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Origin of the Absorption Maxima of the Photoactive Yellow Protein Resolved via Ab Initio Multiconfigurational Methods

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We discuss the role of the protein in controlling the absorption spectra of photoactive yellow protein (PYP), the archetype xanthopsin photoreceptor, using quantum mechanics/molecular mechanics (QM/MM) methods based on ab initio multireference perturbation theory, combined with molecular dynamics (MD) simulations. It is shown that in order to get results in agreement with the experimental data, it is necessary to use a model that allows for a proper relaxation of the whole system and treats the states involved in the electronic spectrum in a balanced way, avoiding biased results due to the effect of nonrepresentative electrostatic interactions on the chromophore.

The photoactive yellow protein (PYP) is a cytosolic globular blue-light photoreceptor belonging to the family of xanthopsins,[1] responsible for the negative phototaxis in some bacteria.[2,3] The chromophore of the protein, the *p*-hydroxycinnamoyl anion, undergoes a trans/cis isomerization of the C_7-C_8 bond when exposed to blue light (absorption maxima $\lambda^a_{max} = 446$ nm) (see Figure 1) and represents the analogue to the well-known cis/trans isomerization present in rhodopsin (Rh). Despite the fact that PYP has been the subject of both experimental[2,3] and theoretical works,[4–12] the role of the protein in the modulation of the absorption maximum remains an open question. Among the many existent studies, only few focused on the analysis of the electronic spectra of the PYP. Molina et al.[4] analyzed the chromophore using high-level ab initio MS-CASPT2//CASSCF techniques in vacuo, while Thompson et al.[5] used TDDFT and a reduced model system optimized using the PM3 and B3LYP levels. Yamada et al.[6] made use of QM/MM calculations based on HF-optimized structures and analyzed the effect of the protein in the isomerization process employing a limited CASSCF method to characterize the low-lying excited state. Groenhoff et al.[7] used QM/MM-based molecular dynamics to study the trans to cis isomerization process employing also a limited CASSCF. The same type of study has been recently carried out on a mutant of PYP in order to assess the role of Arg_{52}.[8] Heyne et al.[9] calculated the spectrum employing ZINDO semiempirical calculations on B3LYP-optimized structures for a reduced model of the protein, while Matsuura et al.[10] analyzed the electronic spectrum at the semiempirical INDO/S method on DFT/AM1-optimized structures using a QM/MM scheme including the full protein. Recently, the effect of the local protein environment was investigated by CC2 and EOM-CCSD calculations for a series of supermolecular complexes, which successively include the amino acids of the chromophore's immediate neighborhood.[11] In this case, the authors had to shift the computed values due to limitations in the employed protein model and quantum chemical method. Gromov et al.,[12] in a further extension of ref. 11, analyzed the electronic spectra of the PYP chromophore in its neutral and anionic forms and the intrinsic factors determining the isomerization of the chromophore. However, none of these works have addressed the problem of studying the effect of the protein on the electronic spectra using a more realistic model where the electrostatic field created by the whole protein is taken into account and the electronic structure method used is able to represent all of the states involved in the electronic spectrum in a balanced way. This is essential to avoid biased results due to the effect of nonrepresentative electrostatic interactions on the chromophore.

It has been shown that a brute force QM/MM scheme based on CASPT2//CASSCF (i.e., geometry optimizations at the CASSCF level and energy computations at the dynamic correlated CASPT2 level) calculations can reproduce the value of the excitation energy (ΔE) for the vertical (Franck−Condon) transitions of biological photoreceptors of different nature[13–15] with enough accuracy to disentangle the factors controlling the color tuning.[15] This protocol is well-known for its ability to describe, in a balanced and accurate way, the ground and excited states of different nature.[16] Moreover, it can appropriately describe regions of energy degeneracy[17] (e.g., conical intersections), which makes it a suitable choice for successfully

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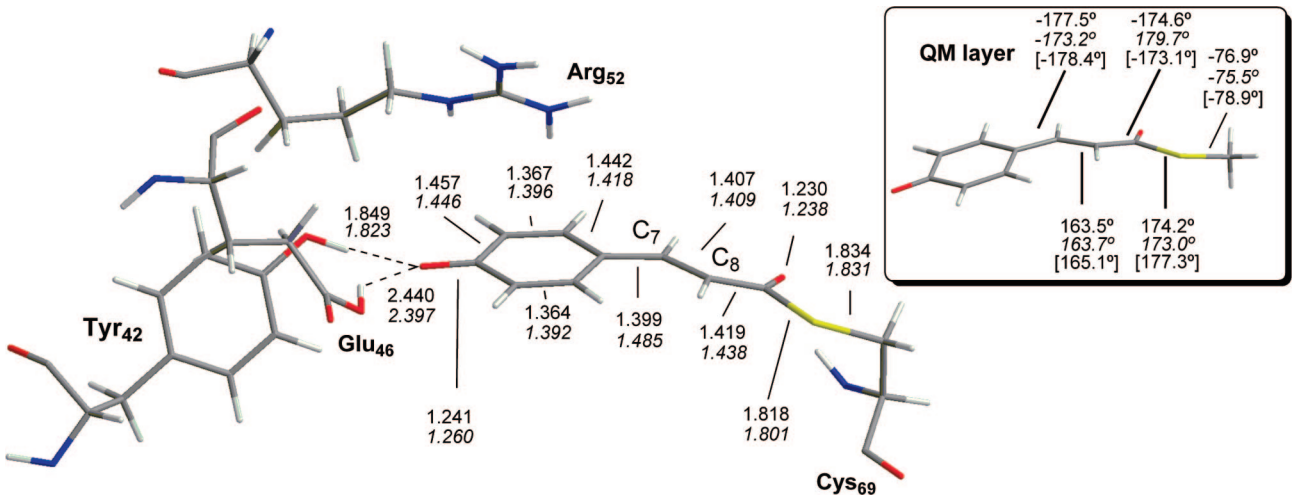

Figure 1. Optimized structure of the PYP chromophore in the ground state. Geometric parameters are compared with the X-ray-derived[22] ones (in brackets, see inset) and those of the in S_1 relaxed structure (in italics).

describing a photochemical reaction path.[18] Here, in order to build a suitable relaxed model for PYP, we address the following basic issues: (i) following previous studies on biological systems,[19–21] the photoreceptor, the protein, the solvent (water), and 6 Na^+ added to ensure a neutral system for a total of 50232 atoms have been built and relaxed using an approach combining QM/MM and MD methods starting from the PDB structure 1NWZ;[22] (ii) to compute the geometrical and electronic structure of PYP, the QM fragment comprising the chromophore has been described using ab initio CASPT2//CASSCF, and the rest of the protein has been treated at the MM level using the OPLS-AA[23] force field; (iii) to disentangle the effect of the protein matrix on the excitation energy, we have computed the change in ΔE values for different reduced models as previously performed for rhodopsin (Rh).[15] Full details on the employed protocols can be found in Supporting Information.

Figure 1 shows the optimized structure obtained at the CASSCF/6-31+G(d) level for the QM subsystem. Glu_{46} and Tyr_{42} (forming a phenolate bonded H-bond network), Arg_{52} (the counterion), and the chromophore bonded Cys_{69} MM residues are also shown. The relevant X-ray torsional parameters are given in the inset. The results point out that the optimized geometry of the PYP chromophore has a quinonic-like character, consistent with a (Mulliken) negative charge distribution in the phenoxy ring and in the thioester fragments of −0.46 and −0.54 au, respectively. This charge distribution is consistent with an electronic structure different from that proposed in previous theoretical/computational[11,12] and experimental works.[24,25] While the lack of agreement with previous computations can be attributed to the different models and electronic structure methods used,[11,12] the origin of the discrepancy with the experimental spectroscopic data[24,25] can be understood in terms of the time scale of the measurements, which appears to be much longer than the time required for the relaxation of PYP out of the Franck−Condon (FC) region. We have carried out a relaxation of the PYP chromophore in the S_1 excited state, showing that at the relaxed structure (0.26 eV more stable than the FC point at the CASSCF level), there has been a charge transfer of −0.13 au from the phenoxy ring to the thioester side, in qualitative agreement with the experimental data (see Supporting Information).

The computed torsion angles are found to be consistent with those derived from the experimental (X-ray crystallographic) structure. To assess the quality of the constructed model, the values of ΔE of the four low-lying singlet electronic states of PYP are compared with the experimental[26] λ^a_{max} (see Table 1).

TABLE 1: Calculated (MS-CASPT2//CASSCF) ΔE (eV), Oscillator Strengths (f), and Charge Transfer (Δq) and Observed Values of the Maximum of Absorption (eV) and Extinction Coefficients ε[26]

	calculated			observed	
state	ΔE	f	Δq^a	ΔE	ε^b
S_1	2.55	1.080	0.108	2.78	45.5
S_2	3.43	<0.001	−0.144		
S_3	4.05	0.002	0.117	3.90[c]	0.6
S_4	4.20	0.001	0.396	4.04	0.2

[a] Calculated as the reduction or increase in negative charge (using Mulliken population analysis) of the thioester fragment upon vertical excitation. The frontier is placed at the isomerizing double bond (see Figure 1 and Supporting Information). [b] Units: ε (10^3 M^{-1} cm^{-1}). [c] Experimentally assigned to a vibrational progression of the 4.04 band.[26]

It is apparent that the calculated excitation energy values compare well with the experimental data with an error for the absorbing state lower than 0.23 eV. The oscillator strengths follow the trend of the observed extinction coefficients. The absorbing state (S_1) is a $\pi \rightarrow \pi^*$ one. The second singlet low-lying excited state (S_2) is a very weak $n \rightarrow \pi^*$ absorbing state, where the lone pair involved corresponds to the phenoxy oxygen and is not observed experimentally. The third and fourth singlet low-lying excited states (S_3 and S_4) are both weak absorbing states. S_3 is essentially a $\pi \rightarrow \pi^*$ state, while S_4 is an $n \rightarrow \pi^*$ state, where the lone pair corresponds to that of the oxygen of the thioester group. Borucki et al.,[26] on the basis of absorption and circular dicroism spectra, have assigned two bands at 307 and 318 nm to an $n \rightarrow \pi^*$ transition, with the latter band being a vibrational progression. Since we have not simulated the CD spectrum, we cannot theoretically elucidate at present whether the main contribution to the 307 and 318 nm observed bands corresponds to one electronic transition with a well-defined vibrational progression or two electronic transitions. These results also differ from the ones reported in refs. 11 and 12 on the basis of a reduced PYP model. Unfortunately the reduced nature of the model and the different quantum chemical treatment prevent a direct comparison with our results.

In order to clarify the role of the protein environment in the modulation of the absorption maximum (i.e. the colour tuning)

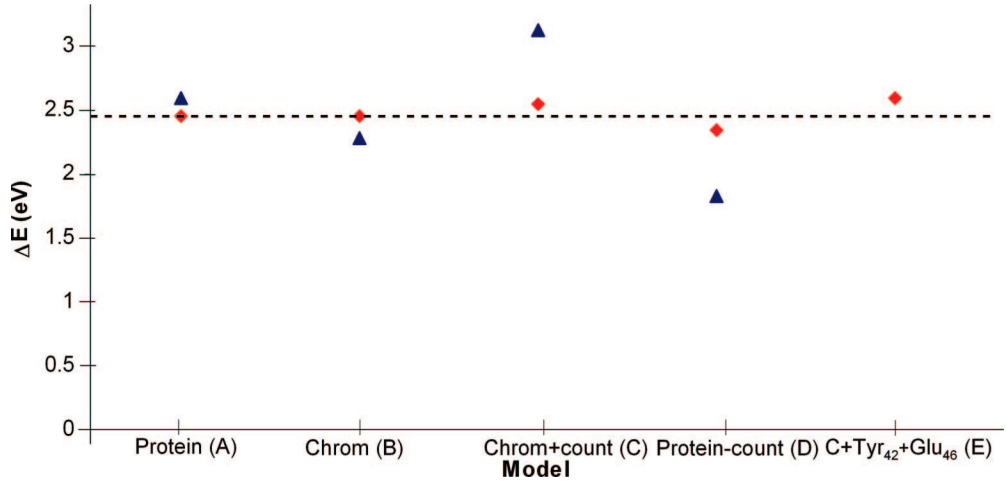

Figure 2. Calculated values, at the CASPT2//CASSCF computing level, of ΔE for the absorbing state (S_1) in PYP (◆) and Rh (▲).[15] Five systems are depicted: Protein (A), bare chromophore (B), chromophore and counterion (C), protein without counterion (D), and C+Tyr_{42}+Glu_{46} (E).

we use the same analysis carried out on Rh[15] and, partially, by Gromov et al.[11] (with reduced models constructed on the basis of X-ray PYP structural parameters). Five model systems are analyzed: (A) "Protein" that indicates the complete PYP model, (B) "Chrom", the bare chromophore, (C) "Chrom+count", that comprises the chromophore and counterion (Arg_{52}) pair, (D) "Protein−count", the complete protein (model A) without the Arg_{52} counterion and, (E) "C+Tyr_{42}+Glu_{46}" that corresponds to the "Chrom+count" model C augmented with the Tyr_{42} and Glu_{46} residues. In Figure 2, we report the values of ΔE for the absorbing state of PYP and Rh for the same four models.

An analysis of the results reveals that the bare chromophore (model B) and the PYP (model A) have a similar ΔE (2.46 and 2.45 eV, respectively). These results are in line with the semiempirical values obtained by Matsuura et al.[10] (2.73 and 2.82 eV, respectively), where the whole protein is included in the QM/MM model, but differ from those of Gromov et al.[11] (2.89 and 3.25 eV (model VII), respectively), which employed reduced PYP models. Model C displays a small blue-shifting effect with respect to model B. The lowest-lying excited state obtained for this model differs from the one reported in ref. 11. The latter shows local contributions of Arg_{52}, which is not described at the QM level in this work. Our result is nevertheless consistent with the fourth low-lying $\pi \rightarrow \pi^*$ excited state reported by Gromov et al.[11] (2.53 and 2.97 (model I), respectively). In contrast to the behavior observed for model C, model D displays a slight red shift. This effect is similar to the one found in Rh, but to a minor extent, pointing out the fact that Arg_{52} plays a minor role in the color tuning.[27] This contrasts with the shift observed in Rh, where the charge distribution of the chromophore is much more localized and charge translocation is more important upon excitation, which, in turn, makes the electrostatic effect of the counterion larger. Interestingly, the small effects caused by the Arg_{52} are appropriately counterbalanced by the rest of the protein, and the resulting S_0-S_1 gap is essentially the consequence of the geometry of the chromophore induced by the protein as if it was in vacuo[13] with a fine-tuning carried out by the protein. This has been previously proposed on the basis of the absorption spectra of models of the PYP chromophore using an electrostatic ion storage ring (ELISA).[28] Inclusion in model C of Tyr_{42} and Glu_{46} residues leads to a more significant blue shift on the S_1 absorbing state (2.60 eV; see Supporting Information) and destabilizes the S_2 state in line with results reported in ref. 11. Moreover, it increases the charge-transfer character of S_1 and its dipole moment, providing a measure of the sensitivity of the chromophore to the molecular environment (see Supporting Information). This effect is counterbalanced by the rest of the protein. This is an indication that in order to represent the effect of the protein on the sensitivity of the chromophore one needs to take into account the whole protein. The fact that reduced models provide ΔE values close to the experimental data cannot be considered satisfactory. In fact, the ultimate target of the construction of our QM/MM PYP model is the simulation of the photochemical reactivity of this photoreceptor. It is in the field created by the full protein (through electrostatic and van der Waals interactions) that the chromophore displays a slightly twisted structure with a partly broken C_7-C_8 double bond (1.407 Å). This suggests that the protein facilitates the trans/cis isomerization.

In conclusion, the role of the protein environment in the electronic spectrum of the PYP chromophore has been investigated, highlighting the need to use a properly equilibrated model. We show that one can reproduce the spectrum of the PYP within 0.25 eV using QM/MM calculations based on high-level ab initio multiconfigurational methods and a suitable receptor model constructed using MD simulations. These results pave the way for a realistic description of the photochemical reaction path.

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Supporting Information Available: Full details of the model building, methodology used, absolute energies, dipole moments, and Cartesian coordinates of the optimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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