α,ω-Diaminoalkanes as Models for Bases that Dicoordinate the Proton: An Evaluation of the Kinetic Method for Estimating Their Proton Affinities

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The effectiveness of the kinetic method for estimating the proton affinities of bases that di-coordinate the proton is evaluated using α,ω-diaminoalkanes as model bases. The proton affinities of these diamines have previously been examined using the equilibrium method and critically evaluated. Calculations using density functional theory at the B3LYP/6-31++G(d,p) level confirm that protonated α,ω-diaminoalkanes have cyclic structures with the proton covalently bound to one of the amino nitrogen atoms and hydrogen-bonded to the other. Furthermore, this cyclic structure persists in the protonated heterodimer ion between an α,ω-diaminoalkane and ammonia (the model reference base); binding of the two bases takes place via a second hydrogen bond between the RNH_3^+ and ammonia. Measuring the proton affinities under several collision energies and extrapolating to zero collision energy yields proton affinities that are smaller than the reference values by −2.8 kcal/mol, on average. Application of the Fenselau correction gives proton affinities that differ from the reference values by ±1.0 kcal/mol. These results indicate that the kinetic method is effective for estimating the proton affinities of molecules that tend to have more than one potential protonation site. Application of this method is particularly suited to biological molecules, such as peptides, where application of the equilibrium method is impossible due to low sample volatility.

Introduction

The gas-phase structures of protonated and metalated amino acids and peptides have received much attention in the past few years.[1] Much of this interest originates from enthusiastic applications of mass spectrometry in the characterization and measurement of biological ions after the advent of electrospray ionization[2] and matrix-assisted laser desorption/ionization (MALDI).[3] An intrinsic understanding of peptide and protein structures can be derived from the affinities of amino acids and oligopeptides for the proton or the metal ion of interest. However, amino acids and peptides are nonvolatile, thus making them unsuitable ligands for the equilibrium method,[4] the conventional technique for measuring relative ion affinities.

The kinetic method, developed by Cooks and co-workers,[5] is an effective method for estimating the relative binding energies of two *similar* bases that bind to a central ion, typically a proton. It is based on measuring the relative abundance of the product ions arising from the dissociation of the complex ion, the ion-bound "heterodimer" of the bases. The logarithmic value of the relative abundance is proportional to the logarithm of the relative rate of dissociation of the two reaction channels, and is used to estimate the relative binding energy of the two ligands for the ion. An attractive feature is that application of the method does *not* require generating a population of the neutral base in the gas phase, thus permitting measurement of relative ion affinities of nonvolatile bases or ligands, such as amino acids and oligopeptides. For the dissociation of a proton-bound heterodimer of B_i and B,

$$[B_i\text{- - -H- - -B}]^+ \rightarrow B + B_iH^+ \quad \text{(rate constant} = k_i) \quad (1)$$

$$\rightarrow B_i + BH^+ \quad \text{(rate constant} = k) \quad (2)$$

where B_i is a reference base whose proton affinity is known, and B is the base for which the proton affinity is being measured. Application of transition state theory[6] leads to

$$\ln(k_i/k) = \ln(Q_i^*/Q^*) + [\epsilon_o - \epsilon_o(i)]/RT_{eff} \quad (3)$$

where Q_i^* and Q^* are the partition functions of the activated complexes; $\epsilon_o(i)$ and ϵ_o are the activation energies; R is the gas constant; and T_{eff} is the effective temperature, a parameter in temperature units that reflects the internal energy of the dissociating heterodimer. Assuming that abundances reflect rate constants and that no reverse activation barriers exist,[5,7]

$$\ln([B_iH^+]/[BH^+]) = \ln(Q_i^*/Q^*) + [PA(i) - PA]/RT_{eff} \quad (4)$$

where $[B_iH^+]$ and $[BH^+]$ are the abundances and PA(i) and PA are the proton affinities of the reference base and the unknown base, respectively. For structurally similar bases, $Q_i^* \approx Q^*$, eq 4 reduces to

$$\ln([B_iH^+]/[BH^+]) \approx [PA(i) - PA]/RT_{eff} \quad (5)$$

A plot of $\ln([B_iH^+]/[BH^+])$ versus the proton affinities of a series of structurally similar, e.g., homologous, reference bases would be linear with a slope of $1/RT_{eff}$ and an x intercept of PA. This approach constitutes the basis for many proton affinity measurements, whose results are typically in good agreement with those measured using the equilibrium method.[4]

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Despite its empirical success, the kinetic method's basic assumptions have generated much interest and discussion, particularly those that were highlighted in recent applications.[8] For biological ligands, the appropriateness of the kinetic method for evaluating their proton affinities is not immediately obvious. An amino acid or peptide typically has more than one potential protonation or metal-ligation site and therefore can potentially di- and even tri-coordinate the central ion.[1] Furthermore, this property makes identification of structurally similar reference bases for these ligands difficult, if not impossible.

A fundamental difficulty of the kinetic method is that its original derivation is based on assumption of thermal equilibrium, a condition unlikely to be applicable to a population of dissociating ions *in vacuo*.[5,8] Norrman and McMahon[8f] reported recently that the T_{eff} measured from a given metastably dissociating heterodimer of protonated nitriles varied inversely with the temperature of the high-pressure ion source in which it was generated and thermally equilibrated. Holmes *et al*.[8g] recently proposed the elimination of the T_{eff} term and discontinuation of its application in the derivation of entropy changes in complexation. Drahos and Vékey,[8b] however, found that T_{eff} correlated well with the mean internal energy of ions dissociating within the analytical time window and not with that of the whole ion population.

Bojesen and Breindahl[8d] were able to show that the relationship

$$\ln([B_iH^+]/[BH^+]) \approx [PA(i) - PA]C \qquad (6)$$

where C is a proportionality constant, could be derived without invoking the assumption of thermal equilibrium for the precursor ions. Applying quantum RRK theory, Craig *et al*.[8e] recently demonstrated that for branching reactions

$$\ln(k_i/k) \propto [\epsilon_o - \epsilon_o(i)] \qquad (7)$$

is an often valid condition even if the precursor ions have an undefined energy distribution.

Given the empirical success of the kinetic method and the lack of a viable alternative method for measuring proton and metal ion affinities of nonvolatile biological molecules, we decided to evaluate the accuracy of the kinetic method for measuring the proton affinities of *model bases* that are *believed to "cyclize" on protonation, and hence be stabilized by intramolecular hydrogen bonding*, and whose *proton affinities have been measured by means of the equilibrium method* and have been independently evaluated. The bonding in these protonated bases serves to mimic di-coordination of amino acids and peptides to the proton,[1a−e,h,i;9] and success or failure of the kinetic method in this evaluation may serve as an indication of the appropriateness of the technique for measuring the proton affinities of amino acids and peptides. In this exercise, α,ω-diaminoalkanes were selected as model bases; our interest in this class of bases was stimulated by the studies of Aue *et al*.[10] and Yamdagni and Kebarle,[11] whose results strongly suggest that protonation of these bases results in di-coordination of the proton by the two amino nitrogen atoms.

We report here the proton affinities of α,ω-diaminoalkanes measured in this exercise using two versions of the kinetic method[5,7] and compare them with reference data.[12] We also report the optimized structures of protonated α,ω-diaminoalkanes and that of the heterodimer of 1,4-diaminoalkane and ammonia using density functional theory (DFT) at the B3LYP/6-31++G(d,p) level of theory. Proton affinities are also calculated and compared with the reference data.

Experimental Section

Experiments were conducted on an atmospheric pressure ionization mass spectrometer of triple quadrupole (QqQ) design (TAGA 6000E, SCIEX, Concord, Ontario, Canada). The electrospray probe was fabricated from an approximately 3-cm long, 33-gauge stainless steel tube (Hamilton, ca. 100 μm i.d.) that had been attached to a length of 1/16 in. o.d. stainless steel tube with epoxy glue. The probe tip was electropolished prior to use. The optimum probe position was established from time to time, but was typically with the tip about 1−2 cm from the interface plate and with the spray off-axis from the orifice. Biassing of the probe tip was achieved via a 50-MΩ current-limiting resistor in series with a high-voltage power supply (Tennelec, model TC 950) set typically between 2.5 and 3.5 kV. The electrospray current was monitored via a custom-built microammeter that could be floated above ground.

Gas-phase proton-bound heterodimers of the amines were generated by means of electrospraying 50/50 water/methanol solutions containing a binary mixture of the amine bases, typically 1 mM per base. To measure the relative abundance of the protonated amine fragment ions, the protonated heterodimer ion was mass-selected in the first quadrupole (Q1), fragmented in q2 via collision with Ar, and the product ions mass-analyzed in Q3 with a dwell time of 10−50 ms per m/z unit. Each α,ω-diaminoalkane unknown base was paired with a minimum of three secondary amines as reference bases. The proton affinities of a number of 1-alkanamines were also measured as a comparison; for these unknown bases, other 1-alkanamines were used as reference bases. Collision-induced dissociation (CID) was performed under constant center-of-mass energies (E_{cm}) for all the pairs; a number of E_{cm} values, ranging from 0.6 to 2.5 eV, were employed. All CID experiments were carried out at a constant collision gas thickness of 1.0×10^{14} atoms cm^{-2},[13] under which single collisions prevailed.

Computational Methods

DFT employing the hybrid B3LYP method (using Becke's three-parameter exchange functional[14] and the correlation functional from Lee, Yang, and Parr[15]) with the 6-31++G(d,p) basis set[16] in Gaussian 98 [17] was used to calculate the optimized geometries and vibrational frequencies of the amines, their protonated ions, and the protonated heterodimers in which ammonia was the reference base. The transition state structure of protonated 1,4-diaminoalkane was found using a combination of the synchronous transit-guided quasi-Newton method (QST2) and the Berny transition-state algorithm in Gaussian 98.[17]

The proton affinity of a base B is the standard enthalpy change, $\Delta H°_{r,298}$, associated with reaction 8:

$$BH^+ \rightarrow B + H^+ \qquad (8)$$

$$\Delta H°_{r,298} = \Delta E_{elec} + \Delta E_{ZPVE}(0) + \Delta E_{int}(298) + 5RT/2 \qquad (9)$$

In eq 9, ΔE_{elec}, $\Delta E_{ZPVE}(0)$, and $\Delta E_{int}(298)$ refer to the changes in electronic energy, zero-point vibrational energy, and thermal energy required to calculate the reaction in eq 8 at 298.15 K, respectively. The constant, $5RT/2$, is the classical estimation of the effect of gaining three translational degrees of freedom ($3RT/2$) for the proton plus RT, the PV term for the proton.

Results and Discussion

Optimized Geometries of Protonated Monomers and Heterodimers. The experimental results of Aue *et al*.[10] and Yamdagni and Kebarle[11]suggest strongly that the proton in a

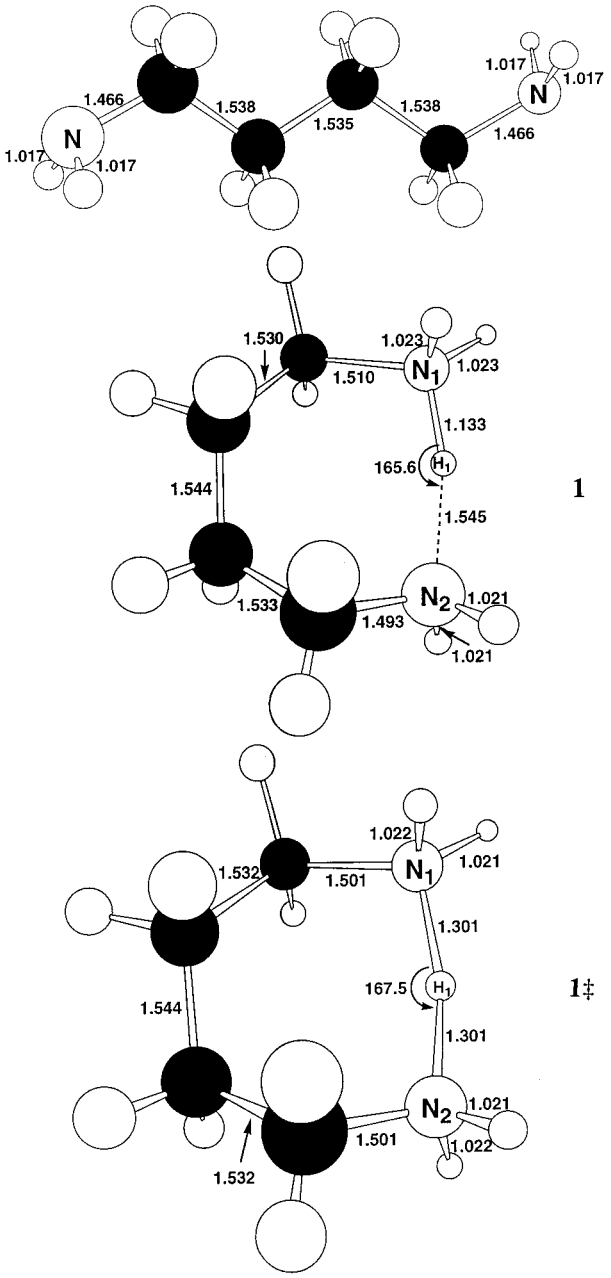


Figure 1. Optimized geometries of neutral and protonated 1,4-diaminobutane, and the transition state structure of interconverting protonated 1,4-diaminobutane isomers: ●, C; ○, H.

protonated α,ω-diaminoalkane ion is di-coordinated to the two amino nitrogen atoms. This interpretation was supported in a theoretical study in which the optimized structures of protonated 1,4- and 1,5-diaminobutane were determined using the SCF/DZP level of theory, although the proton affinity of 1,4-diaminobutane calculated at MP2/TZ2P//SCF/DZP was compared with experimental data referenced to a scale that is currently considered to be questionable.[18]

Since the objective of this study was to evaluate the accuracy of the kinetic method for biological ligands that have multiple potential protonation sites using model ligands that di-coordinated the proton, it was essential that the chosen model bases, α,ω-diaminoalkanes, do indeed di-coordinate the proton and, more importantly, that this di-coordination persists in the protonated heterodimer between the α,ω-diaminoalkane and the reference base. To determine if these conditions are met, we

TABLE 1: Calculated B3LYP/6-31++G(d,p) Energies (in Hartrees), Zero-Point Vibrational Energies, and Thermal Energies (Both in kcal/mol)

base	electronic energies	zero-point energies	thermal corrections
(A) α,ω-diaminoalkanes			
1,2-diaminoethane	−190.54364	69.3	3.5
1,3-diaminopropane	−229.86036	87.2	4.4
1,4-diaminobutane	−269.17835	105.1	5.1
1,5-diaminopentane	−308.49567	122.9	6.0
1,6-diaminohexane	−347.81310	140.7	6.9
(B) protonated α,ω-diaminoalkanes			
1,2-diaminoethane	−190.92137	79.1	3.1
1,3-diaminopropane	−230.25012	96.9	3.7
1,4-diaminobutane	−269.57449	114.6	4.4
1,5-diaminopentane	−308.88737	132.4	5.2
1,6-diaminohexane	−348.20345	150.9	5.9

began with an examination of the optimized geometries of protonated monomers and heterodimers of α,ω-diaminoalkanes.

Preliminary structure optimization studies showed that the neutral α,ω-diaminoalkanes prefer structures in which the amino groups are as far apart as possible. The geometric parameters for all of the α,ω-diaminoalkanes from optimization at B3LYP/6-31++G(d,p) are remarkably invariant with N−H distances of 1.017 Å, C−N distances of 1.465−1.466 Å, and C−C distances of 1.533−1.539 Å (with the exception of ethylenediamine in which the C−C distance is 1.542 Å). We illustrate the geometries by using only one molecule, 1,4-diaminobutane (Figure 1). The electronic, zero-point vibrational and thermal energies are shown in Table 1. Structural information on the other α,ω-diaminoalkanes is given in the Supporting Information.

Protonation has the dramatic effect of producing a cyclic structure, **1**, in which the proton is covalently bound to one of the amino groups and is hydrogen-bonded to the other amino group (Figure 1). For 1,2-diaminoethane, the N−H····N angle is constrained by the ring size to be 123.5°, but as the number of carbon atoms in the ring increases, then this angle also increases and approaches 180°, the preferred value for a hydrogen bond. Protonation results in substantial increases in both C−N distances. The C−NH_3^+ distance is the larger one, with distances between 1.509 and 1.520 Å, whereas the C−NH_2 distance is around 1.494−1.499 Å (except in the most strained ion, $H_2N(CH_2)_2NH_3^+$, where it is 1.471 Å). The changes in C−C distances resulting from protonation at nitrogen are much smaller, with those adjacent to the C−NH_3^+ bonds being shorter than in the neutral α,ω-diaminoalkanes, but by less than 0.01 Å. The C−C bonds adjacent to the C−NH_2 bonds in the protonated α,ω-diaminoalkanes show even smaller changes, but also are decreased relative to those in the parent bases. Conversely, the other C−C distances in the center of the carbon chains are slightly larger in the protonated amines.

The N−H distances in both the NH_3^+ and NH_2 groups in the protonated amines are longer than those in the neutral molecules and the proton involved in the hydrogen bond has the longest bond. In protonated 1,4-diaminobutane the N^+−H distance is 1.133 Å, whereas the N····H distance is 1.545 Å. These compare with a distance of 1.017 Å in the neutral diaminoalkanes.

The transition structure for the intramolecular proton transfer, **1**‡ (Figure 1), has C_2 symmetry with the migrating hydrogen symmetrical between the two nitrogen atoms. The geometric changes within the C_4N_2 backbone on going from the structure at the minimum to the transition structure are all small. The barrier to this rearrangement is very small (0.5 kcal/mol),

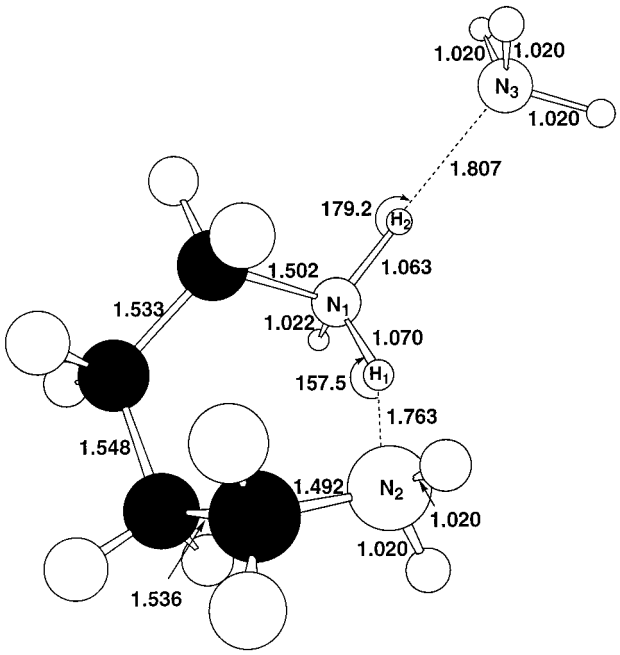
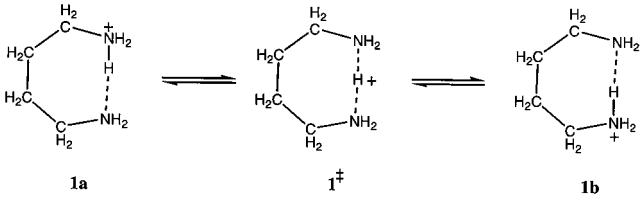


Figure 2. The optimized structure of the protonated heterodimer between 1,4-diaminobutane and ammonia.

indicating that there is rapid interconversion between equivalent structures **1a** and **1b**.



The most stable structure of the protonated heterodimer between an α,ω-diaminoalkane and a reference base was not immediately apparent. Protonation of one of the amino groups, either on the α,ω-diaminoalkane or on the reference amine, results in an RNH_3^+ ion. This has three acidic hydrogen atoms and each can potentially hydrogen bond to one of the two remaining RNH_2 groups. Geometric optimization showed that the lowest energy structure of the heterodimer has two hydrogen bonds, one intramolecular as in the isolated protonated diamine and the other with the reference amine. Figure 2 shows the optimized structure for the heterodimer between protonated 1,4-diaminobutane and ammonia, the latter being selected as the representative reference base for computational efficiency. It is noteworthy that the *same* optimized structure was obtained irrespective of the initially guessed structure. Starting structures included the ammonium cation, NH_4^+, being di-coordinated by the two amino nitrogen atoms of 1,4-diaminobutane and the ammonium ion being attached to only one of the amino hydrogen atoms.

Comparison of the heterodimer structure (Figure 2) with that of the isolated protonated 1,4-diaminobutane in Figure 1 shows the proton between the two nitrogen atoms of the diamine in the dimer ion to have a shorter NH_2-H distance (1.070 Å compared with 1.133 Å) and the hydrogen bond distance to be much longer (1.763 Å compared with 1.545 Å), i.e., the bridging proton is not so extensively shared with the other terminal amino group. The hydrogen between the RNH_3^+ and NH_3 has a slightly shorter N−H bond (1.063 Å) and a slightly longer $NH_3^+\cdots NH_3$ distance (1.807 Å), indicating that this hydrogen bond is weaker than that with the terminal amino group. This is consistent with the higher proton affinity of 1-pentanamine (220.7 kcal/mol) compared with that of ammonia (204.0 kcal/mol).

TABLE 2: Reference Bases for α,ω-diaminoalkanes

reference base	PA (kcal/mol)[a]	α,ω-diaminoalkane 1,2	1,3	1,4	1,5	1,6
1. *N*-methyl methanamine	222.2	•				
2. *N*-methyl ethanamine	225.2	•				
3. *N*-ethyl ethanamine	227.6	•			•	•
4. 2-methyl-N-(2-methyl-propyl)-1-propanamine	229.0		•		•	•
5. *N*-butyl-1-butanamine	231.5		•	•	•	•
6. *N*-(1-methylethyl)-2-propanamine	232.3			•		
7. *N*-(1-methylpropyl)-2-butanamine	234.4		•	•	•	•

[a] NIST evaluated data, see ref 12.

Upon collision activation, the most probable bond fission(s) in the heterodimer occur at the H-bonds, *N3−H2*, *N1−H2*, *N1−H1*, and *N2−H1*. Cleavage of *N1−H1* or *N2−H1* alone will not lead to protonated 1,4-diaminobutane or protonated ammonia product ions; these are only produced upon cleavage of *N3−H2* or *N1−H2*, respectively. Under our experimental conditions, each protonated heterodimer collided, on average, with only one argon atom; this means there was a low probability of multiple activation events. Furthermore, the probability of multiple bond fissions was lowest under the smallest E_{cm}. This is apparently a reason the most accurate PAs were measured at the lowest center-of-mass collision energies. One of the tenets of the kinetic method is that the structure of the protonated monomer and that of the monomer within the protonated heterodimer should be similar. The structural details of the protonated 1,4-diaminobutane in the heterodimer of Figure 2 are very similar to those of the protonated 1,4-diaminobutane monomer shown in Figure 1. It is perhaps not surprising, at least from this perspective, that the kinetic method is applicable in the present case.

Kinetic Method. Table 2 shows the seven secondary amines used as reference bases for the five α,ω-diaminoalkanes examined. Although the secondary amine bases are not members of a homologous series, they are nonetheless structurally very similar and are the best that could be identified given the limited number of evaluated amines with high proton affinities.[12]

The measured PAs of the α,ω-diaminoalkanes at different E_{cm} values, their averages, resultant PAs after corrections, the calculated PAs, and the reference values are listed in Table 3. The measured proton affinities are apparently a function of center-of-mass energies, in accordance with previous observation.[1i,7a] In cases in which binding of the bases to the proton is comparable, such as measurements of PAs of primary amines with other primary amines as reference bases (Table 4), the PAs measured are little affected by collision energies: the average standard deviation of the measurements in different E_{cm} values of the three 1-aminoalkanes is 0.2 kcal/mol, whereas that of the five α,ω-diaminoalkanes is 0.8 kcal/mol.

For the α,ω-diaminoalkanes, the measured PAs are all lower, by an average of 2.8 kcal/mol, than their reference PAs established by the equilibrium method,[4] but are considerably higher than the PAs of their equivalent monoamines[12] as shown in Table 5. The calculated PAs are in good agreement with the reference PAs. These results are in accordance with the expectation that di-coordination of the α,ω-diaminoalkanes is maintained in the dissociating heterodimer between the α,ω-diaminoalkane and the secondary amine.

From Tables 3 and 4, it is readily apparent that the Fenselau correction,[7] irrespective of its assumptions,[8] allows one to derive

TABLE 3: Proton Affinities (PAs) of α,ω-Diaminoalkanes, kcal/mol

base	PAs (E_{cm} in eV)	PA average[a]	PA $E_{cm}=0$[b]	PA Fenselau[c]	PA calc[d,e]	PA ref[f]
1,2	224.7 (0.8), 224.6 (1.5), 224.1 (2.0)	224.5 ± 0.3	225.1	226.6	229.1	227.4
1,3	231.7 (0.6), 231.3 (1.2), 231.0 (2.0)	231.3 ± 0.4	232.0	234.8	237.1	235.9
1,4	237.2 (1.0), 236.0 (1.5), 235.7 (2.0), 234.5 (2.5)	235.9 ± 1.1	238.8	241.3	241.3	240.3
1,5	234.9 (1.0), 233.6 (1.5), 232.6 (2.0), 231.6 (2.5)	232.2 ± 1.4	237.0	239.0	238.6	238.9
1,6	232.8 (1.0), 232.0 (1.5), 231.3 (2.0), 230.5 (2.5)	231.7 ± 1.0	234.3	240.8	237.2	238.9
average deviation from reference PA			−2.8	±1.0	±1.2	

[a] Average of all E_{cm} values and one standard deviation. [b] Extrapolation to $E_{cm}=0$. [c] Fenselau method of correction, see ref 7. [d] Calculated in this study using B3LYP/6-31++G(d,p). [e] Calculated basicities ($\Delta G°$ values) are 220.7, 227.9, 231.6, 229.1, and 227.0 kcal/mol, respectively. [f] NIST evaluated data, see ref 12.

TABLE 4: Proton Affinities (PAs) of 1-Aminoalkanes, kcal/mol

base	PAs (E_{cm} in eV)	PA average	PA $E_{cm}=0$	PA Fenselau	PA ref
1-propanamine	216.0 (0.8), 216.1 (1.2), 215.9 (1.5), 214.9 (2.0)	215.7 ± 0.5	216.9	216.8	219.4
1-hexanamine	221.8 (0.8), 221.7 (1.2), 221.8 (1.5), 221.9 (2.0)	221.8 ± 0.1	221.7	221.7	221.7
1-octanamine	221.1 (0.8), 221.2 (1.2), 221.1 (1.5), 221.1 (2.0)	221.1 ± 0.0	221.1	221.0	222.0

See Table 2 for meanings of the PA columns.

TABLE 5: Reference PAs (kcal/mol) of α,ω-Diaminoalkanes and Their Equivalent 1-Aminoalkanes[12]

α,ω-diaminoalkane	PA	1-aminoalkane	PA
ethylenediamine	227.4	1-propanamine	219.4
1,3-diaminopropane	235.9	1-butanamine	220.2
1,4-diaminobutane	240.3	1-pentanamine	220.7
1,5-diaminopentane	238.9	1-hexanamine	221.7
1,6-diaminohexane	238.9	1-heptanamine	220.7

correctly accurate proton affinities of bases that are known to di-coordinate a proton, using monodentate reference bases.[19] These two tables also show a proposed alternative correction procedure, which involves extrapolating the apparently linear relationship between measured proton affinity and E_{cm} to $E_{cm}=0$, for deriving a best estimate of the correct PA. Figure 3 shows an example of such a correction for 1,5-diaminopentane. The average deviation of the corrected proton affinities from the reference proton affinities using the Fenselau correction was found to be ± 1.0 kcal/mol, whereas that using the proposed extrapolation method was −2.8 kcal/mol. At this juncture, it is perhaps not appropriate to decide, because of the small number of bases considered (limited by the small number of evaluated di-coordinating bases), whether one correction method is more accurate than the other. The positive outcome is that both methods estimate the proton affinities of the α,ω-diaminoalkanes with acceptable accuracies.

Conceptually, the observation that the measured PA of the lowest E_{cm} appears to best approach the real PA (see Table 3) is in accordance with a consideration of the late transition state in which the following equilibrium applies:

$$BH^+ + B_i \overset{K}{\rightleftharpoons} B_iH^+ + B \qquad (10)$$

Provided equilibration of the excess energy within the precursor ion is much more rapid than bond dissociation, then

$$\Delta G_i - \Delta G = -RT_{eff} \ln K \qquad (11)$$

where ΔG_i is the free energy of protonation of B_i and ΔG is that of B. Replacing ΔG with ΔH and ΔS, and ln K with ln(k_i/k),

$$(\Delta H_i - T_{eff}\,\Delta S_i) - (\Delta H - T_{eff}\,\Delta S) = -RT_{eff} \ln(k_i/k) \qquad (12)$$

$$\Delta\Delta H - T_{eff}\,\Delta\Delta S = \Delta PA - T_{eff}\,\Delta\Delta S = -RT_{eff} \ln(k_i/k) \qquad (13)$$

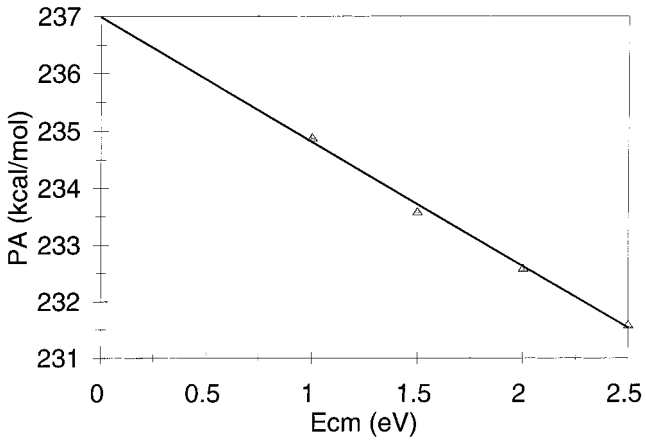


Figure 3. Proton affinity of 1,5-diaminopentane versus E_{cm}.

The $\Delta\Delta S$ value is unlikely to be approximately zero in the present situation where B di-coordinates the proton whereas B_i does not. The $T_{eff}\,\Delta\Delta S$ term, however, can be minimized when T_{eff} is the lowest. Although the actual thermodynamic significance of T_{eff} is subject to question, the parameter is nonetheless a reflection of the internal energy of the ions.[8] On average, a precursor ion that is subject to a more energetic collision (larger E_{cm}) will have a higher internal energy and a higher T_{eff}. This means that the $T_{eff}\,\Delta\Delta S$ term is the smallest when E_{cm} is the lowest in our experiments; as a result, as E_{cm} decreases, ln(k_i/k) or ln([B_iH^+]/[BH^+]) becomes an increasingly accurate estimate of ΔPA. That is, eq 5 becomes increasingly accurate as E_{cm} decreases. For reference bases and unknown bases that are members of a homologous series, such as the 1-alkanamines whose data are shown in Table 4, $\Delta\Delta S$ is very close to zero,[7] and the $T_{eff}\,\Delta\Delta S$ term is approximately zero irrespective of E_{cm}. Consequently ΔPA, and therefore the measured PA of the unknown base, is independent of E_{cm} as shown in Table 4.

The physical equivalence of extrapolating the data to $E_{cm}=0$ is to perform the collision-induced dissociation under an axial potential gradient of zero. Experimentally, this is difficult because the abundances of the product ions are extremely low and become unreliable; thus extrapolation is the only viable means of obtaining accurate abundance ratio of the product ions at $E_{cm}=0$. While no description currently exists that can account for the apparently linear relationship between PA and E_{cm}, the observation that experimentally linearity occurs (Figure 3) simplifies the extrapolation.[20] The attractiveness in this

procedure is that there is no inherent assumption of the precursor ions being in thermal equilibrium;[7] however, the ion energy at $E_{cm} = 0$ is undefined.

We conclude that it is possible to apply the kinetic method to estimate the proton affinities of bases that di-coordinate the proton using reference bases that mono-coordinate. The estimates are most accurate at lowest collision energies. The structure of the protonated 1,4-diaminobutane in a heterodimer of 1,4-diaminobutane and a reference base has been found to be very similar to that of the protonated 1,4-diaminobutane monomer, which is necessary for the kinetic method to be applicable. Despite the inherent conceptual difficulties associated with the kinetic method, it is the only method available (other than bracketing) for nonvolatile bases such as biological ions, many of which contain more than one potential protonation or metalation site. As a consequence of our findings here, we are optimistic that the kinetic method will allow estimation of proton or metal ion affinities of biological molecules, such as amino acids and peptides, to an accuracy of a few kcal/mol.

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Supporting Information Available: Optimized structures of neutral 1,2-diaminoethane, 1,3-diaminopropane, 1,5-diaminopentane and 1,6-diaminohexane; optimized structures of protonated 1,2-diaminoethane, 1,3-diaminopropane, 1,5-diaminopentane and 1,6-diaminohexane; and experimental data used in Table 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Craig et al.[8e] reported that linearity of the $\ln([B_iH^+]/[BH^+])$ versus $PA(B_i)$ plots is limited to precursor ions of high internal energy whereas no such limitation was noted by Drahos and Vékey.[8b] There is no inherent theoretical basis against a linear PA versus E_{cm} relationship.