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Structural Determinants of Gas Phase Basicities of Peptides

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Abstract: Gas phase basicities of more than two dozen peptides have been measured in studies of structural determinants. These were determined using the kinetic method of dissociation of proton-bound dimers with a tandem four-sector mass spectrometer. Basicities of peptides were found to be higher than those of amino acids, and values for polyalanines with residues ranging from 1 to 6 were found to increase with the length of the polymer. Basicities of systematically varied peptides are rank ordered with increments analogous to the proton affinities of the most basic amino acids present in each. The position of the most basic residue was found in a series of tripeptides to have only a small influence (less than 2 kcal/mol) on basicity, in the order: amino terminus > internal > carboxyl terminus. The kinetic method can provide good thermochemical values when it is used with caution, as is discussed in this paper.

INTRODUCTION

Historically the chemistry of peptides has been studied in aqueous solutions. Through the last fifteen years, however, peptides have been brought into the gas phase by plasma desorption, fast atom or ion bombardment, laser desorption and electrospray techniques, which, used in conjunction with mass spectrometry, have provided important information on primary structures[1,2]. Little is known about the physical and chemical properties of peptides in the gas phase. An increased understanding of these properties would be expected to contribute to improved ionization techniques and methods for structural analysis. Comparison of gas phase with solution behavior may also provide a means to access the essential role of solution, crucial

This paper is dedicated to Professor Carl Djerassi.

to all life forms.

Acid and base properties provide a fertile area for exploration. Amino acids, for example, form zwitterions in aqueous solution, while at least one study (of glycine) reports that they exist as non-ionic molecules in the gas phase[3]. The proton affinities of all the gaseous amino acids have been measured, most of them several times with quite different methods and different types of mass spectrometers[4-6]. General agreement is found in these studies, within the experimental uncertainty, with two exceptions, namely glutamate and glutamine. The proton affinities for nineteen of the twenty common eucaryotic amino acids range from about 210 kcal/mol for the least basic amino acid, glycine, to about 232 kcal/mol for histidine. Recent measurements in this laboratory have shown that the proton affinity of the most basic amino acid, arginine, lies well above the others at 245.2 kcal/mol[6].

Protonation of peptides may be more complicated, due to the presence of multiple functional groups that can interact with the proton[7,8]. In the present study basicities of a number of peptides are measured in order to evaluate the effects of different structural features normally encountered in peptides. In subsequent studies the basicities of motifs that signal macromolecular interactions will be evaluated.

The gas phase basicity is defined as the negative free energy change of protonation of a base, while the proton affinity is the negative enthalpy change of protonation. Relative gas phase basicity and proton affinity are usually determined by equilibrium methods that require stable pressure of both a reference base and the unknown base. The low volatility of peptides limits the use of equilibrium methods for their study. Recent studies of proton affinities and acidities of amino acids [3-6,9] and peptides[7,8,10] have employed either the bracketing method or the kinetic method.

The kinetic method measures a thermochemical property from the relative dissociation rate of a dimer ion[11]. It has been applied to determinations of proton affinity[12], gas phase acidity[13], electron affinity[14], and metal ion affinity[15]. In proton affinity work a proton-bound dimer is formed between a reference base B_i and the unknown base B, whose dissociation is dominated by two unimolecular reactions:

$$[B\cdots\cdots H\cdots\cdots B_i]^+ \xrightarrow{k_i} B + B_iH^+ \quad (1a)$$

$$[B\cdots\cdots H\cdots\cdots B_i]^+ \xrightarrow{k} B_i + BH^+ \quad (1b)$$

From the absolute reaction rate theory for unimolecular reactions[16], and with the assumption that reverse barriers are close to zero[11],

$$\ln (k_i/k) = \ln (Q_i^{\neq}/Q^{\neq}) + [PA(B_i) - PA(B)]/RT \quad (2)$$

where k_i and k are the rate constants of the competing reactions (1a) and (1b), and are approximately equal to the relative ion abundance in the mass spectra. $Q_i^{\neq}$ and $Q^{\neq}$ are the partition functions of the activated complex for the formation of $B + B_iH^+$ and $B_i + BH^+$. T is the effective temperature. PA(B) and $PA(B_i)$ are the proton affinities of bases B and B_i. When B and B_i are similar bases, $Q_i^{\neq}=Q^{\neq}$ or $\ln(Q_i^{\neq}/Q^{\neq})=0$, and from equation (2), the proton affinity of an unknown base B can be determined. The difficulty is to find appropriate reference bases that are structurally similar and have proton affinities close to those of peptides. In a previous paper[7] we used as reference bases a series of amines that are structurally dissimilar to peptides but similar among themselves to avoid this difficulty. A very good correlation between the ratios of rate

constants $\ln(k_i/k)$ and proton affinities indicates that $\ln(Q_i^{\neq}/Q^{\neq})$ is roughly constant among the reference bases. However, when collisional activation was used to change the effective temperature, a large entropy effect was found[8], indicating that $\ln(Q_i^{\neq}/Q^{\neq})$ would not be zero. Notice that with the assumption of zero reverse barriers, the term $R\ln(Q_i^{\neq}/Q^{\neq})$ is equal to ΔS, the difference in entropy of protonation between the reference base B_i and the unknown base B. Thus the logarithm of the ratio of rate constants is appropriately related to the difference of gas phase basicity (GB) by

$$\ln (k_i/k) = [GB(B_i) - GB(B)]/RT \quad (3)$$

It has been shown[8] that lowering the internal energy or effective temperature of the dimer ions decreases the importance of entropy. This can be easily understood by examining equation (2). The first term $\ln(Q_i^{\neq}/Q^{\neq})$ on right side of the equation will decrease relatively to the second term as the effective temperature decreases. Therefore, dissociation of metastable ions is preferred over collisionally activated dissociation. In the present work only metastable dissociation was used.

The kinetic method is quite sensitive. It has the potential to differentiate proton affinities as small as 0.1 kcal/mol. Carefully used, it can provide thermochemical data that are consistent with those measured by equilibrium approaches.

EXPERIMENTAL

Experiments were similar to those in the earlier work that determined the proton affinity of arginine and polyglycines[6,7]. All experiments were performed on a JEOL JMS-HX110/HX110 tandem four-sector mass spectrometer with EBEB geometry. The proton-bound dimers were generated by a JEOL fast atom bombardment (FAB) gun operated by at 5 kV. Glycerol and 3-nitrobenzyl alcohol were used as FAB matrices. The spectra were mass-analyzed ion kinetic energy (MIKE) spectra. Proton-bound dimer ions were extracted at 10 kV, selected by the first two sectors (EB) and then allowed to dissociate spontaneously in the third field free region without the use of collision gas (metastable dissociation), and detected immediately after passage through the second electrostatic analyzer. The spectra were averaged profile data of four or five scans with an acquisition time for every scan of about 30 seconds. The selected precursor dimer ions usually had very good intensities relative to the chemical background of the FAB matrix. In the MIKE spectra, ions corresponding to protonated species of one or both of the bases are the only or major products. Ion abundance ratios were calculated from the areas of the peaks.

Amines were purchased from Aldrich Chemical Company (Milwaukee, WI). Most peptides were purchased from Sigma Chemical Co. (St. Louis, MO). GKKGG, GKGKG and KGGGK were synthesized by the Macromolecluar Resources in the Colorado state University (Fort Collins, CO). Typically 0.1 mg of sample was dissolved in 50 μL of 1% trifluoroacetic acid (TFA) solution. In order to optimize the abundance of the cluster ions, extra TFA was added to some solutions.

RESULTS AND DISCUSSION

Polyalanine and Polyglycine

Basicities of several polyglycines were reported earlier[7]. Recently, using collisional activation, we

determined that the entropies of protonation for [Gly]3 and [Gly]4 decrease 10-15 cal/mol·K compared to that of the reference amines[8]. This corresponds to an increase of proton affinity by 4-6 kcal/mol for the peptides. The proton affinities of the amines are about 8 kcal/mol higher than their basicities, which come from the entropy of protonation. Assuming that other peptides exhibit similar entropy effects, proton affinities would be about 13 kcal/mol higher than the basicities. Here we report basicities only and proton affinities can be estimated as above.

A polyalanine series has been examined in the present work (Table 1), using the assumptions discussed in the introduction. Figure 1 shows a typical calibration line, for $[Ala]_4$. The correlation coefficient of linear

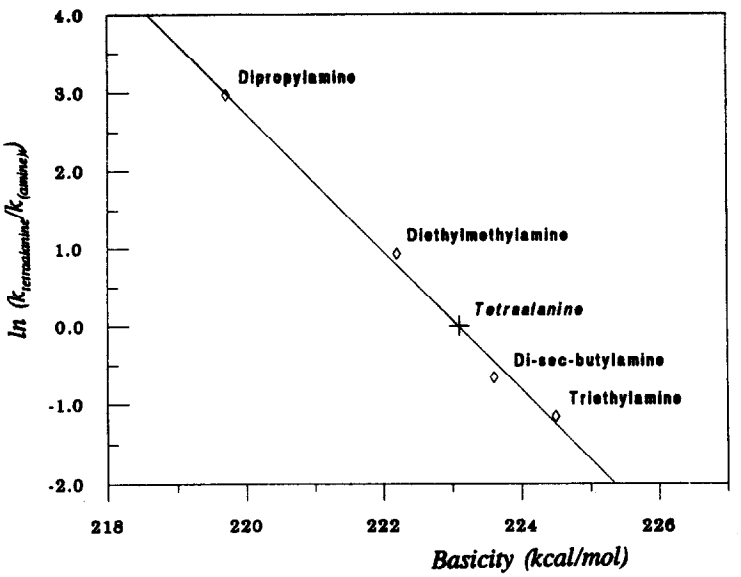

Figure 1. Calibration curve allowing determination of the basicity of tetraalanine $[Ala]_4$.

regression is 0.99. For the polyalanines and other peptides reported in this paper the coefficients range from 0.9 to 0.999. Such good correlations confirm the dependence of the ratios of dissociation rates on relative basicities, and the constancy of $(Q_i^{\bullet}/Q^{\bullet})$ among the reference amines. The uncertainties from experimental data and those from the linear regression are smaller than 0.5 kcal/mol, and the uncertainties of basicities of reference bases in the literature are about ± 0.3 kcal/mol. Therefore the values of basicities of our measurements should be no better than ± 0.8 kcal/mol. For the large peptides $[Gly]_7$, $[Gly]_8$, $[Gly]_{10}$ and $[Ala]_6$, only two references were used and the poor sensitivity for these dimer ions raises the uncertainties to about ± 2.5 kcal/mol.

Equation (3) also allows calculation of the effective temperatures of dimer ions undergoing unimolecular dissociation. The effective temperature reflects the internal energies of metastable ions. For the polyglycines and polyalanines the effective temperatures range from 350 to 600 K. Differences in effective temperatures will influence the accuracies of the relative values.

Figure 2 shows the basicities of polyglycines and polyalanines as a function of polymer length. The two curves are separated roughly by the basicity difference between glycine and alanine. Sperling and

Table 1. Basicities of Polyglycinces and Polyalanines

Peptide	Reference base[a]	Ratio $k_{(Peptide+H)^+}/k_{(Base+H)^+}$	GB[b]
G			**203.7**
GG			**211.3**
GGG			**215.0**
GGGG			**219.3**
GGGGG			**223.8**
GGGGGG			**226.8**
GGGGGGG			**230.2**
GGGGGGGG			**233.0**
GGGGGGGGGG			**237.2**
A			**206.6**
AA			**214.3**
	dimethylamine	18.2/1	212.8
	cyclohexylamine	1.63/1	213.4
	t-amylamine	1/1.84	213.9
	trimethylamine	1/26.2	217.3
AAA			**218.5**
	dimethylaniline	24.6/1	215.4
	trimethylamine	6.04/1	217.3
	diethylamine	3.92/1	217.7
	dipropylamine	1/3.29	219.7
	dibutylamine	1/15.2	220.3
AAAA			**222.9**
	dipropylamine	19.5/1	219.7
	diethylmethylamine	2.76/1	222.2
	di-*sec*-butylamine	1/1.94	223.6
	triethylamine	1/3.18	224.5
AAAAA			**226.2**
	di-*sec*-butylamine	21.1/1	223.6
	triethylamine	15.3/1	224.5
	tripropylamine	1.57/1	226.2
	tributylamine	1/5.12	227.0
AAAAAA			**230.0**
	tributylamine	6.14/1	227.0
	TMG	1/10.1	234.8

a. 1,1,3,3-tetramethylguanidine (TMG).

b. Gas phase basicities (GB) in kcal/mol of reference bases are taken from 19. Gas phase basicities of polyglycines are taken from reference 7. Gas phase basicities of glycine and alanine are taken from reference 19.

Cassady, using the bracketing method in a Fourier transform ion cyclotron resonance mass spectrometer (FTMS), measured the basicities of polyglycines with residues up to six[10]. The basicities determined by them are systematically lower than our values by 2 – 3 kcal/mol, but the relative values of the two sets of data are quite consistent. Differences in the two sets of data may be explained by the differences in the methods and instruments used, and also the uncertainty of temperature in both of the measurements. Increasing basicity and proton affinity have been reported through other polymer series, e.g., polyamines and polyethers[17]. Intramolecular hydrogen bonding or internal solvation of the charge or proton have been suggested for several different kinds of systems[17,18], and could explain the trend in proton affinities.

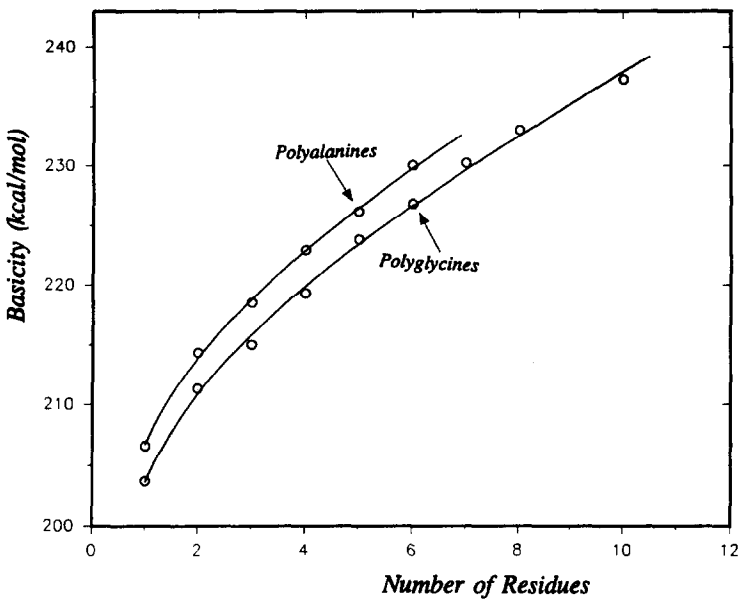

Figure 2. Gas-phase basicities of polyglycines and polyalanines of different chain lengths.

Trends in Tripeptides

Basicity order can be readily determined by applying the kinetic method to a pair of peptides. Figure 3 shows a typical MIKE spectrum of dimer ion $[GGA\cdots\cdots H\cdots\cdots GGV]^+$. The absence of other peaks in the spectrum suggests that no other reactions compete with the hydrogen bond cleavage reactions. The $[GGV+H]^+$ ions are more abundant than $[GGA+H]^+$. Therefore, the basicity of peptide GGV is higher than that of GGA. Basicity orders determined this way for three series of tripeptides are listed in Figure 4. The series of tripeptides XGG, where X stands for an amino acid, was designed to evaluate the effect of residues at the amino terminus position. Similarly the series of GGX and GXA are designed to probe the effects of carboxyl terminus and internal residues, respectively. In all three series the order of basicities of the tripeptides is in the same order as the basicities of the variable amino acid residue. It is obvious that the functional groups of the amino acids contribute to the basicities of the peptides no matter where they are located.

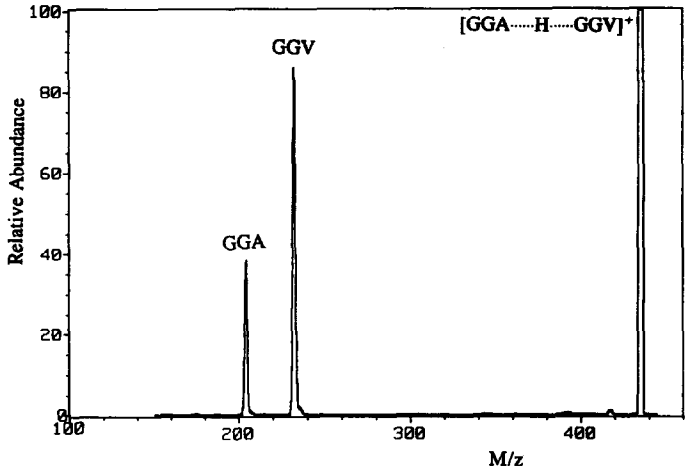

Figure 3. MIKE spectrum of the proton-bound dimer ion $[GGA\cdots\cdots H\cdots\cdots GGV]^+$.

Amino Acid	XGG	GGX	GXA
G	GGG	GGG	GGA
A	AGG	GGA	GAA
V	VGG	GGV	
L	LGG	GGL	GLA
F	FGG	GGF	GFA
Y			GYA
P			GPA

Basicity Increase

Figure 4. Basicity orders of three series of tripeptides determined using the kinetic method.

Basicities and proton affinities of several tripeptides were determined (Table 2). The differences among LGG, GLG, and GGL, in which the position of leucine varies, are less than 2 kcal/mol. Basicities of all three are about 7 kcal/mol higher than leucine (GB=210.3 kcal/mol)[19], which is the most basic residue in each of these three tripeptides. The small differences among the three indicate that the positions of basic residues in peptides are not very important. The amino terminal position has only slightly more influence on the proton affinity than the internal position, followed by the carboxyl terminal position.

Among the functional groups in a peptide, the proton affinity of the amino terminus is similar to that of glycine (NH_2CH_2COOH, PA=211.6 kcal/mol)[19], that of backbone amides is similar to N-methylacetamide ($CH_3CONHCH_3$, PA=212.7 kcal/mol)[19], and that of the carboxyl terminus is similar to acetic acid (CH_3COOH, PA=190.2 kcal/mol)[19]. The proton affinities of the amino and amide groups are very similar.

Table 2. Basicities of Peptides

Peptide	Reference base[a]	Ratio $k_{(Peptide+H)^+}/k_{(Base+H)^+}$	GB[b]
GGL			**217.2**
	t-amylamine	13.7/1	213.9
	dimethylaniline	4.65/1	215.4
	trimethylamine	1.34/1	217.3
	diethylamine	1.01/1	217.7
	dipropylamine	1/14.6	219.7
GLG			**218.2**
	t-amylamine	30.8/1	213.9
	dimethylaniline	9.18/1	215.4
	trimethylamine	3.22/1	217.3
	diethylamine	2.41/1	217.7
	dipropylamine	1/6.27	219.7
LGG			**219.0**
	trimethylamine	13.0/1	217.3
	diethylamine	7.49/1	217.7
	dipropylamine	1/1.81	219.7
	dibutylamine	1/10.5	220.3
AGG			**217.1**
	t-amylamine	14.3/1	213.9
	dimethylaniline	5.29/1	215.4
	trimethylamine	1/1.03	217.3
	diethylamine	1/1.45	217.7
	dipropylamine	1/12.7	219.7
GGA			**216.3**
	t-amylamine	5.57/1	213.9
	dimethylaniline	1.99/1	215.4
	trimethylamine	1/2.14	217.3
	diethylamine	1/2.92	217.7
GGH			**230.0**
	tributylamine	5.30/1	227.0
	TMG	1/15.2	234.8
GGR			**242.6**
	DBU	22.0/1	239.6
	HMPP	1/1.15	242.7
GKKGG			**237.8**
	TMG	9.94/1	234.8
	arginine	2.41/1	237.4
	DBN	1/1.02	237.9
	DBU	1/5.83	239.6
GKGKG			**238.5**
	TMG	20.8/1	234.8
	DBN	2.78/1	237.9
	DBU	1/3.88	239.6
KGGGK			**241.9**
	arginine	125.6/1	237.4
	DBN	82.9/1	237.9
	DBU	5.87/1	239.6
	HMPP	1/1.80	242.7

a. 1,1,3,3-tetramethylguanidine (TMG), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), 1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (HMPP).

b. Gas phase basicities (GB) in kcal/mol of reference bases are taken from 19.

Thus they are both candidates for protonation and internal hydrogen bonding. It is suggested that multiple basic sites interact simultaneously with the proton to delocalize the charge and stabilize the complex.

Some amino acids have strongly basic functional groups in their sidechains, for example, lysine (GB=222.5 kcal/mol)[19], histidine (GB=224.1 kcal/mol)[19], and arginine (GB=237.4 kcal/mol, from reference 6). The basicities of GGH and GGR (Table 2) are higher than those of H and R, respectively, again suggesting multiple interactions with the proton. However, the difference between the basicities of GGH and H is about 6 kcal/mol and between GGR and R is about 5 kcal/mol. These are smaller than the differences between GGL and L (7 kcal/mol) and GGG and G (11 kcal/mol). This trend suggests that when stronger basic sites are present the importance of interaction with the proton by multiple sites decreases and the proton is more localized.

Basicity of the KK Motif

In solution the protease clostripain cleaves proteins specifically at R and KK residues, however, not at a single K site. This suggested to us that KK contiguity increased the basicity to a value nearer that of R. Three pentatpeptides were synthesized in order to test that hypothesis in the gas phase, GKKGG, GKGKG and KGGGK. The measured basicities are listed in Table 2, where the peptide with contiguous KK can be seen to be the least basic of the three. The gas phase basicity of KGGGK may be highest because lysine is located at the amino terminal.

CONCLUSION

Gas phase basicities and proton affinities of peptides are found to depend generally on which amino acids are incorporated and on the size of the peptide. The position of a basic residue in small peptide chains has less influence on the basicity. It is suggested that multiple basic functional groups in the peptide interact with the proton simultaneously, delocalizing the charge and stabilizing the proton-bound peptide. *Ab initio* calculations of glycine and protonated glycine show that more than a dozen conformers exist with only small differences in energy[9,20]. One would expect more conformations for gaseous peptides, and in reality the measured basicities probably represent a large number of conformations.

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