

Chapter 2

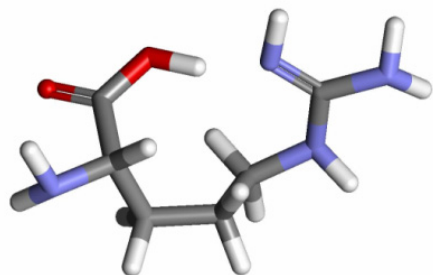
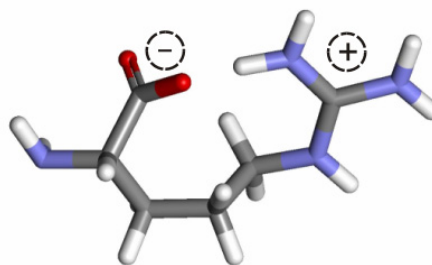
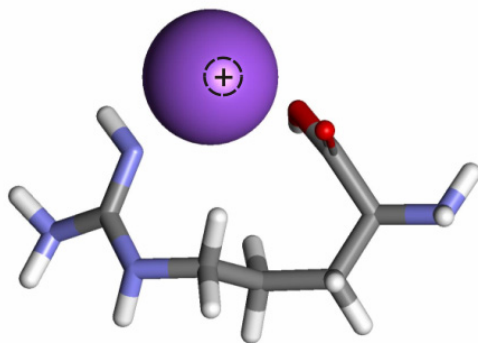
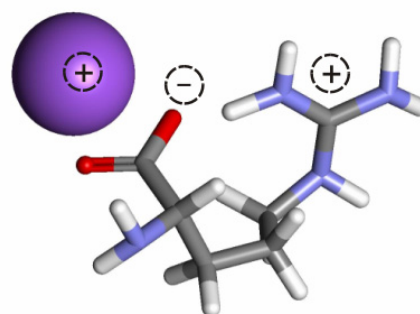
Salt Bridge Stabilization of Charged Zwitterionic

Arginine Aggregates in the Gas Phase

Portions previously published in: Julian R. R.; Hodyss R.; Beauchamp J. L. *J. Am. Chem. Soc.* **2001**, *123*, 3577-3583.

2.1 Introduction

Amino acids are known to exist as zwitterions in solution, but conditions appropriate for stabilization of the zwitterionic form in the gas phase remain debatable. Studies of the gas phase acidity and basicity of glycine with Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry have shown that the glycine zwitterion is unstable by ~ 84 kJ/mol.¹ Many recent calculations confirm that glycine is unlikely to exist in the gas phase as a zwitterion;² furthermore, the zwitterion is not even predicted to be a minima on the potential energy surface. Theory suggests that water molecules can stabilize the zwitterionic form of glycine in the gas phase, with recent calculations suggesting that two water molecules are required.³ In contrast, recent experimental results suggest that the number of water molecules necessary to stabilize glycine as a zwitterion is five.⁴

Arginine **2.1**Zwitterionic Arginine **2.2**Charge Solvated **2.3**Zwitterionic Salt Bridge **2.4**

The gas phase stability of the zwitterionic form of arginine is predicted to be much more favorable. The arginine zwitterion is created by transferring a proton from the C-terminus to the side chain (see **2.1** and **2.2**). The nomenclature indicated for arginine structures **2.1-2.4** will be used in the present work. Arginine is the most basic amino acid (see Table 2.1). This high basicity increases the stability of zwitterionic arginine in the gas phase relative to other amino acids. However, recent experiments have shown that isolated arginine is not a zwitterion. Cavity-ring down laser absorption spectra of jet-cooled arginine do not exhibit a peak corresponding to the calculated carboxylate asymmetric stretch of the zwitterion.⁵ High level computations have confirmed that arginine (**2.1**) is more stable than zwitterionic arginine (**2.2**) in the absence of an

additional charge.⁶ However, theory predicts that the zwitterionic arginine is only less stable than arginine by 4-12 kJ/mol, depending on the level of theory.⁶

Table 2.1 Relative gas phase acidities and basicities.⁷

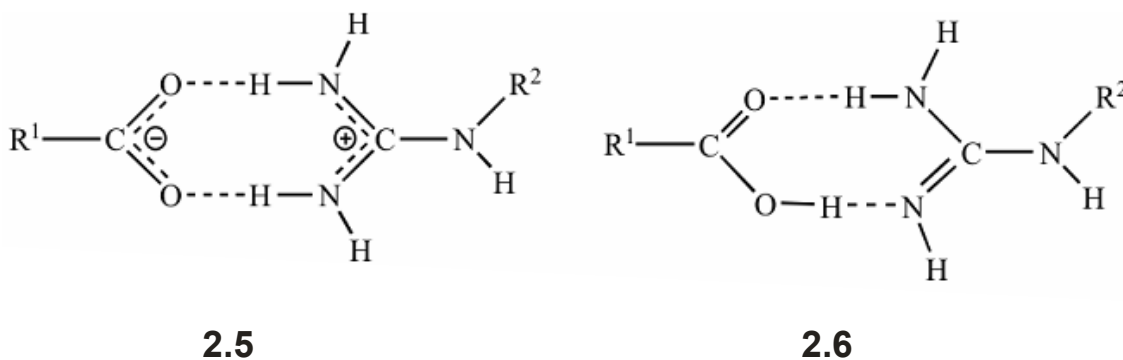
Amino Acid	Gas Phase Acidity (kJ/mol)	Gas Phase Basicity(GB) (kJ/mol)
Glycine	1402(avg.)	852
Lysine	1383	951
Arginine	1360	1007
Histidine	1356	950
HF	1531	--
HCl	1373	--
HBr	1332	--
HI	1294	--
HNO ₃	1330	--

The stability of the zwitterionic forms of amino acids can be substantially modified by specific noncovalent interactions with nearby molecules and ions.⁸ For example, calculations show that copper ions can stabilize zwitterionic glycine.⁹ The addition of a cation to arginine is likely to lead to one of the two structures depicted as **2.3** and **2.4**. Collision induced dissociation spectra obtained with a FT-ICR mass spectrometer suggest that zwitterionic arginine is stabilized by metal cations (K⁺, Rb⁺, and Cs⁺).¹⁰ Related results based on the dissociation of heterodimers also favored structure **2.4** (for K⁺, and Cs⁺).¹¹ Experiments suggest that the protonated arginine dimer exists with one arginine in the zwitterionic state.^{12,13} Ion mobility data for complexes of arginine with Na⁺, Cs⁺, and H⁺ could not discriminate between **2.3** and **2.4** in the case of the metal ions.¹⁴ However, calculations performed in conjunction with these experiments favored the zwitterionic salt-bridge **2.4**.¹⁴

Arginine participates in a variety of specific noncovalent interactions involving ionized functional groups that are easily observed in crystal structures. Crystalline

arginine itself is different from all other amino acids. Typically, amino acids arrange themselves in a peptide-like fashion with the n-terminal amino and c-terminal carboxylate groups aligned with the side chains protruding outward on alternating sides.¹⁵ In contrast, arginine stacks end to end enabling the guanidinium and carboxylate interaction shown in **2.5**.¹⁶ This motif is common in crystal structures containing arginine as illustrated by arginine with acetate⁵ and in the dipeptide Arg-Glu.¹⁷

The stability of **2.5** with various groups R^1 and R^2 in the gas phase has been addressed by several computational approaches. Calculations have been performed on model



systems at various levels of theory, including semi-empirical calculations with PM3 and AM1, density functional theory, and ab initio methods.^{18,19,20} The energetics of **2.5** and **2.6** (where $R^1 = R^2 = \text{CH}_3$) were calculated at the RHF/6-31G** and MP2/6-31G** ab-initio level.¹⁸ Semi-empirical results obtained with AM1 qualitatively reproduced the ab-initio results and both levels of theory predict that **2.5** is not likely to exist in the gas phase, with **2.6** being energetically favored by ~ 18 kJ/mol at the highest level of theory.

The discovery of several new unusually stable aggregates of arginine that are intermolecularly bound by salt bridges is reported here. Quadrupole ion-trap mass spectrometry provides further evidence for the stability of arginine in the zwitterionic state, coordinated to either a cation or anion. Clusters of arginine with sodium,

potassium, lithium, magnesium, chloride, fluoride, bromide, iodide, and nitrate ions are reported. Computational results at the DFT level are used to assign structures to and assess the energetics of particularly prominent clusters. A new method is introduced to investigate the effect of arginine enantiomeric purity on the stability of several of the more prominent clusters reported in this study. Our results are compared and contrasted to the recent report by Cooks and co-workers²¹ of protonated clusters of arginine alone.

2.2 Experimental Methodology

All spectra were obtained using a Finnigan LCQ ion trap quadrupole mass spectrometer without modification. The signal was tuned using the automatic tuning capabilities of the LCQ on the trimer of the protonated arginine clusters. Subsequent tuning on higher order clusters did not increase the signal or change the profile of the cluster distribution. Tuning on the monomer or dimer favors more harsh conditions that lead to the predissociation of the higher order clusters. The particular settings used for all data collection in this paper included source voltage 4.15 kV, capillary voltage 40.47 V, capillary temperature 199.9 °C, and tube lens offset 10 V. For the negative ion mode, source voltage polarity was simply reversed.

Sample concentrations were kept uniformly at ~300 μ M for arginine and ~100 μ M for the additional species of interest, unless otherwise noted. These concentrations are substantially higher than those used for this instrument for analytical purposes (which will not be our focus here). All samples were electrosprayed in an 80:20 methanol/water mixture. Samples were electrosprayed with a flow of 3-5 μ L/min from a 500 μ L Hamilton syringe for optimal signal. Silica tubing with an inner diameter of 12.7

microns was used as the electrospray tip. No acid was added to any of the samples. All chemicals were purchased from Sigma or Aldrich and used without further purification, unless indicated otherwise.

For the study of chiral effects, samples were electrosprayed at ~ 200 μM concentration for each component (the total concentration being ~ 400 μM). D-Arginine HCl was used in conjunction with isotopically labeled L-arginine HCl. The labeled arginine contained two ^{15}N (99+ atom %) at the terminal guanidinium positions. All other conditions were identical to those mentioned previously. The labeled arginine was purchased from Cambridge Isotope and used without further purification.

Initial calculations were performed on the HyperChem 5.1 Professional Suite. Candidate structures were identified with molecular mechanics and submitted to full optimization at the PM3 semi-empirical level. The DFT calculations were carried out using Jaguar 4.0 (Schrödinger, Inc., Portland, Oregon). Full geometry optimization was performed at the B3LYP/6-31G** level of theory.

2.3 Results

Clustering of Electrosprayed Amino Acids and Derivatives. Protonated clusters of arginine are shown in Figure 1.1a. The protonated clusters of arginine fit the distribution $[n\text{Arg}+x\text{H}]^{x+}$ where $n = 1-7$ for $x = 1$, and $n > 11$ for $x = 2$. As shown in the spectrum, the distribution varies uniformly with n , with no indication of clearly favorable structures or “magic numbers”.

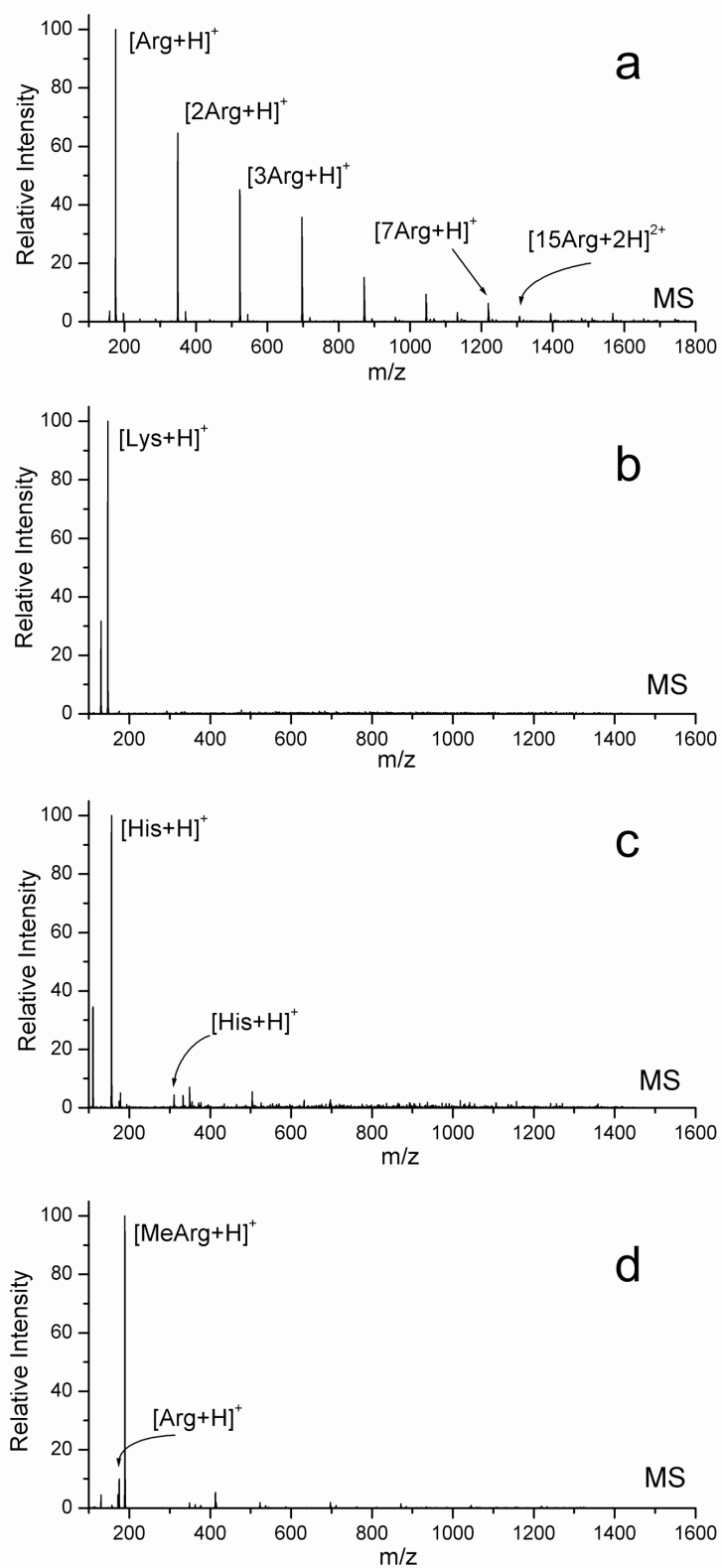


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Figure 2.1 Electrospray mass spectra of basic amino acids and derivatives at 300 μ M concentration. Arginine (a) exhibits extensive noncovalent clusters. Lysine (b) and histidine (c) exhibit no extensive clustering. Arginine methyl ester (MeArg) electrosprayed with arginine (d) shows that MeArg does not cluster with itself or with arginine, demonstrating the importance of the C-terminus in the clustering.

To assure that the arginine clusters were indeed atypical and not the result of electrospray conditions that would lead to the clustering of any small molecule, several other amino acids were run under identical conditions. In particular, lysine and histidine were examined due to the high basicity (Table 2.1)⁷ of these amino acids. The mass spectra of lysine and histidine are shown in Figure 2.1b and 2.1c, respectively. Under conditions where arginine forms extensive clusters, histidine exhibits only a small dimer and no clusters are observed with lysine. Histidine and lysine will cluster at higher concentrations and lower capillary temperatures, but the extent of clustering observed with these milder ion sampling conditions is still less than that illustrated in Figure 2.1a for arginine. The propensity for arginine to cluster more than other amino acids is consistent with past observations.²¹

To probe the origin of the stability of the clusters, a mixture of arginine methyl-ester (MeArg) and arginine was electrosprayed. The spectrum of the mixture is shown in Figure 2.1d. The protonated MeArg peak completely dominates the spectrum, and the intensity of the protonated arginine clusters is dramatically reduced compared to the results in Figure 2.1a. In addition, no clusters are observed for MeArg in a solution

containing no arginine. These results indicate that the C-terminus plays a critical role in the formation of arginine clusters.

Effect of Metal Cations on Clusters of Arginine. In light of previous work^{10,14}, the effect of metal cations on clustering of arginine was examined. The metals were added to the solution in the form of the appropriate chloride salt. The results are summarized in Figure 2.2.

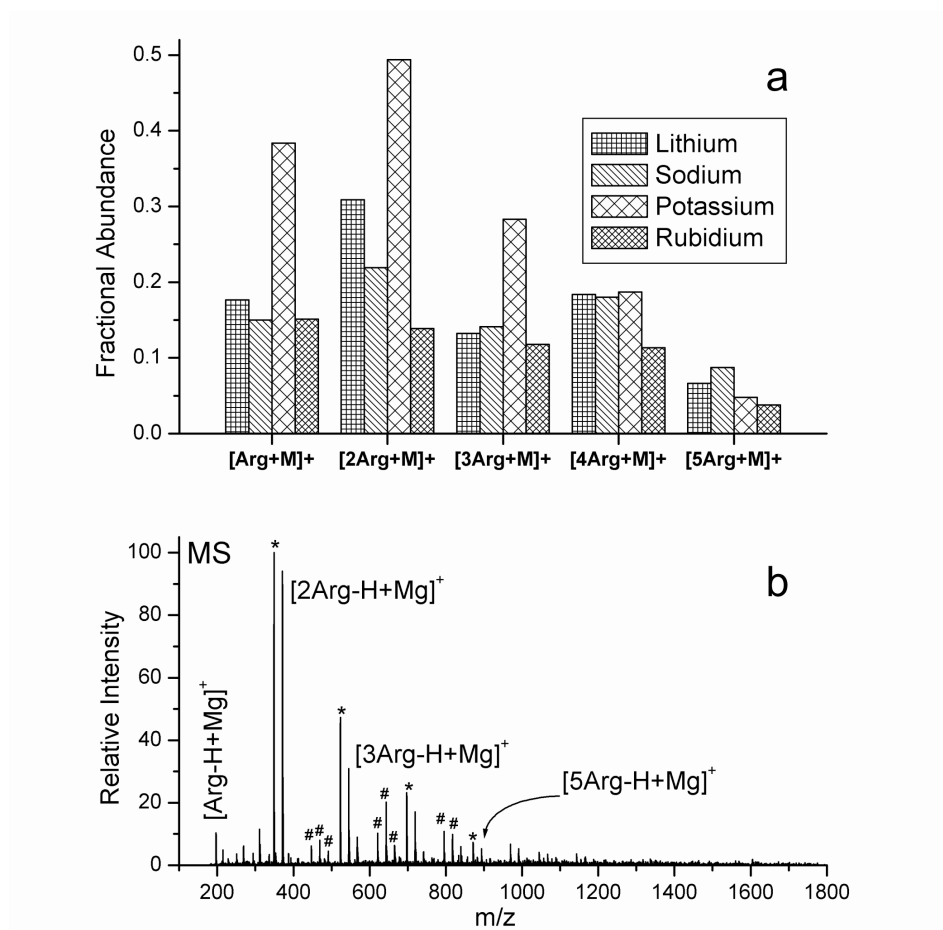


Figure 2.2 (a) Distribution of $[n\text{Arg}+\text{M}]^+$ clusters where $\text{M} = \text{Li}, \text{Na}, \text{K},$ and Rb taken relative to the protonated dimer. (b) Arg/Mg clusters showing the unusual abundance of the dimer. * indicates protonated clusters of Arginine and # indicates clusters of arginine with sulfate.

The results for clusters of arginine with Li, Na, K, and Rb are grouped together in Figure 2.2a. The alkali metal arginine clusters fit a distribution described by $[n\text{Arg}+\text{M}]^+$, where $n = 1-5$ and $\text{M} = \text{Li}, \text{Na}, \text{K}, \text{and Rb}$. Li^+ clusters have a bimodal distribution centered on $n = 2$ and 4. Clusters of arginine with Na^+ fit a similar bimodal distribution. K^+ clusters exhibit a smooth distribution centered on $n = 2$. The distribution changes again for clusters of arginine with Rb^+ , with the intensity of the clusters declining from the monomer. The alkali metal spectra do not suggest that any of the clusters are unusually stable.

The results of mixing arginine with MgSO_4 are shown in Figure 2.2b. The Mg^{2+} clusters are described by $[n\text{Arg}-\text{H}+\text{Mg}]^+$ where $n = 1-5$, resulting in a complex that is singly charged. The dimer ($n = 2$) is particularly favored in this series, exhibiting over twice the intensity of the monomer or the trimer. The structure and stability of this cluster is discussed further below.

Anionic Arginine Clusters. Arginine electrosprayed in negative ion mode yielded the spectrum shown in Figure 2.3a. Anionic clusters of arginine are observed under identical conditions to those producing the cationic clusters (only reversing the polarity). These clusters may be described by $[n\text{Arg}-\text{H}]^-$, where $n = 1-9$, where the net charge on the species is -1 from the loss of one proton. Deprotonated clusters of anionic arginine are observed out to a total of 9 arginines. Apart from the abundant monomer, the distribution of clusters peaks at $n = 4$.

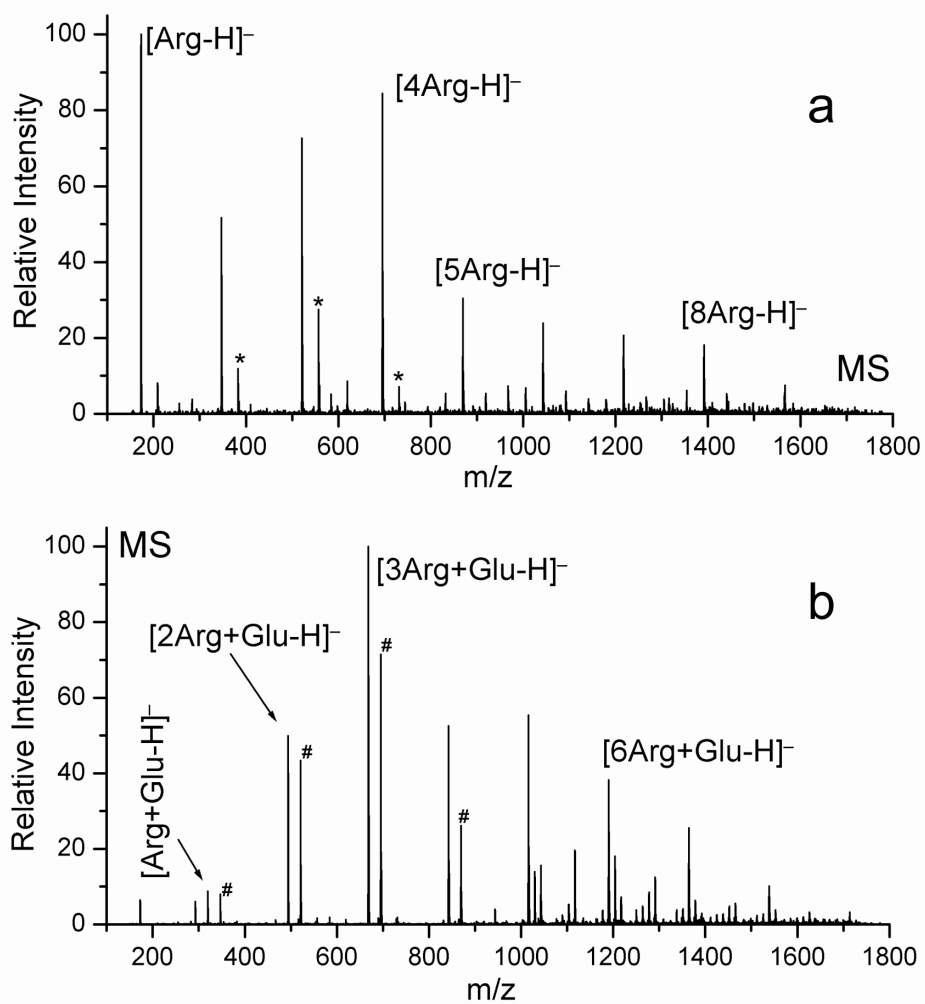


Figure 2.3 Anionic electrospray mass spectra of arginine clusters. (a) Deprotonated arginine clusters. (b) Arg/Glu clusters (150 μM Glu). * indicates clusters of Arg/Cl⁻ (see Figure 2.4) and # indicates deprotonated arginine clusters.

Clusters of arginine with acidic amino acids are generally described by $[n\text{Arg}+x\text{X}-\text{H}]^-$, where $n = 1-9$ for $x = 1$ with $\text{X} = \text{Glu}, \text{Asp}$. The spectrum of a mixture of glutamic acid and arginine is shown in Figure 2.3b. Clusters observed with aspartic acid are nearly identical to the distribution in Figure 2.3b. The presence of an abundant anionic cluster with $n = 3$ is noted and will be discussed further below. Also, there are numerous peaks of low intensity, distributed in the range 1000-1600 m/z . The charge state of these clusters is not within the resolution capabilities of the instrument. However, they appear to be multiply charged higher mass clusters of multiple glutamic acids with multiple arginines.

Anionic clusters of arginine incorporating halide anions were examined by addition of the sodium salt of each halogen to the arginine solution, which was then electrosprayed in negative ion mode. The results are shown in Figure 2.4.

Figure 2.4a shows the spectrum resulting from the addition of fluoride. Interestingly, anionic clusters of arginine incorporating fluoride ions are not observed. The spectrum of clusters observed in the presence of chloride ion is shown in Figure 2.4b. The chloride clusters fit a distribution described by $[n\text{Arg}+x\text{Cl}]^{x-}$, where $n = 1-58$ and $x = 1-6$. Multiply charged clusters are apparent in this case where $x > 1$. The cluster corresponding to the trimer $n = 3$, $x = 1$ is particularly prominent, suggesting unusual stability. Bromide clusters (Figure 2.4c) are described by $[n\text{Arg}+\text{Br}]^-$, where $n = 2-4$. The trimer ($n = 3$) is again the most prominent of the observed clusters. In comparison to chloride, the overall cluster intensity and variety are greatly reduced.

The spectrum for iodide is shown in Figure 2.4d. The iodide clusters are described by $[n\text{Arg}+\text{I}]^-$, where $n = 2, 3$. With iodide, the cluster intensity is reduced again relative to

chloride and bromide. The results for the halogen series demonstrate that chloride has the greatest propensity to form stable clusters with arginine.

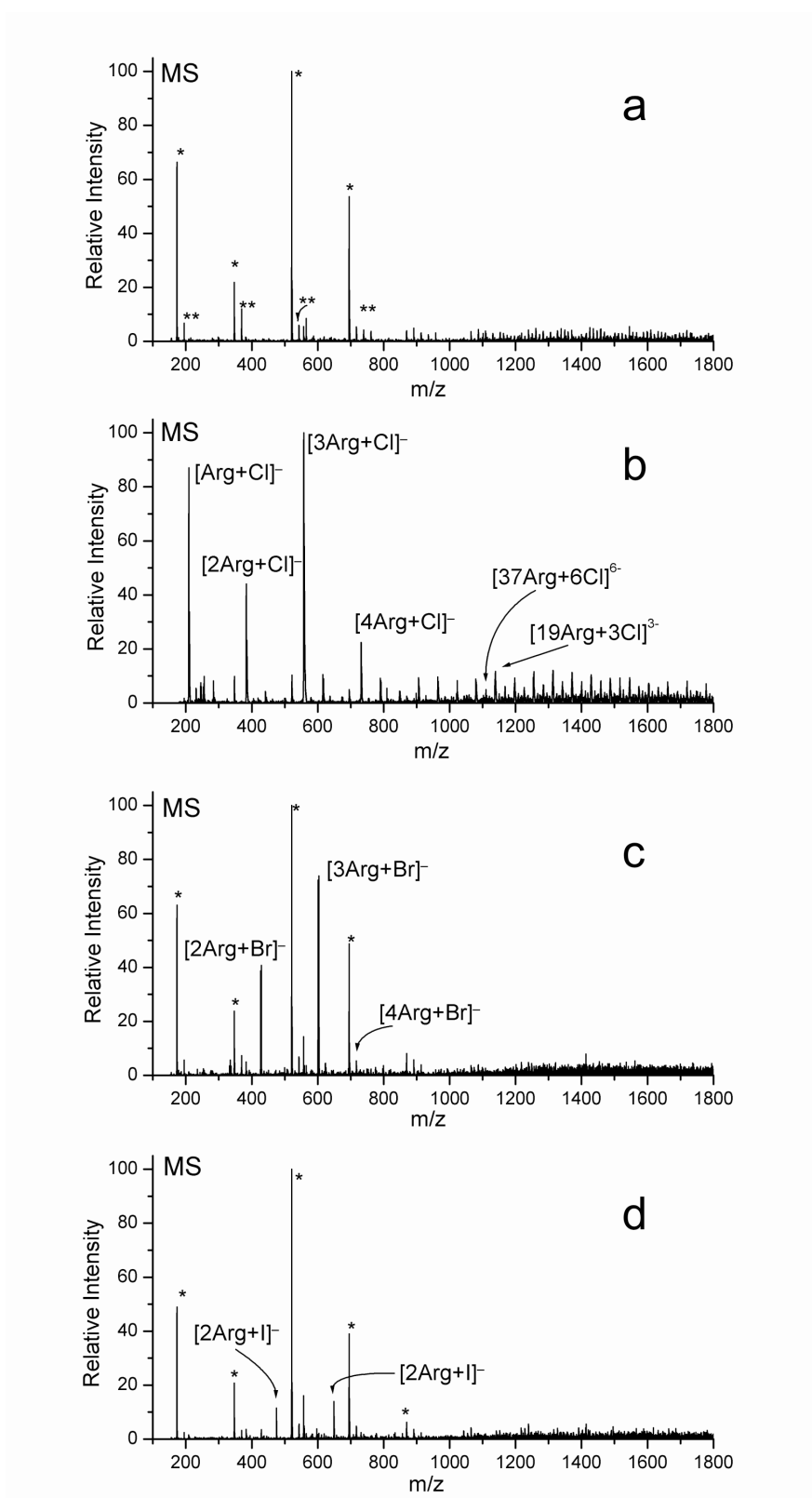


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Figure 2.4 Anionic electrospray mass spectra of Arg/halide clusters. (a) Arginine does not cluster with fluoride. (b) Addition of chloride leads to extensive clustering and “magic” trimer (600 μ M Arg). (c) Arginine clusters to a small degree with the bromide anion, where again the trimer is the primary peak. (d) Arginine forms clusters with iodide, including the trimer, though the clustering is greatly reduced overall. * indicates deprotonated arginine clusters and ** indicates anionic sodiated cluster of Arg.

Chiral Effects. To determine the effect of enantiomeric purity on the stability of the clusters, a mixture of D and L arginine was electrosprayed. The L-arginine was isotopically labeled by two ^{15}N (99+ atom %) at the terminal guanidinium positions to distinguish it from D-arginine in the cluster spectra. The relative concentrations of each enantiomer were approximated by the relative monomer intensities. A binomial distribution was then used to predict the intensities of the observed heterochiral and homochiral species. For example, the dimer should follow the distribution described by LL + DL + LD + DD, where the predicted intensities would be LL=0.25, DL(LD)=0.5, and DD=0.25 for a mixture that was initially 50% of each monomer. Deviation of the observed distribution from this binomial distribution would indicate that cluster stability is dependent on the enantiomeric composition.

Table 2.2 lists the predicted distributions for protonated arginine clusters based on the binomial distribution. The results for the particularly stable NO_3^- trimer are also included (the chloride trimer was not evaluated due to the added isotopic complexity

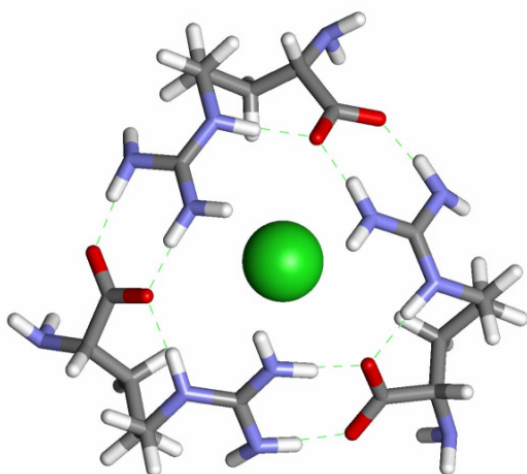
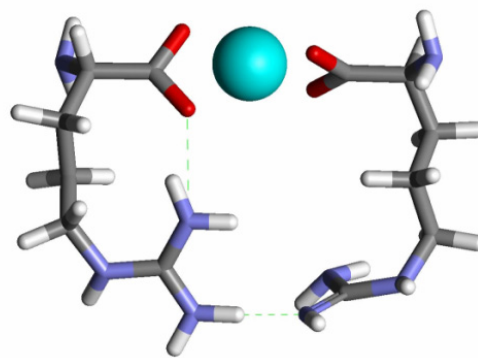
inherent with chloride spectra). The observed distributions do not show a significant deviation from the expected distribution.

Table 2.2 Cluster distributions for an enantiomeric mixture of various protonated arginine clusters and [3Arg+NO₃]⁻. Predicted percentages are based on the expected percent from a binomial distribution of clusters. The initial monomer distribution was 47.7% D-arginine hydrochloride and 52.3% isotopically labeled L-arginine hydrochloride.

Clusters of Arginine from D,L Mixture	Distribution (as % of total)		
	Predicted	Observed	Difference
DD	22.8	21.8	1.0
DL	49.9	49.2	0.7
LL	27.4	28.9	-1.5
DDD	10.9	10.6	0.3
DDL	35.7	33.2	2.5
DLL	39.1	41.5	-2.4
LLL	14.3	14.7	-0.4
DDDD	5.2	5.6	-0.4
DDDL	22.7	19.6	3.1
DDLL	37.3	33.9	3.4
DDL	27.3	29.9	-2.6
LLLL	7.5	10.9	-3.4
DDDDD	2.5	5.0	-2.5
DDDDL	13.5	9.6	3.9
DDDLL	29.7	26.8	2.9
DDLLL	32.5	33.9	1.4
DLLLL	17.8	18.8	-1.0
LLLLL	3.9	5.9	-2.0
DDD*	6.0	5.9	0.1
DDL*	28.0	29.8	-1.8
DLL*	43.5	44.6	-1.1
LLL*	22.5	19.7	2.8

*Results correspond to structure **7** capped with NO₃⁻.

Calculations. DFT calculations at the B3LYP/6-31G** level have been utilized to assess the energetics and structures of the arginine clusters. This level of calculation is feasible for the trimeric clusters, but the size of the remaining observed clusters prohibits the use of higher level calculations. We have found that PM3 calculations will qualitatively reproduce the DFT results, enabling calculations to be performed on larger systems at this level.

**2.7****2.8**

The proposed structure for the anionic trimer (**2.7**) is stabilized by the intermolecular interaction between the guanidinium and carboxylate groups **2.5** that is often observed in crystal structures. The structure allows all three arginines to participate in this highly favored interaction, while allowing for the additional stabilization of an intra-molecular hydrogen bond within each arginine. Higher level DFT calculations carried out at the B3LYP/6-31G** level support structure **2.7**. The charge solvated version of structure **2.7** (with non-zwitterionic arginines) will convert automatically to the zwitterionic version when minimized. Collision induced dissociation (CID) experiments on structure **2.7** demonstrate primarily the sequential loss of neutral arginine.

The calculations on the $[2\text{Arg-H+Mg}]^+$ dimer reveal another geometrical configuration by which the zwitterionic state of arginine is favored. The charge of the dimer is overall +1. This indicates that the one of the arginines is negatively charged and that the other is neutral. The neutral arginine could either be zwitterionic or not. DFT calculations show that the zwitterionic form is more stable by 270 kJ/mol. The geometry is shown in **2.8**, and it can be observed that much of the stabilization comes from the magnesium ion interaction with the carboxylate groups. This highly favorable interaction in which the two carboxylate groups closely approach the cation at nearly right angles to one another is only possible in the dimer and probably accounts for the special stability of this cluster.

Experimental Support for Structure 2.7. It can be noted from the structure for the arginine trimer **2.7** that it has threefold symmetry which might facilitate simultaneous formation of three hydrogen bonds with an appropriate anion. The requirements imposed on this anion would be that it have a size similar to that of the chloride ion and three hydrogen bond acceptor sites that could be aligned with the three available hydrogens from the guanidinium side chains in **2.7**. NO_3^- fits these requirements, with a size ~0.1 angstroms larger than Cl^- . The spectrum for the clusters of arginine with NO_3^- is shown in Figure 2.5. The clusters fit the distribution $[n\text{Arg+NO}_3]^-$, where $n = 1-5$. The most dominant $[n\text{Arg+NO}_3]^-$ cluster corresponds to $n=3$.

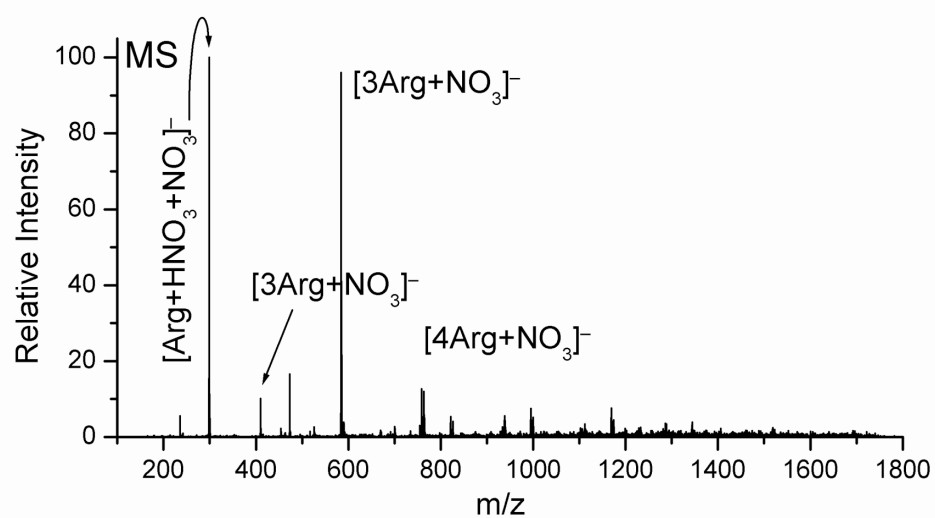


Figure 2.5 Anionic electrospray mass spectra of Arg/NO₃⁻ clusters, exhibiting the trimer as an abundant and unusually stable species, thus supporting the proposed structure (2.7) for the anionic trimer.

2.4 Discussion

Arginine forms extensive supramolecular assemblies in the gas phase, sampled from moderately concentrated solutions by electrospray ionization. The clusters observed in this study are ionic in nature, with the charge bearing group ranging from simple monatomic anions or cations to protonated or deprotonated molecular species. Comparisons with other amino acids show that arginine is unique in its ability to cluster under these conditions. Previous work has shown that arginine may be stable as a zwitterion in the gas phase in the presence of charge.¹⁰⁻¹⁴ It is consistent with all experimental and theoretical results in the present work that salt bridges formed between ions and zwitterionic arginine stabilize the observed clusters.

It has been reported previously that $[4\text{Arg}+\text{H}]^+$ has special stability²¹; however, we find no evidence to support this claim here. The intensity of the cluster corresponding to $[4\text{Arg}+\text{H}]^+$ does not remain unusually abundant with change in concentration, as shown in Figure 2.1a. Although this peak becomes more intense at concentrations several orders of magnitude higher, it still conforms to the surrounding distribution. We attribute the change in the intensity of this cluster to the change in the concentration. We were unable to produce the proposed²¹ planar arrangement of $[4\text{Arg}+\text{H}]^+$ resembling the crystal structure of NaCl with molecular modeling.

The cluster distributions for the alkali metal ions with arginine (Figure 1.2a) are dependent on the particular metal. Na^+ and Li^+ exhibit the same bimodal distribution of clusters, suggesting that these metals form clusters with arginine that are similar in structure. In contrast, clusters of arginine with K^+ exhibit greater intensity, and a different distribution centered on the dimer. This suggests a different series of structures,

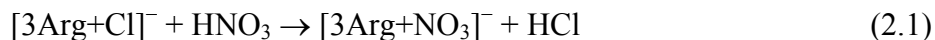
and it is consistent with previous observations where K^+ was found to stabilize the zwitterionic state of arginine better than Na^+ or Li^+ .¹⁰ For Rb^+ the intensity of the clusters is lower and a different distribution is observed. Interestingly, the cluster abundances for Rb^+ are nearly constant from the monomer to the tetramer.

Anionic clusters of arginine with the halides are very different in nature from the cationic clusters discussed thus far. Arginine was not observed to cluster with the fluoride anion (Figure 2.4a). Calculations suggest that this is due to the small size and high gas phase basicity of F^- , which deprotonates the guanidinium group of arginine. The resulting formation of HF would yield a spectrum of deprotonated arginine clusters, consistent with the experimental results shown in Figure 2.4a. The chloride anion is able to form abundant clusters with arginine (Figure 2.4b). The lower gas phase basicity and larger size of the chloride anion make it a better candidate for cluster formation. Both bromide and iodide show decreasing cluster intensity, suggesting that these anions are too large for ideal clustering conditions (Figure 2.4c and Figure 2.4d). While the intensity of the arginine clusters with attached halide ions varies with size much like the alkali metal clusters, the similar cluster distribution observed for each anion suggests a common structure.

Two of the clusters (**2.7** and **2.8**) in this work are unusually abundant when they appear in a cluster distribution, suggesting structures with special stability. Both of these clusters exhibit intensities that are at least double the intensities of the $n+1$ and $n-1$ clusters (where $n = \text{Arg}$). This remains evident over a wide range of concentrations, as may be seen by comparison of Figure 2.3a with Figure 2.4b. In Figure 2.3a trace

impurities lead to the formation of $[n\text{Arg}+\text{Cl}]^-$ clusters (where $n = 1-4$). However, even at very low concentrations, the trimer **2.7** is the most prominent peak.

The anionic trimer shown in **2.7** illustrates the features of zwitterionic arginine that facilitate formation of an unusually stable cluster. The cyclic array of molecules accommodates a nearly planar guanidinium and carboxylate interaction between adjacent arginines. Coulombic attraction and two specific strong hydrogen bonds result from this orientation. In addition, an internal hydrogen bond stabilizes each arginine individually (the lowest energy conformations of arginine itself all contain an internal hydrogen bond).⁶ Each positively charged guanidinium group interacts with the central anion. It is possible to form larger cyclic arrays of arginine around a large central anion, however only at the cost of the intramolecular hydrogen bonds. This suggests an explanation for the lack of a magic tetramer with a larger anion such as iodide.



DFT calculations suggest that reaction (2.1) is exothermic by ~ 8.7 kJ/mol. Assuming a negligible entropic change, this reaction should proceed to the right. When the gas phase acidities of both acids are taken into account (Table 2.1), these calculations indicate that the chloride anion binds more strongly (34 kJ/mol) to the neutral arginine trimer than does the nitrate anion. This result is surprising because the nitrate anion can form three hydrogen bonds to the arginine shell. However, the nitrate anion is larger than the chloride anion, leading to steric constraints that mitigate against the formation of three strong hydrogen bonds between the anion and the arginine shell. The result is a lower binding energy. Although such experiments were not conducted as a part of this investigation, it should be possible to observe processes such as reaction (2.1) in the gas

phase and perhaps even measure equilibrium constants that would further quantify the thermochemistry of these species.

The greatly enhanced abundance of $[3\text{Arg}+\text{NO}_3]^-$ relative to the dimer and tetramer of this series can be attributed to the three hydrogen bonds that can be formed with the guanidinium groups of each arginine. This offers evidence that the arginine trimer is cyclic in nature. DFT calculations further show no barrier for conversion to the proposed zwitterionic state when the cluster initially is comprised of non-zwitterionic arginines. Interestingly, the optimization proceeds by conversion of one arginine to the zwitterionic state, which is then followed by conversion of the remaining arginines to zwitterions.

The dimer of arginine coordinated to divalent magnesium (**2.8**) demonstrates another method of stabilizing zwitterionic arginine. It has been shown that one protonated arginine will stabilize another zwitterionic arginine in the protonated dimer.¹² In structure **2.8**, negatively charged arginine with Mg^{2+} is shown to stabilize the second arginine in the zwitterionic form. All divalent metal cations are likely to coordinate to arginine via the carboxylate ends. The interaction with the carboxylate ends also accounts for the unusual stability of the dimer of this series relative to the trimer and the monomer. It is also possible that other amino acids may be stabilized as zwitterions in a similar divalent metal cation bound dimer.

Experiments with the enantiomers of arginine revealed no enhanced stability for homochiral clusters. As clearly demonstrated above, arginine clusters derive special stability from the strong intermolecular interaction between the guanidinium and carboxylate groups of adjacent molecules. Other interactions are secondary in importance, including those involving the amino group, which remains uncharged in

these clusters. This is consistent with the crystal structure for arginine, in which the amino groups are not charged and there is no preference for homochirality.²² This result is consistent with the proposed structure **2.7**, where the amino groups do not participate in any intra or intermolecular bonding.

2.5 Conclusion

Both theoretical and experimental evidence suggests that the zwitterionic form of arginine can be easily stabilized in the gas phase. It has been shown both by experiment and theory that zwitterionic arginine is very near in energy to arginine, and therefore may be stabilized by nearby charged species.

It has been demonstrated here that it is possible to stabilize the zwitterionic form of arginine in cyclic arrays, which take full advantage of the strong guanidinium and carboxylate interaction. Such arrays are neutral, but are detected by their ability to complex with either an anion or cation. This is well illustrated by the cyclic trimer of arginine bound to an anion (structure **2.7**). The abundance of this species suggests that it is unusually stable, especially when coordinated to NO_3^- , which forms three hydrogen bonds to the guanidinium groups and can be regarded as the crown jewel for capping this structure.

We have developed experimental methodology to examine possible preferences for homochirality in molecular clusters which has broad applications beyond the studies of arginine clusters reported in this paper. The arginine clusters show no preference for homochirality, consistent with structures where the amino group is not involved in cluster

bonding. However, we have examined other systems where there is a pronounced preference for homochirality.²³

Assignment of structures for the larger clusters, e.g., $[50\text{Arg}+6\text{Cl}]^{6-}$ is a formidable problem, with the sheer size of the aggregate rendering impossible all calculations except molecular mechanics. Such enormous clusters offer a glimpse of the chemistry that lies at the interface between the solid state and gas phase. Portions of these clusters may approximate arginine in the crystalline state.

2.6 References

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