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Download date: 22 Dec 2018
Molecular beam and *ab-initio* studies of Photoactive Yellow Protein chromophores: the influence of the thioester functionality and single bond rotation

We report on experimental high-resolution spectroscopic studies in combination with *ab-initio* computational studies that investigate the excited-state dynamics of methyl-4-hydroxycinnamate thioester and 5-hydroxy indan-(1E)-ylidene)acetic acid, derivatives of the Photoactive Yellow Protein (PYP) chromophore. These studies aim to elucidate (a) how the thioester moiety influences the photophysics and photochemistry of the *para*-coumaric acid chromophore, and (b) to what extent rotation of the single bond adjacent to the phenyl ring is involved in the decay dynamics of the electronically excited states. The experimental studies show that sulfur substitution leads to broad, unstructured excitation spectra that contrast sharply with the well-resolved spectra of compounds with an oxygen-based ester. Furthermore, internal conversion to the lower-lying $n\pi^*$ state is absent. The absence of this decay channel is rationalized by quantumchemical calculations that reveal that in the $n\pi^*$ state of the thio compounds the molecule exhibits a large out-of-plane ‘kink’ at the sulfur atom. Franck-Condon simulations of the excitation spectra of the $V(\pi\pi^*)$ state highlight the activity of various vibrational modes in the neutral chromophore and indicate that upon sulfur substitution internal conversion to the ground state occurs at a significantly higher rate. The similarities observed in the excitation spectra and decay dynamics of the locked and unlocked compounds suggest that in the present experiments single-bond torsion does not show up prominently. The conclusion that for the isolated molecule double-bond torsion is dominating the excited-state dynamics is tentatively confirmed by the quantumchemical calculations.

5.1 Introduction

The Photoactive Yellow Protein (PYP)\textsuperscript{1} is a small water-soluble photoreceptor that serves under the category of the Per-Arnt-Sim (PAS) sensory domain\textsuperscript{2} and that was originally isolated from the purple halophilic bacterium \textit{Ectothiorhodospira halophila}.\textsuperscript{1,3} Its chromophore is the deprotonated form of trans-4-hydroxycinnamic acid (\textit{para}-coumaric acid) covalently linked to the Cys69 residue via a thioester linkage and held in place by extensive hydrogen bonding interactions. The free-swimming bacterium \textit{E. halophila} exhibits a negative phototactic response towards blue light\textsuperscript{4} coinciding with the absorption spectrum of PYP which has an absorbance maximum $\lambda_{\text{max}}$ at 446nm.\textsuperscript{1,5} Upon photoexcitation, PYP undergoes a photocycle characterized by a series of intermediates with lifetimes that range from several hundreds of femtoseconds\textsuperscript{6,7} to seconds. Numerous experimental\textsuperscript{7–15} and computational studies\textsuperscript{16–21} of PYP and its chromophore have elucidated the various steps of the photocycle of the protein which have been identified to involve \textit{trans-cis} isomerization of the C$_7$=C$_8$ bond (see Figure 5.3 for numbering of the atoms), protonation of the chromophore, partial unfolding of the protein, and exposure of the binding pocket of the chromophore.

A fundamental understanding of the photophysics and photochemistry of PYP requires detailed knowledge of the intrinsic properties of the lower electronically excited states of its chromophore, and how these properties are modulated by factors such as deprotonation and embedding within the protein. Theoretical calculations have made clear that the relevant electronically excited states involve two $\pi\pi^*$ states and one $n\pi^*$ state.$^{17,19}$ Figure 5.1 displays the contour plots of the relevant molecular orbitals for OM$p$CA and TM$p$CA. The two $\pi\pi^*$ states are in first approximation described by the HOMO $\rightarrow$ LUMO and HOMO $\rightarrow$ LUMO+1 excitations and are designated as the V($\pi\pi^*$) and V'($\pi\pi^*$) states, respectively, while the n$\pi^*$ state arises from the n $\rightarrow$ LUMO excitation. Importantly, these calculations find that the ordering of these states is highly sensitive to whether vertical or adiabatic excitation is considered, and to the finer details of the chemical composition of the chromophore.

For vertical excitation of \textit{para}-coumaric acid (\textit{pCA}) and its methylester, methyl-4-hydroxycinnamate (designated in the following as OM$p$CA) $S_1$, $S_2$, and $S_3$ are associated with the V'($\pi\pi^*$), V($\pi\pi^*$), and n$\pi^*$ state, respectively, but adiabatically the n$\pi^*$ state is the state of lowest energy. In \textit{para}-coumaric thio acid, on the other hand, the n$\pi^*$ state is both vertically and adiabatically the lowest excited state, while the order of the V'($\pi\pi^*$) and V($\pi\pi^*$) states is reversed. Finally, in the anionic form of the thio compounds the lowest excited state is the V($\pi\pi^*$) state that moreover is effectively isolated from the other two states in the sense that it has a much lower excitation energy.
Figure 5.1. Relevant molecular orbitals for the description of electronically excited states of OMpCA and TMpCA.

Experimental high-resolution spectroscopic studies on pCA and OMpCA have contributed considerably to the elucidation of the properties and dynamics of these electronically excited states.\textsuperscript{22–25} One aspect, however, that—from an experimental point of view—has remained elusive in these studies is the role of the thioester linkage. In the present study we therefore combine an experimental and theoretical approach to investigate the electronic manifold of methyl-4-hydroxycinnamate thioester (TMpCA), focusing in particular on the changes in spatial structure upon excitation and how the energy that is put into the system by photon absorption is ultimately dissipated into heat.

The photoisomerization process of the PYP chromophore has been subject of extensive experimental and theoretical studies. There is nevertheless even nowadays still considerable debate on quite fundamental aspects such as which torsional pathways are involved in the isomerization event, what their relative contributions are, and how these contributions depend on environmental conditions. Primary torsional pathways in this respect concern the torsion around the C\textsubscript{7}=C\textsubscript{8} double bond conjugated with the aromatic ring (denoted as $\beta$-torsion) and torsion around the C\textsubscript{4}-C\textsubscript{7} single bond directly connected to the aromatic ring (denoted as $\alpha$-torsion).\textsuperscript{16,19,26} Experimental\textsuperscript{27} and theoretical studies\textsuperscript{26,28} have suggested that both the single bond and double bond are coupled at small excursions of the double bond twist and result in a concerted twist around these bonds leading to isomerization. At the same time, it
would appear from experimental work on chromophores in which torsion of the single bond is restricted\(^\text{27,29}\) that the role of single bond rotation is not as extensive as that it can serve as an alternative to \(\text{C}_7=\text{C}_8\) rotation since the double bond rotation remains the main deactivation channel. PYP derivatives reconstituted with such rotation-locked chromophores do show, however, a significant decrease of up to \(\sim 60\%\) in isomerization yield.\(^\text{27}\) It is clear that our understanding of the role of the \(\alpha\)-torsion would benefit greatly from studies on the excited-state dynamics of isolated chromophores. Such studies provide the intrinsic properties of the chromophores which in the protein environment then become modulated by interactions with neighboring residues. Moreover, they are an ideal starting point for benchmarking quantumchemical calculations. Apart from experimental and theoretical studies of TM\(p\)CA, we have therefore performed the same studies as well for a version of TM\(p\)CA in which the \(\text{C}_4-\text{C}_7\) single bond is locked with an ethane bridge, designated in the following as RL-TM\(p\)CA.\(^\text{30}\)

### 5.2 Experimental and theoretical details

In our studies two-color \((1+1')\) Resonance Enhanced Two-Photon Ionization (RE2PI) spectroscopy has been used to record excitation spectra of supersonically cooled molecules. The employed molecular beam setup and laser systems have been described in detail before\(^\text{25}\) and will therefore only be described insofar as their specific details for the present experiments. Electronic excitation was performed with a dye laser (Sirah Precision Scan) pumped by a 30Hz Nd:YAG laser (Spectra Physics Lab 190). In order to scan the 27700 - 33000 cm\(^{-1}\) frequency region, the dyes DCM and LDS698 were used and the output of the dye laser frequency-doubled by a KDP crystal. Typical excitation energies were between 2-3 mJ per pulse. For ionization from the excited state an argon fluoride (ArF) excimer laser (Neweks PSX-501) with pulse energies of up to 5 mJ was used.

The thiomethyl ester of para-coumaric acid (TM\(p\)CA) and of 5-hydroxy indan-(1E)-ylidene)acetic acid (RL-TM\(p\)CA) were synthesized according to the procedures described previously.\(^\text{27,31}\) For the experiments TM\(p\)CA and RL-TM\(p\)CA samples were heated up to 185°C to generate sufficient vapor pressure and seeded into a supersonic beam of Neon generated by expanding 2 bar of Neon into a vacuum.

Vertical and adiabatic excitation energies were computed using the spin-component scaled version of the approximate coupled-cluster singles-and-doubles model (SCS-CC2). These calculations were performed with the resolution-of-the-identity approximation (RI-CC2) module as implemented in the TURBOMOLE package.\(^\text{32}\) For both geometry optimization and vertical energy calculations the Dunning’s correlation consistent polarized double zeta augmented with the standard auxiliary basis set (aug-cc-pVDZ) was employed. By employing the harmonic approximation with consideration of Duschinsky mixing, Franck-Condon intensities
for vibronic spectra of the TMPpCA and RL-TMPpCA were calculated. To this purpose, ground state and excited state geometries optimizations and normal mode calculations were performed. For the ground state Density Functional Theory (DFT) using the B3LYP hybrid functional and the cc-pVDZ basis set was utilized whereas for the excited state Time Dependent Density Functional Theory (TD-DFT) with the same functional and basis set were employed. These calculations were performed with the Gaussian09 suite of programs. In order to compare with the experimental spectra, a scaling factor of 0.970 was used for the calculated frequencies. To validate the computational results, we have also used the Coulomb-attenuating method with the long-range corrected version of B3LYP (CAM-B3LYP) using the same cc-pVDZ basis set for the ground and excited state calculations as well as for predictions of intensities in vibronic spectra.

5.3 Results and discussion

5.3.1 UV excitation spectra of OMpCA and TMpCA

Figure 5.2 displays the (1+1’) RE2PI excitation spectra of OMpCA and TMpCA in the 290-330 nm region. Previous studies have shown that in the case of OMpCA the initial part of this spectrum should be assigned to the V’(ππ*) state while transitions to the V(ππ*) state start to occur around 34500 cm\(^{-1}\).\(^{22,24,25}\) The spectrum is nicely vibrationally resolved, showing bands with a line width between 2.5-4.8 cm\(^{-1}\) in the region close to the origin transition to the V’(ππ*) state. The excitation spectrum of TMpCA (Figure 5.2b), on the other hand, displays only a broad band system with hardly any indication of vibrational-resolved features.\(^{35}\) Quantumchemical calculations indicate that for this compound the initial part is associated with excitation of the V(ππ*) state.\(^{17}\) In the spectrum of TMpCA we find the onset of absorption in the 29700-30250 cm\(^{-1}\) region, implying a red shift by ~3000 cm\(^{-1}\) with respect to the 0-0 transition to the V’(ππ*) state of OMpCA. Table 5.1 shows that this adiabatic energy difference is in good agreement with the difference in vertical excitation energies calculated for the V’(ππ*) state of OMpCA and the V(ππ*) state of TMpCA (~0.3eV).
Previously, we have shown that the excited-state dynamics of OMpCA involve two decay channels.\textsuperscript{25} Excitation of the “bright” $\pi\pi^*$ state is followed by fast internal conversion to a “dark” state (\textit{i.e.}, a state that cannot be observed directly in RE2PI excitation spectra) which was identified as the $n\pi^*$ state. This internal conversion process occurs on a ps timescale as deduced from the line widths of bands in the excitation spectrum and confirmed by time-resolved RE2PI experiments.\textsuperscript{36} The $n\pi^*$ state, in turn, decays to the ground state with a time constant of tens of nanoseconds as was shown by (1+1’) pump-probe RE2PI experiments.

<table>
<thead>
<tr>
<th></th>
<th>OMpCA</th>
<th>TMPCA</th>
<th>RL-TMPCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>V’($\pi\pi^*$)</td>
<td>4.599 (0.221)</td>
<td>4.513 (0.290)</td>
<td>4.536 (0.381)</td>
</tr>
<tr>
<td>V($\pi\pi^*$)</td>
<td>4.819 (0.627)</td>
<td>4.290 (0.674)</td>
<td>4.133 (0.559)</td>
</tr>
<tr>
<td>n$\pi^*$</td>
<td>5.203 (9 x $10^{-5}$)</td>
<td>4.118 (9.9 x $10^{-5}$)</td>
<td>4.134 (0.032)</td>
</tr>
</tbody>
</table>

\textbf{Table 5.1.} The vertical excitation energies (eV) and oscillator strengths (in parentheses) of the three lower electronic states of OMpCA, TMPCA and RL-TMPCA at the SCS-CC2/aug-cc-pVDZ level.
For TMpCA completely different excited-state dynamics are observed. Firstly, the absence of vibrational resolution in the RE2PI excitation spectrum suggests considerable lifetime broadening of the vibronic levels of the “bright” state. Such a lifetime broadening is surprising as time-resolved studies of neutral TMpCA in solution find an excited-state lifetime of ~4 ps, which would translate into homogeneous line widths of ~1.4 cm⁻¹. The apparent contradiction between the two experiments might be partly reconciled by extensive Franck-Condon activity of low-frequency modes in the gas-phase experiment. However, calculations of the harmonic force field in the ground state indicate that in this state the lowest-frequency mode has a frequency of about 20 cm⁻¹. Assuming that in the excited-state the lowest-frequency mode has a similar frequency, one would still need to conclude that the decay rate of the “bright” state is considerably larger in the gas phase than in solution in order to account for the absence of vibrational resolution. Secondly, time-resolved (1+1’) RE2PI experiments merely show the convolution of the time profiles of the excitation and ionization laser pulses, and thus give no indication for the presence of a longer-living “dark” state. In our experiments the “hot” ground state resulting from internal conversion from an electronically excited state is the only state that cannot be ionized efficiently by one-photon absorption at 193 nm. We therefore conclude that in TMpCA the direct non-radiative decay channel from the “bright” excited state to the ground state dominates the excited-state dynamics. This conclusion is further supported by the RE2PI signal which is orders of magnitude smaller in TMpCA than in OMPpCA.

In order to elucidate the principal reasons for the differences between the excitation spectra of OMPpCA and TMpCA and their excited-state dynamics, it is clear that input is required from quantumchemical calculations. This pertains in particular to the equilibrium geometry of the molecules in the relevant excited states and their harmonic force fields, as well as the associated adiabatic excitation energies as will be discussed in the next section.

5.3.2 Equilibrium characteristics of nπ* and ππ* states of OMPpCA and TMpCA

The substitution of the oxygen atom in the ester linkage by a sulfur atom has the most pronounced influence on the energy of the nonbonding n orbital. As a result, the nπ* state, which is the third vertical excited state in OMPpCA, becomes the lowest state in TMpCA.¹⁷ As yet excited-state geometry optimizations have mainly been performed for the parent compounds p-coumaric acid (pCA) and p-coumaric thio acid (pCTA), respectively, but we notice that structural relaxation reported in¹⁷ has been restricted to in-plane coordinates. Possible out-of-plane geometry changes were not considered. For OMPpCA we find that the molecule behaves in many aspects very similar to pCA. Optimization of the geometry of the nπ* as well as the V’(ππ*) and
V(ππ*) states of OMPCA at the SCS-CC2 level leads to a planar geometry, analogous to the equilibrium geometry of the molecule in the ground state. At its equilibrium geometry the energy of the nπ* state is lowered by 1.001 eV compared to the vertical energy of 5.203 eV, while the energy of the optimized V'(ππ*) state is only lowered by 0.251 eV from its vertical excitation energy. The structure shows that in the nπ* state a significant elongation of the carbonyl C9=O2 double bond (0.189 Å) occurs, resulting in a bond that has considerably more single bond character. Adjacent to the carbonyl moiety, the C9-C9 single bond is contracted and acquires more double bond character. Similar observations have been made in studies on pCA17 and cytosine37 where the geometry changes were explained in terms of “uncoupling” and “recoupling” of the carbonyl π bond to the neighboring π system.

![Figure 5.3. Equilibrium geometry of the nπ* state of TMpCA at the SCS-CC2 level.](image)

In contrast, geometry optimization at the SCS-CC2 level of the nπ* state of TMpCA leads to a structure in which the thioester methyl group is no longer in the plane of the pCTA chromophore, but has undergone a large out-of-plane distortion with an O2=C9=S-C10 dihedral angle of 78.2°. Concurrently with this out-of-plane distortion we find that the bond angles around the C9 atom are changed considerably: the C8-C9-S angle increases by 15.3° while the S-C9=O2 angle decreases by 21.2°. As will be described below, geometry optimizations of the ππ* states at the SCS-CC2 level are hindered by convergence problems. For the ππ* states geometry optimizations have therefore also been performed at the TD-DFT level. In order to be able to compare the CC2 and DFT calculations, the equilibrium geometry of the nπ* state has been determined at the TD-DFT/CAM-B3LYP level as well. The results of these calculations are compatible with the SCS-CC2 results.

The ππ* states are affected by the change in heteroatom as well. In OMPCA the V'(ππ*) state is the lowest ππ* state, both vertically as well as adiabatically. Importantly, the SCS-CC2 calculations indicate that the adiabatic excitation energy of the V'(ππ*) state is only slightly (0.146 eV) above the adiabatic excitation energy of the nπ* state.25 Table 5.1 shows that in TMpCA the order of the two ππ* states for vertical excitation is reversed -the V(ππ*) state becoming lower than the V'(ππ*) state by 0.223 eV- and that the excitation energy of the V(ππ*) state is lowered by 0.309 eV compared to OMPCA. Previously, this shift has been explained in terms of
the stronger electron affinity of the \(-\text{(C=O)-S-CH}_3\) end group compared to the end group \(-\text{(C=O)-O-CH}_3\).\(^{19}\)

In-plane geometry optimization of the \(V(\pi\pi^*)\) state of \(\text{TMpCA}\) at the SCS-CC2 level rapidly converges. However, calculation of the harmonic force field shows that this structure is not a local minimum as it features one imaginary frequency. Similar observations are made when the calculations are performed at the TD-DFT/CAM-B3LYP level. Several attempts have been made to obtain a non-planar unconstrained equilibrium geometry of the \(V(\pi\pi^*)\) state but all failed, primarily because of configurational mixing between the \(n\pi^*\) and \(V(\pi\pi^*)\) states.

![Image](image.png)

**Figure 5.4.** Equilibrium geometry of the \(V(\pi\pi^*)\) state of \(\text{TMpCA}\) at the TD-DFT/B3LYP level.

Previously, we have reported on TD-DFT/B3LYP calculations on \(\text{OMpCA}\).\(^{22}\) From these studies it was concluded that calculations at this level of theory reproduce well the vibrational activity observed in the experimental excitation spectra of the \(V'(\pi\pi^*)\)\(^{22}\) and \(V(\pi\pi^*)\)\(^{25}\) states, even though the energetic ordering of the two states is not predicted correctly. In view of these results, we have therefore reverted for \(\text{TMpCA}\) to calculations at the TD-DFT/B3LYP/cc-pVDZ level. At this level convergence to the equilibrium geometry of the \(V(\pi\pi^*)\) state is easily reached, calculations of the harmonic force field showing that this geometry is indeed a local minimum on the potential energy surface. In agreement with \textit{a priori} expectations based on the SCS-CC2 calculations, the relaxed geometry is found to be non-planar (Figure 5.4). The \(\text{C}_9\text{-S}\) bond is twisted out of plane by \(6.6^\circ\) while the \(\text{O}_2=\text{C}_9\text{-S-}\text{C}_{10}\) dihedral angle is about \(16.1^\circ\). It is gratifying to notice that these results are in good agreement with calculations at the ss-CASSCF level where also an out-of-plane twisted \(\text{C}_9\text{-S}\) bond was obtained.\(^{38}\)

### 5.3.3 Influence of sulfur substitution on spectroscopy and excited-state dynamics

The previous section has shown that replacing the methylester by a thiomethylester has quite a significant influence on the excited-state manifold. In the following we will discuss the experimental observations in the light of these changes. To this purpose we consider first the UV/Vis absorption spectrum of \(\text{TMpCA}\) in n-hexane, which is shown in Figure 5.5a. Simulation of this spectrum using the
calculated vertical excitation energies and oscillator strengths of excited states in vacuo at the SCS-CC2 level leads to an excellent agreement with the experimentally obtained spectrum. At the same time, the simulation indicates that for higher excitation energies the (1+1') RE2PI excitation spectrum displayed in Figure 5.5c very likely contains contributions from transitions to S₂ as well. In fact, the energy difference between the maxima of the two band systems of \(-1250 \text{ cm}^{-1}\) reproduces quite nicely the value of 0.16 eV predicted by the most accurate calculations reported so far and is also in good agreement with the results reported in Table 5.1.

![Figure 5.5.](image)

Figure 5.5. Experimental and theoretically predicted UV/Vis absorption and excitation spectra of TempCA. (a) UV/Vis absorption spectrum in n-hexane. (b) Simulated UV/Vis spectrum at SCS-CC2 level. The solid spectrum has been generated from the stick spectrum by convolution with a Gaussian envelope with a width of 20 nm. (c) Experimental (1+1') RE2PI excitation spectrum. (d) Franck-Condon predicted stick spectrum of the S₁(V(ππ*)) ← S₀ transition.

Nevertheless, the calculations make clear that certainly the initial part of the spectrum arises from Franck-Condon activity in the S₁(V(ππ*)) ← S₀ excitation spectrum. It is therefore instructive to see to what extent the broadness of the spectrum can be explained by Franck-Condon activity. Figure 5.5d displays the simulated Franck-Condon stick spectrum of this transition employing the TD-DFT/B3LYP equilibrium geometries and harmonic force fields of the ground and V(ππ*) states. Importantly, we find that the range of excitation energies for which vibronic activity is predicted is in good agreement with the experimental spectrum, both with respect to the width of the “band” at \(-31500 \text{ cm}^{-1}\) as well as the appearance of the spectrum at higher energies.
Till recently it was assumed for the anionic chromophore that the photoisomerization pathway involved predominantly single- and double bond torsional coordinates. Recent quantumchemical calculations on the V(ππ*) state of the anion of TMpCA hydrogen bonded with two water molecules have shown, however, that an hydrogen out-of-plane (HOOP) coordinate associated with displacements of the H7 and H8 hydrogen atoms is closely coupled to these torsional coordinates. Interestingly, we find in our calculations on the neutral chromophore that a large part of the vibronic activity in the S1(V(ππ*)) ← S0 excitation spectrum derives from normal modes that have significant HOOP character. This is important as it indicates that excitation to the V(ππ*) state intrinsically already “activates” the HOOP coordinate. In a similar vein we notice that it has been suggested in the past that isomerization might involve concerted motions of different bonds. In the solid state such motions, in the form of the bicycle pedal mode of isomerization, have indeed been observed recently using picosecond X-ray crystallography. The present calculations predict significant activity of low-frequency modes at 34 and 68 cm\(^{-1}\) and their combinations that involve such motions. It would thus appear that by excitation to the V(ππ*) state the molecule is in principle also “activated” towards concerted motions.

One of the key differences between the excited-state dynamics observed for OMPCA and TMpCA concerns the internal conversion of the “bright” state to the nπ* state, which is one of the dominant pathways for OMPCA but cannot be detected for TMpCA. On the basis of the present calculations this difference can now be well understood. The internal conversion rate between the two states is determined by their energy difference and the matrix element that couples the two states. As will be shown, OMPCA has in both aspects the more favorable characteristics.

Our and previous calculations show that in OMPCA the nπ* state is S3 for vertical excitation while adiabatically it becomes S1. From this it can be inferred that the nπ* and ππ* states exhibit a conical intersection although it is not known how far above the energy of the nπ* minimum such conical intersections are located. In TMpCA, on the other hand, the nπ* state is S1 both for vertical as well as adiabatic excitation. While it may still occur, a conical intersection with the ππ* states is not directly evident and, if present, can be assumed to occur at higher energies than in OMPCA. Also from a more simple approach in which only adiabatic excitation energies are considered, OMPCA is favored as the energy difference between the relaxed V'(ππ*) and nπ* states in OMPCA is much larger than the energy difference between the relaxed V(ππ*) and nπ* states in TMpCA. The coupling matrix element between the two states contains an electronic and a vibrational factor. With respect to the electronic factor not so much can be said regarding possible differences between OMPCA and TMpCA. However, the vibrational factor clearly favors OMPCA over TMpCA. For the former, our calculations find that the equilibrium geometries of the two states are both planar. For
the latter, on the other hand, the $n\pi^*$ state is significantly distorted from planarity compared to the $\pi\pi^*$ state. At a given excitation energy the overlap of the vibrational wavefunctions of the two states is therefore significantly smaller for TMpCA than for OMpCA.

5.3.4 Influence of single-bond locking on spectroscopy and excited-state dynamics

The (1+1’) RE2PI spectrum of RL-TMpCA is shown in Figure 5.6c. Similar to TMpCA it displays a broad spectrum without any clear vibrational features. The onset of absorption occurs around $\sim 29000 \text{ cm}^{-1}$, some 1000 cm$^{-1}$ lower than in TMpCA. This shift is in good agreement with the UV/Vis absorption spectra of the two compounds in n-hexane (Figures 5.5a and 5.6a) that show a 15 nm red-shifted spectrum for RL-TMpCA. Moreover, the calculations reported in Table 5.1 predict a red-shift of the same magnitude. Finally, we notice that similar red shifts have been observed for the single-bond locked analogues of PYP chromophores with an oxygen ester. Figure 5.6b displays a simulation of the solution spectrum of RL-TMpCA using excitation energies and oscillator strengths reported in part in Table 5.1. Good agreement between experiment and theory is observed, but at the same time one has to conclude that for higher excitation energies -similar to TMpCA- transitions to a different electronic state come into play in the (1+1’) RE2PI excitation spectrum.

![Figure 5.6](image.png)

**Figure 5.6.** Experimental and theoretically predicted UV/Vis absorption and excitation spectra of RL-TMpCA (a) UV/Vis absorption spectrum in n-hexane. (b) Simulated UV/Vis spectrum at SCS-CC2 level. The solid spectrum has been generated from the stick spectrum by convolution with a Gaussian envelope with a width of 20 nm. (c) Experimental (1+1’)
RE2PI excitation spectrum (d) Franck-Condon predicted stick spectrum of the $S_1(V(\pi\pi^*)) \leftrightarrow S_0$ transition.

Figure 5.7. Equilibrium geometries of the (a) $n\pi^*$ state and (b) $V(\pi\pi^*)$ states of RL-TM$p$CA.

Quantumchemical calculations on the equilibrium geometries of the excited states of RL-TM$p$CA lead to similar conclusions as obtained for TM$p$CA, that is, the $n\pi^*$ state is significantly distorted from planarity while the $V(\pi\pi^*)$ state is only slightly non-planar (Figure 5.7). Figure 5.6d shows a simulation of the Franck-Condon activity in the $S_1(V(\pi\pi^*)) \leftrightarrow S_0$ RE2PI excitation spectrum depicted in Figure 5.6c using the TDDFT/B3LYP equilibrium geometries and harmonic force fields of $S_0$ and $S_1$. For TM$p$CA a large fraction of the vibronic activity in the $V(\pi\pi^*)$ excitation spectrum is carried by modes with HOOP character. In the rotation-locked version this activity has been distributed over several out-of-plane modes. The predicted spectrum matches the experimental spectrum up to the region of 30200 cm$^{-1}$ but for higher excitation energies the simulated spectrum decreases in intensity while the RE2PI spectrum still displays considerable absorption. The band with its maximum around $\sim 30700$ cm$^{-1}$ in the RE2PI spectrum is thus attributed to the transition to the $V'(\pi\pi^*)$ state.

With respect to the excited-state dynamics of the $V(\pi\pi^*)$ state a number of interesting observations can be made. Firstly, nanosecond time-resolved pump-probe experiments on RL-TM$p$CA do not give any indication that a long-living state is populated. Apparently, internal conversion to the $n\pi^*$ state is as inefficient for RL-TM$p$CA as it is for TM$p$CA. Such a conclusion is entirely in line with what one would expect on the basis of the considerations discussed above on conical intersections and the energy differences between excited states. Secondly, in both TM$p$CA and RL-TM$p$CA the (1+1') RE2PI excitation spectrum only shows broad features and lacks vibrational resolution, which strongly suggests that in both compounds the excited-state dynamics occur on similar time scales. This is to a certain extent surprising in view of the role of the single-bond torsional coordinate in the photoisomerization process of the anionic species, and the conclusion that the nonradiative deactivation channel in neutral locked compounds is linked to both single- and double-bond twisting. However, the present studies indicate that locking the C$_4$-C$_7$ single bond does not impede the fast dynamics responsible for the lifetime broadening of bands. The RE2PI experiments demonstrate that these dynamics are
determined by internal conversion to the ground state, which in turn suggests that in both compounds the double-bond torsional coordinate is dominantly involved in this internal conversion pathway.

5.3.5 Potential energy surfaces along the double bond torsional coordinate

As yet, quantumchemical calculations on the photodynamics of the V(ππ*) state have almost exclusively focused on the anionic form of the chromophore.\textsuperscript{16,19,21,26,28,40–42} The present experimental studies indicate that for the neutral chromophore the excited-state dynamics are to a large extent determined by motion along the double-bond torsional coordinate. It is therefore of interest to obtain at least a qualitative idea on the topology of the potential energy surfaces of the ground and excited V(ππ*) state along this coordinate. To this purpose we take the B3LYP equilibrium geometry of the ground state as a starting point and generate from it structures along the torsional coordinate. The potential energy surfaces of ground and excited state are then determined by calculation of the TD-DFT/B3LYP energies of the two states at the various geometries. Since all other structural parameters are fixed to their ground-state equilibrium values, these potential energy surfaces are in many aspects only approximate. Similarly, we recognize that the TD-DFT approach is not valid in the neighborhood of near-degeneracies of electronic states. Nevertheless, the calculations should be able to indicate the salient features of the true multi-dimensional surfaces involving this torsional coordinate.

Figure 5.8a displays the potential energy surfaces of the ground and V(ππ*) states in TMpCA along the double-bond torsional coordinate. These surfaces reproduce to a large extent what has been observed before in high-level calculations on the anionic form. In the excited state a shallow energy well is observed for the trans conformer with a relatively small barrier of 0.17 eV along a path that subsequently leads to a crossing with the ground state. In order for photoisomerization to occur, TMpCA has to overcome this barrier which is associated with a twisted structure of some 40°. Furthermore, the V(ππ*) state minimum is located about 0.74eV above the energy of the S\textsubscript{1}/S\textsubscript{0} conical intersection, similar to the value calculated for the anionic form.\textsuperscript{16,19} The potential energy surfaces for RL-TMpCA are shown in Figure 5.8b. Interestingly, we find that for this compound the barrier along the torsional coordinate is slightly lower (0.10 eV) and that it occurs for a smaller value of the torsional angle (20°). Given the low level of theory and the restrictions on the explored conformational space, one should be cautious to give too much value to these differences, but tentatively the suggestion could be put forward that locking of the single-bond rotation enhances the internal conversion rate to the ground state. Such a conclusion would be in agreement with experimental studies on PYP reconstituted with this chromophore.\textsuperscript{27}
Figure 5.8. (a) Potential energy surfaces of the $S_0$ (solid line) and $V(\pi\pi^*)$ states (dotted line) of TM$p$CA and (b) RL-TM$p$CA as a function of the rotation angle around the C$_7$=C$_8$ bond.

5.4 Conclusions

The Photoactive Yellow Protein has become over the years a well-established model to study the conversion of photon energy into biological function. The chromophore which is responsible for light absorption and which initiates the pertaining photocycle is based on para-coumaric acid. This chromophore and its derivatives have therefore been subject of many experimental and theoretical studies. What has received much less attention is the fact that in the protein the chromophore is incorporated as a thioester. In the present study high-resolution spectroscopy and quantumchemical calculations have been employed to determine how such a seemingly small change affects the chromophore. Our results show that the replacement of a normal ester by a thioester has an enormous impact on its photophysics and photochemistry of the chromophore. From a spectroscopic point of view we have found that the well-resolved excitation spectra of the normal ester are replaced by broad featureless spectra, which indicates that the excited-state dynamics occur on a much faster time scale. Franck-Condon simulations have shown that the change in equilibrium geometry upon excitation to the $V(\pi\pi^*)$ state leads to
extensive vibronic activity, and that this might also contribute to the lack of vibrational resolution in the excitation spectra. Interestingly, these calculations also find a significant activity of modes involving HOOP coordinates, which recently have been concluded to be involved significantly in the photoisomerisation pathway of the anionic species. From the quantumchemical calculations it has been concluded that incorporation of the thioester functionality has a large effect on the structure of the molecule in the nπ* state, leading to a large out-of-plane twist of the S-CH3 group. One of the consequences of this large geometry change is that the internal conversion pathway from the “bright” state to the nπ* state is shut down as was demonstrated by pump-probe experiments. Rotation around the single bond adjacent to the phenyl ring is increasingly being considered as one of the coordinates that guides the chromophore along the isomerization pathway. Our studies on a derivative of the chromophore in which this rotational pathway is inhibited have shown that this compound exhibits to a large extent the same properties as the unlocked compound. It can therefore be concluded that in the isolated neutral chromophore internal conversion to the ground state is not dominantly influenced by motion along the single-bond torsional coordinate.

5.5 References

Restriction of the torsional motion of the C₄–C₇ bond by incorporating it in a five-ring can potentially also influence other aspects of the potential energy surface of the excited state. However, as also concluded in the past²⁷,²⁹ it may still serve as a good zeroth-order approximation to assess the role of the single bond in the isomerization dynamics.

Previous studies on OMpCA have shown that under molecular beam conditions the molecule can adopt four different conformations [22,25,43]. The excitation spectra of these conformations have their 0-0 transitions at slightly different...
excitation energies. Since such a conformational heterogeneity is expected as well for TMpCA and will contribute to the broadness of the (1+1’) RE2PI spectrum displayed in Figure 5.1b.


(39) Since out-of-plane optimizations of the V(ππ*) state did not converge we cannot quantify this energy difference in TMpCA. The vertical excitation energies of the nπ* and V(ππ*) states combined with the observation that the relaxation energy of the nπ* state is larger than the relaxation energy of the V(ππ*) state lead to the conclusion, however, that the energy difference between the relaxed V(ππ*) and nπ* states in TMpCA is significantly larger than 0.15 eV.


