The Proton Affinity of Proline Analogs Using the Kinetic Method with Full Entropy Analysis

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The proton affinity of proline analogs, L-azetidine-2-carboxylic acid (Aze), L-proline (Pro), and L-pipecolic acid (Pip), have been measured using the Armentrout modification of the extended kinetic method in a quadrupole ion trap instrument. Experimental values of 223.0 ± 1.5, 224.9 ± 1.6, and 225.6 ± 1.6 kcal/mol have been determined for the 298K proton affinities of Aze, Pro, and Pip respectively. High level theoretical calculations using both MP2 and B3LYP methods at a variety of basis sets were carried out in order to give theoretical predictions for the 298 K proton affinity and gas phase basicity of all three analogs. Recommended values for the gas phase basicity and proton affinity for proline based on our work and other recent determinations are 216 ± 2 and 224 ± 2 kcal/mol. (J Am Soc Mass Spectrom 2002, 13, 72–81)
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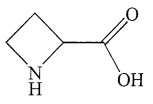
As the molecular building blocks of proteins and peptides, amino acids have been the subjects of great interest and study. We are interested in how subtle changes in molecular structure influence the thermochemical properties of amino acids. Specifically, we are interested in the thermochemical properties of non-protein amino acids (NPAA), which are naturally-occurring amino acids that are not used by humans for protein synthesis. NPAAs are ubiquitous in nature and serve a variety of purposes including nitrogen storage and defense [1, 2]. Some NPAAs are structurally similar to one or more of the protein amino acids and as such can be toxic to humans [3]. NPAAs have been shown to misincorporate into peptides and proteins [4–6] and can compete with their PAA analogs in a variety of biological functions [7–9].

The proline analogs, L-azetidine-2-carboxylic acid (Aze, **1**), L-proline (Pro, **2**), and L-pipecolic acid (Pip, **3**) have been studied in solution [10–15] and by molecular mechanics [16–19].



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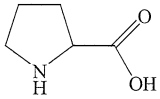

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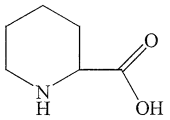

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In addition, proline has been the subject of several recent high-level theoretical [20, 21] and experimental studies [22, 23]. Substitution of Aze for Pro has been shown to drastically alter protein conformation [10, 16–19]. Replacement of Pro by Pip in a nonapeptide substrate for HIV proteinase converts the substrate into a selective inhibitor [11]. As an initial study of the thermochemical properties of NPAAs, we have determined the proton affinity of **1**–**3** using the extended kinetic method in a quadrupole ion trap instrument.

Since its introduction into the field of mass spectrometry, the kinetic method has been used extensively to determine thermochemical properties for a variety of molecules [24–31]. The kinetic method is based on the competitive decomposition of ion-bound dimers by either metastable decomposition or collision-induced dissociation. For example, the ratio of the intensities of AH^+ and BH^+ from decomposition of the proton-bound dimer $[AH^+B]$ can be related to the difference in gas-phase basicity (GB), the negative free energy of protonation, between A and B. In early studies using the kinetic method, entropy effects were assumed to be negligible as long as the structures of A and B were similar [25]. Provided that this requirement was met, it was assumed that the kinetic method was sensitive to differences in proton affinity (PA), the negative enthalpy of protonation, rather than gas-phase basicity.

In the mid-90's, Fenselau and co-workers [27, 30] and Wesdemiotis and co-workers [31] introduced methods to take into account the entropy of dissociation, by performing dissociations at different activation energies. Recently, Armentrout [32] has shown that the approach of Fenselau and Wesdemiotis should be modified in order to eliminate correlation between the variables used to determine the entropy (vide infra).

This work describes the determination of the proton affinities of the proline analogs using the kinetic method with full entropy analysis as well as from high level ab initio and density functional theory calculations. This is the first determination of any kind for the gas-phase thermochemical properties of Aze or Pip. In addition, we have reviewed the literature and present

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recommended values for the gas phase basicity and proton affinity of proline.

Experimental

All experiments were performed in a commercial ion trap mass spectrometer (LCQ-DECA, Finnigan MAT, San Jose, CA) equipped with an external electrospray ionization source. For MS experiments, an ion accumulation time of 50 ms was used. This was increased to 100 ms for MS^2 experiments. ESI and ion focussing conditions were varied in order to optimize the signal of proton-bound dimer ions AHB_i^+. The heated capillary temperature was kept in the range of 100–175 °C in order to maximize dimer formation. Dilute solutions (10^{-4}–10^{-5} M) of a proline analog and a reference base in acidified (1% acetic acid) 50:50 H_2O:CH_3OH were directly infused into the mass spectrometer at flow rates of 5–20 μL/min. The proton-bound dimer ion AHB_i^+ was isolated in the first stage of mass spectrometry at $q_z = 0.250$ with isolation widths of 4–5 aμ. The isolation width was adjusted to maximize ion signal while still maintaining ion isolation.

The proton-bound dimer ions were allowed to undergo collision-induced dissociation with the helium buffer gas at varying activation energies. For these studies, an activation time of 30 ms and activation amplitudes of 15%, 50%, and 85% (3, 10, and 17 $V_{(p\text{-}p,Lab)}$) were used. Total ion intensities for the protonated proline analog and protonated reference base products were obtained from signal averaging 40 scans. Proton affinities and activation entropies for each proline analog were obtained using Armentrout's [32] recent modification of the methods of Wesdemiotis and Fenselau [27, 31]. We used a slightly modified method in which the least-square-fit to the data for the initial plot (plot 1 as described in the following paragraph) is done without the inclusion of the uncertainties in the ratios and proton affinities.

In order to determine the PA and activation entropy from the kinetic method experiment, two plots are needed. The first plot (plot 1) is of the natural log of the ratio of product ion intensities from collision-induced dissociation of the proton-bound dimer, $Ln[AH^+/B_iH^+]$, versus $PA(B_i) - PA_{avg}$, where $PA(B_i)$ is the proton affinity of the reference base and PA_{avg} is the average proton affinity of the set of reference bases used in the determination. The best-fit line to these data obtained from standard regression techniques has a slope of $-1/RT_{eff}$ and intercept of $\{[PA(A) - PA_{avg}] - T\Delta\Delta S\}/RT_{eff}$, where $\Delta\Delta S$ is the average difference in activation entropy between the proline analog channel and the reference base channel. The x-intercept of this best-fit line corresponds to an effective proton affinity in which entropy effects are ignored.

Data is collected at several different activation energies, allowing the contributions of enthalpy and entropy to be separated. The intercepts of the best-fit lines from plot 1 are plotted in a second plot (plot 2) against the negative slope of those lines for the different activation energies. The quantity $[PA(A) - PA_{avg}]$ is obtained from the slope of the best-fit line to the data in plot 2, whereas the y-intercept of this line is $-\Delta\Delta S/R$.

Computational Methods

Proton affinities for Aze, Pro, and Pip were calculated using ab initio and density functional methods using the Gaussian 98W suite of programs [33]. For this study, we used Hartree-Fock [34], Møller-Plesset perturbation theory [34], and hybrid density functional theory (DFT) methods [35]. The B3LYP method comprises the Becke-3 parameter exchange functional [36, 37] and the correlation functional of Lee, Yang and Parr [38]. Optimized geometries, total electronic energies, and harmonic vibrational frequencies for the three amino acids in both their neutral and protonated forms were determined at the following levels of theory: HF/6−31G*, MP2/6−31G*, B3LYP/6−31G*, B3LYP/6−31+G*, and B3LYP/6−311+G*. In addition, Aze and $AzeH^+$ were examined at the MP2/6−31+G* and MP2/6−311+G* levels. For the neutral species, we only investigated canonical structures; that is, zwitterionic forms were not investigated. All structures were verified to be minima by the absence of negative Eigenvalues in the Hessian matrix. Zero-point energy and thermal corrections were obtained from scaled harmonic frequencies. Scaling factors varied with method as recommended by Scott and Radom [39]. Total entropies for all species were also computed. The translational and rotational contributions to the entropy were taken directly from the calculation output, whereas the vibrational contribution was obtained from scaled vibrational frequencies.

Materials

All chemicals were obtained from commercial sources and were used as received. Pipecolic acid and azetidine-2-carboxylic acid were obtained from Sigma (St. Louis, MO). He purity was 99.99%.

Results

Experimental Results

Proton-bound dimers of Aze and a suitable reference base were generated using electrospray ionization from dilute solutions of the two bases in slightly acidified (1% acetic acid) water:methanol solutions. The following reference bases were used: n-butyl amine, i-propyl amine, benzyl amine, pyridine, and c-hexyl amine. Plots of $\ln[AH^+/B_i^+]$ versus $PA(B_i) - PA_{avg}$ at three different activation energies are shown in Figure 1.

The proton affinities for the reference bases are shown in Table 1. For all three analogs, we used the recommended values from the compilation of Hunter and Lias [40] for all reference bases except benzyl

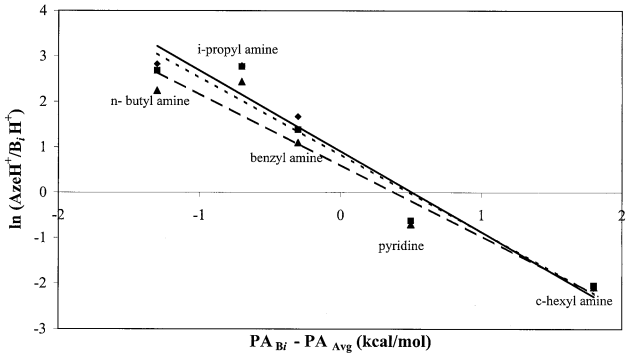


Figure 1. Plot of $Ln[AzeH^+/B_iH^+]$ versus $PA(B_i) - PA_{avg}$ at three different activation energies with best-fit lines. (Filled diamond, single line) 3 V, (filled square, short-dashed line) 10 V, and (filled triangle, long-dashed line) 17.5 V lab.

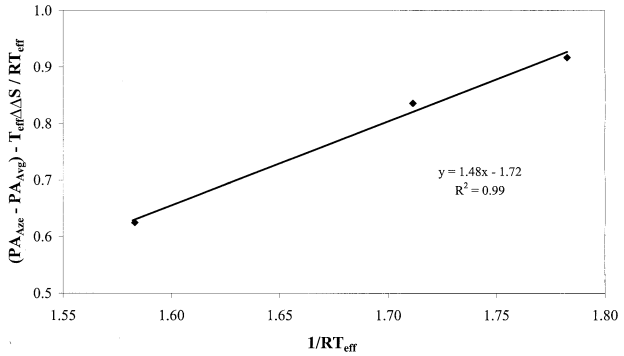


Figure 2. Plot $[(PA_{Aze} - PA_{avg}) - T_{eff}\,\Delta\Delta S]/RT_{eff}$ versus $1/RT_{eff}$ for azetidine-2-COOH.

amine. The compilation lists two primary references for the gas phase basicity for benzyl amine, 210.2 kcal/mol from Taft [41], and 213.4 kcal/mol from Aue and Bowers [42] with the Taft measurement as the recommended value. Based on our measured ratios (see Figure 1), benzyl amine should be more basic than both i-propyl amine and n-butyl amine. Taft's value for the proton affinity of benzyl amine (218.2 kcal/mol) is clearly too low. We therefore used the value of Aue and Bowers of 221.2 kcal/mol for the proton affinity of benzyl amine.

The x-intercepts of the lines shown in Figure 1 correspond to apparent proton affinities for Aze in which entropy effects are ignored. An average of 222.0 kcal/mol is obtained from these data for the apparent proton affinity for Aze. Figure 2 shows a plot of the intercepts of the three lines from Figure 1 versus the negative of slopes of those lines. The slope of the best-fit line to these data is 1.5 ± 0.2 kcal/mol, which, when combined with the average proton affinity of the reference base set (221.5 ± 1.5 kcal/mol), gives the final value of 223.0 ± 1.5 kcal/mol for the proton affinity of Aze.

The final uncertainty for the proton affinity is determined from the root sum square of the uncertainties in the slope of plot 2 and the uncertainty in the average proton affinity of the reference base set. The uncertainty in the average proton affinity is composed of the relative error in the measured quantities and a systematic error in the absolute proton affinity scale. We assign values of $\sqrt{2}$ for the systematic error in the absolute PA scale and $\sqrt{2}/\sqrt{N}$ for the random error, where N is the number of measurements. In this case, N is 5, and the total uncertainty in PA_{avg} is the root sum square of the random and systematic uncertainties, or 1.5 kcal/mol. Combining this with the uncertainty in the slope of plot 2 (0.2 kcal/mol) gives the final uncertainty in the PA of **1**.

Similar experiments were performed in order to redetermine the proton affinity of proline. The following reference bases were used: pyridine, c-hexyl amine, 3-methyl pyridine, and 4-methyl pyridine. The proton affinities for the reference bases are given in Table 1. Figure 3 shows plots of $ln[ProH^+/B_iH^+]$ versus $PA(B_i) - PA_{avg}$, where PA_{avg} for this reference base set is 224.3 ± 1.6 kcal/mol. Figure 4 shows plot 2 for the proline study. An average PA, in which entropy effects are ignored, of 224.2 is obtained from the x-intercepts of the best-fit lines from plot 1. The best-fit line to the data shown in Figure 4 has a slope of 0.6 ± 0.2 kcal/mol, which leads to a final proton affinity for proline of 224.9 ± 1.6 kcal/mol.

Proton-bound dimer ions of pipecolic acid and the following reference bases were investigated using the techniques described above: c-hexyl amine, 3-methyl pyridine, 4-vinyl pyridine, and 4-methyl pyridine. The proton affinities for these compounds are listed in Table

Table 1. Proton affinity of reference bases (kcal/mol)

Base	Proton affinity	reference
$n\text{-}C_4H_9NH_2$	220.2	a
$i\text{-}C_3H_7NH_2$	220.8	a
$C_6H_5CH_2NH_2$	221.2	b
pyridine	222.0	a
$c\text{-}C_6H_{11}NH_2$	223.3	a
3-methyl-pyridine	225.5	a
4-vinyl-pyridine	225.6	a
4-methyl-pyridine	226.4	a

[a]Data from Lias compilation, reference [40].
[b]Data from reference [42].

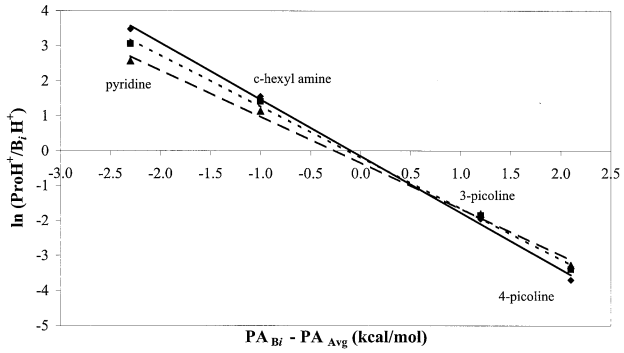


Figure 3. Plot of $ln[ProH^+/B_iH^+]$ versus $PA(B_i) - PA_{avg}$ at three different activation energies with best-fit lines. (Filled diamond, single line) 3 V, (filled square, short-dashed line) 10 V, and (filled triangle, long-dashed line) 17.5 V lab.

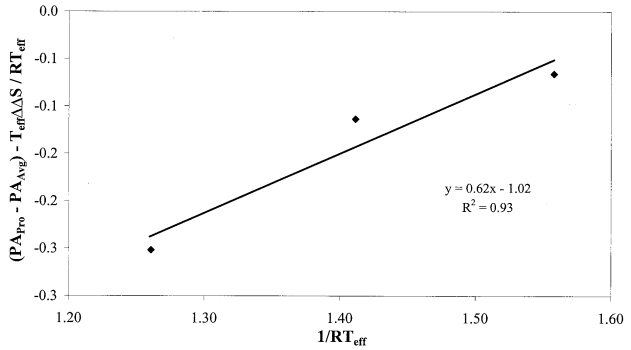


Figure 4. Plot $[(PA_{Pro} - PA_{avg}) - T_{eff}\,\Delta\Delta S]/RT_{eff}$ versus $1/RT_{eff}$ for proline.

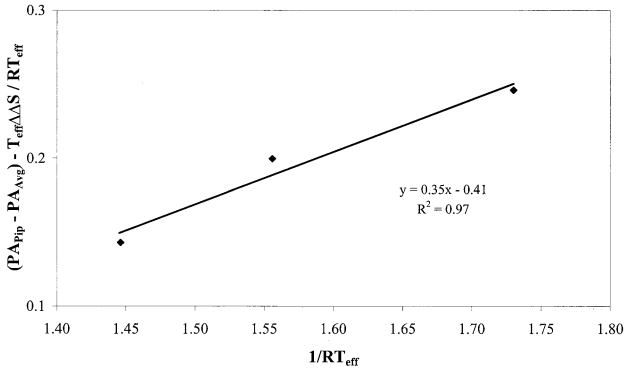


Figure 6. Plot $[(PA_{Pip} - PA_{avg}) - T_{eff}\,\Delta\Delta S]/RT_{eff}$ versus $1/RT_{eff}$ for pipecolic acid.

1. Figure 5 shows the kinetic method plots for three different activation energies. An apparent proton affinity of 225.3 for pipecolic acid, neglecting entropy effects, is derived from these data. Figure 6 shows the entropy work-up for pipecolic acid from which a final proton affinity of 225.6 ± 1.6 kcal/mol is obtained. The uncertainty is the sum of the contributions of the uncertainty in the average PA of the reference base set (±1.6 kcal/mol) and the uncertainty in the slope (±0.1 kcal/mol).

Computational Results

Several low-lying conformers of Aze were examined using HF, MP, and B3LYP methods. Total electronic energies, scaled ZPEs, 298K enthalpies, and absolute entropies for Aze at various levels of theory are given in Table 2. The two lowest energy structures both involve some hydrogen bonding between (I) the hydroxyl hydrogen and the nitrogen atom and (II) the amino hydrogen and the carbonyl oxygen. Figure 7 shows representative structures at the B3LYP/6−311+G* level of theory. The relative stability of these two conformers differs depending on which method/basis set is used as shown in Table 2. Protonated Aze was also examined at the levels described above. A structure with a hydrogen bond between a hydrogen on the nitrogen atom and the carbonyl oxygen was determined to be the global minimum at all levels studied (III, Figure 8). The $AzeH^+$ results are shown in Table 3.

The 298 K proton affinity for Aze was calculated using isodesmic reaction 1 with dimethyl amine as the reference base. The PA values from different levels of theory are

$$\text{Aze} + (CH_3)_2NH_2^+ \rightarrow \text{AzeH}^+ + (CH_3)_2NH \qquad (1)$$

listed in Table 4 along with the calculated ΔS_{base} and derived gas-phase basicity values for Aze. The isodesmic approach using correlated methods (MP2 and B3LYP) gives proton affinities for Aze that are in good agreement with our experimental determination of 223.0 kcal/mol. Uncertainties for the calculated proton affinities at these levels of theory are in the range of 2–3 kcal/mol.

Similar calculations were performed on neutral and protonated proline and pipecolic acid. Total energies, zero-point energies, 298 enthalpies, and total entropies for these species are listed in Tables 2 and 3. Proline has been the subject of several recent theoretical investigations [20, 21]. Our results are in accord with other theoretical predictions in terms of the lowest energy structures for neutral proline. The lowest energy struc-

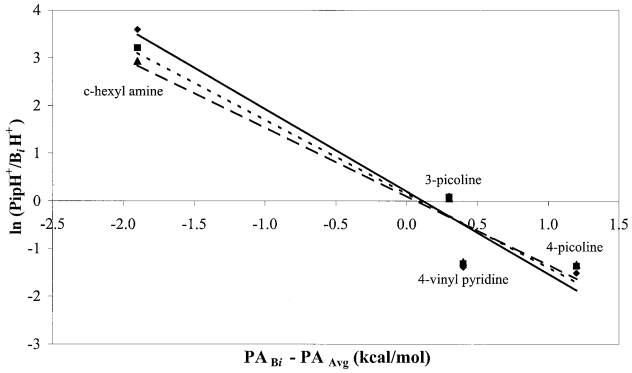


Figure 5. Plot of $\ln[PipH^+/B_iH^+]$ versus $PA(B_i) - PA_{avg}$ at three different activation energies with best-fit lines. (Filled diamond, single line) 3 V, (filled square, short-dashed line) 10 V, and (filled triangle, long-dashed line) 17.5 V lab.

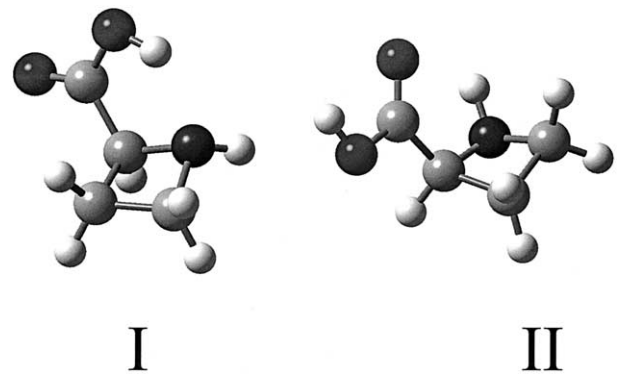


Figure 7. Optimized structures for Aze at the B3LYP/6−311+G* level of theory.

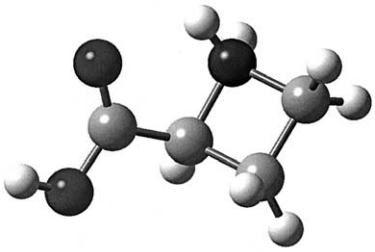
Table 2. Total electronic energies, zero point energies, and 298 K enthalpies (Hartrees) and total entropy (eu) for proline analogs and dimethyl amine

Method	Structure[a]	E_{el}	ZPE[b]	H_{298}[c]	S[d]
Pipecolic acid					
HF/6-31G*	I	−437.80355	0.17246	−437.62185	88.7
HF/6-31G*	II	−437.80562	0.17173	−437.62432	90.6
MP2/6-31G*	I	−439.09842	0.17305	−438.91679	87.8
MP2/6-31G*	II	−439.09597	0.17218	−438.91478	88.7
B3LYP/6-31G*	I	−440.47287	0.17230	−440.29166	87.5
B3LYP/6-31G*	II	−440.47121	0.17143	−440.29047	90.2
B3LYP/6-31+G*	I	−440.49131	0.17161	−440.31071	88.0
B3LYP/6-31+G*	II	−440.49097	0.17084	−440.31079	90.2
B3LYP/6-311+G*	I	−440.59096	0.17129	−440.41068	88.0
B3LYP/6-311+G*	II	−440.59092	0.17057	−440.41102	90.1
Proline					
HF/6-31G*	I	−398.76386	0.14350	−398.61181	87.1
HF/6-31G*	II	−398.76541	0.14296	−398.61365	89.5
MP2/6-31G*	I	−399.92542	0.14375	−399.77379	83.9
MP2/6-31G*	II	−399.92399	0.14330	−399.77250	85.8
B3LYP/6-31G*	I	−401.15371	0.14323	−401.00231	85.9
B3LYP/6-31G*	II	−401.15181	0.14275	−401.00066	86.7
B3LYP/6-31+G*	I	−401.17340	0.14274	−401.02244	85.6
B3LYP/6-31+G*	II	−401.17068	0.14234	−401.01990	87.0
B3LYP/6-311+G*	I	−401.26555	0.14252	−401.11482	85.6
B3LYP/6-311+G*	II	−401.26321	0.14210	−401.11264	87.3
Azetidine-2-COOH					
HF/6-31G*	I	−359.69820	0.11448	−359.57622	80.8
HF/6-31G*	II	−359.70005	0.11404	−359.57837	81.5
MP2/6-31G*	I	−360.72598	0.11396	−360.60494	78.5
MP2/6-31G*	II	−360.72605	0.11374	−360.60508	78.5
MP2/6-31+G*	I	−360.75175	0.11345	−360.63120	78.8
MP2/6-31+G*	II	−360.75062	0.11296	−360.63036	78.8
MP2/6-311+G*	I	−360.89359	0.11416	−360.77227	79.1
MP2/6-311+G*	II	−360.89290	0.11377	−360.77178	80.2
B3LYP/6-31G*	I	−361.80990	0.11372	−361.68889	80.0
B3LYP/6-31G*	II	−361.80854	0.11322	−361.68780	81.5
B3LYP/6-31+G*	I	−361.82900	0.11340	−361.70829	80.1
B3LYP/6-31+G*	II	−361.82702	0.11281	−361.70664	81.7
B3LYP/6-311+G*	I	−361.91341	0.11316	−361.79289	80.1
B3LYP/6-311+G*	II	−361.91180	0.11266	−361.79158	81.7
$(CH_3)_2NH$					
HF/6-31G*	–	−134.23885	0.09071	−134.14271	–
MP2/6-31G*	–	−134.66530	0.09218	−134.56793	–
MP2/6-31+G*	–	−134.67482	0.09175	−134.57786	–
MP2/6-311+G*	–	−134.72395	0.09200	−134.62668	–
B3LYP/6-31G*	–	−135.16285	0.09131	−135.06624	–
B3LYP/6-31+G*	–	−135.17035	0.09102	−135.07401	–
B3LYP/6-311+G*	–	−135.19888	0.09072	−135.10282	–

[a]See text for discussion of structures.
[b]Derived from scaled harmonic vibrational - frequencies; for scaling factors see reference [39].
[c]$H_{298} = E_{el} + ZPE + \Delta ZPE + 5/2$ RT. ΔZPE is derived from harmonic vibrational frequencies; for scaling factors see reference [39].
[d]S_{trans} and S_{rot} taken from Gaussian output, S_{vib} evaluated from scaled vibrational frequencies; for scaling factors see reference [39].

tures involve hydrogen bonding between the hydroxyl hydrogen and the nitrogen lone pair or between the carbonyl oxygen and an amino hydrogen, similar to structures I and II described above for Aze. The lowest energy structures for Pip are also of these forms. Protonated proline and pipecolic acid both adopt structures similar to III with a hydrogen bond between a hydrogen on the nitrogen and the carbonyl oxygen.

Isodesmic reactions with dimethyl amine as the reference base were used to generate predictions for the proton affinity for proline and pipecolic acid. These values are listed in Table 3. As with Aze, the correlated methods give good agreement with our experimental determinations of 224.9 and 225.6 kcal/mol. We should mention that our derived PA for proline of 225.4 at the B3LYP/6−311+G* level does not agree with a recent



III

Figure 8. Optimized structure for $AzeH^+$ at the B3LYP/6−311+G* level of theory.

theoretical study by Toscano and co-workers [21]. They calculate a 298 K PA of 220.9 kcal/mol using B3LYP/6−311++G** calculations. Upon closer examination of the total electronic energies for proline and protonated proline given in their paper, it appears that an arithmetic error was made in the conversion of the 0 K energy difference to the 298 K proton affinity. Taking the total electronic energies for the lowest-energy structures for neutral and protonated proline from their paper gives a ZPE-corrected energy difference of 222.9 kcal/mol. Conversion to a 298 K requires the addition of (1) the difference in integrated vibrational heat capacity between neutral and protonated proline, (2) 3/2 RT for the integrated translational heat capacity of the proton, and (3) an additional RT for conversion of the energy difference to an enthalpy. Since the vibrational frequencies were not listed in the Toscano paper, we cannot evaluate the difference in integrated vibrational heat capacity between Pro and ProH+ and therefore cannot convert their 0 K energy difference to a 298 K PA directly. However, in our work, all 298 K enthalpies are larger than the 0 K energy differences. It appears as if the authors subtracted the thermal correction rather than adding it.

Discussion

Comparison with Previous Results for Proline

The recommended proton affinity and gas-phase basicity of proline according to the Lias compilation are 220.0 and 211.8 kcal/mol respectively [40]. The large discrepancy between our value and the recommended one was initially a cause for concern. Table 5 lists the various

Table 3. Total electronic energies, zero point energies, and 298 K enthalpies (Hartrees) and entropies (eu) for protonated proline analogs and protonated dimethyl amine

Method	E_{el}	ZPE[a]	H_{298}[b]	S[c]
$PipH^+$				
HF/6-31G*	−438.18554	0.18660	−437.98941	90.2
MP2/6-31G*	−439.47413	0.18697	−439.27827	87.2
B3LYP/6-31G*	−440.85040	0.18640	−440.65476	89.2
B3LYP/6-31+G*	−440.86134	0.18598	−440.66608	89.5
B3LYP/6-311+G*	−440.96152	0.18579	−440.76646	89.5
$ProH^+$				
HF/6-31G*	−399.14487	0.15762	−398.97856	87.5
MP2/6-31G*	−400.30033	0.15753	−400.13464	85.0
B3LYP/6-31G*	−401.53197	0.15729	−401.36638	85.3
B3LYP/6-31+G*	−401.54258	0.15705	−401.37721	85.5
B3LYP/6-311+G*	−401.63484	0.15692	−401.46952	86.0
$AzeH^+$				
HF/6-31G*	−360.07483	0.12803	−359.93893	84.1
MP2/6-31G*	−361.09585	0.12757	−360.96087	80.8
MP2/6-31+G*	−361.11263	0.12703	−360.97818	80.6
MP2/6-311+G*	−361.25552	0.12812	−361.11998	80.7
B3LYP/6-31G*	−362.18258	0.12746	−362.04750	82.6
B3LYP/6-31+G*	−362.19302	0.12726	−362.05813	82.3
B3LYP/6-311+G*	−362.27795	0.12724	−362.14307	82.4
$(CH_3)_2NH$				
HF/6-31G*	−134.61353	0.10594	−134.50204	–
MP2/6-31G*	−135.03668	0.10711	−134.92425	–
MP2/6-31+G*	−135.03978	0.10680	−134.92765	–
MP2/6-311+G*	−135.08943	0.10737	−134.97669	–
B3LYP/6-31G*	−135.53403	0.10660	−135.42199	–
B3LYP/6-31+G*	−135.53559	0.10657	−135.42362	–
B3LYP/6-311+G*	−135.56387	0.10628	−135.45215	–

[a]Derived from scaled harmonic vibrational frequencies; for scaling factors see reference [39].
[b]$H_{298} = E_{el} + ZPE + \Delta ZPE + 5/2$ RT. ΔZPE is derived from harmonic vibrational frequencies; for scaling factors see reference [39].
[c]S_{trans} and S_{rot} taken from Gaussian output, S_{vib} evaluated from scaled vibrational frequencies; for scaling factors see reference [39].

Table 4. Derived thermochemical values for proline analogs

Method	Proton affinity[a]	ΔS_{base}(eu)[b]	ΔGB
Pipecolic acid			
HF/6-31G*	225.6	26.7	217.7
MP2/6-31G*	225.2	26.6	217.3
B3LYP/6-31G*	226.6	24.3	219.4
B3LYP/6-31+G*	225.6	24.5	218.3
B3LYP/6-311+G*	226.0	24.5	218.7
Proline			
HF/6-31G*	225.5	28.0	217.2
MP2/6-31G*	224.8	24.9	217.4
B3LYP/6-31G*	227.2	26.7	219.3
B3LYP/6-31+G*	225.2	26.2	217.4
B3LYP/6-311+G*	225.4	25.6	217.8
Azetidine-2-COOH			
HF/6-31G*	222.8	23.4	215.8
MP2/6-31G*	221.7	23.7	214.6
MP2/6-31+G*	220.2	24.2	213.0
MP2/6-311+G*	220.6	24.4	213.3
B3LYP/6-31G*	223.8	23.4	216.8
B3LYP/6-31+G*	222.1	23.8	215.0
B3LYP/6-311+G*	222.2	23.7	215.4

[a]Derived from isodesmic reation 1.
[b]$S(M) + S(H^+) - S(MH^+)$.

experimental determinations of the PA and GB for proline. The recommended value from the NIST website for the proton affinity of proline is taken from the kinetic method determination of Harrison and co-workers [28] with an adjustment for the change in the absolute proton affinity scale [43]. Harrison and co-workers measured proton affinities for proline of 218.5 and 220.5 kcal/mol using ethyl amine and dimethyl amine as reference bases. Of the two different values, Hunter and Lias chose to adjust the lower of the two, taking into account the 1.5 kcal/mol increase in the absolute PA of ethylamine since 1993 [40]. In contrast, in 1997, Harrison and co-workers reevaluated the proton affinities and gas phase basicities for all 20 amino acids and chose to adjust the higher of his two determinations to account for the 1.6 kcal/mol increase in the absolute PA of dimethyl amine, giving a recommended PA of 222.1 kcal/mol [44].

Table 5. Experimental proton affinities and gas phase basicities (kcal/mol) for proline

Gas phase basicity	Proton affinity	Reference
PA measurements		
217.3 ± 1.0[ab]	224.9 ± 1.6	This work
211.8[c]	220.0	5
213.9[c]	222.1	5
211.9[d]	219.9	32
215.9[d]	223.9	31
GB measurements		
214.6-215.0	*222.6-223.0*[d]	44
215.1-219.4	*223.1-227.4*[d]	42
217.7	*225.7*[d]	43
215.0	*223.0*[d]	45
214.3	*222.3*[d]	45
206.7	*214.7*[d]	46
Evaluated quantities		
211.8	220.0	35
214.3	222.1	40
216 ± 2	**224 ± 2**	This work

[a]Italicized entries are derived from the measured value (normal font) by addition/subtraction of $T\Delta S_{base}$.
[b]ΔS_{base} = 25.6 eu derived from B3LYP/6−311+G* calculations.
[c]ΔS_{base} = 27.7 eu from reference [40].
[d]ΔS_{base} = 26.7 eu; average of entropies given in footnotes b and c.

Since the Hunter and Lias compilation, there have been two additional measurements of the PA of proline by Mirza et al. [23] and by Tabet and co-workers [22]. Mirza et al. report a PA of 219.9 kcal/mol based on the extended kinetic method described above. This work used amino acids as reference bases, with proton affinities taken from the Bojeson scale [45], however, the reference PAs were not adjusted to reflect the change in absolute PA scale [43]. This value is therefore probably too low. Tabet and co-workers have also recently remeasured the proton affinities of the 20 PAAs using the extended kinetic method [22]. Their study also used amino acids as reference bases, but only those that have PAs which are consistent across several different PA scales. Using Ser, Leu, Thr, Met, and Trp as reference bases, Tabet and co-workers determined the PA of proline to be 223.9 kcal/mol.

The Harrison determination is the only proton affinity listed in the Lias compilation; all other experiments were for the gas-phase basicity of proline. The recommended value of 211.8 kcal/mol is derived from the PA of Harrison using an entropy difference of −1.7 entropy units between Pro and $ProH^+$ (ΔS_{base} = 27.7 cal mol^{-1} K^{-1}) [40]. Our B3LYP/6−311+G* calculations predict a difference in entropy of 0.4 eu, which leads to a ΔS_{base} for proline of 25.6 cal mol^{-1} K^{-1}. Combining this quantity with our experimental PA of 224.9 ± 1.6 kcal/mol leads to a prediction of GB (Pro) of 217.3 ± 1.9 kcal/mol, where the uncertainty includes an additional 1 kcal/mol uncertainty in ΔS.

Our derived GB value is in excellent agreement with the ICR bracketing study of Amster and co-workers [46] as well as the ICR equilibrium studies of Locke and McIver [47], while it is somewhat higher than a recent bracketing study of Cassady and co-workers [48] and the equilibrium studies of Moetner(Mautner) and Hunter [49]. An examination of Table 5 indicates that the determination of Yamdagni and Kebarle is probably too low [50]. In light of the fact that all but one of the measured GBs listed in the Lias compilation are greater than 214 kcal/mol, we recommend that the accepted value for GB (Pro) be changed to 216 ± 2 kcal/mol, a value that encompasses all of the GB determinations except Kebarle's.

To aid in comparing PA data, we have converted these GB data in Table 5 to PAs by adding a ΔS term of

26.7 cal mol^{-1} K^{-1}, the average of our computational prediction and the entropy given in the Lias compilation [40]. As with GB (Pro), the recommended PA for proline is probably too low, and we recommend a value of 224 ± 2 kcal/mol. This encompasses our value and Tabet's recent determination [22], as well as Harrison's adjusted proton affinity [51], and all of the PAs obtained from conversion of measured GB data.

Agreement Between Theory and Experiment

One of the goals of this research was to establish a level of theory that gives reasonable agreement with our experimental results. In the future we will be investigating amino acids with multiple basic sites. It will be imperative to have a theoretical prediction for the relative basicity of the different sites of these molecules so that we know which site we are accessing in our kinetic method experiment. We will probably not be able to use the most reliable of the currently available theoretical procedures, the compound methods G2 [52], G3[53], CBS-Q [54], and CBS-APNO [55] because of the large size of these amino acids. We will most likely need to resort to lower level MP or DFT methods to get theoretical predictions for our experimental quantities. DFT has been used extensively to calculate the proton affinity of a variety of molecules [56–61] including amino acids [21, 62, 63]. Provided that a suitable basis set is used, DFT has been shown to give reasonable agreement with experimental results and higher level calculations in many cases.

The present study reveals that both MP2 and B3LYP methods reproduce our experimental data. Somewhat surprisingly, the HF/6−31G* calculations also give good agreement with our experimental results for the three analogs. For Aze, we could investigate the effects of basis set size and the addition of diffuse functions on the derived proton affinities. Increasing basis set size from double zeta to triple zeta results in slightly worse agreement at the MP2 level whereas it has no effect at the B3LYP level. Addition of diffuse functions lowers the calculated PAs by approximately 1.5 kcal/mol for both MP2 and B3LYP methods. Based on the comparisons using the 6−31G* basis set, the B3LYP method gives proton affinities that are 1.5 to 2 kcal/mol larger than those from the MP2 method. As the uncertainties in these calculations are probably on the order of ± 2 kcal/mol, all of the levels of theory give satisfactory agreement with our experimental determination.

For the larger analogs, the MP2/6−311+G* calculations were beyond the computational power of our desktop computers. This will also be the case for some of the larger NPAAs, such as arginine analogs, which we wish to investigate in the future. Density functional methods will allow us to study these molecules using relatively large basis sets. Table 4 reveals that for proline and pipecolic acid, B3LYP gives excellent agreement with our experimental results at all three basis sets used. In terms of computational effectiveness, we will use the B3LYP/6−31+G* combination for further studies.

Thermochemical Trends

The primary goal of this study was to determine the effect of ring size on the proton affinity of these amino acids. Proton affinities for the homologous series of heterocyclic nitrogen bases, azetidine, pyrrolidine, and piperidine have been determined by several groups[40] and have recently been studied by high-level theoretical calculations [64]. The Lias compilation recommends values of 224.5, 226.6, and 228 kcal/mol for the 4-, 5-, and 6-membered ring nitrogen heterocycles. The monotonic increase in PA (223.0, 224.9, 225.6 kcal/mol) is also seen in the amino acid analogs, albeit with smaller differences. Replacing the hydrogen alpha to the amine with a COOH group to form the amino acid results in a decrease in PA of ca. 1.5 kcal/mol. This is in good agreement with Harrison's observations in his proton affinity review [44].

Finally, the extended kinetic method allows for the separation of the contributions of enthalpy and entropy to the dissociation of a proton-bound dimer. The entropy obtained from the y-intercept of plot 2 is the average difference in activation entropy between the proline analog channel and the reference base channel. All of our reference bases are primary or secondary amines that should not form intramolecular hydrogen bonds upon protonation. Consequently, the activation entropy difference between the proline analogs (all secondary amines) and the reference bases should be small. The measured values of ΔS are 3.4, 2.0, and 0.8 eu for Aze, Pro, and Pip respectively. The monotonic decrease in entropy differences is of note. These entropies are much smaller than those that have been measured when either the protonated reference bases or the protonated unknown can form intramolecular hydrogen bonds [23]. For example, we have been studying the lysine analogs, Lys, Orn, 2,4-diaminobutanoic acid, and 2,3-diaminopropanoic acid using the extended kinetic method [65]. Preliminary studies give entropy differences on the order of 10–15 eu, much larger than the ones measured here. Therefore it appears that the extended kinetic method can be used as a probe for intramolecular hydrogen bonding.

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