Non-Equilibrium Isomer Distribution of the Gas-Phase Photoactive Yellow Protein Chromophore

Mitra Almasian,[†,‡] Josipa Grzetic,[†] Johanne van Maurik,[†,‡] Jeffrey D. Steill,[†,∥] Giel Berden,[†] Steen Ingemann,[‡] Wybren Jan Buma,*,[‡] and Jos Oomens*,[†,‡,§]

[†]FOM Institute for Plasma Physics Rijnhuizen, Edisonbaan 14, 3439MN Nieuwegein, The Netherlands
[‡]van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands
[§]Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525AJ Nijmegen, The Netherlands

Ⓢ Supporting Information

ABSTRACT: The conjugate base of *para*-coumaric acid, which can be conveniently generated in the gas phase by electrospray ionization (ESI), is a commonly used model system for the chromophore of the photoactive yellow protein. Here we report its gas-phase IR spectrum, which shows that the anion easily adopts a carboxylate structure lying 60 kJ/mol higher in energy than the global minimum phenoxide structure. Generation of the biologically more relevant phenoxide isomer by ESI can be achieved using dry acetonitrile as solvent.
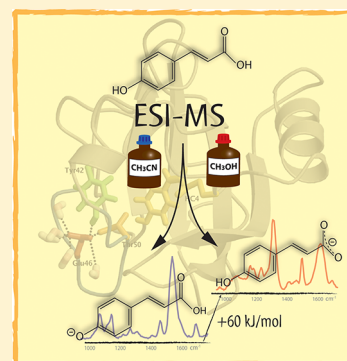
SECTION: Spectroscopy, Photochemistry, and Excited States

The conjugate base of *para*-coumaric acid (*p*-CA, HO—C$_6$H$_4$—CH=CH—COOH) has been under substantial experimental[1−6] and theoretical[7−11] study because it is considered to be a model system for the chromophore in the photoactive yellow protein (PYP).[12,13] In the protein, the anionic chromophore is embedded in a hydrophobic pocket where it is covalently linked to a Cys residue by a thio-ester bond replacing the carboxylic OH of *p*-CA.[13] Spectroscopic studies on PYP in solution revealed that the absorption maxima closely match those of the gas-phase chromophore.[14] In contrast, a substantial blue shift is observed for the chromophore in an aqueous environment, which was interpreted to be evidence of the hydrophobic nature of the protein pocket; it suggests that studies on the anionic chromophore are more relevant to the biological situation when performed in the gas phase instead of in aqueous solution.[15]

Although deprotonation of the chromophore in the protein pocket can occur only at the phenolic OH, isolated *p*-CA possesses two acidic protons so that two isomeric anions can be formed: a carboxylate and a phenoxide anion (see Chart 1). Calculations predict the phenoxide form to be around 60 kJ/mol more stable in the gas phase,[9] which can be rationalized by the two resonance structures efficiently delocalizing the negative charge over the entire molecule. The carboxylate isomer, in contrast, is more stable in aqueous solution, where the charge localized largely on the carboxylate O-atoms is effectively stabilized by H-bonding with protic solvent

Chart 1. Two Isomeric Forms of Deprotonated *trans-para*-Coumaric Acid (*p*-CA): Carboxylate (left) and Phenoxide (right)[a]
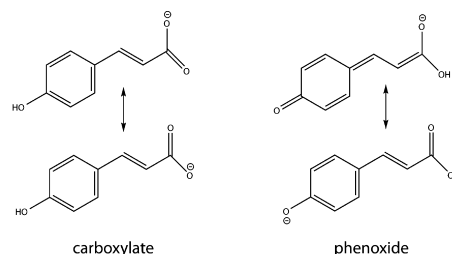
[a]Two resonance forms for the phenoxide isomer efficiently delocalize the charge over the entire molecule, explaining its greater stability in the gas phase.

molecules. These effects are analogous to those discussed in detail for *para*-hydroxybenzoic acid[16] (*p*-HBA, HO—C$_6$H$_4$—COOH), although the difference between the two isomers is 'only' around 30 kJ/mol.

The gas-phase anion of *p*-CA is conveniently generated using electrospray ionization (ESI). It is commonly assumed, particularly for small molecules, that ESI generates the global
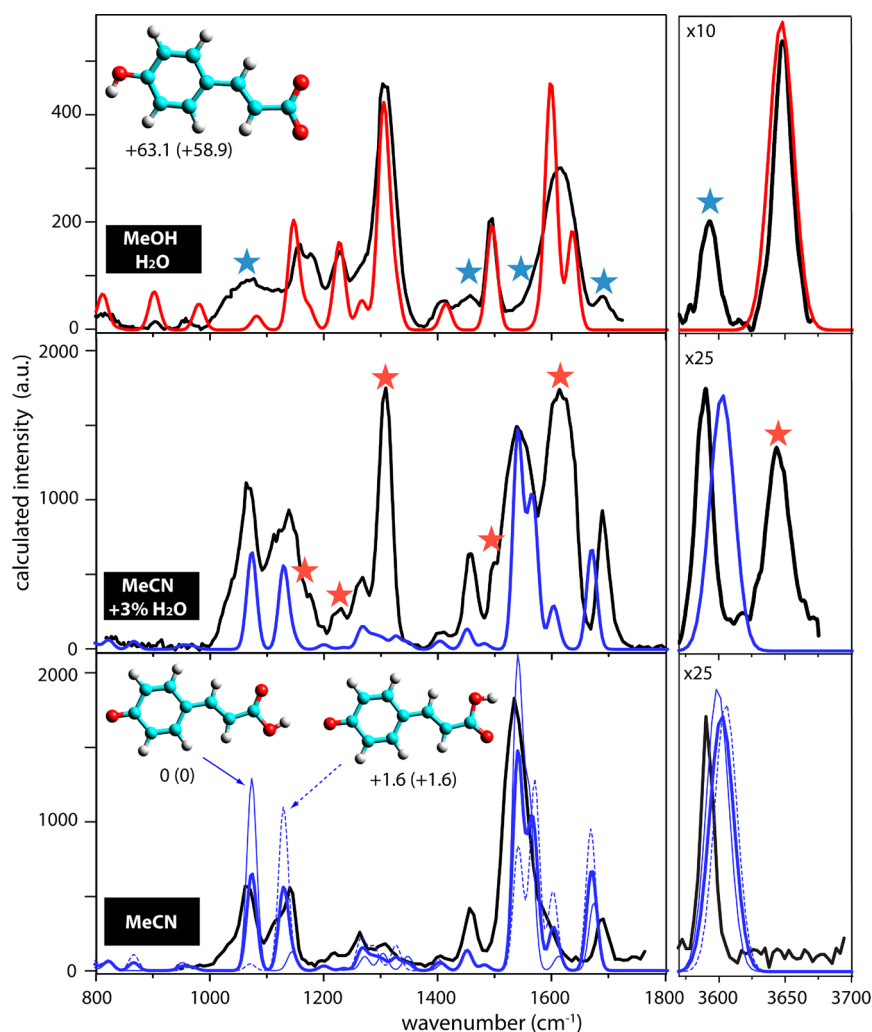
Figure 1. Experimental spectra for the *p*-coumarate anion sprayed from protic (MeOH/H₂O) and aprotic (MeCN) solvents compared with calculated spectra for the carboxylate (red) and phenoxide (blue) isomers of the anion. Relative ZPE corrected energies are given in kilojoules per mole, with Gibbs free energies in parentheses. The cis and trans conformers of the phenoxide isomer are close in energy, and their spectra (thin blue lines) have been averaged (thick). Bands marked with a star have been assigned as originating from contributions of the alternative isomer.

minimum molecular structure — the structure thus being independent of ESI conditions. However, in view of recent studies on the anion of *p*-HBA,[16−18] it is not obvious whether this is true for the *p*-coumarate anion. The deprotonation site of *p*-HBA has been shown to depend on the protic or aprotic nature of the ESI solvent used. IR spectra of the gas-phase anion unambiguously identified the resulting isomeric structure,[18] correcting structural assignments derived from ion−molecule and CID reactions.[17,19] The carboxylate structure prevalent in protic solvents is retained upon ESI and becomes kinetically trapped once in the gas phase. In an aprotic solvent, the carboxylate structure is not stabilized as strongly and ESI is found to produce the global minimum gas-phase structure, that is, the phenoxide anion. Whether analogous effects are at play upon ESI of *p*-CA, where thermodynamics favor the phenoxide isomer still more strongly, is the subject of this study.

A gas-phase UV/vis action spectrum of the *p*-coumarate anion was recorded in an electrostatic ion storage ring (ELISA).[1] Subsequent high-level calculations of the spectrum[9] were found to be at odds with the experimental data. This discrepancy was suggested to arise from complications in generating the mass-spectrometrically indistinguishable phenoxide and carboxylate precursor ions. Molecular-beam UV

spectroscopic studies on neutral *p*-CA[2−4] showed that a (mass-spectrometrically indistinguishable) 'impostor'[3] can readily replace the 'elusive'[2] PYP chromophore during its transfer to the gas phase. To avoid ambiguities in the deprotonation site of *p*-CA, methyl-derivatives can provide isomer specificity, although even studies such as these are not always unambiguous.[1,9] Adequate knowledge of the isomeric structure of *p*-CA in the gas phase and its dependence on ESI conditions is essential to interpret experimental and theoretical investigations of its photochemical properties. Here we present gas-phase IR spectra of the isolated *p*-coumarate anion generated by ESI using a combination of tandem mass spectrometry and tunable infrared lasers.[20]

Various conformers exist for each of the *p*-coumarate isomers, although only a few are of interest in this study. For the carboxylate isomer, two conformers resulting from a 180° rotation around the phenolic C−OH bond are within 1 kJ/mol and have virtually identical calculated spectra (red trace in Figure 1, see also Figure S1 of the Supporting Information). For the phenoxide isomer, two conformers resulting from a cis and trans configuration of the carboxy and vinyl double bonds are also close in energy, but their calculated spectra are subtly different (full and dashed thin blue lines); the thick blue line

represents the averaged spectrum, which is diagnostically different from the carboxylate spectrum. (See Figure S2 of the Supporting Information for higher energy phenoxide conformers.)

IR multiple-photon excitation of deprotonated p-CA (m/z = 163) generates a fragment ion at m/z = 119 due to loss of neutral CO_2. Plotting the yield of this fragment as a function of the laser frequency gives the IR multiple-photon dissociation (IRMPD) spectrum of p-coumarate, shown as the black traces in Figure 1. The spectra in the top, middle, and bottom panels are obtained using as ESI solvent a MeOH/H_2O mixture, MeCN with a small amount (∼3%) of H_2O and dry MeCN, respectively. Clearly, the three experimental spectra are substantially different, indicating that the choice of ESI solvent alters the structure of the analyte. The spectrum obtained from the MeOH/H_2O solution (top panel of Figure 1) is in reasonable agreement with that predicted for the carboxylate isomer represented by the red trace. The characteristic OCO symmetric (1300 cm^{-1}) and asymmetric stretching (1600 cm^{-1}) bands as well as the phenolic CO stretching (1220 cm^{-1}) and COH bending (1150 cm^{-1}) vibrations are clearly identified. The sharp band around 1500 cm^{-1} is due to CC stretching of the aromatic ring. In the 3 μm spectral range, the phenolic OH stretch is recognized at 3650 cm^{-1}. The spectrum features a number of additional bands, marked with a blue star, that cannot be traced back to the carboxylate isomer, appearing with lower intensities near 3600, 1700, 1560, 1400, and 1050 cm^{-1}. We assign these bands to a small amount of phenoxide isomer in the ion population, as suggested by the favorable match with its computed spectrum (blue trace in middle panel). Although the 1560 cm^{-1} feature appears weaker than other phenoxide bands, additional experiments (not shown) indicate that this is due to enhanced IR induced electron detachment.[21,22] In conclusion, the carboxylate isomer, thermodynamically disfavored by 60 kJ/mol, dominates the ion population.

The spectrum of p-CA sprayed from dry MeCN displayed in the bottom panel of Figure 1 is substantially different from that obtained from the MeOH solution (top panel). The thin blue traces in the bottom panel represent the calculated spectra for the trans- and cis-conformers of the phenoxide isomer, the thick blue line representing the averaged phenoxide spectrum. Characteristic bands of the phenoxide isomer due to the conjugated CO stretch of the phenoxide moiety (1550 cm^{-1}), the carboxylic C=O stretch (1680 cm^{-1}), COH bending (1080 or 1125 cm^{-1} depending on conformer), and OH stretching (3590 cm^{-1}) modes are clearly identified in the experimental spectrum. Several weaker bands also match favorably with the calculated phenoxide spectrum. The double-peak structure around 1100 cm^{-1} indicates that trans- and cis-conformers of the phenoxide isomer are both present. An IRMPD spectrum of methyl-4-hydroxycinnamate, the deprotonated methylester of p-CA, further confirms our assignment as the phenoxide structure. (See Figures S3 and S4 of the Supporting Information.)

The spectrum obtained from the MeCN solution with a small amount of H_2O added (middle panel of Figure 1) largely features the same phenoxide IR bands but also bands that cannot be assigned to this isomer, marked with a red star. Frequencies and relative intensities of these bands match those expected for the carboxylate isomer. Adding just a small amount of protic solvent to the ESI solution thus quickly induces the formation of the carboxylate isomer. Note that

drifts in the exact amount of H_2O during the IR scan likely cause fluctuations in the relative abundances of the two isomers.

The observed IR-induced fragmentation, loss of 44 mass units, is independent of the solution conditions used; that is, it is identical for carboxylate and phenoxide isomers, as also observed for deprotonated p-HBA.[18] This suggests that both isomers detach neutral CO_2 upon activation, which raises the question whether both m/z 119 fragment ions possess the same structure. An IRMPD spectrum of this fragment anion (now generated by CO_2 laser irradiation) was therefore recorded using protic and aprotic spray solvents. The two spectra are identical (Figure 2) and can be attributed to the p-
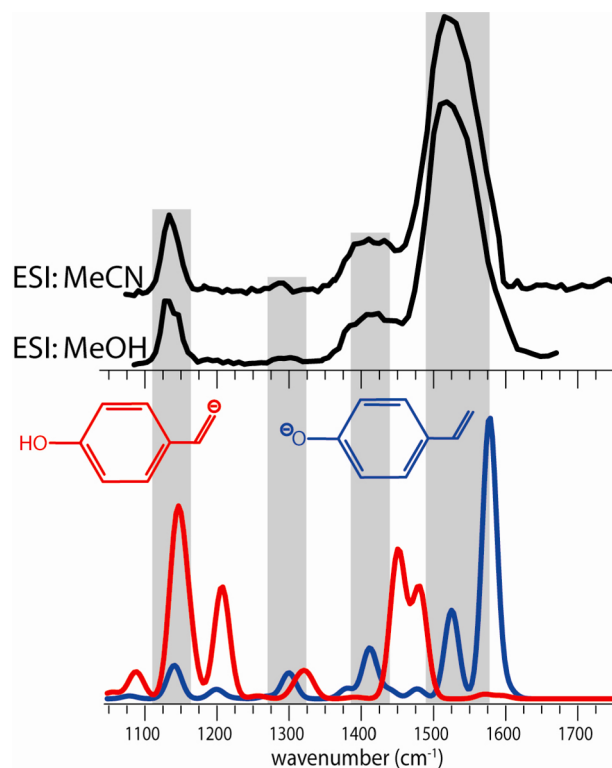

Figure 2. IRMPD spectra of the m/z 119 fragment anion generated by CO_2 laser-induced dissociation of the p-coumarate anion (m/z 163) produced by ESI from a protic solution (MeOH/H_2O) and an aprotic solution (dry MeCN). The spectra are clearly identical and correspond to the spectrum of the *para*-vinylphenoxide anion (blue), as indicated by the vertical shading. The alternative isomer, the *para*-vinylphenol carbanion (red), is higher in energy by 275 kJ/mol and is not formed, suggesting that proton shuttling accompanies CO_2-loss in the dissociation of the carboxylate isomer of p-coumarate.

vinylphenoxide ($H_2C=C-C_6H_4O^-$) isomer based on its calculated spectrum in blue. The shading indicates the match between experiment and theory for four main features, although the strongest feature (due to the unresolved stretching modes of the conjugated CO, aromatic CC, and vinylic CC bonds) is computed at slightly too high frequencies. This assignment is further confirmed by a scan of the OH stretching region, where no band was found (not shown). Apparently, activation and dissociation of the carboxylate p-CA isomer is accompanied by displacement of the phenolic proton. Although the reaction pathways are not accurately known, this suggests that isomer identification solely by CID-MS fragmentation products may be unreliable. Similar rearrangements displacing a

proton upon collisional ion activation have, for instance, been argued to impede the identification of zwitterionic amino acid structures by CID MS.[23]

Comparing these results to those for the conjugate base of *p*-HBA, we thus find a similar behavior despite the approximately two-fold increase in thermodynamic stability (from 34 to 60 kJ/mol) of the phenoxide isomer. Analogous to *p*-HBA, *p*-CA exists in its carboxylate form in protic solvents, and this structure is retained and becomes kinetically trapped in the gas phase upon generation by ESI. The substantial energy barriers in both systems likely prevent gas-phase isomerization at room temperature.[24] The only low-energy isomerization pathway probably involves the noncovalent binding of protic solvent molecules: multiple water (or alcohol) molecules may form a 'proton wire'[25] shuttling the proton in a Grotthuss-like fashion, which is a well-established mechanism in acid−base reactions in aqueous solution.[26−28] Such a mechanism has indeed been suggested recently in computational studies of the micro-hydrated *p*-coumarate anion, which identified local-minima structures for the carboxylate and phenoxide isomers complexed with one and two water molecules.[29] Cyclic structures, connecting the carboxylate and phenoxide moieties, were indeed identified for the doubly hydrated species, although they require a significant distortion of the anion away from the planar geometry; proton wires involving more water molecules would obviously reduce this distortion. In this scenario, the larger separation between the two basic sites in *p*-coumarate as compared with *p*-hydroxybenzoate requires more water molecules to form the wire, raising the entropic barrier to proton shuttling,[27] thus counteracting the effect of increased thermodynamic stability of the phenoxide isomer.

In conclusion, the IR spectra show that ESI of *p*-CA to form the *p*-coumarate anion results in surprisingly high abundances of the carboxylate isomer, disfavored by 60 kJ/mol. The lower-energy and biologically more relevant phenoxide isomer can be formed when using a dry MeCN solution. The addition of just a few percent of water quickly reduces the abundance of the phenoxide isomer.

■ EXPERIMENTAL SECTION

Infrared multiple-photon dissociation (IRMPD) spectra of deprotonated *p*-CA are recorded using an ESI Fourier transform ion cyclotron resonance mass spectrometer (ESI-FTICR-MS) coupled to the IR beamline of the free electron laser (FEL) 'FELIX',[30] as detailed elsewhere.[20]

The anions are produced by ESI in negative ion mode using three different solutions containing 1 mM *p*-CA:

(1) in protic solvent: methanol/H$_2$O (4:3) with 1 mM NaOH added

(2) in aprotic solvent: acetonitrile with 1 mM NaOH added as aqueous solution, giving a water content of about 3%

(3) in aprotic solvent: acetonitrile with about 0.5 mM phenanthridine added as base (no water added).

A Micromass 'Z-Spray' source is used as ESI source and ions are accumulated for a few seconds in an rf hexapole trap before being injected into the ICR cell via a quadrupole deflector and an octopole ion guide.[20,31,32] In the ICR cell, the precursor anion is mass-isolated using a stored waveform inverse Fourier transform (SWIFT) pulse.[33] The ions are subsequently probed by IR photodissociation spectroscopy, scanning the FEL frequency between 800 and 1800 cm^{-1}. The FEL radiation has a bandwidth of about 0.5% of the central frequency and

pulse energies of about 40 mJ per 5 μs pulse.[30] At each wavelength, the ions are irradiated for 3 s with 30 FEL pulses, and a mass spectrum is recorded. Three mass spectra are averaged and the fragmentation yield is computed as the ratio of fragment ion intensities over the combined intensities of fragment and parent ions. This yield is linearly corrected for wavelength-dependent variations in the laser pulse energy and plotted as a function of IR frequency to give an IR spectrum.

To record IR spectra in the hydrogen-stretching range around wavelengths of 3 μm, we coupled the FTICR-MS to the output of a Nd:YAG-pumped (Innolas Spitlight 600) Optical Parametric Oscillator (OPO, LaserVision, Bellevue, WA), which produces 6 ns pulses with energies up to 20 mJ in the 2500−4000 cm^{-1} frequency range. As a result of the weaker transitions in this frequency range (as compared with the mid-IR), longer irradiation times are required. The ions are irradiated for 7.5 s with 75 OPO pulses. To increase the on-resonance dissociation yield, the ions are irradiated for 20 ms with the output of a 30 W CO$_2$ laser directly after each OPO pulse.[34,35]

To determine the isomeric structure of the anion, the experimental spectra are compared with computed vibrational spectra of the anionic isomers in various conformations. Calculations are performed at the B3LYP/6-311++G(d,p) level of theory using Gaussian03, and harmonic frequencies are empirically corrected for anharmonicity using a scaling factor of 0.97 for the 800−1800 cm^{-1} range and 0.952 for the hydrogen stretching range. To facilitate comparison with experimental spectra, stick spectra are convoluted with a 20 cm^{-1} fwhm Gaussian line shape.

para-Coumaric acid and its oxy-ester, methyl-4-hydroxycin-namate, were purchased from Aldrich and ABCR, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Computed spectra for higher energy conformers. Experimental and theoretical spectra for the anionic methyl-ester of *p*-CA, methyl-4-hydroxycinnamate. Table with computed energies for phenoxide and carboxylate conformers of the *p*-coumarate anion. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: joso@rijnhuizen.nl (J.O.), w.j.buma@uva.nl (W.J.B.).

Present Address
‖Sandia National Laboratories, Livermore, California, United States.

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Rocha-Rinza, T.; Christiansen, O.; Rajput, J.; Gopalan, A.; Rahbek, D. B.; Andersen, L. H.; Bochenkova, A. V.; Granovsky, A. A.; Bravaya, K. B.; Nemukhin, A. V.; Christiansen, K. L.; Nielsen, M. B.

Gas Phase Absorption Studies of Photoactive Yellow Protein Chromophore Derivatives. *J. Phys. Chem. A* **2009**, *113*, 9442−9449.

(2) Smolarek, S.; Vdovin, A.; Perrier, D. L.; Smit, J. P.; Drabbels, M.; Buma, W. J. High-Resolution Excitation and Absorption Spectroscopy of Gas-Phase *p*-Coumaric Acid: Unveiling an Elusive Chromophore. *J. Am. Chem. Soc.* **2010**, *132*, 6315−6317.

(3) Smolarek, S.; Vdovin, A.; Tan, E. M. M.; Buma, W. J. Vibrational and Electronic Spectroscopy of the 4-Hydroxystyrene-CO_2 Cluster and Its Hydrate: A *para*-Coumaric Acid Impostor. *J. Phys. Chem. B* **2011**, *115*, 1275−1281.

(4) Ryan, W. L.; Gordon, D. J.; Levy, D. H. Gas-Phase Photochemistry of the Photoactive Yellow Protein Chromophore Trans-*p*-Coumaric Acid. *J. Am. Chem. Soc.* **2002**, *124*, 6194−6201.

(5) Lammich, L.; Rajputaaa, J.; Andersen, L. H. Photodissociation Pathways of Gas-Phase Photoactive Yellow Protein Chromophores. *Phys. Rev. E* **2008**, *78*.

(6) Putschögl, M.; Zirak, P.; Penzkofer, A. Absorption and Emission Behaviour of Trans-*p*-Coumaric Acid in Aqueous Solutions and Some Organic Solvents. *Chem. Phys.* **2008**, *343*, 107−120.

(7) Gromov, E. V.; Burghardt, I.; Koppel, H.; Cederbaum, L. S. Electronic Structure of the PYP Chromophore in Its Native Protein Environment. *J. Am. Chem. Soc.* **2007**, *129*, 6798−6806.

(8) Gromov, E. V.; Burghardt, I.; Hynes, J. T.; Koppel, H.; Cederbaum, L. S. Electronic Structure of the Photoactive Yellow Protein Chromophore: Ab Initio Study of the Low-Lying Excited Singlet States. *J. Photochem. Photobiol.* **2007**, *190*, 241−257.

(9) Zuev, D.; Bravaya, K. B.; Crawford, T. D.; Lindh, R.; Krylov, A. I. Electronic Structure of the Two Isomers of the Anionic Form of *p*-Coumaric Acid Chromophore. *J. Chem. Phys.* **2011**, *143*, 034310.

(10) Leenders, E. J. M.; VandeVondele, J.; Bolhuis, P. G.; Meijer, E. J. Solvation of *p*-Coumaric Acid in Water. *J. Phys. Chem. B* **2007**, *111*, 13591−13599.

(11) Gonzalez, E. M.; Guidoni, L.; Molteni, C. Chemical and Protein Shifts in the Spectrum of the Photoactive Yellow Protein: A Time-Dependent Density Functional Theory/Molecular Mechanics Study. *Phys. Chem. Chem. Phys.* **2009**, *11*, 4556−4563.

(12) Meyer, T. E. Isolation and Characterization of Soluble Cytochromes, Ferredoxins and Other Chromophoric Proteins from the Halophilic Phototrophic Bacterium Ectothiorhodospira Halophila. *Biochim. Biophys. Acta* **1985**, *806*.

(13) Baca, M.; Borgstahl, G. E. O.; Boissinot, M.; Burke, P. M.; Williams, D. R.; Slater, K. A.; Getzoff, E. D. Complete Chemical Structure of Photoactive Yellow Protein: Novel Thioester-Linked 4-Hydroxycinnamyl Chromophore and Photocycle Chemistry. *Biochemistry* **1994**, *33*, 14369−14377.

(14) Nielsen, I. B.; Boye-Peronne, S.; El Ghazaly, M. O. A.; Kristensen, M. B.; Nielsen, S. B.; Andersen, L. H. Absorption Spectra of Photoactive Yellow Protein Chromophores in Vacuum. *Biophys. J.* **2005**, *89*, 2597−2604.

(15) Rajput, J.; Rahbek, D. B.; Aravind, G.; Andersen, L. H. Spectral Tuning of the Photoactive Yellow Protein Chromophore by H-Bonding. *Biophys. J.* **2010**, *98*, 488−492.

(16) McMahon, T. B.; Kebarle, P. Intrinsic Acidities of Substituted Phenols and Benzoic Acids Determined by Gas-Phase Proton-Transfer Equilibria. *J. Am. Chem. Soc.* **1977**, *99*, 2222−2230.

(17) Tian, Z.; Wang, X. B.; Wang, L. S.; Kass, S. R. Are Carboxyl Groups the Most Acidic Sites in Amino Acids? Gas-Phase Acidities, Photoelectron Spectra, and Computations on Tyrosine, *p*-Hydroxybenzoic Acid, and Their Conjugate Bases. *J. Am. Chem. Soc.* **2009**, *131*, 1174−1181.

(18) Steill, J. D.; Oomens, J. Gas-Phase Deprotonation of *p*-Hydroxybenzoic Acid Investigated by IR Spectroscopy: Solution-Phase Structure Is Retained upon ESI. *J. Am. Chem. Soc.* **2009**, *131*, 13570−13571.

(19) Tian, Z.; Kass, S. R. Does Electrospray Ionization Produce Gas-Phase or Liquid-Phase Structures? *J. Am. Chem. Soc.* **2008**, *130*, 10842−10843.

(20) Polfer, N. C.; Oomens, J. Reaction Products in Mass Spectrometry Elucidated with Infrared Spectroscopy. *Phys. Chem. Chem. Phys.* **2007**, *9*, 3804−3817.

(21) IR-induced electron detachment can be monitored by leaking a low pressure of SF_6 into the vacuum of the FTICR, as described in ref 22. Monitoring IR-induced dissociation and detachment simultaneously reveals that the ratio of the two processes is relatively constant with IR frequency. One exception is the 1560 cm^{-1} band, where detachment becomes more favorable than dissociation. Although not understood in detail, this is likely due to peculiarities in the multiple-photon excitation process.

(22) Steill, J. D.; Oomens, J. Action Spectroscopy of Gas-Phase Carboxylate Anions by Multiple Photon IR Electron Detachment/Attachment. *J. Phys. Chem. A* **2009**, *113*, 4941−4946.

(23) Jockusch, R. A.; Price, W. D.; Williams, E. R. Structure of Cationized Arginine (ArgM^+, M = H, Li, Na, K, Rb, and Cs) in the Gas Phase: Further Evidence for Zwitterionic Arginine. *J. Phys. Chem. A* **1999**, *103*, 9266−9274.

(24) Ben-Ari, J.; Etinger, A.; Weisz, A.; Mandelbaum, A. Hydrogen-Shift Isomerism: Mass Spectrometry of Isomeric Benzenesulfonate and 2-, 3- and 4-Dehydrobenzenesulfonic Acid Anions in the Gas Phase. *J. Mass Spectrom* **2005**, *40*, 1064−1071.

(25) Geissler, P. L.; Dellago, C.; Chandler, D.; Hutter, J.; Parrinello, M. Autoionization in Liquid Water. *Science* **2001**, *291*, 2121−2124.

(26) Mohammed, O. F.; Pines, D.; Dreyer, J.; Pines, E.; Nibbering, E. T. J. Sequential Proton Transfer through Water Bridges in Acid-Base Reactions. *Science* **2005**, *310*, 83−86.

(27) Siwick, B. J.; Bakker, H. J. On the Role of Water in Intermolecular Proton-Transfer Reactions. *J. Am. Chem. Soc.* **2007**, *129*, 13412−13420.

(28) Mohammed, O. F.; Pines, D.; Nibbering, E. T. J.; Pines, E. On the Role of Water in Intermolecular Proton-Transfer Reactions. *Angew. Chem., Int. Ed.* **2007**, *46*, 1458−1461.

(29) Zuev, D.; Bravaya, K. B.; Makarova, M. V.; Krylov, A. I. Effect of Microhydration on the Electronic Structure of the Chromophores of the Photoactive Yellow and Green Fluorescent Proteins. *J. Chem. Phys.* **2011**, *135*, 194304.

(30) Oepts, D.; van der Meer, A. F. G.; van Amersfoort, P. W. The Free-Electron-Laser Facility Felix. *Infrared Phys. Technol.* **1995**, *36*, 297−308.

(31) Posey, L. A.; Deluca, M. J.; Johnson, M. A. Demonstration of a Pulsed Photoelectron Spectrometer on Mass-Selected Negative Ions: O^-, O_2^-, and O_4^-. *Chem. Phys. Lett.* **1986**, *131*, 170−174.

(32) Polfer, N. C.; Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. Infrared Spectroscopy of Phenylalanine Ag(I) and Zn(II) Complexes in the Gas Phase. *J. Am. Chem. Soc.* **2006**, *128*, 517−525.

(33) Marshall, A. G.; Wang, T. C. L.; Ricca, T. L. Tailored Excitation for Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Chem. Soc.* **1985**, *107*, 7893−7897.

(34) Settle, R. D. F.; Rizzo, T. R. CO_2 Laser Assisted Vibrational Overtone Spectroscopy. *J. Chem. Phys.* **1992**, *97*, 2823−2825.

(35) Sinha, R. K.; Nicol, E.; Steinmetz, V.; Maitre, P. Gas Phase Structure of Micro-Hydrated [Mn(ClO_4)]^+ and [Mn_2(ClO_4)_3]^+ Ions Probed by Infrared Spectroscopy. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 758−772.