Proton Affinity of Lysine Homologues from the Extended Kinetic Method

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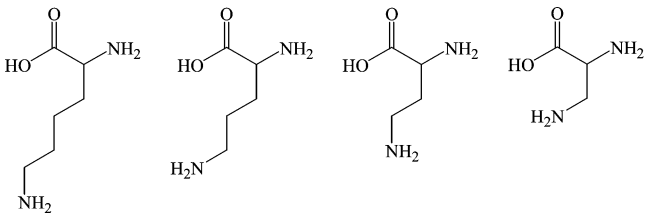
Received: July 3, 2003; In Final Form: October 14, 2003

The proton affinities of lysine (**1**) and its three homologues ornithine (**2**), 2,4-diaminobutanoic acid (**3**), and 2,3-diaminopropanoic acid (**4**) have been determined using two different variants of the extended kinetic method in an electrospray ionization−quadrupole ion trap instrument. A value of 1004.2 ± 8.0 kJ/mol is recommended for the proton affinity for lysine on the basis of this work and previous experimental measurements and theoretical predictions. Values of 1001.1 ± 6.6, 975.8 ± 7.3, and 950.2 ± 7.1 kJ/mol have been determined for the proton affinities of **2**−**4**. These experimental results are supported by hybrid density functional theory calculations at B3LYP/6-311++G**//B3LYP/6-31+G*. An analysis of the derived entropy terms lends support to the notion that these values can be used as a quantitative prediction for the thermodynamic entropy of protonation provided that appropriate error bars are assigned. Finally, for systems in which this entropy term is large, it is essential that the extended kinetic method be used to derive accurate proton affinities.

Introduction

As the building blocks of proteins and peptides, amino acids have been the subject of intense experimental and theoretical study. With the advent of soft ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption (MALDI), it has become possible to investigate the fundamental chemical properties of amino acids and other biologically important molecules using gas-phase ion chemistry techniques in modern mass spectrometers. Determinations of the gas-phase acid/base properties of the 20 protein amino acids (PAAs) were among the first experiments to be performed with these new ion sources.[1−7] Early on it was established that the three amino acids with the highest pK_a values in solution,[8] arginine (Arg), histidine (His), and lysine (Lys, **1**), also have the largest proton affinities (PAs) in the gas phase.[2,3,5,9,10]



1 **2** **3** **4**

In the case of lysine, the large basicity is explained, in part, by its ability to form a strong intramolecular hydrogen bond between the two amino groups when protonated.[8] The pioneering studies of Kebarle,[11] Aue and Bowers,[12] and Moet-ner[13] established that species that can dicoordinate a proton exhibit enhanced PAs. For example, the α,ω-diamines $NH_2(CH_2)_nNH_2$ have PAs that are substantially larger than those of monoamines of similar polarizability.[11−16] Lysine is simply 1,5-diaminopentane that is substituted with a COOH group at the 2-position. The lower homologues of lysine, the nonprotein amino acids (NPAAs) ornithine (**2**), 2,4-diaminobutanoic acid (**3**), and 2,3-diaminopropanoic acid (**4**), should also form strong internal hydrogen bonds when protonated and should therefore have enhanced basicities in the gas phase. We have been studying the intrinsic gas-phase thermochemical properties of nonprotein amino acids in an effort to gain a deeper understanding of the relationship between amino acid structure and thermochemistry.[17−19]

The gas-phase PA of a molecule M is defined as the negative enthalpy of protonation, which is simply the enthalpy of reaction 1. The Gibbs free energy change for reaction 1 is defined as

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

the gas-phase basicity of M, ΔGB. Finally, the entropy change for reaction 1 is the negative entropy of protonation, ΔS_{prot}. Many gas-phase thermochemical techniques such as gas-phase equilibrium experiments and proton-transfer bracketing experiments are sensitive to ΔG(M).[20] To obtain proton affinities from these techniques, two different approaches are taken. The first of these is to use estimates for ΔS from statistical mechanics,[21] group equivalents,[22] or high-level theoretical calculations.[23] A second method involves determining ΔG at various temperatures and using a van't Hoff analysis to extract ΔH and ΔS values directly.[24,25]

An alternative approach is the Cooks kinetic method in which thermochemical information is extracted from the ratio of product ions from the decomposition of proton-bound dimer ions.[26−28] Early applications of the kinetic method sought to minimize the effects of entropy by using reference compounds similar in structure to the unknown compound of interest.[28] For such a case, the transition states for decomposition through the two channels are assumed to have similar entropies of activation, $\Delta S^{\ddagger}$, and therefore, the difference in ΔH between the reference and unknown is assumed to be equal to the difference in ΔG.[28] On the other hand, if the reference compounds are different in structure than the unknown compound, entropy requirements for the two channels may be quite different.

Entropy and enthalpy contributions for these dissociations can be determined directly by using the extended kinetic method,

10.1021/jp0359182 CCC: $27.50
Published on Web 12/09/2003

in which the decomposition is carried out at different collision energies, corresponding to different effective temperatures.[10,29−31] A van't Hoff-like analysis is then carried out to extract enthalpic and entropic contributions to the dissociation directly. The exact quantitative nature of the entropy term from this experiment has been the subject of recent debate.[32,33] The extended kinetic method has been used recently to determine proton affinities and entropies for a variety of compounds, including amino acids.[14,16,17,34−41]

As the lysine analogues **1**−**4** can form internal hydrogen bonds when protonated, they are attractive candidates to investigate the effects of entropy on the dissociation of proton-bound dimer ions containing them. Reference bases were chosen that have no possibility of intramolecular hydrogen bonding to maximize the entropy effects. This approach has been used before to determine proton affinities for amino acids[36] as well as for the α,ω-diamines, which can also form intramolecular hydrogen bonds.[14−16]

We report here a reevaluation of the gas-phase proton affinity of lysine and the first experimental determination of the proton affinities of several of its homologues (**2**−**4**). In addition, the results of high-level hybrid density functional theory calculations are presented that confirm both the experimental proton affinities and the intramolecularly-hydrogen-bonded structures for protonated **1**−**4**. Finally, a discussion of the derived entropy term from the extended kinetic method is presented.

Experimental Section

All experiments were performed in a Finnigan LCQ-DECA instrument using conditions outlined in detail elsewhere.[17] Briefly, dilute solutions (49.5% MeOH/49.5% H_2O/1% HOAc) of a lysine homologue and a reference base of known proton affinity were directly infused into the electrospray ionization source of the LCQ at flow rates in the range of 5−35 μL/min. Solution concentrations were varied to maximize the production of proton-bound dimers of the lysine homologue and the reference base and were usually in the range of 5×10^{-5} to 5×10^{-4} M. Electrospray and ion-focusing conditions were also varied to maximize the ion count for the proton-bound heterodimer. The proton-bound dimer ions were isolated at $q_z = 0.250$ and with a mass width adjusted to maximize the ion signal while isolation was still maintained. The isolated ions were allowed to undergo collision-induced dissociation with the background helium atoms at a variety of activation amplitudes between 15% and 85%, corresponding to laboratory frame energies between 0.75 and 4.25 V. The ratio of protonated lysine homologue to protonated reference base was obtained from the average of 40 individual CID scans. Average ratios were obtained from between 15 and 20 measurements performed on several different days.

Proton affinities and entropy contributions are obtained from the extended kinetic method that has been described in detail elsewhere.[10,30,31] Two plots are generated for the standard extended kinetic method analysis (method I). The first plot (plot 1) is of $\ln[I(\mathrm{Ref}_i\mathrm{H}^+)/I(\mathbf{1H}^+)]$ vs $\mathrm{PA}_i - \mathrm{PA}_{av}$, where PA_i is the proton affinity of reference base i and PA_{av} is the average proton affinity of the set of 3−5 reference bases. Best-fit lines to the data are made at each of the activation energies, and negative values of the intercepts of these lines are plotted vs their slopes in a second kinetic method plot (plot 2). The slope of the best-fit line in plot 2 is $\mathrm{PA}_{AA} - \mathrm{PA}_{av}$, and the intercept is the average difference in activation entropy between the lysine homologue channel and the reference base channels (vide infra).

Proton affinities are also obtained from the same data using the entropy-corrected kinetic method (method II) of Cooks[33] in which $\Delta S[\mathrm{Ref}_i]/R$ (Table 1) is subtracted from $\ln[I(\mathrm{Ref}_i\mathrm{H}^+)/I(\mathrm{AAH}^+)]$, and the resulting ratios are used to make a plot analogous to plot 1. Cooks and co-workers recommend removing the constant entropy of the proton (26 J mol^{-1} K^{-1}) from $\Delta S_{prot}[\mathrm{Ref}_i]$ and using simply the difference in entropy between the neutral molecule and its protonated form. The rest of the analysis remains as outlined above. In this case, the entropy term is used as a prediction for the protonation entropy of the unknown rather than an average difference between the transition-state entropies of the unknown and reference bases.[33]

TABLE 1: Measured Proton Affinities and DDS Terms for the Lysine Homologues

	method 1		method 2	
homologue	PA (kJ/mol)	ΔΔ*S* (J mol^{-1} K^{-1})	PA (kJ/mol)	ΔΔ*S* (J mol^{-1} K^{-1})
1	1006.5 ± 7.2	−77.2 ± 10	1006.5 ± 7.2	−77.3 ± 10
2	1001.1 ± 6.6	−49.6 ± 10	1001.1 ± 6.6	−52.4 ± 10
3	975.8 ± 7.4	−43.5 ± 10	975.8 ± 7.4	−35.9 ± 10
4	950.2 ± 7.2	−49.8 ± 10	950.2 ± 7.2	−48.8 ± 10

Theoretical predictions for proton affinities and gas-phase basicities were also obtained from hybrid density functional theory calculations using the B3LYP functional combinations.[42,43] All calculations were performed using the Gaussian98 suite of programs.[44] Geometries and harmonic vibrational frequencies for the lysine homologues and their protonated forms were calculated at the B3LYP/6-31+G* level. Total electronic energies were obtained from B3LYP/6-311++G** single-point calculations at the B3LYP/6-31+G* geometries. Enthalpies at 298 K were calculated using ZPE and thermal corrections obtained from scaled vibrational frequencies (scale factors were 0.9806 for ZPE and 0.9989 for thermal corrections).[23]

Predictions for the proton affinities of the lysine analogues were computed directly from calculated enthalpies at 298 K according to reaction 2 as well as from isodesmic reaction 3 with ethylenediamine (PA = 951.6 kJ/mol)[45] serving as the reference base. In addition to proton affinities, gas-phase basicities at 298 K (−Δ*G* of protonation) were also calculated for each lysine analogue.

$$\mathrm{Lys} + \mathrm{H}^+ \rightarrow \mathrm{LysH}^+ \quad (2)$$

$$\mathrm{Lys} + \mathrm{NH_2CH_2CH_2NH_3}^+ \rightarrow \mathrm{LysH}^+ + \mathrm{NH_2CH_2CH_2NH_2} \quad (3)$$

Materials

Amino acids were purchased from Sigma (St. Louis) and were used without purification. Reference bases were purchased from Aldrich and were also used without purification.

Results and Discussion

Lysine. Proton-bound dimers of lysine and one of a series of reference bases were generated from electrospray ionization. The following reference bases were used: 1-methylpiperidine, diisopropylamine, triallylamine, triethylamine, *N*,*N*-dimethylcyclohexylamine, and 2,2,6,6-tetramethylpiperidine. The recommended proton affinity values for these compounds are given in Table 2.[45] Figure 1 shows a plot of $\ln[I(\mathrm{Ref}_i\mathrm{H}^+)/I(\mathrm{LysH}^+)]$ vs $\Delta H_{B_i} - \Delta H_{av}$ (method I, closed symbols and solid lines) at three different activation energies, where ΔH_{B_i} is the proton affinity of reference base i and ΔH_{av} is the average proton affinity of the six reference bases used in the study (978.0 kJ/mol). Ratios for all experiments described in this work are given in Table S1 in the Supporting Information along with effective

TABLE 2: Thermochemical Values for Reference Bases

base	PA[a]	ΔS_{prot}[b]	1	2	3	4
pyridine	928.8	2.1				X
exo-2-aminonorbornane	935.1	−5.0				X
phenethylamine	936.4	−5.0				X
N,*N*-dimethylaniline	941.0	2.1				X
3-methylpyridine	943.5	2.1				X
piperidine	954.0	−2.1			X	
4-*tert*-butylpyridine	957.7	2.1			X	
2,6-dimethylpyridine	963.2	2.1			X	
N-methylpiperidine	971.1	5.4	X	X		
diisopropylamine	971.9	−2.1	X			
triallylamine	972.4	5.4	X	X		
triethylamine	982.0	5.4	X	X		
N,*N*-dimethylcyclohexlamine	983.7	5.0	X	X		
2,2,6,6-tetramethylpiperidine	987.0	−2.1	X	X		

[a] Units of kilojoules per mole from ref 45. [b] Entropy for the reaction $M \rightarrow MH^+$ (J mol^{-1} K^{-1}) from ref 45.

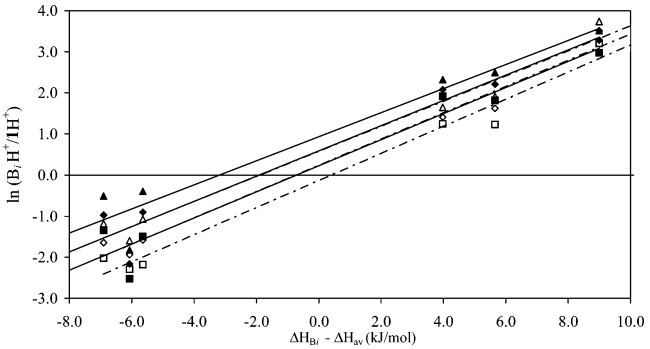


Figure 1. Closed symbols and solid lines: $\ln(B_iH^+/\mathbf{1H}^+)$ vs $\Delta H_{B_i} - \Delta H_{av}$ at activation amplitudes 15% (■), 50% (◆), and 85% (▲). Open symbols and dotted lines: $\ln(B_iH^+/\mathbf{1H}^+) - \Delta S_{B_i}/R$ vs $\Delta H_{B_i} - \Delta H_{av}$ at activation amplitudes 15% (□), 50% (◇), and 85% (△).

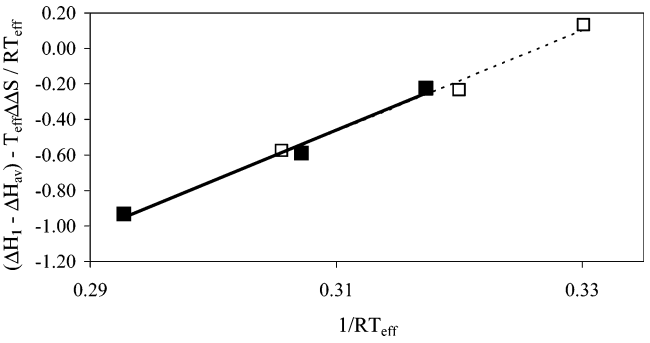


Figure 2. $[(\Delta H_1 - \Delta H_{av}) - T_{eff}\Delta\Delta S/R]/RT_{eff}$ vs $1/RT_{eff}$. The solid symbols and line are from data obtained using method I, and the open symbols and dotted line are from data obtained using method II as described in the text.

temperatures and apparent basicities. The *x*-intercepts of the best-fit lines in Figure 1 give estimates for the proton affinity of lysine ignoring entropy effects on the dissociation of the proton-bound dimer. These "apparent basicities" are in the range of 974.9−977.4 kJ/mol.

Figure 2 shows a plot of $-y_{int}$ of the best-fit lines in Figure 1 vs their slopes. The slope of the best-fit line to the data in Figure 2 is 28.5 kJ/mol, which when combined with the average proton affinity of the six reference bases gives a value for the proton affinity for lysine of 1006.5 ± 7.2 kJ/mol. Table 1 lists the experimentally measured quantities for all four lysine homologues. The uncertainty in the proton affinity for lysine is derived from the root square sum of the uncertainty in the slope of the line (3.4 kJ/mol) and the uncertainty in PA_{av}. The uncertainty in PA_{av} is composed of the relative error in the

TABLE 3: Theoretical Thermochemical Values for Lysine Homologues (hartrees)

compd	E_{elec}	ZPE	ΔH_{therm}	H_{298}	G_{298}
1	−497.190057	0.207566	0.013114	−496.969376	−497.022045
1H$^+$	−497.585217	0.224226	0.011827	−497.349165	−497.397050
2	−457.868014	0.179653	0.011689	−457.676672	−457.725678
2H$^+$	−458.257618	0.194348	0.010693	−458.052576	−458.098595
3	−418.546563	0.153139	0.009499	−418.383926	−418.426553
3H$^+$	−418.927751	0.166507	0.009502	−418.751742	−418.794661
4	−379.223785	0.124367	0.008372	−379.087277	−379.127084
4H$^+$	−379.593974	0.138832	0.008551	−379.442821	−379.483149
6	−190.587850	0.109154	0.010039	−190.468657	
6H$^+$	−190.962481	0.124219	0.009763	−190.828499	

measured quantities and a systematic error in the absolute proton affinity scale. We assign values of 6 kJ/mol for the systematic error in the absolute PA scale and $6/\sqrt{N}$ kJ/mol for the random error, where *N* is the number of measurements.[17,46] In this case, *N* is 6, and the total uncertainty in PA_{av} is the root sum square of the random and systematic uncertainties, or 6.5 kJ/mol. The *y*-intercept of the line in Figure 2 leads to a $\Delta\Delta S$ value of −77.2 ± 10 J mol^{-1} K^{-1}, where the uncertainty comes only from the uncertainty of the intercept of the line in plot 2, which is ≤5 kJ/mol for **1**−**4**. In the following discussion of entropy effects we assign conservative error bars of 10 J mol^{-1} K^{-1} for the entropy term for each of the lysine homologues to account for the use of transition-state entropies as models for thermodynamic entropies of protonation.

Cooks and co-workers have recently suggested an entropy-corrected version of the extended method in which the protonation entropy of the reference bases (minus $\Delta S[H^+]$) is explicitly used in the analysis (method II).[33] This method gives a proton affinity identical to that of method I, but a different entropy term. $\Delta S_i/R$ values for the six reference bases were taken from Hunter and Lias[45] and are listed in Table 2. $\Delta S_i/R$ is subtracted from $\ln[I(Ref_iH^+)/I(LysH^+)]$ to give entropy-adjusted ratios (see Table S1) that are plotted vs $PA_{Ref_i} - PA_{av}$ as shown in the open symbols in Figure 1. Plotting $-y_{int}$ vs the slope of these lines gives the open symbols in Figure 2, which leads to an identical PA of 1006.5 ± 7.2 kJ/mol and a $\Delta\Delta S$ value of −77.3 ± 10 J mol^{-1} K^{-1}.

Theoretical predictions for the proton affinity of lysine were also obtained from density functional theory calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level. Total energies, zero-point energies, thermal corrections, enthalpies, and free energies at 298 K for various conformers of neutral and protonated species investigated in this work are listed in Table S2 in the Supporting Information. Theoretical thermochemical values for the lowest energy conformations of these species are given in Table 3. Total electronic energies were obtained for 10 different lysine conformers, and vibrational frequencies at the B3LYP/6-31+G* level were obtained for the four lowest energy conformers. The lowest energy structure is extended with no internal hydrogen bonding between the two amino groups as shown in Figure 3a.

For protonated lysine, 20 different conformations were investigated. In these studies, the proton was initially placed either on the backbone amino group or on the side chain amino group and the geometry was allowed to optimize. All of the minimum-energy structures for protonated lysine involve strong hydrogen bonding between the amino groups. For all four homologues, low-energy structures were found that had the proton formally residing either on the backbone (α) nitrogen atom or on the side chain ($\beta-\epsilon$) nitrogen atom. The 298 K enthalpy differences between these protonated forms were in the range of 0−16 kJ/mol. We did not perform a full Boltzmann-

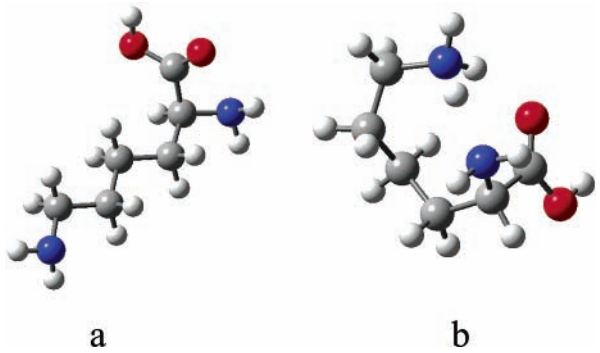


Figure 3. Lowest energy conformations of (a) lysine and (b) protonated lysine calculated at the B3LYP/6-31+G* level of theory.

TABLE 4: Derived Thermochemical Values for Lysine Homologues Obtained from Density Functional Theory Calculations[a]

homologue	PA_{iso}[b] (kJ/mol)	PA (kJ/mol)	GB (kJ/mol)
1	1003.3	1003.9	958.3
2	993.1	993.8	952.9
3	971.9	972.5	940.2
4	939.7	940.3	908.6

[a] All values from the B3LYP/6-311++G**//B3LYP/6-31+G* levl. Zero-point energies and thermal corrections calculated from scaled vibrational frequencies at the B3LYP/ 6-31+G* level. Scaling factors from ref 23. [b] Calculated using a PA of 951.6 kJ/mol for ethylenediamine from ref 45.

weighted analysis of the proton affinity for the lysine homologues as the error in simply using the lowest energy structure for the neutral and cation should be lower than the overall ca. ±8.5 kJ/mol uncertainty in the derived values. The lowest energy conformer that we found for $\mathbf{1H}^+$ has an internal hydrogen bond with N−H distances of 1.1 Å (ϵ-nitrogen atom) and 1.8 Å (α-nitrogen atom) as shown in Figure 3b.

A theoretical prediction of 1003.3 kJ/mol is obtained for the proton affinity of lysine from direct reaction 2, in excellent agreement with our experimental value. A second theoretical prediction for the proton affinity of lysine is obtained from isodesmic reaction 3. Density functional theory calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level give a prediction for the proton affinity of ethylenediamine that is only 0.6 kJ/mol lower than the recommended value from Hunter and Lias (951.5 kJ/mol).[45] Predictions for the proton affinity of each homologue from isodesmic reaction 3 are therefore 0.6 kJ/mol higher than those based on direct reaction 2 as shown in Table 4.

The measured values for the proton affinity for lysine are somewhat higher than the recommended value of 996.0 kJ/mol from Hunter and Lias.[45] This value is based on the original work of several groups,[5,47] including the Fenselau group in their initial report of the extended kinetic method,[10] taking into account the shift in the absolute proton affinity scale.[48] While their method has been modified to give a more reliable estimate of the uncertainties in the derived thermochemical values,[31] the values themselves are unchanged in the modified approach. A recent equilibrium study on the proton affinity of the amide of lysine by Ridge and co-workers[49] suggests that the proton affinity of lysine should be as high as 1009.6 kJ/mol. They also cite agreement with a theoretical estimate of the PA for lysine of 1010.0 kJ/mol from Schaefer and Amster[50] based on calculations on the model compound 1,4-diaminobutane. Maksić and Kovačević calculated the proton affinities of all 20 PAAs at

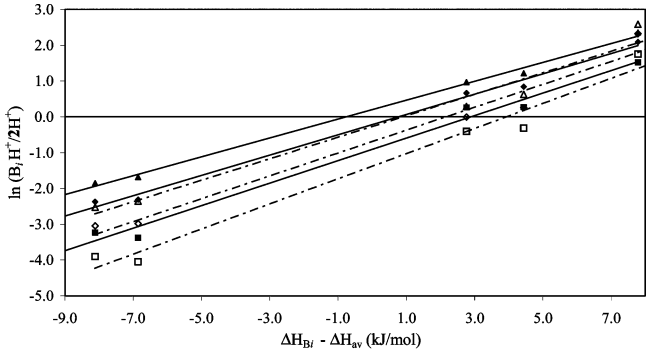


Figure 4. Closed symbols and solid lines: $\ln(B_iH^+/\mathbf{2H}^+)$ vs $\Delta H_{B_i} - \Delta H_{av}$ at activation amplitudes 15% (■), 50% (◆), and 85% (▲). Open symbols and dotted lines: $\ln(B_iH^+/\mathbf{2H}^+) - \Delta S_{B_i}/R$ vs $\Delta H_{B_i} - \Delta H_{av}$ at activation amplitudes 15% (□), 50% (◇), and 85% (△).

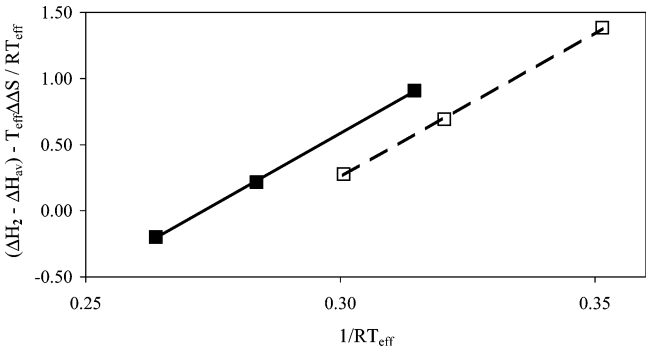


Figure 5. $[(\Delta H_2 - \Delta H_{av}) - T_{eff}\Delta\Delta S/R]/RT_{eff}$ vs $1/RT_{eff}$. The solid symbols and line are from data obtained using method I, and the open symbols and dotted line are from data obtained using method II as described in the text.

the MP2(fc)/6-311+G**//HF/6-31G* level.[51] They report a ZPE-corrected direct PA (i.e., from reaction 2) of 995.0 kJ/mol. On the basis of these prior measurements, theoretical predictions, and values determined here, we recommend a proton affinity of 1004.2 ± 8.0 kJ/mol for lysine.

Ornithine. Similar procedures were carried out to determine the proton affinity of ornithine. The same reference bases were used in the ornithine experiments with the exception of diisopropylamine; PA_{av} for these bases is 979.2 kJ/mol. Figure 4 shows the first kinetic method plot for both method I (solid symbols) and method II (open symbols). Apparent basicities for ornithine are in the range of 979.1−983.2 kJ/mol (see Table S1). The second kinetic method plots for the ornithine experiments are given in Figure 5. The two methods lead to identical proton affinities of 1001.1 ± 6.6 kJ/mol and entropies of −49.6 ± 10 and −52.4 ± 10 J mol^{-1} K^{-1}, respectively.

Density functional theory calculations were also carried out on 9 ornithine conformations and 17 different protonated ornithine conformations, with the proton initially residing on either the backbone or side chain nitrogen atom. As with the lysine calculations, B3LYP/6-31+G* calculations predict that the lowest energy structures for neutral ornithine are extended with no interaction between the two amino groups as shown in Figure S1a of the Supporting Information. The lowest energy conformer for $\mathbf{2H}^+$ has a hydrogen bond that is more equally shared between the two amino groups (r = 1.1 and 1.6 Å for the α- and δ-nitrogen atoms, respectively) as shown in Figure S1b. Proton affinities of 993.1 and 993.8 kJ/mol are predicted for ornithine on the basis of direct reaction 2 and isodesmic reaction 3, in reasonable agreement with our experimental determination.

To the best of our knowledge, there have been no experimental determinations of the proton affinity of ornithine. The gas-phase basicity for ornithine was determined by Amster and co-workers to be identical to that of lysine using proton-transfer bracketing experiments in an FT-ICR instrument.[52] Both compounds were found to have basicities between those of diethylamine and di-*n*-propylamine. On the basis of the adjusted gas-phase basicity scale,[48] these results place the GB for ornithine and lysine between 919.2 and 928.8 kJ/mol. Using their estimate of 69 kJ/mol for $T\Delta S$[52] gives a proton affinity between 988.2 and 997.8 kJ/mol, in good agreement with our experimental and theoretical values.

2,4-Diaminobutanoic Acid and 2,3-Diaminopropanoic Acid. To be useful in an extended kinetic method experiment in our instrument, a reference base must have a basicity in a range such that the ratios of ion intensities are no greater than ca. 30:1. In addition, the proton-bound dimer ion must be able to be isolated with sufficient ion intensity for MS/MS studies and must give only the expected protonated monomer fragments upon CID. We were only able to find three bases: piperidine, 4-*tert*-butylpyridine, and 2,6-dimethylpiperidine, which fit all of these criteria. A proton affinity value of 975.8 ± 7.4 kJ/mol was determined for **3** from methods I and II, respectively. Kinetic method plots for **3** and **4** are similar to those for **1** and **2** and are included in the Supporting Information (Figures S4−S7). Entropy contributions of −43.5 and −35.9 J mol^{-1} K^{-1} are obtained from these experiments.

Unlike lysine and ornithine, the lowest energy structure for neutral **3** at the B3LYP/6-31+G* level does involve the side chain amino group in intramolecular hydrogen bonding. The lowest energy structure contains both a strong hydrogen bond between the hydroxyl oxygen atom and the α-amino nitrogen (r = 1.9 Å) and a weaker interaction between the hydrogen atom on the α-amino group and the γ-nitrogen (r = 2.5 Å) as shown in Figure S2a in the Supporting Information. The lowest energy structure for protonated **3** is similar to those of protonated **1** and **2**, containing a strong intramolecular hydrogen bond between the two amino groups (1.69 Å) as well as a weaker interaction between the hydroxyl hydrogen and the α-nitrogen (r = 2.15 Å) as shown in Figure S2b. Theoretical predictions of 971.9 and 972.5 kJ/mol were determined for the PA of **3** from reactions 2 and 3.

Five reference bases were used to determine the proton affinity of **4**, as listed in Table 1. Apparent basicities for **4** were in the range of 933.9−935.1 kJ/mol. Identical proton affinities of 950.2 ± 7.2 kJ/mol and entropy values of −49.8 ± 10 and −48.8 ± 10 J mol^{-1} K^{-1} were determined for **4** from methods I and II, respectively.

The lowest energy structure for neutral **4** was found to have a strong hydrogen bond between the hydroxyl hydrogen and the β-amino nitrogen (r = 1.77 Å) as shown in Figure S3a in the Supporting Information. The lowest energy structure for the cation contains interactions between the protonated amino group and the carbonyl oxygen (r = 2.01 Å) as well as between the amino groups (r = 2.18 Å) as shown in Figure S3b. Theoretical predictions of 939.7 and 940.6 kJ/mol are obtained for **4**, somewhat lower than our experimental value.

Comparisons with α,ω-Diamines. The proton affinities of the lysine homologues can be compared to those of the α,ω-diamines with n = 2−5, for which the recommended values from Hunter and Lias are 951.6, 987.0, 1005.6, and 999.6 kJ/mol.[45] Since the Hunter and Lias compilation was published, several groups have reexamined the PAs of these species.[14−16] These studies are of mixed opinion as to the efficacy of the kinetic method to determine thermochemical properties for internally-hydrogen-bonded species. Wang et al. showed that the extended kinetic method was able to reproduce the recommended values for the PAs of the diamines with n = 2−6, lending support for the use of the method on the lysine homologues.[14] In addition, they performed high-level density functional theory calculations that lend support to their assertions. In contrast, Holmes and co-workers presented data claiming that, for the larger diamines (n = 3, 4), the kinetic method gives PAs that are too low due to reverse activation barriers.[15] Wesdemiotis and co-workers have recently reexamined the n = 2−4 systems and suggest that the magnitude of this barrier, if present, is small on the basis of the results of MIKES experiments.[16] They finally conclude that the extended kinetic method can be used to accurately measure proton affinities of species that have weak intramolecular interactions when protonated, but that for molecules with strong intramolecular hydrogen bonds the method tends to *slightly* underestimate PAs.

In the present study, the only "known" value with which to compare our PAs is that of lysine. As was discussed earlier, our value is intermediate between Fenselau's measurement[10] and Ridge's estimate based on lysinamide.[49] In addition, our value is in excellent agreement with both our own density functional theory calculations and Schaefer's ab initio estimates.[50] In other work from our laboratory, derived proton affinities are usually toward the upper end of the range of measured values (when known), but tend to be in excellent agreement with theoretical calculations, including species that participate in intramolecular hydrogen bonding.[17−19,38]

As part of a recent study of the proton affinity of another primary diamine, *cis*-1,5-cyclooctanediamine,[38] we remeasured the PA of ethylenediamine (**6**) with the extended kinetic method and the PA of 1,4-diaminobutane (**5**) using the single-reference variant[37,53] of the extended kinetic method with canavanine, an NPAA analogue of arginine,[18] serving as the reference base. In the single-reference method, a calibration curve is generated using canavanine and a series of reference bases. Multiplication of the product ratio $B_iH^+/CavH^+$ by $CavH^+/\mathbf{5}H^+$ gives the desired ratio $B_iH^+/\mathbf{5}H^+$ required for an extended kinetic method analysis. Kinetic method plots for the experiments with **5** and **6** are shown in Supporting Information Figures S8−S11. These experiments lead to a PA of 956.4 ± 6.5 kJ/mol for **6**, in excellent agreement with the recommended value of Hunter and Lias of 951.6 kJ/mol and our density functional calculations as mentioned earlier. In addition, this value is in agreement with the recent measurements of Siu and co-workers[14] and Wesdemiotis and co-workers.[16] We obtained a PA of 1005.6 ± 6.7 kJ/mol for **5**, in good agreement with both the NIST recommended value and with Siu's value of 1009.6 kJ/mol using the Fenselau method.[14] Calculations at B3LYP/6-311++G**//B3LYP/6-31+G* give a value of 1008.3 kJ/mol for the PA of 1,4-diaminobutane (see Table S1). In contrast, our measured PA is 12.5 kJ/mol larger than Wesdemiotis' value[16] and nearly 30 kJ/mol higher than Holmes' value.[15] Cooks, Vekey, and co-workers published a comment criticizing various aspects of the Holmes paper including the lack of an observed energy dependence from changing target gases.[54] The origins of the discrepancies in measured proton affinities are unclear, and experiments are currently being performed in an effort to resolve them. Ultimately, the fact that (1) we can reproduce the Lias values for the PAs of **5** and **6** with both our experimental measurements and our theoretical calculations, (2) our experimental and theoretical values for **1** are in agreement with recent

literature values, and (3) our experimental values for **2**−**4** are in agreement with theoretical calculations lends support to our measurements.

Our results indicate that the ordering of proton affinities in the lysine homologues mirrors that of the diamines, with $n = 4$ and $n = 5$ having nearly the same PA and the two shorter homologues having lower PAs. They also indicate that substitution of a COOH group in the 2 position of a diamine causes little or no change in the PA, except for **3**, where the substitution results in a decrease of almost 12.5 kJ/mol. This result is in contrast to the trend found in other amino acids in which similar COOH substitutions cause a decrease of ca. 8 kJ/mol in PA from the amine to the amino acid.[7] For example, in our recent work on proline analogues the PAs of the amino acids were all 6 kJ/mol less than those of the corresponding heterocyclic amines.[17] The decrease in basicity in the amino acids is presumably due to the electron-withdrawing nature of the COOH group. In the lysine homologues, it appears that these inductive effects are overwhelmed by the stabilization of the cation by intramolecular hydrogen bonding.

Entropy Effects. One of the main goals of this work was to evaluate the nature of entropy effects in the extended kinetic method. The entropy term that arises from the extended kinetic method analysis has been the subject of intense debate over the past few years.[31−33] Cooks and co-workers assert that when the entropy-corrected method is used, the intercept of plot 2 can be used as a *prediction* for $\Delta S_{prot}/R$ of the unknown species,[33] whereas Ervin[32] and more recently Wesdemiotis[16] assert that the intercept is not related to a thermodynamic quantity, but represents only a difference in entropy of the microcanonical density of states at the given activation energy. As the COOH substitution in the lysine homologues is unlikely to cause a large change in the protonation entropy, estimates for ΔS_{prot} for the lysine homologues can be obtained from ΔS_{prot} for the diamines.

Hunter and Lias list several values for ΔS_{prot} for **6** ranging from −18.5 to −55.2 J mol^{-1} K^{-1} (the entropy of the proton (108.7 J mol^{-1} K^{-1}) has been subtracted from ΔS_{prot} to facilitate comparison with the kinetic method data).[45] These values are based on gas-phase equilibrium experiments from Moet-ner,[13] Aue and Bowers,[12] and Kebarle.[11] In general, Moet-ner's values are less negative than values from the other two studies and have been chosen as the recommended values. No explanation as to why these values were chosen over the more negative entropies was given. Similar large ranges of protonation entropies are listed for 1,3-diaminopropane (−47 to −81 J mol^{-1} K^{-1}), **5** (−63 to −100 J mol^{-1} K^{-1}), and 1,5-diaminopentane (−70 to −96 J mol^{-1} K^{-1}). When the entropy-corrected ratios are used in the kinetic method analysis, values for $\Delta\Delta S$ of −49, −36, −50, and −77 J mol^{-1} K^{-1} are obtained as predictions for ΔS_{prot} for **4**−**1**, respectively. Given the uncertainty in the measured entropy values of ca. 10 J mol^{-1} K^{-1} (vide supra), the fact that we are using the diamines as models for the lysine analogues, and the wide range of protonation entropies for the diamine from the literature, the predicted entropy values for **1**−**4** seem reasonable. The entropies for **4** and **1** are within the ranges for **6** and pentanediamine, and those for **2** and **3** are only slightly outside the ranges of propanediamine and **5**. Our kinetic method experiments on **5** and **6** lead to $\Delta\Delta S$ values of −83 and −43 J mol^{-1} K^{-1}, also well within the ranges from the literature. These results seem to be in agreement with Cooks' assertion that the entropy-corrected kinetic method can be used to obtain a quantitative *prediction* for protonation entropies, albeit with somewhat large uncertainties.

While there is some error in the absolute magnitude of the derived entropy term, it is clear that the use of the extended kinetic method is required to obtain reliable proton affinities for species in which intramolecular hydrogen bonding is important. Apparent PAs for **1**−**4** are 976, 980, 958, and 935 kJ/mol. With the exception of **4**, these values are much lower than theoretical estimates. In our study of the PAs of **5** and **6** apparent basicities of ca. 981 and 942 kJ/mol were obtained, 25 and 10 kJ/mol lower than the recommended PA values.

Conclusions

The proton affinities for four lysine homologues have been determined using two different versions of the extended kinetic method in a quadrupole ion trap mass spectrometer. The proton affinities for the lysine homologues follow a trend similar to that of the related α,ω-diamines, with the two longer homologues having nearly the same PA and the two shorter homologues having PAs that decrease monotonically. An analysis of the derived entropy terms lends support to the notion that these values can be used as a quantitative prediction for the thermodynamic entropy of protonation provided that appropriate error bars are assigned. Finally, for systems in which this entropy term is large, it is essential that the extended kinetic method be used to derive accurate proton affinities.

Acknowledgment. Funding for this work was generously provided by the Camille and Henry Dreyfus Foundation, the Thomas F. and Kate Miller Jeffress Memorial Trust, the Research Corporation Cottrell College Research Award, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and The College of William and Mary. O.E.S., K.E.C., and K.L.C. acknowledge the Howard Hughes Medical Institute for summer fellowships through the Undergraduate Biological Sciences Education Program.

Supporting Information Available: Structures for the lowest energy conformers of **2**, **2H**$^+$, **3**, **3H**$^+$, **4**, and **4H**$^+$, kinetic method plots for **3**−**6**, raw and entropy-corrected ion intensity ratios for the extended kinetic method experiments for **1**−**6**, and calculated thermochemical values and optimized structures for all molecules studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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