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Gas-phase acidities of lysine homologues and proline analogs from the extended kinetic method

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Dedicated to Professor Alex Harrison for his many important contributions to gas-phase thermochemistry.

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ABSTRACT

The absolute gas-phase acidities (ΔH_{acid}) for three lysine homologues (ornithine (Orn), 2,4-diaminobutanoic acid (Daba) and 2,3-diaminopropanoic acid (Dapa)) and 2 proline analogs (azetidine-2-carboxylic acid (Aze) and pipecolic acid (Pip)) were determined using the extended kinetic method in an electrospray ionization–triple quadrupole instrument. The gas-phase acidities of the three lysine homologues (1415 ± 10, 1419 ± 7, and 1418 ± 8 kJ/mol for Orn, Daba, and Dapa, respectively) are the same as that of lysine (1416 ± 7 kJ/mol) obtained from earlier studies within error limits. The two proline analogs are less acidic (1425 ± 13 and 1432 ± 11 kJ/mol for Aze and Pip) than the lysine analogs, but have the same acidity as proline (1430 ± 7 kJ/mol). Experimental acidities are supported by density functional theory calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level, which give predictions for the acidities from an isodesmic reaction with acetic acid as the reference bases. Agreement between theory is excellent (within 5 kJ/mol) for Daba, Dapa, Aze, and Pip. The computed acidity for Orn is 9 kJ/mol higher than the measured acidity, but is still within the error limits. As the difference in acidities within the sets of analogs is smaller than the absolute error bars, relative acidities (ΔG_{acid}) were obtained using kinetic method ratios with 3-OH-benzoic acid as the reference acid. Relative acidity (ΔG_{acid}) orderings of Dapa > Lys > Daba > Orn and Pro > Pip > Aze were obtained.

1. 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 fast atom bombardment (FAB) [1], electrospray ionization (ESI) [2] and matrix-assisted laser desorption (MALDI) [3], 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 (PAA) were among the first experiments to be performed with these new ion sources [4–9].

The gas-phase proton affinity, PA, and gas-phase acidity (ΔH_{acid}) of a molecule M are defined as enthalpy changes for Eqs. (1) and (2), respectively:

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

$$M \rightarrow [M{-}H]^- + H^+ \quad (2)$$

The Gibbs free energy changes for these reactions are the gas-phase basicity and ΔG_{acid} respectively. GA and PA values for gas-phase molecules can be obtained from a variety methods including gas-phase equilibrium and bracketing techniques [10]. Both of these methods require that the analyte of interest is volatile enough to allow for the introduction of neutral vapor into the mass spectrometer. An alternative approach that can be used with non-volatile species 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 [11–13]. Entropy and enthalpy contributions for these dissociations can be determined directly by using the extended kinetic method, in which the decomposition is carried out at different collision energies, corresponding to different effective temperatures [14–17]. A Van't Hoff-like analysis is then carried out to extract enthalpic and entropic contributions to the dissociation directly.

We have been studying the intrinsic gas-phase thermochemical properties of non-protein amino acids (NPAAs) in an effort to gain a deeper understanding of the relationships between amino acid structure and thermochemistry [18–21]. NPAAs are naturally occurring compounds that are not coded for in RNA [22,23]. They are ubiquitous in the plant and fungi kingdoms and serve a variety of functions ranging from nitrogen storage to defense from predators [24–36]. Many NPAAs are structurally similar to one or more of the PAAs and therefore serve as attractive candidates with

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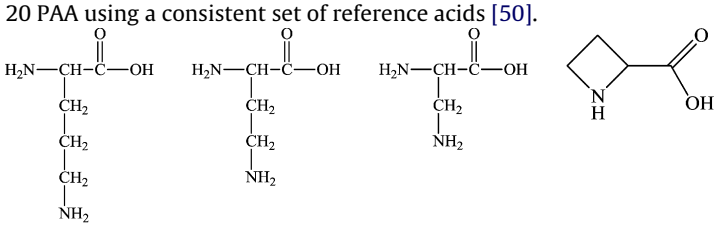
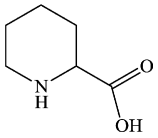
which to study the subtle interplay between structure and energetics. We have recently determined proton affinities for proline analogs [18], lysine homologues [19] and for oxyanalogs of amino acids involved in the urea cycle [21] using the extended kinetic method in a quadrupole ion trap instrument. These studies were aided by a robust literature on experimental and theoretical proton affinities of the 20 PAAs upon which we could base our comparisons [4,7–9,37–46]. In contrast, there have been far fewer studies of the gas-phase acidities of amino acids [5,46–52]. We recently completed a comprehensive study of the gas-phase acidities of all 20 PAA using a consistent set of reference acids [50].



Orn (**1**) Dapa(**2**) Dapa (**3**) Aze (**4**)



Pip (**5**)

With a set of internally consistent acidity values for the PAAs available, we are now in the position to extend our PA measurements to GAs of a set of NPAA analogs. We report here the first experimental determination of the GA for the lower homologues of lysine: ornithine (Orn, **1**), 2,4-diaminobutanoic acid (Daba, **2**), 2,3-diaminopropanoic acid (Dapa, **3**) and the 4- and 6-membered ring analogs of proline: azetidine-2-carboxylic acid (Aze, **4**) and pipecolic acid (Pip, **5**).

2. Experimental

All experiments were performed in a Thermo TSQ Quantum Discovery triple quadrupole instrument. Briefly, dilute solutions of an amino acid and one of a series of reference acids of known acidity in slightly basic (10% NH_4OH) 80:20 methanol:water were directly infused (flow rates 5–35 μl/min) into the electrospray ionization source of the TSQ. Solution concentrations were varied in order to maximize the production of proton-bound dimers of the deprotonated amino acid and the deprotonated reference acid 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 in Q1 at a resolution of 0.7 amu and were allowed to pass into the Q2 collision cell. The isolated ions were allowed to undergo collision-induced dissociation with argon gas maintained at a pressure of 0.3 mTorr. Product ion spectra were recorded at collision energies between 0 and 30 V (lab). The ion intensities of each primary product and any secondary products were recorded and were analyzed using standard extended kinetic method (KM) techniques. Secondary product ion intensities were added to the corresponding primary product intensities before undergoing KM analysis.

Gas-phase acidities (ΔH_{acid}) and entropy contributions (ΔS_{prot}) were obtained from the extended kinetic method that has been described in detail elsewhere [14,16,17]. The normal implementation of the kinetic method requires two plots, the first of which (plot 1) is of $\ln\left(I_{[ref\text{-}H]^-}/I_{[AA\text{-}H]^-}\right)$ vs. $GA_{Ai} - GA_{avg}$, where $I_{[ref\text{-}H]^-}$ and $I_{[AA\text{-}H]^-}$ are the intensities of the deprotonated reference acid and amino acid products, GA_{Ai} is the gas-phase acidity (ΔH_{acid}) of the *i*th reference acid, and GA_{avg} is the average gas-phase acidity of the set of *i* reference acids. 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 $(GA_{AA} - GA_{avg})$, and the intercept is the average difference in deprotonation entropy between the deprotonated amino acid channel and the reference acid channels over R ($\Delta S_p/R$).

In our analyses, plot 2 is used to gauge the quality of the data, and to aid in choosing an appropriate range of activation energies, but is not ultimately used to obtain acidities and entropies. Rather we use the Orthogonal Distance Regression (ODR) method as implemented in the ODR-pack program of Ervin and Armentrout [53]. In this method all intensity ratios of *m* reference acids at *n* collision energies are analyzed simultaneously. A total of *n* lines are generated and forced to cross at a single isothermal point, which gives the gas-phase acidity and de-protonation entropy for the amino acid in question. This method gives a more realistic estimation of the errors in the derived quantities by using Monte Carlo simulations to determine isothermal points from randomly perturbed intensity ratios. For these studies, we used a window of ±8 kJ/mol in the reference acidity values and a window of ±0.05 for the ln(ratio) values. Acidity values and entropies are reported with error bars corresponding to 95% confidence. The ODR workup also generates effective temperature values for each activation voltage. All kinetic method plots shown in this manuscript are generated using the ODR-derived effective temperatures rather than using the traditional best-fit method.

Predictions for ΔH_{acid} for all amino acids studies were also obtained from hybrid density functional theory calculations using the B3LYP functional combinations [54,55]. All calculations were performed using the Gaussian98 suite of programs [56]. Geometries and harmonic vibrational frequencies for the lysine analogs and their protonated forms were calculated at the B3LYP/6-31+G* level. Zero-point energy (ZPE) and thermal corrections were obtained from un-scaled harmonic vibrational frequencies. As the recommended scaling factors for B3LYP frequencies for ZPE and thermal corrections are 0.98 and 0.99, respectively [57], we chose to use unscaled frequencies for deriving ZPE and thermal corrections. Total energies for the lowest-energy conformers were obtained using single-point energy calculations at the B3LYP/6-311++G** level and were combined with ZPE, thermal corrections, and a PV work term to give 298 K enthalpy values. Total entropies were taken from the Gaussian 98W output without scaling.

Predictions for the gas-phase acidities of the amino acid analogs were computed directly from calculated enthalpies at 298 K according to reaction 3. We chose this level

$$AAH \rightarrow [AA\text{-}H]^- + H^+ \quad (3)$$

of theory based on previous work on proton affinities of amino acids [18,19] in which the B3LYP/6-311++G**//B3LYP/6-31+G* method gave nearly quantitative agreement with experimental PAs for a variety of nitrogenous bases including dimethyl amine, ethylene diamine and glycine [39]. Whereas this method performs well for proton affinity studies, it does not give quantitative agreement with experimental acidities for even simple carboxylic acids such as acetic acid. This method gives an absolute acidity for HOAc that is 10 kJ/mol too low (1446 kJ/mol vs. 1456 kJ/mol) [58]. In our PAA acidity study, we found that whereas our chosen method does not give absolute acidity values that agree with experimental results, the *relative* acidity values predicted from this method are in

Table 1
Supplementary thermochemical values (kJ/mol).[a]

Acid	ΔH_{acid} [b]	Orn	Daba	Dapa	Aze	Pip
Phenylacetic acid	1429 ± 8.8	X	X	X	X	X
Benzoic acid	1423 ± 9.2	X	X	X	X	X
2,5-Dimethylbenzoic acid	1420 ± 8.4	X			X	X
3-Hydroxybenzoic acid	1417 ± 8.8	X	X	X	X	X
4-Fluorobenzoic acid	1410 ± 8.8	X	X	X		

[a] X indicates that the given acid was used as a reference acid in the kinetic method experiment for the lysine or proline analog.
[b] Values in kJ/mol from Ref. [58].

excellent agreement with experimental acidities [50]. Therefore, we use isodesmic reaction (4):

$$AAH + CH_3COO^- \rightarrow [AA\text{-}H]^- + CH_3COOH \quad (4)$$

to calculate the relative acidity between our amino acid and acetic acid. This relative acidity can be combined with the known acidity of acetic acid to give an isodesmic prediction for acidity of the amino acid.

3. Materials

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

4. Results and discussion

4.1. Lysine analogs

4.1.1. Ornithine

Proton-bound dimer anions of deprotonated Orn and several deprotonated reference acids were generated using electrospray ionization of dilute solutions of the two acids in basic (10% ammonium hydroxide) 80:20 methanol:water solutions. The following reference acids had acidities that gave product ion ratios in the appropriate range for kinetic method analysis (ca. 25:1–1:25): 4-fluorobenzoic acid, 3-hydroxybenzoic acid, 2,5-dimethylbenzoic acid, benzoic acid, and phenylacetic acid. The literature acidities for all of the reference acids used in this study are given in Table 1 [58]. Proton-bound heterodimer ions were isolated in Q1 of the triple quadrupole instrument and allowed to undergo collision-induced dissociation (CID) in Q2 with argon target gas with activation voltages ranging from 0 to 30 V (lab). Ion intensities for both primary anionic products and any secondary products were recorded and used in an extended kinetic method analysis as described in Section 2. Experimental and theoretical gas-phase acidity values for **1–5** are given in Table 2. Seven different activation energies between 6 and 24 V (lab) were used for the kinetic method workup for ornithine. Fig. 1 shows the first kinetic method plot for four of these activation energies (6, 12, 18, 24 V, others omitted for clarity). Figs. S1–S4 in Supporting Information show kinetic method plots for the other amino acids investigated in this study and Tables S1–S5 give measured ratios for all kinetic method studies performed in this work. The fits to the data were obtained from the ODR-derived effective temperatures and isothermal point. ODR analysis including all seven activation energies gives a derived acidity of 1415 ± 10 kJ/mol and a ΔS_p of −10 ± 9 J K^{-1}. This derived entropy term is a prediction for the difference in entropy between Orn and [Orn-H]$^-$ and does not include the entropy of the proton. This value is rather small and suggests that the conformational spaces of neutral and deprotonated Orn should be similar. In contrast, the protonation entropy for lysine from our previous study was in the range of −80 J K^{-1} [19].

Theoretical predictions for the gas-phase acidity for Orn were also obtained using hybrid density functional theory. As Orn has a long, flexible side chain, finding the global minimum conformation for ornithine required consideration of many low-energy conformers. We used the GMMX search algorithm in PCModel to generate all low-lying conformations that lie within 30 kJ/mol of the MMX minimized structure. These conformers were used as starting structures for progressively larger ab initio and density functional theory calculations. Ultimately, geometries and harmonic vibrational frequencies for both neutral and deprotonated Orn were obtained using the B3LYP/6-31+G* level of theory. Single point energies were determined for each conformer at the B3LYP/6-311++G** level. Total electronic energies, ZPE/thermal corrections and 298 K enthalpies are shown in Table S6 for all neutral and deprotonated amino acids in this study. Figs. S1–S8 of Supporting Information show the structures for low lying conformers for **1–5** and their deprotonated forms. Combining the 298 K enthalpies for Orn, [Orn-H]$^-$ and H$^+$ gives a prediction for the gas-phase acidity for Orn of 1414 kJ/mol. Using isodesmic reaction (4) gives a more accurate prediction of 1424 kJ/mol for the GA of Orn. The uncertainty in this value is not straightforward to calculate, but is certainly no less

Table 2
Experimental and theoretical gas-phase acidities for amino acids (kJ/mol).

Analog	ΔH_{acid}	ΔH_{acid}, theory[a]
Lysine	1416 ± 7[b]	1415[b]
Ornithine	1415 ± 10	1424
Daba	1419 ± 7	1417
Dapa	1418 ± 8	1420
Azetidine-2-carboxylic acid	1425 ± 13	1427
Proline	1431 ± 9[b]	1430[b]
Pipecolic acid	1432 ± 11	1427

[a] All values from the B3LYP/6-311++G**//B3LYP/6-31+G* level of theory. Zero point energy and thermal corrections calculated from un-scaled vibrational frequencies at the B3LYP/6-31+G* level of theory. Calculated isodesmically using ΔH_{acid} of 1456 kJ/mol for acetic acid from Ref. [58].
[b] From Ref. [50].

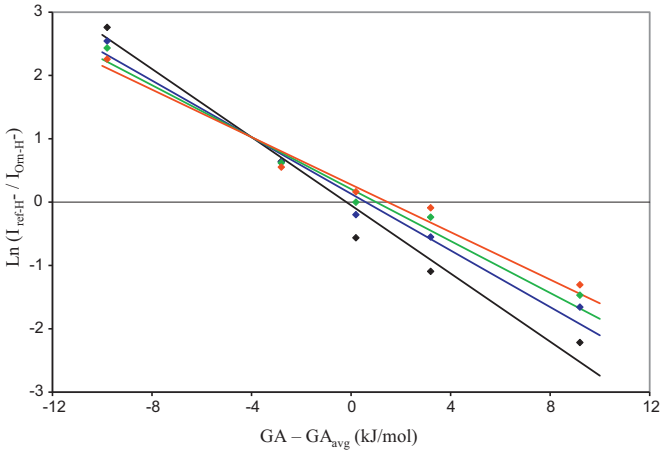


Fig. 1. Plot of $\ln(I_{[ref\text{-}H]^-}/I_{[Orn\text{-}H]^-})$ vs. $GA_{Ai} - GA_{avg}$ at collision energies between 6 V and 24 V (lab).

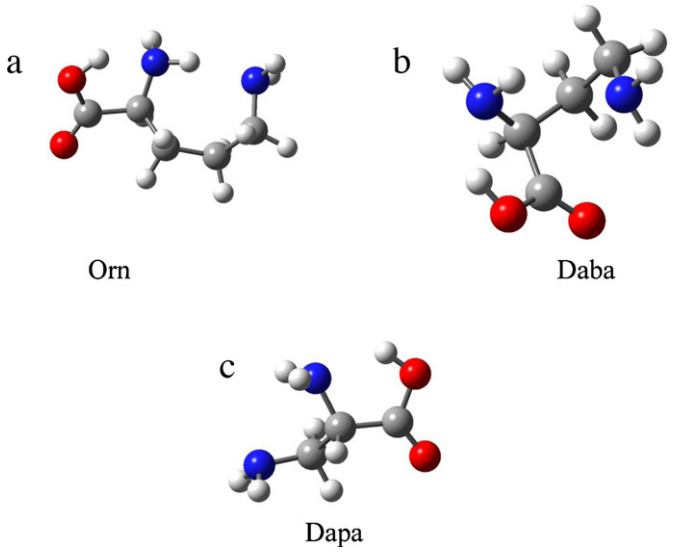


Fig. 2. Lowest energy structures of Orn, Daba, and Dapa calculated at the B3LYP/6-31+G* level of theory.

than ±8–12 kJ/mol. Given this uncertainty, the experimental and theoretical acidities agree within their error limits.

Examining the lowest energy structures for Orn and $[Orn\text{-}H]^-$ shown in Figs. 2a and 3a, shows that both molecules exhibit strong intramolecular hydrogen bonding. The hydrogen bonding scheme in neutral ornithine, in which the carboxyl hydrogen is involved in a hydrogen bond with the amino nitrogen atom (1.87 Å), is similar to the low-energy arrangement for lysine found by Paizs and co-workers [44]. This arrangement allows for a significant interaction between amino groups on the terminus and the side chain. Gronert et al. also recently completed a comprehensive study of the PA of all 20 PAAs [59]. In their study they argue for using computed free energies to determine the most stable conformer. Their lowest free-energy structure for lysine has an extended side chain with no N—N hydrogen bonding. We find similar behavior for ornithine in that the second lowest enthalpy conformer (ΔH_{298} = 5 kJ/mol above the global minimum, Fig. S1) is an extended structure that has a slightly lower free energy (ca. 0.8 kJ/mol) than that of our minimum enthalpy conformer.

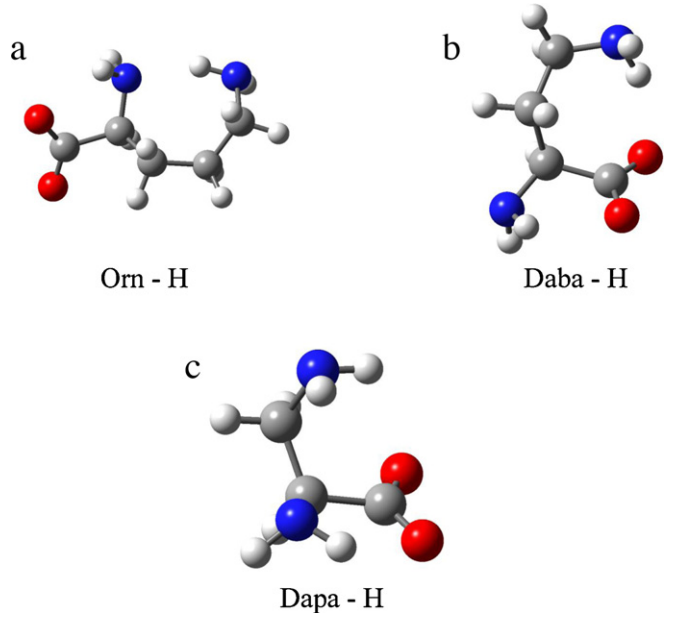


Fig. 3. Lowest energy structures of $[Orn\text{-}H]^-$, $[Daba\text{-}H]^-$, and $[Dapa\text{-}H]^-$ calculated at the B3LYP/6-31+G* level of theory.

The lowest energy structure for deprotonated ornithine also exhibits strong hydrogen bonding between the amino groups (r = 2.04 Å) as well as an interaction between the N-terminus and the carboxylate oxygen atom (r = 2.2 Å). A different conformer in which the two amino groups are each interacting with the carboxyl oxygen atoms (Fig. S1) is only 0.6 kJ/mol less stable (ΔH) than the minimum energy structure. We found 12 different structures that lie within 5 kJ/mol of the minimum structure, all of which should be present in a room temperature sample of ions (Fig. S5). All of these conformers are compact with significant hydrogen bonding and are similar to the minimum enthalpy neutral Orn structure. The derived entropy term from the kinetic method experiment is $-9\,J\,K^{-1}$, which implies that there is not a large geometry change between the neutral and the anion. The fact that the computed structures for both neutral and deprotonated ornithine contain significant hydrogen bonding is consistent with the experimentally derived entropy term.

4.1.2. Daba and Dapa

Similar experiments were performed in order to determine the gas-phase acidities of Daba and Dapa. 4-fluorobenzoic acid, 3-hydroxybenzoic acid, benzoic acid, and phenylacetic acid were used as references for both studies and kinetic method plots for both Daba and Dapa are shown in Figs. S1 and S2. ODR analysis gives acidities of 1419 ± 7 and 1418 ± 8 for Daba and Dapa, respectively. In addition, protonation entropy values of −4 and $-9\,J\,K^{-1}$ were derived from this data. Again, these small entropy values suggest a similar conformational spaces between the neutral and deprotonated amino acids. Theoretical acidities of 1417 and 1420 kJ/mol were obtained from isodesmic reaction (4) for Daba and Dapa and are in excellent agreement with the experimental acidities.

Figs. 2 and 3 show the lowest energy structures for neutral and deprotonated Daba and Dapa. As with Orn, these amino acids form strong intramolecular hydrogen bonds in both their neutral and deprotonated forms. The lowest energy conformer for Daba (Fig. 2b) exhibits the same OH—NH_2 hydrogen bonding scheme (r = 1.92 Å) as Orn, but in this case, the side chain prefers to form weak interactions with both the N-terminus (r = 2.50 Å) and the carboxylic acid oxygen (r = 2.64 Å). The conformer with a similar structure to the global minimum in Orn was found to be 1.3 kJ/mol higher in energy (Fig. S6). The lowest energy extended structure is 8.5 kJ/mol higher in energy and also higher in free energy (Fig. S6). The lowest energy structure for Dapa is similar to that of Orn with a strong OH—NH_2 hydrogen bond (r = 1.92 Å) and a weaker NH—NH_2 hydrogen bond (r = 2.31 Å). The structure similar to the lowest energy Daba structure is 0.3 kJ/mol higher in energy (Fig. S7). The side chain of Dapa is too short to support a truly extended structure.

The structures for deprotonated Daba and Dapa are similar to each other in that the side chain amino group is interacting with the carboxyl oxygen rather than the terminal amino group. In deprotonated Daba, the carbonyl oxygen forms hydrogen bonds with both the side chain (r = 2.00 Å) and terminal (r = 2.25 Å) (terminal) amino groups. The conformer with NH—N hydrogen bonding is 2 kJ/mol higher in energy (Fig. S6). Deprotonated Dapa has a moderate hydrogen bond between the carboxyl oxygen atoms and the terminal amino group (r = 2.03 Å). The side chain amino groups forms weak interactions with the carboxyl oxygen (r = 2.78 Å) and the terminal amino group (r = 2.53 Å).

4.1.3. Trends

The gas-phase acidity of lysine was determined in our previous study on the gas-phase acidity of PAAs to be 1416 ± 7 kJ/mol using the extended kinetic method in a quadrupole ion trap instrument

Table 3
Measured intensity ratios at 15 V (lab) collision energy.

Analog	$\ln\left(I_{[3\text{-hydroxybenzoic acid-H}]^-}/I_{[AA\text{-H}]^-}\right)$
Lysine	0.82
Ornithine	0.64
Daba	0.76
Dapa	0.95
Azetidine-2-carboxylic acid	2.47
Proline	3.25
Pipecolic acid	2.89

[50]. This value is in excellent agreement with a previous determination by O'Hair et al. using the single reference kinetic method (1412 kJ/mol) [5]. As can be seen from Table 2, the acidities of all for homologues are the same within error. This is in contrast to the PAs of these compounds in which the PAs of lysine and ornithine are similar (ca. 1004 and 1001 kJ/mol) [7,44,59–62] but those of Daba and Dapa are much lower (976 and 950 kJ/mol) [19]. We used three of the same reference acids for the determination of the GAs for all analogs and can therefore use the experimentally-determined product ion ratios to give relative apparent gas-phase acidities (ΔG_{acid}) for **1–3**. Table 3 shows product ln(ratio) at 15 V for 3-OH-benzoic acid, benzoic acid and phenyl-acetic acid with **1–5**. All three references give the same ordering in apparent (ΔG_{acid}), Dapa > Daba > Orn. Our GA value for lysine (1416 kJ/mol) was obtained in an ion trap instrument so a direct comparison with ratios from our previous work is not possible. We therefore re-measured product ion ratios for the proton-bound dimer ion of deprotonated lysine and deprotonated 3-hydroxybenzoic acid. The ln(ratio) for this dimer at 15 V is 0.82, whereas the ln(ratio) for the lower homologues are 0.64, 0.76, and 0.95, which makes the final relative (ΔG_{acid}) ordering Dapa > Lys > Daba > Orn. Thus, even in the apparent acidity ordering, there is no direct trend in acidity with side-chain length. One might have expected that since the preferred low-energy structures for both the neutral and anionic forms of **1–3** involve cyclic intramolecular hydrogen bonding that one would see a systematic effect in the acidity with increasing side-chain length as was seen in the PAs of these species. These results indicate that the acidity of these species is dominated by the enhanced stability of the anions due to this intermolecular hydrogen bonding but that systematic inductive effects are small by comparison. Previous studies have shown that these inductive effects are small in analogous length simple carboxylic acids, e.g., the acidities propanoic acid, butanoic acid, pentanoic acid and hexanoic acid are the same within error (ca. 1449 kJ/mol) [58].

4.2. Proline analogs

4.2.1. Aze and Pip

Similar kinetic method studies were carried out for the 4- and 6-membered ring analogs of proline, azetidine-2-carboxylic acid (Aze), and pipecolic acid (Pip). First kinetic method plots for Aze and Pip are shown in Figs. S3 and S4. 3-Hydroxybenzoic acid, 2,5-dimethylbenzoic acid, benzoic acid, and phenyl acetic acid were used for both studies. ODR derived values of 1425 ± 13 and 1432 ± 11 were obtained for the acidities Aze and Pip. In addition, protonation entropies of -9 ± 10 and 2 ± 18 were derived for Aze and Pip were derived from the ODR analysis. These studies indicate that Aze and Pip have the same acidity as proline from our previous study (1431 ± 9 kJ/mol) [50] and from a recent re-evaluation by Bouchoux et al. (1426 kJ/mol) [46] within error.

Conformational analysis for the proline analogs was more straightforward that for the lysine homologues in that the ring structures limit the number of low-energy conformers. Fig. 4 shows the lowest energy conformers for Aze, Aze-H, Pip, and Pip-H. The two neutral structures are the same as those reported by Armentrout and co-workers in their studies of the alkali metal ion affinities of Aze and Pip [63] in which the carboxyl OH group is hydrogen bonding with the ring nitrogen atom (r_{OH-N} = 1.96 Å for Aze and 1.94 Å for Pip). This hydrogen bonding scheme is also the same as that for proline as obtained in a comprehensive study of low-energy conformer of aliphatic amino acids by Bouchoux [46]. The lowest energy conformers for the anions involve NH—O hydrogen bonding (r = 2.19Å in Aze-H and 2.21 Å in Pip-H). Isodesmic reaction (4) predicts that the acidities of Aze and Pip are both 1427 kJ/mol. Additional low energy structures for neutral and deprotonated **4** and **5** are shown in Fig. S8.

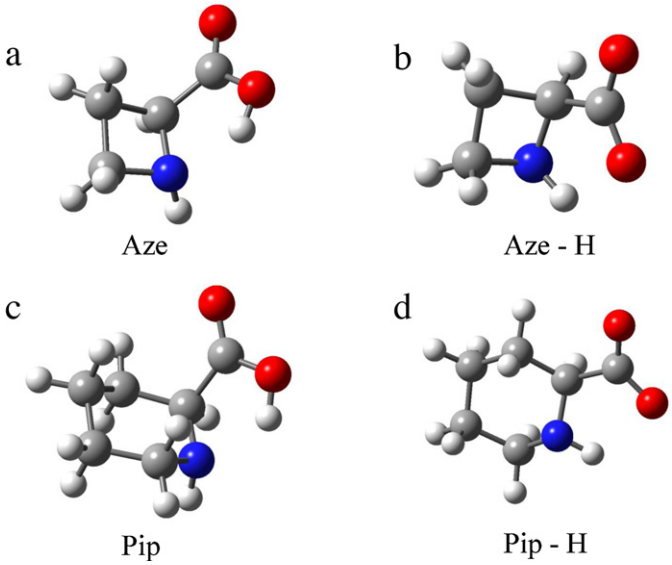


Fig. 4. Lowest energy conformers of Aze, $[Aze\text{-}H]^-$, Pip, and $[Pip\text{-}H]^-$ calculated at the B3LYP/6-31+G* level of theory.

4.2.2. Trends

As with the lysine homologues, the difference in the measured acidity between Aze and Pip is smaller than the error limits of the measurements. The relative apparent (ΔG_{acid}) ordering for all three analogs was obtained from ln(ratio) values using 3-OH benzoic acid as the reference acid. Ln(ratio) values of 2.47, 3.25, and 2.89 were obtained for Aze, Pro and Pip, respectively at 15 V collision energy (lab), which leads to a relative (ΔG_{acid}) ordering of Pro > Pip > Aze, that is, proline is the weakest acid. The relative PA values for proline analogs [18] are in a different order: Pip > Pro > Aze, which is consistent with differing inductive effects from increased ring size. Interestingly, the alkali-metal affinities for the three analogs give an ordering Pro > Pip > Aze [63], which is a result of the enhanced stability of zwitterion form proline molecule.

5. Conclusions

Gas-phase acidity values for three lower lysine homologues and two proline analogs were determined using the extended kinetic method in an electrospray ionization–triple quadrupole instrument. Measured ΔH_{acid} values of 1415 ± 10, 1419 ± 7 and 1420 ± 8 kJ/mol were determined for Orn, Daba, and Dapa. These values are in agreement with isodesmic acidities obtained at the B3LYP/6-311++G**//B3LYP/6-31+G* level of theory. A relative acidity (ΔG_{acid}) ordering of Dapa > Lys > Daba > Orn was obtained using measured ratios with 3-hydroxybenzoic acid as the reference acid. Acidities for the 4- and 6-membered ring analogs of proline, Aze and Pip, were also determined using this method. Experimental acidities of 1425 ± 13 and 1432 ± 11 kJ/mol are in

excellent agreement with the theoretical prediction that both analogs have the same acidity of 1427 kJ/mol. A relative acidity (ΔG_{acid}) ordering of Pro > Pip > Aze was obtained using 3-OH-benzoic acid as the reference base. This study represents the first investigation of the gas-phase acidity of these non-protein amino acids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2011.12.017.

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