J|A|C|S
COMMUNICATIONS

Published on Web 02/08/2006

Electronic Spectroscopy of Cold, Protonated Tryptophan and Tyrosine

Oleg V. Boyarkin, Sébastien R. Mercier, Anthi Kamariotis, and Thomas R. Rizzo*

Laboratoire de Chimie Physique Moléculaire, Ecole Polytechnique Fédérale de Lausanne, EPFL SB ISIC LCPM, Station 6, CH-1015 Lausanne, Switzerland

Received December 10, 2005; E-mail: thomas.rizzo@epfl.ch

The spectroscopic study of biological molecules in the gas phase can provide insight into the photophysics of these same species in solution. Levy and co-workers were the first to measure highly resolved electronic spectra of tryptophan and several analogues in the gas phase by seeding the molecules in a supersonic expansion.[1] The cooling afforded by the expansion greatly simplifies the spectrum such that features from individual stable conformers can be identified and their photophysical properties studied individually.[2] Similar approaches have been applied to study a variety of biological molecules ranging from individual amino acids[3−6] and nucleic acid bases[7−10] to small peptides.[11−13] While most of this work has been performed on neutral species, most biological molecules are charged in solution, and the presence of a nearby charge can strongly influence their photophysics.[14,15] We report here a method to obtain electronic spectra of charged biological molecules produced in the gas phase by electrospray and cooled to ~10 K in a radio frequency (RF) ion trap. As a first demonstration of this method, we measure the electronic spectra of cold, protonated tryptophan ($TrpH^+$) and tyrosine ($TyrH^+$).

A few recent studies have used photofragment spectroscopy to measure electronic spectra of protonated tryptophan in the gas phase.[16,17] Weinkauf and co-workers observed only broad vibronic bands in the spectrum of $TrpH^+$ in a liquid nitrogen cooled Paul trap,[16] in contrast to neutral tryptophan, which exhibits well-resolved spectral features at low temperature.[1] It was not entirely clear at the time if the broad features were inherent to the photophysics of $TrpH^+$ or due to incomplete cooling since the RF field can drive collisional heating in a quadrupole trap. In recent time-resolved, pump−probe photofragment studies, Kang et al. have observed that $TrpH^+$ exhibits a 380 fs decay after 266 nm excitation, while under the same conditions, protonated tyrosine decays with a time constant of 22 ps.[18] They attribute the difference in lifetimes between these two species to a difference in curve crossing between the $\pi\pi^*$ state responsible for the UV absorption and a dissociative $\pi\sigma^*$ state.[19,20]

The key element in our approach to study the spectroscopy and dynamics of biomolecular ions in the gas phase is the use of a linear, 22-pole RF ion trap that allows cooling the species of interest to temperatures near 10 K.[21] As illustrated in Figure 1, we produce protonated amino acids in the gas phase by nanoelectrospray, draw them into vacuum through a glass capillary with metallized ends, and collect them in an RF hexapole trap. After 50 ms collection time, we pulse the ions out of the hexapole, select a particular mass in a first quadrupole stage, bend them 90° with a static quadrupole, and guide them into the 22-pole ion trap which is mounted on a closed cycle refrigerator and maintained at 6 K. We inject a pulse of helium into the trap, giving it sufficient time to equilibrate to the temperature of the trap housing before the arrival of the approaching ion packet. After a 40 ms delay to allow for the ions to cool, an ultraviolet laser pulse is sent through the trap, exciting the ions to the lowest $\pi\pi^*$ state in the region of 35 000 cm^{-1}. We detect spectroscopic transitions by measuring the fragments that

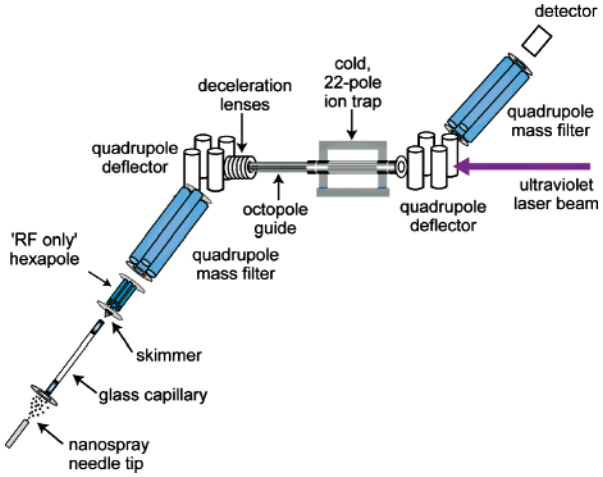

Figure 1. Schematic of tandem mass spectrometer with a cooled, 22-pole ion trap.

result when some portion of the electronically excited ions dissociate. Both parent and fragment ions are released from the trap and passed through an analyzing quadrupole before being detected and counted. We generate a spectrum by monitoring the number of ions corresponding to a particular product fragment as a function of the laser wavenumber. The trapping cycle is repeated 20 times per second, while the laser fires at 10 Hz repetition rate. The parent ion signal recorded between laser shots is used to normalize the data, removing slow fluctuations in the nanospray source.

Figure 2 shows an electronic spectrum of $TrpH^+$ obtained by monitoring the fragment signal at *m*/*z* 188 amu (corresponding to the loss of NH_3) as a function of UV excitation wavenumber. The upper panel shows the spectrum obtained at ambient temperature, while the lower panel shows the spectrum when the trap is cooled to 6 K. The spectrum of Figure 2b shows a broad first band at 35 100 cm^{-1} followed by a second band peaking at 35 850 cm^{-1}. Compared to the spectrum of Weinkauf and co-workers,[16] the features we measure are slightly narrower, which is what one might expect if the ions in our experiment were colder. There is also a difference in the relative intensity of the second feature, which is higher in their work. This may arise from the fact that they measure an action spectrum by collecting all of the fragment ions produced by a variety of channels, while we record an individual fragment mass channel. If the branching ratio for dissociation into the various product channels changes as a function of the excitation energy, we might see a decrease in signal with increasing frequency, while they would not.

For comparison, the electronic spectrum of $TyrH^+$ is shown in Figure 3. In contrast to the case of protonated tryptophan, the spectrum of cold, protonated tyrosine shows sharp, well-resolved features. The lowest energy band origin, which occurs at 35 083 cm^{-1}, is shifted 403 cm^{-1} lower than that of the neutral molecule.[5]

10.1021/ja058383u CCC: $33.50

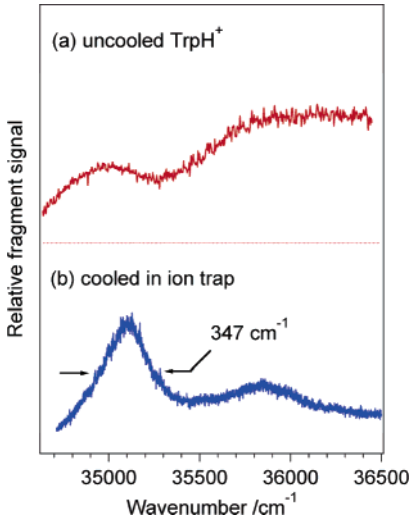

Figure 2. Electronic photofragmentation spectra of (a) uncooled $TrpH^+$; (b) $TrpH^+$ that has been cooled in the 22-pole ion trap. The product fragment corresponding to *m*/*z* 188 amu was monitored in each case.

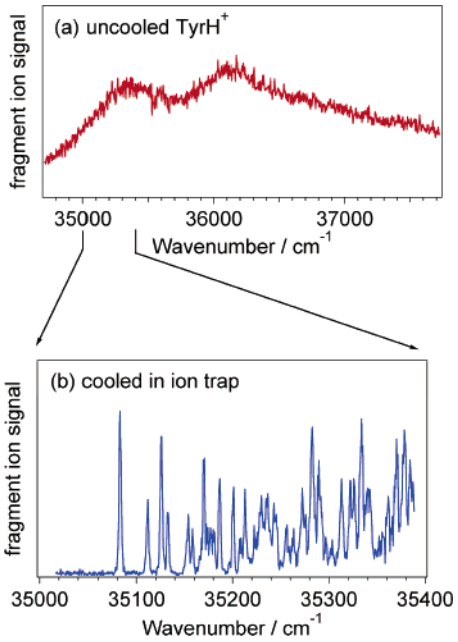

Figure 3. Electronic photofragmentation spectra of (a) uncooled $TyrH^+$; (b) $TyrH^+$ that has been cooled in the 22-pole ion trap. The product fragment corresponding to *m*/*z* 136 amu was monitored in each case.

Since the laser resolution is on the order of 0.05 cm^{-1}, the 2.7 cm^{-1} width of individual peaks should reflect the rotational band contour together with any broadening that may come from the finite lifetime of the excited state. Although we have not yet performed a full analysis of this spectrum, which will require data from hole-burning studies, it is clear that several stable conformers must be contributing. DFT calculations at the B3LYP/6-31++G** level indicate the presence of several low frequency motions of the amino acid backbone, but not nearly enough to account for the observed number of features if only a single conformer were present.

The lack of clear hot-band features to the red of the lowest energy band origin indicates that the vibrational temperature of the ions must be $\leq$10 K.

The low temperature determined from the protonated tyrosine spectrum implies that the $TrpH^+$ molecules responsible for the spectrum of Figure 2b must also be cold since the two spectra were obtained under nearly identical conditions. This, in turn, implies that individual vibrational features in the electronic spectrum of $TrpH^+$ are broadened by a fast decay from the initially excited electronic state. While this is in qualitative agreement with the time-resolved measurements of Kang et al.,[18] the broadening we observe is significantly greater than the 13 cm^{-1} implied by the 380 fs decay that they measure. This difference could, in principle, come from the superposition of spectra of several conformers, although DFT calculations indicate that there should be at most two conformers populated under the conditions of our experiment. If the latter is true, our spectra imply that the lowest $\pi\pi^*$ state of $TrpH^+$ decays on a time scale of <100 fs.

Given that the fluorescence lifetime of neutral tryptophan is on the order of nanoseconds, even in solution, our result emphasizes the extreme sensitivity of the excited state dynamics to the influence of charge in its local environment. This suggests that the local "dielectric constant" of the medium surrounding the indole chromophore should play a major role in determining the fluorescence lifetime.

The significance of this result is much broader than tryptophan and tyrosine spectroscopy. The ability to cool biomolecular ions in the gas phase to low internal temperatures opens up the possibility of applying spectroscopic techniques to a wide range of large molecules that can be produced in the gas phase by electrospray.

Acknowledgment. We are grateful to Prof. Dieter Gerlich for his help in designing our 22-pole ion trap, and Dr. Rainer Beck for helpful technical advice. We also thank the École Polytechnique Fédérale de Lausanne and the Fonds National Suisse (Grant No. 200020-103666/1) for their generous support of this work.

References

(1) Rizzo, T. R.; Park, Y. D.; Peteanu, L. A.; Levy, D. H. *J. Chem. Phys.* **1986**, *84*, 2534−2541.
(2) Rizzo, T. R.; Park, Y. D.; Levy, D. H. *J. Chem. Phys* **1986**, *85*, 6945−6951.
(3) Snoek, L. C.; Robertson, E. G.; Kroemer, R. T.; Simons, J. P. *Chem. Phys. Lett.* **2000**, *321*, 49−56.
(4) Snoek, L. C.; Kroemer, R. T.; Hockridge, M. R.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1819−1826.
(5) Grace, L. I.; Cohen, R.; Dunn, T. M.; Lubman, D. M.; de Vries, M. S. *J. Mol. Spectrosc.* **2002**, *215*, 204−219.
(6) Carney, J. R.; Zwier, T. S. *J. Phys. Chem. A* **2000**, *104*, 8677−8688.
(7) Nir, E.; Grace, L.; Brauer, B.; de Vries, M. S. *J. Am. Chem. Soc.* **1999**, *121*, 4896−4897.
(8) Plutzer, C.; Kleinermanns, K. *Phys. Chem. Chem. Phys.* **2002**, *4*, 4877−4882.
(9) Kim, N. J.; Kang, H.; Park, Y. D.; Kim, S. K. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2802−2805.
(10) Mons, M.; Dimicoli, I.; Piuzzi, F.; Tardivel, B.; Elhanine, M. *J. Phys. Chem. A* **2002**, *106*, 5088−5094.
(11) Dian, B. C.; Longarte, A.; Mercier, S.; Evans, D. A.; Wales, D. J.; Zwier, T. S. *J. Chem. Phys.* **2002**, *117*, 10688−10702.
(12) Unterberg, C.; Gerlach, A.; Schrader, T.; Gerhards, M. *J. Chem. Phys.* **2003**, *118*, 8296−8300.
(13) Chin, W.; Dognon, J. P.; Piuzzi, F.; Tardivel, B.; Dimicoli, I.; Mons, M. *J. Am. Chem. Soc.* **2005**, *127*, 707−712.
(14) Callis, P. R.; Vivian, J. T. *Chem. Phys. Lett.* **2003**, *369*, 409−414.
(15) Marquezin, C. A.; Hirata, L. Y.; Juliano, L.; Ito, A. S. *Biopolymers* **2003**, *71*, 569−576.
(16) Nolting, D.; Marian, C.; Weinkauf, R. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2633−2640.
(17) Talbot, F. O.; Tabarin, T.; Antoine, R.; Broyer, M.; Dugourd, P. *J. Chem. Phys.* **2005**, *122*, 74310−74310.
(18) Kang, H.; Jouvet, C.; Dedonder-Lardeux, C.; Martrenchard, S.; Gregoire, G.; Desfrancois, C.; Schermann, J. P.; Barat, M.; Fayeton, J. A. *Phys. Chem. Chem. Phys.* **2005**, *7*, 394−398.
(19) Sobolewski, A. L.; Domcke, W.; Dedonder-Lardeux, C.; Jouvet, C. *Phys. Chem. Chem. Phys.* **2002**, *4*, 1093−1100.
(20) Sobolewski, A. L.; Domcke, W. *Chem. Phys. Lett.* **1999**, *315*, 293−298.
(21) Gerlich, D. *Adv. Chem. Phys.* **1992**, *82*, 1−176.

JA058383U