VIP Radical Cations

DOI: 10.1002/anie.200804101

Structure of the Observable Histidine Radical Cation in the Gas Phase: A Captodative α-Radical Ion**

*Jeffrey Steill, Junfang Zhao, Chi-Kit Siu, Yuyong Ke, Udo H. Verkerk, Jos Oomens, Robert C. Dunbar, Alan C. Hopkinson, and K. W. Michael Siu**

Protein-based radicals play crucial roles in some of the greatest biosynthetic challenges in nature, including photosynthesis and substrate oxidation.[1] Radical centers have been located on aromatic and sulfur-containing amino acid residues, as well as glycine residues.[1a] Invariably these charged or neutral radical species are generated through involvement of an adjacent metal cofactor. The positions of charge and spin in the radical cations are paramount for reactivity modulation and proton-coupled electron transfer, but obtaining structural details is difficult even for the simplest models.[1b,2] Experiments in vacuo permit the investigation of intrinsic properties of radical cations in the absence of a reactivity-modulating environment. Radical cations of amino acids and peptides have been produced in vacuo by one-electron transfer in collision-induced dissociations (CIDs) of a ternary complex system comprising copper(II), an auxiliary ligand, and the amino acid or peptide. Such ternary complexes are efficiently generated by electrospray ionization,[3] and probed downstream by using mass spectrometry (MS). Under appropriate conditions, CID of the complex yields the radical cation of the amino acid or peptide that can be isolated and trapped for spectroscopic interrogation.[4] Herein, we report the first infrared multiple photon dissociation (IRMPD) spectroscopic experiments on a prototypical amino acid radical cation, $His^{\bullet+}$, and its ternary complex ion.

In a recent article, Ke et al.[5] showed that, by judicious choice of the auxiliary ligand, $His^{\bullet+}$ of different stabilities are formed through CID of the ternary complex ion. In particular, the use of 2,2′:6′,2″-terpyridine (tpy) as the ligand leads primarily to a $His^{\bullet+}$ that is stable on the MS timescale and can be isolated and fragmented at a subsequent MS stage; by contrast, employing acetone as the ligand results in a metastable $His^{\bullet+}$ and only its fragment ions are observed. Furthermore, the former, relatively stable $His^{\bullet+}$ fragments by losing a water molecule to give $[b_1\text{-H}]^{\bullet+}$ and then CO to give $[a_1\text{-H}]^{\bullet+}$, whereas the latter, metastable $His^{\bullet+}$ dissociates spontaneously by losing first CO_2 to give the 4-ethaniminoimidazole radical cation, which then loses methanimine to give the 4-methyleneimidazole radical cation. Density functional theory (DFT) calculations at the (unrestricted) UB3LYP/6-311++G(d,p) level of theory predicted five low-energy $His^{\bullet+}$ structures. Scheme 1 shows these structures with additional, new information on the barriers against their interconversions (see Figures S2 and S3 in the Supporting Information for details). Ke et al.[5] postulated that the stable and metastable $His^{\bullet+}$ are **His5** (the structure at the global minimum) and **His2**, respectively. **His5** is a captodative[6] α-radical ion that differs from the canonical **His1** structure in having the α-CH hydrogen migrated to the imino nitrogen of the imidazole ring; **His2** is best described as a 4-ethaniminoimidazole radical cation solvated by CO_2. **His2**–**His5** are all unconventional structures, and experimental verification of the $His^{\bullet+}$ structure is highly desirable for confirmation of the key roles played by spin and charge delocalization in $His^{\bullet+}$ stabilization.

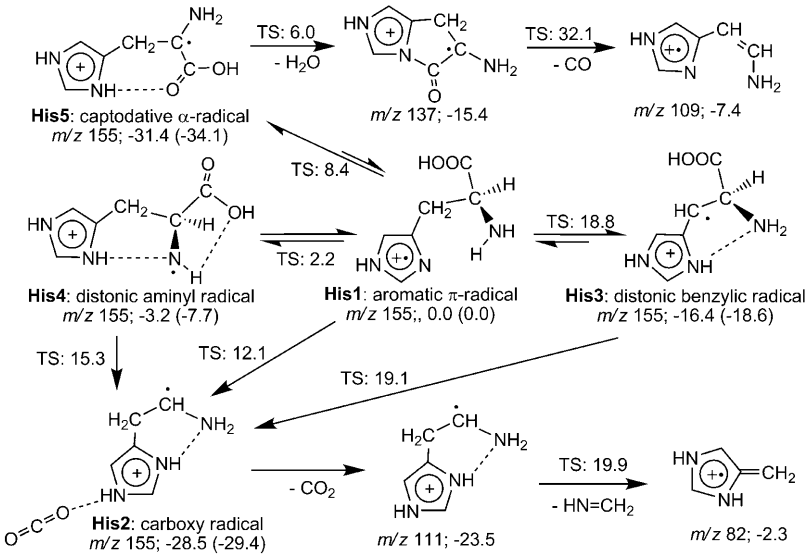
Figure 1 compares the experimental IRMPD spectrum collected for $His^{\bullet+}$ with the DFT-predicted IR spectra of **His1**–**His5**. It is apparent that only one predicted IR spectrum, that of **His5**, resembles the measured IRMPD spectrum. In particular, **His5** is the only isomer predicted to exhibit two bands, 1596 and 1653 cm^{-1}, which are assigned as NH_2 scissoring and C=O stretching, respectively, that match the 1606 and 1666 cm^{-1} bands in the IRMPD spectrum. The lack of a strong band at around 1780–1790 cm^{-1} in the IRMPD spectrum rules out the presence of a significant fraction of **His3** and **His4**. Similarly, **His1** can be ruled out by the presence of the doublet, 1606 and 1666 cm^{-1}, and the absence of spectroscopic details in the region of 1077–1320 cm^{-1}. **His2** can be eliminated by the absence of peaks at around 810–820 cm^{-1} and by the low endothermicity against loss of the solvating CO_2 (5 kcal mol^{-1}).[5]

We interpret the excellent match between the experimental IRMPD spectrum and the predicted IR spectrum of **His5** to indicate that **His5** is the only abundant species present. This degree of selectivity is feasible as **His5** is positioned at the bottom of a deep well on the potential-energy surface of $His^{\bullet+}$. The barriers against **His5** converting into the other His isomers and dissociating into $[b_1\text{-H}]^{\bullet+}$ are high (Scheme 1),

[*] Dr. J. Zhao, Dr. C.-K. Siu, Y. Ke, Dr. U. H. Verkerk, Prof. A. C. Hopkinson, Prof. K. W. M. Siu
Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street
Toronto, ON M3J 1P3 (Canada)
E-mail: kwmsiu@yorku.ca
Dr. J. Steill, Dr. J. Oomens
FOM Institute for Plasma Physics
3430 BE Nieuwegein (The Netherlands)
Prof. R. C. Dunbar
Chemistry Department, Case Western Reserve University
Cleveland, OH 44106 (USA)

[**] This study was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada and made possible by the facilities of the Shared Hierarchical Academic Research Computing Network (SHARCNET: www.sharcnet.ca). The skillful assistance of the FELIX staff is gratefully acknowledged.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804101.



Scheme 1. Isomerization and dissociation of His$^{\bullet+}$. Relative enthalpies at 0 K, $\Delta H°_0$/kcal mol^{-1}, are evaluated at the UB3LYP/6-311++G(d,p) level of theory (UBMK/6-311++G(d,p) values). TS = transition structure (kcal mol^{-1}).

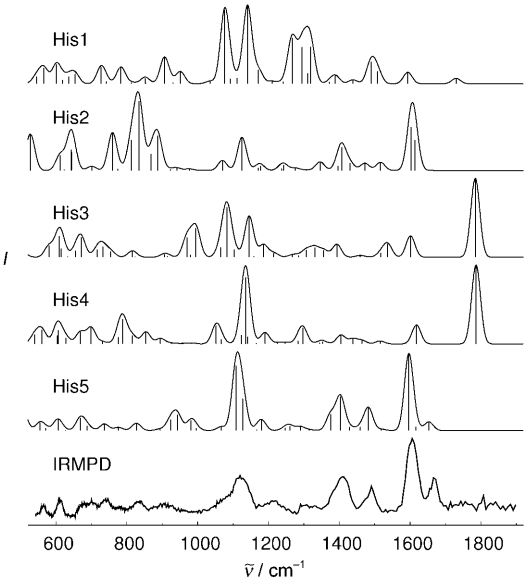


Figure 1. IRMPD spectrum of His$^{\bullet+}$ and theoretical IR spectra of **His1**–**His5** calculated at the UB3LYP/6-311++G(d,p) level of theory.

thereby "trapping" His$^{\bullet+}$ in this isomeric structure once it has fallen into the potential energy well.

His5 is a captodative radical ion[7] that formally has its unpaired electron at the α carbon and its charge on the protonated imidazole ring. The NH_2 group is a powerful electron-donating (dative) group and the C=O$^{\delta+}$···H-imidazole$^+$ is a strong electron-withdrawing (captor) group; these features account for the extraordinary stability of **His5** (Scheme 1). **His5** can be readily formed from canonical **His1** because of a low energy barrier of 8.4 kcal mol^{-1},[5] which can easily be overcome in dissociation of the precursor complex ion, [Cu^{II}(tpy)(His)]$^{\bullet2+}$, to give His$^{\bullet+}$.

Histidine can exist in the precursor complex in two forms—canonical or zwitterionic—thereby resulting in a charge-solvated (CS) or salt-bridged (SB) complex. The structures and predicted IR spectra of the lowest-energy isomers of the CS and SB complexes are shown in Figure 2, along with the IRMPD spectrum. (Please note: according to DFT calculations using UB3LYP, **CS** is lower in enthalpy than **SB-1** by only 0.1 kcal mol^{-1}.[5] It has, however, been argued that the DFT method UBMK may be more accurate than UB3LYP in energy calculations for radical ions;[8] using UBMK increases the difference to 1.9 kcal mol^{-1} (and to 1.3 kcal mol^{-1} in free energy, see Figure S1 in the Supporting Information)). It is readily apparent that the IRMPD spectrum resembles much more closely the predicted IR spectrum of **CS**; however, the high wavenumber shoulder of the intense IRMPD peak at 1660 cm^{-1} and the low intensity band at 917 cm^{-1} may indicate the presence of a minor fraction of **SB-1** (the bands being assigned as the C=O stretching and the imidazole N–H out-of-plane bending at 1653 and 921 cm^{-1}, respectively). An estimate, using the C=O stretching band for **CS** at 1762 cm^{-1} and **SB-1** at 1653 cm^{-1} as gauges for comparison with the IRMPD spectrum, puts the presence of the SB form at a maximum of 20%. Although the grounds for this intensity comparison are debatable, the result is in

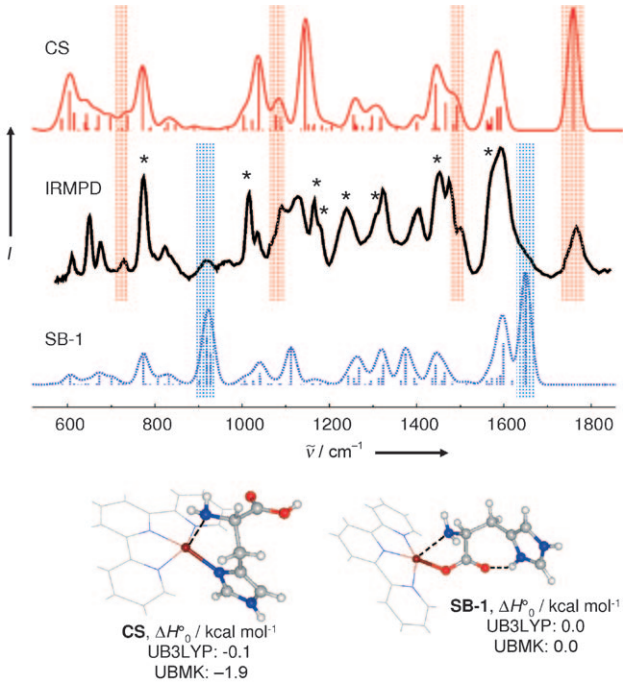


Figure 2. IRMPD spectrum and calculated IR spectra of the charge-solvated (CS) and salt-bridged (SB) coordination mode of [Cu^{II}(tpy)-(His)]$^{\bullet2+}$. Bands marked by ⋆ are assigned to the auxiliary ligand tpy. Relative energies are evaluated at the UB3LYP (UBMK) levels of theory by using the 6-311++G(d,p) basis set.[5]

accordance with that of Ke et al.,[5] which gave the SB form at about 14% based on tandem MS results. The absence of strong bands at 1687 and 1352 cm^{-1} in the IRMPD spectrum indicates the absence of **SB-2**, the salt-bridged complex in which the zwitterionic histidine is in an enolate form (see Figure S1 in the Supporting Information).

Thus all results point to a canonical His structure within the precursor CS complex that dissociates to give **His1**, which then isomerizes to **His5**. This study provides the first direct evidence of the captodative structure for the radical ion of an amino acid and confirms deductions based on DFT calculations and tandem MS experiments.[5] Presumably IRMPD spectroscopy will also be invaluable in shedding light on the structures of peptide radical ions, which tend to have a large number of low-lying isomers separated by relatively high energy barriers. This knowledge is fundamental to investigations of intrinsic properties of these ions and understanding some of the most challenging and important biochemical processes.

Experimental Section

IRMPD spectroscopy was performed at the FOM-Institute for Plasma Physics "Rijnhuizen" in Nieuwegein (The Netherlands) by using the Free Electron Laser for Infrared eXperiments (FELIX) facility.[4,9] Metal complexes were prepared in 1:1 water/methanol solutions by mixing copper(II) perchlorate, tpy, and histidine (Sigma–Aldrich, Zwijndrecht, The Netherlands) to a final concentration of 1 mM $[Cu^{II}(tpy)(His)]^{\bullet 2+}$. A laboratory-built Fourier transform ion cyclotron resonance mass spectrometer equipped with a Z-spray (Micromass UK Ltd.) electrospray ionization source and an octopole ion guide was used to generate and isolate the precursor ion, $[Cu^{II}(tpy)(His)]^{\bullet 2+}$, by using a stored waveform inverse Fourier-transform (SWIFT) pulse. In this setup, collisional heating of ions during transfer into the ICR cell was avoided by applying a potential switch on the octopole ion guide.[10a] $His^{\bullet +}$ was generated by exposing the precursor ion to a SORI pulse.[10b,c] The spectra shown in Figure 1 and 2 have been power corrected for the leveling off of laser intensity in the high wavenumber range. Geometry optimizations and harmonic vibrational frequencies were calculated using the Gaussian 03 suite of programs[11] at the UB3LYP/6-311++G(d,p) level of theory. A scaling factor of 0.976, known to be appropriate for DFT comparisons with IRMPD spectra, was applied.[12] Band wavenumbers were convoluted by using Gaussian profiles with a full-width-at-half-maximum of 30 cm^{-1}. See the Supporting Information for further experimental details.

Received: August 20, 2008
Published online: November 10, 2008

Keywords: collision-induced dissociation · density functional calculations · gas-phase chemistry · radical ions

[1] a) J. Stubbe, W. A. van der Donk, *Chem. Rev.* **1998**, *98*, 705–762; b) F. Himo, P. E. M. Siegbahn, *Chem. Rev.* **2003**, *103*, 2421–2456, and other papers in this issue on radical enzymology; c) V. L. Davidson, *Acc. Chem. Res.* **2008**, *41*, 730–738.

[2] a) H. D. Connor, B. E. Sturgeon, C. Mottley, H. J. Sipe, Jr., R. P. Mason, *J. Am. Chem. Soc.* **2008**, *130*, 6381–6387; b) R. Sibert, M. Josowicz, F. Porcelli, G. Veglia, K. Range, B. A. Barry, *J. Am. Chem. Soc.* **2007**, *129*, 4393–4400; c) C. Shih, A. K. Museth, M. Abrahamsson, A. M. Blanco-Rodriguez, A. J. Di Bilio, J. Sudhamsu, B. R. Crane, K. L. Ronayne, M. Towrie, A. Vlcek, Jr., J. H. Richards, J. R. Winkler, H. B. Gray, *Science* **2008**, *320*, 1760–1762.

[3] For reviews, see: a) A. C. Hopkinson, K. W. M. Siu in *Principles of Mass Spectrometry Applied to Biomolecules* (Eds.: J. Laskin, C. Lifshitz), Wiley, Hoboken, **2006**, pp. 301–335; b) F. Tureček, *Mass Spectrom. Rev.* **2007**, *26*, 563–582.

[4] For a review of the IRMPD spectroscopic technique and its applications, see a) N. C. Polfer, J. Oomens, *Phys. Chem. Chem. Phys.* **2007**, *9*, 3804–3817; b) J. Oomens, B. G. Sartakov, G. Meijer, G. von Helden, *Int. J. Mass Spectrom.* **2006**, *254*, 1–19.

[5] Y. Ke, J. Zhao, U. H. Verkerk, A. C. Hopkinson, K. W. M. Siu, *J. Phys. Chem. B* **2007**, *111*, 14318–14328.

[6] A. K. Croft, C. J. Easton, L. Radom, *J. Am. Chem. Soc.* **2003**, *125*, 4119–4124.

[7] P. C. Burgers, J. L. Holmes, J. K. Terlouw, B. van Baar, *Org. Mass Spectrom.* **1985**, *20*, 202–206.

[8] a) D. Moran, R. Jacob, G. P. E. Wood, M. L. Coote, M. J. Davies, R. A. J. O'Hair, C. J. Easton, L. Radom, *Helv. Chim. Acta* **2006**, *89*, 2254–2272; b) I. K. Chu, J. Zhao, M. Xu, S. O. Siu, A. C. Hopkinson, K. W. M. Siu, *J. Am. Chem. Soc.* **2008**, *130*, 7862–7872.

[9] J. J. Valle, J. R. Eyler, J. Oomens, D. T. Moore, A. F. G. van der Meer, G. von Helden, G. Meijer, C. L. Hendrickson, A. G. Marshall, G. T. Blakney, *Rev. Sci. Instrum.* **2005**, *76*, 023103.

[10] a) N. C. Polfer, J. Oomens, D. T. Moore, G. Von Helden, G. Meijer, R. C. Dunbar, *J. Am. Chem. Soc.* **2006**, *128*, 517–525; b) J. W. Gauthier, T. R. Trautman, D. B. Jacobson, *Anal. Chim. Acta* **1991**, *246*, 211–225; c) S. H. Guan, A. G. Marshall, *Int. J. Mass Spectrom. Ion. Processes* **1996**, *158*, 5–37.

[11] Gaussian 03 (Revision D.01): M. J. Frisch et al., see the Supporting Information for the full reference.

[12] a) N. C. Polfer, J. Oomens, R. C. Dunbar, *Phys. Chem. Chem. Phys.* **2006**, *8*, 2744–2751; b) N. C. Polfer, J. Oomens, R. C. Dunbar, *ChemPhysChem* **2008**, *9*, 579–589.

