Experimental and ab Initio Studies of the Gas-Phase Basicities of Polyglycines

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Abstract: The gas-phase basicities of polyglycines, Gly_n (n = 1–6), were determined by proton transfer reactions in a Fourier transform ion cyclotron resonance mass spectrometer. The basicities were found to increase as the peptide chain length increased: 206.2, 215.3, 218.9, 225.0, 225.3, and 227.4 kcal/mol for n = 1–6, respectively. Comparable results, but with some deviations, were obtained using the kinetic method of collision-induced dissociation on a proton-bound dimer. The large change in basicity between glycine and diglycine suggests significant differences in the strength of intramolecular hydrogen bonding in their protonated forms. This is consistent with ab initio Hartree–Fock calculations, which were carried out using the 6-31G* basis set for glycine, diglycine, and their protonated species. Full optimization of geometry yielded eight minimum-energy structures: glycine (**1**), protonated glycines at the amino nitrogen (**2**) and the carbonyl oxygen (**3**), diglycine (**4**), and protonated diglycines at the amino nitrogen (**5**), the amide carbonyl oxygen (**6**), the amide nitrogen (**7**), and the carboxyl carbonyl oxygen (**8**). The ideal-gas basicities for the relevant protonations at 298.15 K and 1 atm, calculated after inclusion of zero-point energy and temperature changes in enthalpy and entropy, were found to be 207.5 (**1** → **2**), 194.3 (**1** → **3**), 214.0 (**4** → **5**), 212.9 (**4** → **6**), 196.9 (**4** → **7**), and 188.3 (**4** → **8**) kcal/mol. For the glycines, more accurate electronic energies were obtained with geometry optimization in the larger 6-31+G(d,p) basis, including electron correlation corrected to the fourth order in the Møller–Plesset perturbation treatment. Scaling the zero-point energy with a factor of 0.91 was also found to improve the calculated basicities. The best theoretical estimates of basicities corresponding to protonation at the terminal amine were 206.3 and 215.2 kcal/mol for the respective glycine and diglycine; these numerical values are in excellent agreement with the experimental gas-phase basicities. The corresponding theoretical and experimental proton affinities are also presented. Detailed analysis is made on the structural features that affect the relative stabilities of the various molecular species. In addition, several topics relevant to the theoretical and experimental determinations of basicity are discussed.

Introduction

Proton transfer is a fundamental process in the chemistry of peptides. In solution, the locations of basic sites affect the internal hydrogen bonding of peptides, influencing three-dimensional structure and biological activity. The acid–base properties of a peptide also impact physicochemical considerations such as solubility, hydrophobicity, and electrostatic interactions.[1] In the gas phase, protonation is the dominant ionization pathway during the analysis of peptides by mass spectrometry.[2] For desorption ionization techniques such as fast atom bombardment (FAB),[3] both solution- and gas-phase proton transfer occur during ion formation[4] and more basic molecules have greater ionization efficiencies.[5] In addition, the elucidation of peptide sequence information by mass spectrometry involves the dissociation of protonated peptide ions. The location of the proton affects fragmentation patterns and, consequently, the structural information obtained from mass spectrometry.[6-8]

Major thermodynamic parameters of proton transfer are gas-phase basicity (GB), which corresponds to the negative of the Gibbs free energy change ($-\Delta G$) for the protonation reaction 1,

$$M + H^+ \rightarrow MH^+ \qquad (1)$$

and proton affinity (PA), which is the negative of the enthalpy ($-\Delta H$) of protonation. Gas-phase basicity and proton affinity measurements increase our understanding of the intrinsic properties of molecules in the absence of solvents. Many investigations have determined the gas-phase basicities of organic materials,[9-12] but few studies have involved biomolecules.[13-18] In particular,

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with the exception of two very recent investigations,[19,20] peptides have not been the focus of gas-phase basicity research. The reason for this is that the low volatilities of peptides have prohibited mass spectrometry studies involving the measurement of proton transfer equilibrium constants,[9,10] which is the most common method for determining gas-phase basicities. The present study employs deprotonation reactions of protonated polyglycine ions (Gly_nH^+) with reference compounds (B) of known basicity to bracket the gas-phase basicity of the polyglycine, reaction 2.

$$Gly_nH^+ + B \rightarrow BH^+ + Gly_n \qquad (2)$$

The presence or absence of reaction 2 gives an upper or lower limit (respectively) on the basicity of the polyglycine. The determination of gas-phase basicities with deprotonation reactions is an established technique,[21] but it has not been used extensively for biomolecules because it requires a mass spectrometer that allows both the facile production of protonated molecular ions by desorption ionization techniques (e.g., FAB) and ion/molecule reaction studies. These instruments, such as our external source Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR),[22,23] have only become readily available within the past few years.

The present study focuses on the gas-phase basicities of polyglycines, Gly_n (n = 1–6), containing one through six residues. Glycine is the simplest amino acid, with no side chains to complicate the bonding of the proton. In addition, polyglycines can be considered as the backbone of peptides, with more complex species being formed by the addition of functional groups at methylene sites. Therefore, polyglycines are a logical choice for initial studies that probe the gas-phase basicities of peptides. Basicity information was obtained by both deprotonation reactions and collision-induced dissociation (CID) experiments.

To complement the experimental studies, ab initio molecular orbital calculations[24] were carried out on molecular species related to the two smallest members: glycine (NH_2CH_2COOH) and diglycine ($NH_2CH_2CONHCH_2COOH$). The ab initio calculations provided information on the structures and energetics of protonation. In order to obtain calculated values at a level commensurate to experimental accuracy, extended basis sets and geometry optimizations were employed and effects of electron correlation, zero-point energy, and temperature change (from 0 K to 298.15 K) were included to the extent allowed by the available computing resources. As compared with pioneering ab initio treatments on these systems,[25] the present study represents a marked advance in the level of theory applied to the computation of electronic energies. This work also differs from the more recent ab initio studies on glycine[26,27] in two respects: first, by including larger species (diglycine) in the high-level theoretical treatment, and second, by calculating the gas-phase basicities at laboratory temperature for direct comparisons with experiments. This study resembles more closely a recent experimental and theoretical study of the gas-phase basicities of β-lactams and azetidines.[18] With respect to the most recent semiempirical AM1 calculations of the basicities of glycine and diglycine,[20] the present ab initio high-level calculations yield much more accurate structures and energies.

Experimental Methods

All experiments were performed using a Bruker CMS-47X Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR) equipped with an external ion source and a 4.7 T superconducting magnet.[23] Protonated polyglycine ions were produced in the external source using a Phrasor Scientific FAB gun[28] which employs a 6–10 kV beam of xenon or argon atoms and ions. The polyglycines were dissolved in glycerol, which was doped with trifluoroacetic acid as necessary to increase ion abundance. Water or methanol was also added to the glycerol matrix to increase the solubility of hexaglycine.

Ions were transferred from the external source into the FT-ICR cell by electrostatic focusing. Three stages of differential pumping allowed a matrix/bombardment gas pressure in the source of 10^{-4} Torr, while the base pressure in the cell remained at 1×10^{-9} Torr. Following ion transfer, thermalization of the trapped ions was achieved by admitting a pulse of argon into the cell vacuum chamber via a General Valve Corp. Series 9 pulsed solenoid valve. The pulse pressure reached a maximum of 10^{-5} Torr. At least 0.5 s was allowed for collisional cooling of the ions[29] and for the argon to be pumped away before monitoring ion/molecule reactions. Additional experiments were performed with xenon and sulfur hexafluoride as the collision gas, as discussed in the Results and Discussion section.

The Gly_nH^+ were mass selected by resonant frequency ejection techniques[30] and allowed to react with static pressures of reagent gases in the range of $(3–30) \times 10^{-8}$ Torr. All reactions were studied to greater than 80% completion except in cases of prohibitively slow reactions. Overall rate constants (k_{all}) were determined by observing the pseudo-first-order change in reactant ion intensity as a function of time at a constant pressure. Except as noted below, all first-order decay plots were linear, suggesting the predominance of ground-state reactant ions. In cases where protonation was in all competition with another primary reaction pathway (e.g., dimer formation), the rate constant for the protonation pathway (k_{proton}) was obtained from a plot of the relative protonated base (BH^+) intensity as a function of time using eq 3.[31]

$$[BH^+]_t/[Gly_nH^+]_0 = (k_{proton}/k_{all})[1 - \exp(-k_{all}t)] \qquad (3)$$

Pressures were measured with an ionization gauge that was calibrated using reaction 4,

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3 \qquad (4)$$

with a rate constant of 1.335×10^{-9} cm^3 molecule^{-1} s^{-1}, as the reference.[32] Pressures were corrected for the reactant gas ionization efficiency,[33] which involved polarizabilities calculated by atomic hybrid parameter procedures.[34] Reported reaction efficiencies are the ratio of the experimental rate constant (k_{proton}) to the collisional rate constant that was obtained using the average dipole orientation model[35] (k_{ADO}): reaction efficiency = (k_{proton}/k_{ADO}). The FT-ICR cell was maintained at room temperature (*ca.* 298 K) during all experiments.

Collision-induced dissociation experiments[36] utilized argon collision gas at pressures of $(1–10) \times 10^{-7}$ Torr. The collision energy was varied from 0 to 150 eV (laboratory).

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Table I. Reaction Efficiencies for the Proton Transfer Reactions of Protonated Polyglycine Ions

		reaction efficiency for $Gly_nH^+ + B \rightarrow Gly_n + BH^+$					
reference compd (B)	GB,[a] kcal/mol	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$
ammonia	199.7	0.00	0.00	-[b]	-	-	-
acetamide	202.5	0.00	0.00	-	-	-	-
pyrrole	206.1	0.01	0.01	-	-	-	-
		—[c]					
aniline	206.4	0.24	0.01	-	-	-	-
2-fluoropyridine	207.9	0.34	0.01	0.02	-	-	-
N,*N*-dimethylformamide	209.5	0.33	0.04	0.01	-	-	-
tert-butylsulfide	210.9	0.54	0.05	0.03	-	-	-
3-methylaniline	211.5	0.68	0.04	0.04	0.01	-	-
3-fluoropyridine	212.9	0.76	0.03	0.02	0.01	0.01	-
allylamine	214.8	0.61	0.07	0.06	0.05	0.05	0.02
			—				
benzylamine	215.9	0.82	0.61	0.08	0.07	0.03	0.04
n-propylamine	217.0	-	0.66	0.08	0.06	0.03	0.06
isopropylamine	218.1	-	0.79	0.08	0.04	0.03	0.07
				—			
pyridine	219.8	-	0.85	0.11	0.02	0.05	0.05
cyclohexylamine	220.6	-	1.07	0.29	0.09	0.07	0.05
N,*N*-dimethylaniline	223.1	-	-	0.47	0.08	0.05	0.09
2-methylpyridine	224.7	-	-	0.64	0.06	0.05	0.05
					—	—	
diethylamine	225.3	-	-	0.71	0.23	0.12	0.08
piperidine	225.9	-	-	0.57	0.22	0.14	0.08
di-*n*-propylamine	226.9	-	-	0.78	0.44	0.15	0.06
							—
di-*n*-butylamine	227.8	-	-	0.89	0.60	0.29	0.14
1-methylpiperidine	229.5	-	-	-	0.51	0.16	0.22
triethylamine	232.1	-	-	-	0.65	0.39	0.17
tributylamine	234.6	-	-	-	0.78	0.65	0.63

[a] Reference compound gas-phase basicities were obtained from ref 12 or were obtained from ref 11 and adjusted to the scale of ref 12. [b] The "-" indicates that no experiment was performed. [c] The "—" signifies the break between slow and facile proton transfer for each Gly_nH^+.

All reagents were used as received except for multiple freeze–pump–thaw cycles that were needed to remove non-condensable gases from reference compounds. Polyglycine samples were obtained from Sigma Chemical Co.

Computational Methods

Ab initio self-consistent-field (SCF) molecular orbital calculations were carried out for glycine (Gly) and diglycine (Gly_2) and their respective protonated species ($GlyH^+$ and Gly_2H^+) using the Gaussian 90 program on an IBM ES/9121/480 at Miami University and the Gaussian 92 program on a CRAY Y-MP8/864 at the Ohio Supercomputer Center.[37,38] For convenience, Gly and $GlyH^+$ will be referred to collectively as glycines and Gly_2 and Gly_2H^+ as diglycines.

To find the stable structures, the Hartree–Fock (HF) procedure was used in a split-valence-plus-polarization (6-31G*) basis. All geometries were full optimized. Electron correlation, where incorporated, was estimated from results of Møller–Plesset (MP) perturbation treatment.[24] For comparisons with the gas-phase basicity data measured at room temperature, zero-point energies and statistical thermodynamic properties at 298.15 K and 1 atm were calculated at the HF/6-31G* level for all species.

In the interest of better accuracy, a larger basis set 6-31+G(d,p), which is equivalent to 6-31G* supplemented with p functions on hydrogens and diffuse s and p functions on heavy atoms, was explored on the glycines at the HF level for geometry optimization and at the MP4 level for electronic energy. This basis set is too large to be practical for the diglycines.

Considering that the primary goal of these calculations was to determine the theoretical gas basicities and the relative ease of protonation at the available sites, searches for stable structures were confined to the lowest-energy conformers of the neutral and protonated species. As the starting geometry for a neutral species, a cis N—C—C=O between the amine and carbonyl groups, a cis O=C—O—H for the carboxylic group, and a trans O=C—N—H for the amide group were chosen. The starting geometry for a protonated species usually began with the most stable structure found for the neutral species. Optimization was carried out without symmetry or geometric constraints until the molecular energy reached a minimum, accompanied by all positive eigenvalues of the Hessian matrix.[38] Usually the optimized geometry obtained at a lower level (in a STO-3G or 3-21G basis) was used as the starting geometry at a higher level [in a 6-31G* or 6-31+G(d,p) basis]. The final lowest-energy geometry was further confirmed as a stationary point on the molecular potential energy surface by a normal-mode vibrational frequency calculation that yields all real frequencies.

Searches for some of the stable protonated diglycines were not as straight forward as described above. When starting with the optimized geometry of the neutral species and the added proton on the amino or amide nitrogen, a stable O-protonated species at the original carbonyl oxygen cis to the nitrogen was invariably produced, which represents a migration of the proton to the amide carbonyl or carboxyl carbonyl oxygen. In order to find the N-protonated species, direct searches were made in the 6-31G* basis with starting geometries at nearly trans positions between the terminal amino C—N and the amide C=O bonds. These initial geometries led to three stable species: two with protonations at the amino N and one at the amide N.

Results and Discussion

Experimental Gas-Phase Basicities from Deprotonation Reactions. The gas-phase basicities of Gly_n, $n = 1$–6, were obtained by monitoring reaction 2. The reference compounds employed, their gas-phase basicities, and the measured reaction efficiencies are shown in Table I. Table II summarizes the gas-phase basicity and proton affinity values obtained. With the exception of a very recent study,[20] deprotonation reactions of ions generated by FAB have not been used to obtain gas-phase basicities. Therefore, an in-depth discussion of several factors involved in these measurements will be given here.

Gas-phase basicities were assigned by determining the "break point" between exoergic and endoergic deprotonation reactions

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Table II. Experimental and Theoretical Gas-Phase Basicities and Proton Affinities of Polyglycines (kcal/mol)

	expt GB			"exptl" PA			
Gly_n, n =	deprotonation reactions	kinetic method	calcd GB[a]	deprotonation reactions[b]	kinetic method[b]	calcd PA[a]	lit PA[c]
1	206.2 ± 2.2	*d*	206.4	213.5	*d*	213.6	213.9[e] 216.3[f] 211.7[g] 215.4[h]
2	215.3 ± 2.5	215.3	215.2	223.6	223.4	223.5	227.1[i] 224.5[h]
3	218.9 ± 2.9	220.2	*j*	227.2	228.5	*j*	230.6[i] 226.0[h]
4	225.0 ± 2.3	225.0	*j*	233.3	233.3	*j*	234.8[i] 226.0[h]
5	225.3 ± 4.4	227.3	*j*	233.6	235.6	*j*	239.4[i] 228.1[h]
6	227.4 ± 4.6	230.8	*j*	235.7	239.1	*j*	242.0[i]

[a] Details for the ab initio values are provided in Table VI and the text. [b] Experimental GB values were converted to PAs using entropy terms obtained from ab initio calculations for n = 1 and 2. [c] Literature values were adjusted to the PA scale of ref 12. [d] The kinetic method could not be performed for glycine because glycine did not form proton-bound dimers with the reference compounds. [e] From ref 15. [f] From ref 14. [g] From ref 16. [h] From ref 20. Values for n = 2–5 assume protonation is on the terminal amine with hydrogen bonding to the amide carbonyl. [i] From ref 19. [j] Ab initio calculations were only performed on n = 1 and 2.

using the reaction efficiencies. For proton transfer reactions that are not sterically hindered, efficiency is small for endoergic and thermoneutral reactions, but increases steadily as ΔG becomes negative for exoergic reactions.[39,40] An efficiency of 0.10 (*ca.* 10% of all collisions resulting in proton transfer) has been employed as the break between slow (endoergic) and facile (exoergic) deprotonation. The gas-phase basicity of each polyglycine was assigned at this point. This criterion has been utilized previously[40] and is necessary because slightly endoergic reactions may occur, but in low efficiencies, in FT-ICR studies. This is particularly important for the reactions under study here because a static pressure of the neutral Gly_n is not present in the FT-ICR cell. Therefore, if a slightly thermodynamically unfavorable proton transfer does occur, there is no possibility of a reverse reaction to regenerate Gly_nH^+.

The experimental rate constants were reproducible to at least ±20% for reactions with efficiencies of ≥0.10. For reactions with lower efficiencies, a greater variability occasionally existed. This is because a small amount of BH^+ may form via proton transfer from background ions (chemical noise), resulting in the measured proton transfer rates being slightly high. This effect is minor for fast processes where abundant BH^+ is produced from reaction 2, but it is more noteworthy for slow reactions and for reactions involving highly basic reference compounds. This is particularly true for n = 6, where FAB yielded Gly_nH^+ in roughly 3–10 times lesser abundance than for n = 1–5, thus leading to a lower signal-to-noise ratio. This effect was minimized by using ion ejection techniques to isolate each Gly_nH^+ as cleanly as possible, but without inducing kinetic excitation. Thus, while the efficiencies reported for some slow reactions in Table I may be slightly inflated, we do not believe that the assignment of gas-phase basicity values was affected. However, background protonation must be monitored carefully in deprotonation reaction studies and this is an additional reason for employing an efficiency criterion of 0.10 before assigning a gas-phase basicity value.

The gas-phase basicities of the reference compounds (shown in Table I) were obtained using a revised basicity ladder recently published by Meot-Ner and Sieck.[12] In their study, the enthalpies of proton transfer equilibria were measured using variable-temperature pulsed high-pressure mass spectrometry. We converted these proton affinity values at 298 K to gas-phase basicities using entropy terms calculated from rotational symmetry changes, as shown in eq 5,

$$-T\Delta S(B \rightarrow BH^+) = RT \ln[\sigma(BH^+)/\sigma(B)] \quad (5)$$

plus the entropy term for a free proton (7.76 kcal/mol).[9-11] These values are referenced to a gas-phase basicity of 199.7 kcal/mol for ammonia at 298 K. For compounds that were not included in this revised ladder, gas-phase basicities were obtained from the literature[11] and adjusted to the new scale. As compared to the previous ladder,[11] the revised ladder[12] has increased basicity values by 4–8 kcal/mol in the upper portion of the scale, which is the region employed in this work. The absolute gas-phase basicity ladder is still in a state of fluctuation and other changes may be reported which impact the absolute gas-phase basicity values found in this paper. The relative values between various Gly_n and the reference compounds should remain constant, however.

As shown in Table II, the uncertainty in assignment of the gas-phase basicities is 2–3 kcal/mol for Gly_n, n = 1–4. These uncertainties were determined from the basicity range of the two bracketing reference compounds and from the uncertainties associated with the reference compound gas-phase basicities. The reference basicities are often known to ±0.2 kcal/mol, although the error limits are ±2 kcal/mol for some compounds.[11] As a conservative measure, ±2 kcal/mol has been included in all reported uncertainties to account for errors that may have been induced during the conversion of literature values to the Meot-Ner and Sieck[12] scale. For n = 5 and 6, the uncertainties in Table II have also been expanded slightly because the break between slow and facile proton transfer is less apparent than it is for n = 1–4.

The data in Table I indicate that the break between slow and fast proton transfer is less pronounced as the polyglycine chain length increases. This is consistent with larger peptides having a greater possible number of protonation sites, with various structures having slightly different basicities. For example, our ab initio results indicate that the most energetically favorable protonation site on diglycine is the terminal amine group with H bonding to the amide carbonyl oxygen. For Gly_nH^+, n = 3–6, more than one amide group is present and the energetics of protonation at sites involving the various oxygens may differ only slightly. While less likely to be problematic, recent ab initio studies[41,42] suggest that several glycine conformers exist with energies only a few kilocalories per mole above the lowest energy conformation, including a species that is only 0.5 kcal/mol higher in energy than the most stable conformer.[42] This effect should be even more pronounced for larger polyglycines.

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(41) Jensen, J. H.; Gordon, M. S. *J. Am. Chem. Soc.* **1991**, *113*, 7917.

(42) Czászár, A. G. *J. Am. Chem. Soc.* **1992**, *114*, 9568.

Another possible explanation for the less pronounced rate change with larger polyglycines is that, as the chain length increases and folding becomes feasible, steric factors affect the kinetics of deprotonation reactions leading to lower rate constants for slightly exoergic reactions. The effects of steric hindrance on proton transfer are not well understood; for example, *tert*-butylated pyridines exhibit slow exoergic reactions[43] but *tert*-butylated benzenes do not.[40] In addition, with alkylated pyridines, the incidence of low rate constants for exoergic reactions is most pronounced when both the ion and the neutral are hindered.[43] The role of steric hindrance in our studies of peptide protonation is unknown; however, the bases employed here are generally small, which will minimize these effects.

During these studies, care was be taken to ensure that Gly_nH^+ did not have excess kinetic energy that would obscure the measured rate constants. Both FAB ionization and the process of transferring ions from the source into the FT-ICR cell may impart excess energy to ions. It is important to note, however, that the ion lifetimes were seconds to minutes; very little dissociation occurred, suggesting that the ions arrived in the cell with minimal kinetic energy. Therefore, the transfer process may minimize the population of kinetically excited ions because ions with kinetic energies sufficient to overcome the 1 V trap potential of the cell are not retained. In addition, Gly_nH^+ were subjected to thermalizing collisions with an inert gas following their introduction into the cell. This is an established technique for cooling kinetically excited ions.[29] Several Gly_nH^+ reactions were studied with no inert gas pulse and with collisional-cooling employing argon, xenon, and sulfur hexafluoride. (Thermalization efficiency increases with the size and complexity of the quenching neutral.[29]) The pressures and collision delays were varied to ascertain the optimal conditions for obtaining a thermal ion population, as judged based on the magnitude of the rate constant and the linearity of the first-order kinetics plots. As expected, collisional cooling had the greatest effect for reactions that were near thermoneutral. Each of the three collision gases gave similar results. A 10^{-5}-Torr peak pressure for the inert gas, followed by a 0.5-s collision delay, was found to adequately thermalize the ions. At this point, the rate constant had plateaued and was unaffected by further increases in either the pressure or the delay. Therefore, these conditions, which correspond to 60–100 collisions, were employed in our experiments.

Experimental Gas-Phase Basicities from Collision-Induced Dissociation of Proton Bound Dimers. For the reference compounds listed in Table I, reactions with Gly_nH^+ occurred by only two dominant pathways: proton transfer (reaction 2) and formation of a proton-bound dimer, reaction 6. Reaction 6 was

$$Gly_nH^+ + B \rightarrow Gly_nHB^+ \qquad (6)$$

not observed to an appreciable extent for glycine. It is most pronounced with the larger polyglycines, which have more bonds available to disperse energy and thus limit dissociation of the dimer. This process is very dependent on energetics and is observed in the greatest abundance when the reaction is near thermoneutral. As proton transfer becomes increasingly exoergic, the amount of excess internal energy in this dimer (which can be considered as the reaction intermediate for the proton transfer process) increases; thus, its rate of dissociation increases and the observed dimer intensity decreases. In fact, the magnitude of dimer formation can serve as a rough indicator of the energetics of the proton transfer reaction.

Dimer formation, reaction 6, is also pressure dependent. If proton transfer is slightly endoergic, increasing the pressure of either the reference compound or an inert gas increases the observed Gly_nHB^+ intensity because of collisional stabilization. In contrast, if the proton transfer reaction is exoergic, increasing the pressure gives a decreased Gly_nHB^+ intensity; here, the combination of excess internal energy and increased pressure facilitates CID of the dimer. Dimer formation also decreased if Gly_nH^+ were not collisionally cooled and if kinetic energy was imparted to Gly_nH^+ by application of on-resonance pulses at the ion's cyclotron frequency. This provides additional evidence that excess energy will lower the observed intensity of the dimer.

The presence of Gly_nHB^+ in the mass spectra is fortuitous because it allows gas-phase basicities of polyglycines to be determined by an additional method employing collision-induced dissociation of the dimer. This technique, which is known as the "kinetic method", was developed by Cooks and co-workers[44] and assumes that when a dimer, such as Gly_nHB^+, is excited it will fragment to form either Gly_nH^+ or BH^+, with the more basic species preferentially retaining the proton at low dissociation energies. The kinetic method has been used to order the basicities of a variety of compounds, including amino acids,[17] and has recently been used by Wu and Fenselau to determine the proton affinities of polyglycines by high-energy CID.[19] However, the kinetic method must be employed cautiously because its accuracy is only confirmed in cases where the two species comprising the dimer have very similar structures. In addition, the method assumes that the sites of H bonding in the dimer are the same as the protonation sites of the free compounds. For multifunctional biomolecules, this supposition may not be valid. Also, structural rearrangements may occur during the CID process. Due to the assumptions of the kinetic method, Cooks and co-workers[45] have recently stated that "its use is justified only by the fact that it provides the desired information in cases where alternatives do not exist." In fact, this technique is the only alternative for some highly basic biomolecules, where deprotonation reactions are not feasible due to a lack of volatile reference compounds of appropriate basicity. The method has the additional advantages of requiring only a small amount of sample (which need not be pure) and being easy to implement in all tandem mass spectrometers. Therefore, a secondary goal of our present research is to assess the accuracy of the kinetic method for peptides by comparing polyglycine gas-phase basicities obtained with the kinetic method to values acquired using the established technique of deprotonation reaction bracketing.

Low-energy CID experiments were performed on all Gly_nHB^+ that were produced from the reference compounds. Table II includes gas-phase basicities obtained from the kinetic method. Our assignments assume that the dominant lower energy CID product is Gly_nH^+ when $GB(Gly_n) > GB(B)$ and that BH^+ dominates at lower CID energies when $GB(B) > GB(Gly_n)$. The gas-phase basicity of each Gly_n is considered to be bracketed between the two compounds where the predominant lower energy CID product changed from Gly_nH^+ to BH^+.

For Gly_n, $n = 2$ and 4, deprotonation reactions and the kinetic method yielded identical gas-phase basicities. For $n = 3$, 5, and 6, the values obtained from the kinetic method were higher than those obtained from deprotonation reactions by 1.3, 2.0, and 3.4 kcal/mol, respectively. This is within the experimental error limits of the techniques, thus the significance is unclear.

Ab Initio Structural Analysis. In Figures 1 and 2 the HF/6-31G* minimum-energy structures are shown for the neutral and protonated glycines (**1–3**) and diglycines (**4–8**). The relative HF/6-31G* energies in kilocalories per mole are given in the figure captions. The corresponding molecular figures with atom

(43) Meot-Ner (Mautner), M.; Smith, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 862.

(44) (a) McLuckey, S. A.; Cameron, D.; Cooks, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 1313. (b) Nourse, B. D.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Processes* **1991**, *106*, 1991. (c) Brodbelt-Lustig, J. S.; Cooks, R. G. *Talanta* **1989**, *36*, 255.

(45) Majumdar, T. K.; Clairet, F.; Tabet, J.-C.; Cooks, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2897.

(46) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithica, 1960. The van der Waals radii for the relevant atoms are 1.2 Å for H, 1.5 Å for N, and 1.4 Å for O.

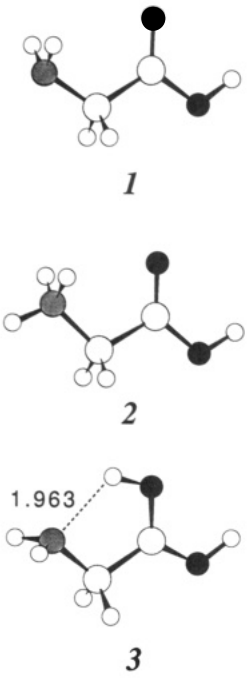

Figure 1. HF/6-31G* minimum-energy structures for glycine (**1**) and protonated glycines (**2** and **3**). The relative energies (kcal/mol) are 0.00 (**1**), –223.33 (**2**), and –209.52 (**3**). Each important hydrogen bond is shown with a dotted line; the bond distance is given in Å. The atoms are identified by shading (H, none; C, light; N, medium; O, dark) or size (H, small; C, large).

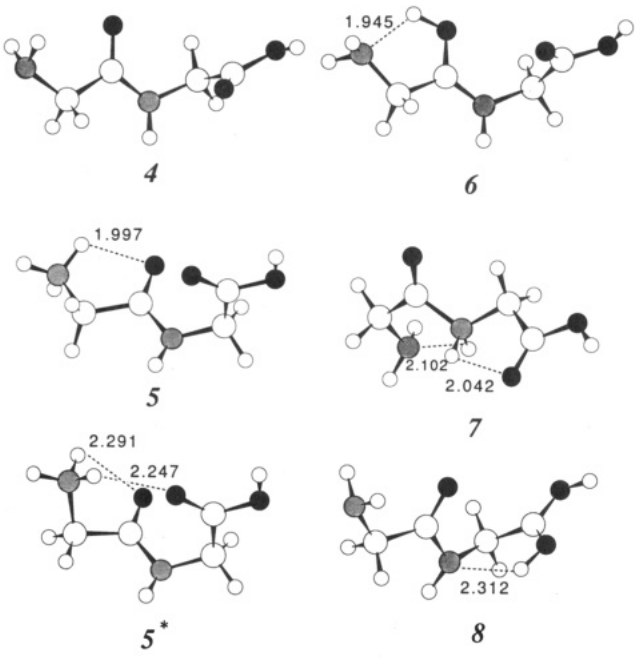

Figure 2. HF/6-31G* minimum-energy structures for diglycine (**4**) and protonated diglycines (**5**, **5***, **6**, **7**, and **8**). The relative energies (kcal/mol) are 0.00 (**4**), –230.76 (**5**), –230.29 (**5***), –229.07 (**6**), –212.66 (**7**), and –203.86 (**8**). Each important hydrogen bond is shown with a dotted line; the bond distance is given in Å. The atoms are identified by shading (H, none; C, light; N, medium; O, dark) or size (H, small; C, large).

labels and optimized geometrical parameters (bond lengths, bond angles, and dihedral angles) are provided as supplementary material (Figures S-1 and S-2 and Tables S-I and S-II).

For the glycines, the results are consistent with previous ab initio findings.[26,27] For the diglycines, however, there is both agreement and disparity between our results (**5**–**8** of Figure 2) and the recently published semiempirical AM1 results (**IIa**–**IIe**).[20] The ab initio species **5**, **6**, and **7** bear qualitative similarities to the AM1 species **IIa**, **IIb**, and **IId** in both shape and relative energies. The energy difference between **5** and **6** is comparable to that between **IIa** and **IIb** (1.69 vs 2.1 kcal/mol). Favorable comparison is also obtained between the pair **5** and **7** and the pair **IIb** and **IId** (18.10 vs 11.5 kcal/mol). Nonetheless, the ab initio **5*** and **8** are missing from the AM1 list. As for the AM1 **IIe**, no analogous stable structure was found during the HF/6-31G* geometry optimization searches with geometries starting at 180° ± 10° for the dihedral angle $H_3N—C—C=O$. Finally, a structure resembling the AM1 **IIc** was identified as a transition-state species from our preliminary HF/STO-3G calculations.

Table III. Selected Intramolecular Nonbonded Interactions (A···B)[a]

					atomic charge (e)	
type	structure	A···B	distance (Å)	population (e)	A	B
C	**3**	N···H	1.963	0.053	–0.93	0.56
D	**5**	O*···H	1.997	0.039	–0.61	0.50
E	**5***	O···H	2.247	0.022	–0.63	0.50
F	**5***	O*···H	2.291	0.018	–0.57	0.48
G	**6**	N···H	1.945	0.050	–0.92	0.55
H	**7**	O···H	2.042	0.035	–0.54	0.49
I	**7**	N*···H	2.102	0.040	–0.92	0.50
J	**8**	N*···H	2.312	0.020	–0.83	0.54

[a] See Figures 1 and 2 and Appendix.

An analysis of the structures in Figures 1 and 2 is given in the Appendix. Emphasis is placed on the structural features that discern the relative stability of the various molecular species. In particular, the more important nonbonded interactions (A–J) are described in detail because their roles are not as well-known as resonance, conjugation, and new bond formation commonly ascribed to a peptide and its protonation. Interactions of types A and B are present in most structures; those in glycine are used as examples. Types C–J (shown with dotted lines in the figures) are stronger interactions due to shorter nonbonded distances[46] and larger Mulliken overlap populations; they are summarized in Table III.

Factors Governing Protonation Sites. From the structural analysis on the species **1**–**8**, preference to a particular protonation site X appears to depend on (a) the stabilization brought to the molecular system in forming the new X–H bond, (b) the additional stability arising from conjugation with atoms bonded to X, and (c) the strength of the intramolecular H bonding with another atom Y in Y···HX.

The preferred protonation site for glycine, **1**, is found to be the amino nitrogen instead of the carboxyl carbonyl oxygen. This indicates that stabilization due to bond formation in $–NH_3^+$ in **2** far outweighs that of $–C(OH)_2^+$ in **3**, inspite of conjugation and H bonding in the latter. The H-bonding N···HO in **3**, however, could be the reason that the HF/6-31G* energy difference between **2** and **3** (*ca.* 14 kcal/mol) is smaller than the usual difference (*ca.* 25 kcal/mol) found experimentally between amines and carboxylates.[11] An explanation would be that protonated carboxylates lack the extra stability enjoyed by the protonated glycine (**3**) from H bonding.

Consistent with glycine, the preferred protonation site in diglycine, **4**, is again the amino nitrogen. Two stable conformers **5** and **5*** differing by only 0.47 kcal/mol are found; both structures possess the expected stability of an ammonium ion plus extra contribution from H bonding of the type O···HN. Protonation at the amide carbonyl oxygen in **6** has the combined advantage of conjugation in $[–C(OH)NH–]^+$ and a very strong N···HO bonding which yields an energy only 1.69 kcal/mol above that of the most stable protonated diglycine **5**. The less favored protonation sites are the amide nitrogen and the carboxyl carbonyl oxygen, resulting in **7** and **8**, at substantially higher HF/6-31G* energies (18.16 and 26.90 kcal/mol) than **5**. Again, it is interesting to note that the energy difference between the two O-protonated diglycines **6** and **8** (*ca.* 25 kcal/mol) is comparable to the difference in experimental protonation energies between formamide and

Table IV. HF/6-31G* Energies and Thermodynamic Properties[a]

		E_{SCF} (au)	E_{ZP} (kcal/mol)	$E-E_0$ (kcal/mol)	S (cal/(mol·K))
Gly	(1)	−282.831096	54.38	3.34	73.47
GlyH$^+$	(2)	−283.186989	64.21	3.44	74.89
GlyH$^+$	(3)	−283.164985	63.05	3.20	72.03
Gly_2	(4)	−489.645401	92.33	5.95	99.00
Gly_2H^+	(5)	−490.013141	102.31	5.90	97.25
Gly_2H^+	(5*)[b]	−490.012349			
Gly_2H^+	(6)	−490.010444	101.50	5.75	95.99
Gly_2H^+	(7)	−489.984294	101.59	5.85	98.01
Gly_2H^+	(8)	−489.970276	100.73	5.82	95.58

[a] See text and Figures 1 and 2. E and S are at 298.15 K and 1 atm. [b] Thermodynamic properties were not calculated because this species was not used in the basicity determination.

Table V. Electronic Protonation Energies ($-\Delta E_{elec}$) for Glycine[a]

			$-\Delta E_{elec}$ (kcal/mol)	
basis	method[c]	$E_{elec}(1)$ (au)[b]	1 → 2	1 → 3
6-31G*	HF	−282.831 096	223.33	209.52
	MP2	−283.596 549	222.69	205.11
	MP3	−283.613 672	223.58	207.99
	MP4	−283.651 270	223.64	206.40
6-31+G(d,p)	HF	−282.858 138	222.16	210.67
	MP2	−283.664 908	220.08	204.48
	MP3	−283.681 442	221.83	208.45
	MP4	−283.723 172	221.16	205.95

[a] See text and Figure 1. [b] From the listed $-\Delta E_{elec}$ and $E_{elec}(1)$, $E_{elec}(2)$ and $E_{elec}(3)$ may be deduced. [c] Calculation at the HF optimized geometry in the basis indicated.

formic acid (*ca.* 20 kcal/mol).[11] The extra stability (*ca.* 5 kcal/mol) in the case of diglycine could be due in part to a stronger H-bonding G in **6** relative to J in **8**.

Gas-Phase Basicities from ab Initio Calculations. In this study a systematic procedure is employed to calculate basicity from ab initio data. The necessary data and results are presented in Tables IV–VI for the respective protonation reactions involving glycine and diglycine,

$$\mathbf{1} \rightarrow \mathrm{I}: \quad \mathrm{Gly}(\mathbf{1}) + \mathrm{H}^+ \rightarrow \mathrm{GlyH}^+(\mathrm{I}) \tag{7}$$

$$\mathbf{4} \rightarrow \mathrm{J}: \quad \mathrm{Gly_2}(\mathbf{4}) + \mathrm{H}^+ \rightarrow \mathrm{Gly_2H}^+(\mathrm{J}) \tag{8}$$

where I = **2**, **3**, and J = **5**, **6**, **7**, **8**.

Table IV lists the HF/6-31G* energies and relevant thermodynamic properties[24] at 298.15 K and 1 atm. Terms include SCF energies (E_{SCF}), which represent electronic energy (E_{elec}) in a Hartree–Fock model, zero-point energies (E_{ZP}), standard energy change from 0 to 298.15 K ($E-E_0$), and standard entropy at 298.15 K (S). Note that at 0 K:

$$E_0 = E_{elec} + E_{ZP} \tag{9}$$

At 298.15 K, there are contributions from translational, rotational, and vibrational motions to E and S:

$$E = E_0 + E_{trans} + E_{rot} + E_{vib} \tag{10}$$

$$S = S_{trans} + S_{rot} + S_{vib} \tag{11}$$

where $E_{trans} = E_{rot} = 1.5RT = 0.89$ kcal/mol. Statistical thermodynamic expressions for the other components in eqs 9–11 can be found in standard references.[24,47] Harmonic vibrational frequencies (ν_i) are used in computing E_{ZP}, E_{vib}, and S_{vib}.

Table V presents electronic protonation energies for the glycines using the Møller–Plesset perturbation approach from first to fourth orders (HF, MP2, MP3, and MP4).[24] The main purpose is to obtain better electronic energy by including correlation energy, E_{corr}:

$$E_{elec} = E_{SCF} + E_{corr} \tag{12}$$

Here $E_{corr} = 0$ for the HF calculation. The protonation energy is defined as the negative of change in electronic energy, $-\Delta E_{elec}$. Results are shown using the 6-31G* basis at the HF/6-31G* geometries and the 6-31+G(d,p) basis at the HF/6-31+G(d,p) geometries.

The protonation energies in Table V generally exhibit an alternating pattern in the convergence of the following Møller–Plesset series: HF > MP3 > MP4 > MP2. This is consistent with the previous findings on the protonation energies of NH_3 and H_2O computed with the 6-31+G(2d,2p) basis at the MP2/6-31+G(d,p) geometries.[48] For the glycine systems of interest here, a MP2 single-point calculation at the HF optimized geometry can seriously overestimate correlation. At the MP4 level, fluctuations in the series appear to stabilize. Thus the MP4 results may be considered most reliable. Based on the two basis sets used, changes in electronic energy from HF to MP4 appear to be much more pronounced for the O-based protonation (**1** → **3**) as compared with the N-based protonation (**1** → **2**). The larger basis set, 6-31+G(d,p), seems to accommodate a larger correlation effect.

The substantially different corrections for electron correlation in glycines due to different protonation sites may be rationalized on the basis of electronic structure. Correlation for the N-based protonation is expected to be minor because the change is simply from a nonbonding electron pair on N to a more localized bonding pair in the N–H bond. The effect on the O-based protonation should be greater as there are two changes: one from a π electron pair in C=O to a more localized σ bonding pair in O—H and the other from a nonbonding electron pair on the adjacent O to a partially localized π electron pair in the conjugated C—O bond. Changes in correlation energy (ΔE_{corr}) for the N- and O-based protonations, 1.00 and 4.72 kcal/mol calculated in the 6-31+G(d,p) basis, support this explanation.

Separate correlation calculations for the O-based protonations of diglycine (**4** → **6** and **4** → **8**) were performed to a partial fourth order (MP4SDQ) by including single, double, and quadruple virtual-orbital substitutions. At present, a complete MP4 treatment for diglycines, by adding triple substitutions to those in MP4SDQ, would require an unreasonably large amount of computer time. A comparison between patterns of convergence in the MP series (from HF to MP2, MP3, and MP4SDQ) among the three O-based protonations (including **1** → **3** of glycine) shows close similarity both in trend and magnitude. In view of this favorable comparison, ΔE_{corr} for the N- and O-based protonations in glycine, −0.31 and +3.12 kcal/mol calculated in the 6-31G* basis, may be used later as "constants" for improving the ΔE_{elec} values of polyglycines calculated in the same 6-31G* basis. (Obviously, the ideal approach would be, for example, to calculate E_{elec} using a basis larger than 6-31G* at a geometry optimized at the MP2 level given the right computing environment).

The thermodynamic equations and steps required to calculate ideal-bas basicities from ab initio data are summarized below.[24,47]

$$\Delta G = \Delta H - T\Delta S \tag{13}$$

$$\Delta H = E(\mathrm{MH}^+) - E(\mathrm{M}) - E(\mathrm{H}^+) + \Delta(\mathrm{PV})$$

$$= \Delta E_{SCF} + \Delta E_{corr} + \Delta E_{ZP} + \Delta(E - E_0) - 1.48 \text{ kcal/mol} \tag{14}$$

$$-T\Delta S = -T[S(\mathrm{MH}^+) - S(\mathrm{M}) - S(\mathrm{H}^+)]$$

$$= -T[S(\mathrm{MH}^+) - S(\mathrm{M})] + 7.76 \text{ kcal/mol} \tag{15}$$

where

$$\Delta E_{SCF} = E_{SCF}(\mathrm{MH}^+) - E_{SCF}(\mathrm{M}), \text{ etc.} \tag{16}$$

(47) Levin, I. N. *Physical Chemistry*, 3rd ed.; McGraw Hill: New York, 1988; Chapter 22.

(48) Del Bene, J. E.; Shavitt, I. *J. Phys. Chem.* **1990**, *94*, 5514.

Table VI. Ab Initio Calculations of Ideal-Gas Basicity of Glycine and Diglycine at 298.15 K and 1 atm[a]

A. Ab Initio Energies (kcal/mol)

		glycine		diglycine			
level	data	1→2	1→3	4→5	4→6	4→7	4→8
6-31G*//6-31G*	ΔE_{SCF}	−223.33	−209.52	−230.76	−229.07	−212.66	−203.86
	ΔE_{ZP}	9.83	8.67	9.98	9.17	9.26	8.40
	$\Delta(E-E_0)$	0.10	−0.14	−0.04	−0.20	−0.10	−0.13
	ΔH	−214.88	−202.47	−222.30	−221.58	−204.98	−197.07
	$-T\Delta S$	7.34	8.19	8.28	8.66	8.06	8.78
	ΔG	−207.54	−194.28	−214.02	−212.92	196.92	188.29
	ΔE_{ZP}(scaled)	8.95	7.89	9.08	8.34	8.43	7.64
MP4/6-31G*//6-31G*[b]	$\Delta E_{SCF}+\Delta E_{corr}$	−223.64	−206.40	(−231.07)	(−225.95)	(−212.97)	(−200.74)
MP4/6-31+G(d,p)//6-31+G(d,p)	$\Delta E_{SCF}+\Delta E_{corr}$	−221.16	−205.95				

B. Basicity (kcal/mol)

	glycine		diglycine			
protonation site	N	O	N	O*	N*	O
calculated						
6-31G*//6-31G*	207.5	194.3	214.0	212.9	196.9	188.3
6-31G*//6-31G* (scaled)	208.7	191.9	214.9	213.8	197.8	189.1
MP4/6-31G*//6-31G* (scaled)[b]	207.9	191.2	(215.2)	(210.6)	(198.1)	(185.9)
MP4/6-31+G(d,p)//6-31+G(d,p) (scaled)	206.3	191.5				
experimental	206.2		215.3			

[a] See text for details. [b] From Table V, the ΔE_{corr} for glycines are −0.31 and +3.12 kcal/mol for the N- and O-protonations. The same ΔE_{corr} values are used for diglycines. Because of this approximation, the diglycine values are enclosed in parentheses.

$$-E(H^+) + \Delta(PV) = -2.5RT \quad (17)$$

$$TS(H^+) = (1.5\ ln\ M_H - 2.5\ ln\ T - 1.1649)RT \quad (18)$$

In Table VI, the HF/6-31G* values for all the relevant terms are presented. Three additional entries for quantities that would improve the calculated basicities are shown. First, ΔE_{ZP} based on harmonic frequencies (ν_i) alone is generally known to be too large and a scale factor of 0.91 was applied.[24,49] The ν_i in the $\Delta(E-E_0)$ and $-T\Delta S$ terms are not similarly scaled, however, because vibrational contributions to these terms are negligible. [Note that $\Delta(E-E_0) = \Delta E_{vib}$ due to $\Delta E_{trans} = \Delta E_{rot} = 0$.] Furthermore, the ν_i are nonlinear parameters in ΔE_{vib} and ΔS_{vib} and scaling would not be as simple as the linear scaling applied to ΔE_{ZP}. Second, to incorporate electron correlation at the MP4/6-31G* level, ΔE_{corr} equaling −0.31 and 3.12 kcal/mol for the respective N- and O-protonations of glycines were applied to the ΔE_{SCF} of all species to obtain better estimates of ΔE_{elec}. Those for diglycines are enclosed in parentheses because the ΔE_{corr} values were calculated for glycines and not for diglycines. Finally, the most accurate ab initio values obtained for the glycines to date, the MP4/6-31+G(d,p) values for ΔE_{elec}, are presented. These ΔE_{elec} values would be combined with the 6-31G* values for the other terms in calculating the basicity of glycine. [Separate calculations on the molecular species involved in this study indicate that values for the ΔE_{ZP}, $\Delta(E-E_0)$, and $-T\Delta S$ terms resulting from the 3-21G and 6-31G* bases for a given species are quite similar. Thus, the thermal quantities for a given species may be considered transferrable among different bases to save the large computing cost over frequency calculations using a larger basis such as 6-31+G(d,p).]

Basicities for the six protonation reactions are presented for the 6-31G* results (with and without scaling) and the MP4/6-31G* results (with scaling). Finally, the MP4/6-31+G(d,p) results (with scaling) are given for glycines only. The best theoretical values, under the conditions described in this work, are 206.3, 191.5, 215.2, 210.6, 198.1, and 185.9 kcal/mol for the respective protonations. Note in particular that the ab initio basicities for glycine and diglycine (206.3 and 215.2 kcal/mol) agree with the measured values (206.2 and 215.3 kcal/mol) to within experimental errors.

The present procedure of calculating basicity is the most complete to date. Previous ab initio basicity calculations omitted different aspects of this process,[18,25-27] primarily in steps associated with electron correlation and with translational, rotational, and vibrational motions. One reason could be the lack of adequate computing resources demanded by the MP and frequency calculations for large molecules when extended basis sets such as 6-31G* and 6-31+G(d,p) are used. However, comparisons with experimental gas-phase basicities clearly indicate that an accurate ab initio calculation of this quantity must include steps beyond Hartree–Fock and geometry optimization to obtain contributions from electron correlation, molecular vibrations, and thermal motions. Detailed analysis of the various aspects of the calculations further confirms the advantages of using a larger basis set for better electronic energies and applying a scaling factor for better estimates of zero-point energy.

Correlation of Basicity and Structure. The ab initio results indicate that the most stable structure for $GlyH^+$ involves protonation of the amino nitrogen (**2**). This is consistent with our experimental and theoretical gas-phase basicities that are in the range found for amines as opposed to carboxylic acids. [For example, GB(methylamine) = 205.7 kcal/mol,[12] while GB(acetic acid) = 181.7 kcal/mol.[11]] In addition, CID experiments performed in our laboratory on $GlyH^+$ indicate that the dominant low-energy product is $NH_2{=}CH_2^+$. This is analogous to the immonium ions, $NR_2{=}CH_2^+$, that are found in alkylamine CID spectra[50] and provides additional evidence for N-protonation. Water loss, which would be expected if the carboxylate group were protonated, was not observed in the CID spectra.

Diglycine is considerably more basic than glycine. The experimental results place the gas-phase basicity of diglycine at 9.1 kcal/mol more than that of glycine, while the theoretical results suggest that it is 8.9 kcal/mol higher (calculated at the theoretical levels shown in Table VI). This is a significant increase that cannot be explained simply by the additional charge-induced dipole interactions of a larger chain. (For example, the gas-phase basicity of *n*-hexylamine is only *ca.* 1.0 kcal/mol higher than that of *n*-propylamine.[11]) Instead, this large basicity increase suggests structural differences between the protonated forms of glycine and diglycine. The ab initio results are in agreement, showing that while the preferred protonation site of both species

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is the terminal amino nitrogen, intramolecular H bonding to the nearby carbonyl oxygen is much stronger for diglycine. Low-energy CID experiments are also consistent with internal interaction of the proton for diglycine: Gly_2H^+ dissociates to yield $GlyH^+$ (loss of the N-terminal $NH_2CH{=}C{=}O$) as the dominant lowest energy product, with $NH_2{=}CH_2^+$ only becoming prominent at higher energies. However, the CID results should not be viewed as definitive because proton migration may occur under CID conditions.

There is considerable evidence in the literature to support our conclusions about the importance of amide carbonyl in the structure of Gly_2H^+. A recent experimental and theoretical study of the gas-phase basicities of several β-lactams and acyclic amides found that the amide oxygen is the most basic site, with internal bonding (cyclization) affecting the basicity.[18] In addition, intramolecular H bonding involving amide groups is known to occur in solution where it is involved in establishing folding patterns for proteins[51] and other long-chain amides.[52] This type of interaction has been proposed for gas-phase peptide ions.[7,53,54] Intramolecular H bonding also increases the gas-phase basicities of a variety of multifunctional molecules,[55] including polyethers.[56]

The gas-phase basicities of polyglycines continue to increase as additional residues are added to the peptide. A 3.6-kcal/mol increase is seen for n between 2 and 3, while an increase of 6.1 kcal/mol is found in going from $n = 3$ to $n = 4$. There is a marked leveling in basicity between $n = 4$, 5, and 6, with hexaglycine being only 2.4 kcal/mol more basic than tetraglycine. Although ab initio calculations were not performed on $n = 3$–6, it is most probable that the protonation of these peptides also involves terminal nitrogen protonation with intramolecular H bonding to an amide oxygen (but not necessarily at the N-terminal carbonyl). The CID spectra of the larger polyglycines are complex, but the lowest energy processes involve primarily cleavages related to the amide groups, suggesting (but not proving) that the proton may be coordinated at these sites. In addition, semiempirical calculations and metastable ion decomposition studies[54] of lithiated Gly_3 are consistent with Li^+ coordination to the three carbonyl oxygens. Therefore, it is possible that larger polyglycines involve proton coordination at multiple amide groups. Such an effect would increase the basicities as additional intramolecular H bonding occurs, but the basicity should level off when steric factors hinder further coordination. This is consistent with our experimental gas-phase basicities.

Other factors may also play a role in increasing the gas-phase basicities as the polyglycine chain length increases. As noted by Bursey and co-workers,[7] ion–dipole solvation of interior protons should enhance the basicities of larger peptides. In addition, charge-induced dipole interactions between a charged site and a polarizable alkyl group are known to increase the basicities of various organic compounds.[57] In particular, long-chain amides show a small continuous increase in gas-phase basicity from n-propyl- to n-decylamine that is believed to result from the coiling of the polarizable alkyl chain to bring it into proximity with the charged amino group.[9] Analogous charge-induced dipole interactions may occur for polyglycines.

Experimental and Theoretical Proton Affinities. Gas-phase proton transfer properties of molecules are often expressed in terms of proton affinity ($-\Delta H$ of reaction 1). The ab initio data presented in Tables IV–VI are equally applicable to the calculations of proton affinity a 298.15 K and 1 atm. Corresponding to the theoretical best estimates of the gas-phase basicities cited earlier, the proton affinities are 213.6 (**1** → **2**), 199.7 (**1** → **3**), 223.5 (**4** → **5**), 219.3 (**4** → **6**), 206.2 (**4** → **7**), and 194.7 (**4** → **8**) kcal/mol.

An advantage of doing theoretical calculations on proton affinity and gas-phase basicity is the ease with which statistical thermodynamic equations can be applied to the calculated structures of neutral and protonated species. Unfortunately, this is not the case when an experimental proton affinity must be derived from a measured gas-phase basicity. The thermodynamic relation between the two quantities is given by eq 19,

$$PA = GB - T\Delta S \qquad (19)$$

for which the experimental value of ΔS is often unavailable. When protonation does not induce substantial structural changes, ΔS is frequently approximated using the rotational symmetry changes pertaining to the site of protonation.[9-12] (This approach assumes that the protonation site is known.) In the case of the N-based protonation in glycine (**1** → **2**) and diglycine (**4** → **5**), for example, $-T\Delta S(Gly_n \rightarrow Gly_nH^+) \approx 0.65$ kcal/mol using eq 5. Yet, the ab initio value (Table IV) for this term is –0.42 kcal/mol for glycine, differing both in sign and magnitude, and 0.52 kcal/mol for diglycine. A further breakdown of ΔS into different components shows contributions of 3 and –1% translation, 1 and 4% rotation, and 96 and 98% vibration for the respective glycine and diglycine. Analysis on the other protonations reveals a similar pattern in contributions, which implies that the vibrational contribution determines the magnitude of this term. This finding is in contradiction to the frequent assumption that the rotational contribution is most important.[9-12] Fortunately, the term in question, $-T\Delta S(Gly_n \rightarrow Gly_nH^+)$, is small in comparison with the $TS(H^+)$ term attributed to the free H^+ (7.76 kcal/mol in eq 15).

In light of the ab initio data for ΔS, the practice of using rotational symmetry calculations to convert gas-phase basicity to proton affinity may be inaccurate for the species considered here. A better approach is to use the ab initio $-T\Delta S$ (e.g., 7.34 and 8.28 kcal/mol for protonations of glycine and diglycine in Table VI) to convert experimental gas-phase basicity to "experimental" proton affinity. The data in Table II include "experimental" proton affinities calculated from eq 19 using our experimental gas-phase basicities and the ab initio $-T\Delta S$ values. (These values are referenced to the Meot-Ner and Sieck[12] scale in which the proton affinity of ammonia is 208.3 kcal/mol at 298 K.) Since no ab initio values were available for Gly_n and Gly_nH^+, $n = 3$–6, the ab initio $-T\Delta S$ obtained for diglycine was employed for $n = 2$–6. This assumes that the most stable protonated structures of $n = 3$–6 resemble the structure of Gly_2H^+. It is also noteworthy that the $-T\Delta S$ term for diglycine (8.28 kcal/mol) is larger than that of glycine (7.34 kcal/mol); it has been shown experimentally that $-T\Delta S$ increases when protonation results in a significant structural change due to factors such as intramolecular H bonding.[55,56]

Comparisons to Literature Proton Affinities. Table II includes a summary of proton affinity values found in the literature for polyglycines. To facilitate comparison, all literature values were adjusted to the proton affinity ladder of Moet-Ner and Sieck.[12] For glycine, our experimental proton affinity of 213.5 is in excellent agreement with two equilibrium constant measurement studies that yielded values of 213.9 kcal/mol[15] and 216.3 kcal/mol.[14] This is important because equilibrium constant measurements are regarded as the most accurate method for determining basicities.[11] In addition, our result agrees, within experimental error, with values of 211.7 kcal/mol obtained by Amster and co-workers[16] from reactions of laser-desorbed glycine species and

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215.4 kcal/mol obtained by Wu and Lebrilla[20] from reactions of FAB-generated $GlyH^+$. As discussed above, our experimental results also agree with values obtained by ab initio calculations. These comparisons suggest that our deprotonation reaction studies of FAB-generated ions yield accurate gas-phase basicity and proton affinity values.

In addition to the results presented here, the basicities of polyglycines have been the subject of two recent investigations.[58] Wu and Fenselau[19] have reported on the proton affinities of Gly_n, $n = 2$–8 and 10. These values were obtained by the kinetic method employing high-energy CID in a sector mass spectrometer; the reference bases were alkyl and aromatic amines similar to those used in our study. Wu and Lebrilla[20] have probed the gas-phase basicities and proton affinities of Gly_n, $n = 1$–5, using a procedure similar to our own involving ion–molecule reactions of Gly_nH^+ generated by FAB. All three studies agree that the basicities rise steadily as the length of the peptide chain increases. Our values are generally intermediate to the values obtained from the other two studies. The kinetic method results of Wu and Fenselau[19] and of our own study are somewhat higher than the two sets of deprotonation reaction results, with the deviation between the kinetic method and the ion–molecule reaction values increasing as n becomes larger. Further comparisons of these two approaches for obtaining gas-phase basicity information on biomolecules are warranted.

Concluding Remarks

In addition to providing specific conclusions about polyglycine basicity, several general aspects of this work are important. Deprotonation reactions involving FAB-generated ions were found to yield gas-phase basicity values that agree with other experimental methods and with ab initio calculations. This versatile technique should be applicable to many peptides and low-volatility biomolecules. Also, due to the complex nature of peptide protonation, the combination of experimental and ab initio research was shown to be especially meaningful. In addition, the ab initio data obtained on geometry, charge distribution, and energy provides important information on the selectivity of protonation sites for glycine and the simplest peptide, diglycine. The knowledge and experience gained from this in-depth theoretical study should help in the formulation of computational strategies for future studies of larger peptides.

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Appendix: Detailed Ab Initio Structural Analysis

Geometries of the glycines (**1**–**3**) all have a plane of symmetry (the C_s point group). The symmetry plane builds upon the main chain (N—C—C—O), bisects the amino and the α-methylene groups, and includes the carboxylic group (O=C—O—H) in an eclipsed conformation between the C=O and O—H bonds. The various planar segments have facilitated nonbonded interactions between oxygen and hydrogen:

A: O···H, 1,4 interaction in O=C—OH

B: O···(H,H), bifurcated 1,5 interactions in H_2N—C—C=O

Typical values are those for the neutral glycine, Gly (**1**); the O···H distance and overlap populations are 2.277 and 0.009 e for A and 2.777 Å and 0.003 e for B. The respective total atomic charges on A and B are –0.56 and –0.56 e on O and +0.47 and +0.35 e on H. In view of the negligible overlap populations, A and B may be considered simply as attractive electrostatic interactions.

Upon protonation at the amino nitrogen of glycine, $GlyH^+$ (**2**), there are conspicuous changes in atomic charges and lengthening of the C—N and N—H bonds. The driving force for this protonation is the N—H bond formation in producing the exceptionally stable ammonium ion group, $-NH_3^+$, as nonbonded interactions in **2** are essentially unchanged from those in **1**.

The protonated glycine at the carboxyl carbonyl oxygen, $GlyH^+$ (**3**), however, introduces dramatic changes in the CO bond lengths from 1.188 and 1.330 Å in the carboxylic O=C—OH of **1** to two nearly equal lengths of 1.252 and 1.261 Å in the conjugated HO—C—OH of **3**. The protonation also has introduced a new nonbonded interaction between nitrogen and hydrogen in a nearly planar, ring-like structure:

C: N···H, 1,5 interaction in N—C—C—OH

The nonbonded distance of 1.963 Å and population of 0.053 e in C are significantly shorter and larger, respectively, than those in A and B above. The respective total charges on N and H, –0.93 and +0.56 e, are also larger. These are characteristic features of intramolecular H bonding, a stronger attraction than the electrostatic interactions discussed earlier.

On going from the neutral glycine to diglycine, Gly_2 (**4**), most bond lengths, bond angles, and nonbonded interactions present in the monomer stay about the same. The important new feature is the appearance of the amide group which, in this case, is the nearly planar peptide bond O=C—NH that binds the two glycine fragments. As a consequence of the resonance O=C—NH ↔ O—C=NH, the bond lengths C=O and C—N become respectively longer and shorter and the negative charges on O and N become respectively greater and smaller than those in the monomer. The corresponding values are 1.198 Å, 1.365 Å, –0.60 e, and –0.77 e in **4** versus 1.188 Å, 1.439 Å, –0.56 e, and –0.82 e in **1**. As for the nonbonded interactions, **A** and **B** in glycine carry over to diglycine. **B** is now modified to HN*—C—C=O and H_2N—C—C=O*. The asterisks are used to distinguish the atoms in the peptide linkage (O*=C*—N*) from those in the terminal amine and carboxylic groups.

The preferred protonation site for diglycine is again the amino nitrogen. The two stable structures, Gly_2H^+ (**5**) and Gly_2H^+ (**5***), differ mainly in the dihedral angle N—C—C*—N* (–168.9 and 123.1°). In addition to stabilization from forming $-NH_3^+$ as in **1** → **2**, several new nonbonded attractions emerge:

D: O···H, 1,5 interaction in HN—C—C*=O*

E: O···H, 1,8 interaction in HN—C—C*—N*—C—C=O

F: O···H, 1,5 interaction in HN—C—C*=O*

D in **5** is much stronger than B in **2** as evidenced by an increase of 7.43 kcal/mol in the HF/6-31G* protonation energy of diglycine from that of glycine. E in **5*** is extraordinary in its function—it brings the two termini of the dipeptide to proximity. The F in **5*** is very similar to D but weaker due to a longer nonbonded distance (2.291 Å vs 1.997 Å); this may well be the

(58) Three groups working independently in this area first presented their results at the 40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, May 31–June 5, 1992. Authors of these presentations are: (a) Sperling, B. D.; Cassady, C. J.; (b) Wu, J.; Lebrilla, C. B.; (c) Wu, Z.; Fenselau, C.

reason for **5*** being higher in energy than **5**. E and F together appear to drive the rotation about the N*—C bond in **4** to a more folded skeleton in **5***.

Protonation at the amide carbonyl oxygen results in Gly_2H^+ (**6**). The most significant change is the transformation of the peptide bond O=C—NH in **4** to the conjugated linkage HO—C—NH in **6** with nearly equal CO and CN bond lengths and a small increase and decrease in the negative charges on O and N. The respective bond distances and charges in **6** are 1.270 Å, 1.291 Å, –0.64 e, and –0.73 e. An additional source of stabilization comes from H bonding,

G: N···H, 1,5 interaction in N—C—C*—O*H

Protonation at the amide nitrogen would destroy the stable, planar peptide bond in diglycine, which would be energetically unfavorable. Interestingly, a stable species Gly_2H^+ (**7**) has been identified—this involves a nearly trans orientation between the amine and the amide carbonyl groups. (The dihedral angle N—C—C*—N* is –32.3°.) The structure is stabilized by two adjacent H-bonding ring-like structures:

H: O···H, 1,5 interaction in HN*—C—C=O

I: N···H, 1,5 interactions in N—C—C*—N*H

Like **5***, H and I together bring about a more folded structure for **7**.

The last protonated site is the carboxyl carbonyl oxygen, Gly_2H^+ (**8**). The change in structure for the protonation **4** → **8** in diglycine closely resembles that for **1** → **3** in glycine. The C in **3** is now modified to one involving an amide nitrogen

J: N···H, 1,5 interaction in N*—C—C—OH

J is substantially weaker than C because of a larger separation (2.312 Å) and smaller population (0.020 e).

In summary, the strength of nonbonded attractions A···B may be ordered roughly as follows:

$$B, A < J, F, E < H, D < I, G, C$$

The ranking is essentially based on the interatomic distance and Mulliken population in the atom pair A···B. The total charges on A and B and the relative stability of the protonated species in which they appear are also taken into consideration. Note especially that in a protonation reaction, the transferred proton is invariably involved in the formation of a stronger H bond (e.g. types C–J). The exception is the N-protonated glycine, **2**, where molecular symmetry (C_s) results in two symmetry equivalent H atoms in $-NH_3^+$, leading to larger O···H distances and weaker H-bonding interactions (e.g., type B).

Supplementary Material Available: Figures S-1 and S-2 (HF/6-31G* minimum-energy structures and relative energies) and Tables S-I and S-II (HF/6-31G* optimized geometrical parameters) for glycines and diglycines (6 pages). Ordering information is given on any current masthead page.