UV and IR laser spectroscopy of isolated molecular structural dynamics
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Chapter 6

High-resolution excitation and absorption spectroscopy of gas phase p-coumaric acid: unveiling an elusive chromophore

ABSTRACT

The high-resolution absorption spectrum of para-coumaric acid (pCA), the chromophore of the photoactive yellow protein, is measured in a supersonic molecular beam and in a matrix of liquid helium nanodroplets. Conformational heterogeneity is examined with UV-depletion experiment leading to the assignment of the s-cis, s-trans, OH-syn and OH-anti conformers. Additional IR-depletion experiments are conducted in order to confirm these assignments. The electronic structure of excited states is analyzed. The first clear evidence for a low-lying nπ* state in pCA is provided.

6.1 Introduction

Light drives many important processes of life, ranging from photosynthesis to vision, from phototropism to phototaxis, and from circadian rhythms to other photoperiodicities. Nature utilizes to this purpose small, light absorbing molecules (chromophores) enclosed within a protein that transform light energy into a cellular response. After photon absorption by the chromophore, the protein undergoes a series of reactions via a number of intermediates, and ultimately finds itself in its initial state, the whole process thus forming a photocycle. One of the most extensively studied photoreceptors is the photoactive yellow protein (PYP), originally discovered in the Halorhodospira halophila bacterium [1] where it is responsible for the negative phototaxis towards harmful blue light [2]. PYP has over the years become a model system for studies in photochemistry and photobiology [3] because of its small size (14 kDa), its water solubility, its excellent chemical and photostability, and the simplicity with which crystals can be grown.
The photocycle of PYP is believed to be initiated with the photoinduced trans-to-cis isomerization of the para-coumaric acid (pCA) chromophore. The excited state properties of pCA (Chart 1) thus have been the subject of a large number of experimental [4] and theoretical [5] studies aiming to elucidate the primary steps of this photocycle. One of the appealing aspects of gas-phase studies is that they provide data on the isolated chromophore, thereby allowing for an assessment of the role of the protein environment, and providing the most direct link to quantum chemical calculations. However, until now pCA has remained out of reach from high-resolution spectroscopy [6] and only derivatives like p-vinyl phenol [7] and the oxy-ester methyl-4-hydroxy cinnamate (OMpCA) have been studied [8]. Here we report the first electronic excitation and absorption spectra of pCA together with its conformational analysis.

Chart 6.1. Chemical structure of relevant pCA conformations. The phenolic OH can adopt a syn or anti configuration.

6.2 Experimental and theoretical methods

In our experiments pCA (Aldrich) was heated to 155 °C and expanded with Ne gas at a pressure of 2.5 bars via a 0.5 mm nozzle into a vacuum to create a supersonic expansion. Electronic spectra of cold and isolated molecules were recorded by monitoring the number of ions at the parent mass created by two-color Resonance Enhanced 2-Photon Ionization (R2PI) as a function of the excitation laser frequency. The two-color R2PI excitation spectrum was measured over the range of 32200-35600 cm\(^{-1}\) using a frequency-doubled Nd:YAG (Spectra Physics Lab 190) pumped dye laser (Sirah Precision Scan) operating at 30 Hz. The excitation pulse energy was typically 1 mJ. The laser beam was partially focused by a lens with a focal length of 1 m that was placed at a distance of 0.5 m from the intersection with the molecular beam. The counter-propagating ionization beam was produced by an ArF excimer (Lumonics EX-700) laser (193 nm) and was introduced simultaneously with the excitation pulses. The ionization beam with pulse energies of 2-4 mJ was also partially focused by a system of lenses.
For the investigation of conformer-specific spectra UV- and IR-depletion experiments were performed. The additional pump laser pulses were introduced 100 ns before the excitation-ionization beam sequence and were generated by an excimer-pumped (Lambda Physik CompexPro 205) dye laser (Lumonics HD500). The frequency of the excitation laser was fixed at bands that were associated exclusively with a single conformation (32608 and 32654 cm\(^{-1}\) for the syn and anti configurations of the \(s\)-cis conformer, and 32901 cm\(^{-1}\) for the \(s\)-trans conformer). While scanning the wavelength of the pump beam and recording the signal produced by the probe, we observe a depletion of the probe signal as a result of the depopulation of the ground state each time the pump laser is in resonance with a transition of the selected conformer. The pulse energies and focusing conditions for excitation and ionization beams in these experiments were identical to those used in the REMPI experiments. In case of the UV-depletion experiments the pulse energy of the pump beam was 1.5 mJ. It was also partially focused by a lens of focal length of 1 m placed at a distance of 0.5 m from the ionization volume. For the IR-depletion experiments the pump beam in the range of 3422-3760 cm\(^{-1}\) and with an energy per pulse of 1.7 mJ was produced by difference frequency mixing with the first harmonic of the Nd:YAG laser in a LiNbO\(_3\) crystal. The beam was partially focused by a lens with a focal length of 0.3 m that was placed at a distance of 0.2 m from the intersection with the molecular beam.

The electronic absorption spectrum in helium nanodroplets was obtained in the setup described in detail previously [9]. Helium droplets consisting on average of 2700 atoms were formed by expansion of helium at 30 bars from a 5 µm nozzle cooled to 17 K. In these experiments the \(p\)CA sample was heated to a temperature of 50 °C. These conditions ensured that a single molecule pickup process dominated and that dissociation of molecular clusters contributed negligibly to the observed signal. Two laser beams were used to obtain the ion depletion spectrum. The probe beam was provided by an amplified Ti:Sapphire femtosecond laser system (CPA-1000, Clark-MXR) (~780 nm, 1 mJ/pulse) and served to set up a nonresonant multiphoton ionization signal. This beam was focused with a 350 mm focal length lens, the exact position of the focal point chosen in such a way that the difference between the ion signals with and without depletion beam was maximized. The depletion beam was generated by the frequency-doubled output of a 20 Hz Nd:YAG pumped (Spectra Physics Pro 250) dye laser (Sirah Precision Scan) with pulse energies of 2 mJ. This beam was focused at the helium nanodroplet stream with a 350 mm focal length lens and preceded the femtosecond probe beam by delays up to 360 ns. Ions were detected mass-selectively in a TOF mass spectrometer. Absorption spectra have been obtained by monitoring the mass of the \(p\)CA molecular ion.

Calculations of ground state geometries and their normal modes were performed with density functional theory using the B3LYP hybrid functional and a Gaussian basis set of triple zeta valence quality with polarization functions (\(\text{def-TZVP}\)). Vibrational
frequencies were scaled by the factor of 0.958. Geometry optimization and normal mode calculations for electronically excited states were performed with the second-order approximate coupled-cluster approach with resolution-of-the-identity approximation (RI-CC2). In these calculations the correlation-consistent valence polarized triple zeta basis set (cc-pVTZ) was employed. All optimizations were done without imposing symmetry restrictions. For each optimized geometry a normal mode vibrational analysis was performed to ensure that it corresponded to a minimum on the potential energy surface. Adiabatic and vertical ionization energies for each electronic state were calculated at the RI-CC2/cc-pVTZ level of theory. The calculations were performed with the TURBOMOLE 5.7 suite of programs [10]. Franck-Condon intensities for vibronic spectra of conformers were calculated in the harmonic approximation and taking into account the Duschinsky effect, using an in-house developed program [11] based on the recursion relations for Franck-Condon integrals [12].

6.3 Results and discussion

Figure 6.1 shows the full excitation spectrum obtained in the 32500-34600 cm\(^{-1}\) range.

![Figure 6.1. Two-color R2PI excitation spectrum of p-coumaric acid.](image)

Starting from \(~32590\) cm\(^{-1}\) the spectrum shows a dense manifold of lines that initially have a width of 3-4 cm\(^{-1}\) and broaden up with higher excitation energies. In view of the results obtained for OMPCA [8], one suspects that the spectrum is actually built up from contributions from several conformations. This idea is confirmed by UV depletion spectroscopy [13] which results are presented on Figure 6.2. It allows us to distinguish three distinct conformations with electronic origins at 32588, 32609, and 32896 cm\(^{-1}\). The similarity of these results with those obtained for OMPCA suggests that the lower two transitions are associated with s-cis conformations in which the phenolic OH adopts
either a syn or anti configuration, while the third one derives from one of the syn or anti s-trans conformations. Spectral congestion prohibited an unambiguous identification of the partnering s-trans conformation.

Figure 6.2. Two-color RE2PI excitation and UV-depletion spectra of p-coumaric acid. (a) Excitation (lower panel) and UV-depletion spectra in 32500-33500 cm⁻¹ range probing at (see arrows) 32608 (blue), 32654 (green), and 32901 cm⁻¹ (red). Stick spectra in depletion spectra show Franck-Condon simulations of the V'(ππ*) excitation spectrum of s-cis (blue and green) and s-trans (red) pCA. (b) High-resolution UV-depletion spectra of the electronic origin regions of each conformation.

IR depletion spectroscopy and DFT calculations at the B3LYP/def-TZVP level confirm these assignments. Conformation-specific IR ion dip spectra are depicted in Figure 6.3 where they are compared with spectra calculated for s-cis and s-trans conformations. Using a scaling factor of 0.958, the calculations find a phenolic OH stretch frequency of 3647 cm⁻¹ for both s-cis and s-trans conformations, irrespective of the syn/anti configuration. For the carboxylic OH group, on the other hand, frequencies of 3590 (s-cis) and 3597 cm⁻¹ (s-trans) are calculated. Experimentally, frequencies of 3586 and 3593 cm⁻¹ are observed for the carboxylic OH, while all conformations have their phenolic OH
at 3652 cm\(^{-1}\). Experiment and theory are thus in excellent agreement if the excitation spectra are assigned as described above.

Figure 6.3. IR ion depletion spectra obtained for ionization via the 32608 (blue-bottom), 32654 (green-middle), and 32901 cm\(^{-1}\) (red-top) resonances compared with quantum chemical predictions for the ground state IR absorption spectrum of s-cis (light cyan) and s-trans (dark cyan) pCA using a frequency scaling factor of 0.958.

Previous calculations at the EOM-CCSD level predict for pCA and OMPCA that in the Franck-Condon region accessed from the ground state the lower two excited singlet states are the \(^1\)A(V') (S\(_1\), HOMO \(\rightarrow\) LUMO+1 transition) state having a small oscillator strength, and the \(^1\)A(V) (S\(_2\), HOMO \(\rightarrow\) LUMO+1 transition) (\(\pi\pi^*\)) state with a large oscillator strength [5c,8]. At this level, however, the calculation of harmonic force fields is not possible. We have therefore resorted to calculations at the CC2 level. Previous studies [5d] have shown - and our calculations confirm this - that at this level the ordering of the \(^1\)A(V') and \(^1\)A(V) is reversed. Vertical excitation energies of 4.40 and 4.04 eV and oscillator strengths of 0.18 and 0.69 are predicted for the \(^1\)A(V') and \(^1\)A(V) states, respectively. Nevertheless, we find that the vibrational activity observed in the experimental spectra can only be reproduced satisfactorily using the equilibrium geometry and force field of the \(^1\)A(V') state. Simulations show that the major feature distinguishing the two states is the much stronger activity of the lowest energy in-plane bending mode (92 cm\(^{-1}\)) in the spectrum of the V state (Figure 6.4). The experimental spectra lack this activity, while the rest of the vibrational activity in the experimental excitation spectrum matches nicely that predicted for the V' state (Figure 6.2a).
One notable exception is a ~9 cm\(^{-1}\) progression superimposed on each band that is most clearly visible in high-resolution spectra of the origin regions of the three conformations (Figure 6.2b). This progression is present in the excitation as well as depletion spectra which proves that these low-frequency bands do not derive from hot bands, but are associated with excited-state vibrational levels. Since the only candidates for such low-frequency modes are out-of-plane twisting and butterfly modes, we conclude that upon excitation pCA adopts a non-planar geometry.

The spectra discussed so far have been obtained using 193 nm to ionize the molecule after excitation. Remarkably, we find that one-color R2PI does not result in
measurable ion signals. In fact, even in a two-color 1+1’ scheme and using ionization wavelengths down to 216 nm no resonance enhancement is seen. A possible explanation could be that ionization only proceeds efficiently if the ionic manifold is accessed at high vibrational energies [14]. This in turn would imply that ionization does not take place from an electronically excited state with limited vibrational content as suggested by the excitation spectrum, but from a highly vibrationally excited state. Ab initio calculations predict that the adiabatic excitation energy of the $1^1A^\prime\prime(n\pi^*)$ state is actually lower than that of the $V'$ and $V$ states [5b,c] (Figure 6.5). All pieces of the puzzle thus fall into place if we assume that after excitation of the $V'$ state fast internal conversion takes place to the $1^1A^\prime\prime(n\pi^*)$ state. This conclusion is further supported by calculations of the vertical ionization energy from the $1^1A^\prime\prime(n\pi^*)$ state. We find a value of 6.5 eV (~191 nm), consistent with the observed onset of ionization (the calculated ionization energies from the optimized $V'$ and $V$ states are 4.1 eV (~303 nm) and 4.4 eV (~283 nm), respectively). The present experiments thus provide the first clear evidence for a low-lying $n\pi^*$ state in pCA and its derivatives that previously had been postulated by ab initio calculations.

![Figure 6.5](image-url)

*Figure 6.5. Results of calculations of electronic states of pCA for different isomers.*
UV-depletion experiments performed on $p$CA in helium nanodroplets [15] instead of molecular beam conditions are in line with a fast decay of the $V'(\pi\pi^\ast)$ state into the low-lying $n\pi^\ast$ state. In these experiments a multiphoton ionization signal was set up using an amplified Ti:Sapphire fs laser, while a counter-propagating tunable ns laser beam was used to perform UV-depletion spectroscopy. When the ionization laser was preceded by resonant UV absorption, the ion signal was depleted (Figure 6.6). Comparison of the helium nanodroplet spectrum with the molecular beam spectrum shows that the two spectra display the same features, but that the helium droplet spectrum is displaced by 700 cm$^{-1}$ to lower energies. Importantly, we notice that the line widths of the resonances in the helium droplet spectrum are not noticeably smaller, even though the rovibrational temperature of the sample is much lower (0.38 K). This suggests that they are intrinsic and determined by lifetime broadening.

The above observations suggest that after absorption of the UV photon the $p$CA molecule decays to a state from which ionization is no longer possible. Our experiments show that this cannot be the ground state. Furthermore experiments with a high intensity, tightly focused fs beam were also performed in order to identify any fragments created by the UV pump beam. They proved that the $p$CA ion signal is not depleted because of fragmentation upon internal conversion to the ground state. We thus must conclude that another stable state is populated. Experimentally, we could vary the delay between pump and probe laser up to 1 µs, and found that during this delay the signal was not recovered. This implies that a state is populated with a long lifetime. Because of its extremely low oscillator strength, the $n\pi^\ast$ state is the most logical candidate.

![Figure 6.6. $p$CA RE2PI spectrum under molecular beam conditions (lower) and ion dip spectrum in a helium nanodroplet matrix (upper).](image)
6.4 Summary

In summary, we have observed for the first time the excitation and absorption spectra of the lowest excited singlet state of para-coumaric acid. Analysis of the vibrational activity in these spectra leads to the conclusion that this state should be assigned as the $V'(\pi\pi^*)$ state, and that upon excitation the molecule undergoes significant out-of-plane geometry changes. In the $V'(\pi\pi^*)$ state fast internal conversion takes place to a state that on the basis of the observed ionization requirements is identified as the $1^1A'(n\pi^*)$ state. Photoexcitation of the para-coumaric acid chromophore in PYP triggers a cascade of follow-up reactions that lead to light-induced signal transduction, one of the central issues in photobiology. The spectroscopic and dynamic properties of para-coumaric acid unveiled in the present study may thus serve as an excellent point of reference for further elucidation of the fascinating photochemical and photophysical properties of PYP in particular, and those of photosensory proteins in general.

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References


