Proton Affinities of Lysine and Histidine: A Theoretical Consideration of the Discrepancy between Experimental Results from the Kinetic and Bracketing Methods

Andrey A. Bliznyuk,* Henry F. Schaefer III,† and I. Jonathan Amster

Contribution from the Department of Chemistry, University of Georgia, Athens, Georgia 30602

Received September 8, 1992

Abstract: *Ab initio* quantum chemical computations in conjunction with semiempirical methods have been applied to the proton affinities of the amino acids lysine and histidine. The data obtained show that lysine is much more basic than histidine. The results reported here agree well with recent bracketing measurements of the proton affinities but contradict other measurements made by the kinetic method. The reason for this disagreement is discussed.

I. Introduction

Hydrogen bonding (H-bonding) plays a central role in the structure and energetics of biopolymers, such as the helical structure of DNA and the conformations of proteins.[1] While such interactions involve mainly neutral molecules, H-bonds involving ions are also relevant to biological systems. For example, many of the processes in the active sites of enzymes involve proton transfer reactions.[2] Knowledge of the gas-phase proton affinity (PA) values of biomolecules will provide insight into intramolecular forces. The correlation between the strength of H-bonds and the proton affinity (PA) of the components forming this bond has been established by numerous experiments.[3,4]

Knowledge of the pK_a values of ionizable groups of biomolecules is also important for interpreting the binding of a substrate or inhibitor to a protein. They are of particular interest for understanding the mechanism of enzyme activities.[5] However, the experimental determination of individual pK_a values is difficult in complex systems. Theoretical approaches to calculating relative and/or absolute pK_a values require knowledge of the gas-phase PA's.[6]

It is useful therefore to have a reliable and accurate values of the PA's of amino acids. The experimental determination of their gas-phase basicities dates from 1979[7] when the PA of six of the most thermally stable amino acids were determined. Early measurements of the gas-phase basicities of amino acids used equilibrium methods with high-pressure mass spectrometry or ion cyclotron resonance spectrometry.[7] However, these techniques require a volatile, thermally stable sample. Unfortunately, most amino acids and peptides are neither volatile nor thermally stable, and so other methods are required to obtian these important thermodynamic quantities.

Recently, the absolute and relative PA's of all 20 amino acids have been reported.[8-10] In two of these studies,[8,9] the kinetic approach[11] was applied for the determination of PA values. In one study,[10] the laser desorption/chemical ionization technique was used to allow the gas-phase basicity (GB) and PA to be determined by the bracketing method.[12,13] For many of the amino acids, the results from the two methods are in a good agreement with each other, and with the previous results.[7] But comparison of the PA values for lysine (Lys) and histidine (His) shows a great difference. Indeed, the PA's of these amino acids according to the kinetic method[8,9] are similar (PA(Lys) = 228.7 kcal/mol, PA(His) = 230.5 kcal/mol) with those of Lys slightly less basic. But the results of the bracketing study[10] suggest that Lys is significantly more basic than His, PA(Lys) = 242.6 kcal/mol and PA(His) = 228.5 kcal/mol.

In this paper, we describe results of a theoretical investigation of the proton affinities of lysine and hystidine. Our approach is to use high-level theoretical methods to characterize the model compounds 1,4-diaminobutane and 5-methylimidazole. Using these results in combination with semiempirical predictions, we estimate the proton affinities of lysine and hystidine. This work not only allows the determination of the absolute and relative PA's of the amino acids, but it also provides insight into the relative merits of the two above-mentioned experimental techniques for making such measurements.

II. Choice of the Model

It is well-known that *ab initio* methods using large basis sets and incorporating electron correlation give accurate PA values.[14-17] Unfortunately, the Lys and His (see Figures 1 and 2) are too big to allow computations at the most desirable levels of theory. Therefore, the appropriate selection of models becomes important.

(a) Lysine. Recent 6-31G computations of the neutral and protonated forms of L-2,4-diaminobutyric acid[18] (this amino acid has two CH_2 groups instead of four in Lys) showed that the most

* Permanent address: Institute of Bioorganic Chemistry, Lavrentjev Avenue 8, Novosibirsk, 630090, Russia.

† Author to whom correspondence should be addressed.

(1) Stryer, L. *Biochemistry*; W. H. Freeman: New York, 1988.
(2) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: New York, 1985.
(3) Meot-Ner, M.; Sieck, W. *J. Am. Chem. Soc.* **1986**, *108*, 7525–7529.
(4) Zeegers-Huyskens, T. *J. Mol. Struct.* **1988**, *177*, 125–141.
(5) Yadav, A.; Jackson, R. M.; Holbrook, J. J.; Warshel, A. *J. Am. Chem. Soc.* **1991**, *113*, 4800–4805.
(6) Lim, C.; Bashfold, D.; Karplus, M. *J. Phys. Chem.* **1991**, *95*, 5610–5620.
(7) Meot-Ner, M.; Hunter, E. P.; Field, F. H. *J. Am. Chem. Soc.* **1979**, *101*, 686–689. Locke, M. J.; McIver, R. T., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 4226–4232.
(8) Briendahl, T.; Bojesen, G. *Proceedings of the 12th International Mass Spectroscopy Conference*; Amsterdam, WeA-1347, 1991.
(9) Wu, Z.; Fenselau, C. *Rapid Common. Mass. Spectrom.* **1992**, *6*, 403–405.
(10) Gorman, G. S.; Speir, J. P.; Turner, C. A.; Amster, I. J. *J. Am. Chem. Soc.* **1992**, *114*, 3986–3988.
(11) McLucky, S. A.; Cameron, D.; Cooks, R. D. *J. Am. Chem. Soc.* **1981**, *103*, 1313–1317.
(12) Speir, J. P.; Gorman, G. S.; Amster, I. J. *Anal. Chem.* **1991**, *63*, 65–69.
(13) Speir, J. P.; Amster, I. J. *Anal. Chem.* **1992**, *64*, 1041–1045.
(14) Del Bene, J. E. *J. Comp. Chem.* **1985**, *6*, 296–301.
(15) De Frees, D. J.; McLean, A. D. *J. Comp. Chem.* **1986**, *7*, 321–333.
(16) Del Bene, J. E.; Shavitt, I. *J. Phys. Chem.* **1990**, *94*, 5514–5518.
(17) Komornicki, A.; Dixon, D. A. *J. Chem. Phys.* **1992**, *97*, 1087–1094.
(18) Fugler-Domenico, L.; Russel, C. S.; Sapse, A. M. *Struct. Chem.* **1990**, *1*, 379–384.

0002-7863/93/1515-5149$04.00/0

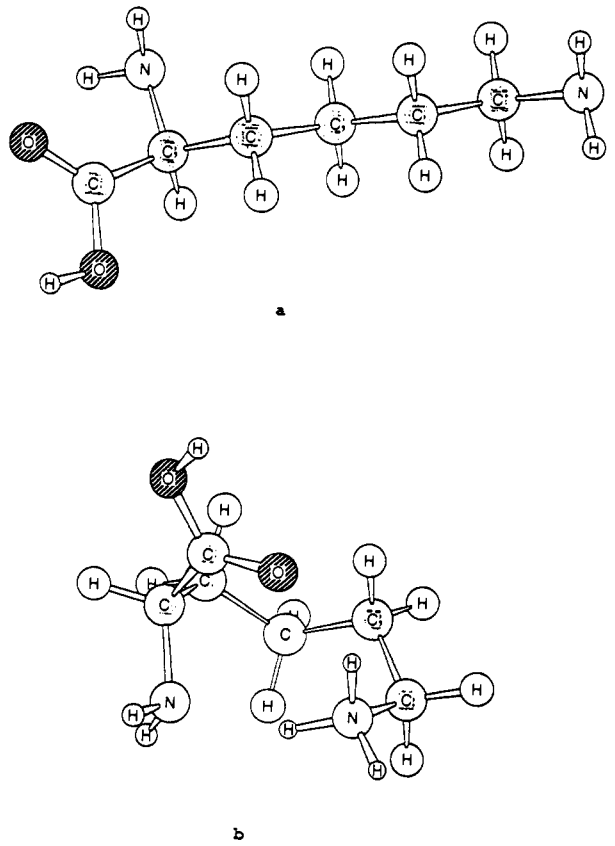


Figure 1. The most stable neutral (a) and protonated (b) forms of lysine, according to semiempirical methods.

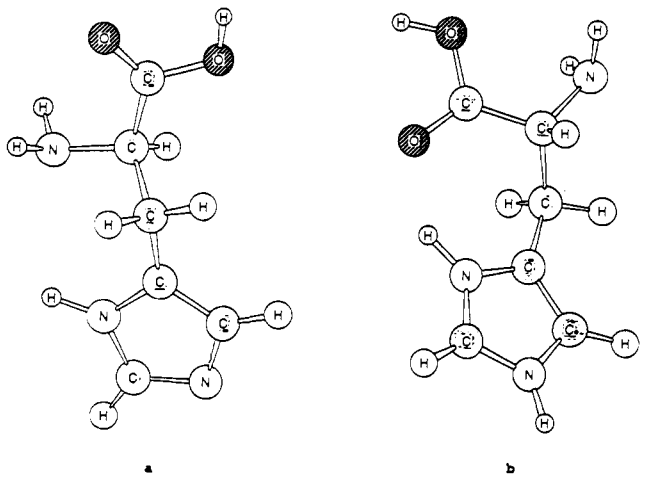
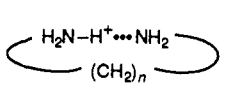


Figure 2. The most stable conformations of histidine obtained by semiempirical methods: (a) neutral form, (b) protonated form.

stable protonated conformation is the cyclic one with a hydrogen bond of the type



For the neutral form the 6-31G self-consistent-field (SCF) method predicts the extended conformations without H-bonds to be the lowest in energy. Similar results were obtained for neutral γ-aminobutyric acid, which also has a $-(CH_2)_2-NH_2$ tail (6-31G computations).[19] The 4-31G investigation of neutral β-alanine[20] (tail $-CH_2-NH_2$) predicts the conformation with H-bond –C=O···HNH– to be 1.2 kcal/mol lower than the extended one. But it is well-known that the 4-31G basis set has a tendency to overestimate the dissociation energies of hydrogen-bonded systems (see, for example, ref 21).

Table I. The Proton Affinities (kcal/mol) of Lys and Two Diamino Compounds, Obtained by Semiempirical Methods

molecule	MNDO/M	AM1	PM3
1,4-diaminobutane	221.6	224.6	222.8
1,5-diaminopentane	220.2	223.8	221.6
lysine	223.4	226.2	223.4

In accordance with the above considerations, we can conclude that the energy of the protonated conformation of Lys should be mainly determined by the formation of the intramolecular cyclic H-bond. The energy of the neutral form seems to be attributed to the most stable conformation of the $-(CH_2)_4-NH_2$ tail. From this point of view, the choice of the 1,5-diaminopentane ($NH_2(CH_2)_5NH_2$) molecule as a model for the determination of the Lys PA is obvious. But we can take the next step in the direction of reducing the size of our model and choose 1,4-diaminobutane ($NH_2(CH_2)_4NH_2$). This step is justified by two arguments. First, it is obvious, that both 1,5-diaminopentane and 1,4-diaminobutane in the cyclic protonated conformations are free from the strain (this is not true for 1,3-diaminopropane; see, for example, ref 22). Second, the experimental PA values of these molecules differ by only 0.5 kcal/mol, and this difference is within the experimental error (±1.5 kcal/mol).[23]

The PA values evaluated using semiempirical approximations are listed in Table I. The MNDO,[24] AM1,[25] and PM3[26] methods were used for calculations of the neutral species. For the protonated molecules, the MNDO/M[27] approach, which is known to be superior in the description of H-bonded systems,[28-30] was used instead of MNDO. The geometries were optimized by the EF method[31] up to the norm of gradients below 0.05 kcal/(mol Å) (kcal/(mol rad.)) using a modified version of the MNDO-85 program.[32] The PA values were calculated assuming $\Delta H_f(H^+) = 367.2$ kcal/mol.[33] The most stable conformations of the neutral and protonated forms of Lys are shown in Figure 1. The slightly higher value of the PA in comparison with the diamino species is attributed to the additional stabilization effect of the COOH group. However, according to the data in Table I, this effect is small, about 1–2 kcal/mol. Thus we can conclude that the choice of 1,4-diaminobutane as a model of Lys in the prediction of its PA may underestimate the actual PA value by 1–2 kcal/mol.

(b) Histidine. In Table II the PA values of His, 5-methylimidazole (5-MeIm), and imidazole (Im) obtained by semiempirical methods are listed. The most stable conformations of His and $HisH^+$ are shown in Figure 2. From the energetic data of Table II, the COOH group has a strong effect on the PA due to formation of the intramolecular H-bond C=O···H—N(Im) (see Figure 2). It should be noted that this H-bond is far from linear. The O–H–N

(19) Fugler-Domenico, L.; Russel, C. S.; Sapse, A. M.; Debler, E. A. *J. Mol. Struct. (Theochem)* **1988**, *168*, 323–333.
(20) Ramek, M. *J. Mol. Struct.* **1990**, *208*, 301–355.
(21) Hobza, P.; Zahradnik, R. *Intermolecular Complexes*; Elsevier: Amsterdam, 1988.
(22) Dian, X.; Scheiner, S. *J. Am. Chem. Soc.* **1992**, *114*, 5849–5856.
(23) Lias, S. G.; Liebman, J. E.; Levin, R. D. *J. Chem. Phys. Ref. Data* **1984**, *13*, 695–808.
(24) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899–4906.
(25) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
(26) Stewart, J. J. P. *J. Comp. Chem.* **1989**, *9*, 209–220.
(27) Voityuk, A. A.; Bliznyuk, A. A. *Theor. Chim. Acta* **1987**, *72*, 223–228.
(28) Bliznyuk, A. A.; Voityuk, A. A. *J. Mol. Struct. (Theochem)* **1988**, *164*, 343–348.
(29) Khalil, M.; Woods, R. J.; Weaver, D. F.; Smith, V. H. *J. Comp. Chem.* **1991**, *11*, 584–593.
(30) Vasiliev, V. V.; Bliznyuk, A. A.; Voityuk, A. A. *Int. J. Quantum Chem.*, submitted for publication.
(31) Baker, J. *J. Comp. Chem.* **1986**, *7*, 385–395.
(32) Bliznyuk, A. A.; Voityuk, A. A. *Zh. Struct. Khim.* **1986**, *27*(4), 190–191.
(33) Olivella, S.; Urpi, F.; Vilarrasa, J. *J. Comp. Chem.* **1984**, *5*, 230–236.

Table II. The Proton Affinities (kcal/mol) of Histidine, 5-Methylimidazole (5-MeIm), and Imidazole (Im), Obtained by Semiempirical Methods

molecule	MNDO	AM1	PM3
Im	213.6	221.7	219.8
5-MeIm	214.2	223.9	221.8
histidine	219.6[a]	225.7	225.3

[a] MNDO/M results.

angle is 145.9° (MNDO/M), 134.2° (AM1), and 142.2° (PM3). We can expect that the MNDO/M method, which was parametrized to describe linear H-bonds,[27] overestimates slightly the H-bond energy. Thus, an energy of about 4–5 kcal/mol should be a good estimate of the PA difference between His and 5-MeIm.

There is some uncertainty in the value of Me group influence to Im PA. According to the STO-3G[34] and 4-31G[35] SCF methods the difference in PA of 5-MeIm and Im is about 4–4.8 kcal/mol. This value agrees well with the experimental data of ref 23, 4.6 kcal/mol. But the more recent experimental estimation gives only 1–2.5 kcal/mol for this difference,[35,36] and the latter result agrees with the data of Table II.

In our opinion, the 5-MeIm molecule may reasonably be chosen as a model of His, taking into consideration the 4–5 kcal/mol underestimation of the PA value.

III. Ab Initio Methods

Molecular geometries were optimized using a double-ζ plus polarization (DZP) basis[37,38] with a set of d functions on N (a_d = 0.8) and C (a_d = 0.75) and p functions on H (a_p = 0.75). Then the harmonic vibrational frequencies of the molecular species were evaluated. Finally, the Huzinaga–Dunning triple-ζ plus double polarization (TZ2P) basis set[37,39] was used for single-point calculation. In the TZ2P basis set the numbers of basis functions for 1,4-$(NH_2)_2$Bu, 1,4-$(NH_2)_2$Bu-H^+, 5-MeIm, and 5-MeImH^+ are 252, 261, 198, and 207 respectively. The polarization exponents were a_d(C) = 1.5, 0.375; a_d(N) = 1.6, 0.4; a_p(H) = 1.5, 0.375. In addition to the self-consistend-field (SCF) method used here, the second-order perturbation theory (designed MP2 here)[40] was used in conjunction with the DZP and TZ2P basis sets. All computations were carried out using the TURBOMOL programs of Ahlrichs and co-workers.[41]

The PA of a base B at 298 K is defined as $-\Delta H_r(298)$ for the gas-phase reaction $B + H^+ \rightarrow BH^+$. Using the standard thermodynamic scheme[42] we can write

$$PA = -\Delta H_r(298) = \Delta E_e(0) + \Delta E_v(0) + \Delta(\Delta E_v(298)) + {}^5/_2RT \quad (1)$$

where E_e is the electronic energy, evaluated at the SCF or MP2 level; $E_v(0)$ is the zero-point vibrational energy (harmonic frequencies were obtained using the DZP basis set and reduced by 0.91 according to ref 43); $\Delta E_v(298)$ is the change of vibrational energy during heating from 0 to 298 K; and ${}^5/_2RT$ is the classical estimation of the effect of losing three translational degrees of freedom (${}^3/_2RT$) during protonation, plus the PV term (RT). The Δ refers to the difference between reagents and products.

According to prior data,[44,45] we may expect that our approach (MP2/TZ2P//DZP) will give results better than MP2/6-31G(d,p)//6-31G(d) and of approximately the same quality as MP2/6-311+(2d,2p)//6-31G(d). The theoretical ΔE_e values for the protonation reactions of NH_3 and CH_3NH_2 are compared in Table III. In Table IV the H-bond energies of the NH_4^+–NH_3 complex at several levels of theory are listed. Indeed, as follows from these tables, our results are generally close to the data obtained with the larger 6-311+G(2d,2p) basis set. It should be noted that the additional expansion of the basis set and/or using more sophisticated correlated methods ordinarily gives insignificant improvement in the PA[16] or H-bond energy[51] (about 1 kcal/mol).

Thus, according to the above consideration, we can expect that the computations on the MP2/TZ2P//DZP level of theory used here permit us to estimate the absolute PA values with a precision better than 2 kcal/mol.

Table III. Theoretical ΔE_e Values (kcal/mol) for the Reaction $M + H^+ \rightarrow MH^+$, for M = NH_3, CH_3NH_2

level of theory	NH_3	CH_3NH_2
6-31G(d,p)//6-31G(d)	219.0[a]	230.3[d]
DZP//DZP	217.0[b]	228.5[b]
6-31+G(d,p)//6-31G(d)	216.5[c]	227.8[d]
6-311G(2d,2p)//6-31G(d)	218.5[a]	230.0[d]
TZ2P//DZP	216.7[b]	228.7[b]
6-311+G(2d,2p)//6-31G(d)	216.1[c]	228.0[d]
MP2/6-31G(d,p)//6-31G(d)	219.9[a]	229.8[d]
MP2/DZP//DZP	216.1[b]	226.8[b]
MP2/6-31+G(d,p)//6-31G(d)	214.8[c]	225.1[d]
MP2/6-311G(2d,2p)//6-31G(d)	215.4[a]	225.9[d]
MP2/TZ2P//DZP	212.8[b]	224.0[b]
MP2/6-311+G(2d,2p)//6-31G(d)	211.6[a]	222.7[d]
PA(MP2/TZ2P//DZP)[e]	205.0 (204.0)[f]	216.2 (214.1)[f]

[a] Reference 46. [b] This work. [c] Reference 47. [d] Reference 14. [e] Calculated from eq 1. [f] Experimental value of ref 36. The old experimental data[36] were used here, instead of the new,[48] because the results of precise *ab initio* calculations are much closer to the previous data, at least for ammonia.[15]

Table IV. The Hydrogen Bond Energy (kcal/mol) of the NH_4^+–NH_3 Complex[a]

level of theory	H bond energy
6-31G(d,p)//6-31G(d)	26.2[b]
DZP//DZP	24.1[c]
6-31+G(d,p)//6-31G(d)	23.6[b]
6-311G(2d,2p)//6-31G(d)	24.2[b]
TZ2P//DZP	22.4[c]
6-311+G(2d,2p)//6-31G(d)	22.1[b]
MP2/6-31G(d,p)//6-31G(d)	30.3[b]
MP2/DZP//DZP	28.8[c]
MP2/6-31+G(d,p)//6-31G(d)	27.0[b]
MP2/6-311G(2d,2p)//6-31G(d)	29.0[b]
MP2/TZ2P//DZP	26.9[c]
MP2/6-311+G(2d,2p)//6-31G(d)	25.9[b]
ΔH_f(MP2/TZ2P//DZP)[d]	25.8
experiment[e]	26.0

[a] Evaluated as $E_e(NH_3) + E_e(NH_4^+) - E_e(NH_4^+–NH_3)$. [b] Reference 49. [c] This work. [d] From an equation similar to (1), but with $4RT$, corresponding to the loss of three additional rotational degrees of freedom. [e] Experimental value of ref 50.

IV. Results

(1) 1,4-Diaminobutane. (a) Neutral Form. To our knowledge, the only previous *ab initio* study of 1,4-diaminobutane is the 3-21G data of ref 52. However, the authors of ref 52 did not discuss the relative stabilities of the different conformations, and

(34) Mo, O.; Yanez, M.; Eguero, J. *J. Org. Chem.* **1987**, *52*, 1713–1720.
(35) Catalan, J.; de Paz, J. L. G.; Yanez, M.; Claramunt, R. M.; Lopez, C.; Elguero, J.; Aniva, F.; Quian, J. H.; Taagerepa, M.; Taft, R. W. *J. Am. Chem. Soc.* **1990**, *112*, 1303–1312.
(36) Lias, S. G.; Bartmess, J. E.; Liebman, J. E.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1.
(37) Huzinaga, S. *J. Chem. Phys.* **1965**, *42*, 1293–1302.
(38) Dunning, T. H. *J. Chem. Phys.* **1970**, *53*, 2823–2833.
(39) Dunning, T. H. *J. Chem. Phys.* **1971**, *55*, 716–723.
(40) Hehre, W. J.; Radom, L.; Schleyer, P. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley: New York, 1986.
(41) Ahlrich, R. A.; Bar, M.; Haser, M.; Horn, H.; Kolmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165–169.
(42) Del Bene, J. E.; Metter, H. D.; Frisch, M. J.; Luke, B. J.; Pople, J. A. *J. Phys. Chem.* **1983**, *87*, 3279–3282.
(43) Grev, R. S.; Janssen, C. L.; Schaefer, H. F. *J. Chem. Phys.* **1991**, *95*, 5128–5132.
(44) Scuseria, G. E.; Schaefer, H. F. *J. Chem. Phys.* **1989**, *90*, 3629–3636.
(45) Grev, R. S.; Schaefer, H. F. *J. Chem. Phys.* **1989**, *91*, 7305–7306.
(46) Frisch, M. J.; Del Bene, J. E.; Raghavachari, K.; Pople, J. A. *Chem. Phys. Lett.* **1981**, *83*, 240–242.
(47) *The Carnegie-Mellon Quantum Chemistry Archive*, 3rd ed.; Whiteside, R. A., Frisch, M. J., Pople, J. A., Eds.; Carnegie-Mellon University, 1983.
(48) Meot-Ner, M.; Sieck, W. *J. Am. Chem. Soc.* **1991**, *113*, 4448–4460.
(49) Del Bene, J. E. *J. Comp. Chem.* **1987**, *8*, 810–815.
(50) Meot-Ner, M. *J. Am. Chem. Soc.* **1992**, *114*, 3312–3322.
(51) Del Bene, J. E.; Shavitt, I. *Int. J. Quantum Chem. Symp.* **1989**, *23*, 445–452.
(52) Politzer, P.; Elmingavwi, I. M.; Lane, P. *J. Mol. Struct. (Theochem)* **1989**, *201*, 117–128.

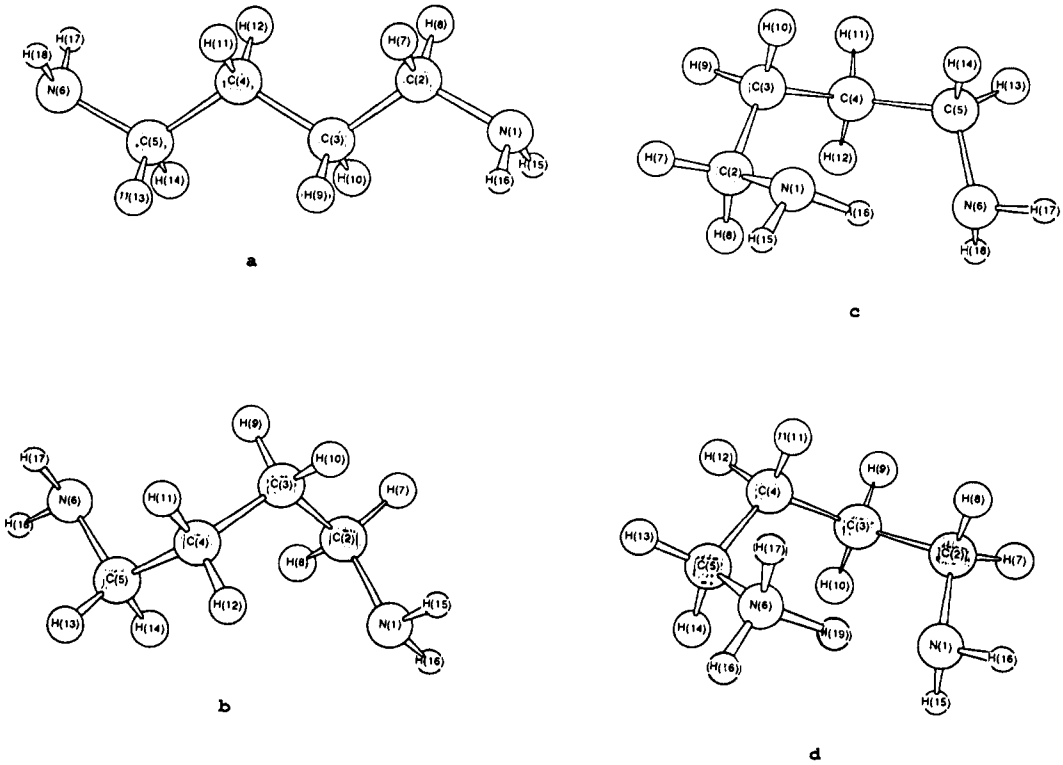


Figure 3. Conformations of 1,4-diaminobutane considered here: (a) trans form, (b) gauche, (c) H-bonded, (d) protonated.

one of the possible gauche forms seems to have been arbitrarily chosen. The AM1 computations[53] predict the trans form to be the most stable. Our preliminary MNDO, AM1, and PM3 calculations give the same result. But according to an earlier 4-21G investigation of 1,2-diaminoethane, the H-bonded gauche form is the most stable conformer.[54] Close agreement was obtained for this molecule by the MM3 method.[55] Moreover, after comparing these data with those of more extensively studied aliphatic acyclic amines, the picture becomes unclear. For example, the MM2 method predicts the trans conformation of amines to be the most stable[56] due to partial delocalization of the nitrogen lone pair along the N–C bond. The *ab initio* 6-31G(d) calculation with inclusion of correlation via the MP2 and MP3 levels also showed the trans form to be the lowest in energy.[57] But according to the recent data of nonempirical 3-21G+d(N) computations,[58] the trans and gauche forms of *n*-propylamine have comparable energies, and the latter structure is the most stable for *n*-butylamine due to interaction of the nitrogen lone pair with hydrogen in one of the C–H bonds. The 6-31G data for amino acids[18,19] also predict the gauche conformation of the tail to be the most stable.

Therefore, in this work, three neutral forms of 1,4-diaminobutane were studied. The first one is the trans C_{2h} form (Figure 3a); the next one is the gauche form (Figure 3b) in which two interactions of the nitrogen lone pairs with C–H bonds are possible; and the last one is the H-bonded form (Figure 3c). The energies and selected geometrical parameters of these conformations, obtained at the DZP level of theory, are shown in Table V. According to our results the trans form is the most stable one.

Table V. The Total Energies (au) and Geometrical Parameters (Distances, Å; Angles, deg) of the Neutral and Protonated Forms of 1,4-Diaminobutane, Obtained at the DZP SCF Level of Theory

parameter	trans form	gauche form	H-bonded form	protonated form
energy	−267.406007	−267.404422	−267.402627	−267.802380
C_2N_1	1.456	1.460	1.456	1.475
C_3C_2	1.531	1.528	1.530	1.529
C_4C_3	1.529	1.533	1.537	1.539
C_5C_4	1.531	1.528	1.532	1.528
N_6C_5	1.456	1.460	1.463	1.501
$N_1C_2C_3$	115.61	111.79	112.91	112.50
$C_2C_3C_4$	113.11	115.40	118.17	117.25
$C_3C_4C_5$	113.11	115.40	118.01	117.31
$C_4C_5N_6$	115.61	111.79	112.59	112.13
$N_1C_2C_3C_4$	180.0	−57.74	−78.73	70.97
$C_2C_3C_4C_5$	180.0	−59.76	70.28	−76.85
$C_3C_4C_5N_6$	180.0	−57.81	−81.64	73.84
N_1N_6	6.301	4.617	3.107	2.764

This agrees well with the conclusions from *ab initio* 6-31G(d),[57] AM1,[53] and our preliminary semiempirical calculations.

(b) Protonated Form. For the protonated conformation of 1,4-diaminobutane, the AM1 method[53,59] predicts the distorted chair form to be the most stable one. Our preliminary semiempirical computations (MNDO/M, PM3) also give the same result. According to the *ab initio* computations of the protonated form of L-2,4-diaminobutyric acid, the conformation with the distorted chair form of the tail has the lowest energy.[18] Thus, only this form was calculated. The DZP energy and selected geometrical parameters are listed in Table V.

(2) 5-Methylimidazole. (a) Neutral Form. There are two possible orientations of the Me group for 5-MeIm. The first has the C–H bond directed toward the C (designed C–H(C) here), and the second has the C–H bond directed toward the N atom (C–H(N)) as shown in Figure 4, a and b. In the case of 4-MeIm the choice of the most stable conformation can be made using simple chemical considerations. As shown from the results of nonempirical computations,[35] the conformation C–H(N) has the

(53) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075–8086.
(54) Van Alsenoy, C.; Sram, K.; Ewbank, J. D.; Schafer, L. *J. Mol. Struct. (Theochem)* **1986**, *136*, 77–91.
(55) Schmitz, L. R.; Allinger, N. L. *J. Am. Chem. Soc.* **1990**, *112*, 8075–8086.
(56) Profeta, S., Jr.; Allinger, N. L. *J. Am. Chem. Soc.* **1985**, *107*, 1907–1918.
(57) Schmitz, L. R.; Allinger, N. L.; Profeta, S., Jr. *J. Comp. Chem.* **1988**, *9*, 460–464.
(58) Batista de Carvalha, L. A. E.; Teieira-Dias, J. J. C.; Fausto, R. *Struct. Chem.* **1990**, *1*, 553–542.
(59) Dannenberg, J. J.; Vilson, L. K. *J. Phys. Chem.* **1988**, *92*, 5635–5639.

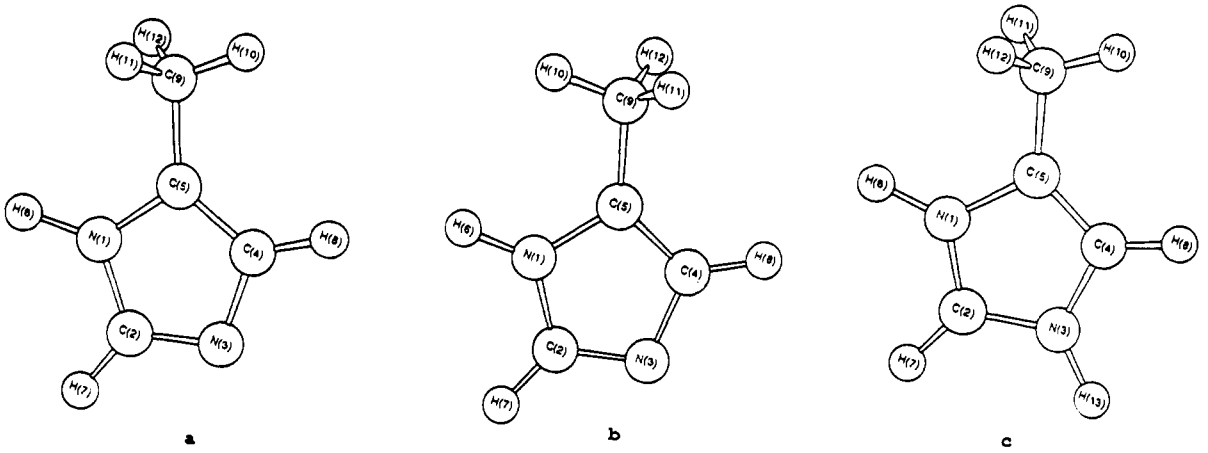


Figure 4. Conformations of 5-methylimidazole: (a) C–H(C) form, (b) C–H(N) form, (c) protonated form.

Table VI. The Total Energies (au) and Geometrical Parameters (Distances, Å; Angles, deg) of the Neutral and Protonated Forms of 5-Methylimidazole (5-MeIm) from the DZP SCF Method

parameter	C–H(C)	C–H(N)	protonated form
energy	−263.907375	−263.905362	−264.295037
N_1C_2	1.356	1.355	1.317
C_2N_3	1.289	1.290	1.311
N_3C_4	1.376	1.375	1.387
C_4C_5	1.357	1.358	1.347
C_5N_1	1.377	1.375	1.390
C_9C_5	1.496	1.501	1.496
$N_1C_2N_3$	112.04	111.90	108.03
$C_2N_3C_4$	105.29	105.38	109.32
$N_3C_4C_5$	110.85	110.83	107.15
$C_4C_5N_1$	104.59	104.52	105.47
$C_5N_1C_2$	107.22	107.37	110.03
$N_1C_5C_9$	122.99	122.85	122.54
$H_{10}H_8$	2.788		2.823
$H_{10}H_6$		2.520	

lowest energy due to the interaction of the nitrogen lone pair with the hydrogen atom of the CH_3 group. But for 5-MeIm, the situation is not so well-defined. The energy and selected geometrical parameters of both forms obtained at the DZP level of theory are listed in Table VI. One sees that the structure with the C–H directed toward the C atom has the lowest energy. The geometrical parameters corresponding to the two possible orientations of the methyl group are similar. The only significant difference occurs for the C_5–C_9 bond length. In our opinion, the increase of this bond length can be attributed to the H_{10}–H_6 repulsion. Evidently, this is also the reason for the relative destabilization of the C–H(N) structure. Our preliminary semiempirical calculations also predict the conformation C–H(C) to be the most stable one.

(b) Protonated Form. Following the results obtained for the neutral molecule, only one protonated conformation (designate C–H(C)) was considered. The DZP total energy and geometrical parameters are shown in Table VI.

(3) Proton Affinity. The results for the most stable conformations of the molecules investigated are summarized in Table VII. The PA's predicted for 5-MeIm and 1,4-diaminobutane are 228.2 and 239.9 kcal/mol, respectively. There is no experimental value for 5-MeIm, because the 4-MeIm isomer is slightly more stable. According to the 4-31G results[35] this energy difference is 1.2 kcal/mol. Thus, the value of 227 kcal/mol agrees well with the recent experimental PA of 4-MeIm, 226.2 kcal/mol,[35] and is slightly higher than previous results, 224.8 kcal/mol[60] and 224.4 kcal/mol.[23] The PA obtained for 1,4-diaminobutane (239.9 kcal/mol) may be compared with the experimental value of 237.6 kcal/mol.[23]

The theoretical PA values for the model molecules are reliable, as was expected, and may slightly overestimate the actual PA values. This overestimation can be partially attributed to the existence of other conformations close in energy, leading to the lower experimental values of the PA. As follows from this consideration and the data of Table III, this overestimation is systematic, so that the relative values of the proton affinities should be more accurate.

V. Discussion

From the data presented above, we estimate the PA of His as 232.7 kcal/mol and the PA of Lys as 241.4 kcal/mol. The theoretical PA value for His agrees well with the conclusions from both experimental techniques.[8-10] However, there is a large difference between the PA of Lys obtained here, and that derived from kinetic measurements.[8]

With the kinetic method, relative PA values are obtained from measurements of the intensities of the products of the dissociation of a proton bound complex, as in the scheme

$$B_1{-}H^+{-}B_2 \xrightarrow{k_1} B_1H^+ + B_2$$
$$B_1{-}H^+{-}B_2 \xrightarrow{k_2} B_2H^+ + B_1$$

The difference in proton affinities ΔPA (PA(B_1) − PA(B_2)) is given by the expression

$$\Delta PA = -\Delta E^{\ddagger} = kT \ln(k_1/k_2) = kT \ln([B_1H^+]/[B_2H^+]) \quad (2)$$

where $[B_1H^+]$ and $[B_2H^+]$ refer to the intensities of the peaks in a mass spectrum, and $\Delta E^{\ddagger}$ is the difference in activation energies for the two reaction channels. This method, postulated by Cooks,[11] is derived from transition state theory and depends on several assumptions. One key assumption is that no reverse activation energy barrier exists for the dissociation of the H-bonded complex, so that $\Delta PA = -\Delta E^{\ddagger}$. For the amino acids described in this paper, this is true only if the molecules in the H-bonded complex, in the neutral form, and in the protonated form have approximately the same structure. If this is not so, than we can expect that a barrier corresponding to the changing of conformation will appear on the potential energy surface. In such a case, the $-\Delta E^{\ddagger}$ will not equal ΔPA, as shown in Figure 5.

It is well-known that diamino species change conformation dramatically after protonation due to internal H-bond for-

(60) Meot-Ner, M.; Liebman, J. E.; Del Bene, J. E. *J. Org. Chem.* **1986**, *51*, 1105–1112.
(61) Laider, K. J. *Theories of Chemical Reaction Rates*; McGraw Hill Book Company: New York, 1969.

Table VII. The Proton Affinities of 5-Methylimidazole (5-MeIm) and 1,4-Diaminobutane

value[a]	5-MeIm	5-MeImH$^+$	1,4-$(NH_2)_2$Bu	1,4-$(NH_2)_2H^+$
DZP//DZP	−263.907375	−264.295037	−267.406007	−267.802380
MP2/DZP//DZP	−0.937376	−0.930596	−0.999129	−1.003556
$E_v(0)$[b]	66.57 (60.58)	75.79 (69.97)	112.56 (102.43)	123.56 (112.44)
$\Delta E_v(298)$	1.43	1.45	3.13	2.51
TZ2P//DZP	−263.944221	−264.332328	−267.443946	−267.838754
MP2/TZ2P//DZP	−1.067186	−1.055238	−1.139441	−1.139443
PA(MP2/TZ2P//DZP)	228.2		239.9	

[a] $E_v(0)$, $\Delta E_v(298)$, and PA are in kcal/mol, while other energetic quantities are in au. [b] The zero-point vibrational energies scaled by 0.91 are given in parentheses.

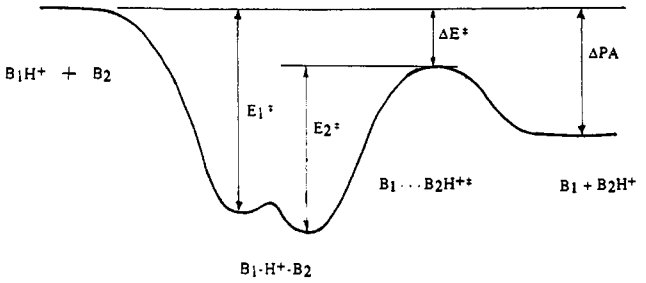


Figure 5. A schematic representation of the potential energy surface for the dissociation of the H-bonded dimer B_1–H^+–B_2 into two bases, with alteration of the B_2H^+ cation structure.

mation.[62-64] The same is true in the case of Lys, as showed by our results, and for other amino acids containing the aliphatic amino group.[18,20] The enthalpy of cyclization for diamino molecules is about 18 kcal/mol.[64] Of course, the actual underestimation of the Lys PA by kinetic measurements should be less, due to the interaction of B_1 with B_2H^+ * in the activated complex.

Moreover, according to semiempirical calculations, histidine also changes conformation following protonation (see Figure 2). The difference in energy between HisH$^+$ in its most stable conformation and HisH$^+$ constrained to the conformation of the neutral His is 0.6 (AM1), 1.9 (MNDO/M), and 2.7 kcal/mol (PM3). In this case, the kinetic approach should also underestimate the PA, but not so dramatically as for Lys.

(62) Yamadagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1973**, *95*, 3504–3510.
(63) Aue, D. H.; Webb, H. M.; Bowers, M. T. *J. Am. Chem. Soc.* **1973**, *95*, 2699–2701.
(64) Meot-Ner, M. *Acc. Chem. Res.* **1984**, *17*, 186–193.

The PA of Lys obtained here (241.4 kcal/mol) agrees well with the experimental value of ref 10 (242.6 kcal/mol). In comparison, the PA measured by the kinetic method is 228.7 kcal/mol. Figure 5 suggests that the difference between the two values represents the reverse activation barrier to proton transfer to lysine. The difference between the two measurements, 14 kcal/mol, is approximately equal to the energy released in forming an intramolecular hydrogen bond in a diamine of similar ring size.[64] These findings suggest that the reverse activation barrier to proton transfer can be determined by comparing measurements obtained by both the bracketing and kinetic methods.

VI. Conclusion

This paper presents the estimated proton affinities of the amino acids lysine and histidine. *Ab initio* methods including electron correlation were used in combination with semiempirical approaches. The results show that the kinetic method[8] should not be used for measurements of the proton affinities of amino acids for which intramolecular H-bonding occurs, because the basic assumption of this method may not be valid for such systems. Measurements of proton affinities of peptides, where intramolecular H-bonding is highly likely for the protonated species, will best be accomplished by other techniques, such as the bracketing method.[10]

Acknowledgment. The authors are grateful for financial support by the National Science Foundation, CHE-9024922 (I.J.A.) and CHE-8718469 (H.F.S.). We are very grateful to Drs. Mingzuo Shen and Richard Remington for many valuable discussions.