Gaseous Arginine Conformers and Their Unique Intramolecular Interactions

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Extensive ab initio calculations were employed to characterize stable conformers of gaseous arginine, both the canonical and zwitterionic tautomers. Step-by-step geometry optimizations of possible single-bond rotamers at the B3LYP/6-31G(d), B3LYP/6-31++G(d,p), and MP2/6-31++G(d,p) levels yield numerous structures that are more stable than any known ones. The final electronic energies of the conformers were determined at the CCSD/6-31++G(d,p) level. The lowest energies of the canonical and zwitterionic structures are lower than the existing values by 2.0 and 2.3 kcal/mol, respectively. The relative energies, rotational constants, dipole moments, and harmonic frequencies of the stable conformers remain for future experimental verification. The conformational distributions at various temperatures, estimated according to thermodynamic principles, consist almost exclusively of the newly found structures. One striking feature is the occurrence of blue-shifting hydrogen bonds in all six of the most stable conformers. A unique feature of important conformations is the coexistence of dihydrogen and blue- and red-shifting hydrogen bonds. In addition to the hydrogen bonds, the stereoelectronic effects were also found to be important stabilization factors. The calculated and measured proton affinities agree within the theoretical and experimental uncertainties, affirming the high quality of our conformational search. The theoretical gas-phase basicity of 245.9 kcal/mol is also in good agreement with the experimental value of 240.6 kcal/mol. The extensive searches establish firmly that gaseous arginine exists primarily in the canonical and not the zwitterionic form.

1. Introduction

The structures and properties of amino acids, the elemental building blocks of proteins, are of intrinsic interest. Of the 20 natural amino acids, the conformational study of arginine is the most challenging because of its large number of rotational degrees of freedom. Arginine is also the most basic natural amino acid because of its extremely basic guanidine side chain. It is speculated that the zwitterionic form of arginine exists in the gas phase. Consequently, arginine has been the subject of many intensive studies.[1−18] In their extensive study of the potential energy surface of gaseous arginine, Rak et al.[5] applied a simple genetic algorithm to vary selected geometrical parameters and performed geometry optimizations from the trial structures at the semiempirical PM3 level of theory, followed by MP2 and B3LYP geometry optimizations of the PM3 structures to provide the lowest-energy conformers of canonical, zwitterionic, and protonated arginine. Several new structures were identified in their study, and the lowest-energy canonical structure was found to be more stable than the lowest-energy zwitterionic structure by 4.0 kcal mol^{-1} with the CCSD/6-31++G(d,p) energies. The calculated proton affinity of 256.3 kcal/mol and gas-phase basicity of 247.8 kcal/mol of arginine were in reasonable agreement with the measured values of 251.2 and 240.6 kcal/mol,[19] respectively. These results were nicely confirmed in recent computations using extended atomic orbital basis sets (up to 1380 functions) and complete-basis extrapolations, as well as the theory of coupled-cluster single and double excitations with approximate inclusions of triple excitations to treat the electron correlation effects {CCSD(T) and CCSD[T]}.[6]

As noted by Rak et al., however, the discrepancies between the experimental and theoretical values of the proton affinity and gas-phase basicity were larger than could be explained by the inaccuracy in the CCSD/6-31++G(d,p) energies. The harmonic approximation of molecular vibrations as well as an incomplete exploration of the conformational space were suggested as possible deficiencies of the computational approach.[5] The latter deficiency is a serious shortcoming because of the critical importance of the most stable conformers in determining the properties of gaseous arginine. It also casts doubt on whether the lowest-energy zwitterionic structure was found by the same incomplete search. Therefore, even the very basic question of whether gaseous arginine exists in the canonical or zwitterionic form might have not been properly answered, despite the fact that all recent computational studies concluded that the most stable tautomer had the canonical form.[3−5] Similarly, it was shown that the IR−CRLAS experiment[2] could not provide a conclusive answer because of the spectral overlap of the canonical and zwitterionic structures in the observed IR spectrum range.[5] Consequently, it is necessary to carry out a more thorough and reliable investigation to

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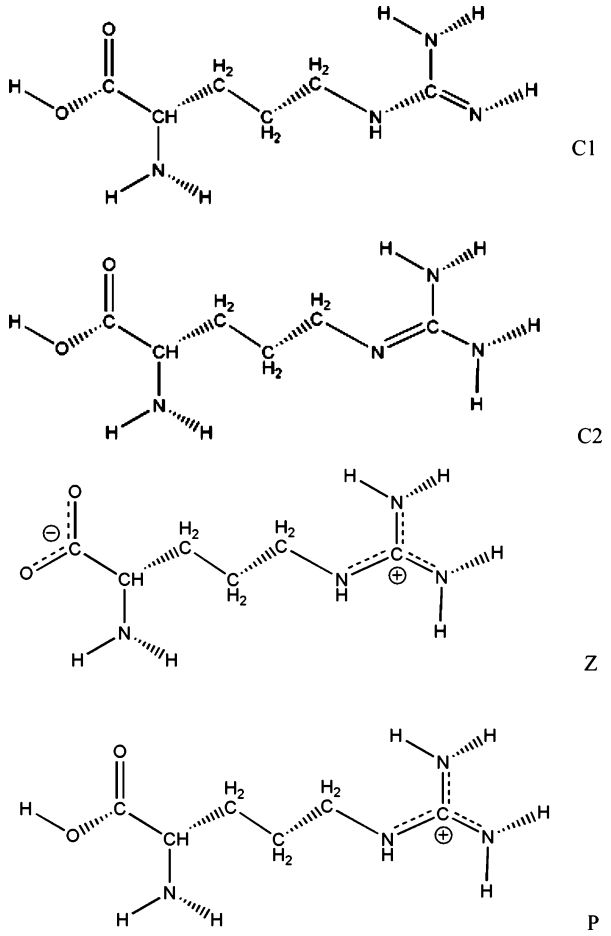

Figure 1. Structures of neutral and protonated arginine considered in this study.

determine the most stable conformers of the canonical and zwitterionic tautomers. Only then can one determine convincingly whether the gaseous arginine structure is indeed the canonical form.

The inability to locate the most stable conformer in the study by Rak et al. can be attributed to their initial search by the semiempirical PM3 method, which is prone to miss some important low-energy conformers.[20] Moreover, they omitted rotational degrees of freedom of the C—NH_2 and C═NH bonds of the guanidine group (see Figure 1), which might be a serious limitation. Here, we employed a more systematic and thorough search by varying all rotational degrees of freedom and performing ab initio calculations to characterize the conformational topology of gaseous arginine. A series of local minima of the canonical, zwitterionic, and protonated arginine structures were obtained by step-by-step ab initio geometry optimizations of possible single-bond rotamers. With the new structures, the calculated proton affinity and gas-phase basicity of arginine are in improved agreement with the measured values. To facilitate experimental verifications by future IR measurements, characteristic vibrational frequencies and intensities for the most important neutral arginine structures are reported here. Moreover, the vertical ionization energies of the conformers were also calculated.

It has long been known that intramolecular hydrogen bonds are important factors in determining relative structural stability,[21,22] and the role of hydrogen bonds involving amino acids has recently been widely researched.[23−25] It is also known that stereoelectronic effects endow nucleic acids and carbohydrates with conformational stability.[26] For example, stereoelectronic effects were found to contribute to the conformational stability of collagen.[27] Herein, the Atoms in Molecules (AIM)[28] and Natural Bond Orbital (NBO)[29] theories were employed to analyze the effect of weak intramolecular interactions on the stability of low-energy conformers. Unique features of hydrogen bonds were found for the most stable conformers. Moreover, the effect of stereoelectronic interactions on the stability of conformers of amino acids has been revealed.

2. Theoretical Methods

The planar structures of neutral and protonated arginines are shown in Figure 1. In principle, the full conformational space of gaseous arginine can be explored through a systematic variation of all rotational degrees of freedom. As a dihedral angle can change from 0° to 360°, typically, 60° increments for asymmetrical dihedral angles and 120° increments for symmetrical dihedral angles are required to ensure a complete scan of the potential energy surface.[30,31] However, it suffices to consider syn- or anti-periplanar arrangements corresponding to 0° and 180° torsions for the C···OH and C═NH groups. As a result, taking the three neutral arginine tautomers as examples, the numbers of trial structures obtained by allowing for all combinations of every internal bond rotamers are $2 \times 6 \times 3 \times 6 \times 6 \times 6 \times 6 \times 3 \times 3 \times 2$, $2 \times 6 \times 3 \times 6 \times 6 \times 6 \times 6 \times 2 \times 3 \times 3$, and $3 \times 3 \times 6 \times 6 \times 6 \times 6 \times 3 \times 3 \times 3$, respectively. To avoid losing important low-energy conformers, these 1 994 544 trial structures would have to be optimized by ab initio or DFT calculations—an impossible task. Clearly, efficient minimum-energy search algorithms are required to explore the complex conformational space of this molecule. Unfortunately, mathematically speaking, there is no numerical approach that can both efficiently and reliably locate the global minimum.[32] Therefore, one has to rely on some physical insight to scan the potential energy surface efficiently.

The relative energies of different conformers in a given tautomer are determined mainly by the interplay between intramolecular interactions such as hydrogen bonds and the associated internal strains. As the intramolecular interactions are weak, the global-minimum conformers cannot exhibit major steric clashes. Indeed, all seven of the most stable conformers of phenylalanine determined by ab initio calculations of all 648 trial structures[33] were located by optimizing only 58 possible structures selected by excluding rotamers exhibiting major steric clashes.[34] Therefore, only a small subset of the above 1 994 544 trial structures might be needed to locate the most stable arginine conformers. Moreover, the global minimum is the structure with the best overall balance of intramolecular interactions and internal strains. It is improbable for the global minimum to have several rotational degrees of freedom that deviate significantly from the stable structure. In other words, starting from a set of local minima obtained by considering a few structural degrees of freedom, one can expect to find the global minimum by sequential searches of local minima for every additional rotational degree of freedom. We expect that this procedure should yield conformers with energies very close to the global minimum (say, within a range of 2−3 kcal/mol).

On the basis of the above observation, our step-by-step search for conformers proceeded as follows: All rotamers obtained by varying the four (three) leftmost degrees of freedom of canonical (zwitterionic) arginine as shown in Figure 1 were optimized with B3LYP/6-31G(d) calculations.[35−37] The starting rotamers for the three tautomers of neutral arginine were obtained from the conformers C2, C3, and Z3 found by Rak et al.,[5] respectively. The total numbers of trial rotamers were 216,

216, and 54, leading to 61, 48, and 9 local minima, respectively. All of the stable geometries obtained were then used to generate the trial structures when scanning the next rotational degree of freedom. The process was repeated until all of the remaining degrees of freedom were scanned. In this process, a total of 5348 and 542 trial structures were optimized for the canonical and zwitterionic species, respectively. Optimizing such a large number of trial structures might be overcautious but appears to be necessary for ensuing reliability, as the optimizations of some rotamers obtained by rotating high-lying local minima yielded low-energy conformers as a result of substantial geometry relaxations. Starting from C5[5] instead of C2 with the same scanning procedure yielded the same set of most stable conformers, as discussed in the Results section, confirming the reliability of the searching methodology.

We also considered another two zwitterionic structures with the α-amino group protonated. Many local minima on the potential energy surface were located for these tautomers. However, they all had much higher energies than the global minimum of the zwitterionic arginine.

The most stable 100 canonical, 13 zwitterionic, and 50 protonated structures were subjected to further geometry optimizations at the B3LYP/6-31++G(d,p) and MP2/6-31++G-(d,p) levels of theory in order to ascertain their structural accuracy and stability. Unlike the cases for some aromatic[24,33] and smaller aliphatic amino acids,[38,39] for which there are virtually no differences between the B3LYP/6-31++G(d,p) and MP2/6-31++G(d,p) structures, the differences in the B3LYP/6-31++G(d,p) and MP2/6-31++G(d,p) structures of arginine are noticeable. An average difference in the dihedral angle associated with the guanidine side chain for some 28 selected structures was 3°. An average MP2/6-31++G(d,p) energy difference for 15 low-energy canonical conformers for the B3LYP/6-31++G(d,p) and MP2/6-31++G(d,p) structures was 1.1 kcal/mol, with the largest difference of 1.7 kcal/mol. The final energies for 25 conformers of interest were determined at the coupled-cluster level of theory with single and double excitations (CCSD)[40] and the 6-31++G (d,p) basis set at the optimal MP2 geometries, as MP2 is usually superior to B3LYP for treating hydrogen-bonded systems.[22,41] The zero-point vibrational corrections and thermal contributions to the energy and entropy were determined at the B3LYP/6-31++G(d,p) level of theory with the B3LPY geometries. Scaling factors of 0.9614[42] and 0.9813[5] for the frequencies above and below 2000 cm^{-1}, respectively, are used when discussing the simulated IR spectra. No imaginary frequency was observed for any of the conformers examined, confirming that the optimized structures were true local minima.

The vertical ionization energy (VIE) is defined as the energy difference between the neutral and ionized species at the minimum-energy geometry of the neutral species. It is known that values of the VIE obtained with the B3LYP functional and high-quality basis sets are typically very reliable.[43] For comparative purpose, both the B3LYP/6-31G++(d,p) and CCSD/6-31G++(d,p) methods were used to calculate the VIEs of neutral arginine.

The equilibrium populations of neutral and protonated arginine in the gas phase at different temperatures were calculated according to the principles of statistical thermodynamics.[44,45] Using the respective ab initio data for the various conformers, that is, moments of inertia, vibrational frequencies, and electronic energies, the partition functions can be calculated, and the equilibrium contents of various conformers at the given temperature can be determined.[46]

The proton affinity (PA) of arginine is defined as the negative of the enthalpy change for the gas-phase reaction

$$\mathrm{Arg(g)} + \mathrm{H^+(g)} \rightarrow \mathrm{ArgH^+(g)}$$

whereas the gas-phase basicity (GB) is the negative of the Gibbs free energy change. Both PA and GB are usually measured at $T = 298$ K. The enthalpy of H^+, $H(H^+)$, is the sum of the translational energy of H^+ and the PV work from the reaction and is calculated to be $H(H^+) = E + PV = {}^5/_2RT = 1.48$ kcal/mol. A value of $G(H^+) = H(H^+) - TS(H^+) = -6.28$ kcal/mol is used for the proton free energy.[47] The values of the thermodynamic functions for a species were obtained through weighted averaging over its structures.[5]

The majority of all calculations were carried out on our PC Cluster in Hefei, China, with the Gaussian 98 software package[48] and MOLPRO.[49] The CCSD/6-31G++(d,p) calculations were performed with the Tensor Contraction Engine (TCE) of the NWChem 4.7 software package[50] and were carried out on an Intel Itanium2 cluster in the Environmental Molecular Sciences Laboratory (EMSL), Richland, WA.

3. Results and Discussions

The approach we used to locate the low-energy conformers was fruitful. Eleven canonical and four zwitterionic conformers were found to have lower energies than their respective lowest-energy conformers published previously. With the newly found structures, the agreements between the theoretical and experimental proton affinity and gas-phase basicity values are improved. The identification of new stable conformers demonstrates that the simple genetic algorithm[51] search based on the PM3 method and restricted conformational space[5] was not sufficiently robust, as it missed the majority of the lowest-energy structures.

3.1. Conformers and Energies. The most stable structures of neutral arginine are displayed in Figure 2, and those for protonated arginine are shown in the Supporting Information. The geometries were obtained by MP2/6-31++G(d,p) optimizations, because MP2 typically performs much better for hydrogen-bonded systems than DFT.[22,41] The labeling letters c, z, and p refer to the canonical, zwitterionic, and protonated structures, respectively. Both c and z are neutral species. The numeral following the letter indicates the stability of the neutral or protonated species ordered according to ascending electronic energy.

The relative energies of the most stable conformers of neutral arginine are listed in Table 1. The electronic energies were determined at the CCSD/6-31++G(d,p) level. A higher level of theory and larger basis sets were not attempted, but CCSD-(T) calculations with very large basis sets for five canonical and three zwitterionic conformers provided basically the same relative stabilities as the CCSD/6-31++G(d,p) approach used here.[6] In fact, as shown in Table 1, even CCSD/6-31G(d,p) provided acceptable relative stabilities. MP2/6-31++G(d,p) also represented the energy ordering of the most stable conformers reasonably well, but provided less accurate relative energies than the CCSD/6-31G(d,p) results. Taking the global minimum as the reference, the relative MP2/6-31++G(d,p) energies of other canonical conformers were overestimated, whereas those of the zwitterionic conformers were underestimated. On the other hand, the CCSD/6-31G(d,p) method overestimated the relative energies of both the canonical and zwitterionic conformers. The ordering in terms of the B3LYP/6-31++G(d,p) energies was found to be misleading. This is why 100 canonical, 13

TABLE 1: Relative Electronic Energies (E)[a] of the Most Stable Canonical and Zwitterionic Conformers of Neutral Arginine Calculated by the B3LYP, MP2, and CCSD Theories with the 6-31++G(d,p) Basis Set and the Relative CCSD Energies Corrected for the Contributions of the Zero-Point Energy, $\Delta E_{0,vib}$, Enthalpy $\Delta H_{298,corr}$, and Free Energy ($\Delta G_{298,corr}$)[b,c]

structure	E^{B3LYP}	E^{MP2}	E^{CCSD}	$E_0{}^{CCSD\,d}$	$E^{CCSD}+\Delta E_{0,vib}$	$E^{CCSD}+\Delta H_{298,corr}$	$E^{CCSD}+\Delta G_{298,corr}$
c1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c2	0.649	0.588	0.440	0.579	0.373	0.482	−0.306
c3	0.766	1.151	0.460	0.650	0.650	0.661	0.481
c4	−2.739	1.377	0.496	1.002	−0.068	0.184	−0.718
c5	−2.749	1.494	0.604	1.187	−0.051	0.232	−0.774
c6	0.702	1.334	0.962	1.405	0.727	0.904	−0.035
c7	−1.744	2.616	1.064	2.129	1.000	1.136	0.666
c8	−0.983	3.223	1.536	2.701	1.127	1.457	0.525
c9	2.021	2.613	1.744	2.053	1.871	1.906	1.449
c10	2.260	2.762	1.762	2.266	1.238	1.501	0.675
c11	2.336	2.638	1.887	2.809	1.685	1.900	0.943
c12	−1.020	3.622	1.971	2.814	1.877	1.999	1.528
c20	−0.431	3.862	2.790	3.293	2.244	2.514	1.299
z21	0.891	2.438	3.694	5.298	3.407	3.076	4.063
z22	1.230	2.736	3.828	5.166	3.284	3.017	3.582
z23	2.932	2.898	4.076	5.387	4.535	4.082	5.695
z24	3.898	5.209	5.745	7.024	5.438	5.349	5.636
z25	0.823	5.298	6.013	7.212	5.237	5.312	5.498

[a] The electronic energies of c1 calculated at the B3LYP/6-31++G(d,p), MP2/6-31++G(d,p), and CCSD/6-31++G(d,p) levels are −606.5970620, −604.8532647, and −604.9076207 au, respectively. [b] All quantities are given in kcal/mol. [c] c12 and c20 are the two most stable canonical conformers, and z25 is the most stable zwitterionic conformer identified previously.[5] [d] $E_0{}^{CCSD}$ refers to the energies calculated at the CCSD/6-31G(d,p) level of theory.

zwitterionic, and 50 protonated structures were optimized at the B3LYP/6-31++G(d,p) and MP2/6-31++G(d,p) levels of theory.

One important finding of the current study is that 11 minimum-energy structures were found to be more stable than the previously published global minimum,[5] denoted here as c12. Indeed, the CCSD/6-31++G(d,p) energy of c1 is 2.0 kcal/mol lower than that of c12. As shown in Figure 2, the structures of c1 and c2, or those of c4 and c5, are very similar, differing only in the rotation of an amino group about the C10−N12 bond. (c5 was identified as the global minimum in a yet-to-be-published study by Wang, Ohanessian, and Wesdemiotis, and our new results are being used to obtain an improved sodium affinity of Arg by these authors.[18]) It is important to notice that the most stable canonical forms correspond to the structure C2 from Figure 1, i.e., they have the N9 atom deprotonated and the side chain terminated by two amino groups attached to C10.

A few stable zwitterions were also found, but their total number was much smaller than for the canonical tautomer. Four new zwitterionic conformers were found to be more stable than the previously known zwitterion,[5] which is labeled z25 throughout this article. Indeed, z21, our most stable zwitterionic species, is 2.3 kcal/mol more stable than z25. The lowest-energy canonical conformer, c1, is 3.7 kcal/mol more stable than the most stable zwitterionic counterpart, z21. Clearly, arginine takes the canonical form in the gas phase.

The energies of the six most stable protonated conformers can be found in the Supporting Information, of which p1, p3, and p5 were known previously, corresponding to P1, P2, and P3 of ref 5, respectively. Our search does not reveal a new global minimum, but the new second-lowest-energy conformer, p2, is 2.7 kcal/mol more stable than P2 of ref 5. It is noted that both the most stable zwitterions and protonated conformers do not carry a protonated α-amino group.

The differences in the electronic energies of the most stable arginine conformers are very small. Thus, the zero-point vibrational energies and thermal effects might significantly affect the gas-phase equilibrium compositions. Because of the small zero-point energies and significant entropic contributions, c5 and c4 are the two most stable neutral conformers in the free energy scale. Similarly, z22 is the most stable zwitterion in the free energy scale, but its free energy is 4.4 kcal/mol higher than that of c5.

3.2. Conformational Distribution. Molecular partition functions were determined using the CCSD/6-31++G(d,p) energies, MP2 structural parameters, and B3LYP/6-31++G(d,p) harmonic frequencies. The percent shares of different structures in the gas-phase sample of neutral arginine at 98, 198, 298, and 443 K are reported in Table 2. Because of the small energy differences for the lowest-energy conformers, the neutral arginine is a genuine multiconformer system even at 98 K, as there are four conformers each with more than 5% concentration that could be observed experimentally. The structurally similar c4 and c5 are the dominant conformers at 98 K, with a combined population of 79%. With increasing temperature, the population of c4 and c5 decreases, and the populations of many higher-free-energy conformers increase. However, the concentration of any zwitterionic structure is well below 1%. Thus the system is practically pure canonical. The system also consists practically of only the new conformers found in this work, as the total concentration of the previously published conformers is less than 4% even at 443 K. The room-temperature compositions of the protonated species are also included in Table 2. The previously known global minimum remains the dominant structure, and the newly identified second-most stable conformer, p2, contributes 4% to the population.

It should be pointed out that the anharmonic effect can significantly influence the relative abundance of conformers with very similar free energies.[52] That is, the relative abundances of c4 and c5 might be highly approximate because of the harmonic approximation. To solve this problem accurately, one would have to use the state-of-the-art DQMC approaches of Clary and others.[52,53] Unfortunately, arginine is too large to be treated with this method at this time. However, the dominance of the c4 and c5 populations at low temperature should not be affected by the harmonic approximation, as their free energies are significantly below those of other conformers.[53]

3.3. Proton Affinity and Gas-Phase Basicity. The experimental values of the proton affinity (PA) and gas basicity (GB) of arginine at 298 K are 251.2 and 240.6 kcal mol^{-1},

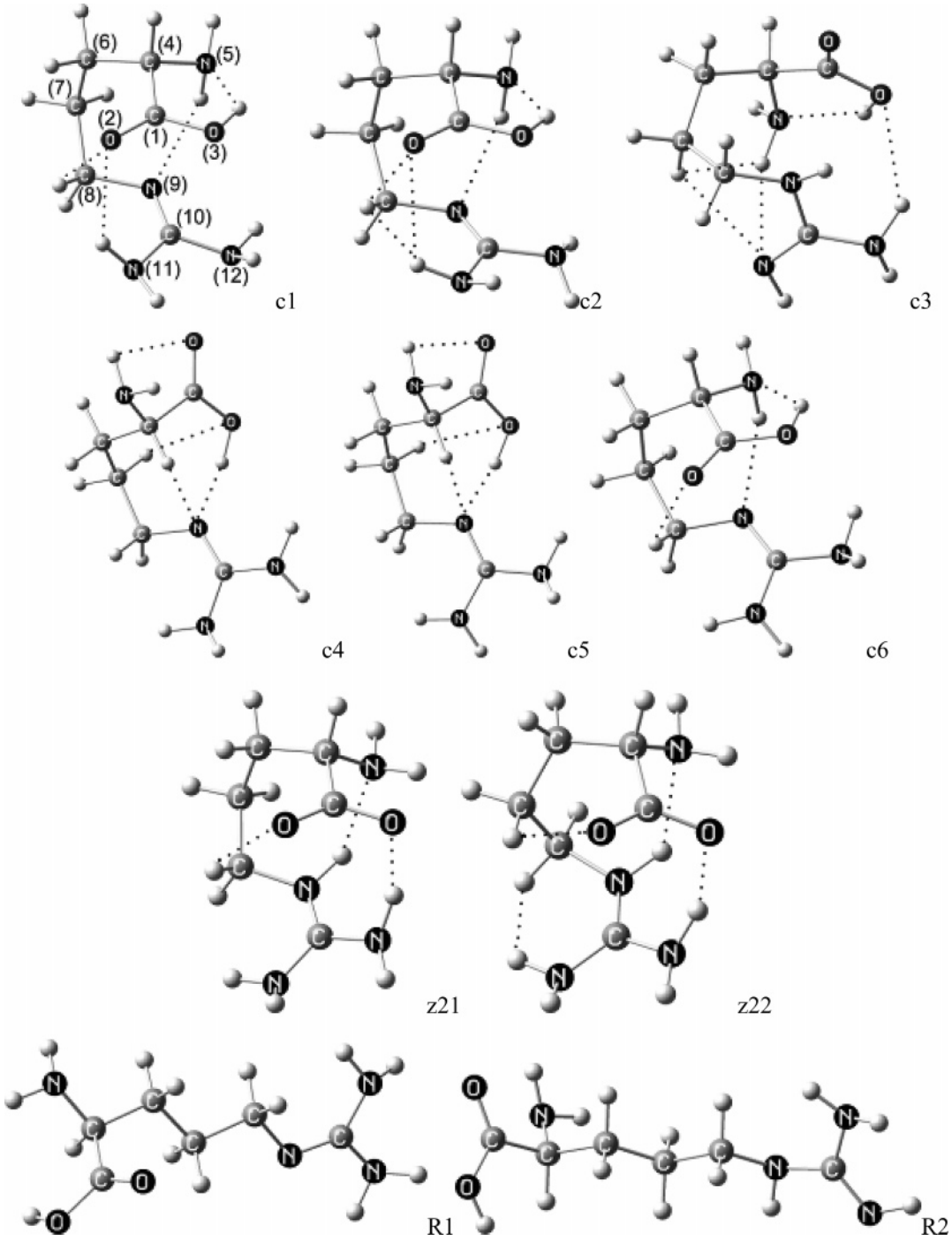

Figure 2. MP2 geometries for the most stable conformers of neutral arginine and for two reference structures. c1−6 are canonical structures, z21 and z22 are zwitterionic structures, and R1 and R2 are the two reference structures. The dotted lines indicate the intramolecular hydrogen bonds.

respectively.[19] Our theoretical values of the PA and GB are 253.9 and 245.9 kcal/mol, respectively.

As there are approximations in our theoretical approach, such as the ideal gas assumption, the harmonic oscillator−rigid rotor model, and an approximate solution of the electronic Schrödinger equation, as well as experimental challenges, such as clustering reactions of protonated molecules with polar neutral molecules and difficulties in attaining thermodynamic equilibrium, the agreement between the theoretical and experimental PA results is excellent, as a difference of 2.7 kcal/mol is within the theoretical and experimental uncertainties. This claim is supported by the benchmark calculations of ref 5 for glycine, a simpler and more rigid amino acid, for which the conformational space was fully explored.[20] The calculated values of the PA and GB for glycine are 214.3 and 206.5 kcal/mol, respectively, and the corresponding experimental data are 211.9 and 203.7 kcal/mol,[19] i.e., the computations lead to 2.4 and 2.8 kcal/mol overestimations for the PA and GB, respectively, of glycine. In the case of arginine, the overestimation of the GB value is larger by an additional 2.5 kcal/mol. This can be attributed to additional sources of error in determining the entropy contribution, e.g., neglecting the tendency toward molecular pair formation due to dipole−dipole interactions, the effects of a nonharmonic vibration potential, and the coupling of the low-frequency vibrational modes and the rotational components in the computational model,[54] and the formation of stable charged aggregates of arginine in the experiment.[15] Therefore, we believe

TABLE 2: Percent Shares of the Neutral or Protonated Arginine Conformers in Their Respective Equilibrium Mixtures at Various Temperatures

	98 K	198 K	298 K	443 K
		Neutral Forms		
c1	14.7	10.6	7.6	5.3
c2	5.0	11.8	12.7	11.6
c3	0.6	2.6	3.4	3.3
c4	39.8	31.4	25.6	19.8
c5	38.8	33.3	28.1	22.4
c6	0.9	5.3	8.1	9.3
c7	0.1	1.3	2.5	3.2
c8	0.1	1.3	3.1	4.9
c9	–	0.2	0.7	1.3
c10	–	1.0	2.4	4.0
c11	–	0.4	1.5	3.1
c12	–	0.1	0.6	1.2
c13	–	–	0.2	0.4
c14	–	0.1	0.4	1.1
c15	–	–	0.2	0.5
c16	–	–	0.1	0.4
c17	–	0.2	0.9	2.6
c18	–	0.2	0.9	2.4
c19	–	–	0.2	0.5
c20	–	0.1	0.8	2.4
z21	–	–	–	–
z22	–	–	–	0.1
z23	–	–	–	–
z24	–	–	–	–
z25	–	–	–	–
		Protonated Forms		
p1			94.6	
p2			4.2	
p3			0.1	
p4			0.2	
p5			0.7	
p6			0.1	

that the theoretical and experimental GBs of arginine also agree within the model uncertainties.

3.4. Vibrational Spectra. The simulated IR spectra with a Gaussian broadening of 10 cm^{-1} for the conformers c2, c3, c4, and z21 are shown in Figure 3. The IR spectra for c1, c5, c6, z22, z23, and z24 are provided in the Supporting Information. The IR−CRLAS spectrum of arginine at 443 K was measured by Saykally et al. in the 1550−1750 cm^{-1} range, and two transitions at 1666 and 1693 cm^{-1} were observed.[2] Considering the theoretical uncertainty as indicated by the suggested scaling factors of 0.9614[42] and 0.9813,[5] both observed transitions can be identified in several conformers of c1−6. Moreover, the two transitions can also be found in z21−23. This observation is in support of the assessment by Rak et al.[5] that the frequency range is not unique for the canonical conformers. Similarly, a frequency range of 1750−1850 cm^{-1} recommended by Rak et al. is not a distinct feature of the canonical structures either. Only the 2250−2750 cm^{-1} region can be said to be characteristic for zwitterions. The 10 most stable canonical conformers do not offer intense transitions in the region. This frequency range corresponds to the NH stretching of the terminal amino group involved in hydrogen bonding with the carboxylate residue (see Figure 2). Observation of such a vibration would indicate the presence of the zwitterion. However, the absence of such vibrations does not exclude the presence of zwitterions, as the corresponding frequency for z23 is 2840 cm^{-1}, which overlaps with some vibrational modes of the canonical conformers.

Intramolecular interactions have a strong influence on the frequencies of vibrational stretching modes involving hydrogen. Taking the OH stretching mode as an example, its frequency varies from 2760 to 3322 cm^{-1} among the six most stable conformers because of various hydrogen-bond configurations, whereas the corresponding frequency is about 3651 cm^{-1} for the reference structure R1, which has no hydrogen bond. The exceedingly large range of frequencies is due to intramolecular interactions. Because of the large number of hydrogens in the molecule, it is difficult to identify any single characteristic vibrational mode. Overall, the real tests of the computational results might have to rely on the measurement of the full vibrational spectrum.

It is interesting to note that all six of the most stable conformers located in our calculations have unconventional blue-shifting hydrogen bonds. It can be inferred then that the blue-shifting hydrogen bonds play an important role in the conformational stability of arginine. All blue-shifting hydrogen bonds involve HC stretches in X···HC, where X = N, O. As shown in Figure S3 of the Supporting Information for some blue-shifted vibrational modes, most of the blue shifts are about 50 cm^{-1} or less, as in the cases of c2 and c3, but some large blue shifts of

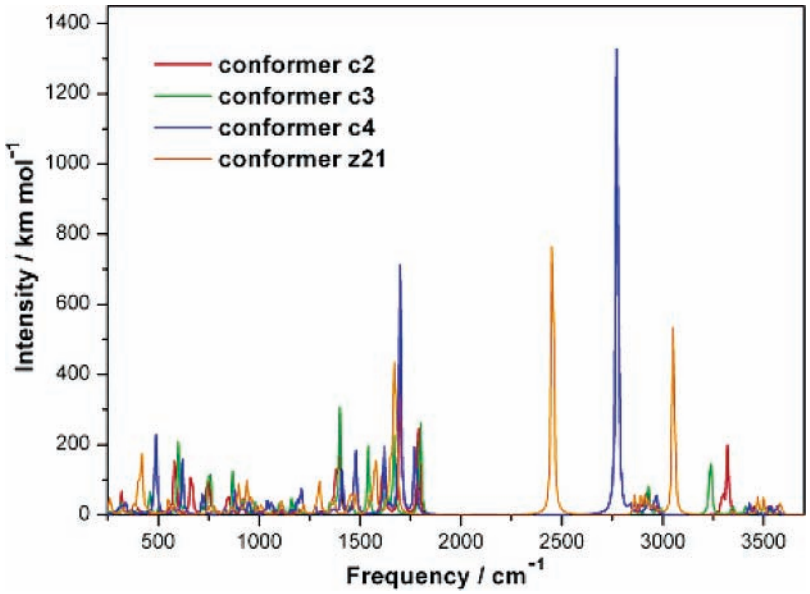

Figure 3. Simulated IR spectra of the canonical conformers, c2−4, and the zwitterionic conformer, z21, of gaseous arginine.

TABLE 3: Selected Geometrical Characteristics of Hydrogen Bonds in the Most Stable Conformers of Neutral Arginine Optimized at the MP2/6-31++G(d,p) Level

structure[a]	hydrogen bond	X···H distance (Å)	X···HY valence angle (deg)	type[b]
c1	N(5)···HO(3)	1.90	124.9	▲
	N(9)···HN(5)	2.17	130.2	▲
	O(2)···HC(8)	2.62	114.0	■
	O(2)···HN(11)	2.36	129.6	▲
c2	N(5)···HO(3)	1.93	123.8	▲
	N(9)···HN(5)	2.15	133.2	▲
	O(2)···HC(8)	2.54	121.4	■
	O(2)···HN(11)	2.63	116.1	▲
	C(8)H···HN(11)	1.92	114.4, 120.9	●
c3	O(3)···HN(11)	2.47	131.1	▲
	N(5)···HO(3)	1.86	127.1	▲
	N(12)···HN(5)	2.43	156.8	▲
	N(12)···HC(7)	2.61	112.2	■
	C(7)H···HN(5)	2.08	106.5, 121.7	●
c4	O(2)···HN(5)	2.50	97.4	▲
	O(3)···HC(7)	2.65	108.2	■
	N(9)···HO(3)	1.67	173.4	▲
	N(9)···HC(4)	2.47	112.6	■
c5	O(2)···HN(5)	2.50	97.0	▲
	O(3)···HC(7)	2.67	107.7	■
	N(9)···HO(3)	1.67	173.8	▲
	N(9)···HC(4)	2.48	112.8	■
c6	N(5)···HO(3)	1.91	124.9	▲
	N(9)···HN(5)	2.22	127.4	▲
	O(2)···HC(8)	2.64	113.0	■

[a] See Figure 2 for atom labels. [b] ▲ denotes a red-shifting hydrogen bond, ■ denotes a blue-shifting hydrogen bond, and ● denotes a dihydrogen bond.

more than 150 cm^{-1} are observed for the N(9)···HC(4) bonds of c4 and c5.

3.5. Intramolecular Interaction. Intramolecular interactions are important factors in determining relative conformational stability and other properties. Because of the structural flexibility of arginine and the presence of 14 H atoms, multiple hydrogen bonds can be formed within one conformer. The existence of a hydrogen bond is usually determined by a geometric criterion of taking a cutoff distance of 2.8 Å for near-atom interactions. The hydrogen bonds identified by applying this criterion to the most stable canonical conformers of arginine are listed in Table 3. As nitrogen and oxygen atoms are good hydrogen-bond acceptors and O−H and N−H groups are good proton donors, various hydrogen bonds can be formed between these groups. These hydrogen bonds are characterized by a red shift of the stretching NH or OH modes. As in some other amino acids,[33] the hydrogen bond between the carboxyl hydrogen and the nitrogen in the α-amino group is the strongest. Overall, the N···HO distance is the shortest, being around 1.9 Å or less.

The tendency to form a hydrogen bond between the C−H groups and the carbonyl oxygen or the nitrogen in the α-amino group is also evident. This type of hydrogen bond should be weaker than the hydrogen bonds involving two electronegative heavy atoms, and the X···HC (X = N, O) distance is around 2.5 Å. However, X···HC hydrogen bonds can be found in all six of the most stable conformers, as reported in Table 4. IR analysis shows that all of the X···HC hydrogen bonds are blue-shifted,[55] i.e., the CH stretching vibration is higher than that in the reference conformers.

The origin of a blue-shifting hydrogen bond can be explained properly by a viewpoint that both the blue- and red-shifting hydrogen bonds X···HY are governed by the same competing effects: hyperconjugation, which increases the population of an antibonding orbital and elongates the HY bond, and rehybridization, which shortens the HY bond by increasing the s character of a hybrid orbital of Y and strengthening the X−H bond.[56] With the natural bond orbital (NBO) analysis[29,57] of c2 illustrated in Figure 4, the increases in the s character of both the O−H and C−H bonds are obvious, indicating the proper rehybridization effect. However, in the O−H···N hydrogen bond, the rehybridization effect is completely overshadowed by the dominating effect of hyperconjugation [reflected by the large $E(2)$ value of 12.9 kcal/mol], resulting in an elongated O−H bond and a red-shifting hydrogen bond. On the other hand, in the C−H···O hydrogen bond, the rehybridization prevails over the weak hyperconjugation [the $E(2)$ value is only 0.4 kcal/mol], resulting in a shortened C−H bond and a blue-shifting hydrogen bond.

As shown in Table 3, there are also dihydrogen bonds between the C−H and N−H groups in c2 and c3. The distance between the two hydrogen atoms is around 2.0 Å, and the angle between the hydrogen-bond donor and acceptor (120.9° and 121.7° for c2 and c3, respectively) is larger than the angle between the hydrogen-bond acceptor and donor (114.4° and 106.5° for c2 and c3, respectively), which all coincides with

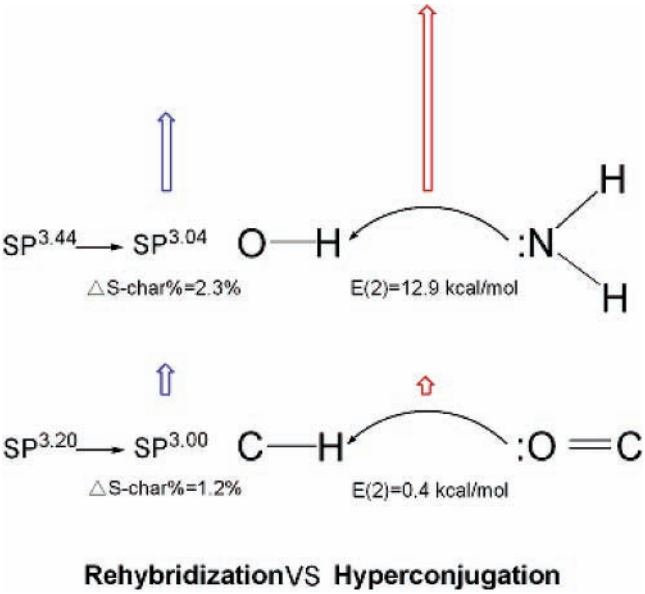

Figure 4. Diagrammatic sketch illustrating the origin of the frequency shifts of H bonds in the c2 conformer of arginine. The lengths of the hollow arrows represent the relative magnitudes of two competing effects: rehybridization (left) and hyperconjugation (right). SP^x is the hybridization of the HY bond. ΔS-char% is a percentage change in the s character measured relative to the reference conformer R1. The change results from rehybridization upon hydrogen bonding. $E(2)$ is the stabilization energy determined by the second-order perturbation approach. It quantitatively reflects the hyperconjugative interaction as determined from the NBO analysis.

TABLE 4: Selected Characteristics of Stereoelectronic Effects in Canonical Conformers of Arginine Optimized at the MP2/6-31++G(d,p) Level

structure	stereoelectronic interaction: donor	stereoelectronic interaction: acceptor	$E(2)$ (kcal/mol)	interaction distance (Å)
c1	(π)N9−C10	(π*)C1−O2	1.73	2.86
c2	(π)N9−C10	(π*)C1−O2	1.12	3.03
c3	(n)N9	(π*)C1−O2	4.06	2.68
c6	(π)N9−C10	(π*)C1−O2	1.43	2.78
c9	(n)N9	(π*)C1−O2	2.93	2.80
c10	(π)N9−C10	(π*)C1−O2	1.87	2.63
c11	(n)N9	(π*)C1−O2	3.72	2.68

the geometry rules found for dihydrogen bonds.[58] This type of dihydrogen bond is unconventional, as the C atom is more electronegative than the H atom, and was found recently in a relatively rare valine conformer.[59] As seen in Table 3, there are five hydrogen bonds in c2 and c3, each with three red-shifting, one blue-shifting, and one dihydrogen hydrogen bonds. c2 and c3 are two other examples of the coexistence of dihydrogen and red- and blue-shifting hydrogen bonds in a single molecular structure. Unlike the previously noted valine conformer,[59] c2 and c3 are relatively stable because of the structural flexibility of arginine. c2 and c3 should also be easily detected experimentally as they are populous in the equilibrium mixture. It can be further inferred that, with significant structural flexibility in larger biomolecules, the coexistence of dihydrogen, C−H-related blue-shifting, and normal red-shifting hydrogen bonds should be a common phenomenon and might have important effects on conformational stability and other properties.

As the geometric criteria cover only one of hydrogen-bond features, the AIM theory and NBO theory were also adopted to provide a more comprehensive analysis of the hydrogen bonds. All hydrogen bonds listed in Table 4 have bond critical points and can be considered as the true AIM hydrogen bonds, but the O···H−N bonds are very weak.[28,60] In the NBO analysis, the hydrogen bonds are also confirmed by the interactions between the proton-donor antibonding orbital and the corresponding proton-acceptor lone electron pair (red- and blue-shifting hydrogen bonds) or bonding orbitals (dihydrogen bonds).

More analysis from the energetic point of view was made to ascertain the coexistence of three different hydrogen bonds in c2 and c3. Taking c2 as an example, the hydrogen-bond energy can be estimated with some simple relationships between the hydrogen-bond strength and the properties of the hydrogen-bond critical point. Using the relationship between the hydrogen-bond energy (E_{HB}) and the potential energy at the AIM hydrogen-bond critical point (V_{CP}),[61] $E_{HB} = {}^1/_2 V_{CP}$, the energies of the five hydrogen bonds in c2 were found to be $E_{O-H\cdots N} = -8.8$ kcal mol^{-1}, $E_{N-H\cdots N} = -4.1$ kcal mol^{-1}, $E_{N-H\cdots O} = -1.6$ kcal mol^{-1}, $E_{C-H\cdots O} = -1.9$ kcal mol^{-1}, and $E_{N-H\cdots H-C} = -2.7$ kcal mol^{-1}. In addition, a similar energy for the N−H···H−C dihydrogen bond of -2.8 kcal mol^{-1} is obtained with the Grabowski expression $E_{HB} = -336.22\rho_b + 1.93$ kcal mol^{-1} for dihydrogen-bonded complexes, where ρ_b is the charge density at the hydrogen-bond critical point.[62] The coexistence of three different hydrogen bonds in c2 as well as in c3 is therefore further confirmed.

In addition to hydrogen bonds, influential stereoelectronic effects were also noticed in the most stable arginine conformers according to the AIM and NBO analyses, and these are included in Table 4. Stereoelectronic effects are usually defined as a mixing of the bonding orbital of an electron pair with the antibonding orbital σ^* of an adjacent polar bond (C−X, where X = N or O).[63] In arginine, the stereoelectronic interaction occurs between the nitrogen atom on the δ-C and the carbon atom in the carbonyl group. The NBO analysis reveals two types of the N···C stereoelectronic interactions in the stable arginine conformers. One is the $n-\pi^*$ interaction originated from the overlap of the n_N electron lone pair and the π^*_{CO} orbital, as illustrated in Figure S4 of the Supporting Information. The other is a $\pi-\pi^*$ interaction originating from the overlap of the π_{NC} and the π^*_{CO} orbitals. The $\pi-\pi^*$ stereoelectronic interaction is of the same order of magnitude as the energy of the C−H···O hydrogen bond.

TABLE 5: Vertical Ionization Energies (VIEs), Dipole Moments, and Rotational Constants of the 25 Most Stable Conformers of Arginine

	VIE (eV)			rotational constants (GHz)		
structure	B3LYP	CCSD	dipole (D)	*A*	*B*	*C*
c1	8.320	8.398	2.791	1.191	0.844	0.719
c2	8.337	8.458	2.983	1.173	0.850	0.731
c3	8.542	8.628	3.431	1.137	0.873	0.706
c4	8.468	9.216	8.718	1.421	0.549	0.484
c5	8.460	9.210	8.694	1.442	0.541	0.472
c6	8.325	8.498	3.208	1.195	0.802	0.668
c7	8.575	8.990	8.600	1.388	0.597	0.454
c8	8.572	9.084	8.223	1.397	0.590	0.451
c9	8.548	8.660	3.004	1.181	0.821	0.676
c10	8.269	8.504	4.229	1.207	0.790	0.636
c11	8.480	8.539	2.817	1.160	0.857	0.696
c12	8.560	9.176	8.311	1.594	0.524	0.445
c13	8.655	8.731	3.955	1.142	0.853	0.694
c14	8.537	9.029	9.675	2.063	0.408	0.360
c15	8.539	8.829	6.966	1.522	0.521	0.498
c16	8.800	8.968	7.334	1.504	0.520	0.499
c17	8.724	8.924	8.225	1.484	0.455	0.406
c18	8.397	8.571	2.265	1.117	0.861	0.717
c19	8.526	8.868	8.825	1.977	0.465	0.439
c20	8.517	9.189	8.185	1.413	0.567	0.446
z21	8.060	8.521	6.202	1.250	0.881	0.679
z22	8.073	8.583	6.509	1.227	0.906	0.716
z23	8.211	8.231	5.421	1.265	0.884	0.778
z24	8.149	8.427	4.950	1.344	0.817	0.696
z25	7.685	8.036	9.427	1.339	0.718	0.587

Summarizing briefly, the coexistence of the three types of hydrogen bonds has been confirmed in the stable conformers of arginine. All of the stable conformers adopted a configuration with the carbon side chain bent to make the guanidino plane neighboring the carboxyl plane and resulting in multiple intramolecular interactions. Such behavior is expected to be common in large biomolecules.

3.6. Vertical Ionization Energy. The values of the vertical ionization energy (VIE) for the 25 most stable conformers of neutral arginine were determined at the B3LYP/6-31++G(d,p) and CCSD/6-31++G(d,p) levels of theory, as reported in Table 5, together with the dipole moments and rotational constants for the MP2/6-31++G(d,p) geometries. The average values of the VIE for the 25 conformers at the B3LYP/6-31++G(d,p) and CCSD/6-31++G(d,p) levels of theory are 8.41 and 8.73 eV, respectively. The differences between the B3LYP/6-31++G(d,p) and CCSD/6-31++G(d,p) results are usually larger for conformers with larger dipole moments. The CCSD/6-31++G(d,p) calculations typically predict higher VIEs for conformers with larger dipole moments. For conformers with comparable dipole moments, the VIEs for canonical structures are usually higher than those for zwitterions. The experimental VIE of gaseous arginine has not yet been measured, but the experimental VIE value of 9.10 eV for guanidine[64] is close to the theoretical VIE value for arginine, suggesting that the first ionization of arginine is likely the detachment of a delocalized π electron from the guanidine group. The VIEs, dipole moments, rotational constants, and IR spectra of gaseous arginine can be measured[65−68] to test the computational predictions of this work.

4. Summary

A structural search based on the B3LYP/6-31G(d) geometry optimizations led to a series of new low-energy structures that are more stable than the most stable respective conformers identified previously. The results demonstrate a failure of the prescreening by a semiempirical method in a constrained conformational space. Clearly, a more thorough exploration of

conformational space using a relatively high level of theory is required to identify the most stable conformers. Compared to the impossible task of optimizing all possible trial structures, the segmented step-by-step approach proposed here is reasonably efficient and computationally tractable. It is also reliable, as two different sets of initial structures led to the same set of the most stable conformers. Therefore, the proposed approach might be helpful to researchers facing the challenging problem of conformational degrees of freedom in other biologically important molecules.

The CCSD/6-31++G(d,p) energy of the most stable canonical conformer is 3.7 kcal/mol lower than that of its zwitterionic counterpart. Thermal effects contribute substantially to the relative stability of different conformers because of the near-degeneracy of their electronic energies. The relative conformational stabilities calculated at the CCSD/6-31G(d) and MP2/6-31++G(d,p) levels are acceptable, but the B3LYP/6-31++G(d,p) results are misleading. Small but noticeable differences between the B3LYP/6-31++G(d,p) and MP2/6-31++G(d,p) structures were observed. The conformational distributions at 98, 198, 298, and 443 K were calculated. Gaseous arginine is a genuine multiconformer system, but consists of purely canonical structures. An improved agreement between the theoretical and experimental room-temperature proton affinities and gas-phase basicities was found. The relative energies, dipole moments, rotational constants, vertical ionization energies, and IR spectra remain for future experimental verification.

The intramolecular hydrogen bonds were analyzed by the AIM and NBO theories, as well as conventional geometrical criteria. Multiple hydrogen bonds are common in the most stable conformers. All X···HY (X, Y═N, O) hydrogen bonds are red-shifting, and all X···HC hydrogen bonds are blue-shifting. Less conventional dihydrogen bonds, NH···HC, were also found. In particular, the coexistence of dihydrogen and red- and blue-shifting hydrogen bonds was found in the second and third most stable conformers, which are amenable to experimental explorations because of their high populations. Stereoelectronic interactions with magnitudes comparable to weak and medium-strong intramolecular hydrogen bonds are also common in the most stable conformers. The intramolecular hydrogen bonds strongly influence the stretching mode vibrations, with a frequency shift in the range between -890 cm^{-1} and $+160$ cm^{-1}.

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Supporting Information Available: Supplementary data to Table 1 for conformers c13−19. Energies and structures for the most stable conformers of protonated arginine. Cartesian coordinates of the MP2/6-31++G(d,p)-optimized geometries of the most stable canonical, zwitterionic, and protonated structures of arginine and the two reference structures. Simulated IR spectra of the neutral arginine conformers c1, c5, and c6 and z22, z23, and z24. Representative vibrational modes with blue-shifting hydrogen bonds. Stereoelectronic effect illustrated by the $n-\pi^*$ interaction originating from the overlap of the n_N electron lone pair and the π^*_{CO} orbital. Complete citations for refs 48 and 50. This material is available free of charge via the Internet at http://pubs.acs.org.

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