Chemical Physics Letters 652 (2016) 184–189

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Research paper

Computational study on the color change of 3′-hydroxyechinenone in the orange carotenoid protein

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ARTICLE INFO

Article history:
Received 1 March 2016
Revised 30 March 2016
In final form 18 April 2016
Available online 19 April 2016

Keywords:
Carotenoid
Absorption spectrum
Pigment–protein interaction
TD-DFT calculation

ABSTRACT

The orange carotenoid protein, which contains 3′-hydroxyechinenone (hECN), changes color from orange to red when irradiated with blue-green light. In this study, the origins of the color change have been investigated. The conformation of hECN in the red form is more planar than that in the orange form; consequently, the absorption band is red-shifted on conversion from the orange form to the red form. Another source of the red shift is that the electrostatic field generated by the protein in the red form stabilizes the excited state better than that generated by the protein in the orange form.

1. Introduction

The orange carotenoid protein (OCP) is involved in the cyanobacterial photoprotective mechanism. In this mechanism, excess light energy is dissipated as heat, allowing regulation of the excitation energy arriving at the photosynthetic reaction center [1–3]. The orange color of OCP originates from the S_0–S_2 absorption of a carotenoid, 3′-hydroxyechinenone (hECN; Fig. 1), which is non-covalently bound to the protein. When the inactive orange form of OCP (OCP^o) is illuminated with intense blue-green light, it is converted to the active red form (OCP^r) and then binds to phycobilisome, quenching the excited state of a light-harvesting pigment, bilin [3,4]. The relative position and orientation of the N- and C-terminal domains dramatically change upon conversion of OCP^o to OCP^r, and such structural changes of protein are crucial for the binding to phycobilisome [4–8]. The change in color of OCP from orange to red corresponds to a red shift of the absorption band [9–11]. Although the change in the S_0–S_2 excitation energy of hECN is not crucial for the photoprotective mechanism, the spectral shift may be favorable because blue-green light is preferentially absorbed by OCP^o rather than by OCP^r. Transient spectroscopic studies have indicated that the excited-state absorption and stimulated emission also differ between OCP^o and OCP^r [9–12]. These results suggest that both the conformation and environmental interactions of hECN differ between OCP^r and OCP^o. The absorption band of OCP^r is broader than that of OCP^o, suggesting that the structural fluctuations of OCP^r are largely due to the exposure of the carbonyl group of hECN to aqueous solvent [12]. Although a number of spectroscopic studies have been reported for OCP^o and OCP^r [4,5,8–12], the origins of the differences in the absorption spectra of OCP^o and OCP^r remain unclear.

The excitation energy of a pigment can be modulated by changing the protein environment. For example, the protonated Schiff base of retinal exhibits a wide spectral shift in various mutants of human cellular retinol binding protein [13]. The variation in the excitation energy has been explained in terms of the electrostatic interaction between the pigment and protein by computational investigations. To clarify the environmental effects on the absorption spectrum of a pigment–protein complex with computational methods, atomic resolution structures are needed. Three crystal structures have been determined for the orange form of OCP that contain hECN [14] or an analogous carotenoid [7,15], while no structure analyses have been reported for the red form. Recently, it has been reported that the OCP fragment consisting of a part of the N-terminal domain alone forms a carotenoid-protein complex with hECN or an analogous carotenoid. Furthermore, the complex exhibits an absorption spectrum quite similar to that of full-length OCP^r [4,7]. The crystal structure of the complex has been determined, and the conformation of the carotenoid is different from that observed in OCP^o [7]. The results of biochemical experiments also indicate that hECN is accommodated in the N-terminal domain of OCP^r [5,6,8]. Therefore, the conformation and position of hECN in OCP^r may be similar to those in the N-terminal fragment of OCP. In the present study, a computational approach has been used to clarify the origins of the spectral change accompanying the OCP^o-to-OCP^r conversion.

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http://dx.doi.org/10.1016/j.cplett.2016.04.062
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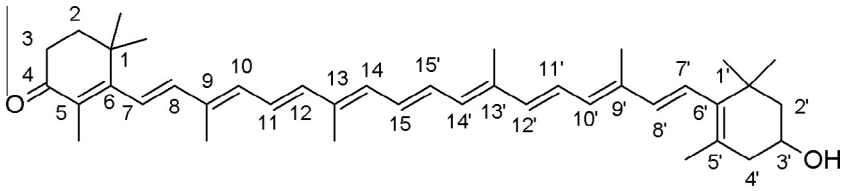

Fig. 1. Structural formula of hECN showing atom labeling.

2. Computational details

2.1. Molecular dynamics (MD) simulations

The initial structures for the models of the orange and red forms containing hECN were constructed using the crystal structures of OCP^{o} (PDB code: IM98 [14]; chain A) and of the N-terminal fragment containing canthaxanthin (PDB code: 4XB4 [7]; chain B), respectively. Canthaxanthin in the latter crystal was replaced with hECN (see Supplementary material). The AMBER ff14SB [16] and TIP3P [17] force field parameters were used for protein and water, respectively, while the GAFF parameter set [18] was used for hECN with slight modifications. Electrostatic potential (ESP) charges were used for the hECN atomic charges [19] calculated at the MP2 [20]/6-311G** [21] level. The simulation cell was a rectangular box consisting of one hECN-protein complex, Na^+ ions (added for charge neutralization), and water molecules. Periodic boundary conditions were applied. The van der Waals parameters for Na^+ were taken from Ref. [22]. Long-range electrostatic interactions were evaluated with the particle mesh Ewald (PME) method [23]. Firstly, energy minimization was performed on hECN and the protein in an equilibrium configuration of solvent and ions. Then, the temperature was increased slowly to 298 K. The NVT ensemble was used for the production run with the temperature being regulated at (298 ± 1) K using the Andersen thermostat [24]. The geometry of hECN was fixed at the optimized structure during all MD runs. The SHAKE algorithm [25] was applied to the covalent bonds to hydrogen atoms in the protein. After equilibration for 1 ns, a 2 ns trajectory was collected with a time step of 2 fs. The MD simulations were performed with the AMBER14 package [26], and trajectory analysis was carried out with cpptraj [27]. Details of the simulation systems and the set-up procedures are given in Supplementary material.

2.2. Molecular orbital (MO) calculations

The geometry of hECN in the model was optimized by a quantum mechanics/molecular mechanics (QM/MM) method with mechanical embedding. In this scheme, hECN was modeled by density functional theory (DFT) at the B3LYP [28,29]/6-31G* [30] level and the remaining part of the model was treated with the above mentioned force fields. The initial structure of the model was obtained by energy minimization of the initial structure by MM or a random selection of snapshots from the MD trajectory. Each model consisted of hECN and those protein residues and water molecules within 15 Å from hECN. The average numbers of water molecules included were 221 and 552 for the orange and red forms, respectively. During optimization, the positions of the protein and water were fixed. The geometry of free hECN was also optimized at the B3LYP/6-31G* level. The excitation energies were computed using time-dependent (TD)-DFT (CAM-B3LYP [31]/6-31G*) method. The effects of the protein and water molecules on the excitation energy were included by modeling them as point charges. The relative permittivity was assumed to be 2.0. The ESP charges in the S_0 and S_2 states were computed at the energy minimum at the CAM-B3LYP/6-311G** level of theory, and these charges were used to evaluate the differences in the interaction energy in post-trajectory analysis. All the quantum mechanical and QM/MM calculations were performed in Gaussian09 [32]. Electrostatic potentials generated by the protein and water were evaluated with the ESPF module of MOLCAS8 [33].

3. Results and discussion

3.1. Effects of the conformation of hECN

For carotenoids and linear conjugated polyenes, the energy of the 0–0 band in the S_0–S_2 absorption is linearly correlated with the conjugation length [34]. According to this relationship, the effective conjugation length of hECN is calculated to be 9.8 [35], which is shorter than the nominal value of twelve (eleven C=C bonds and one C=O) or the empirical conjugation length parameter of 10.5 (=9 + 0.5 × 2 + 0.5; the number of endocyclic C=C and that of C=O were multiplied by 0.5) [9]. The reduction of the effective conjugation length is attributed to the deviation from the planarity in the linkage between the linear polyene part and the terminal rings. To examine stable conformations of hECN, the potential energy curves with respect to the C5—C6—C7—C8 (ϕ 5–6–7–8) and C5′—C6′—C7′—C8′ (ϕ 5′–6′–7′–8′) torsion angles were computed by a relaxed scan at the B3LYP/6-31G* level. As shown in Fig. 2(a) and (b), the potential energy curve has two minima around ±45° corresponding to the s-cis forms and a local minimum at −160 for ϕ 5–6–7–8 (or +170° for ϕ 5′–6′–7′–8′), which corresponds to the s-trans form. The s-cis conformers are more stable than the s-trans conformer although the energy difference (3–6 kJ mol^{-1}) is small. For both ϕ 5–6–7–8 and ϕ 5′–6′–7′–8′, the potential energy curve is not represented by the typical torsional profile for C=C—C=C systems with a periodicity of two. Instead the s-cis minima deviate from a planar structure. To examine the influence of the torsion angle on the excitation energy, TD-DFT calculations were performed at each geometry, and these are plotted in Fig. 2(c) and (d). The dependence on ϕ 5–6–7–8, which involves the enone part, is larger than that on ϕ 5′–6′–7′–8′. The torsion-angle dependence is rather large in the vicinity of the s-cis minima at ±∼45°, suggesting that the twisting motion about the C6—C7 or C6′—C7′ bond may broaden the absorption band.

Leverenz et al. examined the structures of hECN and analogous carotenoids, which differed from hECN in the substitution pattern at the 3′- and/or 4′-positions, in the crystals of OCP or its N-terminal fragment [7]. The conformations of the carotenoids in OCP^{o} are similar in the three crystal structures: the C5—C6—C7—C8 moiety is in the s-trans form while the C5′—C6′—C7′—C8′ moiety is in the s-cis form [7,14,15]. The linear polyene moiety is not planar but bowed, and each C—C bond is slightly twisted. Such distortions, however, have no significant influence on the excitation energy [36]. In the N-terminal fragment of OCP, the carotenoid adopts a different conformation; the C5—C6—C7—C8 moiety is in the s-cis form while the C5′–C6′–C7′–C8′ moiety is in the s-trans form, and the linear polyene moiety is almost planar [7]. The conformations of hECN in the OCP^{o} and OCP^{r} models were examined by