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High-Resolution Excitation and Absorption Spectroscopy of Gas-Phase *p*-Coumaric Acid: Unveiling an Elusive Chromophore

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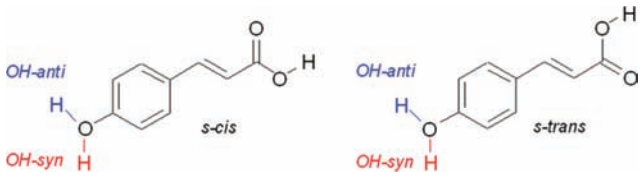
Light drives many important processes of life, ranging from photosynthesis to vision, from phototropism to phototaxis, and from circadian rhythms to other photoperiodicities. Nature utilizes to this purpose small, light absorbing molecules (chromophores) enclosed within a protein that transform light 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 toward 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 (*p*CA) chromophore. The excited state properties of *p*CA (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 *p*CA 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 (OM*p*CA) have been studied.[8]

Chart 1. Chemical Structure of Relevant *p*CA Conformations[a]



[a] The phenolic OH can adopt a *syn* or an *anti* configuration.

Here we report the first electronic excitation and absorption spectra of *p*CA together with its conformational analysis. In our experiments *p*CA (Aldrich) was heated to 155 °C and seeded into a supersonic expansion of Ne. 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 two-photon ionization (RE2PI) as a function of the excitation laser frequency. For the ionization step a counterpropagating ArF excimer laser beam (193 nm) was used that overlapped in time with the excitation laser.[9]

Figure 1a shows the excitation spectrum obtained in the 32 500−33 500 cm^{-1} range.[10] Starting from ∼32 590 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 OM*p*CA,[8] one suspects that the spectrum is actually built up from contributions from several conformations. This idea is confirmed by UV depletion spectroscopy[11] which allows us to distinguish three distinct conformations with electronic origins at 32 588, 32 609, and 32 896 cm^{-1}. The similarity of these results with those obtained for OM*p*CA suggests that the lower two transitions are associated with s-*cis* conformations in which the phenolic OH adopts either a *syn* or an *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.

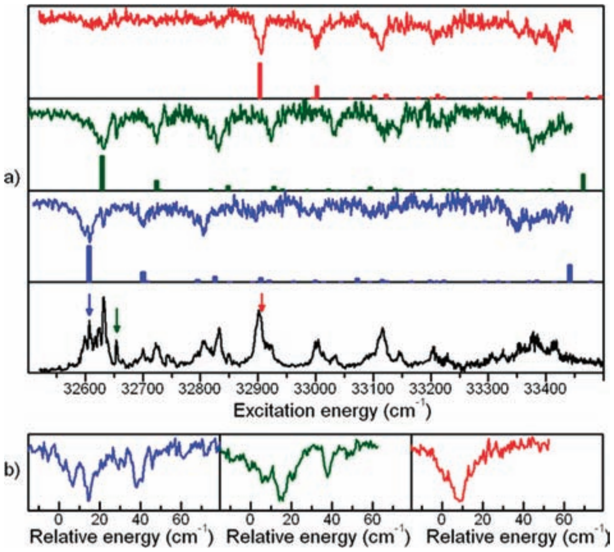


Figure 1. Two-color RE2PI excitation and UV-depletion spectra of *p*-coumaric acid. (a) Excitation (lower panel) and UV-depletion spectra in 32 500−33 500 cm^{-1} range probing at (see arrows) 32 608 (blue), 32 654 (green), and 32 901 cm^{-1} (red). Stick spectra in depletion spectra show Franck−Condon simulations of the V′($\pi\pi^*$) excitation spectrum of s-*cis* (blue and green) and s-*trans* (red) *p*CA. (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 2 where they are compared with spectra calculated for s-*cis* and s-*trans* conformations.[9] Using

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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.

Electronic structure calculations on *p*CA and OM*p*CA predict that in the Franck−Condon region accessed from the ground state S_1 corresponds to the $V'(\pi\pi^*)$ state having a small oscillator strength, while S_2 corresponds to the $V(\pi\pi^*)$ state with a large oscillator strength.[5c,8] Simulations of the vibronic spectra 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. 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 1a). One notable exception is an ∼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 1b). 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 *p*CA adopts a nonplanar geometry.

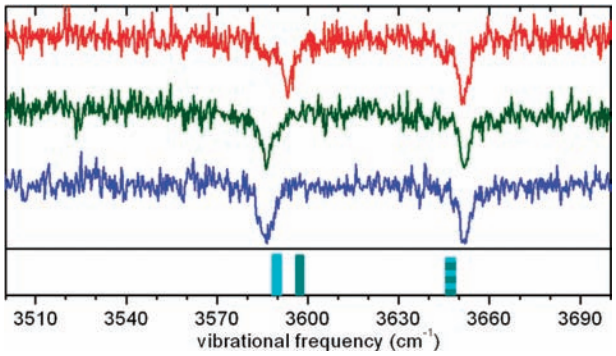

Figure 2. IR ion depletion spectra obtained for ionization via the 32 608 (blue), 32 654 (green), and 32 901 cm^{-1} (red) resonances compared with quantum chemical predictions for the ground state IR absorption spectrum of s-*cis* (cyan) and s-*trans* (dark cyan) *p*CA using a frequency scaling factor of 0.958.

The spectra discussed so far have been obtained using 193 nm to ionize the molecule after excitation. Remarkably, we find that one-color RE2PI 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.[12] 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 $1^1A''(n\pi^*)$ state is actually lower than that of the V′ and V states.[5b,c] 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 $1^1A''(n\pi^*)$ state. This conclusion is further supported by calculations of the vertical ionization energy from the $1^1A''(n\pi^*)$ state. We calculate a value of 6.5 eV (∼191 nm), consistent with the observed onset of ionization.[13] The present experiments thus provide the first clear evidence for a low-lying $n\pi^*$ state in *p*CA and its derivatives that previously had been postulated by *ab initio* calculations.

UV-depletion experiments performed on *p*CA in helium nanodroplets[14] instead of molecular beam conditions are in line with a fast decay of the $V'(\pi\pi^*)$ 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.[9] The resulting ion dip spectrum is depicted in Figure 3. Comparison 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.

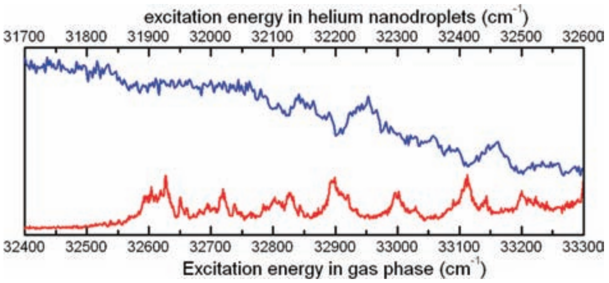

Figure 3. *p*CA RE2PI spectrum under molecular beam conditions (red) and ion dip spectrum in a helium nanodroplet matrix (blue).

In summary, we have observed for the first time the excitation and absorption spectra of the lowest excited singlet state of *p*-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 *p*-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 *p*-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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Supporting Information Available: Experimental and theoretical details, the full 32 500−34 600 cm^{-1} experimental excitation spectrum, and Franck−Condon simulations of $S_0 \rightarrow V'(\pi\pi^*)$ and $S_0 \rightarrow V(\pi\pi^*)$ excitation spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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