The stabilization of arginine's zwitterion by dipole-binding of an excess electron

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(Received 17 December 2004; accepted 12 January 2005; published online 2 March 2005)

The arginine parent anion was generated by a newly developed, infrared desorption-electron photoemission hybrid anion source. The photoelectron spectrum of the arginine anion was recorded and interpreted as being due to dipole binding of the excess electron. The results are consistent with calculations by Rak, Skurski, Simons, and Gutowski, who predicted the near degeneracy of arginine's canonical and zwitterionic dipole bound anions. Since neutral arginine's zwitterion is slightly less stable than its canonical form, this work also demonstrates the ability of an excess electron to stabilize a zwitterion, just as ions and solvent molecules are already known to do.
 [DOI: 10.1063/1.1864952]

Zwitterions strongly influence the structure and function of peptides and proteins. For this reason, it is important to understand zwitterion formation in amino acids themselves. All of the 20 common, naturally occurring amino acids can form zwitterions in solution due to the stabilizing effects of solvation and/or counter ions. As isolated (gas phase) molecules, however, it now seems clear that none of these amino acids form zwitterions. Because arginine possesses the largest proton affinity[1] among the amino acids in this group, it would have been the most likely candidate for doing so, and indeed, there was an experimental report[2] suggesting that gaseous arginine exists as a zwitterion. Subsequent experimental[3] and computational[4–8] studies, however, demonstrated that arginine is not a zwitterion in the gas phase. Since then, several experimental studies have demonstrated *bona fide* zwitterion formation in amino acids due to complexation with neutral molecules and/or with charges.[9–12]

Specifically, Gutowski, Simons, and co-workers[6] calculated the relative energies of the canonical versus the zwitterionic tautomers of neutral arginine, finding the zwitterionic form to be less stable than the canonical (non-zwitterionic) form by 3–4 kcal/mole. A subsequent larger scale computation by Simons and co-workers[7] confirmed these results. Gutowski, Simons, and co-workers[8] also investigated the effect of excess electron attachment to arginine. While both canonical and zwitterionic arginine were found to form dipole bound anions by virtue of their large dipole moments, the zwitterionic arginine anion was stabilized by 7.3 kcal/mole, while the canonical arginine anion was stabilized by only 3.7 kcal/mole. This resulted in the near degeneracy of the zwitterionic and canonical arginine anion tautomers. The greater stabilization of the zwitterionic anion tautomer over the canonical anion tautomer is due to neutral zwitterionic arginine having a larger dipole moment than neutral canonical arginine. The quasidegeneracy of the two anion tautomers is, of course, not perfect. The calculation found the zwitterionic form of the anion to be more stable than the canonical form of the anion by 0.4 kcal/mole, although this energy difference is within the uncertainty of the calculation. These authors predicted vertical detachment energies (VDE) of 0.32 eV and 0.14 eV for the zwitterionic and the canonical anions, respectively. Thus, while the total energies of the two anions were predicted to be essentially the same, their VDE values were predicted to differ significantly.

Here, we report our study of the arginine molecular anion and the measurement of its vertical detachment energy. The most imposing hurdle facing the study of free biological molecules is getting them into the gas phase, because while some of them can be vaporized thermally without decomposition, most are essentially involatile. Studying anions of biomolecules in the gas phase presents its own challenges. While the electrospray method was a major advance, its anions tend to have lost a hydrogen atom and/or to be multiply charged. In the present study, we are interested in obtaining and studying the parent anion of arginine in the gas phase. As most often practiced, matrix assisted laser desorption ionization (MALDI) also has difficulty providing parent anions of biomolecules. To solve this problem, we developed a new source which is a hybrid of two existing techniques, pulsed laser infrared desorption and pulsed laser photoelectron emission. The work of de Vries[13] and of Boesl[14] provided valuable guidance to us in implementing infrared desorption and photoelectron emission techniques, respectively, although the earlier work of Schlag and co-workers had underpinned them both.[15,16] Our hybrid anion source functions as follows. Pulses of helium are coordinated with infrared pulses (from the fundamental frequency of a Nd:YAG laser) which strike a slowly translating, biomolecule-coated metal rod, which itself sits very near but slightly below the pulsed gas nozzle. The absorption of infrared light causes the desorption of intact neutral biomolecules into the gas phase due to ultrafast heating of the sample itself. (In the deVries configuration, a thin layer of biomolecules is coated on a graphite bar which itself acts as an infrared absorber, in turn leading to desorption by ultrafast heating.) Coordinated with the

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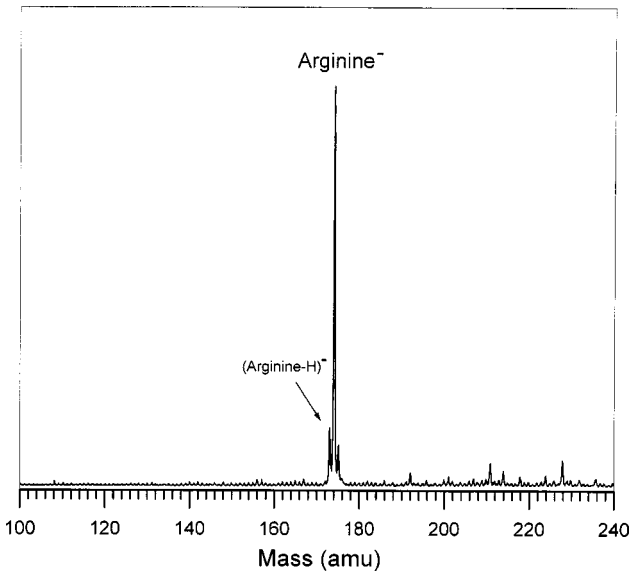

FIG. 1. A mass spectrum showing the arginine parent anion and its dehydrogenated anion, both of which were generated by our infrared desorption/photoelectron emission hybrid ion source.

foregoing are visible (or ultraviolet) pulses of light from another Nd:YAG laser that strike a nearby metal wire, mounted parallel to the sample rod. This results in a large burst of electrons which are unleashed into the momentary cloud of neutral biomolecules in the presence of cooling helium collisions. This leads to electron attachment and the formation of intact (parent) anions of biomolecules in the gas phase. This technique is not MALDI *per se*, but it is a laser assisted desorption technique for making ions.

We choose arginine as the first test of our hybrid source's ability to make parent anions of difficult-to-vaporize biomolecules. (Arginine has been thermally vaporized,[3] but doing so is on the edge of feasibility.) Figure 1 shows a typical mass spectrum that resulted. It is dominated by the parent anion of arginine, with a weaker intensity deprotonated arginine one amu lower in mass. The peak one amu higher in mass is due to the carbon-13 isotope pattern of arginine. Generally, the mass spectrum is quite clean with remarkably little fragmentation. Since our hybrid source is pulsed, we utilized our pulsed photoelectron spectrometer to measure the photoelectron spectrum of the arginine anion. Photoelectron spectroscopy is governed by the energy-conserving relationship, $h\nu = \mathrm{EBE} + \mathrm{EKE}$, where $h\nu$ is the photon energy, EBE is electron binding energy, and EKE is electron kinetic energy. Knowing the photon energy and measuring the electron kinetic energy, one determines the electron binding energies of the observed transitions. Our pulsed photoelectron spectrometer consists of a pulsed anion source (described above), a linear time-of-flight mass spectrometer for mass selection, another Nd:YAG laser for photodetachment, and a magnetic bottle for electron energy analysis. The resolution of our magnetic bottle, electron energy analyzer is at least 50 meV at EKE = 1 eV.

The photoelectron spectrum of the arginine anion was measured with both 1064 nm (1.165 eV) and 355 nm (3.49 eV) photons, with no significant differences observed between the two spectra. The 1064 nm photoelectron spectrum

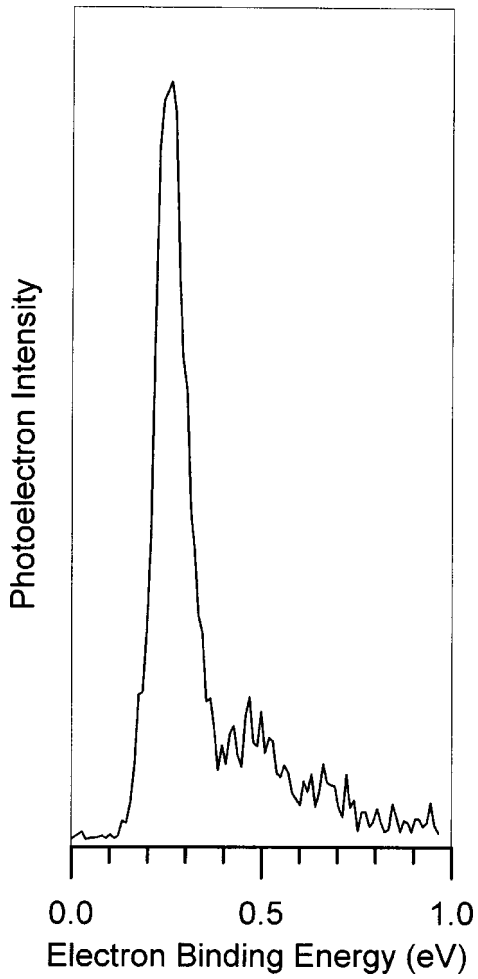

FIG. 2. The photoelectron spectrum of the arginine parent anion recorded with 1064 nm photons.

of the arginine anion is presented in Fig. 2. The VDE extracted from this spectrum is 0.256 ± 0.03 eV. There is also a weak but reproducible shoulder at EBE = 0.18 eV on the low EBE side of the main peak. There are at least two additional weak peaks on the high EBE side of the main peak, one located at 0.47 eV and the other at 0.67 eV. The first of these is separated from the center of the main peak by 0.21 eV, while the second of these is separated from the first by 0.20 eV. We interpret these as vibrational features deriving from arginine. Since these energy differences correspond to 1600–1700 cm^{-1}, it is likely that they represent excitations of C=N vibrations.[6] The arginine anion photoelectron spectrum was calibrated against that of Cu^-.

The arginine anion we observed is very unlikely to be arginine's valence anion. Calculations by Gutowski, Simons, and co-workers[8] did not find a stable arginine valence anion. Results from electron transmission spectroscopy suggest that amino acids do not form stable valence anions.[17,18] Moreover, calculations on glycine[19] and on betaine[20] also fail to find stable valence anions. As we discovered about fifteen years ago with our study of $(H_2O)_2^-$, dipole bound anions have distinctive photoelectron spectral signatures.[21] The photoelectron spectra of dipole bound anions are dominated by single, narrow peaks at low electron binding energies with one or more considerably weaker intensity peaks (character-

istic of their indigenous molecular vibrations) to their high EBE sides. Another example of the photoelectron spectral signature of dipole bound anions can be seen in the photoelectron spectrum[22] of $(HF)_2^-$ and in its theoretical analysis and modeling.[23] The dominant peak in the photoelectron spectrum of the arginine anion has all the characteristics of a dipole bound anion, and we interpret it as such.

As mentioned above, the calculations of Gutowski, Simons, and co-workers[8] found two, quasidegenerate dipole bound anions. These were deemed to be due to electron attachment to canonical (neutral) arginine, i.e., the lowest energy tautomer, and to its higher energy (net neutral) zwitterionic tautomer. Their prediction for the VDE of the zwitterionic anion tautomer is 0.32 eV. The VDE of the dominant peak in our spectrum is 0.26 eV. Their prediction for the VDE of the canonical anion is 0.14 eV. The EBE of the shoulder on the low EBE side of the main peak in our spectrum is 0.18 eV. Since this weak feature is subject to peak-pulling effects by the neighboring dominant peak, its true EBE may be slightly smaller than 0.18 eV. We interpret the dominant peak in the photoelectron spectrum as being due to the zwitterionic dipole bound anion and the weaker shoulder as probably being due to the canonical dipole bound anion. While the agreement between theory and experiment is quite good, it is interesting to note that the predicted VDE value is larger than the measured value in one case, yet smaller or perhaps about the same in the other. Also, the dissimilarity of the photoelectron intensities of the two species speaks to the extent to which the two dipole bound anions are truly degenerate. The implication is that the zwitterionic anion is the more stable of the two, just as theoretically predicted.

Many biological molecules and assemblies of their molecules have substantial dipole moments and thus are subject to forming dipole bound anions. The example of arginine and its anions shows the potential for electron-dipolar interactions to reverse the order of stability among neutral tautomers versus their anionic tautomers. More generally, this and related work[12] hints at the energetically significant role that excess electron interactions may play in biological systems.

We thank U. Boesl, P. Burrow, M. deVries, M. Gutowski, K. Kleinermanns, and E. Schlag for stimulating discussions. This work was supported by the National Science Foundation under Grant No. CHE-0211522.

[1] T. Wyttenbach, M. Witt, and M. T. Bowers, J. Am. Chem. Soc. **122**, 3458 (2000).
[2] W. D. Price, R. A. Jockusch, and E. R. Williams, J. Am. Chem. Soc. **119**, 11988 (1997).
[3] C. J. Chapo, J. B. Paul, R. A. Provencal, K. Roth, and R. J. Saykally, J. Am. Chem. Soc. **120**, 12956 (1998).
[4] Z. B. Maksic and B. Kovacevic, J. Chem. Soc., Perkin Trans. 2 **2**, 2623 (1999).
[5] P. Skurski, M. Gutowski, R. Barrios, and J. Simons, Chem. Phys. Lett. **337**, 143 (2001).
[6] J. Rak, P. Skurski, J. Simons, and M. Gutowski, J. Am. Chem. Soc. **123**, 11695 (2001).
[7] R. J. Gdanitz, W. Cardoen, T. L. Windus, and J. Simons, J. Phys. Chem. A **108**, 515 (2004).
[8] P. Skurski, J. Rak, J. Simons, and M. Gutowski, J. Am. Chem. Soc. **123**, 11073 (2001).
[9] R. A. Jockusch, W. D. Price, and E. R. Williams, J. Phys. Chem. A **103**, 9266 (1999).
[10] B. A. Cerda and C. Wesdemiotis, Analyst (Cambridge, U.K.) **12**, 657 (2000).
[11] R. R. Julian, R. Hodyss, and J. L. Beauchamp, J. Am. Chem. Soc. **123**, 3577 (2001).
[12] S.-J. Xu, J. M. Nilles, and K. H. Bowen, J. Chem. Phys. **119**, 10696 (2003).
[13] G. Meijer, M. S. deVries, H. E. Hunziker, and H. R. Wendt, J. Chem. Phys. **92**, 7625 (1990).
[14] U. Boesl, C. Baessmann, G. Drechsler, and V. Distelrath, Eur. Mass Spectrom. **5**, 455 (1999).
[15] H. V. Weyssenhoff, H. L. Selzle, and E. W. Schlag, Z. Naturforsch. Teil A **40A**, 674 (1985).
[16] J. Lindner, J. Grotemeyer, and E. W. Schlag, Int. J. Mass Spectrom. Ion Processes **100**, 267 (1990).
[17] K. Aflatooni, B. Hitt, G. A. Gallup, and P. D. Burrow, J. Chem. Phys. **115**, 6489 (2001).
[18] P. D. Burrow and G. A. Gallup, private communication, 2004.
[19] M. Gutowski, P. Skurski, and J. Simons, J. Am. Chem. Soc. **122**, 10159 (2000).
[20] J. Rak, P. Skurski, and M. Gutowski, J. Chem. Phys. **114**, 10673 (2001).
[21] K. H. Bowen and J. G. Eaton, in *The Structure of Small Molecules and Ions*, edited by R. Naaman and Z. Vager (Plenum, New York, 1988), pp. 147–169.
[22] J. H. Hendricks, H. L. de Clercq, S. A. Lyapustina, and K. H. Bowen, J. Chem. Phys. **107**, 2962 (1997).
[23] M. Gutowski and P. Skurski, J. Chem. Phys. **107**, 2968 (1997).