On the interaction of electrons with betaine zwitterions

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Betaine is a permanent zwitterion. The molecular betaine anion has been generated in a hybrid, infrared desorption-electron photoemission source and its photoelectron spectrum recorded. The photoelectron spectrum of the betaine anion is characteristic of a dipole bound anion, and its vertical detachment energy was measured to be 0.29 ± 0.03 eV. Calculations by Rak, Skurski, and Gutowski [J. Chem. Phys. **114**, 10673 (2001)] had found the betaine anion to be a dipole bound anion with a vertical detachment energy of 0.28 eV. We also measured the vertical detachment energy of deprotonated betaine to be ~1.9 eV. © *2005 American Institute of Physics*.
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Of the 20 amino acids utilized by nature to synthesize proteins, all of them form zwitterions in solution, and yet, as isolated (gas phase) neutral molecules, none do. Nevertheless, many of these same amino acids can be induced to form zwitterions in the gas phase when they are complexed with neutral molecules and/or with charges, thereby mimicking the stabilizing effects of solvation and counter ions, respectively.[1–4] There are, however, other amino acids which form zwitterions as isolated molecules, and betaine is an example. Betaine is the methylated version of zwitterionic glycine, $(CH_3)_3N^+CH_2COO^-$. Since nitrogen is fully methylated, no proton is available for transferring to its $-COO^-$ end, and betaine remains a zwitterion permanently, i.e., betaine is a zwitterion in its lowest energy, neutral state. While not used to make proteins, betaine is nonetheless biologically important. It is synthesized naturally in both plant and animal tissue. It plays a metabolic role in the oxidation of choline, and it helps to control osmotic pressure in cells.[5] Commercially, it is sold as a feed supplement for livestock, as an enrichment agent for human food, and as a biodegradable surfactant.

We have been interested in exploring the interaction of excess electrons with zwitterions of amino acids. In one study, we investigated the interaction of electrons with hydrated clusters of glycine, phenylalanine, and tryptophan,[4] while in another we examined the stabilization of arginine's zwitterion due to its interaction with (solvation by) an excess electron.[6] In the former case, where several water molecules did the lion's share of the work inducing zwitterion formation, the excess electron probably also played a significant role. In the latter case, where zwitterionic arginine (neutral) sits higher in energy than its nonzwitterionic (canonical) counterpart (also net neutral), interaction with excess electrons brought their corresponding anions into near degeneracy. In both studies, the interaction of excess electrons with the dipole moments of zwitterions resulted in the formation of dipole bound anions.

Not requiring an outside stabilizing interaction to coax it into its zwitterionic state, betaine is an ideal model system for studying excess electron binding by amino acid zwitterions. For this reason, Rak, Skurski, and Gutowski[7] have conducted *ab initio* calculations on betaine and its anion. They found neutral (zwitterionic) betaine to possess a single stable conformer with a dipole moment of 11.5 D. For the anionic state of betaine, they found a dipole bound anion with a vertical detachment energy of 2261 cm^{-1} (0.28 eV), and they modeled its expected photoelectron spectrum. Here we report our study of the betaine molecular anion by photoelectron spectroscopy and the measurement of its vertical detachment energy (VDE).

The most imposing obstacle facing the study of free biological molecules is getting them into the gas phase intact, 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 betaine 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 have developed a new source which is a hydrid of two existing techniques, pulsed laser infrared desorption and pulsed laser photoelectron emission. The work of deVries[8] and of Boesl[9] provided the most direct guidance to us in implementing infrared desorption and photoelectron emission techniques, respectively, although the earlier work of Schlag, Grotemeyer, Selzle, and co-workers had underpinned them both.[10,11]

Our hybrid source functions as follows (see Fig. 1). Pulses of helium are coordinated with infrared pulses (via the fundamental frequency of a Nd:YAG laser) which strike a slowly translating, biomolecule-coated rod or bar, which itself sits very near but slightly below the pulsed gas nozzle. This is similar to both the deVries[8] and the Kleinermanns[12] configurations, in which graphite bars are routinely used as

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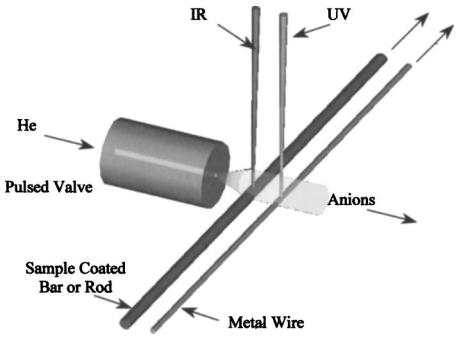

FIG. 1. Schematic of our infrared desorption/photoelectron emission hybrid anion source.

substrates for thinly applied biomolecule samples. Upon infrared irradiation, intact neutral biomolecules are desorbed into the vacuum due to ultrafast heating of the graphite substrate under the thin film of biomolecule sample. In our experiments with betaine, we have utilized copper metal rods sometimes and graphite bars at other times. In the former case, our samples are thickly applied, with the copper rod functioning only as a mechanical support and not as an infrared absorber. In the latter case, we have applied thin layers of sample to graphite bars. Coordinated with the 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 and moving with it. This results in a strong burst of electrons which are hurled into the momentary puffs of desorbed neutral biomolecules, all in the presence of cooling helium collisions. The result is electron attachment and the formation of both intact (parent) and fragment anions of biomolecules in the gas phase, assuming that the parent anion exists for a given molecule. These are then accepted into the spectrometer. Thus, while this technique is not MALDI *per se*, it is a laser assisted desorption technique for making ions.

Making betaine anions has been the most stringent test, thus far, of our hybrid source's ability to prepare parent anions of difficult-to-vaporize biomolecules. Since each betaine molecule is a zwitterion, the intermolecular forces between them makes betaine especially involatile. Nevertheless, we were able to prepare betaine parent anions with both copper rods and graphite bar substrates as described above. Interestingly, however, the betaine parent anion signals were stronger and their mass spectra cleaner when we used the metal rod. Experiments using graphite bars as sample substrates gave low intensities of the betaine anion and significant intensities of many different anions, possibly due to foreign materials that had absorbed onto its surface. It is important to realize, however, that our optimization of substrate materials and other source conditions is still in its infancy. Generally, we found that lower infrared laser power worked better than higher powers, and that 1.5–2.5 mm was an optimal diameter for the IR laser spot where it hit the sample. Some conditions also generated anions of betaine minus a hydrogen atom, i.e., deprotonated betaine.

Since our hybrid anion source is pulsed, we utilized our pulsed photoelectron spectrometer to measure the photoelectron spectrum of the betaine anion. Photoelectron spectrometry 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[13] consists of a pulsed anion source (described above), a linear time-of-flight mass spectrometer for mass selection, a second Nd:YAG laser for photodetachment, and a magnetic bottle for electron energy analysis. The resolution of our mass spectrometer is ~600, while the resolution of our magnetic bottle, electron energy analyzer is ~50 meV at EKE=1 eV.

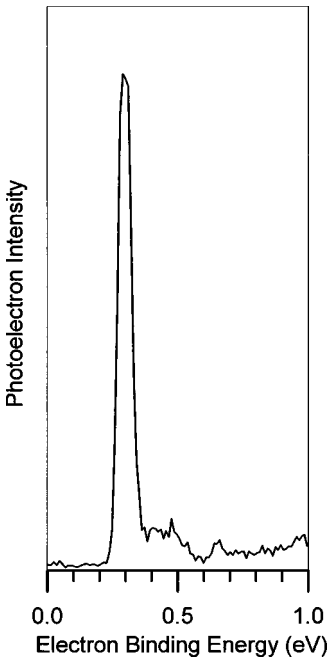

FIG. 2. The photoelectron spectrum of the betaine parent anion recorded with 1064 nm photons.

The photoelectron spectrum of the betaine 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 of the betaine anion is presented in Fig. 2. The vertical detachment energy extracted from this spectrum is 0.29 ± 0.03 eV. 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 0.66 eV. The first of these is separated from the center of the main peak by 0.18 eV, while the second of these is separated from the first by 0.19 eV. The vertical detachment energy of the deprotonated betaine was measured to be ~1.9 eV. Both photoelectron spectra were calibrated against that of Cu^-.

The betaine anion that we observed is very unlikely to be betaine's valence anion. The calculations of Rak, Skurski, and Gutowski[7] did not find a stable betaine valence anion. Results from electron transmission spectroscopy also suggests that most amino acids do not form stable valence anions.[14,15] Indeed, the photoelectron spectrum of the betaine anion has all of the characteristics of a dipole bound anion. As we discovered in our studies of $(H_2O)_2^-$, $(HF)_2^-$, $[CH_3CN(H_2O)]^-$, uracil anion, arginine anion, and others,

dipole bound anions have distinctive photoelectron spectral signatures.[6,16–18] Their spectra are dominated by single, narrow peaks at low electron binding energies with one or more considerably weaker intensity peaks (characteristic of their indigenous molecular vibrations) to their high EBE sides. This is unlike any other pattern in anion photoelectron spectroscopy. Based on our results, the betaine anion is interpreted to be a dipole bound anion. The two weaker peaks are due to excited vibrations of neutral betaine. Since the energy differences between the main peak and the first vibrational peak as well as between the first and second vibrational peaks both correspond to $\sim$1450 cm^{-1}, it is likely that they are both due to H-C-H bending in CH_3 and CH_2 groups.

The calculations of Rak, Skurski, and Gutowski[7] predict the betaine anion to be the dipole bound anion of the betaine zwitterion with a vertical detachment energy of 0.28 eV. We agree that it is a dipole bound anion, and we find, in very good agreement with their predicted value, that its vertical detachment energy is 0.29 ± 0.03 eV. They furthermore predict that the photoelectron spectrum of the betaine anion will exhibit only very weak vibrational structure, and that too is consistent with our results. There is, however, a minor discrepancy between theory and experiment in regard to which vibrational frequencies and thus modes will be most apparent in the spectrum. Theory predicted the weak vibrational features to be related to modes with frequencies around 650–850 cm^{-1}, while we observed frequencies around 1450 cm^{-1}. Interestingly, Rak and co-workers[7] commented in their paper that the largest discrepancy between computed and measured frequencies were found for modes that describe H-C-H bending in CH_3 and CH_2 groups, i.e., $\sim$1450 cm^{-1}. One should recognize, however, that using photoelectron spectroscopy to measure vibrational transitions in a molecule with so many modes can be problematic due to issues of peak overlap and resolution. Overall, however, the agreement between theory and experiment in this study is excellent.

Many biological molecules and assemblies of their molecules have substantial dipole moments and thus are subject to forming dipole bound anions.[19–21] The example of betaine and its anion hints at the energetically significant role that excess electron interactions may play in biological systems.

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