Gas-Phase Zwitterions in the Absence of a Net Charge

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The ground state for neutral, isolated molecules in the gas phase can be zwitterionic under appropriate conditions. Quantum chemical calculations show that increasing the basicity of the basic component of a zwitterion leads to enhanced stability for the charge-separated state, which can lead to a ground-state zwitterion. Density functional theory calculations show that methylation of the side chain of arginine is sufficient to induce a ground-state zwitterion. The results for the stepwise methylation of arginine are given, and clearly illustrate enhanced zwitterion stabilization with increasing basicity. In protonated systems, guanidinylation of the N-terminus of arginine yields a salt bridge or charge-stabilized zwitterion structure. The enhanced basicity of guanidino versus amino groups is responsible for the charge separation in this case, which is not observed to be the ground state for protonated arginine itself. These results indicate that charge separation can be favorable in the gas phase and are discussed in light of future experimental efforts.

Introduction

The study of fundamental molecular properties in the gas phase is important because the intrinsic nature of a molecule can be directly probed.[1] For example, recent studies have shown that isolated amino acids are not zwitterionic in the gas phase,[2] whereas in solution amino acids are known to be zwitterions. When these two pieces of information are combined, it is clear that (in solution) the zwitterionic state is induced by the presence of solvent molecules. Further studies have elucidated other means by which zwitterionic states can be favored, including through the addition of diffuse proximal charges,[3] electrons,[4] or a few solvent molecules[5] or through noncovalent clustering.[6] In each of these examples, charge separation is stabilized by an external factor. However, examples of isolated small molecules that are ground-state zwitterions[7] in the gas phase are very rare.[8] In fact, even the addition of a proton is typically insufficient to stabilize a ground-state zwitterion in a salt bridge type structure for small isolated ions.[9]

The only known ground-state gas-phase zwitterion has an unusual structure which is resonantly stabilized in the charge-separated state.[8] However, it remains unknown whether there are other molecules that are intrinsically stable as ground-state zwitterions in the gas phase. Furthermore, no methods for systematically altering zwitterionic stability have been explicitly examined. The amino acids have received much attention with regard to zwitterion stability.[3−6] Inspection of the current results reveals that arginine forms the most stable zwitterion, with the ground-state being just a few kilocalories per mole lower in energy.[10,11] It has been suggested that the greater basicity of arginine (relative to the other amino acids) leads to the enhanced stability of the zwitterionic state. From the standpoint of chemical intuition, this makes good sense because the central issue in zwitterion formation and stabilization involves simple acid/base chemistry. To form a stable zwitterion in the gas phase, the Coulombic energy gained from the interaction of oppositely charged groups must exceed the difference in basicity between the protonated base and the deprotonated acid. Therefore, enhancing the basicity of the base or increasing the acidity of the acid should theoretically lead to greater stabilization of the zwitterionic state. The central issue then becomes whether the acid/base chemistry of a molecule can be changed sufficiently to stabilize the charge-separated state.

Herein we explicitly test the effect of basicity on zwitterion stability for small molecules in the absence of any net charges or intermolecular interactions. It is shown that methylation of the side chain of arginine enhances the basicity sufficiently to stabilize charge separation in the gas phase. The effects of adding up to four methyl groups are investigated. It is found that the addition of three methyl groups leads to the formation of the most stable zwitterion. The roles of steric hindrance and hydrogen bonding in zwitterion formation are also discussed. Additionally, it is shown that guanidinylation of the N-terminus of arginine is sufficient to stabilize charge separation when the molecule is protonated. Both of these chemical modifications increase the basicity of the molecule, confirming the hypothesis that enhanced basicity leads to enhanced zwitterion stabilization. The results are discussed in relation to future experiments.

Methodology

Candidate structures were generated using both chemical intuition and the Boltzmann jump approach in Cerius[2], where torsions are varied randomly and evaluated using molecular mechanics. Low-energy structures were submitted to full minimization at the PM3 semiempirical level. The lowest energy semiempirical structures were initially minimized again utilizing the hybrid functional B3LYP with the 6-31G** basis set. Additionally, low-energy structures that have been calculated previously for arginine were modified by methylation or guanidinylation and subjected to minimization at this level of theory if the modification could be made without disrupting the existing network of hydrogen bonding. Thus, the conformational space of this system has been extensively searched in both the present and past work. Final structures were obtained by full minimization at the B3LYP/6-311++G** level of theory. Frequencies (and zero-point energies) were also calculated at this level of theory to verify that all structures represent

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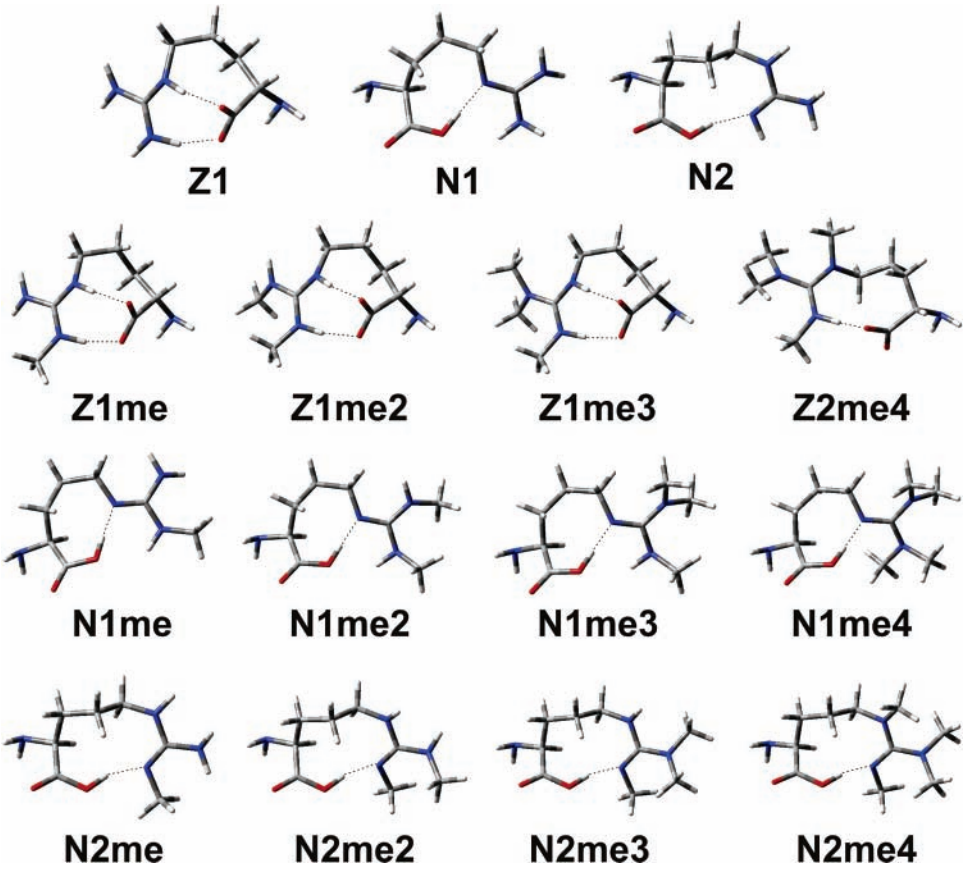

Figure 1. Minimized structures for arginine and methylated derivatives. Dashed lines are hydrogen bonds.

TABLE 1: Gas-Phase Proton Affinities

molecule	proton affinity[15,a]	molecule	proton affinity[15,a]
ammonia	204	guanidine	235.7
methylamine	214.9	*N,N,N′,N′*-tetramethylguanidine	246.5
dimethylamine	222.2	imidazole	225.3
trimethylamine	226.8	*N*-methylimidazole	229.3

[a] All values are in kilocalories per mole.

true minima on the potential energy surface. Single-point calculations at other levels of theory were performed for comparison with the results obtained using density functional theory (DFT). MP2 single-point calculations were performed with the 6-31++G** basis set.

The molecular mechanics calculations were carried out using Cerius[2] 3.0 (Molecular Simulations Inc.) with the Dreiding force field[12] and charges from charge equilibration.[13] PM3 semi-empirical calculations were carried out using CACHe Worksystem Pro 5.04 (Fujitsu, Inc., Beaverton, OR). All of the remaining high-level electronic structure calculations were carried out using the Gaussian 03 suite of programs.[14]

Results and Discussion

Alkylation enhances the basicity of nitrogen-containing bases. As shown in Table 1, the sequential methylation of ammonia leads to a stepwise increase in proton affinity. The same effect is observed when other bases are methylated. Therefore, methylation is a simple and effective method for enhancing basicity in the gas phase. Of the naturally occurring amino acids, arginine is the most basic by a significant amount. Despite this fact, the ground state for arginine is not zwitterionic, although there is a zwitterionic structure just a few kilocalories per mole higher in energy. It should be possible to further increase the basicity of arginine through methylation of the side chain, which should lead to significant stabilization of the zwitterionic structure.

TABLE 2: Relative Energies for Methylated Arginine Derivatives[a]

molecule	B3LYP/ 6-311++G**	MP2	ZPE corrected[b]	dipole[c]
Z1	0	0	0	9.10
N1	−1.98	−1.37	−1.62	7.77
N2	−1.76	−1.11	−1.04	7.89
Z1me	0	0	0	8.95
N1me	0.59	1.95	0.38	8.08
N2me	0.59	0.53	0.76	7.59
Z1me2	0	0	0	9.40
N1me2	1.11	2.44	0.76	8.14
N2me2	2.08	2.52	1.94	7.59
Z1me3	0	0	0	9.64
N1me3	3.69	5.14	3.22	8.25
N2me3	2.40	3.45	2.03	7.87
Z2me4	0	0	0	10.35
N1me4[d]				6.26
N2me4	−2.90	−2.13	−2.59	8.21

[a] All energies are in kilocalories per mole. Each energy is given relative to that of the zwitterionic molecule. [b] Zero-point energy obtained at the B3LYP/6-311++G** level. [c] Given in debyes. [d] This structure has no local minimum corresponding to a zwitterionic state and represents a constitutional isomer different from Z2me4. N1me4 is 0.50 kcal/mol lower in energy than N2me4.

Neutral Molecules. We have calculated the structures and relative energies for a series of methylated arginine derivatives. The structures are shown in Figure 1, and the calculated energies are summarized in Table 2. After an extensive search of conformational space, the lowest energy structures for the zwitterionic and canonical forms of these arginine derivatives were found to be similar to those for arginine itself. Thus,

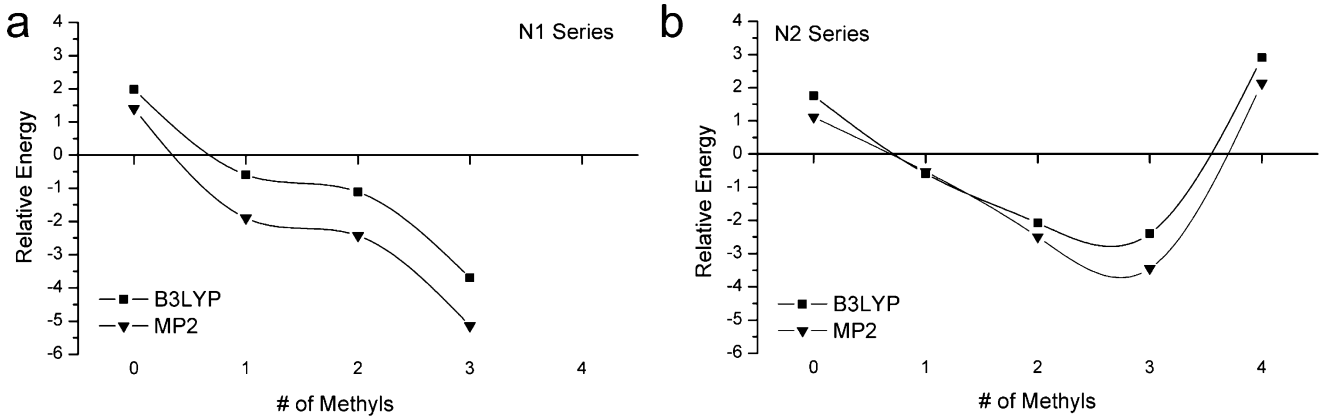

Figure 2. Energetic stabilization vs number of methylations. Negative numbers indicate that the zwitterionic structure is favored. All values are in kilocalories per mole.

structures Z1me, Z1me2, and Z1me3 are very similar to Z1, which has been described previously.[10] These results are logical, given that three methyls can be added to Z1 without disrupting the hydrogen-bonding network. The situation changes on addition of the fourth methyl, where a hydrogen bond must be broken and a different structure, Z2me4, is more energetically stable. This conformational rearrangement and requisite loss of a hydrogen bond is important with respect to zwitterion stability as we shall describe in further detail below. The canonical structures N1 and N2 have also been described previously,[10] but upon methylation the lowest energy conformation switches back and forth between the N1 and N2 series.

The calculated energy difference (1.98 kcal/mol) between Z1 and N1 is in good agreement with results obtained previously (1.82 kcal/mol) utilizing similar levels of theory.[10] However, it should be noted that coupled cluster theory suggests a greater difference in energy, with N1 being lower in energy by 3.97 kcal/mol.[10] Consequently, it appears that DFT methods may overestimate zwitterion stability relative to that of canonical structures in some cases by ∼2 kcal/mol. The energetics for the various N1 and N2 structures relative to the corresponding Z structures versus the number of added methyl groups are plotted in Figure 2. The addition of the first three methyl groups each leads to further stabilization of the zwitterionic form for both structure series. In fact, Z1me3 is ∼3.7 kcal/mol more stable than N1me3 and ∼2.4 kcal/mol more stable than N2me3. This indicates that *N*,*N*,*N*′-trimethylarginine will exist as a zwitterion in the ground state, even if the zwitterion stability is overestimated by 2 kcal/mol. These results suggest that enhanced basicity leads to stabilization of the zwitterionic state. Other effects, such as steric repulsion and hydrogen bonding, also play important roles in determining overall isomeric stability and are responsible for the shape of the curves in Figure 2. However, these effects are typically secondary with respect to zwitterion stability except under certain conditions which are stated below.

Upon addition of the fourth methyl group, the relative zwitterion stability decreases despite an increase in basicity. There are several factors that influence this result. First, the increase in basicity is smaller with each consecutive methylation (see Table 1); however, we do not believe that this is the controlling factor. Second, and more importantly, the reduced zwitterionic stability is due to the loss of an intramolecular hydrogen bond. Comparison of the Z1 and N1/N2 structures reveals that each of the Z1 structures contains an additional intramolecular hydrogen bond relative to the corresponding N1/N2 structure. The addition of the fourth methyl group concomitantly reduces the number of hydrogen bonds to one for both the zwitterionic and canonical structures. The loss of the hydrogen bond significantly reduces the stability of the Z2me4 structure relative to N2me4. It is therefore apparent that hydrogen bonding can also play an important role in zwitterion stability, particularly when the number of hydrogen bonds differs between the zwitterionic and canonical structures. Nevertheless, if the N−H bond length for Z2me4 is locked and the methyls are removed, minimization yields a structure ∼6.8 kcal/mol higher in energy than N2. Recalling that Z2me4 is only 2.9 kcal/mol higher in energy than N2me4, it can be inferred that methylation stabilizes the zwitterionic state by ∼4 kcal/mol for Z2me4, which (in the absence of hydrogen-bonding effects) would be in good agreement with the trend shown in Figure 2b. The other canonical structure, N1me4, represents a different constitutional isomer for which we found no corresponding zwitterionic state.

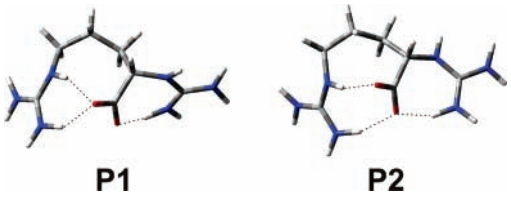

Figure 3. Two lowest energy structures for the protonated arginine derivatives. Dashed lines are hydrogen bonds.

Protonated Molecules. Another chemical modification that greatly increases basicity is guanidinylation.[16] As seen in Table 1, guanidine is ∼30 kcal/mol more basic than ammonia. Therefore, guanidinylation of the amino group of arginine will greatly enhance the basicity of this secondary basic site, which becomes important with respect to zwitterion stabilization when the molecule is protonated. A protonated molecule may exist in a "charge-solvated" state, where appropriate groups noncovalently solvate the proton, or in a charge-separated "zwitterionic" state, which can also be described as a salt bridge. In the case of the salt bridge, two basic sites are required for stability with respect to the charge-solvated structure. Previous work has demonstrated that protonated arginine does not exist as a salt bridge structure in the gas phase,[3b,10] although theory has also shown that other ions are capable of inducing salt bridge structures.[3]

The two lowest energy structures for protonated guanidinylated arginine are shown in Figure 3 and labeled P1 and P2. Although both structures are salt bridges, P2 is 1.68 kcal/mol higher in energy than P1 at the B3LYP/6-311++G** level of theory. All of the charge-solvated trial structures that were generated at lower levels of theory minimized to salt bridge structures when DFT was employed. Thus, in contrast to

arginine, guanidinylated arginine strongly prefers the zwitterionic state when protonated. This is a second example where greater basicity leads to enhanced zwitterionic stabilization. These results are in good agreement with previous work emphasizing the importance of proton affinity in alkali-metal-cationized structures.[3] Other structural changes with respect to Z1 are also worth noting. Although P2 is structurally similar to Z1, it is not the lowest energy structure in this case. P1 is slightly more favorable energetically because the side chain is less strained. The worst dihedral overlap is 29.9° for P1 and 12.2° for P2. The more relaxed conformation in P1 is achieved through bifurcation of the two hydrogen bonds between the side chain and the carboxylate. Thus, the same total number of intramolecular hydrogen bonds exist in both structures, but the more relaxed side chain allows P1 to be energetically favored.

Conclusion

Ab initio quantum chemical calculations have revealed that isolated small molecules can exist in the gas phase as charge-separated or zwitterionic molecules in the ground state. This can be achieved by appropriate selection of strongly basic groups, which are better able to compete for protons with the deprotonated acids that are inherently part of a zwitterion. Methylation of the side chain of arginine, which is a known posttranslational modification,[17] is sufficient to favor the zwitterionic state. Although not explicitly tested herein, stronger acidity should also lead to the stabilization of zwitterionic states. For salt bridge structures to be favored in protonated molecules, two sufficiently basic sites must be present. Alkylguanidines are shown to be superior to alkylamines in this regard.

The results presented herein suggest the common assumption that charge separation is inherently unfavorable in the gas phase may need to be revisited, even for molecules in the absence of a net charge. In attempts to confirm predictions made by theory, various experiments have searched for energetically favored zwitterionic states in the gas phase. Typically, these experiments offer indirect evidence and are performed on ions, but recently, experiments capable of directly probing the charge distribution on neutral molecules have been described.[18] Given the large differences in dipole moments between zwitterionic and canonical structures, it should be possible to experimentally test the results predicted by theory in the present work.

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Supporting Information Available: Fully minimized structure coordinates (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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