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# Excited state dynamics of Photoactive Yellow Protein chromophores elucidated by high-resolution spectroscopy and *ab initio* calculations

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We report on experimental high-resolution spectroscopic studies in combination with advanced theoretical calculations that focus on the excited-state dynamics of various forms of the chromophore of the Photoactive Yellow Protein (PYP), and the dependence of these dynamics on conformational and isosteric structure, as well as the biological environment. Three-colour nanosecond multiphoton ionization pump–probe studies confirm and extend previous conclusions that the dominant decay channel of the lowest excited  $\pi\pi^*$  state (the so-called  $V'$  state) of methyl-4-hydroxycinnamate is picosecond internal conversion to the adiabatically lower  $n\pi^*$  state, and enable us to resolve apparent contradictions with picosecond pump–probe studies. Comparison of multiphoton ionization and laser induced fluorescence excitation spectra leads to the assignment of the hitherto elusive excitation spectrum of the  $V(\pi\pi^*)$  state. Complexation of methyl-4-hydroxycinnamate with water radically changes the excited-state dynamics; internal conversion to the  $n\pi^*$  state is absent, and bond isomerization channels instead play a prominent role. Excited states of the thio-ester compound, the form in which the chromophore is present in PYP, have till the present study remained out of reach of gas-phase studies. The excitation spectra obtained here show a broad, almost structureless band system, giving evidence for enhanced nonradiative decay channels. The gas-phase results will be discussed in the context of results from ultrafast studies on these two chromophores in solution.

## Introduction

Light is used ubiquitously in nature to drive important processes such as photosynthesis, but also as a means to perceive and respond to environmental changes as occurs in vision, phototropism and phototaxis.<sup>1</sup> Common to all these processes is that absorption of light triggers conformational changes in a light-sensitive signaling protein that ultimately lead to a response at the cellular level. Nature uses to this

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purpose small chromophores embedded in the protein whose photochemical response leads to large-scale structural changes of the entire protein.

Photoisomerization is one of the most widely used methods to translate light absorption into biological function.<sup>1</sup> Ideally, excitation of the chromophore leads exclusively to the desired photoresponse. However, in practice, the electronic states that are accessed upon photon absorption are also subject to a large number of competing processes such as internal conversion to other electronically excited states, vibrational relaxation, and solvation relaxation. It is therefore important to determine how the geometrical and electronic structure of such chromophores changes upon light absorption, how these changes are influenced by specific groups and how these groups steer the chromophore towards an optimal biological functioning, and how coupling of the chromophore to the protein and a solvent influences its photochemical and photophysical properties.

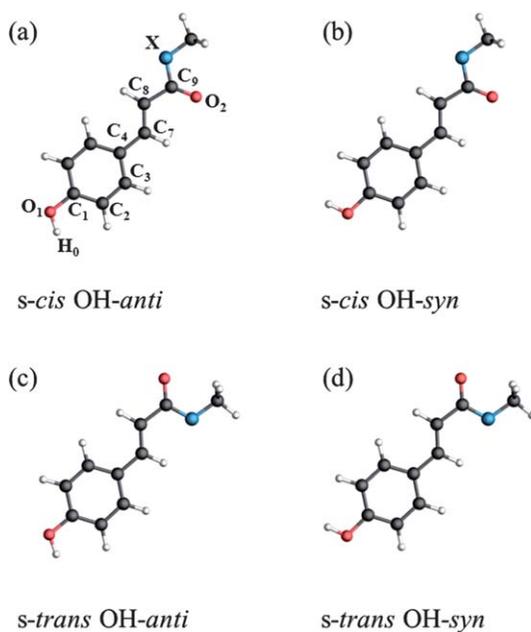
The Photoactive Yellow Protein (PYP) is one of the proteins used in nature for light-induced signalling. PYP was originally discovered in the *Ectothiorhodospira halophila* bacterium.<sup>2,3,4</sup> It utilizes isomerisation of a chromophore derived from *trans*-4-hydroxycinnamic acid (*para*-coumaric acid (*pCA*)) to initiate a photocycle that ultimately leads to a negative phototaxis towards blue light,<sup>5</sup> thereby protecting the bacterium from its potential harmful effects. PYP is in many aspects an ideal system for the study of biological photoreceptors. It is relatively small (125 residues, 14 kDa), robust towards chemical changes, and can easily be crystallized. As a result, its *modus operandi* has been the subject of many experimental and theoretical studies.<sup>6–9</sup> Although nowadays a comprehensive description of the structural changes occurring during the photocycle of PYP has been acquired,<sup>10</sup> many aspects of the initial processes in the photoexcited states are still matter of debate. There is therefore considerable interest in the photochemical and photophysical properties of the PYP chromophore, and, in particular, on how to relate the properties of the isolated chromophore to its properties when it is part of the protein.

The most simple representation of the PYP chromophore is *pCA*. Gas-phase studies of *pCA* have for a long time not been possible.<sup>11–13</sup> Recently, we succeeded in recording the first excitation and absorption spectra under supersonic beam conditions. These studies enabled the identification of the lowest electronically excited state and the geometry changes associated with electronic excitation.<sup>14</sup> *Ab initio* calculations on *pCA*<sup>15–17</sup> indicate that three states are important for the description of the lower excited singlet manifold: the  $V(\pi\pi^*)$  state, in a one-configuration picture described by the HOMO  $\rightarrow$  LUMO + 1 excitation, the  $V(\pi\pi^*)$  state described by the HOMO  $\rightarrow$  LUMO excitation, and the  $n\pi^*$  state described by the  $n \rightarrow$  LUMO excitation. For vertical excitation from the ground state these states are respectively  $S_1$ ,  $S_2$ , and  $S_3$ . However, calculation of the adiabatic excitation energies shows that in that case the  $n\pi^*$  state has the lowest excitation energy, although the order of the two  $\pi\pi^*$  states remains unchanged.<sup>15</sup>

In the protein *pCA* is (i) present in its anionic form, (ii) covalently linked to the Cys69 residue *via* a thioester bond, and (iii) forms hydrogen bonds on the phenolate side with the Glu46 and Tyr42 residues. Theoretical studies have therefore focused on how the properties of the lower excited singlet states depend on the anionic nature of the chromophore in the protein, the role of the sulphur atom, and the influence of hydrogen bonding interactions.<sup>18–23</sup> From these studies it has become clear that each of these aspects has its own consequences for the excited state manifold. Replacing the oxygen atom by a sulphur atom makes the

$n\pi^*$  state both vertically as well as adiabatically the lowest excited singlet state, and reverses the order of the  $V'(\pi\pi^*)$  and  $V(\pi\pi^*)$  states. In the anion of the thio-compounds the  $V(\pi\pi^*)$  state is the lowest excited singlet state, the energy differences with the other two excited states becoming much larger. Hydrogen bonding gives rise to pronounced energetic shifts.

In recent years a number of gas-phase spectroscopic studies have been performed aimed at elucidating the properties of *pCA*-related compounds more similar to the form of the chromophore in the protein, including gas-phase studies on the anion.<sup>24–26</sup> The ester functionality influences the structural and excited-state properties of the chromophore; studies of molecular systems like methyl-4-hydroxycinnamate (historically indicated as *OMpCA*, see Scheme 1) in which this functionality is incorporated are therefore important to assess the influence of the protein environment. LIF<sup>27</sup> and REMPI<sup>28</sup> studies on *OMpCA* show vibrationally resolved excitation spectra that indicate that the geometry changes of the molecule upon excitation are relatively small, in good agreement with theoretical predictions for the  $V'(\pi\pi^*)$  state. The LIF and REMPI studies show a striking influence of hydrogen bonding. In the *OMpCA*-H<sub>2</sub>O cluster the origin transition to the  $V'(\pi\pi^*)$  state is shifted by 641 cm<sup>-1</sup>, and its intensity is very much enhanced with respect to the bare molecule. REMPI studies on the  $V'(\pi\pi^*)$  state of *pCA* concluded that internal conversion to the  $n\pi^*$  state is an important decay channel.<sup>14</sup> The same conclusion was reached for the  $V'(\pi\pi^*)$  state of *OMpCA*.<sup>28</sup> The observed intensity enhancement of transitions in the water cluster has therefore been interpreted in



**Scheme 1** Molecular structure of compounds studied and employed atom labelling. Methyl-4-hydroxycinnamate corresponds to X=O and is designated in the text as *OMpCA*, the thio ester corresponds to X=S and is referred to in the text as *TMpCA*. Drawn are the four low-energy conformations that have been identified in previous molecular beam experiments on *OMpCA*: (a) *s-cis* OH-*anti*, (b) *s-cis* OH-*syn*, (c) *s-trans* OH-*anti* and (d) *s-trans* OH-*syn*.

terms of a reduced internal conversion rate to the  $n\pi^*$  state. Recent ps time-resolved REMPI studies on *OMP*CA and *OMP*CA–H<sub>2</sub>O suggest on the other hand an explanation in terms of excited-state isomerisation.<sup>29</sup>

A principal goal of the present work is to determine the importance of the various possible decay channels of the  $V'(\pi\pi^*)$  state of *OMP*CA. We furthermore want to determine how complexation with water influences the electronic properties of the chromophore, and how this in turn is reflected in the excited-state dynamics. In the protein the  $V(\pi\pi^*)$  state is the lowest excited singlet state. Studies of its properties are therefore important. Although in the LIF study on *OMP*CA some bands were tentatively assigned as transitions to the  $V(\pi\pi^*)$  state, its structural and dynamic properties have so far remained unclear. Here, we will identify its excitation spectrum and study its decay channels. Finally, we will study the methyl-4-hydroxycinnamate thio ester (*TMp*CA) and report for the first time its excitation spectrum under molecular beam conditions. Theory predicts that in *TMp*CA the  $V(\pi\pi^*)$  state is the first excited  $\pi\pi^*$  state. Yet, under solution conditions *OMP*CA and *TMp*CA show rather similar excited-state dynamics.<sup>30,31</sup> Comparison of the gas-phase excitation spectra of *OMP*CA and *TMp*CA will enable us to assess how the electronic manifold changes by sulphur substitution, and how solute–solvent interactions influence the excited-state dynamics.

## Experimental details

In our studies two- and three-colour Resonance Enhanced MultiPhoton Ionization (REMPI) spectroscopy has been used to record excitation and depletion spectra of molecules cooled in a supersonic free jet expansion. The molecular beam setup employed for these measurements has been described in detail before.<sup>32,33</sup> Briefly, the setup makes use of a pulsed nozzle construction in which the sample is placed in an oven that is heated to the temperature required to generate a high enough vapour pressure of the sample. A molecular beam is then produced by mixing the vapour with a rare gas, in the present experiments Ne at a pressure of 2 bar, and expanding this mixture into a differentially pumped vacuum with a pulsed nozzle (General Valve Iota One system). The nozzle has an orifice of 0.5 mm and is kept 5 °C higher in temperature than the oven to avoid clogging of the nozzle. After expansion, the molecular beam is skimmed with a conical skimmer (2 mm) and enters the ionization chamber where mass-resolved ion detection is performed using a reflectron time-of-flight mass spectrometer (R.M. Jordan Co.). Clusters of chromophores with water are generated by mixing the carrier gas with small amounts of water vapour.

A two-colour (1 + 1') Resonance Enhanced Two-Photon Ionization (RE2PI) scheme has been employed to record excitation spectra. In these experiments a frequency-doubled dye laser (Sirah Precision Scan) pumped by a 30 Hz Nd:YAG laser (Spectra Physics Lab 190) was used for electronic excitation. A series of dyes including DCM, Rhodamine 640, Rhodamine 610, Rhodamine 590, Pyromethen 597 and Fluorescein 548, in combination with frequency-doubling by a KDP crystal, enabled us to cover the frequency region from 30000 to 36500 cm<sup>-1</sup>. Typical excitation energies were 2–3 mJ per pulse. The second colour was used to ionize electronically excited states and was produced by an ArF excimer laser (Neweks PSX-501) with typical pulse energies of 5 mJ per pulse. Both laser beams were introduced in a counter-propagating fashion into the spectrometer without

further focusing. In the two-colour ( $1 + 1'$ ) RE2PI experiments these two laser systems were synchronized in time using a delay generator (Stanford Research Systems DG535). The same delay generator was used to study the decay dynamics of excited states by recording the decay of the RE2PI signal as a function of the time delay between excitation and ionization.

To obtain conformation-specific spectra we have utilized UV-UV depletion spectroscopy.<sup>34</sup> UV-UV depletion spectroscopy monitors the signal from a probe laser that is fixed at the frequency of one particular band in the excitation spectrum, and is associated exclusively with a single conformation. Scanning the pump laser then leads to a depletion of this 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. In the present study we used either one- or two-colour RE2PI to generate a probe signal. To generate the probe signal in the one-colour RE2PI scheme the Nd:YAG pumped dye laser system described above was used. In the two-colour RE2PI scheme electronic excitation occurred with a dye laser system consisting of a dye laser (Lumonics HD500) pumped by an excimer laser (Lambda Physik CompexPro 205) operating on XeCl. The output of this dye laser was frequency-doubled with a KDP or BBO crystal using an autotracking system (INRAD AutoTracker II), and was introduced co-linearly with the 193 nm ionization beam into the spectrometer. Typical pulse energies employed for  $S_1 \leftarrow S_0$  excitation were 0.5–1.0 mJ. Depletion of the ground state took place in these three-colour experiments with the Nd:YAG pumped dye laser system. Depletion and probe beams were introduced in a counter-propagating fashion into the spectrometer with a typical time delay of 160 ns. For the absolute determination of the wavelength of both dye lasers a Burleigh WA-4500 wavemeter was used. Since in previous studies<sup>27,28</sup> the wavelength had been calibrated in an indirect manner, the excitation frequencies reported here differ slightly from those reported previously.

Methyl-4-hydroxycinnamate was obtained from ABCR with a purity of 95% and used without further purification. The thiomethyl ester derivative of *para*-coumaric acid was synthesised according to the procedure described previously.<sup>35</sup> In our experiments *OMP*CA and *TM**p*CA the samples were heated up to 165 °C and 185 °C, respectively, in a glass bottle placed in the oven assembly.

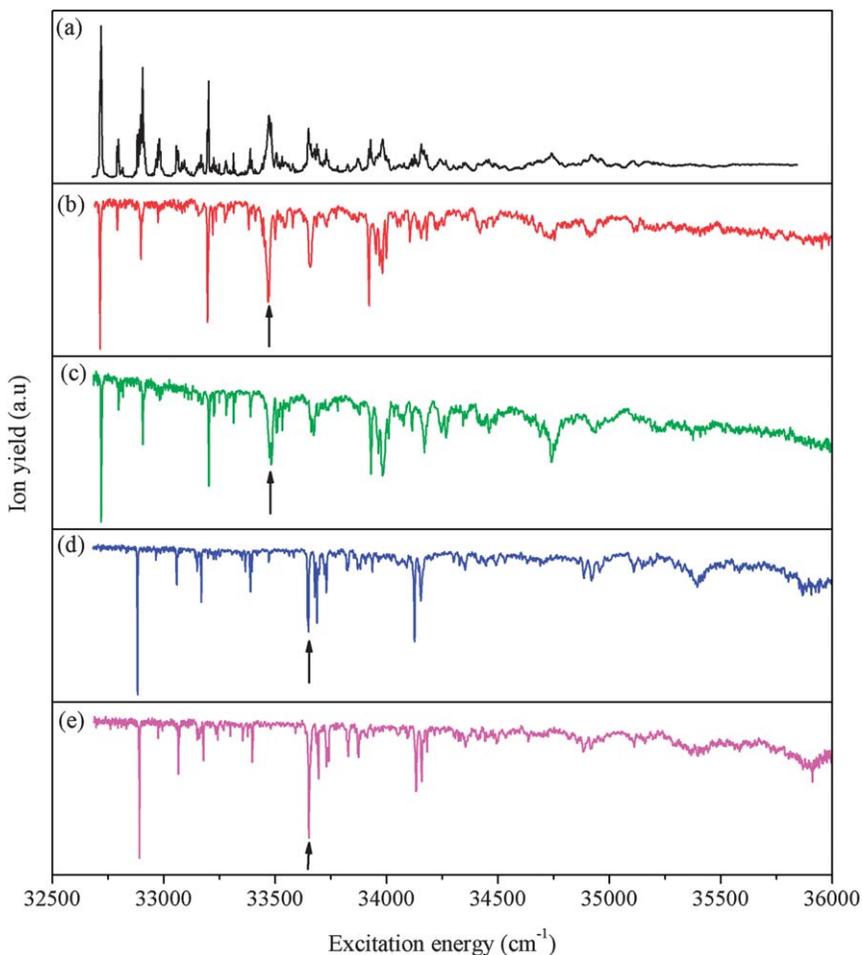
## Theoretical details

Using the spin-component scaled version of the approximate coupled-cluster singles-and-doubles model (SCS-CC2),<sup>36,37</sup> the vertical and adiabatic excitation energies for the excited states of interest of the investigated molecules systems were calculated. The geometry of each structure was optimized in  $C_s$  symmetry in the respective electronic state ( $S_0$ – $S_3$ ) using the RICC2 module<sup>38,39</sup> as implemented in the TURBOMOLE package.<sup>40</sup> For all calculations the cc-pVTZ basis set,<sup>41</sup> together with the standard auxiliary basis sets,<sup>42</sup> was employed.

## Results and discussion

### Excited state dynamics of *OMP*CA

The ( $1 + 1'$ ) RE2PI excitation spectrum of *OMP*CA in the 32700–35700  $\text{cm}^{-1}$  excitation region is shown in Fig. 1a. This spectrum shows a considerably better



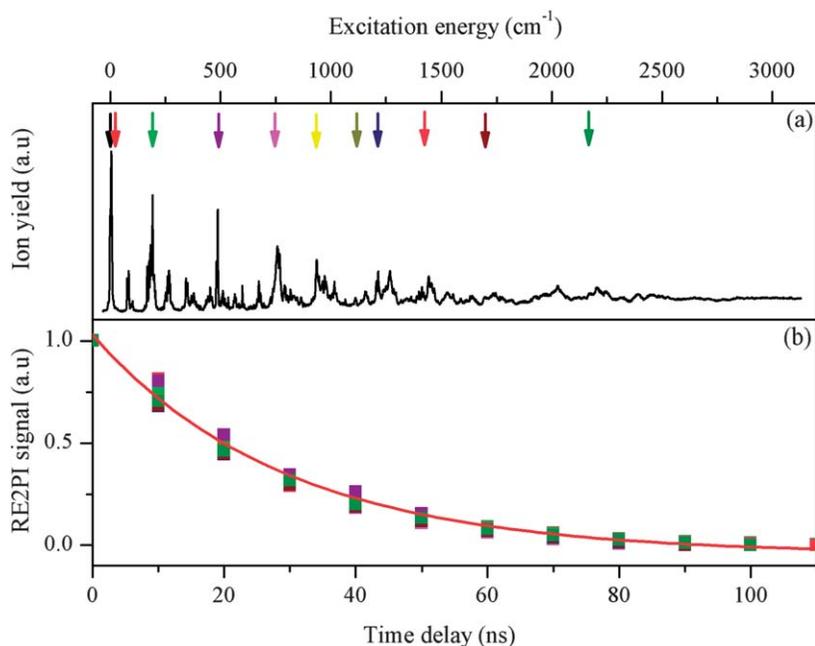
**Fig. 1** (a)  $(1 + 1')$  RE2PI spectrum of OMpCA and UV-UV depletion spectrum of the (b) *s-cis* OH-*syn* (c) *s-cis* OH-*anti* (d) *s-trans* OH-*anti* (e) *s-trans* OH-*syn* conformers. The bands indicated with a black arrow in the depletion spectra are assigned to the  $S_2 \leftarrow S_0$  origin transitions (see text).

resolution than the previously reported one-colour RE2PI excitation spectrum.<sup>28</sup> Moreover, well-resolved vibrational activity is observed up to higher excitation energies. The increase in resolution can be attributed to the fact that the  $(1 + 1')$  RE2PI signal is significantly larger than the  $(1 + 1)$  RE2PI signal under the same excitation intensity conditions. As a result, lower laser intensities can be used for the excitation step in the two-colour scheme as compared to the one-colour scheme. Indeed, comparison of the line widths in the LIF spectrum with the line widths in the one-colour RE2PI spectrum gave in the past reason to believe that it is hard to avoid partial saturation in one-colour RE2PI.<sup>28</sup> As will be argued below, the observation that the two-colour signal is much stronger than the one-colour signal provides direct evidence for the conclusion that the electronic state that is excited is not the same state from which ionization takes place.

OMpCA can adopt four different conformations under our molecular beam conditions (see Scheme 1). To investigate to what extent the excited-state

dynamics depend on the finer details of the molecular structure, it is necessary to obtain the excitation spectrum of each of the conformers separately. Such conformation-specific excitation spectra obtained by UV-UV depletion spectroscopy are reported in Fig. 2b–2e for the *s-cis* OH-*syn*, *s-cis* OH-*anti*, *s-trans* OH-*anti* and *s-trans* OH-*syn* conformer, respectively, with  $S_1(V(\pi\pi^*)) \leftarrow S_0$  origin transitions located at  $32711.0 \pm 0.2 \text{ cm}^{-1}$ ,  $32715.9 \pm 0.2 \text{ cm}^{-1}$ ,  $32879.5 \pm 0.2 \text{ cm}^{-1}$ , and  $32887.7 \pm 0.2 \text{ cm}^{-1}$ . Interestingly, we find that the line widths of the transitions associated with the *s-cis* conformers are systematically larger than those of the *s-trans* conformers. As a result, the excitation spectra of the *s-trans* species show well-resolved bands up to higher excitation energies than the *s-cis* species.

The difference in line widths might indicate that the *s-cis* and *s-trans* conformers have different excited-state lifetimes. The spectral resolution of our experiments, however, does not enable us to observe single rotational transitions. The widths of the vibronic bands are thus in principle determined by a combination of homogeneous lifetime broadening and inhomogeneous rotational broadening, and do not allow us to determine the excited-state lifetime directly from the band widths. Comparison of the widths in the  $(1 + 1')$  RE2PI spectrum with those in the UV-UV depletion spectra show that the bands in the former spectrum are systematically broader. For example, when the origin bands are fitted with a Lorentzian line shape we find in the UV-UV depletion spectra widths of  $3.2 \pm 0.1$ ,  $3.3 \pm 0.2$ ,  $2.0 \pm 0.1$ , and  $2.3 \pm 0.1 \text{ cm}^{-1}$ , while in the  $(1 + 1')$  RE2PI spectrum they have widths of  $3.3 \pm 0.2$ ,  $4.8 \pm 0.1$ ,  $2.5 \pm 0.3$ , and  $2.7 \pm 0.3 \text{ cm}^{-1}$ .



**Fig. 2** (a) Excitation spectrum of OMPcA. The energy scale has been shifted to the excitation energy of the  $S_1 \leftarrow S_0$  origin transition of the *s-cis* OH-*syn* conformer at  $32711.0 \text{ cm}^{-1}$ . (b) Normalized decays of the  $(1 + 1')$  RE2PI signal at various excitation energies above the origin of the *s-cis* OH-*syn* conformer: 0.0, 4.3, 191.1, 486.1, 753.9, 936.4, 1111.7, 1204.6, 1417.6, 1703.1, and  $2169.0 \text{ cm}^{-1}$ .

This increase suggests that inhomogeneous rotational broadening plays a non-negligible role in determining the overall width. The spectral width of our laser is smaller than the line width. If the band is inhomogeneously broadened, the probe step in the UV-UV depletion experiments monitors selectively only part of the unresolved rotational transitions. This, in turn, leads to narrower lines when the pump frequency is scanned in depletion spectra than in the  $(1 + 1')$  RE2PI spectrum where this selection is absent. We thus conclude that the lifetimes of 1.5 and 2.5 ps for the *s-cis* and *s-trans* conformations, respectively, as determined from the Lorentzian fits of the UV-UV depletion spectra should be considered as lower limits for the lifetime.

The conclusion that the lifetime of the  $V'(\pi\pi^*)$  state is in the low picosecond regime is in agreement with time-resolved studies by picosecond UV-UV pump-probe spectroscopy.<sup>29</sup> In these studies it was reported that the lifetime of the *s-cis* and *s-trans* conformations is 8 and 9 ps, respectively, with an uncertainty of 3 ps.<sup>43</sup> Both the line width measurements and the time-resolved studies lead us to expect that studies of the decay of the  $(1 + 1')$  RE2PI signal as a function of the time delay between excitation and ionization lasers will merely show the convolution of their ns time profiles. Fig. 2 shows that the opposite is actually true. In this Figure the normalized  $(1 + 1')$  RE2PI signal decay is plotted for several excitation energies up to  $\sim 2200 \text{ cm}^{-1}$  above the  $S_1(V'(\pi\pi^*)) \leftarrow S_0$  origin transition of the *s-cis* OH-*syn* conformer. In all cases the decay can be fitted very well with a mono-exponential decay with a time constant of  $29.3 \pm 1.6 \text{ ns}$ . The only way to reconcile these apparently contradictory observations is to conclude that excitation from the ground state to the optically bright state is followed by a fast internal conversion to another state with a ns lifetime. Importantly, our experiments demonstrate that this state can still be ionized. It therefore must be an electronically excited state that does not show up directly in the RE2PI excitation spectrum. In the unlikely scenario that internal conversion would occur to a hot ground state species -which in that case would need to decay to some other species on the observed time scale of ns- ionization would energetically still be possible, but would be precluded because of the highly unfavourable vibrational overlap between the initial neutral and the final ionic states (*vide infra*).

*Ab initio* calculations on *pCA* predict that for vertical excitation the  $n\pi^*$  state is  $S_3$ , but that adiabatically it is the lowest electronically excited state.<sup>15,16</sup> CC2

**Table 1** Vertical and adiabatic excitation energies (eV) of the lower electronically excited states of the *s-cis* OH-*anti* conformer of OMP*CA* and the OMP*CA*-H<sub>2</sub>O cluster calculated at the SCS-CC2 /cc-pVTZ level. Oscillator strengths for vertical excitation are given in parentheses

	OMP <i>CA</i>		OMP <i>CA</i> + H <sub>2</sub> O	
	Vertical	Adiabatic	Vertical	Adiabatic
$V'(\pi\pi^*)$	4.599 (0.221)	4.348	4.523 (0.437) <sup>a</sup>	4.216
$V(\pi\pi^*)$	4.819 (0.627)	4.585	4.718 (0.437) <sup>a</sup>	4.522
$n\pi^*$	5.203 ( $9 \times 10^{-5}$ )	4.202	5.240 ( $9 \times 10^{-5}$ )	4.236

<sup>a</sup> Upon complexation with water the excited-state characters of  $S_1$  and  $S_2$  are no longer exclusively associated with the HOMO  $\rightarrow$  LUMO + 1 and HOMO  $\rightarrow$  LUMO excitations as they are in the  $V'(\pi\pi^*)$  and  $V(\pi\pi^*)$  states of the bare chromophore, but become strongly mixed.

calculations reported in Table 1 show that in *OMpCA* the  $V'(\pi\pi^*)$ ,  $V(\pi\pi^*)$ , and  $n\pi^*$  states have the same state ordering.<sup>44</sup> We therefore conclude that the excitation spectrum displayed in Fig. 1 shows transitions to the  $V'(\pi\pi^*)$  and possibly the  $V(\pi\pi^*)$  states. Transitions to the  $n\pi^*$  state should be present at lower excitation energies but have so far escaped detection because of the small oscillator strength of the  $n\pi^* \leftarrow S_0$  transition. The calculations thus strongly suggest that internal conversion to the  $n\pi^*$  state is responsible for the ps decay of the excited bright state. The  $n\pi^*$  state, in turn, decays with a time constant of 30 ns to the ground state.

In the present experiments with ns lasers we observe decay of the pump-probe REMPI signal on a ns time scale. This would seem to be at odds with the results of the ps pump-probe studies in which a decay of the ionization signal is observed on a ps time scale.<sup>29</sup> Consideration of the experimental conditions under which both experiments have been performed reveals, however, that the ps studies actually provide further evidence that internal conversion takes place to the  $n\pi^*$  state. In the ps experiments the ionization laser frequency was fixed at 32590  $\text{cm}^{-1}$  (4.041 eV). For excitation of the *s-cis* conformers at 32710  $\text{cm}^{-1}$  the total energy is therefore barely above (150  $\text{cm}^{-1}$ ) the adiabatic ionization energy (65154  $\text{cm}^{-1}$ ).<sup>28</sup> After internal conversion from the  $V'(\pi\pi^*)$  state the  $n\pi^*$  state is populated. For this state we calculate a vertical ionization energy to  $D_0$  of 5.751 eV, which is much higher than the employed 4.041 eV photons. Franck-Condon arguments thus tell us that in the ps experiments ionization from the  $n\pi^*$  state will be highly inefficient.<sup>45</sup> A second aspect that should be taken into account is the ionic state to which ionization takes place.<sup>46,47</sup> In a one-configuration picture the ground state  $D_0$  of the ion can be described as a  $\pi^{-1}$  state. One thus expects efficient ionization to  $D_0$  from the  $V'(\pi\pi^*)$  and  $V(\pi\pi^*)$  states, but not from the  $n\pi^*$  state. The latter state, instead, will preferentially ionize to the  $n^{-1}$  state, one of the electronically excited states of the ion, but this state is not accessible in the ps experiments. Both arguments thus imply that the ps experiments are “blind” to the hot  $n\pi^*$  states created after internal conversion. What is therefore observed in the ps experiments is the decay of the electronically excited state that can be ionized (in this case the  $V'(\pi\pi^*)$  and/or  $V(\pi\pi^*)$  state), but not the electronic state to which it decays.

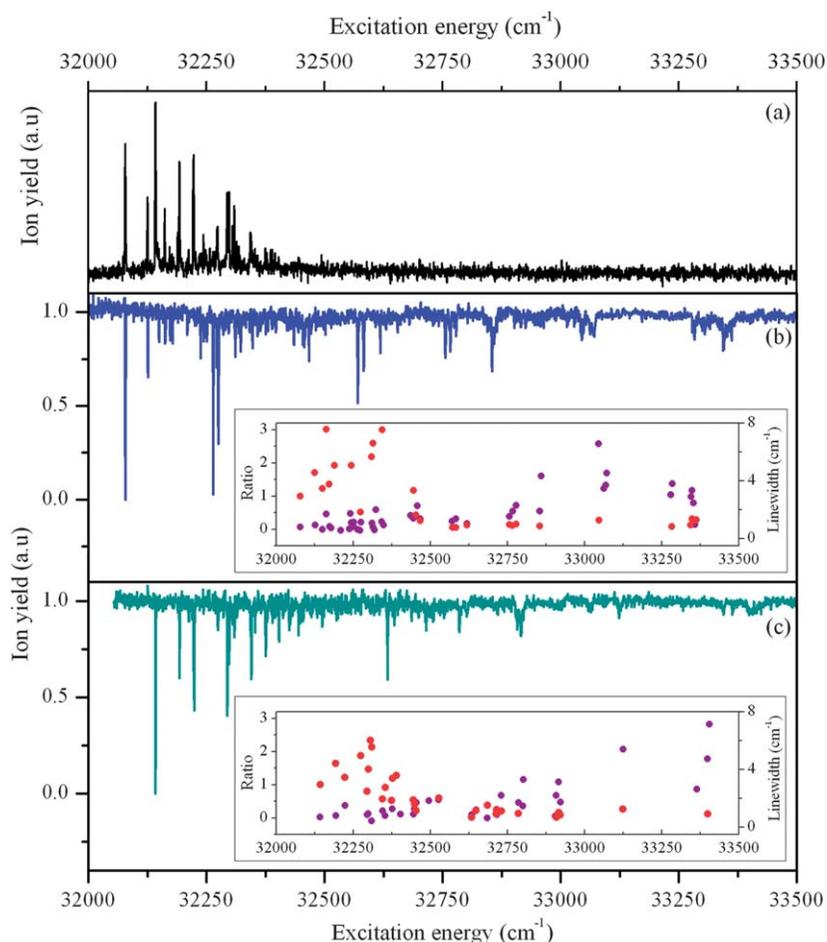
In the ns experiments, on the other hand, the ionization laser frequency is fixed at 51813  $\text{cm}^{-1}$  (6.424 eV), which according to our calculations is enough for efficient ionization of hot  $n\pi^*$  states. In fact, the situation and numbers are rather similar to the previously reported situation for *pCA* for which ionization with 193 nm was necessary to observe any REMPI signal at all.<sup>14</sup> In the ns pump-probe experiments on *OMpCA* we therefore observe a similar fast decay of the initially excited  $V'(\pi\pi^*)$  state as seen in the ps experiments, although this time not in the time domain but in the frequency domain. However, since there is enough energy for efficient ionization in the ns experiments, we are not “blind” to the hot  $n\pi^*$  states and can observe their time evolution.

On the basis of *ab initio* calculations on the potential energy surfaces of ground and excited states along the  $C_7=C_8$  double bond isomerization coordinate (see Scheme 1 for atom numbering), it has been suggested that a barrierless *trans-cis* isomerization process of the double bond *via* a conical intersection of the  $S_1$  and  $S_0$  state might contribute to the nonradiative decay dynamics of  $S_1$ .<sup>29</sup> The present results indicate that it is unlikely that such a process determines dominantly the

decay of  $S_1$ , even though we recognize that in the ns experiments we are “blind” to the hot ground-state species that would be created in such a process.<sup>48</sup> On the other hand, our calculations indicate that the  $V'(\pi\pi^*)$  state has a stable minimum on its potential energy surface, which would preclude a barrierless isomerization process.

### Excited state dynamics of *OMpCA-H<sub>2</sub>O*

The  $(1 + 1')$  RE2PI excitation spectrum detected at the mass of the *OMpCA-H<sub>2</sub>O* cluster is displayed in Fig. 3a. Overall, the spectrum reproduces the spectrum detected with LIF,<sup>27</sup> although the relative intensity of the origin band of the *s-trans* conformers appears somewhat low. Plots of the ratio of band intensities in the LIF and RE2PI spectra (not shown) do not show any systematic trends, and therefore do not give reason to believe that there is a fundamental difference in the non-radiative decay channels of the *s-cis* and *s-trans* conformers. Fig. 3b and 3c show



**Fig. 3** (a) RE2PI excitation spectrum of *OMpCA-H<sub>2</sub>O* and UV-UV depletion spectra of (b) *s-cis* of *OMpCA-H<sub>2</sub>O* and (c) *s-trans* *OMpCA-H<sub>2</sub>O*. The insets of panels (b) and (c) show the ratio of band intensities in the RE2PI and depletion spectra (red) as well as their line width (purple).

three-colour UV-UV depletion spectra obtained at probe excitation energies of 32078.0 and 32142.0  $\text{cm}^{-1}$ , respectively, associated with the  $S_1 \leftarrow S_0$  origin transitions of the *s-cis* and *s-trans* conformers.<sup>49,50</sup> Careful analysis of these origin transitions show that they are actually composed of two close-lying transitions that we associate with the OH-*syn* and OH-*anti* conformations, thereby confirming the conclusions drawn in the LIF hole burning experiments.<sup>28</sup> Compared to the spectra of the bare molecule, the band shape of the origin transitions in the water cluster changes significantly. Fits with a Gaussian line shape now give better results than fits with a Lorentzian line shape, and the bands have a much smaller FWHM, which appears to be determined by inhomogeneous rotational broadening.

Interestingly, the intensities of the one- and two-colour RE2PI signals are rather similar for the OMPCA-H<sub>2</sub>O cluster, in contrast to the situation for the bare molecule where the two-colour signal is considerably enhanced. In the first instance one might suspect that this is due to the decrease of the ionization energy of the cluster compared to the bare molecule (0.442 eV),<sup>28</sup> which could lead to a more efficient ionization of the  $n\pi^*$  state in the cluster. However, experiments in which the decay of the REMPI signal is monitored as a function of the delay between excitation and ionization show a signal that follows the convolution of the time profiles of the two lasers and does not give any indication of a long-living state. This conclusion is in agreement with the ps pump-probe studies in which lifetimes of  $930 \pm 200$  and  $700 \pm 200$  ps were reported at the *s-cis* and *s-trans* origin transitions.

We thus conclude that in the cluster the internal conversion pathway to the  $n\pi^*$  state is blocked near the vibrationless level, and that the dominant decay channel of the  $V'(\pi\pi^*)$  state is internal conversion to the ground state. Further support for these conclusions is found in the results of *ab initio* calculations on the vertical and adiabatic excitation energies of the lower electronically excited states in the OMPCA-H<sub>2</sub>O cluster (Table 1). We find that the two  $\pi\pi^*$  states are red-shifted -the red shift of the  $V'$  state being twice as large as that of the  $V$  state- whereas the  $n\pi^*$  state undergoes a blue shift. Such shifts are consistent with the general rule that solvation by proton-donating solvents causes a red shift of  $\pi\pi^*$  transitions, while  $n\pi^*$  states are blue-shifted by the increased stabilisation of the ground state compared to the excited state.<sup>51</sup> As a result of the opposing shifts of the  $\pi\pi^*$  and  $n\pi^*$  states, the adiabatic excitation energy of the  $n\pi^*$  state in the cluster becomes *higher* than that of the  $V'(\pi\pi^*)$  state, thereby explaining the experimentally observed absence of the  $V'(\pi\pi^*) \rightarrow n\pi^*$  internal conversion pathway.

Fig. 2 and 3 show that complexation of OMPCA with water changes the general features of the RE2PI excitation spectrum of the chromophore considerably. For the bare molecule extensive vibrational activity is observed but in the water cluster such activity vanishes  $\sim 400 \text{ cm}^{-1}$  above the origin. The UV-UV depletion spectra reported in Fig. 3b and 3c, on the other hand, show vibrational activity up to much higher excitation energies. The loss in signal intensity in the RE2PI spectrum is therefore not due to a loss in absorption intensity, but should be attributed to the onset of a non-radiative decay channel to a state with a significantly reduced ionization cross section.<sup>52,53</sup> In agreement with this conclusion we find that the excitation spectrum that is obtained when the two UV-UV depletion spectra are added correlates well with the RE2PI spectrum reported for ps laser excitation.<sup>29</sup>

Importantly, however, the higher spectral resolution of the present experiments gives us access to conformation-specific excitation spectra and decay dynamics. In order to obtain further information on the threshold of the decay channel and on its conformational dependence we have plotted in Fig. 3b and 3c the ratio of band intensities in the RE2PI and depletion spectra, normalizing this ratio to 1.0 at the electronic origin transitions of the *s-cis* and *s-trans* conformers. The same Figures show the line widths of transitions in the depletion spectra. The dependence of both the intensity ratio as well as the line width on the excitation energy clearly indicates that at higher excitation energies an additional decay channel opens up. For the *s-cis* conformers this occurs  $\sim 400\text{--}500\text{ cm}^{-1}$  above the origin, for the *s-trans* conformers at slightly higher excess energies ( $\sim 600\text{ cm}^{-1}$ ).

*A priori* various pathways come to mind as possible candidates for such a decay channel. What is important to bear in mind when considering these channels is the experimental observation that the state to which decay takes place cannot be ionized. As will be argued below, this condition excludes any mechanism that produces OMpCA in electronically excited states such as photodissociation, internal conversion to the  $n\pi^*$  state, and conformational changes in electronically excited states. Photodissociation of the complex into water and OMpCA leads to electronically excited OMpCA, which subsequently can be ionized by the ionization laser. One would thus still be able to observe OMpCA-H<sub>2</sub>O resonances, albeit not in the mass channel of the complex but in that of the bare molecule. Mass-resolved wavelength scans of the excitation region of interest monitoring the OMpCA mass do not show such resonances, and we therefore have to conclude that photodissociation is not responsible for the increased decay rate.

In the bare molecule the excited-state dynamics are dominated by internal conversion to the  $n\pi^*$  state. *Ab initio* calculations and RE2PI decay measurements show that in the complex this channel is blocked near the vibrationless level (*vide supra*). Table 1 indicates that in the cluster the adiabatic excitation energy of the  $n\pi^*$  state is only slightly higher than that of the  $V'(\pi\pi^*)$  state and one might therefore assume that internal conversion comes into play at higher excitation energies. In such a case the decay rate of  $V'(\pi\pi^*)$  levels is enhanced, leading to broader bands, but one is not able to explain the loss of signal in the RE2PI spectrum since the  $n\pi^*$  state populated after internal conversion can still be ionized. Using a similar reasoning one can exclude mechanisms based on bond isomerization in the electronically excited state without further decay to the electronic ground state.

Radiationless decay mechanisms that involve internal conversion to the electronic ground state, on the other hand, are in excellent agreement with our experimental observations as they produce hot ground state molecules that cannot be ionized by single-photon absorption, and thus lead to a loss of signal in the RE2PI spectrum. One of these mechanisms is *trans-cis* isomerization of the C<sub>7</sub>=C<sub>8</sub> double bond *via* a conical intersection between S<sub>1</sub> and S<sub>0</sub> as suggested previously in the ps pump-probe experiments.<sup>29</sup> In recent years it has become clear from theoretical studies on the primary isomerization pathway of the chromophore that the *trans-cis* isomerization process not only involves torsion around the C<sub>7</sub>=C<sub>8</sub> double bond, but also other vibrational coordinates, most notably torsion around the C<sub>4</sub>-C<sub>7</sub> single bond.<sup>19,22,54,55</sup> Single-bond isomerization in the excited state might thus as well accelerate internal conversion to the ground state. In fact, femtosecond upconversion studies on the viscosity dependence of

the excited-state dynamics of neutral OMPCA have been interpreted as indicating that in OMPCA isomerization occurs *via* a concerted motion of double and single bonds.<sup>31</sup>

Our experiments show that in the bare molecule *trans-cis* isomerization is of minor importance while it dominates the excited-state dynamics of the OMPCA-H<sub>2</sub>O cluster, implying that *trans-cis* isomerization is facilitated considerably by complexation with water. Such a conclusion finds further support in previous experimental results and in the results of our calculations. Molecular beam IR absorption experiments have shown that water complexation decreases the phenolic OH stretch frequency by 156 cm<sup>-1</sup> and leads to a large increase of its transition dipole moment.<sup>28</sup> Because of the hydrogen bonding, we anticipate that in the cluster a quinone-like electronic configuration, in which the double bond character of the C<sub>7</sub>=C<sub>8</sub> bond is reduced, gains importance. Inspection of the equilibrium geometry of the V'(ππ\*) state in the absence and presence of water indeed reveals that upon complexation the C<sub>7</sub>=C<sub>8</sub> double bond length increases by 0.006 Å, and that in the benzene ring the C<sub>2</sub>-C<sub>3</sub> and C<sub>5</sub>-C<sub>6</sub> bond lengths decrease while the other bond lengths in the ring increase slightly. Interestingly, the C<sub>4</sub>-C<sub>7</sub> bond length remains unaffected. Furthermore, in agreement with the EOM-CCSD data,<sup>27</sup> a comparison of the computed oscillator strengths on the CC2 level for the bare and complexated OMPCA indicate a strong mixing of the V and V' state for the OMPCA-H<sub>2</sub>O cluster. The change of excited state character upon complexation also involves an increase of the antibonding character with respect to the C<sub>7</sub>=C<sub>8</sub> bond, which promotes the *trans-cis* isomerization pathway. In a zeroth-order view on the isomerization process one might thus say that water complexation leads to loss of double bond character of the C<sub>7</sub>=C<sub>8</sub> double bond, which in turn lowers the barrier associated with torsion around this bond and thereby facilitates access to geometries where internal conversion to the electronic ground state is enhanced.

### The S<sub>2</sub> (V(ππ\*)) state of OMPCA

Up to this point, we have only considered the S<sub>1</sub> (V'(ππ\*)) state as the carrier of the RE2PI spectra. Yet, *ab initio* calculations<sup>15,19,27</sup> indicate that in the excitation energy range explored in Fig. 1, transitions to the S<sub>2</sub> (V(ππ\*)) state are expected to be present as well, certainly when taking into account that the V(ππ\*) ← S<sub>0</sub> oscillator strength is predicted to be larger than the oscillator strength of the V'(ππ\*) ← S<sub>0</sub> transition. In the following we will argue that the RE2PI spectrum indeed contains contributions from transitions to both states. To this purpose we will consider the line intensities in the RE2PI and LIF excitation spectra, and the Franck-Condon factors predicted for the V'(ππ\*) ← S<sub>0</sub> and V(ππ\*) ← S<sub>0</sub> transitions. Fig. 4 displays both the (1 + 1') RE2PI excitation spectrum measured in the present study and the previously reported LIF spectrum.<sup>27</sup> Comparison of the two spectra leads to the conclusion that some 700 cm<sup>-1</sup> above the lowest origin transition the intensities in the two spectra start to become quite different. The UV-UV depletion spectra displayed in Fig. 1 show that concurrently the bands become broader.

Further input is provided by the Franck-Condon factors reported in ref. 27 for the V'(ππ\*) ← S<sub>0</sub> transition. Here it was found that relatively small changes in geometry occur upon excitation to the V'(ππ\*) state. Accordingly, the vibronic

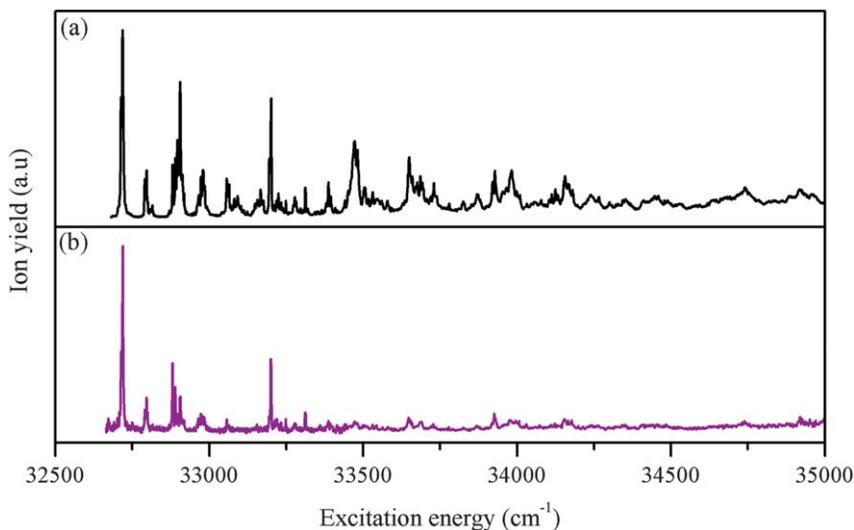


Fig. 4 (a)  $(1 + 1')$  RE2PI and (b) LIF excitation spectrum of OmpCA.

activity for the  $V'(\pi\pi^*) \leftarrow S_0$  transition is rather limited and actually follows quite nicely the activity observed in the LIF spectrum. The differences between LIF and RE2PI spectra therefore are not caused by a loss in fluorescence intensity as would occur, for example, if an additional non-radiative channel would open up  $700\text{ cm}^{-1}$  above the vibrationless level of the  $V'(\pi\pi^*)$  state. Instead, the appropriate interpretation should be that in the RE2PI spectra transitions to another electronic state are visible which are much weaker in the LIF spectrum. Based on the predicted excitation energy of the  $V(\pi\pi^*)$  state, we associate these transitions with the  $V(\pi\pi^*)$  state. On the basis of line widths and deviations from expected Franck–Condon intensities we tentatively assign the  $V(\pi\pi^*) \leftarrow S_0$  origin transition of the *s-cis* OH-*syn*, *s-cis* OH-*anti*, *s-trans* OH-*anti*, and *s-trans* OH-*syn* conformers to the  $33466.7 \pm 0.2$ ,  $33473.9 \pm 0.2$ ,  $33645.3 \pm 0.2$ , and  $33650.8 \pm 0.2\text{ cm}^{-1}$  bands in their UV-UV depletion spectra (see Fig. 1). A coarse comparison of the observed vibronic activity in the  $S_2 \leftarrow S_0$  part of the single-conformation excitation spectra (Fig. 1) with the activity predicted in ref. 27 shows a reasonable agreement. A more detailed inspection reveals that they contain a mixture of broad and more narrow transitions. The narrow transitions most likely are associated with  $S_1$  vibronic transitions that obtain transition intensity by vibronic coupling to the  $S_2$  state.

In particular in the UV-UV depletion spectra of the *s-cis* OH-*syn* and *s-cis* OH-*anti* conformers, a reasonable strong transition is observed at about  $484\text{ cm}^{-1}$  from the origin which does not fit the predicted Franck–Condon intensity.<sup>56</sup> However, dispersed emission spectra recorded at these excitation energies<sup>27</sup> have shown that in the emission spectra unpredicted activity is present at similar vibrational energies as well. The transition should thus be assigned to a  $S_1 \leftarrow S_0$  vibronic transition for which the intensity apparently is not correctly predicted by the Franck–Condon calculations.

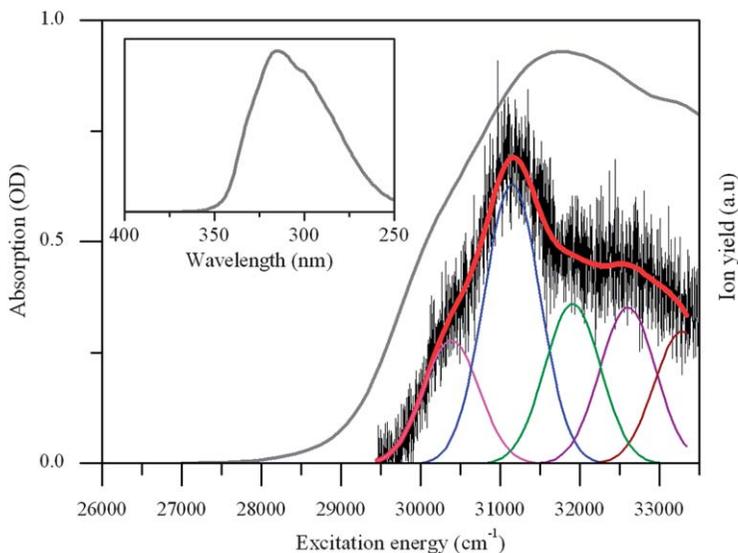
Although the vibronic activity in the RE2PI spectra can now be well understood, the intensity differences between the RE2PI and LIF spectra require some

further attention. Kasha's rule tells us that, in the absence of  $S_2$  decay channels other than internal conversion to  $S_1$ , line intensities in the two spectra should be the same. The observation that this is not the case implies that the  $S_2$  state has an additional – in this case dominating – decay channel that brings the molecule into a state from which it can be ionized, but from which radiative decay to the ground state is less efficient. The logical candidate for this pathway is direct internal conversion to the  $n\pi^*$  state. This conclusion is further supported by the observation that for transitions assigned to  $S_2$  the RE2PI signal decays with a similar time constant as for transitions assigned to  $S_1$  (see Fig. 2).

Now that the  $S_2 \leftarrow S_1$  spectrum has been identified in the bare chromophore, one might wonder where the  $S_2 \leftarrow S_1$  spectrum is located for the  $OMpCA-H_2O$  cluster. Table 1 indicates that the energy difference between the origin transitions of  $S_1$  and  $S_2$  should be roughly  $600\text{ cm}^{-1}$  larger in the cluster than in the bare molecule. Although a number of broader transitions are present in this part of the spectrum (see Fig. 3), there are no other indications in terms of intensity or other criteria that would warrant to assign them as transitions to  $S_2$ .

### Excited state dynamics of $TMpCA$

The  $(1 + 1')$  RE2PI jet-cooled excitation spectrum of  $TMpCA$  is shown in Fig. 5. In contrast to  $OMpCA$  for which the excitation spectrum shows well-resolved vibrational structure, the spectrum of  $TMpCA$  only displays a broad band system with little structure. The band system has an onset at about  $29\,700\text{ cm}^{-1}$  and a first clearly discernible inflection point at about  $30\,400\text{ cm}^{-1}$ . The red shift of  $\sim 3000\text{ cm}^{-1}$  with respect to the excitation spectrum of  $OMpCA$  is in the range expected from theoretical calculations<sup>19</sup> which predict that the vertical excitation energies of



**Fig. 5**  $(1 + 1')$  RE2PI excitation spectrum of  $TMpCA$  (black). The red spectrum is the spectrum obtained by fitting the experimental spectrum with five Gaussian bands. The grey spectrum is the absorption spectrum recorded for  $TMpCA$  dissolved in *n*-hexane at room temperature. A more extended absorption spectrum under such conditions is displayed in the inset of the figure.

the  $V'(\pi\pi^*)$  state in *OMpCA* and the  $V(\pi\pi^*)$  state in *TMpCA* differ by 0.1 to 0.3 eV. To exclude that imperfect cooling in the molecular beam is responsible for the observed broadening, we have performed various experiments in which molecular beam conditions such as the carrier gas (He, Ne, or Ar), backing pressure, and heating temperature of the sample have been varied. However, none of these experiments gave rise to better resolved excitation spectra. The broadening of the bands is therefore an intrinsic property of *TMpCA* and not due to experimental conditions. Band broadening is indicative of a short-lived excited state, a conclusion that finds further support in the RE2PI signal intensities which are considerably lower for *TMpCA* than for *OMpCA*. In this respect our experimental observations parallel what is observed in REMPI spectroscopic studies on nucleobases and base pairs where efficient coupling of excited states to the ground state *via* conical intersections lead to broad, unresolved REMPI excitation spectra.<sup>57</sup>

Although the spectrum shows little structure, it is clear that it cannot be represented by a single Gaussian or Lorentzian band. A coarse inspection of the inflections in the spectrum suggests that it is composed of at least four bands. To obtain further information on possible values of band widths and energy spacings we have fitted the spectrum with a sum of Gaussian bands. In view of the limited resolution we have taken in the first instance a minimal approach and assumed that all of these bands have the same width and are equally spaced from each other. We then find that the spectrum can be well fitted (see red spectrum in Fig. 5) with the sum of five Gaussian bands with a width of  $352.5 \pm 5.6 \text{ cm}^{-1}$  and with a spacing of  $725 \pm 40 \text{ cm}^{-1}$ . In Fig. 5 the jet-cooled excitation spectrum of *TMpCA* is compared with the absorption spectrum recorded in a solution of n-hexane at room temperature. The solution spectrum is in good agreement with spectrum reported before for *TMpCA* in an aqueous buffer solution<sup>30</sup> albeit that the n-hexane spectrum shows somewhat more resolution and is displaced to slightly higher excitation energies. Comparison of the jet-cooled and solution-phase spectra shows that the bands in the jet-cooled spectrum are considerably more narrow and confirms our conclusion that the band widths in the former spectrum are not determined by the cooling conditions in the molecular beam.

Calculations predict that upon going from *OMpCA* to *TMpCA* significant changes occur in the ordering of the lower electronically excited states.<sup>15,19</sup> The  $V'(\pi\pi^*)$  and  $V(\pi\pi^*)$  state are reversed, the  $V(\pi\pi^*)$  state becoming the lowest excited  $\pi\pi^*$  state, while the  $n\pi^*$  state is now both vertically as well as adiabatically the lowest excited singlet state. It is thus reasonable to conclude that the large differences between the  $(1 + 1')$  RE2PI excitation spectra of *OMpCA* and *TMpCA* are associated with differences in the structural and dynamic properties of the  $V'(\pi\pi^*)$  and  $V(\pi\pi^*)$  states. A further interesting aspect that appears from the calculations is that they predict that the oscillator strengths for the two  $\pi\pi^*$  states are quite different for the two compounds. At the EOM-CCSD level the oscillator strength of the  $V(\pi\pi^*) \leftarrow S_0$  transition in *OMpCA* is about seven times as large as that of the  $V'(\pi\pi^*) \leftarrow S_0$  transition, in *TMpCA* both transitions have roughly the same oscillator strength. The  $(1 + 1')$  RE2PI spectrum depicted in Fig. 5 therefore most likely contains contributions from transitions to both states, and one should be cautious to give too much weight to the Gaussian fit discussed above. Nevertheless, the low-energy part of the spectrum definitely indicates that a  $\sim 725 \text{ cm}^{-1}$  vibrational mode plays a prominent role in the spectrum and that much smaller band widths than  $350 \text{ cm}^{-1}$  cannot be accommodated by the spectrum (however, *vide infra*).

Although we cannot rigorously exclude it, it seems unlikely that the band width of  $350\text{ cm}^{-1}$  can be attributed entirely to lifetime broadening since it would imply an excited-state lifetime on the order of a few tens of femtoseconds. More likely is an explanation that combines extensive Franck–Condon activity of low-frequency modes in the electronically excited state with lifetime broadening. For *OMpCA* we have observed that internal conversion to the  $n\pi^*$  state dominates the excited-state dynamics. One might therefore speculate that in *TMpCA* internal conversion to the  $n\pi^*$  state is enhanced, leading to larger line widths. Such a scenario in which bands with a width on the order of tens of wavenumbers are observed in jet-cooled excitation spectra due to internal conversion to another long-living excited state are not without precedence.<sup>58</sup> However, measurements of the decay of the  $(1 + 1')$  RE2PI signal in *TMpCA* do not give any evidence for a possible decay to a longer-living state.<sup>59</sup>

The considerations discussed above, in combination with the photochemical properties of the  $V(\pi\pi^*)$  state appearing from theoretical and experimental studies, suggest that photoisomerization is the most likely candidate for the observed broadening. In this respect it is worth noticing that calculations we performed on the ground and excited states of *TMpCA* readily identify the  $725\text{ cm}^{-1}$  mode active in the spectrum as a mode that has a large contribution of double bond torsion and to a minor extent of single bond torsions. The same calculations enable us to make a rough estimate of the minimum lifetime of the excited state compatible with the observed  $350\text{ cm}^{-1}$  width. To this purpose we assume that the width is caused by unresolved Franck–Condon activity of a low-frequency mode, and that the transitions to each of these vibronic levels are lifetime-broadened. The calculations indicate that the minimal spacing in such a Franck–Condon progression is at least  $20\text{ cm}^{-1}$ , implying that the transitions should have a similar width to become indistinguishable.<sup>60</sup> Transient absorption studies of the excited-state dynamics of the neutral form of *TMpCA* in aqueous solutions report an excited-state lifetime of  $3.7\text{ ps}$ ,<sup>30</sup> which corresponds to a homogeneous line width of  $1.4\text{ cm}^{-1}$ . Such a line width clearly is incompatible with the gas-phase excitation spectrum. The present results thus suggest that the radiationless decay rate to the ground state is substantially larger in the gas phase than in solution. Assuming that this decay rate is closely related to bond isomerization processes, this implies that solvation impedes these isomerization processes.

## Conclusions

Understanding the photophysics and photochemistry of photoactive proteins requires a profound understanding of the structural and dynamical properties of the lower excited states of the chromophore that initiates the photocycle, and the influence exerted on these primary steps by external factors like substituents and solvent molecules. The present work has provided a detailed view on the properties of electronically excited states of chromophores of the Photoactive Yellow Protein. Nanosecond RE2PI excitation studies in combination with pump–probe studies of the first optically bright state in *OMpCA* (the  $V(\pi\pi^*)$  state) provide unambiguous evidence that internal conversion to the adiabatically lower-lying  $n\pi^*$  state is an important decay channel in the ps decay of this state. Although there are formally no dark states in multiphoton ionization, the present study has

emphasized the importance of the energy of the photon used for ionization of the molecule from an excited state, in particular when electronically excited states are studied that are not the lowest excited state, and/or when ionization to the ground ionic state has a low cross section on account of the electronic character of the excited and ionic states. Comparison of RE2PI and LIF excitation spectra and consideration of the Franck-Condon activity visible in conformation-specific excitation spectra has led us to propose that the excitation spectrum of the  $S_2$  state of OMPCA (the  $V(\pi\pi^*)$  state) starts some  $760\text{ cm}^{-1}$  higher in energy than the  $S_1 \leftarrow S_0$  excitation spectrum. The differences between the RE2PI and LIF excitation spectra indicate that internal conversion to the  $n\pi^*$  state is also for  $S_2$  the dominant decay channel.

Complexation of OMPCA with water leads to large differences in the excited-state dynamics of the  $V(\pi\pi^*)$  state, and has a large impact on isomerization pathways. The  $V(\pi\pi^*) \rightarrow n\pi^*$  internal conversion pathway is completely shut down near the bottom of the  $V(\pi\pi^*)$  well, and appears to remain shut down for higher excitation energies as well. This observation has been rationalized by *ab initio* calculations which show that complexation with water lowers the excitation energy of the  $\pi\pi^*$  states and increases that of the  $n\pi^*$  state to such an extent that the  $V(\pi\pi^*)$  state becomes the lowest excited singlet state in the OMPCA- $H_2O$  cluster. In agreement with previous ps pump-probe experiments<sup>29</sup> we find that at excess vibrational energies of some  $400\text{--}600\text{ cm}^{-1}$  an additional radiationless decay channel to the ground state, which we associate with isomerization, opens up. In the present experiments we cannot determine the final distribution over the various possible single- and double-bond isomers in the electronic ground state. At the same time it is clear that such information would provide key information on salient details of the potential energy surface of the excited state. We are therefore presently setting up experiments similar to the ones reported in ref. 53 which will enable us to probe the ground state after internal conversion from the excited state.

It is interesting to notice that both transient absorption<sup>30</sup> and fluorescence upconversion<sup>31</sup> experiments on neutral OMPCA in aqueous solution conclude that the excited state has a lifetime of 2–3 ps, and that both find no evidence for the presence of a long-lived excited state. This demonstrates that under such conditions internal conversion to the  $n\pi^*$  state is indeed not a competitive process, in agreement with the present results. Moreover, for excitation of excited-state levels above the barrier of  $400\text{--}500\text{ cm}^{-1}$  in the *s-cis* conformers and  $600\text{ cm}^{-1}$  in the *s-trans* conformers, the measured lifetime is in good agreement with the lifetime determined in the ps pump-probe experiments (faster than 8 ps)<sup>29</sup> and with our line width measurements. In view of the conclusion of the present studies that the excited-state dynamics of OMPCA are affected by complexation with hydrogen-bonding solvents, it would be of interest to perform similar time-resolved experiments on OMPCA in non-hydrogen-bonding solvents. The present studies suggest that under such conditions the excited state might have a longer lifetime.

The present study has reported the first jet-cooled excitation spectrum of TMPCA. In striking contrast with the excitation spectrum of OMPCA, the excitation spectrum of TMPCA is broad and almost featureless, and is shifted by about  $3000\text{ cm}^{-1}$  to lower energies. These differences have been rationalized in terms of the excited-state dynamics associated with the  $V(\pi\pi^*)$  and  $V(\pi\pi^*)$  states. In view of the different electronic nature of the two states, such differences are not

unexpected. What is actually more surprising – given that the two chromophores have a different lowest excited  $\pi\pi^*$  state – is that in solution OMpCA and TMpCA have quite similar excited-state lifetimes (2.1 and 3.7 ps, respectively). These observations once more highlight the importance of solute–solvent interactions for the excited-state dynamics of these two chromophores, and indicate that further time-resolved studies on OMpCA and TMpCA exploring a wider range of solvents would be useful.

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