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Structure of phytochromobilin in the P_r and P_{fr} forms: SAC-CI theoretical study

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Abstract

Phytochrome, an important photoreceptor in green plants, contains a cofactor, phytochromobilin, which shows photo-isomerization to respond as a sensor of light. The SAC-CI method was applied to the absorption spectra of several structural isomers of phytochromobilin and successfully identified the structure of the key isomers, P_r and P_{fr} forms which have long been discussed. The P_r and P_{fr} forms of the phytochromobilin are identified to be ZZZasa and ZZEass isomers, respectively, in their protonated forms. The results also indicated that the structures of the lumi-R and meta-R_a forms, the intermediates during the photochemical cycle, would be ZZEasa isomers.

A biliprotein phytochrome is one of the most important photoreceptors [1] and controls the photo-morphogenic processes in plants. Phytochrome exists in one of two photo-interconvertible forms: physiologically inactive P_r and active P_{fr} forms which absorb light in the red (λ_{max} = 668 nm, 1.86 eV) and in the far-red (λ_{max} = 730 nm, 1.70 eV) regions, respectively [2]. The absorption of light initiates the photo-isomerization of phytochromobilin (PΦB, Fig. 1) included in phytochrome. Several transient intermediates between the P_r and P_{fr} forms have also been detected and monitored by UV/vis spectroscopy [3]. Resonance Raman spectroscopy [4–9] was used for studying the structure of PΦB. Kneip et al. [9] proposed that PΦB in the P_r form is in ZZZasa (C_5-Z, C_{10}-Z, C_{15}-Z, C_5-anti, C_{10}-syn, C_{15}-anti) structure, while Andel III et al. [6] reported that the P_r and P_{fr} forms are ZEZaas and ZEEaaa isomers, respectively. However, the crystal structure of the phytochrome has not yet been obtained.

In such a situation, a reliable theoretical study of the absorption spectra would provide useful information for the relationship between the structure and the absorption spectrum. We apply here the SAC-CI (symmetry-adapted cluster-configuration interaction) method [10–14] to study the excited states of PΦB isomers. The SAC-CI method treats the electron correlations for the ground and excited states in a balanced way and has been established as a reliable tool for calculating the transition energies and the properties of the excited states involved [13,14]. The SAC-CI method has been successfully applied to various biological molecules including porphyrins, heme, etc. [13,14]. The SAC-CI code is now available in the GAUSSIAN 03 package [15].

We have examined three models for the photo-isomerization, Model A1, A2, and B, as shown in Fig. 2. The Models A1 and A2 are based on the Resonance Raman study by Kneip et al. [9]. For Model A2, we also referred to a study by Lippitsch et al. [16] in which a rotation around a single bond (C_{14}–C_{15}) was also suggested (Hula Twist). Model B is based on the Resonance Raman study by Andel III et al. [6].

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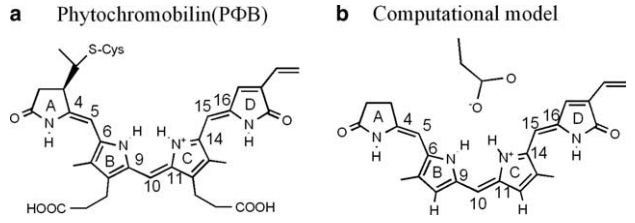

Fig. 1. (a) Structure of phytochromobilin, PΦB, in ZZZasa (C_5-Z, C_{10}-Z, C_{15}-Z, C_5-anti, C_{10}-syn, C_{15}-anti) form. (b) Computational model of PΦB (ZZZasa) with the aspartate residue that mimic the chromophore-binding site of phytochrome.

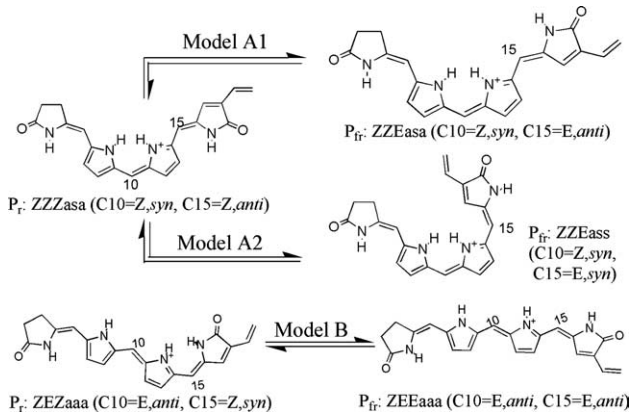

Fig. 2. Models of the photo-isomerization of phytochromobilin.

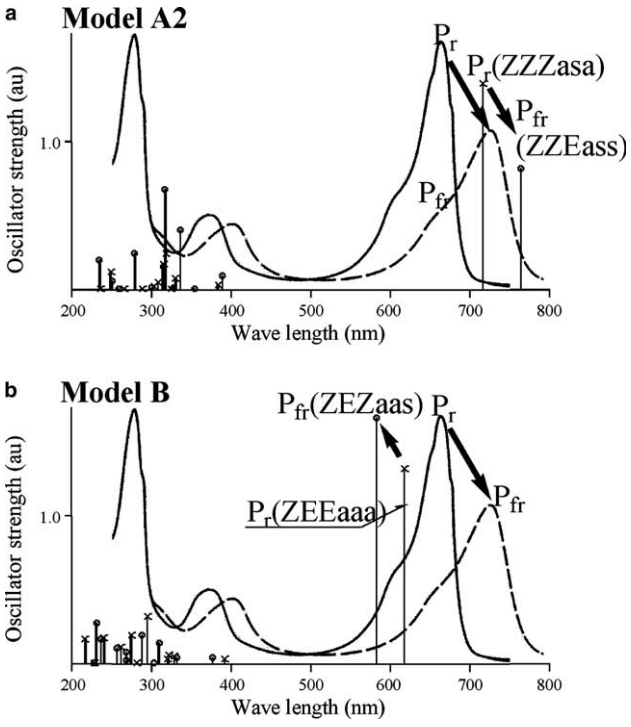

Fig. 3. (a) SAC-CI spectra for Model A2: ZZZasa (×) and ZZEass (○) isomers. (b) SAC-CI spectra for Model B: ZEZaas (×) and ZEEaaa (○) isomers.

Fig. 1b shows the computational model. The substituents that do not conjugate with the π-electrons were replaced by the hydrogen atoms. We included a propanoic acid that mimics an acidic residue. Most of the recent experimental studies concluded that the ring C of PΦB is protonated [5,6,9]. This indicates that an acidic residue stabilizes the protonated chromophores, as observed in C-phycocyanin [6,9,17]. The protonation state and the geometry were calculated with the DFT [18] (B3LYP [19]). The basis sets employed were VDZP plus diffuse functions (6-31+g(d)).

For all the isomers in Models A1 and A2, the protonated forms $(P\Phi B\text{-}H)^+$-$(Asp)^-$ are more stable than the neutral forms (PΦB)-(Asp-H) by 4.5 and 5.4 kcal/mol, respectively. These results agree with the experimental findings [5,6,9]. However in Model B, the neutral forms of ZEZaas and ZEEaaa isomers are slightly more stable than the protonated ones by 0.7 and 3.4 kcal/mol, respectively. Therefore, we performed the SAC-CI calculations for both the protonated and neutral forms of ZEZaas and ZEEaaa isomers.

The experimental absorption spectra of the P_r and P_{fr} forms [2] are shown in Fig. 3. After the photo-isomerization $P_r \rightarrow P_{fr}$, the peak at 668 nm (1.86 eV) shows a red-shift of 0.16 eV with decreasing the absorption intensity. In the higher-energy region, there are several peaks as seen in Fig. 3, but the strong one at ~300 nm is ascribed to the aromatic protein residues [2].

Single-point SAC-CI calculations were performed for the structures described above. The basis sets used were of DZ quality. Huzinaga's (63/5)/[5121/41] sets [20] were adopted for C and N atoms, and Huzinaga's (4)/[31] set [21] were for H atoms. For the negatively charged oxygen atoms in the aspartate, single p-type anion function ($\alpha = 0.059$) [22] was augmented. From our experience, polarization functions gave only minor corrections to the excitation energies of tetrapyrrolic compounds like porphyrins [23]. The active space for the SAC-CI calculations included all valence orbitals. The 1s orbitals and their corresponding virtual orbitals were treated as the frozen orbitals. The perturbation selection of the excitation operators [13] was carried out with the energy thresholds of 5×10^{-6} and 5×10^{-7} a.u. for the ground and excited states, respectively. The results are summarized in Table 1, and the SAC-CI spectra for Models A2 and B are compared with the experimental spectra in Fig. 3.

As shown in Fig. 3, the SAC-CI results clearly show that the spectral change of Model A2 is very close to that of the experiment. The amount of the red-shift is calculated to be 0.11 eV, which is very close to the experimental value (0.16 eV). The calculated excitation energies for ZZZasa and ZZEass structures are 1.73 and

Table 1
Transition energy of the first excited state of phytochromobilin (PΦB) isomers

Isomer	SAC-CI		Exptl.	
	E_{ex}[a]	Osc.[b]	E_{ex}[a]	Osc.[b]
P_r form of Models A1 and A2				
ZZZasa	1.73 (0.0)[h]	1.31	1.86[d] (0.0)[h]	S[c]
P_{fr} form of Model A1				
ZZEasa	1.71 (−0.02)[h]	1.25	1.80[e] (−0.06)[h], 1.87[f] (+0.01)[h]	
P_{fr} form of Model A2				
ZZEass	1.62 (−0.11)[h]	0.77	1.70[g] (−0.16)[h]	M[c]
P_r form of Model B				
ZEZaas	2.00 (0.0)[i]	1.21		
P_{fr} model of Model B				
ZEEaaa	2.13 (+0.13)[i]	1.53		

[a] Excitation energy in eV unit.
[b] Oscillator strength.
[c] 'S' and 'M' denote strong and medium, respectively [2].
[d] P_r form [2].
[e] Lumi-R form [24].
[f] Meta-R_a form [2].
[g] P_{fr} form [2].
[h] Relative energy from ZZZasa isomer.
[i] Relative energy from ZEZaas isomer.

1.62 eV, respectively, which are in reasonable agreement with the experiment [2]. The oscillator strength of ZZZasa and ZZEass structures is 1.31 and 0.77 a.u., respectively, and the change in the spectral intensity was also reproduced.

On the other hand, the SAC-CI results for Models A1 and B could not explain the experimental spectra. In Model A1, the excitation spectrum of ZZEasa isomer is similar to that of ZZZasa, and the amount of the red-shift is calculated to be only 0.02 eV. The result for Model B (neutral form) is much more different from the experimental spectra, as illustrated in Fig. 3b. The change in the oscillator strength was also to the opposite direction. The P_{fr} form has the intensity larger than that of the P_r form. We also carried out the SAC-CI calculations for the protonated ZEZaas and ZEEaaa isomers. However, the excitation energy obtained were 1.61 and 1.80 eV, respectively, which also could not account for the experimental spectral changes. From these results, we conclude that protonated ZZZasa and ZZEass isomers are assigned to the P_r and P_{fr} forms of PΦB, respectively.

The UV/vis spectroscopy [3,24] and time-resolved circular dichroism (TRCD) [25] studies discovered the lumi-R and meta-R_a states as the intermediate states between the P_r and P_{fr} forms. The experimental peak maximums of lumi-R (1.80 eV) and meta-R_a (1.87 eV) states are very close to that of P_r form [24]. The C_{15}=C_{16} rotation is so far accepted as the primary step of the photoisomerization [26,27]. Our present result showed that the structure differences between the P_r and P_{fr} forms are both in the C_{15}=C_{16} rotation from Z- to E-conformation and in the C_{14}–C_{15} rotation from anti- to syn-conformation. Therefore, ZZEasa isomer is a possible candidate for the lumi-R or meta-R_a forms. The calculated excitation energy for ZZEasa isomer was 1.71 eV, which was by 0.02 eV smaller than that of ZZZasa isomer, P_r form. This is consistent to the experimental observation [24].

The present and previous studies [13,14,23,28–35] indicate that the reliable theoretical spectroscopic studies as provided by the SAC-CI method could be useful and powerful means for clarifying the electronic mechanisms, structural chemistries, reactivities, and the electronic origins of the various phenomena in photobiology.

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