Cooperative Salt Bridge Stabilization of Gas-Phase Zwitterions in Neutral Arginine Clusters

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Both theory and experiment support a nonionic structure for monomeric arginine, with the zwitterionic structure predicted to be less stable by only 2.8 kcal/mol. We report here first principles quantum mechanics calculations (at the B3LYP/CCPVTZ(−F)$^{2+}$//B3LYP/6-31G** level) which indicate that the neutral arginine dimer and trimer have all arginine in the zwitterionic state. The zwitterionic tautomer of the dimer is 10 kcal/mol more stable than the best nonionic form, even though each forms six hydrogen bonds involving both guanidinium groups interacting with the carboxylate groups. The most stable form of the neutral trimer of arginine has all arginine in the zwitterionic state, with a total of 12 hydrogen bonds involving interactions of the guanidinium and carboxylate groups between adjacent molecules. The additional coulomb energy derived from the salt bridges in the zwitterionic tautomers of the dimer and trimer more than compensates for the energetic cost of generating zwitterionic arginine. This is the first case in which small amino acid clusters are predicted to be stable in the zwitterionic form through self-solvation in the absence of a net charge.

Introduction

While amino acids are known to exist as zwitterions in solution, the general assumption that zwitterions do not exist in the gas phase has been the subject of recent debate from both experimental and theoretical approaches. Glycine has received much attention because it is the simplest amino acid. Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometry has demonstrated that glycine is unstable as a zwitterion by ∼20 kcal/mol.[1] Ab initio calculations confirm that glycine is unlikely to exist in the gas phase as a zwitterion.[2,3] However, glycine also has the lowest proton affinity of the amino acids, making it the worst candidate for a gas-phase zwitterion. On the other hand, the guanidinium group of arginine (Arg) gives it the highest proton affinity of the amino acids, making Arg the best candidate for a gas-phase zwitterion.

The first studies on Arg by Williams and co-workers suggested that isolated Arg might exist in the zwitterionic form.[4] However, further experimental[5] and theoretical[6] studies indicate that the isolated Arg monomer is not a zwitterion in the gas phase. On the other hand several recent experiments, supported by theory, suggest that Arg in the presence of a net charge may exist in the zwitterionic state.[7] These studies are supported by recent calculations indicating that the attachment of an electron to glycine reduces the instability of the glycine zwitterion from 20 to 9 kcal/mol.[8]

Compared to other amino acids, Arg possesses a high propensity to form abundant clusters when electrosprayed into the gas phase.[9,10] Recent studies suggest that this clustering ability is due to the salt bridges formed by association of the guanidinium group of one Arg with the carboxylate group of another.[10] The resulting clusters are detected by their ability to associate with either a cation or an anion, suggesting that isolated neutral analogues of such clusters might be stable.

We report here first principles quantum mechanical calculations (density functional theory (DFT) at the B3LYP/CCPVTZ(−F)$^{2+}$//B3LYP/6-31G** level) of the structures for the neutral dimer and trimer of Arg and assess the energetics for both the zwitterionic and nonionic tautomers of each cluster. Predictions for the structures of larger clusters and implications for the gas-phase structures of peptides and proteins are made on the basis of these results.

Methods

Candidate structures were identified by two methods. Extensive MD simulations with simulating annealing were performed to identify candidate structures. The second approach consisted of minimizing structures assembled according to chemical intuition, followed by simulated annealing on each of these structures. In general, the second approach yielded much better results. The lowest energy conformers were submitted to higher levels of theory. Candidate structures were also evaluated at the PM3 semiempirical level.

The DFT calculations were carried out using Jaguar 4.0 (Schrödinger, Inc., Portland, OR). Full geometry optimization was performed at the B3LYP/6-31G** level of theory, followed by single point calculations at the B3LYP/CCPVTZ(−F)$^{2+}$ level for selected structures. Zero-point energies and vibrational frequencies were obtained from the analytical Hessian for the optimized structure at the B3LYP/6-31G** level.

The molecular dynamics (MD) calculations were carried out using Cerius^2 from Molecular Simulations Inc. The MD calculations used the DREIDING force field[11] (with the exponential-six form of the van der Waals potentials) and charges from charge equilibration.[12]

Semiempirical PM3 MNDO-type calculations were carried out using the HyperChem 5.1 Professional Suite (Hypercube, Inc., Gainesville, FL). Predicted vibrational intensities were calculated at this level of theory.

Results and Discussion

Dimers of Arginine. After an extensive search of conformational space, the lowest energy conformer for the neutral Arg

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TABLE 1: Heteroatom Separation in Hydrogen Bonds of Arginine Dimers[a]

bond[b]	DZ1	DZ2	DN1	DN2
a	2.74	2.68	2.97	2.59
b	2.72	2.79	2.57	2.88
c	2.73	2.82	2.98	2.94
d	2.73	2.74	2.98	2.94
e	2.72	2.74	2.57	2.88
f	2.74	2.69	2.97	2.59

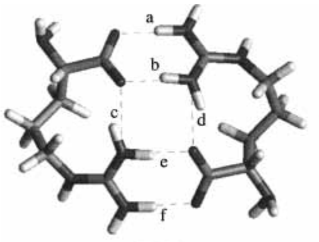
[a] All lengths are given in angstroms. [b] As defined by the pattern in structure **DZ1**.

TABLE 2: Calculated Electronic Energies for Arginine Dimers[a]

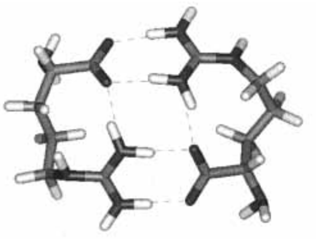
structure	zero-point energy	B3LYP/ 6-31G**[b]	zero-point corrected [b]	B3LYP/ CCPVTZ(-F)$^{2+}$ [b]	zero-point corrected[b]
DZ1	281.7	0	0	0	0
DZ2	282.3	2.3	2.9	na	na
DN1	280.7	14.6	13.6	11.6	10.5
DN2	280.6	13.4	12.3	10.4	9.3

[a] All energies are given in kcal/mol. [b] Energies are given relative to structure **DZ1**.

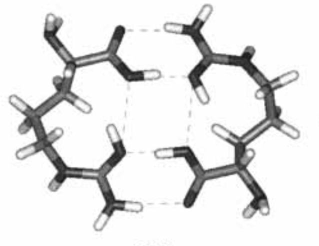
dimer that we have been able to identify is structure **DZ1**. This structure is composed of two zwitterionic Arg and has C_2 symmetry. The Arg are arranged in a head to tail fashion with the guanidinium group of one molecule interacting with the carboxylate of the neighboring Arg. Two salt bridges are formed by the interacting zwitterions, accompanied by six hydrogen bonds. This structure is not surprising given that guanidinium/carboxylate interactions are observed in both the crystal structure[13] of Arg and in the lowest energy conformer[6b] of the zwitterionic Arg monomer in the gas phase.



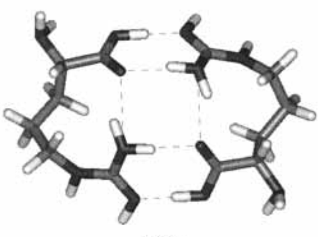
DZ1



DZ2



DN1



DN2

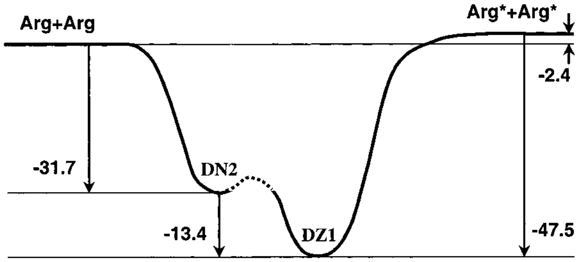


Figure 1. Reaction coordinate diagram for the dimerization of arginine as determined at the DFT B3LYP/6-31G** level in kcal/mol. Arg represents the neutral ground state of arginine. Arg* is the lowest energy zwitterionic conformation of arginine. The transition barrier to convert **DZ1** to **DN2** was not calculated and is represented by a dashed line; however, this barrier is expected to be very small. The monomeric structures were taken from ref 6b (it should be noted that the actual difference between the separated monomers is probably closer to 5.6 kcal/mol as indicated in ref 6b).

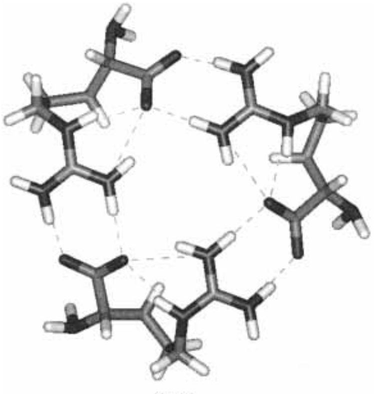
A second zwitterionic structure, **DZ2**, exists in a metastable state as a local minimum on the potential energy surface, only ∼2 kcal/mol higher in energy (Table 2). This structure is formed from **DZ1** by flipping one guanidinium while leaving the interacting carboxylate unchanged, leading to C_1 symmetry.

There are three metastable nonionic tautomers of **DZ1**. Two of these (**DN1** and **DN2**) are symmetrical nonionic tautomers. Structure **DN1** has both interior hydrogens placed on the carboxylate groups, while structure **DN2** has the exterior hydrogens placed on the carboxylate groups. The relative energetics of the nonionic and zwitterionic arginine dimers are shown in the reaction coordinate diagram at the B3LYP/6-31G** level in Figure 1. All four dimers contain six hydrogen bonds (Table 1), but the nonionic structures lack the coulomb attraction derived from the salt bridges in **DZ1**. The result (Table 2) is that the nonionic tautomers are less stable than **DZ1** by 10 to 12 kcal/mol. Furthermore, starting from a structure with one salt bridge interaction, the dimer minimizes without barrier to **DZ1**. This suggests that the creation of one salt bridge stabilizes the other in a cooperative manner. Thus we conclude that Arg can solvate itself, stabilizing the zwitterionic state that predominates in solution.

For the gas phase, similar calculations on the monomer indicate that the zwitterion is less stable than the nonionic form by 2.8 kcal/mol.[6] Therefore, the coulomb attraction derived from the two salt bridges provides about 18 kcal/mol to compensate the conversion of neutral Arg to the zwitterionic form. Structure **DZ1** is stabilized by 12 kcal/mol as a result of this coulomb attraction.

Interestingly, it should be possible to confirm the preferred state of the arginine dimer experimentally. The asymmetric CO stretch for the nonionic tautomers is calculated to fall in the range of 1900 to 2200 cm^{-1}. The predicted intensity of these bands (2049.55, 2024.42, and 2134.39 cm^{-1}, 2056.54 cm^{-1}) should be sufficient for experimental absorption measurements. The zwitterionic tautomers have no predicted vibrations in this range (ranging from 1798 to 2789 cm^{-1}). A list of all frequencies and selected intensities is provided as Supporting Information.

Trimers of Arginine. A particularly stable anionic Arg trimer has previously been identified experimentally.[10] After extensive conformational searching, we find the neutral Arg trimer to have a very similar structure, **TZ1**.Again the cyclic structure is



TZ1

dominated by guanidinium/carboxylate interactions, with each Arg maintaining two intramolecular hydrogen bonds. This structure has C_3 symmetry. There are a variety of nonionic tautomers of **TZ1**. Only the two symmetrical cases were studied at the DFT level of theory, one with the exterior hydrogens transferred to the carboxylate groups and the other with the interior hydrogens transferred to the carboxylate groups. In both cases, we find that the nonionic tautomers convert without barrier to the zwitterionic state. PM3 calculations indicate that all other tautomers are bracketed energetically by the two symmetrical cases. Restricting the lowest energy tautomer to be nonionic yields an energy 31 kcal/mol above **TZ1**.

The cyclic array of salt bridges in **TZ1** allows for a variety of additional long-range coulomb interactions. **TZ1** demonstrates much greater stability relative to the nonionic trimer than is the case with the dimers. This observation is a result of the larger cyclic salt bridge in **TZ1**, which allows for more favorable synergistic interactions between the salt bridges and reduced coulomb repulsion between like charges. The results suggest that these long-range interactions help to stabilize the zwitterionic state of Arg. Or put another way, the greater the number or Arg present, the easier it becomes to self-solvate charged groups. The extreme example of this is found in crystal structures, where even glycine is stabilized in the zwitterionic state.[14] This suggests that it should be possible to stabilize all of the amino acids as zwitterions through self-solvation, provided a sufficient cluster size is attained. For Arg, the critical cluster size is the dimer. The results presented here suggest that all Arg clusters larger than the dimer will consist of zwitterionic Arg and that the primary bonding pattern will be dominated by interactions between guanidinium and carboxylate groups.

Several computational studies have addressed the stability of gas-phase salt bridges by analyzing isolated salt bridge pairs in the gas phase. When isolated, the nonionic hydrogen-bound tautomers are always lower in energy.[15−17] The results presented here suggest that in a large protein, the synergistic interaction of an array of salt bridges may enhance their stability in the gas phase. The complex molecular environment surrounding a candidate gas-phase salt bridge (for example between the side chains of arginine and aspartic acid) in a protein must be carefully analyzed to identify stabilizing factors. The presence of other charges, salt bridges, hydrogen bonding, or any combination of these possibilities may be sufficient to stabilize a salt bridge in a protein in the gas phase.

Conclusions

Arg prefers the zwitterionic form when clustered together with at least one other Arg even in the absence of solvent or net charge. The structure for the gas-phase dimer (**DZ1**) of Arg is defined by two salt bridges, where the guanidinium group of one Arg interacts with the carboxylate of another. Two intramolecular hydrogen bonds and four intermolecular hydrogen bonds additionally bind the juxtaposed arginines. Although the nonionic tautomers of the dimer are local minima on the potential energy surface and maintain all of the hydrogen bonds, they lack the additional favorable Coulombic interactions. The neutral Arg trimer exhibits a similar structural motif. However in this case, the nonionic tautomers convert without barrier to the zwitterionic structure shown in **TZ1**.

Molecular clusters provide an interesting bridge between the gas- and solid-phase properties of molecules. Given sufficient size, the molecular clusters will begin to exhibit solid-phase characteristics, such as the stabilization of zwitterionic salt bridges through self-solvation. This principle is not limited to Arg and should be general in nature, although the particular results will vary from molecule to molecule. For instance, similar studies suggest that serine is stabilized as a zwitterion in the gas phase at ∼8 serines.[18] Experimentally, these serine octamers also exhibit a chiral preference, also a property commonly associated with the solid phase.

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Supporting Information Available: A list of vibrational frequencies and xyz coordinates for all structures are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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