Gas-Phase Basicity Measurements of Dipeptides That Contain Valine

Greg S. Gorman and I. Jonathan Amster*

Contribution from the Department of Chemistry, University of Georgia, Athens, Georgia 30602

Received December 17, 1992

Abstract: Gas-phase basicities of 22 dipeptides that contain valine were measured by a double bracketing method in a Fourier transform ion cyclotron resonance spectrometer. Matrix-assisted laser desorption was used to generate protonated peptide ions which were reacted with reference compounds to bracket the gas-phase basicity. In addition, neutral peptide molecules were formed by substrate-assisted laser desorption and reacted with protonated reference ions to confirm the assignment of the gas-phase basicity. The rate of proton transfer between the protonated molecule of alanylvaline and six reference compounds was measured to examine the behavior of both exoergic and endoergic reactions. Gas-phase basicities of most of the dipeptides were found to be nearly equal to that of their most basic amino acid residue. The results are consistent with an intramolecular hydrogen bond between the N-terminus nitrogen and the amide carbonyl oxygen of a dipeptide. Furthermore, the results suggest that inductive effects cause an increase in the strength of the intramolecular hydrogen bond that parallels the increase in the basicity of the C-terminus amino acid residue. Dipeptides VF and VY are more basic than their constituent amino acids. These data and molecular mechanics calculations suggest that these two peptides are stabilized by an electrostatic interaction between the N-terminal ammonium ion and the polarizable electrons of the aromatic side chain of the C-terminus.

Introduction

Determinations of gas-phase basicity ($-\Delta G°$ of protonation) and proton affinity ($-\Delta H°$ of protonation) of small organic molecules have been the subject of extensive studies because of the fundamental significance of the proton transfer reaction. Such studies have examined the relationship of molecular structure and basicity through investigations of substituent effects, electronic and steric interactions, and the effects of other intramolecular forces on the measured gas-phase basicity and proton affinity of a molecule.[1-5] Gas-phase basicity (GB) and proton affinity (PA) values have been compiled for more than 800 compounds.[6] Recently, there has been widespread interest in measuring these values for amino acids and peptides.[7-14] These compounds are the building blocks of proteins and knowledge of their fundamental acid/base properties will be useful for correlating physical properties, such as pK_a and binding equilibrium constants, with protein structure.

Most reported values of relative GB and PA are based on a measurement of the equilibrium concentrations of two compounds (A and B) and their protonated forms (AH^+ and BH^+), shown in eq 1. Several different techniques have been used to make equilibrium measurements of these thermochemical values, including ion cyclotron resonance[4,15,16] and high-pressure mass spectrometry.[17-20] With these methods, the equilibrium constant for the reaction of eq 1 is determined from measurements of ion intensities and pressures of the neutral species. The flowing-afterglow technique has also been used to determine equilibrium constants by measuring the rates of the forward and reverse reactions of eq 1.[21-23] For all of these methods, relative gas-phase basicity (ΔGB, defined as $-\Delta G°$ of proton transfer) is derived from the measured equilibrium constant by eq 2, in which K_{eq} is the equilibrium constant of eq 1, R is the universal gas constant, and T is the temperature at which the equilibrium is measured.

$$AH^+ + B \rightleftarrows A + BH^+ \quad (1)$$

$$\Delta GB = -RT\ln(K_{eq}) = -RT\ln([A][BH^+])/([B][AH^+]) \quad (2)$$

A drawback of equilibrium methods is that these techniques are restricted in application to compounds that are volatile and stable at the temperature of the experiment (300–600 K). The nonvolatility and thermal lability of many biologically important compounds, such as amino acids and peptides, make it difficult to obtain gas-phase thermochemical data with these methods. For such samples, nonequilibrium methods must be applied. In

* Author to whom correspondence should be addressed. Ph: (706) 542-2001. FAX: (706) 542-9454.

(1) Yamdagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1976**, *98*, 1320–1324.
(2) Wolf, J. F.; Staley, R. H.; Koppie, I.; Taagepera, M.; McIver, R. T., Jr.; Beauchamp, J. L.; Taft, R. W. *J. Am. Chem. Soc.* **1977**, *99*, 5417–5429.
(3) Bowers, M. T.; Aue, D. H.; Webb, H. M.; McIver, R. T., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 4314–4315.
(4) Aue, D. H.; Webb, H. M.; Bowers, M. T. *J. Am. Chem. Soc.* **1973**, *95*, 2699–2701.
(5) Yamadagni, R.; Kebarle, P. J. *J. Am. Chem. Soc.* **1973**, *95*, 3504–3510.
(6) Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Chem. Phys. Ref. Data* **1984**, *13*, 695–808.
(7) Wu, Z.; Fenselau, C. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 403–405.
(8) Isa, K.; Omote, T.; Amaya, M. *Org. Mass Spectrom.* **1990**, *25*, 620–628.
(9) Bojensen, G. *J. Am. Chem. Soc.* **1987**, *109*, 5557–5558.
(10) Briendahl, T.; Bojensen, G. *Proceedings of the 12th International Mass Spectrometry Conference*, Amsterdam, WeA-B47.
(11) Gorman, G. S.; Speir, J. P.; Turner, C. A.; Amster, I. J. *J. Am. Chem. Soc.* **1992**, *114*, 3986–3988.
(12) Kinser, R. D.; Ridge, D. P. *Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics*, Nashville, TN, May 19–24, 1991; pp 31–32.
(13) Sperling, B. D.; Cassidy, C. J. *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*, Washington, DC, June 1–5, 1992; pp 1220–1221.
(14) Lebrilla, C. B.; Wu, J. *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*, Washington, DC, June 1–5, 1992; pp 83–84.
(15) Taft, R. W. In *Proton Transfer Reactions*; Caldin, E. F., Gold, V., Eds.; Chapman and Hall: London, 1975; p 31.
(16) Wight, C. A.; Beauchamp, J. L. *J. Phys. Chem.* **1980**, *84*, 2503–2506.
(17) Kebarle, P. *Annu. Rev. Phys. Chem.* **1977**, *28*, 445–476.
(18) Mautner, M. *J. Phys. Chem.* **1980**, *84*, 2716–2723.
(19) Mautner, M. J.; Sieck, L. W. *J. Am. Chem. Soc.* **1991**, *113*, 4448–4460.
(20) Mautner, M. J.; Sieck, L. W. *Int. J. Mass Spectrom. Ion Processes* **1991**, *109*, 187–208.
(21) Roche, A. E.; Sutton, M. M.; Bohme, D. K.; Schiff, H. I. *J. Chem. Phys.* **1971**, *55*, 5480–5484.
(22) Bohme, D. K.; Mackay, G. I.; Schiff, H. I. *J. Chem. Phys.* **1980**, *73*, 4976–4986.
(23) Mackay, G. I.; Bohme, D. K. *Int. J. Mass Spectrom. Ion Phys.* **1978**, *26*, 327–343.

0002-7863/93/1515-5729$04.00/0

the study reported here, we have used the bracketing method[24-29] to measure GB values for 22 dipeptides. With the bracketing technique, the compound of interest is allowed to react with a series of reference compounds with well-characterized GB and PA values. The basis of this method is that observation of rapid proton transfer (eq 1, forward direction) demonstrates a negative free energy of reaction and that GB(B) > GB(A). For compounds in which the entropy of proton transfer is negligible, one can also conclude that PA(B) > PA(A). When proton transfer is slow, it is not observed to occur during the experimental time frame and it can be concluded that the reaction is endoergic. In this work, we use double bracketing measurements in which we independently observe both the forward and reverse reactions of eq 1, thereby establishing the thermodynamic endpoint of the proton transfer reaction with great certainty.

The kinetic method[30-32] has received considerable attention as a technique for measuring proton affinities of amino acids,[7-10] peptides,[13,33] and crown ethers.[34] With this method, a proton-bound complex of the compound of interest and a reference compound are allowed to dissociate and the ratio of the product ion intensities is related in a quantitative manner to the relative proton affinities of the two compounds. Although many of the proton affinity values of amino acids determined by the kinetic method are in good agreement with values obtained by bracketing methods, there are a few notable exceptions. One of the assumptions of the kinetic method is a zero reverse activation energy barrier for the dissociation of the proton-bound complex. Recent ab initio calculations of the amino acid lysine and its protonated counterpart find a substantial reverse activation energy barrier (14 kcal/mol) for proton transfer to this amino acid.[35] The calculations yield a proton affinity value that closely agrees with the value determined by the bracketing method and suggest that values obtained by the kinetic method[7-9] are in error by an amount equal to the reverse activation energy. In general, the kinetic method is not applicable to compounds which can form an intramolecular hydrogen bond. As hydrogen bonds are likely to occur in peptide ions such as those examined here, the bracketing method is the most reliable technique for determining the gas-phase basicities of peptides.

In this paper, we present data for the gas-phase basicities of 22 combinations of valine-containing dipeptides, measured by the bracketing technique. These values are compared to those of their constituent amino acids, for which the gas-phase basicities and proton affinities have been previously reported.[11] Results of the measurements of the rate constants of both exoergic and endoergic proton transfer reactions of a dipeptide are presented to establish that the rate of proton transfer depends on the free energy of the reaction in the expected manner. These results are the first of a planned systematic study of the gas-phase basicities of all 400 dipeptides that can be formed from the 20 common α-amino acids.

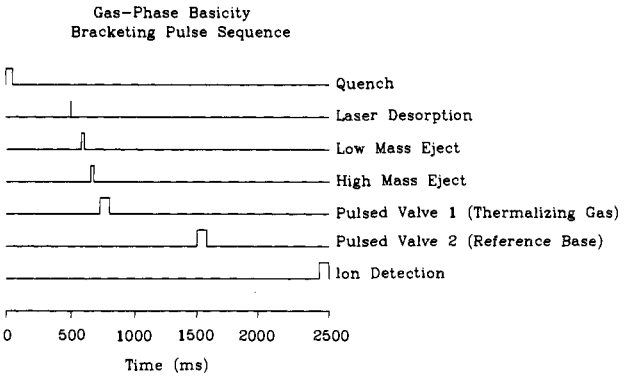


Figure 1. FTICR experimental sequence for a bracketing experiment in which a proton is transferred from the peptide to a reference compound. All ions are removed from the cell during the quench pulse. Protonated peptide ions are formed by MALDI. Ejection pulses are used to remove all other ions from the cell, for example, matrix ions. Pulsed valve 1 admits argon to thermalize the peptide ion. Pulsed valve 2 admits the reference compound. Ion detection is delayed to allow pulsed gases to be pumped away.

Experimental Section

All experiments were performed with a Fourier transform mass spectrometer designed and constructed in this laboratory. Details of this instrument have been described elsewhere.[36] Features relevant to these experiments are an electromagnet (Walker Scientific, Worcester, MA), operated at a field strength of 1 T, and a gas inlet system consisting of two pulsed valves (General Valve, Fairfield, NJ) and a sapphire sealed leak valve (Varian Associates, Lexington, MA). For bracketing experiments, the reference bases and the gases for collisional thermalization were admitted through pulsed valves. For measurements of the proton transfer rate constants, reference bases were introduced through a leak valve to maintain a constant background pressure. The protonated molecules of the reference compounds were generated by electron impact ionization of the volatile gas admitted through a pulsed valve. Pulse sequence timing, ion detection, and signal processing were controlled by a data system (IonSpec, Irvine, CA).

The peptides used in this study were obtained commercially (Sigma Chemical Co., St. Louis, MO) and used without further purification. For bracketing studies, proton transfer was examined both from a protonated peptide molecule to a reference compound and from a protonated reference compound to a neutral peptide molecule. For the former reaction, protonated peptide molecular ions were produced using matrix-assisted laser desorption–ionization (MALDI) by focusing 337-nm radiation (N_2 line) from an excimer laser (Questek, Model 2110, Billerica, MA) onto the sample probe at a power density of 10 MW/cm^2.[37] Samples were prepared for MALDI experiments by electrospraying[38] the equivalent of 1000 monolayers of a 100:1 mixture (sinapinic acid:peptide) of the sample dissolved in methanol onto a 1.3-cm-diameter Macor probe tip coated with 75 nm of Au/Pd. The sequence of events in MALDI bracketing experiments is shown in Figure 1. Standard Fourier transform ion cyclotron resonance spectrometry (FTICR) ejection techniques were used to remove any other ions formed during the desorption step from the analyzer cell. The protonated molecular ions were then collisionally thermalized (40–60 collisions/ion) with Ar, admitted through a pulsed valve, to reduce the population of excited-state ions before allowing reactions with the neutral reference bases. Reference bases were admitted through a second pulsed valve and allowed to react with the protonated peptide ions stored in the analyzer cell. The time dependence of the pressure of a typical reference compound admitted through the pulsed valve is shown in Figure 2. An ion undergoes 15 collisions on average with a reference compound, as determined by numerical integration of a pressure versus time plot. Ion detection occurs 1000 ms after the pulsed addition of the reference compound, at which time the pressure has returned to its background level.

(24) Munson, M. S. B. *J. Am. Chem. Soc.* **1965**, *87*, 2332–2336.
(25) Beauchamp, J. L.; Holtz, D.; Woodgate, S. D.; Patt, S. L. *J. Am. Chem. Soc.* **1972**, *94*, 2798–2807.
(26) Ridge, D. P. *J. Am. Chem. Soc.* **1975**, *97*, 5670–5674.
(27) Polley, C. W.; Munson, B. *Int. J. Mass Spectrom. Ion Phys.* **1978**, *26*, 49–60.
(28) DeFrees, D. J.; McIver, R. T., Jr.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 3334–3338.
(29) Smith, D.; Adams, N. G.; Lindinger, W. *J. Chem. Phys.* **1981**, *75*, 3365–3370.
(30) McLuckey, S. A.; Cameron, D.; Cooks, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 1313–1317.
(31) Nourse, B. D.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Processes* **1991**, *106*, 249–272.
(32) Cooks, R. G.; Kruger, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1279–1281.
(33) Wu, Z.; Fenselau, C. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 863–866.
(34) Liou, C. C.; Brodbelt, J. S. *J. Am. Chem. Soc.* **1992**, *114*, 6761–6764.
(35) Bliznyuk, A. A.; Schaeffer, H. F.; Amster, I. J. *J. Am. Chem. Soc.*, in press.
(36) Speir, J. P.; Gorman, G. S.; Cornett, D. S.; Amster, I. J. *Anal. Chem.* **1991**, *63*, 65–69.
(37) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A–1203A.
(38) McNeal, C. J.; Macfarlane, R. D.; Thurston, E. L. *Anal. Chem.* **1979**, *51*, 2036–2039.

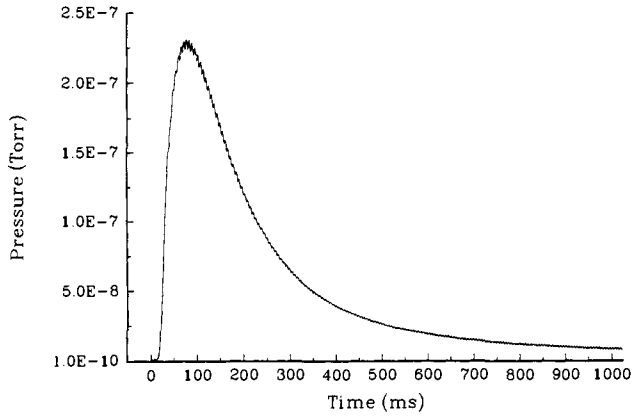


Figure 2. Plot of the time dependence of the pressure of a reference gas, ammonia, admitted through a pulsed valve during a bracketing experiment. Numerical integration of this plot indicates that ions in an ICR cell undergo 15 collisions with the reference gas.

For the reverse proton transfer reaction, desorption of peptide neutral molecules was accomplished using the substrate-assisted laser desorption technique (SALD).[39] With this method, an organic substrate was formed by applying 500 monolayers of a UV-absorbing compound, sinapinic acid, to the probe tip. One thousand monolayers of a dipeptide were deposited on top of the sinapinic acid substrate. The electrospray method was used to apply the thin films evenly over the entire 1.3-cm^2 surface of the probe tip. The sample was irradiated with a pulse of 337-nm light from an excimer laser at an irradiance of 1 MW/cm^2. The light was absorbed by the substrate, causing the desorption of intact, neutral peptide molecules. The laser-desorbed peptide neutrals were allowed to react with the protonated molecule of a reference compound. The occurrence or nonoccurrence of a proton transfer reaction between various reference bases and the peptides in these reactions was monitored by standard FTICR methods.

For rate constant measurements, MALDI was used to generate the protonated molecule of alanylvaline, which was collisionally thermalized and allowed to react with a reference compound. The volatile reference base was admitted into a vacuum system through a leak valve to maintain a constant background pressure, which was monitored with an ion gauge. Pressure readings were corrected for the ion gauge sensitivity of each gas by the method of Found and Dushman.[40,41] The ion gauge was calibrated by measuring the reaction rate of proton transfer between $C_2H_5^+$ and NH_3, which has a rate constant of 2.0×10^{-9} cm^3/molecule·s.[42] The measured pressure, after correction for sensitivity for a specific gas, was found to be 1.9 times lower than the true pressure due to the proximity of the ion gauge to the vacuum pump. The factor of 1.9 was used to adjust all pressure measurements for rate constant determinations. The duration of the reaction between a protonated peptide and a reference base was varied between 1 and 5 s for the rate constant measurements. Triplicate measurements were made at intervals of 500 ms, and the standard deviation of the measurement at each time point was calculated. The time dependence of the intensity of the protonated molecule of a peptide was monitored to determine a pseudo-first-order rate constant. A second-order rate constant was calculated by dividing the first-order rate constant by the density of the reference compound, which was derived from the pressure measurement.

Results and Discussion

Bracketing Measurements. The use of SALD and MALDI to establish the basicity of a dipeptide is demonstrated by the data in Figure 3. Protonated 3-fluoropyridine, when allowed to react with laser desorbed dipeptide AV, produces the mass spectrum shown in Figure 3a. Proton transfer is observed, indicating that the peptide is more basic than 3-fluoropyridine. The protonated molecule of ethylamine is not observed to transfer a proton to AV under these conditions, as shown in Figure 3b, suggesting that AV is less basic than this reference base. From these data, it can be inferred that the basicity of AV falls between that of 3-fluoropyridine and ethylamine. This assignment is confirmed by the data in Figures 3c and 3d in which the protonated molecule of AV is allowed to react with 3-fluoropyridine and ethylamine. No proton transfer is observed with 3-fluoropyridine, confirming that it is less basic than AV, while proton transfer is observed with ethylamine, as this compound is more basic than the dipeptide. Using this strategy, the gas-phase basicities (350 K) of 22 dipeptides containing valine have been measured. Their values are listed in Table I and found to fall in the range of 211–228 kcal/mol. In both the SALD and MALDI bracketing experiments, the originally formed protonated ions are collisionally thermalized before reaction. However, the laser-desorbed peptides are collisionally thermalized only in the MALDI experiment, as there is no effective way to subject the laser-desorbed neutrals to thermalizing collisions in a SALD experiment. Since the MALDI and SALD bracketing results are in agreement for all the dipeptides in this study, we contend that any shift in the free energy of proton transfer caused by nonthermal peptide neutrals lies within experimental error.

Proton Transfer Rate Measurements. We assign the transition between rapid and slow proton transfer as the zero point of the *free energy* of the reaction, which is not necessarily equal to the zero point of the *enthalpy* of the reaction. The dependence of the rate of proton transfer on free energy has been established experimentally.[22,43] A consequence of this dependence is that entropy can drive endothermic reactions at the collision rate, if the free energy of proton transfer is negative. Although this behavior has been demonstrated in a high-pressure mass spectrometer,[43] it has not been confirmed in the low-pressure environment of an FTICR. In a recent study in which the methods reported here were applied to a determination of the gas-phase basicities and proton affinities of amino acids, it was established that gas-phase basicity is a thermodynamic property that is bracketed by this type of experiment, on the basis of the differences observed between measurements of lysine and leucine.[11] Further evidence for entropy-driven reactions in FTICR will be the subject of a separate paper.[44]

In the bracketing studies reported here, the occurrence or nonoccurrence of proton transfer between a peptide and a reference compound is examined after a protonated ion has been allowed to react with a brief pulse of a neutral compound. A fast reaction is inferred if proton transfer is observed to occur, while the absence of proton transfer is interpreted as a slow reaction. These assumptions were tested by measuring the rate of proton transfer for the reactions of 3-fluoropyridine (GB = 211.3 ± 1.5 kcal/mol), ethylamine (GB = 213.2 ± 1.5 kcal/mol), isopropylamine (GB = 216.3 ± 1.5 kcal/mol), pyridine (GB = 218.4 ± 1.5 kcal/mol), *tert*-butylamine (GB = 220.5 ± 1.5 kcal/mol), and trimethylamine (GB = 223.3 ± 1.5 kcal/mol) with the protonated molecule of alanylvaline, AV. A plot of the time dependence of the intensity of the protonated peptide ion that is allowed to react with a static pressure of the reference compounds is shown in Figure 4. A linear dependence is observed for a decrease in the log of the intensity of the protonated peptide ion with time, as expected for a pseudo-first-order reaction. The rate constants of these reactions, calculated from the slopes of the plots and the pressures of the reference compounds, are $1.1 \pm 0.1 \times 10^{-9}$ cm^3/molecule·s (trimethylamine, *tert*-butylamine, pyridine), $9.3 \pm 0.4 \times 10^{-10}$ cm^3/molecule·s (isopropylamine), $8.2 \pm 0.5 \times 10^{-10}$ cm^3/molecule·s (ethylamine), and $2.0 \pm 0.2 \times 10^{-10}$ cm^3/molecule·s (3-fluoropyridine). The ratio of the measured rates to the collision rates for the six reaction pairs are plotted as a function of the GB of the reference compound in Figure 5. Most of the reactions occur near the collision rate, indicating exoergic thermochemistry. A dramatic decrease in the rate of proton

(39) Speir, J. P.; Amster, J. A. *Anal. Chem.* **1992**, *64*, 1041–1045.
(40) Found, C. G.; Dushman, S. *Phys. Rev.* **1924**, *23*, 734–743.
(41) Dushman, S.; Young, A. H. *Phys. Rev.* **1945**, *68*, 278.
(42) Su, T.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Phys.* **1973**, *12*, 347–356.
(43) Mautner, M. *J. Phys. Chem.* **1991**, *95*, 6580–6585.
(44) Gorman, G. S.; Amster, I. J. *Org. Mass Spectrom.*, submitted for publication.

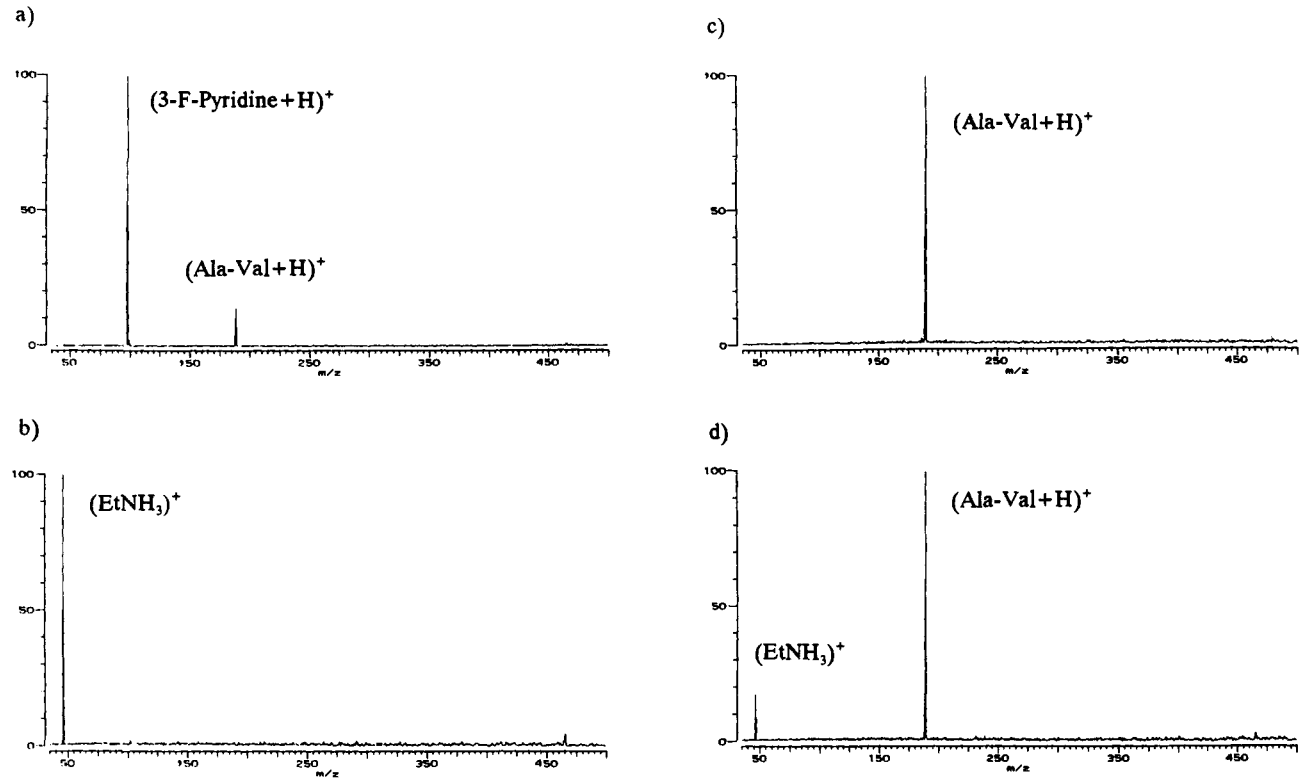


Figure 3. Mass spectra demonstrating bracketing measurements of the gas-phase basicity of alanylvaline. SALD is used to desorb peptide neutral molecules and allowed to react with the protonated molecule of (a) 3-fluoropyridine and (b) ethylamine. Proton transfer is observed only for the former, suggesting that the GB of the dipeptide lies between those of 3-fluoropyridine and ethylamine. MALDI is used to form the protonated molecule of alanylvaline, which is allowed to react with (c) 3-fluoropyridine and (d) ethylamine. Proton transfer is observed only in the latter reference compound, confirming the assignment of the GB of the dipeptide.

Table I. Comparison of Dipeptide and Amino Acid Basicities

					reference compounds	
peptide[a]	GB(dipeptide)	GB(N)[b]	GB(C)[c]	ΔGB[d]	lower	upper
VG	212.3 ± 2.5	212.3 ± 2.5	200.7 ± 1.7	0 ± 3.5	3-fluoropyridine	ethylamine
GV	212.3 ± 2.5	200.7 ± 1.7	212.3 ± 2.5	0 ± 3.5	(211.3 ± 1.5)	(213.2 ± 1.5)
VD	212.3 ± 2.5	212.3 ± 2.5	210.3 ± 2.6	0 ± 3.5		
DV	212.3 ± 2.5	210.3 ± 2.6	212.3 ± 2.5	0 ± 3.5		
VS	212.3 ± 2.5	212.3 ± 2.5	210.3 ± 2.6	0 ± 3.5		
AV	212.3 ± 2.5	212.3 ± 2.5	212.3 ± 2.5	0 ± 3.5		
VA	214.8 ± 3.1	212.3 ± 2.5	212.3 ± 2.5	2.5 ± 4.0	ethylamine	isopropylamine
VV	214.8 ± 3.1	212.3 ± 2.5	212.3 ± 2.5	2.5 ± 4.0	(213.2 ± 1.5)	(216.3 ± 1.5)
VL	214.8 ± 3.1	212.3 ± 2.5	214.8 ± 3.1	0 ± 4.4		
LV	214.8 ± 3.1	214.8 ± 3.1	212.3 ± 2.5	0 ± 4.4		
YV	217.4 ± 2.6	217.4 ± 2.6	212.3 ± 2.5	0 ± 3.7	isopropylamine	pyridine
FV	217.4 ± 2.6	217.4 ± 2.6	212.3 ± 2.5	0 ± 3.7	(216.3 ± 1.5)	(218.4 ± 1.5)
MV	219.5 ± 2.6	219.5 ± 2.6	212.3 ± 2.5	0 ± 3.7	pyridine	*tert*-butylamine
VM	221.9 ± 2.9	212.3 ± 2.5	219.5 ± 2.6	2.4 ± 3.9	*tert*-butylamine	trimethylamine
VW	221.9 ± 2.9	212.3 ± 2.5	221.9 ± 2.9	0 ± 4.1	(220.5 ± 1.5)	(223.3 ± 1.5)
VY	221.9 ± 2.9	212.3 ± 2.5	217.4 ± 2.6	4.5 ± 3.9		
VF	221.9 ± 2.9	212.3 ± 2.5	217.4 ± 2.6	4.5 ± 3.9		
PV	221.9 ± 2.9	221.9 ± 2.9	212.3 ± 2.5	0 ± 4.1		
VP	223.7 ± 1.9	212.3 ± 2.5	221.9 ± 2.9	1.8 ± 2.9	trimethylamine	diethylamine
VE	223.7 ± 1.9	212.3 ± 2.5	223.7 ± 1.9	0 ± 2.7	(223.3 ± 1.5)	(224.1 ± 1.5)
VK	226.0 ± 3.4	212.3 ± 2.5	226.0 ± 3.4	0 ± 4.8	diethylamine	dipropylamine
KV	226.0 ± 3.4	226.0 ± 3.4	212.3 ± 2.5	0 ± 4.8	(224.1 ± 1.5)	(227.9 ± 1.5)

[a] Key to one letter code for the amino acids: A = alanine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, K = lysine, L = leucine, M = methionine, P = proline, S = serine, V = valine, W = tryptophan, and Y = tyrosine. [b] GB(N) = GB of N-terminal amino acid; GB value from ref 2. [c] GB(C) = GB of C-terminal amino acid. [d] ΔGB = GB(dipeptide) – GB (most basic amino acid residue). [e] GB values of the reference compounds at 350 K are shown in parentheses and are from ref 2.

transfer is found to occur between reference compounds 3-fluoropyridine and ethylamine. Based on the known dependence of the rate of proton transfer with the free energy of the reaction,[22,43] the data strongly suggest that the $\Delta G = 0$ point lies between these two reagents. The bracketing measurements performed with pulsed reagents also suggest that the GB of AV falls between that of 3-fluoropyridine and ethylamine. This demonstrates that by using the pulsed introduction of a neutral compound, monitoring the occurrence/nonoccurrence of proton transfer is a valid method to distinguish fast versus slow proton transfer. The kinetic data presented here show that the reaction rate constant must be greater than 2×10^{-10} cm^3 molecule^{-1} s^{-1} in order to observe proton transfer to neutrals introduced in a brief pulse using the experimental conditions reported here.

General Trends of Gas-Phase Basicity. A comparison of GBs of the dipeptides with the basicities of their amino acid constituents

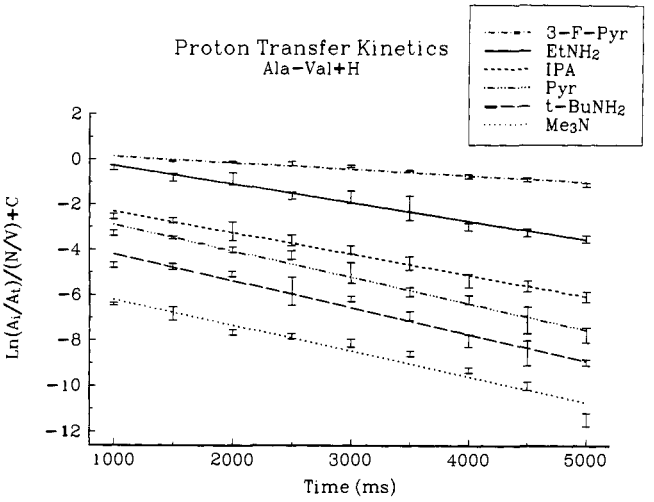
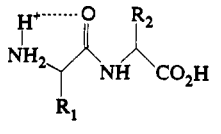


Figure 4. Time plots of the disappearance of the protonated molecule of AV undergoing proton transfer to six reference compounds. The ordinate represents the natural logarithm of the ratio of the initial abundance of protonated AV to the abundance at the time indicated along the absicca. This value is scaled by the number density (N/V) of the neutral gas so that the slopes of all the plotted lines can be related by the same proportionality constant to the rate constant for proton transfer. For clarity, a constant has been added to each data set to separate the lines. The actual intercepts of all the lines fall between 0 and −1. Error bars represent ±1 standard deviation, derived from triplicate measurements at each time point.

reveals an unexpected trend. The GB of a dipeptide is approximately equal to that of its most basic amino acid residue regardless of the position of the amino acid in the peptide. This can be seen from an examination of the data in the last column of Table I, which lists the difference between the GB of a dipeptide and that of its most basic amino acid constituent. For a majority of the dipeptides, this difference is close to zero. With the exception of lysine and perhaps tryptophan, which are discussed below, the nitrogen of the α-amine group is the most basic site in the amino acids that compose the dipeptides examined in this study, that is, the side chain groups of most of the peptides do not contain sites that are more basic than the α-nitrogen. The amide functional group of a dipeptide is expected to be less basic than its N-terminus amine group since amines are more basic than amides of comparable polarizability. For example, *n*-propylamine is 13 kcal/mol more basic than methylformamide and diethylamine is 9 kcal/mol more basic than dimethylacetamide.[45] Consequently, one would expect that dipeptide basicities should parallel those of their N-terminus amino acids. For the dipeptides studied here, this is certainly not the case. Ten of the dipeptides, including GV, DV, VM, VY, VF, VW, VP, VE, and VK, have C-terminus amino acid residues that are more basic than their N-terminus amino acid. (A key to the one-letter amino acid codes is provided at the bottom of Table I.) The measured GBs of this subset of dipeptides is 5–15 kcal/mol higher than that of their N-terminus amino acids. For many dipeptides, this trend can be explained by an enhancement in hydrogen bonding between the nitrogen at the N-terminus and the carbonyl oxygen of the amide group for the dipeptide (structure **1**) versus the strength of this bond in the free amino acid. Recent work by Kinser and



1

(45) Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*, 695–808.

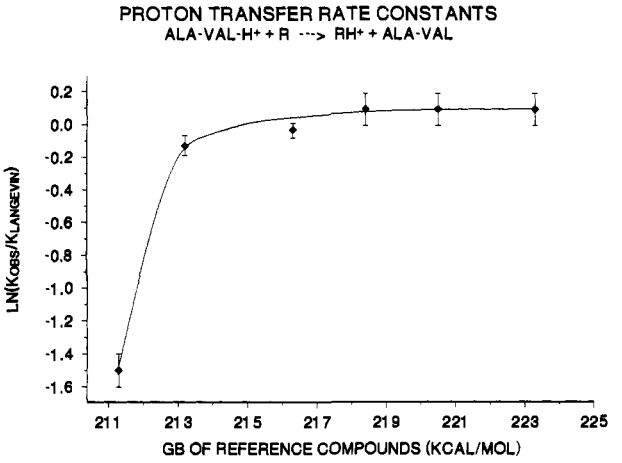


Figure 5. Plot of the rate constants for proton transfer of AV to five reference compounds versus their gas-phase basicities. Error bars indicate ±1 standard deviation from an average of three measurements. A solid line is included only to guide the eye.

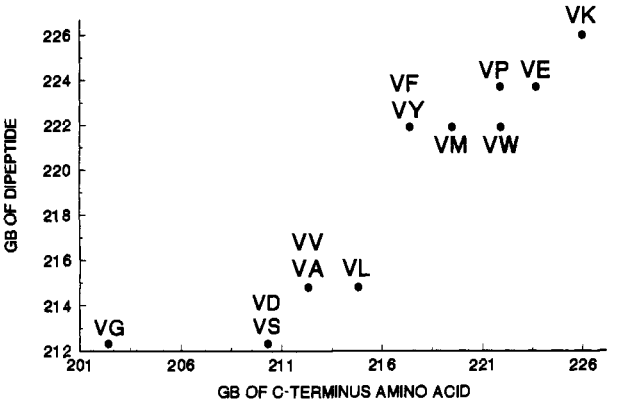


Figure 6. Gas-phase basicities of dipeptides with a valine N-terminus plotted versus gas-phase basicities of their C-terminal amino acids. The gas-phase basicity of valine is 212.3 kcal/mol.

Ridge on glycine and glycinamide have shown an increase in the proton affinity of the amide over the free amino acid by 13 kcal/mol. They have performed calculations on the structures of the protonated molecules of glycine and its amide and found that the distance between the proton on the N-terminus nitrogen and the carbonyl oxygen is shorter for glycinamide (1.750 Å) than for glycine (1.930 Å), consistent with a stronger hydrogen bond for the amide. A hydrogen bond was not present in the neutral forms of these compounds. A stronger hydrogen bond accounts for the enhanced basicity of the amide over the free amino acid.[46] Lebrilla and Wu have reported similar findings in a study of glycine and glycine peptides, based on both measurements and semiempirical calculations.[14] The results of the work presented here are also consistent with a stronger intramolecular hydrogen bond, illustrated by the dotted line in structure **1**, in the N-terminus amino acid of a dipeptide compared to that of its free amino acid. For example, glycylvaline is 12 kcal/mol more basic than glycine. In addition, the data show that for a fixed amino acid at the N-terminus, the basicity of the dipeptides increases with an increase in the basicity of its C-terminus amino acid. This trend is apparent from a comparison of the GB values of dipeptides with a valine N-terminus, as illustrated in Figure 6. For dipeptides with C-termini that are less basic than valine (VG, VS, and VD), the GBs of the peptides are equal to that of valine (212.3 kcal/mol). For dipeptides in which the C-termini have the same basicity as valine (VV and VA), the GBs are slightly higher than that of

(46) Kinser, R. D. Ph.D. Thesis, University of Delaware, 1993.

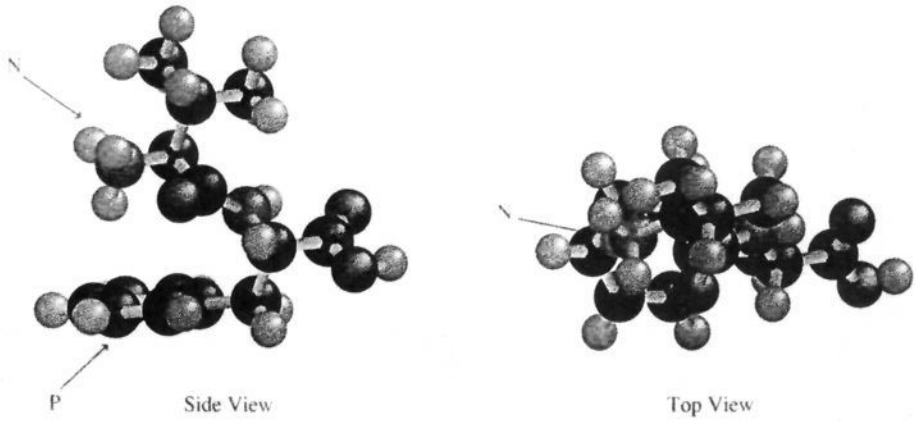


Figure 7. Two views of the most stable configuration of the protonated molecule of valanylphenylalanine, determined by molecular mechanics calculations. The ammonium group, indicated by the label N, can be seen to be oriented over the phenyl ring, indicated by the label P.

valine. For dipeptides in which the C-termini are more basic than valine (VL, VF, VY, VM, VW, VP, VE, and VK), the GBs increase as the basicities of their C-terminus amino acid increase. The data are consistent with an increase in the strength of the intramolecular hydrogen bond of structure **1** as the basicity of the C-terminus amino acid increases and suggest an inductive effect. However, in the case of VF and VY, the increase in basicity makes the dipeptides significantly more basic than their constituent amino acids. This suggests an additional stabilizing interaction, which is discussed below. One explanation of the general trend is that the more basic amino acids have more electron density at their nitrogen atoms and form amide bonds in dipeptides with enhanced electron density at the amide carbonyl oxygen. The more nucleophilic carbonyl oxygen can, in turn, form a stronger hydrogen bond, as in structure **1**. An alternate interpretation of these results is that the site of protonation lies within the most basic amino acid residue, even when these are C-terminus residues. This interpretation seems unlikely as the amide functionality is the most basic site for the C-terminus residue of most of the dipeptides, and an amide functional group is less basic than the primary amine group of an N-terminus, as discussed above.

It would be surprising if the inductive effect of C-terminus amino acids increased the stability of a dipeptide so that the GB was always the same as that of its C-terminus amino acid. In fact, this generalization can be seen to be only a close approximation to the observed behavior, as 6 of the 22 dipeptides are more basic than their most basic amino acid constituent. For dipeptides VV, VP, and VM, the increase in the basicity of the dipeptide seems less significant than the experimental error of the measurement. However, we can be certain that these dipeptides are all more basic than their C-terminus amino acids, based on bracketing measurements, as they fall in a bracket that is higher in basicity than that of their constituent amino acids. The values of experimental error listed reflect uncertainties in the assignment of the *value* of gas-phase basicity but not in the relative order of basicity.

This trend in gas-phase basicities suggests a general rule that dipeptide basicity is equal to that of the most basic residue. However, this broad generalization obscures more subtle effects such as through-space interactions of the site of protonation with the side chains of amino acids, as discussed below, and influence of steric factors. For example, GV is found to be 11.6 kcal/mol more basic than glycine, but VV is only 2.5 kcal/mol more basic than valine. It seems that the inductive effect of the valine C-terminus residue on the intramolecular hydrogen bond illustrated in structure **1** is much greater when the N-terminus is glycine than when it is valine. This may be an example of a steric effect caused by the R_1 group of structure **1**. Steric compression when R_1 is isopropyl, as in the case of valine, versus hydrogen, as in the case of glycine, may reduce the stabilization energy of structure **1** for valine versus that for glycine.

Side-Chain Intramolecular Interactions. Dipeptides VF and VY are more basic than their constituent amino acids (4.5 ± 3.9 kcal/mol). This increase can be rationalized as resulting from the interaction of the charge on the ammonium group at the N-terminus with the polarizable electrons in the aromatic ring of the C-terminus amino acid side chain. Calculations of the most stable configurations of VF and F were made via molecular mechanics using the Tripos force field, which includes terms for forces resulting from electrostatic interactions.[47] The lowest energy configuration of dipeptide VF is shown in Figure 7. The ammonium group of its N-terminus is located directly over the aromatic ring. In contrast, the amino acid phenylalanine cannot attain such a favorable, stabilizing configuration. In Figure 8, we see the results of a similar calculation for protonated phenylalanine. The ammonium group lies close to one edge of the ring and so its interaction with the electrons in the phenyl ring is reduced compared to that of the dipeptide. For this reason, VF is more basic than its constituent amino acids because the *stabilizing electrostatic interaction is significant only in the case* of a dipeptide. FV is found to be no more basic than phenylalanine itself, consistent with the arguments above, since the interaction of the ammonium terminus with the phenyl ring will be the same as for phenylalanine. The advanced arguments for the enhanced basicity of VF compared to that of FV or of phenylalanine itself would also apply to tyrosine and dipeptides which contain tyrosine and would explain the enhanced basicity of VY relative to that of tyrosine.

The basicity of VW is found to be the same as that of tryptophan. If the site of protonation was the amine group at the N-terminus of the dipeptide, then the resulting ammonium group would interact with the benzene ring of the indole group in a similar fashion to that described above for VF, according to molecular mechanics calculations, which also show that this interaction would not be possible for the amino acid itself. This suggests that the basicity of dipeptide VW should be greater than that of tryptophan, if the site of protonation for these molecules was the N-terminus amine group. Since the experiments do not show such an increase in basicity, the results suggest that the proton may be located in the indole side chain of tryptophan. If the side chain is protonated, then the position of the amino acid in the peptide is not expected to have an effect on its basicity.

(47) The calculations used SYBYL and MM3(91) programs, available from Tripos Associates, Inc., 1699 South Hanley Road, Suite 303, St. Louis, MO 63144.

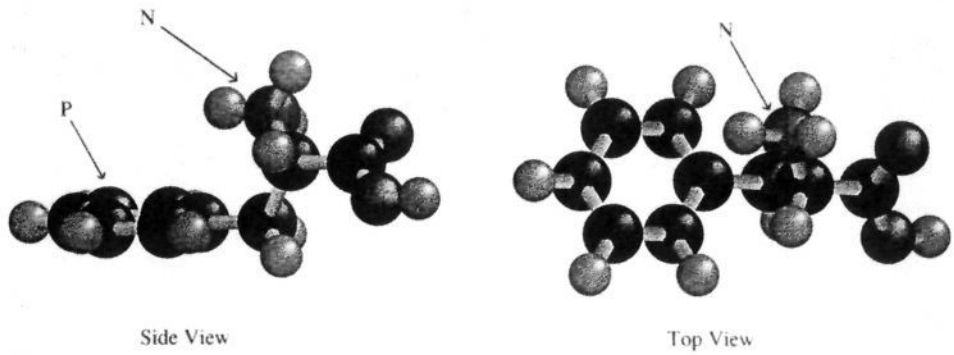


Figure 8. Two views of the most stable configuration of the protonated molecule of phenylalanine, determined by molecular mechanics calculations. The ammonium group is indicated by the label N, and the phenyl group by the label P.

Also of note is that both of the dipeptides VK and KV (K = lysine) have the same basicity as lysine. Lysine is the second most basic amino acid[11] because the amine group on its side chain forms an intramolecular hydrogen bond with the amine group attached to its central carbon.[11,35,48] This hydrogen bond is expected to occur in dipeptide KV, and so it is not surprising that KV has the same GB value as lysine. However, for VK, the nitrogen of the α-amine group of lysine has transformed into an amide nitrogen, reducing its basicity and reducing the likelihood of its participation in an intramolecular hydrogen bond. The large GB value of VK suggests that the nitrogen of the lysine side chain has formed a strong hydrogen bond to some other atom in the dipeptide. The oxygen of the amide carbonyl and the nitrogen at the N-terminus both have sufficient electron densities to form a strong hydrogen bond, but entropic considerations slightly favor the oxygen atom as the site of protonation, which would form a ring composed of 10 atoms versus 11 atoms for hydrogen bonding to the N-terminus nitrogen.

The dipeptides used in this study are composed of valine combined with 13 of the 20 common α-amino acids. The selection of this subset (22 of 39 possible combinations) of valine-containing dipeptides was based on their commercial availability. The broad range of basicities possible for the amino acids is well represented by the constituents of dipeptides studied here and includes amino acids of low basicity (Gly, Asp, Ser, Ala, Val, and Leu), intermediate basicity (Phe, Tyr, Met, Pro, and Trp), and high basicity (Lys and Glu). Dipeptides containing the most basic amino acid, arginine, were not studied. Arginine is more basic than any of the reference bases that can be used with the methods employed here, and so only a lower limit of basicity would be established for such compounds in any case. Recent work by Fenselau suggests that amidine derivatives have proton affinities that exceed that of arginine,[7] and so these may be useful for future studies of peptides containing this highly basic amino acid. The low volatility of amidine compounds will require a modification of the experimental approach used in these studies, such as laser desorption of both the peptide and the amidine compound.

(48) Campbell, S.; Beauchamp, J. L.; Rempe, M.; Lichtenberger, D. L. *Int. J. Mass Spectrom. Ion Processes* **1992**, *117*, 83–99.

Conclusions

The results presented here demonstrate the utility of substrate-assisted and matrix-assisted laser desorption methodologies for studies of the gas-phase chemistry of nonvolatile compounds. With these techniques, peptides can be desorbed either as neutral molecules or as protonated ions and thus take the role of either the ion or the molecule in an ion–molecule reaction. Further studies will be directed at experiments in which both the peptide and the reference base are desorbed by independent lasers in order to utilize the highly basic, low volatility amidine compounds, which can be used to bracket arginine and arginine-containing peptides.

Acknowledgment. Financial support by the National Science Foundation (CHE-9024922) is gratefully acknowledged. The authors wish to thank Mr. Eugene Stewart and Dr. Philip Bowen of the University of Georgia Computational Center for Molecular Structure and Design for their assistance in performing molecular mechanics calculations.