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Is Arginine Zwitterionic or Neutral in the Gas Phase? Results from IR Cavity Ringdown Spectroscopy

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It is commonly thought that amino acids and peptides exist in a neutral configuration (protonated carboxylic acid and deprotonated amine) in the gas-phase[1,2] because zwitterionic charge separation is unfavorable in the absence of solvation. However, a recent report suggested that the most stable form of gaseous arginine is actually zwitterionic.[3] In this proposed configuration, the guanidine in the side chain serves as an intramolecular proton acceptor, whereas the carboxylic acid serves as the donor (cf. Figure 1). We know of no previous direct experimental evidence that can support this intriguing claim and have accordingly performed a spectroscopic study of jet-cooled arginine using the novel technique of infrared cavity ringdown laser absorption spectroscopy (IR-CRLAS). Our results confirm that arginine indeed exists in the neutral configuration in a supersonic molecular beam.

The characterization of isolated gas-phase zwitterions is an important objective that has thus far proved elusive. Detailed spectroscopic information would facilitate understanding of the structure and energetics of these systems, providing data that could be used to better parametrize biomolecular potential models.[4] Moreover, such studies constitute an essential starting point for investigating salient details of biomolecular solvation. In infrared spectroscopy experiments, one could unambiguously identify the neutral form of arginine by the presence of carbonyl stretch bands at ca. 1700 cm^{-1} and the zwitterion by carboxylate asymmetric and symmetric stretches at ca. 1500−1600 cm^{-1}.

IR-CRLAS is a new ultrasensitive direct absorption technique that has recently been used to study a variety of molecular systems.[5−7] The method employs a set of highly reflective mirrors that form an optical cavity into which an absorbing sample can be placed. The sample absorption is determined by monitoring the exponential intensity decay of laser radiation coupled into the cavity. Resonant absorption will attenuate more light on each pass than does the passive cavity, leading to a faster decay of the light intensity. By measuring the time constant of the exponential decay, one can directly extract the absolute absorbance of the sample. Tuning the light over a given frequency range will thus produce an absorption spectrum. Ultrahigh sensitivity results from the combination of a large path length and insensitivity to fluctuations in the total intensity.

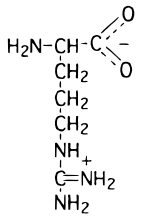

Figure 1. Zwitterionic form of arginine. Notice that this is not the standard zwitterion associated with biomolecules. For this system, the guanidine in the side chain is the base, as opposed to the backbone amine of the amino acid.

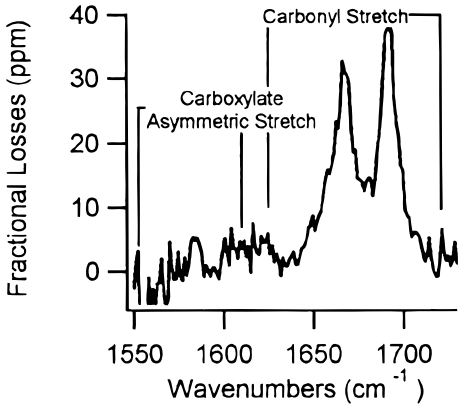

Figure 2. IR-CRLAS spectrum of jet-cooled arginine in the carbonyl and carboxylate asymmetric stretch region. The absence of a band near 1600 cm^{-1} confirms a small population of the zwitterion.

Gas-phase arginine was produced using a heated, pulsed, 4-inch-slit molecular beam source, described previously.[8] The free base form of L-arginine (CAS 74-79-3) was obtained from Sigma. No additional sample preparation was used. Maintaining the source at a temperature ca. 170 °C optimized the arginine signal. After the experiment was run, a mass spectrum and FTIR spectrum (taken in a KBr pellet) of the sample remaining in the source as well as the residue collected on a microscope slide downstream of the source confirmed that the sample in the molecular beam did not undergo significant decomposition.

The IR-CRLAS spectrum of arginine in the 1550−1750 cm^{-1} region revealed two peaks near 1700 cm^{-1} (cf. Figure 2; 1666 cm^{-1}, 1693 cm^{-1}), corresponding to a carbonyl stretch of a carboxylic acid and confirming that neutral arginine was present in our molecular beam. The fact that several peaks were observed in this region can be explained by the presence of several nearly isoenergetic conformers. Different structures led to distinct local environments and vibrational frequencies. Similar patterns have been found in matrix isolation studies of other amino acids.[9] Scans near 1600 cm^{-1} showed no peaks. Consequently, a significant population of zwitterions cannot be present in our molecular beam, assuming that the carboxylate mode intensity of the zwitterionic form of arginine is comparable to the carbonyl mode intensity of the neutral.

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Table 1. Intensity Calculation Results for the Model System of Neutral and Zwitterionic Glycine

	intensity (km/mol)	
level	C═O s (neutral glycine)	COO^- asym s (zwitterionic glycine)
HF/6-31G	298.6	441.8
HF/6-311G*	327.3	555.8
MP2/6-311G*	206.5	405.7

Although the frequencies of the carboxylate symmetric and asymmetric stretches are well-known from both experiment and theory,[10] the intensities of these modes have not been reliably determined. This is due to the fact that many quantum chemical programs do not provide fast, accurate intensities for large systems, since extremely large basis sets are required to precisely describe the molecular orbitals and thus the harmonic force field.[11] For example, infrared intensities determined by Hartree−Fock methods often produce errors on the order of 10−50%.[12]

To estimate the carboxylate mode intensities in arginine, we performed *ab initio* calculations on the more tractable system of glycine at a variety of levels.[13] We argue that the side chain of arginine does not significantly influence the magnitude of the carboxylate mode intensities since the first part of the side chain is saturated and contributes a minimal amount of electron density to the carboxylate group. The results summarized in Table 1 show that the intensity of the carboxylate asymmetric stretch of the zwitterion is of similar order as that of the carbonyl stretch of the neutral amino acid. Therefore, we conclude that given a comparable population of neutral and zwitterionic configurations, we would be able to detect both carbonyl stretch and carboxylate asymmetric stretch features.

IR-CRLAS directly provides absolute band intensities, which we combine with an *ab initio* vibrational intensity in a simple Beer's law analysis to obtain the absolute number density of a species within our molecular beam. As discussed in the previous paragraph, we used *ab initio* carboxylate mode intensities for glycine to estimate the lower bound for the number density of neutral arginine in our molecular beam ($2.1 \times 10^{12}/cm^3$ with Hartree−Fock level intensities; $3.3 \times 10^{12}/cm^3$ with MP2 level intensities). Assuming that we would be able to identify a carboxylate band having a minimum integrated intensity a factor of 7 less than the neutral carbonyl band and a baseline noise level of 1 ppm, we estimate an upper bound for the number density of zwitterionic arginine as $3 \times 10^{10}/cm^3$, or 1% of the neutral density.

Arginine thus exists predominately in the neutral configuration in our molecular beam, a conclusion that was not necessarily expected in light of recent results published in the literature. However, the fact that we did not detect arginine as a zwitterion is perhaps not surprising, given that we began with neutral arginine in our heated molecular beam source and that there is presumably a significant barrier to proton transfer from the carboxylic acid to the amine. Several calculations have suggested that addition of as few as two to five water molecules is sufficient to stabilize zwitterionic amino acids with respect to the corresponding neutral forms in the gas phase.[14,15] We are currently investigating the role of water in determining the charge structure of arginine.

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