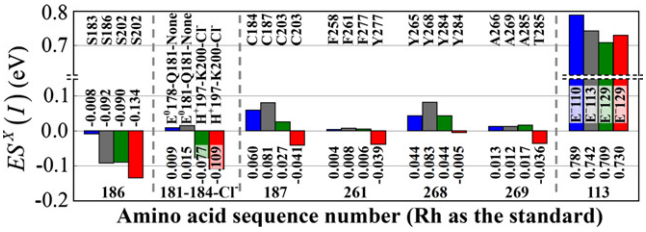

Fig. 3. Amino acid sequences important for the color tuning. The names of the residues and their ES contributions to the excitation energies ($ES^X(I)$, in eV) in HB (blue), Rh (grey), HG (green), and HR (red). The superscripts of E and H denote the charge states of the amino acids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

These amino acid positions may be grouped into four, depending on their roles. The first group of amino acids works to discriminate HB from the others. The largest contribution comes from the position 186. It is very interesting that the common amino acid, serine, gives red-shift contributions of 0.09–0.13 eV in Rh, HG, and HR, while that in HB is very small. In Ser186 of Rh and in Ser202 of HG and HR, the OH directions are parallel to the retinal π skeleton, which results in a spectral red shift, since they create positive ESP around the Schiff-base part. On the other hand, the OH group in HB is perpendicular to the π skeleton, so that its effect is negligible. These OH directions are controlled by the hydrogen-bonding networks and so tight. This machinery we found for HB and Rh [14] is actually common to all the visual pigments.

The second one is the Cl^- binding site which is composed of the positions 181, 184, and Cl^- ion. As shown in Fig. 3, the overall contribution of the Cl^- binding site is the spectral red shift in both HG and HR, although the Cl^- itself has a blue-shift contribution. The positions 181 and 184 in HG and HR are positively charged histidine and lysine, respectively, which have red-shift contributions of 0.05–0.14 eV. In contrast, HB and Rh have neutralized glutamate and glutamine at the positions 181 and 184, respectively, which have almost no contributions.

The third group (positions 187, 261, 268, and 269) characterizes HR. At the positions 261 and 269, HR has dipolar Tyr277 and Thr285, while HB, HG, and Rh have non-polar phenylalanine and alanine [5]. The OH groups in HR align such that the O atoms are closer to the retinal chromophore, since the ESP created by the retinal PSB is positive around the β-ionone side. These OH orientations provide negative ESP on the β-ionone side of the chromophore, which decreases the absorption energy.

The last group (position 113) is the counter-residue of the PSB. The amount of the shift is HB > Rh > HR > HG. Since this effect is so large, it should be described with the QM model as in the AS model.

Thus, we found that the color tuning is regulated by the amino acids at the specific positions in the amino acid sequence. This fact may have some genetic origins.

The present theoretical study has thus shed light on the physical and chemical origins of the color tuning behind the existing experimental findings.

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