Gas-Phase Basicities and Sites of Protonation of Glycine Oligomers (GLY_n; n = 1–5)

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Abstract: The gas-phase basicities (GB) of glycine and its oligomers (GLY-GLY, GLY-GLY-GLY, GLY-GLY-GLY-GLY, GLY-GLY-GLY-GLY-GLY) are determined by bracketing measurements using an external source quadrupole Fourier transform mass spectrometry instrument. The GB of glycine is found to be 206.4 ± 2 kcal/mol. The larger oligomers have similar basicities corresponding to 213.5 ± 2 kcal/mol for diglycine, 215.0 for tri- and tetraglycine, and 217.1 for pentaglycine. Various base sites are examined on diglycine using the semiempirical AM1 molecular orbital calculation method. It is proposed that the most basic sites for glycine and diglycine are similar and correspond to the protonation of the terminal amine with hydrogen bonding to the adjacent carbonyl oxygen. The increase in GB between the two oligomers is due to the increased hydrogen-bonding interaction when the carboxylic acid is converted to an amide.

The hydrogen-bonding interactions of specific base sites in large biological macromolecules are fundamentally important. In peptides and proteins, these interactions play an active role in the secondary (and tertiary) structures, as well as in the function of these compounds. In mass spectrometry, the ionization methods used for the analysis of peptides nearly all rely on some form of cation (usually proton) coordination. Thus the determination of intrinsic gas-phase basicities is important in both biological chemistry and analysis.

Earlier methods for the determination of gas-phase basicities have relied exclusively on the volatility of some of the amino acids.[1-5] However, the relative involatility of peptides and many amino acids makes it difficult to obtain gas-phase thermochemical information for these compounds. Recent techniques such as laser desorption[6-13] and fast atom bombardment[14,15] (FAB) have made it possible to routinely produce and trap ions of bioorganic molecules from nonvolatile precursors in an ion cyclotron resonance (ICR) cell.[16,17] In this report, we present gas-phase basicity (GB) values for glycine and some of its oligomers (GLY-GLY, GLY-GLY-GLY, GLY-GLY-GLY-GLY, GLY-GLY-GLY-GLY-GLY). We also show the utility of the external source quadrupole Fourier transform mass spectrometry instrument (QFTMS) for determining the GB values of peptides. Ions are produced in a secondary ion mass spectrometry (SIMS, Cs^+) source and injected into the analyzer cell where they are allowed to react with a neutral base of known GB. Experimental results are compared with theoretical results calculated by the semiempirical method AM1[18,19] to determine the sites of protonation and intramolecular hydrogen bonding interactions in protonated peptide molecules.

Experimental and Theoretical Procedures

Experimental Procedure. Experiments are performed in an external source quadrupole Fourier transform mass spectrometry[20,21] instrument constructed in our laboratory. Detailed descriptions of the instrument are provided in earlier publications.[22,23] Proton-transfer reactions have not been performed with this particular instrumentation. Most of the proton exchange reactions performed in ICR involve ions which are produced within the cell.[24-26] In the experiments described here, ions are produced in the external source and injected into the analyzer cell. A secondary ion mass spectrometry (SIMS, Cs^+) source is used to produce ions from a glycerol/thioglycerol matrix. An rf-only quadrupole guides ions from the source into the analyzer cell. Because of the long time scale of the technique, matrix interference (from matrix derived ions) is minimal, and the signal due to the amino acid/petides is the most abundant.[23] We attribute the lack of matrix ions to the relatively fast decomposition rates of matrix cluster ions (between 10^{-6} and 10^{-3} s). These decomposition rates are slow in the time scale of sector instruments (i.e., 10^{-6} s) but fast in the time scale of the FTMS instruments (i.e., $>10^{-3}$ s).

Reactions are allowed to proceed between the protonated amino acid and the neutral reference base. The instrument contains a split manifold system which allows us to introduce reactant gases into both the source and the analyzer chamber. This can be performed by either pulsing the reference base (via a General Valve Corp. pulsed valve) or keeping it at a steady-state background pressure (via a Varian leak valve). For all the

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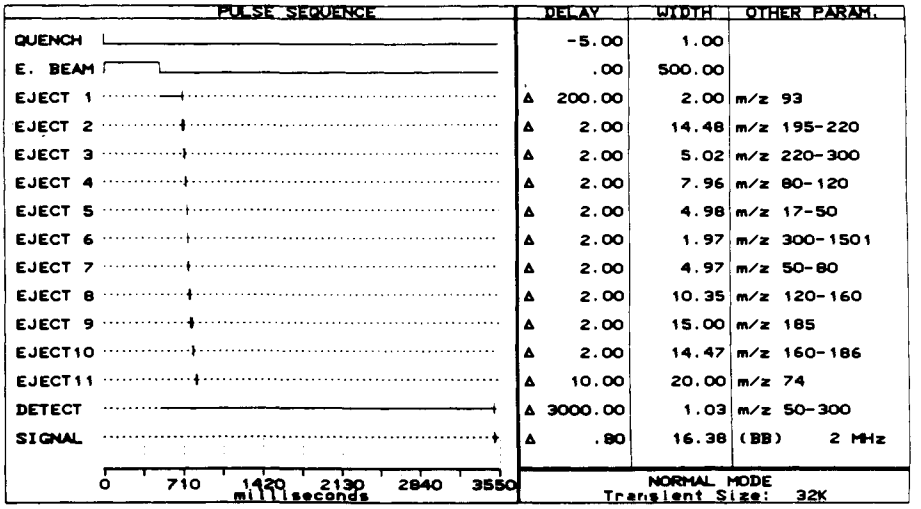

Figure 1. A typical pulse sequence of a proton-transfer reaction experiment in the external source FTMS instrument. The quench pulse clears the analyzer cell of ions from the previous experiment. The electron beam pulse turns on the acceleration voltage of the Cs^+ gun as well as the radiofrequency on the quadrupole rods to transmit the ions. The ejection pulses clear the analyzer cell of all ions except the protonated peptide. The detection pulse excites the ions for the detection.

experiments in this report, however, the reference base is introduced as a steady-state background gas with pressures between 1×10^{-9} and 3×10^{-8} Torr, as measured by a calibrated ion gauge. The ion gauge was calibrated using published proton-transfer rate constants for several reactions including tripropylamine with tributylamine and triethylamine with tripropylamine.[27] An empirical method was then used to determine the relative ion gauge sensitivities of all reference bases.[28,29]

To determine the reactivity of the protonated parent, the species is isolated after the injection period by a series of ejection pulses. Figure 1 illustrates a typical pulse sequence used during the experiments. The last ejection pulse eliminates the protonated reference base which is formed from the reaction of the neutral base with other protonated species (e.g., matrix ions). The reaction times used to calculate the rates begin from the end of the last ejection pulse. The protonated ions are then allowed to undergo proton-transfer reactions with the background pressure of base.

The trapped ions often contain some translational energy both from the ion transport (between the source and the analyzer cell) and from the ejection pulses. Experiments were performed to determine the effects of translational cooling by pulsing in argon gas before the proton exchange reaction. This caused the rate to increase by 30–40% but never more than 50%. Since the uncertainty in the calculated rates is already 30–40%, the translational energy obtained by the ion does not alter the overall results and the assignment of GB values. For this reason, all rate constants reported were obtained without collisional cooling.

Theoretical Calculations. Calculations are performed on a Vax 750 using the molecular orbital program package AMPAC.[18,19] For all calculations, the Hamiltonian AM1 is used. Full geometry optimization is performed, and the values reported, unless otherwise indicated, correspond to the lowest energy structures found.

Results

Experimental Results. Gas-phase basicity (GB) is defined as the negative of the free-energy change ($GB = \Delta G°$) associated with the coordination of a proton (reaction 1, where A = amino acid/peptide, B = reference base). In the present study, the

$$A + H^+ = AH^+ \quad (1)$$

protonated peptides are reacted with a series of gaseous neutral bases (reference bases) whose basicities are linked to a known basicity scale (reaction 2).

$$AH^+ + B = A + BH^+ \quad (2)$$

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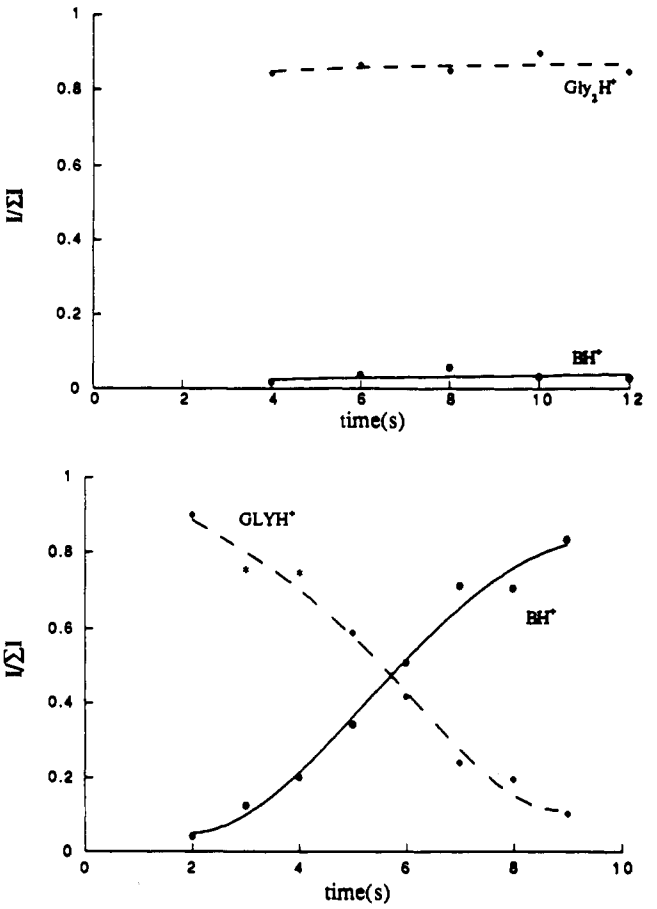

Figure 2. Representative time plots of proton-transfer reactions. The reaction of GLY_2H^+ with 2,4-dimethyl-1,3-pentadiene is a slow (3.0×10^{-11} cm^3 molecule^{-1} s^{-1}) reaction (2a) indicating an endothermic reaction. The reaction of glycine with 2,4-dimethyl-1,3-pentadiene is a fast reaction (1.6×10^{-9} cm^3 molecule^{-1} s^{-1}) indicating an exothermic reaction (2b).

Representative time plots are shown in Figure 2, corresponding to a slow (2a) and a very rapid (2b) reaction. Rapid reactions occur near the collision rates (10^{-9} cm^3 molecule^{-1} s^{-1}) and are indicative of exothermic reactions. From the decay curves, we calculate directly the rate constants for the respective proton-transfer reactions.

Results for the reaction of the protonated peptides with several reference bases are tabulated in Table I. The absolute rate

Table I. Selected Rate Constants[a] for Proton-Transfer Reactions of Protonated Glycine Oligomers with Standard Referene Bases

reference base and gas-phase basicities (300 K)	GLY	$(GLY)_2$	$(GLY)_3$	$(GLY)_4$	$(GLY)_5$
pyrrole					
204.4[b]	0.023				
aniline					
205.8[b]	0.25				
dimethylformamide[c]					
206.9	1.6				
2,4-dimethyl-1,3-pentadiene[c]					
208.2	1.6	0.030	0.0206	0.042	0.038
3-fluoropyridine[b]					
211.6	2.5	0.39	0.24	0.22	0.33
cyclopropylamine[c]					
21.20	1.8	0.084	0.059	0.23	0.23
allylamine[c]					
213.0	1.7	0.011	0.074	0.039	0.085
dimethylacetamide[c]					
214.1	3.0	1.2	0.23	0.21	0.17
n-propylamine[c]					
215.8	2.2	6.4	1.2	1.2	0.66
isobutylamine[c]					
216.8	2.8	1.3	1.4	0.93	0.50
sec-butylamine[c]					
217.4	4.4	2.5	2.0	2.0	2.3
di-*n*-propylamine[c]					
226.0	2.4	2.1	1.3	1.4	1.3
tri-*n*-propylamine[c]					
232.5				1.5	1.8

[a] All rate constants × 10^{-9} cm^3 $molecule^{-1}$ s^{-1}. [b] Gas-phase basicity values are obtained directly from ref 30 and adjusted to the temperature of 300 K. [c] The relative gas-phase basicity values are obtained from ref 25, adjusted to fit the scale of ref 30, and adjusted to 300 K.

constants have errors of 40% due mainly to the pressure calibration of the ion gauge and the use of empirically calculated response factors for the respective bases. The listed GB's are adjusted using the scale recently revised by Moet-Ner and Sieck at 600 K and adjusted to 300 K.[30] In assigning the GB values of the reference bases, isobutene is used as the absolute GB standard. Three bases on the Meot-Ner and Sieck (M–S) scale are used in this report (pyrrole, aniline, and 3-fluoropyridine). Their temperature-adjusted GB values are used directly. The other reference bases are fixed to the M–S scale by obtaining their relative GB values from the Lias, Liebman, and Levin (LLL) scale[25] and adjusting these values with a base present in both scales and having GB values in the proximity of the reference bases. The temperature of 300 K is the reaction temperature chosen since the analyzer source filament is not used during the experiment and the instrument is maintained at ambient temperature.

All the peptides in this investigation have been reacted with all the listed reference bases (Table I) to provide multiple overlapping and confirming reactions. Protonated glycine reacts with compounds at least as basic as dimethylformamide (206.9 kcal/mol). The total ion abundance remains relatively unchanged during the reaction, indicative of actual proton-transfer reactions between the protonated amino acid and reference base. Conversely, pyrrole does not react with protonated glycine to any significant degree. The proton-transfer reaction with aniline (GB 205.8) is slow but still noticeable in the decay plots. The large change in the rate constants between aniline and dimethylformamide is used to assign a gas-phase basicity value for glycine between aniline and dimethylformamide, 206.4 ± 2 kcal/mol (Table II). This value is in line with that reported by Locke and McIver (207.0 kcal/mol on the MS scale)[1,2] and Moet-Ner, Hunter, and Field (205.8 kcal/mol),[3] but higher than that reported by Gorman, Speir, Turner, and Amster (202.4 kcal/mol).[31,32]

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Table II. Intrinsic Gas-Phase Basicities of GLY_n (n = 1–5) Determined by Production of Protonated Peptide in the External Source and Reaction with a Gaseous Reference Base in the Analyzer Cell of an External Source FTMS Instrument

	GB_{exp} (300 K), kcal/mol
$GLY + H^+ \rightarrow GLYH^+$	206.4 ± 2
$GLY_2 + H^+ \rightarrow GLY_2H^+$	213.5 ± 2
$GLY_{3,4} + H^+ \rightarrow GLy_{3,4}H^+$	215.0 ± 2
$GLY_5 + H^+ \rightarrow GLY_5H^+$	217.1 ± 2

The latter value, however, has uncertainties of 3.2 kcal/mol which would still make it consistent with our results.

All the higher glycine oligomers of this study (GLY_n, n = 2–5) are found to have similar gas-phase basicities. They do not undergo observable proton-transfer reactions with compounds having basicities as low as 208.2 kcal/mol (2,4-dimethyl-1,3-pentadiene) but instead undergo rapid proton-transfer reactions with bases at least as basic as *n*-propylamine (215.8 kcal/mol). The reactions of the polyglycines with pyrrole, aniline, and dimethylformamide were performed although no rate constants are listed. These reactions were extremely slow (less than or equal to 10^{-11} cm^3 $molecule$ s^{-1}). Rate constants having this order of magnitude have larger relative errors since the reactions are extremely slow, allowing proton-transfer reactions to occur between background ions and the reference bases. Rapid reactions (near collision rates) are more accurate since fast proton-transfer reactions between the protonated peptide and the reference base are more readily observed. Protonated diglycine transfers the proton at near-collision rates with dimethylacetamide. It undergoes very slow proton transfer with 2,4-dimethyl-1,3-pentadiene, cyclopropylamine, and allylamine. The reaction of 3-fluoropyridine with all the peptides appears unusually fast compared to the other reference bases with similar basicities. The calculation of the rate constants for the reactions of 3-fluoropyridine may be affected by the empirical method employed to obtain relative ion gauge sensitivities. A linear equation relating the compound's polarizability with its relative sensitivity has been used to calibrate the ion gauge.[29] It is known, however, that the correlation of relative sensitivities of halogen-containing compounds differs from that of either nitrogen- or oxygen-containing compounds.[29]

We assign the basicity of diglycine to be 213.5 ± 2 kcal/mol, between allylamine and dimethylacetamide. A general decrease in the rates of proton transfer is observed in the reation of dimethylacetamide with peptides larger than diglycine. There is a further increase (albeit smaller) between the GB of di- and triglycine. Proton-transfer rate constants corresponding to collision rates are observed between the reaction of protonated tri-/tetraglycine with *n*-propylamine. This allows us to assign a value of 215.0 ± 2 kcal/mol for the gas-phase basicity of tri- and tetraglycine. We assign a slightly higher gas-phase basicity for pentaglycine (217.1 ± 2 kcal/mol), but the difference between penta- and tri-/tetra- is really below the experimental error of our technique. Part of the problem is the ability of protonated pentaglycine to react moderately with several bases having similar GB's.

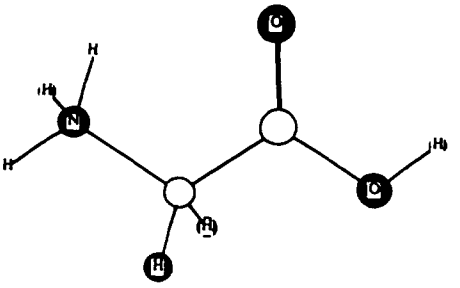
Side reactions are observed with certain combinations of reference bases and peptides. One side reaction, dimerization, occurs when the GBs of the reacting compounds are similar. For example, the protonated peptide GLY_3H^+ forms a mixed dimer with *n*-propylamine (reaction 3). These reactions, however,

$$GLY_3H^+ + n\text{-}C_3H_7NH_2 \rightarrow [GLY_3\cdots H\cdots C_3H_7NH_2]^+ \quad (3)$$

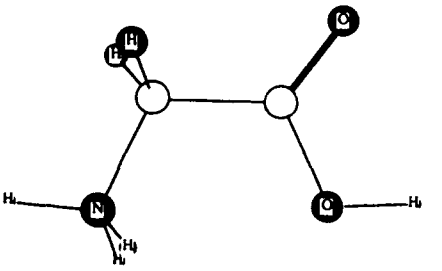
proceed slower than the proton-transfer reactions and account

(32) These GB values, with the exception of Amster's, have been calculated using the values for the reference base aniline from ref 30 adjusted to the temperature cited in ref 25. The actual GB values cited have been further adjusted to 300 K.

Chart I



Ia (0.0 Kca/mole)



Ib (0.6 Kca/mole)

for less than 20% of the total ion abundance. They are included in the calculation of the rate constants with the assumption that the abundance of the mixed dimer stays constant during the course of the proton-transfer reactions.

Another side reaction observed is fragmentation. This occurs more often with the larger glycine oligomers. This reaction, albeit slow, sometimes interferes with the determination of the proton-transfer reaction since the fragments are often weakly basic and will thus readily transfer protons to the neutral base. For example, fragmentation reactions occur between cyclopropylamine and tetra- and pentaglycine which accounts for the larger rate constants compared to the smaller oligomers. Fragmentation reactions are particularly important in slow reactions where they can sometimes be competitive with proton transfer. In any case, reactions involving reference bases with much greater basicities than the peptide (i.e., >4 kcal/mol) produce proton-transfer reactions which are faster than the fragmentation reactions.

Theoretical Results. Molecular orbital calculations using the AM1 Hamiltonian represent a "new generation" of semiempirical treatment. This method has been designed to deal specifically with hydrogen-bonding interactions.[18,19] Limitations of this technique are known. However, for compounds which are structurally similar, errors in calculating heats of formation of protonated and nonprotonated species do not necessarily transfer into errors in proton affinities. Deficiencies in calculating heats of formations presumably occur in both protonated and nonprotonated forms, thus cancelling in the calculation of proton affinities. Indeed, in a recent calculation to obtain the proton affinities of 99 widely dissimilar compounds, an overall uncertainty of only 3.1 kcal/mol was obtained.[33]

The theoretical heats of formation, as calculated by AM1, of various protonated glycine and diglycine structures are shown in Chart I. The lowest energy structure for protonated glycine corresponds to structure Ia. This configuration is slightly more stable than the other rotamer (Ib) by only 0.6 kcal/mol. Recent high level ab initio calculations predict a difference of 4.6 kcal/mol between the two structures. The inclusion of MP2 decreases the difference further to 3.7 kcal/mol so that the true value may actually be close to the value obtained by AM1.[34] The hydrogen-bonding interaction is not expected to be strong for protonated glycine as evidenced by the small entropy change reported by Moet-Ner, Hunter, and Field.[3] From the lowest energy glycine structure (ΔH_f = 64.7 kcal/mol) and the experimental value for the proton (ΔH_f = 367.2 kcal/mol),[35] we calculate a theoretical proton affinity value for glycine of 201.6 kcal/mol.

The heats of formation of protonated diglycines and their corresponding structures are shown in Chart II. The lowest heat of formation is obtained for the protonated terminal amine with hydrogen bonding to the adjacent carbonyl oxygen (IIa). Because the three hydrogen atoms on the amine are equivalent, charge is distributed equally over them. Hydrogen-bonding interaction is most favorable when two of the atoms interact with the adjacent amide oxygen. Protonation of the amide oxygen with hydrogen bonding to the terminal nitrogen is higher in energy 3.1 kcal/mol (IIb). Protonation of the amide oxygen with hydrogen bonding to the adjacent carbonyl oxygen is higher in energy by 2.3 kcal/mol (IIc). This value is expected to decrease when the molecule is lengthened to a triglycine, allowing the proton to be coordinated by two carbonyl amides. Protonation of the amide nitrogen in diglycine is higher in energy by 11.5 kcal/mol (IId). The theoretically calculated proton affinity of diglycine, using the lowest energy neutral structure (−137.5 kcal/mol) and the lowest energy protonated species, is 206.8 kcal/mol which makes it 5.2 kcal/mol more basic than glycine.

Hydrogen-bonding interactions are important for the diglycine and account for the increase in basicity. Rotating the protonated amino group by 180° (IIe) increases the heat of formation by 6.3 kcal/mol or equivalent to the difference in theoretical proton affinity values between glycine and diglycine. This hydrogen-bonding interaction is also evident in the bond distance between the carbonyl oxygen and the nearest hydrogen on the amine. For protonated glycine (Ia) this distance is 2.69 Å, but for the protonated diglycine (IIa) the corresponding O–H distance is 2.56 Å.

Discussion

Although the experimental absolute gas-phase basicities (and proton affinities) presented in this report may further change owing to more accurate methods for determining the absolute standards, the relative GB between glycine and its oligomers should remain relatively constant. After this paper was submitted, Sperling and Cassady[36] and Wu and Fenselau[37] reported proton affinities of glycine oligomers consistent with the gas-phase basicities presented in this report. The differences in gas-phase basicities between glycine and its oligomers are significant but point to the absence of strong long-range interactions.

Assignment of experimental proton affinity ($-\Delta H$ of reaction 1) involves determining the entropy change in the protonation reaction (reaction 1). This includes entropy loss due to coordination of the proton ($\Delta S(H^+)$) as well as changes in entropy in going from nonprotonated to protonated amino acids ($\Delta S(A \rightarrow AH^+)$). The first term is well known and contributes, in the form of $T\Delta S$, to the total free energy by at least 7.8 kcal/mol (at 300 K). The second term requires knowledge of the site of protonation and the types of intramolecular coordination in both the product and the reactants. For simplicity, we assume that the protonation of glycine at the amino group is not accompanied by strong interactions with the carbonyl group. This assumption is supported by the MO calculation which shows a decrease in NH–O distance when the carboxylic group is converted to an amide by the presence of the second glycine group.

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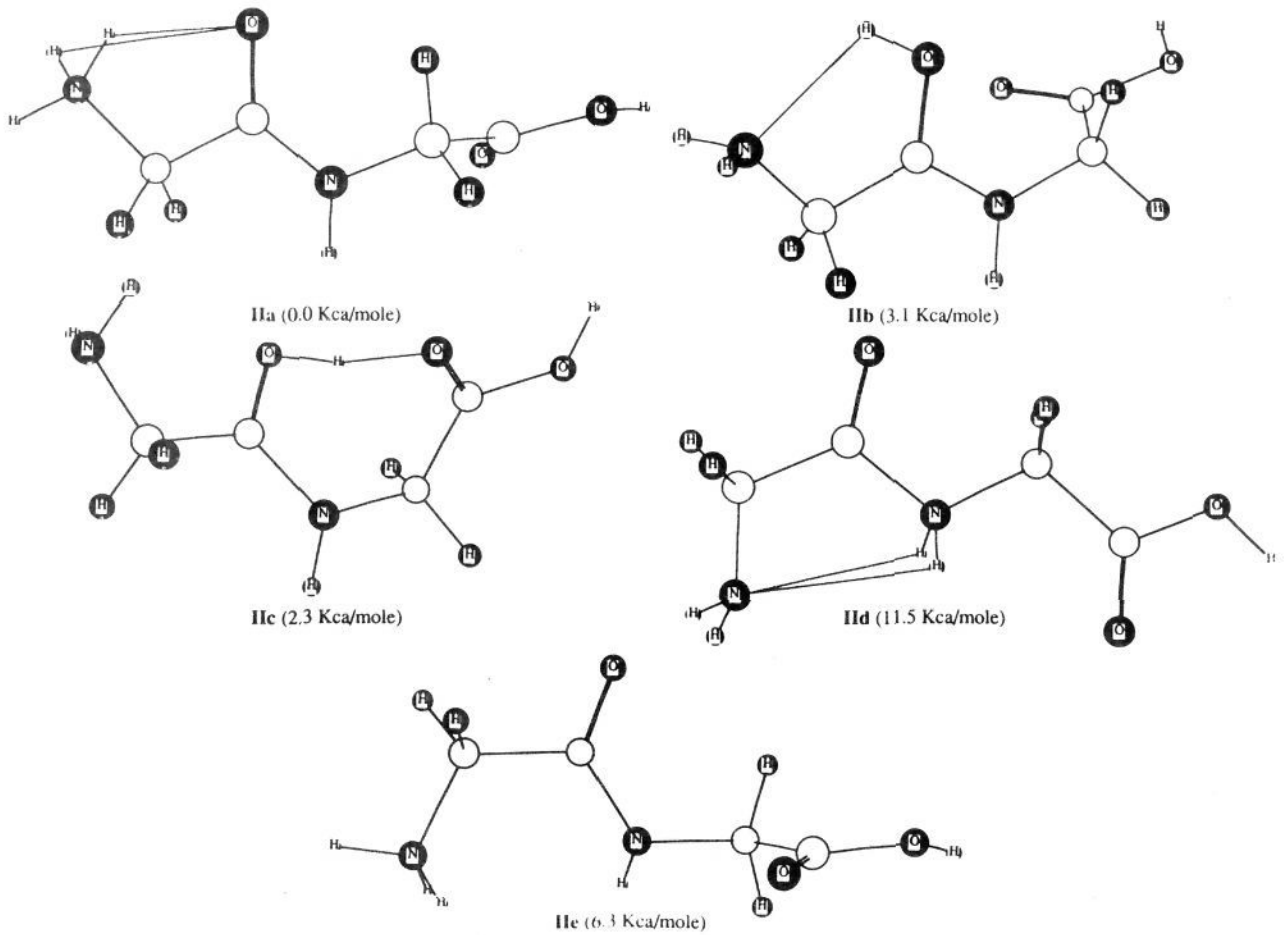
(34) Jensen, F. *J. Am. Chem. Soc.* **1992**, *114*, 9533.
(35) Stull, D. R.; Prophet, J. *JANAF Thermochemical Tables*; NSRDS-NBS37, 1971.
(36) Sperling, B. D.; Cassady, C. J. The 40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington DC, May 31–June 5, 1992.
(37) Wu, Z.; Fenselau, C. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 863.

Chart II



Similarly, rotation of the amino group to inhibit NH–O interactions in glycine (Ib) increases the energy by only 0.6 kcal/mol. In contrast, the analogous rotation with diglycine (IIe) increases the energy by 6.3 kcal. Thus, one can assume that glycine is essentially monofunctional and that the total contribution of entropy (the second term) varies between the product and the reactant by less than 1 kcal/mol. In light of the larger deviation associated with this method, we neglect a net effect of structure on entropy so that $\delta\Delta G = \delta\Delta H$. Given this assumption, we obtain an experimental proton affinity of 215.4 ± 2 kcal/mol for glycine.

The site of protonation on the neutral gas-phase glycine molecule is the terminal amino group. In other words, neutral glycine does not behave as a zwitterion in the gas phase. This has been determined by Locke and McIver by comparing the gas-phase basicity and acidity of glycine to amines and carboxylic acids, respectively.[1] Determining the site of protonation is more difficult with the larger oligomers of glycine. The similarity in the gas-phase basicities of the polyglycines (GLY_n, $n > 1$) could be indicative of either a common protonation site or similar intrinsic basicities of various sites. The lowest energy configurations of protonated diglycine, calculated by AM1, correspond to structures IIa and IIc. The lowest energy structure, protonation on the terminal amine with hydrogen bonding to the adjacent amide oxygen (IIa), is more favorable than the other, which involves binding exclusively to the carbonyl oxygen atoms (IIc), by only 2.3 kcal/mol. There is, however, additional experimental evidence to support the protonation of exclusively the terminal amine. Kinser, Ridge, and Uggerud have determined the basicity of glycine amides and have found that the relative gas-phase basicity increases by similar amounts to the increase we observe between glycine and diglycine.[38] Because another carbonyl group is not present in the molecule, replacing the carboxylic acid with an amide increases the basicity simply by making the carbonyl oxygen more basic and similarly enhancing hydrogen bonding between the amino nitrogen and the amide oxygen.

Table III. Estimated Proton Affinities of GLY_n (n = 1–5) Obtained from the GB Values by Assuming Protonation on the Terminal Amine with No Interaction between the Proton and the Neighboring Carbonyl Amide

peptide	proton affinity, kcal/mol
GLY	215.4
GLY_2	222.4
$GLY_{3,4}$	223.8
GLY_5	226.9

Assigning proton affinity values ($-\Delta H$) for the polyglycines is similarly complicated by the presence of multiple base sites with similar intrinsic basicities. For polyfunctional groups the change in entropy due to cyclization is large and may increase the value of the proton affinity by an additional 4 to 7 kcal/mol depending on the size of the ring formed.[26] Values are listed for proton affinities of the glycine oligomers (Table III) which neglect intramolecular interactions such as coordination of the protonated amine to the carbonyl amide via hydrogen bonding. The inclusion of these interactions would raise the proton affinity by another 4–7 kcal/mol depending on the interaction. For example, protonation of diglycine to produce IIa is entropically closest to the protonation of ethylenediamine. Both protonated species produce a five-membered ring, although it is understood that the

(38) Kinser, R. D.; Ridge, D. P.; Uggerud, E. The 39th ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 19–24, 1991.

Table IV. Estimated Proton Affinities of GLY_n (n = 2–5) Obtained from the GB Values by Assuming Protonation on the Terminal Amine with Hydrogen-Bonding Interaction between the Proton and the Neighboring Carbonyl Amide

peptide	proton affinity, kcal/mol
GLY_2	224.5 ± 4
$GLY_{3,4}$	226.0 ± 4
GLY_5	228.1 ± 4

N–H–O bond in IIa is weaker than the N–H–N bond in ethylenediamine. The estimated contribution of the $T\Delta S$ term for this interaction at 300 K is 11.0 kcal/mol.[26] Protonation of the carbonyl oxygen atoms to produce a seven-membered ring as in IIc is estimated to have a contribution as $T\Delta S$ of 13.9 kcal/mol. If we assume that protonation occurs to form primarily IIa, then the estimated *experimental* proton affinities (Table IV) would be 224.5 kcal/mol for diglycine, 226.0 kcal/mol for tri-/tetraglycine, and 228.1 kcal/mol for pentaglycine. A large error bar (±4) must, however, be included to account for the differences between ethylenediamine and IIa and the formation of other dicoordinated species such as IIc.

In the idealized proton-transfer reaction between glycine and the polyglycines (reaction 4), we find reasonable agreement in

$$GLYH^+ + GLY_2 \rightarrow GLY + GLY_2H^+ \qquad (4)$$

$$\Delta H_{exptl} = -7.1 \text{ to } -10.1 \text{ kcal/mol}, \ \Delta H_{theor} = -5.2 \text{ kcal/mol}$$

the proton affinity values between experimental and theoretical results. There exists some discrepancy between calculated and experimental proton affinities. Much of this is due to the determination of the experimental proton affinity and the tendency of AM1 to underestimate the stability of most protonated amines. The latter, however, is systematic and should cancel when comparing similar amines.

Conclusion

Determination of the proton affinities of glycine and its oligomers generally illustrates the difficulty of obtaining proton affinity ($-\Delta H$) values compared to the gas-phase basicity ($-\Delta G$) values of peptides. Until more is known about intramolecular interactions, we suggest that values be reported as gas-phase basicities for these polyfunctional compounds.

Although there are large discrepancies between absolute values of theoretical and experimental proton affinities, there is reasonable agreement between theory and experiments within a group of similar compounds. The use of AM1 is useful in obtaining insight into the intramolecular interactions of these molecules. It can always be argued that it would be more suitable to perform ab initio calculations. Kinser, Ridge, and Uggerud[38] and Bouchonnet and Hoppilliard[39] are currently performing high level ab initio calculations on selected amino acids and their amides. However, calculations on diglycines and larger oligomers using high level ab initio techniques may still be too expensive and time consuming.

There has also been a general question concerning the mobility of the proton in gas-phase protonated peptides. Since the difference in the theoretical proton affinity values of IIa and IIb is small, one can assume that even a small amount of vibrational excitation can make the proton extremely mobile. The amount of internal energy obtained by the ion will naturally depend on the method of ionization. In methods where fragmentation reactions occur, sufficient mobility of the proton should also occur, allowing many different base sites to be populated. Any mechanism for proton migration will most likely involve the amide oxygen atoms. For example, starting with proton coordination on the terminal amine and the amide oxygen (IIa), the molecule can convert to IIb and to IIc and on to other structures similar to IIc. This migration would almost certainly occur during collisionally activated dissociation (CAD) which means that the most basic site of protonation may not necessarily govern the fragmentation reactions.

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(39) Bouchonnet, S.; Hoppilliard, Y. *Org. Mass Spectrom.* **1992**, *27*, 71.