Photobiology

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Protein-Induced Color Shift of Carotenoids in β-Crustacyanin**

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Abstract: *β-Crustacyanin (β-CR) is a pigment protein responsible for the blue color of lobsters. We show using correlated ab initio calculations how the protein environment tunes the chromophores of β-CR through electrostatic and steric effects.*

Understanding how the color of light-absorbing molecules depends on their specific chemical environments is central for elucidating the molecular mechanisms of photobiological systems.[1] The accuracy of computational methods has now reached a level that allows the color-tuning mechanisms to be quantitatively addressed.[2] We study here the light-absorbing properties of carotenoids (CRT) in β-crustacyanin (β-CR), a protein responsible for the blue color of lobsters (*H. gammarus*).[3] Upon cooking, their color changes to red as a result of denaturation of the β-CR and release of the chromophore from the protein. The color tuning of β-CR has intrigued scientists for more than half a century,[3] but the origin of this large protein-induced color shift still remains unclear.[4] β-CR comprises two stacked astaxanthin (AXT) CRTs (Figure 1) that absorb at $\lambda = 580$–590 nm (2.10–2.14 eV),[4a,5] while in organic solvents, the AXT absorbs at $\lambda = 472$ nm (2.63 eV).[6] We show for the first time by using correlated calculations how this drastic color change arises from changes in the molecular and electronic structure of the chromophore due to the protein surroundings.

In 2002, the X-ray structure of β-CR was resolved at a 3.2 Å resolution,[4a] revealing that the two AXT molecules bind noncovalently at a distance of 7 Å from each other at the heterodimeric subunit interface (Figure 1). The CRT rings are coplanar with the polyene chain, which was suggested to extend the conjugation of the molecule and to perturb the electronic ground state.[4a] The AXTs form hydrogen bonds with histidine residues, thus suggesting that a dipole moment

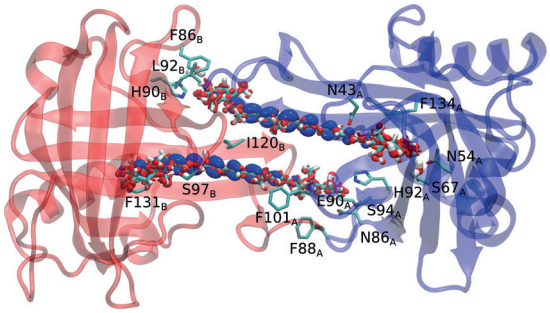

Figure 1. The structure of β-CR and its two stacked AXT chromophores, showing the frontier orbitals involved in the photoexcitation and key protein residues that affect the color tuning.

is induced in the chromophore.[7] The structural studies also showed that the AXTs are bent in the binding pocket, which also could contribute to the large color shift through a planarization and polarization mechanism.[4f]

Quantum chemical predictions of the excited states of CRTs are particularly challenging due to the long polyene chain,[8] which can lead to spin contamination, triplet instability, and the multireference character of the ground state. Previous calculations on AXT at the time-dependent density functional theory level were not able to quantitatively explain the color shift of AXT in β-CR.[4a–e] In the present hybrid quantum mechanics/classical mechanics (QM/MM) study, we employ correlated calculations of vertical excitations energies (VEEs) at the adiabatic diagrammatic correction to the second order (ADC(2)).[9] We show that the large protein-induced shift of AXT in β-CR arises from destabilization of the ground state relative to the excited state due to electrostatic polarization and steric constraint of the chromophore in the protein-binding pocket. The large-scale computations are possible due to the gain in computational speed provided by the employed reduced virtual space (RVS) approach.[10]

We obtain a VEE of 2.34 eV (530 nm) for the first excited state of AXT in β-CR, which agrees rather well with the experimental absorption maximum of 2.10–2.14 eV (580–590 nm; Table 1),[4a,5,6] The chemical environment of the other CRT (AXTB) forms weaker hydrogen bonds to the protein residues, thus resulting in a slightly higher VEE of 2.40 eV (517 nm). We also find a weakly allowed second excited state at 3.10–3.15 eV (ca. 400 nm). When the AXT chromophore is optimized in vacuum, the VEEs blue-shift to 2.53–2.62 eV, which agrees well with the excitation energies of 2.45–2.63 eV for CRTs in *n*-hexane (Table 1).[6] Based on recent experiments[11] and ensemble averaged AXT spectra, we find that vibronic effects do not significantly contribute to these spectral shifts of AXT (Figure S6). The calculations show that the s-*cis* conformer of AXT is 4 kcal mol^{-1} more stable in the gas-phase relative to the all-*trans* conformer found in the

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Table 1: VEEs of β-CR.[a]

QM region	VEE [eV] Protein	VEE [eV] Isolated	VEE Shift [E_{el}/E_{str}]	*Exp.* [eV]
AXTA + AXTB	2.34	2.44	0.10/0.18	2.10–2.14[a]
	2.40	2.50	0.10/0.12	
	3.10	2.85	0.25/0.59	
	3.15	2.92	0.23/0.52	
AXTA	2.33	2.47	0.14/0.15	–
	3.13	3.12	0.01/0.32	
AXTB	2.38	2.49	0.11/0.13	–
	3.08	2.86	0.22/0.58	
AXT *opt.*	ES1	2.62 (s-*cis*)		2.63[b]
	ES2	3.44 (s-*cis*)		
	ES1	2.53 (*trans*)		
	ES2	3.33 (*trans*)		

[a] "Protein" and "Isolate " denote VEEs obtained for AXT with or without the protein environment, respectively, by using the protein-optimized structures of the AXTs. "VEE Shift" refers to the electrostatic (Eel) and steric (Estr) shifts, as defined in the Supporting Information. Exp. refers to the absorption maximum measured for [b] β-CR and [c] AXT in *n*-hexane.

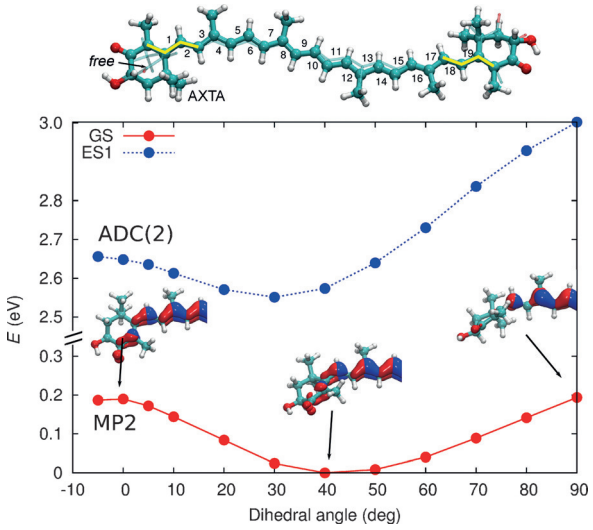

Figure 2. A relaxed dihedral scan of the AXT in the ground state (MP2/def2-TZVP) with VEEs at the ADC(2)/def2-TZVP level. The scan mimics the steric effect of the protein. The planarization extends the π system of the conjugated polyene to the ring. GS = ground state ES1 = excited state.

protein (Figure 2 and Table S5 in the Supporting Information), which imposes a planar orientation of the CRT rings relative to the polyene chain. In the gas-phase structure, the torsion angle between the ring and the conjugated chain is 38°, while we obtain values of 170–180° for the corresponding torsion angles for the QM/MM optimized protein structure, which is consistent with the torsion angle of 43–53° and 170–177° for experimental structures of isolated CRTs[12] and β-CR, respectively (Table S1). The structural changes induce a red-shift of 0.15 eV on the VEEs due to a larger destabilization of the ground state relative to the excited state. By performing a dihedral scan of the CRT rings, we find that the ring orientation red-shifts the absorption band by approximately 0.1 eV (Figure 2). A similar tuning effect has been suggested for sensory rhodopsin II, where the β-ionone ring is coplanar with the retinyl side chain.[13] Moreover, we find that the alignment of the ring plane with the polyene chain slightly increases the π-electron delocalization (Figure 2), as also suggested previously.[4a]

The protein environment imposes an electrostatic red-shift of 0.10–0.25 eV on the AXTs, which is consistent with experimental data[7b,14] and increases the ground-state dipole moment of the CRT by a few debye. To probe the electrostatic effect of the protein environment, we performed VEE calculations in which the charge distribution of the nearby protein residues was systematically removed (Figure 3 and Table S3). The calculations neglect effects arising from structural relaxation, which might lead to an underestimation of the shifts. The calculations suggest that the overall electrostatic shift arises from a fine-tuned balance between red- and blue-shifting contributions. For example, residue Glu-90_A has a blue-shifting effect of 0.2 eV on AXTB, but this effect is quenched by red-shifting contributions from water molecules, for example. Most of the protein residues that are in hydrogen-bonding contact with the AXT seem to impose a small blue-shift.

We studied differences in the VEEs by QM/MM calculations on monomeric and dimeric models (Table 1) to investigate whether the color shift arises from exciton coupling of the chromophores as suggested in experimental studies.[7b,15] The obtained values indicate that the exciton coupling imposes an energetic splitting of approximately 0.004–0.02 eV, and thus has a very small effect on the overall tuning, which is in agreement with semi-empirical and[4d] embedding calculations,[4e] and recent experiments.[11] In contrast, transition–dipole interaction models yield an overestimated exciton coupling of 0.49 eV.[15]

In summary, we show how β-CR, a protein that is responsible for the color of blue lobsters, tunes the color of its two bound CRTs from 2.6 eV in the gas phase to 2.3 eV in our protein models. Our results are in good agreement with the experimental absorption maxima at 2.6 eV and 2.1 eV for the AXT in *n*-hexane and in β-CR, respectively. We treated the 192 atoms of the dimeric protein-embedded chromophores quantum mechanically and coupled this to an MM description of the protein environment through a QM/MM methodology. Due to the large size of the chromophore, the calculations do not consider the quantum mechanical polarization effects of the protein surroundings, which might further influence the electrostatic tuning contribution.[16]

We show that the bathochromic shift of AXT arises approximately 50% (0.15–0.23 eV) from electrostatic effects, 50% (0.15 eV) from steric contributions, and less than 1% (0.004–0.02 eV) from exciton coupling between the two chromophores. The calculations provide detailed insights into the photophysical properties of the CRTs and show how the surrounding protein residues contribute to the biological color tuning mechanism by imposing balanced polarization and planarization effects on the chromophore.[4f]

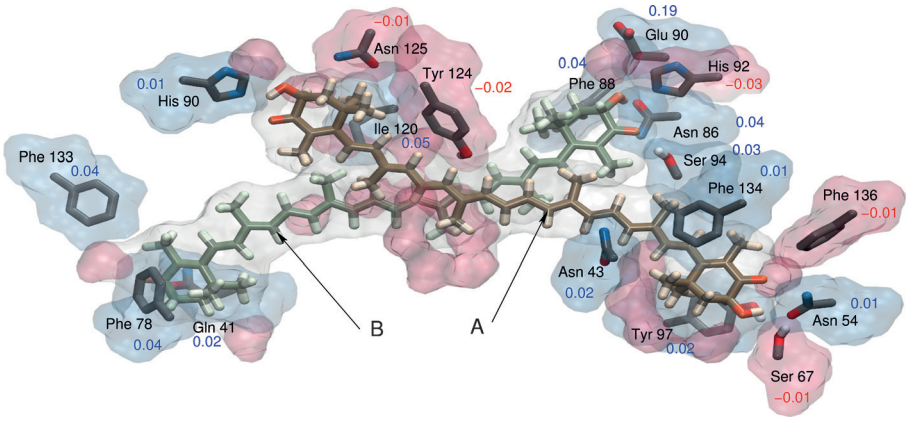

Figure 3. Individual red-shifting (red) and blue-shifting (blue) amino acid contributions to the VEEs of AXTA and AXTB.

Experimental Section

A QM/MM model of β-CR was built based on PDB ID: 1GKA.[4a] The system was classically relaxed for 10 ns at $T = 310$ K with a 1 fs integration time step by using the CHARMM27 force field,[17] followed by QM/MM relaxation for 0.5 ps and geometry optimization. The QM region comprised the two chromophores ($N = 192$ atoms) at the B3LYP/def2-SVP level,[18] with the remaining system treated at the MM level. Vertical excitation energies were calculated at the CC2[19] and ADC(2)[9]/def2-TZVP[20] levels by using the RVS approach,[10a] with virtual orbitals more than 50 eV above the HOMO omitted from the correlation calculation. The calculations were performed by using CHARMM/Q-Chem,[21] NAMD2,[22] and TURBOMOLE v. 6.5.[23] VMD was used for visualization.[24] Details of the methods can be found in the Supporting Information.

Keywords: biochromophores · computational chemistry · crustacyanin · quantum chemsitry · spectral tuning

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