Gas-Phase Protonation Thermochemistry of Arginine

Guy Bouchoux,* Sylvain Desaphy, Sophie Bourcier, Christian Malosse, and Rosa Ngo Biboum Bimbong

Laboratoire des Mécanismes Réactionnels, Ecole Polytechnique, 91128 Palaiseau, France

Received: October 3, 2007; In Final Form: December 11, 2007

The gas-phase basicity (GB), proton affinity (PA), and protonation entropy ($\Delta_pS°(M) = S°(MH^+) - S°(M)$) of arginine (Arg) have been experimentally determined by the extended kinetic method using an electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass spectrometer. This method provides GB(Arg) = 1004.3 ± 2.2 (4.9) kJ·mol^{-1} (indicated errors are standard deviations, and in parentheses, 95% confidence limits are given). Consideration of previous experimental data using a fast atom bombardment ionization tandem sector mass spectrometer slightly modifies these estimates since GB(Arg) = 1005.9 ± 3.1 (6.6) kJ·mol^{-1}. Lower limits of the proton affinity, PA(Arg) = 1046 ± 4 (7) kJ·mol^{-1}, and of the "protonation entropy", $\Delta_pS°(Arg) = S°(ArgH^+) - S°(Arg) = -27 \pm 7$ (15) J·mol^{-1}·K^{-1}, are also provided by the experiments. Theoretical calculations conducted at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p) level, including 298 K enthalpy correction, predict a proton affinity value of ca. 1053 kJ·mol^{-1} after consideration of isodesmic proton-transfer reactions with guanidine as the reference base. Computations including explicit treatment of hindered rotations and mixing of conformers confirm that a noticeable entropy loss does occur upon protonation, which leads to a theoretical $\Delta_pS°(Arg)$ term of ca. −45 J·mol^{-1}·K^{-1}. The following evaluated thermochemical parameter values are proposed: GB(Arg) = 1005 ± 3 kJ·mol^{-1}; PA(Arg) = 1051 ± 5 kJ·mol^{-1}, and $\Delta_pS°(Arg) = -45 \pm 12$ J·mol^{-1}·K^{-1}.

I. Introduction

Arginine is an amino acid of fundamental importance in the biochemistry of peptides, particularly owing to its acidobasic properties. It is, for example, well-known that, at physiological pH, protein chains are predominantly protonated at the arginine residues. Moreover, it has recently emerged that arginine residues may also act as general-base catalysts in enzymatic processes.[1] Acidobasic properties of arginine residues also play a significant role in the sequencing of peptide by mass spectrometric methods.[2] Accordingly, in the gas phase, arginine appears to be preferentially protonated on the highly basic guanidine group of its side chain.[3] As a consequence, the major fragmentations of protonated arginine (ammonia and guanidine losses) have been interpreted by the dissociations of structures involving protonation on a nitrogen atom of the side chain.[4−9] Similarly, the arginine residue strongly orients the protonation of peptides and consequently determines the subsequent fragmentation reactions of protonated peptides.[2,3] Accordingly, the effective sequestration of the added proton(s) by the side chain of arginine residues is at the origin of a number of pecularities of the fragmentations of protonated peptides.[2] The knowledge of the protonation characteristics (i.e., structure and thermochemistry) of arginine and its derivatives in the gas phase is consequently crucial in the understanding of the mass spectrometry results.

The thermochemical quantities associated with the concept of basicity in the gas phase of a given molecule M are defined by considering the deprotonation reaction of the MH^+ ion:[10]

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

The proton affinity, PA(M), and gas-phase basicity, GB(M), of the molecule M are the standard enthalpy, $\Delta_1H°$, and standard Gibbs free energy, $\Delta_1G°$, of reaction 1, respectively, generally given for a room temperature of 298 K.[15,16] It is a custom, for a species M, to call the "protonation entropy" the difference in absolute entropies between its protonated and neutral forms.[15,16] Even if it is not, strictly speaking, a finite difference, this term is noted $\Delta_pS°(M)$, i.e.

$$\Delta_pS°(M) = S°(MH^+) - S°(M) \quad (2)$$

and consequently, the entropy of reaction 1, $\Delta_1S°$, is related to the protonation entropy $\Delta_pS°(M)$ by eq 3.

$$\Delta_1S° = S°(H^+) - \Delta_pS°(M) \quad (3)$$

A very small number of studies have been devoted so far to the gas-phase protonation thermochemistry of arginine itself.[11−14] Qualitative basicity ordering of the most common naturally occurring amino acids was done from examination of the decomposition of protonated dimers of two different amino acids produced by fast atom bombardment, FAB.[11] These earlier studies showed that arginine is the most basic amino acid of the series. A few years later, Gorman et al.[12] and Bojesen and Breidahl[13] demonstrated that arginine is more basic than tributylamine and 1,1,3,3-tetramethylguanidine, respectively. Considering the presently accepted gas-phase basicity values of tributylamine and 1,1,3,3-tetramethylguanidine,[15,16] a lower limit of ∼997 kJ·mol^{-1} can be derived from these experiments for the gas-phase basicity of arginine. The first, and presently lone, tentative experimental determination of the gas-phase basicity of arginine was done by Wu and Fenselau[14] in 1992.

* To whom correspondence should be addressed. Phone: 33 1 69 33 48 42. Fax: 33 1 69 33 48 03. E-mail: bouchoux@dcmr.polytechnique.fr.

10.1021/jp709677c CCC: $40.75
Published on Web 02/21/2008

The authors used the simple kinetic method[17] and, in their original work, proposed a proton affinity estimate of 1026 kJ·mol^{-1}. The data from the Wu and Fenselau experiments[14] were interpreted differently by Hunter and Lias,[15] who considered that the experiments provided gas-phase basicity rather than proton affinity information and thus assigned to arginine a gas-phase basicity value of 1006.6 kJ·mol^{-1}. In recent years, it has been amply demonstrated that the simple kinetic method is not adapted to systems where strong hydrogen bond(s) appear in the protonated species.[18−23] In such circumstances the use of an "extended" version of the kinetic method is preferred. The underlying reason for the deficiencies of the simple kinetic method is related to situations where a significant entropy loss occurs during protonation. This is precisely what is expected in the case of arginine because of the existence of two basic sites, namely, the α-amino group and the guanidine moiety; the design of new experiments using more sophisticated methods is therefore essential.

On the other hand, theoretical computation conducted at various levels of sophistication[24−27] leads to proton affinity, PA(Arg), situated between 1042[24] and 1072[25] kJ·mol^{-1}. Such a large range of values is clearly unsatisfactory, and the origin of these discrepancies should be understood.

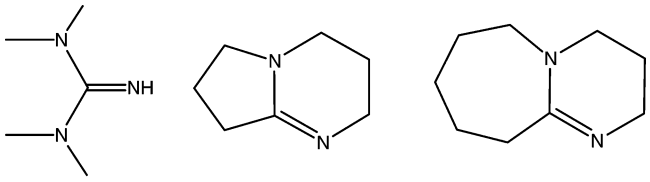
Finally, no experimental or theoretical estimate of the protonation entropy of arginine, $\Delta_p S°(\text{Arg}) = S°(\text{ArgH}^+) - S°(\text{Arg})$, is presently available. A simple comparison between the evaluated[14,15] GB(Arg) = 1006.6 kJ·mol^{-1} and the 1042−1072 kJ·mol^{-1} proton affinity range[24,25] gives a $\Delta_p S°$(Arg) situated between −8 and −109 J·K^{-1}·mol^{-1}. Indeed, a negative value of $\Delta_p S°$(Arg) is in agreement with a strong intramolecular hydrogen bond in the protonated form of arginine; however, a more precise assignement of this entropic term is obviously desirable.

The aim of the present study is to answer these questions by providing new experimental and theoretical approaches of the gas-phase protonation thermochemistry of arginine. The extended kinetic method is used to obtain both PA(Arg) and $\Delta_p S°$(Arg) estimates and to deduce a confident GB(Arg) value. Density functional calculations at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p) level corrected to a temperature of 298 K and isodesmic proton-transfer reactions with reference to guanidine were considered to compute the 298 K proton affinity of arginine. Theoretical considerations on the protonation entropy including corrections due to hindered rotation and entropy of mixing complete this investigation.

II. Methods

ESI-MS−MS experiments were carried out in a Waters Q-TOF Premier mass spectrometer working in the MassLynx 4.1 environment. The cone voltage was set at ∼50 V, while the capillary voltage was varied between 3.0 and 3.7 kV to optimize the conditions for obtaining the maximum intensity of protonated dimers. Typical values for the other source parameters were sampling cone −90 V, extraction cone −5 V, and ion guide −4.2 V. The pulse velocity in the T-wave apparatus was 300 m/s, and the source temperature was set to 80 °C. CID-MS−MS spectra were obtained using argon as the collision gas at a pressure of 1.5×10^{-3} mbar. Experimental data have been collected at several different collision energies in the laboratory frame, E_{lab}, of protonated dimers. It has been considered that the kinetic energy of the ions entering the gas cell is related to the voltage difference between the ion guide and the gas cell. This voltage difference is simply given by the sum of the static offset value (so-called "collision energy") and the ion guide value. Practically, the range of explored E_{lab} values extends from 4 to 40 V. The center of mass collision energy, E_{cm}, has been calculated by the usual conversion expression: $E_{cm} = E_{lab} m_{target}/(m_{target} + m_{ion})$. A scan rate of 1 s/scan was used for all experiments with a data acquisition duration of 40 s for each energy step. The spectra were acquired at several times for a period of 3 months. Sample solutions were prepared in a 50/50 mixture of methanol and an aqueous solution of ammonia adjusted to pH 10 and dissolved to achieve typically a concentration of 10^{-4} M for both the amino acid and the reference bases.[28] All solutions were infused at a flow rate of 0.1−1.0 μL·mn^{-1} with a CIL Cluzeau (Courbevoie, France) syringe. Three reference bases B_i have been used to produce the relevant proton-bound heterodimer $[MHB_i]^+$ (where M stands for arginine): TMG (1,1,3,3-tetramethylguanidine), DBN (1,5-diazabicyclo[4.3.0]non-5-ene), and DBU (1,8-diazabicyclo-[5.4.0]undec-7-ene). The samples, bases, and solvents of HPLC grade were purchased from Sigma-Aldrich (St Quentin Fallavier, France) and used as received without any further purification. The collision-induced dissociations of the mass-selected $[MHB_i]^+$ ions were examined by the kinetic method; i.e., the natural logarithm of the fragment ion abundances $y_i = \ln([MH]^+/[B_iH]^+)$ has been correlated with the proton affinity of the reference base B_i, PA(B_i). The $[MH]^+$ and $[B_iH]^+$ intensities were evaluated by summing the fragment ion abundances of each protonated species. This procedure is essentially correct if no further excitation energy is given to the produced fragment ions in the T-wave collision cell. This is expected with the collision gas pressure and wave pulse height and velocity used in our experiments[29] and was checked by controlling that an increase in argon pressure does not lead to a noticeable change in the relative ion abundances. The results discussed below correspond to y_i determined at several typical E_{cm} values situated between 0.5 and 4.0 eV. The data were analyzed by using the ODRPACK program for weighted orthogonal distance regression.[21,30]

SCHEME 1



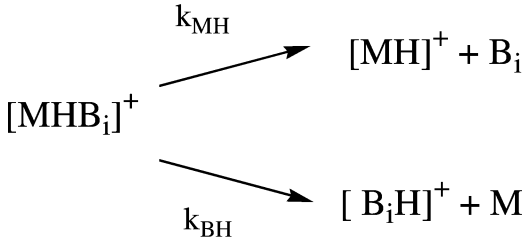
TMG DBN DBU

Molecular orbital calculations have been conducted under the density functional theory formalism using the Gaussian 03 suite of programs.[31] Geometries were optimized at the B3LYP/6-31G(d) and B3LYP/6-31+G(d,p) levels, which is one of the most reasonable approaches taking into account the electron correlation effects for systems containing ca. 10 heavy atoms as considered here. Improved energies were obtained by single-point calculations at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p) level. Enthalpies at 298 K were calculated using thermal corrections obtained from unscaled B3LYP/6-31G(d) vibrational frequencies. This level of theory has also been utilized in the entropy calculations except for the internal rotations, which were treated as hindered rotors by a simplified procedure described below (see section III).

III. Results and Discussion

Extended Kinetic Method. As extensively described in the literature,[10,17−23] the kinetic method allows the determination

of the protonation thermochemistry from the branching ratio of the two possible dissociation routes of a proton-bound dimer $[MHB_i]^+$, i.e.



Consideration of the absolute rate theory and two simplifying assumptions, namely, (i) that the ratio of peak intensities $[MH]^+/[B_iH]^+$ is equal to the ratio of rate constants k_{MH}/k_{B_iH} and (ii) that the transition structures of each dissociation channel are nearly identical to their products in structure and in energy, leads to eq 4, where the $G°$ values are individual Gibbs free energies

$$y = \ln([MH]^+/[B_iH]^+) = [G_T°(M) + G_T°(B_iH^+) - G_T°(MH^+) - G_T°(B_i)]/RT \quad (4)$$

and T is an "effective temperature" related to the excitation energy of the dissociating $[MHB_i]^+$ species.[33] Equation 4 may be expressed in various forms, among which are eqs 5 and 6,

$$y = [GB_{298}(M) - GB_{298}(B_i) + (T - 298)\Delta_{MB_i}S_{298}° + \Delta H_{298\rightarrow T}° + T\Delta S_{298\rightarrow T}°]/RT \quad (5)$$

$$y = [PA_{298}(M) - PA_{298}(B_i) + T\Delta_{MB_i}S_{298}° + \Delta H_{298\rightarrow T}° + T\Delta S_{298\rightarrow T}°]/RT \quad (6)$$

where the gas-phase basicities, GBs, or proton affinities, PAs, at 298 K are considered and where $\Delta_{MB_i}S_{298}° = S_{298}°(MH^+) + S_{298}°(B_i) - S_{298}°(M) - S_{298}°(B_iH^+)$ (or, in terms of protonation entropies, $\Delta_{MB_i}S_{298}° = \Delta_pS_{298}°(M) - \Delta_pS_{298}°(B_i)$. The terms $\Delta H_{298\rightarrow T}°$ and $\Delta S_{298\rightarrow T}°$ are thermal corrections for enthalpy and entropy, respectively, which, because of the structural similarities of $MH^+ + B_i$ on one hand and $M + B_iH^+$ on the other, are generally assumed to cancel to zero.[10,15,32] Obviously, in this hypothesis, eqs 5 and 6 reduce to

$$y = [GB_{298}(M) - GB_{298}(B_i) + (T - 298)\Delta_{MB_i}S]/RT \quad (7)$$

$$y = [PA_{298}(M) - PA_{298}(B_i) + T\Delta_{MB_i}S]/RT \quad (8)$$

at the "effective" temperature T.

To deduce the thermochemical parameters $GB_{298}(M)$, $PA_{298}(M)$, and $\Delta_pS_{298}°(M)$ by the kinetic method, a set of experiments involving different reference bases B_i is considered. Moreover, a controlled excitation energy may be imparted to the precursor ions $[MHB_i]^+$, thus allowing the variation of the effective temperature T. Equations 7 and 8 may be applied to experiments conducted at a single or variable excitation energies, i.e., at a single or variable effective temperatures T. The kinetic method is called "simple" when it uses experiments conducted at only one excitation energy or "extended" if it uses experiments done at variable excitation energies. Obviously, when the term $\Delta_{MB_i}S$ is different from zero as expected for arginine, only the use of the extended version may provide both $PA_{298}(M)$ (or $GB_{298}(M)$) and $\Delta_{MB_i}S$.

A straightforward method for extracting thermochemical information from the extended kinetic method consists in using a set of experimental observables y_{ij} obtained from n_j experiments differing in the adduct ion activation conditions and, for each j, from the n_i points corresponding to the number of reference bases B_i.[21,23,24] Expressing eq 8 by

$$y_{ij} = \Delta_{MB_i}S°_{iso}/R + [PA_{298}(M) - PA_{298}(B_i)]/RT_j \quad (9)$$

the y_{ij} vs $PA_{298}(B_i)$ points may be fitted by a set of regression lines $(y_{ij})_{calcd} = y_0 + b_j(x_0 - x_i)$ intersecting in a common point of coordinate $x_0 = PA_{iso}(M)$ and $y_0 = \Delta S_{iso}°/R$. This point of intercept, called the "isothermal"[23,24] or "isoequilibrium"[21] point, may be localized by a statistical treatment of eq 9, based on a least-squares regression analysis which takes into account simultaneously all the $[n_i, n_j]$ data points, and leads to $PA_{iso}(M)$ and $\Delta_{MB_i}S°_{iso}/R$.[21,29]

In theory, $PA_{iso}(M)$ and $\Delta S_{iso}°$ are equal to $PA_{298}(M)$ and to the mean value of the entropy terms $\langle\Delta_{MB_i}S°\rangle$, respectively. However, using model systems, Ervin and Armentrout[21] and Drahos et al.[20,22] reported results predicting that the proton affinities and protonation entropies determined by the extended kinetic method would present systematic errors seemingly related to the size of the protonation entropies. The extent of these systematic deviations has been delineated in a recent study by considering the experimental data obtained for a large set of bi- or tridentate bases.[23] The main conclusions of these studies are that the extended kinetic method provides generally an underestimate of the proton affinity PA(M) and of the absolute value of $\Delta_pS°(M)$. Fortunately, the positive point is that the corresponding gas-phase basicity GB(M) is correctly estimated by the isothermal extended kinetic method.[20−23] Accordingly, when a lot (35) of experiments are considered, a mean deviation of only 2.4 kJ·mol^{-1} is reported between $GB_{iso}(M) = PA_{iso}(M) - 298(S°_{298}(H^+) - \Delta_pS_{iso}°)$ and the accepted literature data.[23]

Experimental Gas-Phase Basicity of Arginine. Our approach consists in producing $[MHB_i]^+$ adducts in an electrospray source, with M = arginine and B_i a reference base. The ratio of peak intensities $[MH]^+/[B_iH]^+$ obtained at various collision energies is treated by the extended kinetic method following eq 9 for several reference bases B_i. In the upper part of the gas-phase basicity scale explored here, only three reference bases were available, namely, TMG, DBN, and DBU (Scheme 1).

It is important to emphasize how the protonation thermochemistry of these reference bases has been obtained since the precision on these quantities governs the validity of our results. The gas-phase basicities of TMG, DBN, and DBU have been determined by the equilibrium method using a Fourier transform ion cyclotron resonance mass spectrometer.[34,35] In their original experiments Decouzon et al.[34] used tri-*n*-propylamine and tri-*n*-butylamine as reference bases to anchor the gas-phase basicity of a variety of amidines and guanidines. Using GB(tri-*n*-propylamine) = 949.4 kJ·mol^{-1} and GB(tri-*n*-butylamine) = 955.2 kJ·mol^{-1}, the authors deduced GB(TMG) = 982.8 kJ·mol^{-1}, GB(DBN) = 993.3 kJ·mol^{-1}, and GB(DBU) = 1002.9 kJ·mol^{-1}. Several years later, it appeared that the gas-phase basicity scale had to be expanded in its upper part, thus leading to substantial changes in the previous GB determinations.[10,36,37] In 1998, considering the new GB(tri-*n*-butylamine) value of 967.6 kJ·mol^{-1}, Hunter and Lias[15,16] re-evaluated the gas-phase basicities of TMG, DBN, and DBU to 997.4, 1005.9, and 1015.5 kJ·mol^{-1}, respectively. Finally, Raczynska et al.[35] recently reconsidered the basicity of a number of molecules containing the imino group. In the region below 995 kJ·mol^{-1}, the authors anchored the GB scale to a larger set of reference bases (di- and trialkylamines, pyridines, and pyrrolidines) than previously. For stronger bases, they extended the ladder, step

TABLE 1: Thermochemical Data Relevant to Reference Bases B_i[a]

B_i	$PA_{298}(B_i)$[d]	$\Delta_pS°(B_i)$	$GB_{298}(B_i)$[c]
TMG[b]	1032.2	−5.8	998.0
DBN[b]	1040.8	0	1008.4
DBU[b]	1050.6	0	1018.2

[a] PA and GB in kJ·mol^{-1}, $\Delta_pS°$ in J·mol^{-1}·K^{-1}. [b] TMG = 1,1,3,3-tetramethylguanidine, DBN = 1,5-diazabicyclo[4.3.0]non-5-ene, and DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene. [c] From ref 35. [d] $PA_{298} = GB_{298} + (298 \times 10^{-3})(108.8 - \Delta_pS°)$ kJ·mol^{-1}.

by step, up to GB ≈ 1080 kJ·mol^{-1} by using inter-related proton-transfer reactions and the corresponding relative basicities. No less than 18 equilibrium proton-transfer reactions involving TMG, DBN, and DBU were considered. The resulting GB values (998.0, 1008.4, and 1018.2 kJ·mol^{-1} for TMG, DBN, and DBU, respectively, as reported in Table 1) are associated with a standard deviation of about 1.5 kJ·mol^{-1}.

TMG, DBN, and DBU are monodentate bases where only one basic site, the imino nitrogen, is expected to be protonated in the conditions of equilibrium proton transfer. No significant entropy change is consequently expected between the neutral and the protonated forms of these molecules. One exception however arises with TMG since a change of symmetry point group occurs after protonation. The protonation entropies, $\Delta_pS(B_i) = S°(B_iH^+) - S°(B_i)$, were consequently assumed to be zero for DBN and DBU and equal to $-R$ ln 2 for TMG. These figures lead to the proton affinity values indicated in the second column of Table 1.

In preamble to the direct application of the extended kinetic method, it is interesting to follow the evolution of ln($[MH]^+/[B_iH]^+$) as a function of the excitation energy or, equivalently, of the effective temperature T_j of the activated adduct $[MHB_i]^+$. It offers another angle of view of the entropy effect related to the protonation of arginine. Examples of the evolution of ln($[MH]^+/[B_iH]^+$) with the center of mass collision energy E_{cm} measured using the three reference bases B_i are presented in Figure 1. Qualitatively, the three curves tend toward a negative asymptote at high E_{cm} values. Following eq 9, the increase in T results in an asymptotic limit equal to $\Delta_{MB_i}S/R$. Figure 1 thus demonstrates a negative $\Delta_{MB_i}S = \Delta_pS(Arg) - \Delta_pS(B_i)$ term, i.e., a negative protonation entropy for arginine, $\Delta_pS(Arg)$, since the protonation entropies of the reference bases, $\Delta_pS(B_i)$, are essentially negligible for DBN and DBU and even negative for TMG (see Table 1).

The $[MH]^+/[B_iH]^+$ ratio obtained at various collision energies, i.e., at various effective temperatures T, is treated by the kinetic method in its extended form to deduce both $PA_{iso}(Arg)$ and $\Delta_pS°_{iso}(Arg)$ using eq 9. A number of ln($[MH]^+/[B_iH]^+$) points, corresponding to E_{cm} values situated between 0.5 and 4.0 eV by steps of 0.5 eV, were selected and used in the orthogonal distance regression (ODR) method[30] to locate the isothermal point. The relevant data are presented in Table 2. To illustrate the quality of the data, three examples of linear fits, corresponding to E_{cm} = 0.5, 1.5, and 4.0 eV, are presented in Figure 2.

The ODR treatment of the set of 24 experimental points leads to PA_{iso} and ΔS_{iso} values, deduced from the isothermal point coordinates, equal to 1043.9 ± 1.9 (4.0) kJ·mol^{-1} and −22 ± 4 (9) J·mol^{-1}·K^{-1}, respectively (indicated errors are standard deviations, and in parentheses, 95% confidence limits are given). Using the mean value of $\Delta_pS(B_i)$ (i.e., −1.9 J·mol^{-1}·K^{-1}) we derive for arginine a protonation entropy equal to $\Delta_pS(Arg)$ = −24 ± 4 (9) J·mol^{-1}·K^{-1}. It must be recalled here that the application of the extended kinetic method leads to proton affinity and protonation entropy associated with systematic errors.[20−23] This is particularly true when the protonation entropy is significantly negative. It has been observed, for example, that the proton affinity values of 1,3-propanediamine and 1,3-aminopropanol given by the extended kinetic method were underestimated by no less than 7 and 9 kJ·mol^{-1}, respectively.[23] Concerning the protonation entropy, in both cases, the value

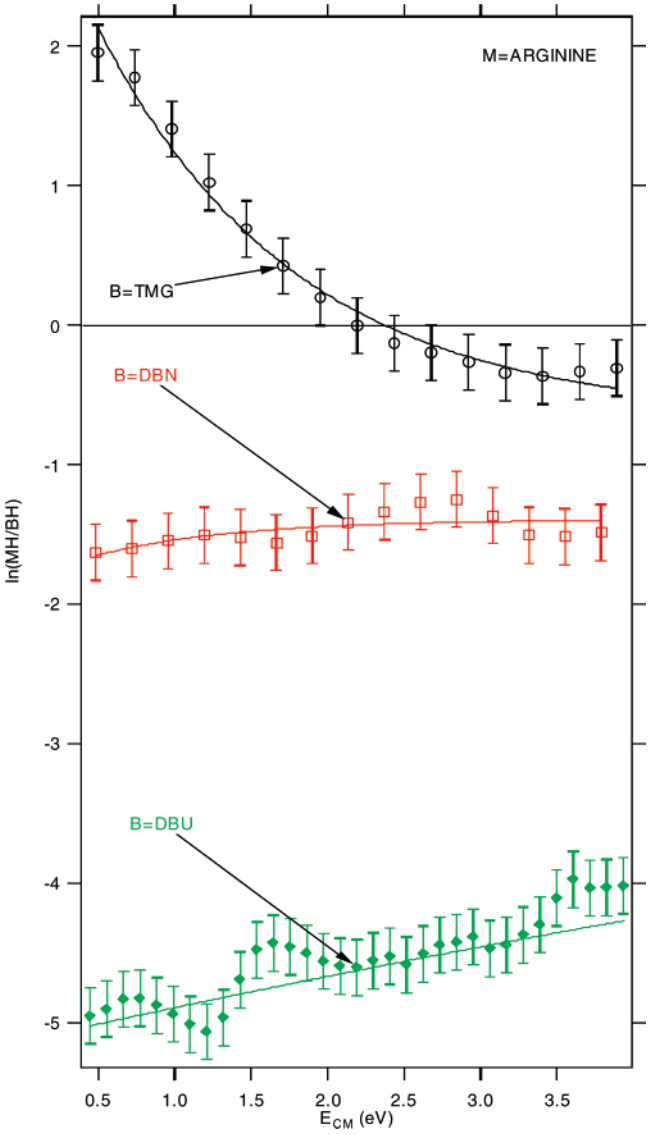


Figure 1. Evolution of ln(MH/B_iH) as a function of the center of mass collision energy E_{CM} (solid lines are exponential fits presented only for graph visibility purposes).

TABLE 2: Center of Mass Collision Energy, E_{cm}, and ln(MH/B_iH) for the Three Reference Bases B_i = TMG, DBN, and DBU, M Being Arginine

	ln(MH/B_iH)		
E_{cm} (eV)	TMG	DBN	DBU
0.5	2.30	−1.63	−4.90
1.0	1.40	−1.55	−5.00
1.5	0.68	−1.53	−4.48
2.0	0.20	−1.45	−4.55
2.5	−0.15	−1.40	−4.58
3.0	−0.28	−1.40	−4.45
3.5	−0.35	−1.40	−4.10
4.0	−0.50	−1.40	−4.00
FAB[a]	3.25	−0.18	−3.38

[a] Experimental data from ref 14.

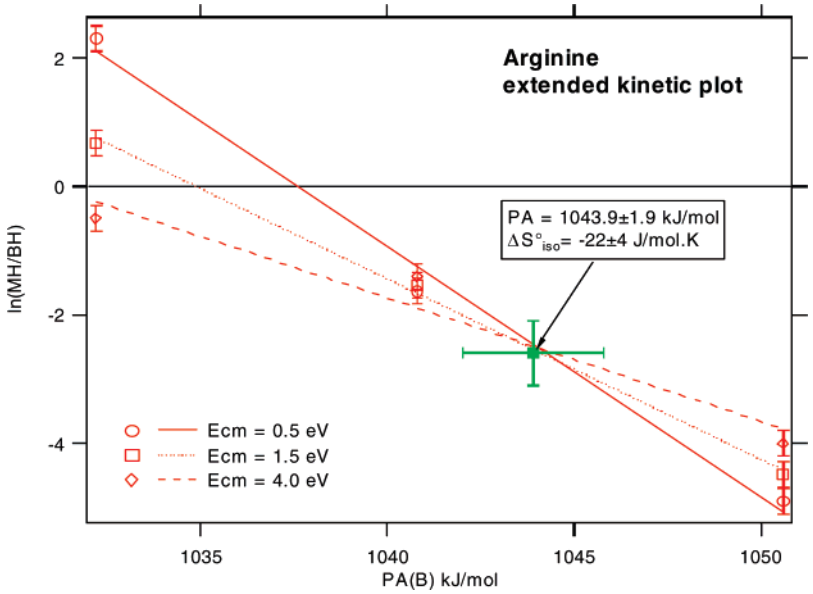


Figure 2. Kinetic method plot illustrated for three center of mass energies. The isothermal point has been determined by the ODR method from eight temperature values (see Table 1).

deduced from ΔS_{iso} was equal to ca. -23 J·mol^{-1}·K^{-1}, while the literature values are -49 and -43 J·mol^{-1}·K^{-1} for 1,3-propanediamine and 1,3-aminopropanol, respectively. Considering these limitations, the experimentally determined PA(Arg) and $\Delta_p S$(Arg) values of 1044 kJ·mol^{-1} and -24 J·mol^{-1}·K^{-1}, respectively, should be considered a priori as lower limits for the proton affinity and the absolute value of the protonation entropy of arginine. However, as recalled before, another important observation concerning the particularities of the extended kinetic method is that the resulting gas-phase basicity is accurately determined.[21−23] Thus, combining the experimental PA_{iso}(Arg) and $\Delta_p S$(Arg) values obtained here by the extended kinetic method, we deduce more confidently a gas-phase basicity of arginine, GB_{298}(Arg), equal to 1004.3 ± 2.2 (4.9) kJ·mol^{-1}.

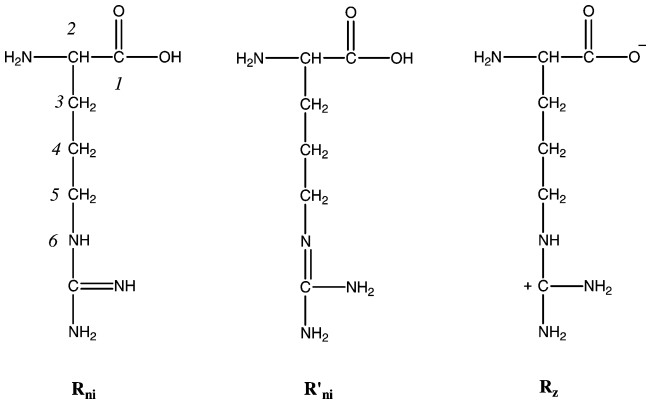
It is interesting to compare these results with the previous observation by Wu and Fenselau.[14] These authors were able to measure the $[MH]^+$ and $[B_iH]^+$ peak intensities (M = Arg, and B_i = TMG, DBN, and DBU) from the dissociations of metastable $[MHB_i]^+$ ions produced in an FAB ion source and traveling an EB−EB sector mass spectrometer. Since their data were obtained without activation of the precursor ions and are thus characterized by only one effective temperature *T*, the simple kinetic method alone may be used. The data of Wu and Fenselau[14] are recalled in the last line of Table 2. A linear correlation is found between $\ln([MH]^+/[B_iH]^+)$ and PA(B_i) with an *x* intercept equal to 1040.9 ± 0.8 (7.2) kJ·mol^{-1} and a slope corresponding to an effective temperature of 335 ± 17 K. Following eq 8, the *x* intercept corresponds to $PA_{298}(M) + 335[\Delta_p S(M) - \Delta_p S(B_i)]$, and since the latter entropy term is negative, it is not surprising to see that the Wu and Fenselau[14] *x* intercept is lower than the PA(M) given above by the extended kinetic method. Another means to take into account the Wu and Fenselau[14] experiments is to join their experimental points to ours in a common set of data and treat this overall set by the ODR method. This procedure may be justified by the fact that, in eqs 4−9, the mode of formation of the adduct ions $[MHB_i]^+$ and the experimental constraints associated with the measurement of the fragment ion intensities are expected to be taken into account in the empirical effective temperature *T*. This global treatment leads to PA_{iso} = 1047.7 ± 2.6 (5.5) kJ·mol^{-1}, $\Delta_p S$(Arg) = -31 ± 6 (12) J·mol^{-1}·K^{-1}, and GB(Arg) = 1005.9 ± 3.1 (6.6) kJ·mol^{-1}. Although the standard deviations and 95% confidence limits are slightly extended, the results concerning the gas-phase basicity of arginine are still coherent.

In summary, it may be concluded from the experiments that the gas-phase basicity of arginine is close to 1005 kJ·mol^{-1}. The existence of a negative protonation entropy is clearly indicated by the evolution of $\ln([MH]^+/[B_iH]^+)$ as a function of the excitation energy and by the extended kinetic method results. The actual, averaged, value is equal to $\Delta_p S$(Arg) = -27 ± 7 (15) J·mol^{-1}·K^{-1}. Considering the absolute value of this entropy term as a lower limit, this implies that the minimum value for the proton affinity of arginine is PA(Arg) = 1046 ± 4 (7) kJ·mol^{-1}. It is now interesting to compare these experimental data with expectations based on quantum chemical calculations.

Theoretical Proton Affinity of Arginine. In recent years, several studies have reported computational investigations on the tautomers and conformers of neutral arginine with various successes.[24−27,38−40] The encountered difficulties were due to the very large number of conformer possibilities conjugated with the uncertainties on the energy calculations offered by the necessarily low level of theory which is generally used in the first selection of the low-energy conformers. The most recent studies[27,39] on neutral arginine clearly illustrate this aspect since they present no less than 11 new canonical and zwitterionic forms which are lower in energy by 8−10 kJ·mol^{-1} than the precedingly identified structures. According to these investigations, the zwitterion R_z is less stable than the nonionic form R_{ni} by ca. 15 kJ·mol^{-1} (Scheme 2).[25,27,39,40] This confirms previous conclusions, even though the most stable conformations were not necessarily correctly identified, and is consistent with the fact that arginine exists in a nonzwitterionic form when vaporized in a supermolecular beam.[41] The two possible tautomeric forms of the guanidine moiety, R_{ni} (−NHC(NH$_2$)=NH) and R_{ni}' (−N=C(NH$_2$)$_2$), seem to present similar stabilities[25,27] (Scheme 2). However, in their recent theoretical study, Ling et al.[27] found that the most stable conformers of neutral arginine pertain generally to the R_{ni}' tautomeric form thus having one imino nitrogen and two NH$_2$ groups in the guanidine moiety.

Ling et al.[27] showed that, at the B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p) level, the two most stable forms (in terms of electronic energies and 298 K enthalpies) of neutral arginine are structures C4 and C5 (Figure 3). These two

SCHEME 2



conformers present an *anti*-HOCO arrangement and a bifurcated $NH_2\cdots O{=}C$ hydrogen bond in the amino acid moiety (a situation similar to that found in glycine and in other amino acids[42]). Additional stabilization is brought by another hydrogen bond involving the imino nitrogen of the guanidine group and the hydroxyl hydrogen. Conformers C4 and C5 differ only in the relative arrangement of the two amino groups of the guanidine moiety. At the MP2/6-31++G(d,p) and CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) levels, the energy order of the various investigated species is changed. The most stable conformer becomes structure C1 (Figure 3); however, structures C4 and C5 are situated only ~2 kJ·mol^{-1} above.[27] Conformer C1 is also characterized by an *anti*-HOCO arrangement, but by contrast with C4 and C5, the stabilization in the amino acid part is offered by one hydrogen bond between the hydrogen of the hydroxyl group and the nitrogen atom of the α-amino group (a conformation less stable in the case of glycine[42]). Another hydrogen bond involving the imino nitrogen of the guanidine group and one H of the α-amino group participates in the overall stabilization of this conformer.

The structure of the most stable conformation of protonated arginine reached a consensus more easily.[8,25−27,43,44,54] This structure (denoted P1 in Figure 3) is stabilized by two internal hydrogen bonds, one between the H(N) of the protonated guanidine and the nitrogen atom of the α-amino group and the second between one H of the NH_2 group of protonated guanidine and the oxygen of the carbonyl function.

Having identified the most stable neutral and protonated structures of arginine, the corresponding 298 K proton affinity may be deduced from quantum chemical calculations of the enthalpy of the reaction $ArgH^+ \rightarrow Arg + H^+$. Using their computational results, Ling et al.[27] devised a 298 K proton affinity value of 1062 kJ·mol^{-1} and a gas-phase basicity of 1028.8 kJ·mol^{-1}, but they did not precisely state how these figures were obtained. On the other hand, Bleiholder et al.[26] investigated the proton affinities of the 20 naturally occurring α-amino acids. The authors calculated for arginine 0 K proton affinities of 1056.8 and 1046.8 kJ·mol^{-1} at the B3LYP/6-31+G(d,p) and G2(MP2) levels, respectively;[26] consideration of the 298 K corrections (see later, Table 3) leads to PA_{298}(Arg) equal to 1063.1 and 1053.1 kJ·mol^{-1}. However, if the authors correctly identified the protonated form P1 (Figure 3) as the most stable, the neutral structure they used in their computation consists in a $-NHC(NH_2){=}NH$, R_{ni} tautomer, demonstrated to be less stable than their R_{ni}' (Scheme 2) counterpart.[27] In fact, we found that this conformer is situated 7.4 kJ·mol^{-1} above C4 at the B3LYP/6-31+G(d,p) level. The PA(Arg) estimates of ~1063−1053 kJ·mol^{-1} based on the Bleiholder et al.[26] calculations may be consequently overestimated.

To complete these previous results, we have reconsidered the proton affinity of arginine by using a larger basis set in the DFT calculations and isodesmic correction on both DFT and CCSD computations. The geometries of structures C1, C4, C5, and P1 were optimized at the B3LYP/6-31+G(d,p) and B3LYP/6-31++G(d,p) levels. Corrections to the zero-point vibrational energy and to the 298 K enthalpy were also calculated at these levels. Furthermore, single-point energy calculations were done by using the more extended 6-311+G(3df,2p) basis set on the B3LYP/6-31+G(d,p)-optimized structures. The results are gathered in Table 3 and compared with the CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) data of Ling et al.[27] To test the reliability of the computation, we also consider, at various levels of theory, a related reference base: guanidine, for which the experimental proton affinity is equal to 987.4 kJ·mol^{-1}.[51] From the values reported in Table 3, the theoretical proton affinity of guanidine calculated at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p), B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p), B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p), and CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) levels is equal to 996.1, 995.9, 995.6, and 996.0 kJ·mol^{-1}, respectively. An overestimate of the proton affinity of guanidine, by no less than 8 kJ·mol^{-1} with respect to the experimental value, is consequently given by theory at the level considered here. It may be noted that a comparable observation has been made by Hao et al.,[53] who proposed on the basis of CCSD(T)/aug-cc-pVTZ calculations a proton affinity value of 985 kJ·mol^{-1} for guanidine. It is consequently expected that the PA(Arg) deduced from computational data summarized in Table 3 should also be overestimated since the protonation site is of comparable structure in arginine and guanidine. In other words, a proper theoretical approach of the proton affinity of arginine would be to correct the crude enthalpy estimate based on the $ArgH^+ \rightarrow Arg + H^+$ reaction by considering the isodesmic reaction: $ArgH^+$ + guanidine → Arg + guanidine$-H^+$. The resulting isodesmic PA(Arg) values are reported in the last column of Table 4.

Under these circumstances, the theoretical B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p) isodesmic proton affinities calculated using the neutral arginine forms C1 and C4 are equal to 1055.0 and 1041.9 kJ·mol^{-1}, respectively (Table 3). The CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) calculations provide PA values of 1052.7 and 1053.4 kJ·mol^{-1} for neutral arginine conformers C1 and C4. It consequently appears that, at all the levels of theory considered in Table 3, protonation of conformer C1 (the structure predicted to be more stable at the MP2 and CCSD levels) corresponds to a proton affinity of ca. 1053 kJ·mol^{-1}. Unexpectedly, the proton affinity of conformer C4 or C5 (the structures predicted to be more stable at the B3LYP/6-31++G(d,p) level) is strongly sensitive to the level of theory used. This effect is not observed for guanidine, and this observation suggests that the discrepancy originates from the existence of internal hydrogen bonds in protonated arginine. It is generally argued that MP2 or coupled-cluster correlated levels of theory are superior to B3LYP in treating hydrogen-bonded systems.[27,52] On this basis, the proton affinity values calculated at the CCSD//6-31++G(d,p)//MP2/6-31++G(d,p) level, i.e., a common figure close to 1053 kJ·mol^{-1} for C1, C4, and C5, should be preferably retained.

It is now important to underline that the extended theoretical investigation of Ling et al.[27] clearly demonstrates that a mixture of conformers should be considered to interpret the experimental data obtained at 298 K. Considering the electronic energies, the lowest energy conformers in the ~10 kJ·mol^{-1} range are up to seven at the B3LYP/6-31++G(d,p)//B3LYP/6-31++G-

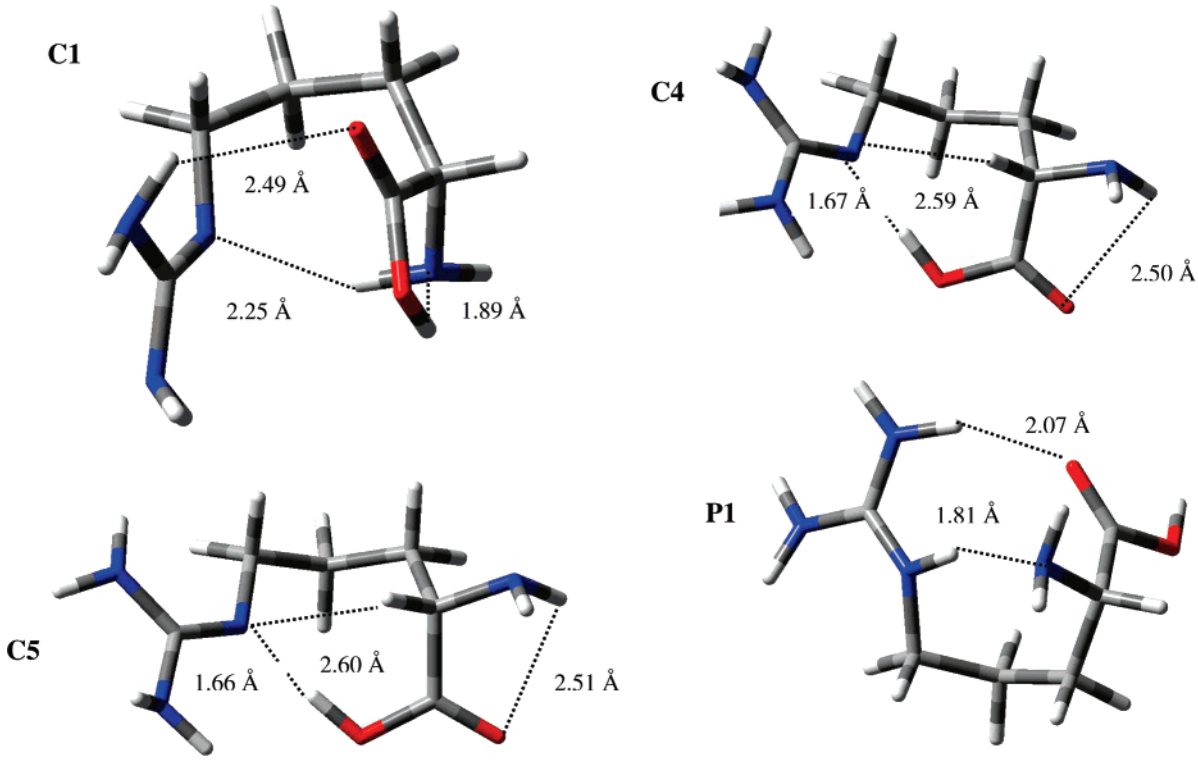


Figure 3. B3LYP/6-31++G(d,p) geometries of the most stable conformers of neutral (C1, C4, and C5) and protonated (P1) arginine.

TABLE 3: Total Energy, Zero-Point Vibrational Energy, and Thermal Contribution to Enthalpy at 298 K (hartrees)[a]

level[b]		C1	C4	C5	P1	guanidine	guanidine−H^+
b+	total energy	−606.598171	−606.601964	−606.601920	−607.014864	−205.396459	−205.786696
b++		−606.598743	−606.602566	−606.602525	−607.015366	−205.396730	−205.786897
bdf		−606.777958	−606.782459	−606.782368	−607.184211	−205.460780	−205.850841
CCSD		−604.907621	−604.906830	−604.905868	−605.323144	−204.827906	−205.218127
b+	ZPVE	0.223445	0.222529	0.222392	0.236695	0.075928	0.088384
b++		0.223401	0.222500	0.222358	0.236651	0.075928	0.088384
b+	$H_{0\rightarrow 298}$	0.237234	0.236725	0.236634	0.250899	0.0814240	0.094637
b++		0.237199	0.236701	0.236607	0.250865	0.081424	0.094637

[a] 1 hartree = 2625.5 kJ·mol^{-1}. [b] b+ = B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p), b++ = B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p), bdf = B3LYP/6-311+G3df,2p)//B3LYP/6-31+G(d,p), and CCSD = CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) from ref 27 for C1, C4, C5, and P1.

TABLE 4: Calculated Isodesmic[a] Proton Affinities of the Most Stable Forms of Arginine (kJ·mol^{-1})

level[b]	PA(C1)	PA(C4)	PA(C5)
b+	1055.7	1044.4	1044.3
b++	1055.6	1044.2	1044.2
bdf	1055.0	1041.9	1041.9
CCSD	1052.7	1053.4	1053.7

[a] PA(Arg) is given by the 298 K enthalpy of the reaction ArgH^+ + guanidine → Arg + guanidine−H^+ plus the experimental value of PA(guanidine) = 987.4 kJ·mol^{-1}. [b] b+ = B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p), b++ = B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p), bdf = B3LYP/6-311+G3df,2p)//B3LYP/6-31+G(d,p), and CCSD = CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) from ref 27 for C1, C4, C5, and P1.

(d,p) level and to twelve at the CCSD/6-31++G(d,p)//MP2/6-31++G(d,p) level of theory. The experimentally determined proton affinity may be defined as the averaged quantity PA_{av}:

$$PA_{av} = \sum_{1}^{N} x_i PA_i \qquad (10)$$

where PA_i and x_i are the proton affinities and the molar fractions, respectively, of each of the N conformers. Assuming a Boltzmannn distribution of the conformers, the individual populations x_i are given by

$$x_i = \exp(-G_i/RT) \Big/ \sum_{1}^{N} \exp(-G_i/RT) \qquad (11)$$

with G_i representing the individual Gibbs free energies.

The precise knowledge of the Gibbs free energy of the various identified conformers of arginine is hardly attainable, mainly because of the difficulties to correctly estimate the entropy of the low vibrational frequencies (see below) and to treat anharmonic effects.[42] Using the crude Gibbs free energies provided by the Gaussian 98 software, i.e., without consideration of the above-mentioned limitations, Ling et al.[27] proposed a rough conformer distribution at several temperatures. Using their x_i values calculated at 298 K and the isodesmic individual proton affinities, the average proton affinity, PA_{av}, converges toward a common value of ∼1054 kJ·mol^{-1}.

Comparison of these theoretical estimates, close to 1053 kJ·mol^{-1}, with our experimental determination of 1044 kJ·mol^{-1} shows a difference of ca. 10 kJ·mol^{-1}. This observation is not surprising since the proton affinity obtained by the extended kinetic method is known to be underestimated[20−23] as recalled before.

Considerations on the Protonation Entropy of Arginine. It is well-known that the vibrational contribution to entropy is particularly sensitive to low frequencies.[32,42] Unfortunately, the corresponding degrees of freedom generally present large-amplitude motions where the classical harmonic approximation to the potential energy profile does not hold. Computation of thermodynamic properties based on the harmonic oscillator approximation is thus not adapted to species containing hindered rotations. For a correct prediction of the thermodynamic properties of species containing hindered rotations, a complete investigation of the torsional potentials is required. Moreover, these calculations should be done for all the conformations expected to be populated at the considered temperature. Due to the inherent cost of such a formidable task in the case of arginine, a more suitable approach is to introduce corrections based on reasonable approximation as discussed below.

The treatment of the hindered rotor associated with a torsional potential of the form $V(\phi) = 1/2V_0(1 - \cos n\phi)$, with V_0 the barrier of rotation, n the number of minima of the potential, and ϕ the dihedral angle, has been done by Pitzer et al.[45] In this study, the resulting contribution to entropy, $S_{hind}°$, has been expressed as a function of V_0 and the reduced moment of inertia of the rotation I_{red}. Calculation of the contribution to entropy of each individual rotation, 1−2, 2−3, 3−4, 4−5, and 5−6 (see Scheme 2 for the numbering convention), has been done here for neutral and protonated arginine at variable V_0 values. The results are illustrated in Figure 4a for neutral arginine using the equilibrium geometry of structure C4. It is noteworthy that quasi-identical curves are obtained for conformer C1 and protonated arginine P1 since the changes of a reduced moment of inertia between these structures are negligible. Another consequence is the fact that the decrease in $S_{hind}°$ as a function of V_0 follows almost exactly the same analytic curve. It is therefore convenient to summarize the above calculations by considering the averaged entropy loss $S_{hind}° - S_{free}°$ versus V_0 as depicted in Figure 4b.

As far as the protonation entropy, $\Delta_pS°(M) = S°(MH^+) - S°(M)$, is concerned the major event is the change in rotational barriers V_0 between the neutral and protonated forms M and MH^+. The principal contributions to the stability of the most stable conformations C1, C4, and P1 are internal hydrogen bonds. The maximum energy required to rotate around the 1−2, 2−3, 3−4, 4−5, and 5−6 σ bonds is thus related to the breaking of some specific hydrogen bonds. In the case of the neutral conformer C4 or C1, this bond involves one hydrogen of the acid moiety or of the α-amino group and the imino nitrogen of the guanidine group. For the protonated structure P1, the critical hydrogen bond is situated between the hydrogen of the protonated imino group and the nitrogen of the α-amino group. An estimate of the energy of these two kinds of hydrogen bonds may be proposed on the basis of simpler bimolecular systems. Most of the neutral gaseous AH···A dimers exhibit enthalpies of dissociation in the very narrow range 15−20 kJ·mol^{-1}.[46,47] Considering that, in conformer C4, the donor moiety is a carboxylic acid, the true value of the hydrogen bond energy is probably closer to the upper limit of 20 kJ·mol^{-1} for this conformer. Turning now to protonated arginine, an estimate of the internal hydrogen bond energy may be obtained by using the empirical relationship proposed by Mautner[48] to calculate the enthalpies of dissociation of $AH^+\cdots B_i$ complexes. In the case of proton-bonded nitrogen bases, this relationship reduces to $\Delta H° = (97 \pm 3) - (0.25 \pm 0.05)[PA(A) - PA(B_i)]$ (kJ·mol^{-1}). If we use PA(A) = PA(guanidine) = 987.4[51] kJ·mol^{-1} and $PA(B_i)$ = PA(glycine) = 885[16] kJ·mol^{-1}, we derive $\Delta H° = 72 \pm 8$ kJ·mol^{-1}. The change in rotational barrier V_0 from 20 to ca. 70 kJ·mol^{-1} when passing from neutral C4 (or C1) to protonated arginine P1, which may be suggested from the above considerations, is in line with other results. Accordingly, it has been observed during conformational analysis of α,γ-disubstituted molecules, such as diamines or amino alcohols, and their protonated counterparts that V_0 is generally in the 10−30 kJ·mol^{-1} range while it rises to 60−100 kJ·mol^{-1} in the corresponding protonated forms.[49,50]

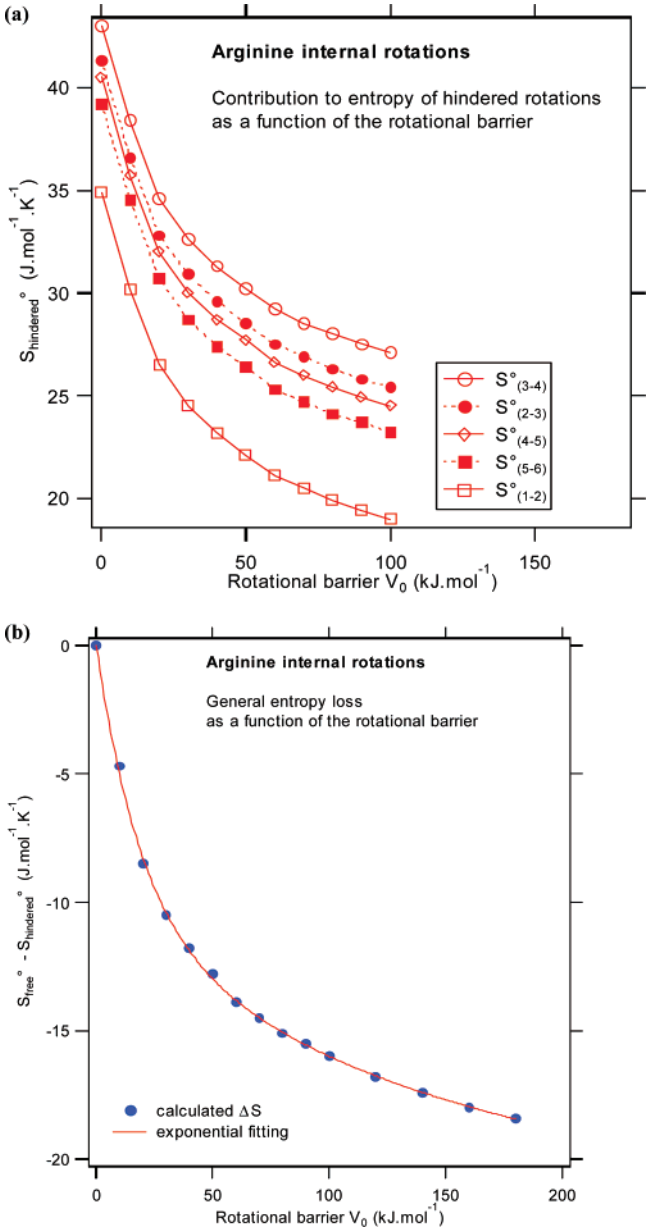


Figure 4. Influence of the rotational barrier V_0 upon the corresponding contribution to vibrational entropy in the hindered rotor approximation of Pitzer: (a) individual contributions of the five internal rotors in C4, (b) averaged entropy difference $S_{free}° - S_{hind}°$ for each internal rotor of C4.

Considering the data of Figure 4, a change in rotational barrier V_0 from 20 to 70 kJ·mol^{-1} induces a decrease in entropy of 6 J·mol^{-1}·K^{-1}. If this amount is supposed to be identical for the five rotations, a contribution to the protonation entropy of −30 J·mol^{-1}·K^{-1} is predicted due to the hindrance of internal rotation induced by the protonation. If we assume uncertainties of ±10 and ±20 kJ·mol^{-1} on the V_0 barriers in the neutral and protonated arginine, respectively, a total uncertainty of ±7 J·mol^{-1}·K^{-1} on this entropy loss estimate results.

To fully estimate the protonation entropy, $\Delta_p S°$, the second point to consider is the entropy of mixing since several conformers are expected to be populated at 298 K, particularly for the neutrals as recalled in the preceding paragraph. For a mixture of *N* distinguishable conformers, the entropy of mixing is given by the expression

$$\Delta S_{mix}° = -R \sum_{1}^{N} x_i \ln x_i \qquad (12)$$

where x_i is the molar fraction of each component of the mixture (see eq 10). In fact, the magnitude of the entropy of mixing is not very sensitive to the actual proportions x_i of the components.[32] To a first approximation, one may thus assume that we are dealing with a set of *N* equally populated conformations; then the entropy of mixing reduces to $-R \ln(1/N)$. The number of conformations significantly populated, *N*, may be approached using a cutoff energy of ca. 6−10 kJ·mol^{-1}. Accordingly, in this energy range ∼95% of the conformer population is generally recovered[42] if their entropies are not too much different. In the 6−10 kJ·mol^{-1} energy range, the number of identified arginine conformers is situated between 3 and 12 depending upon the level of theory used (either B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p) or CCSD/6-31++G(d,p)//MP2/6-31++G(d,p)).[27] The resulting entropy of mixing is consequently situated between 9 and 21 J·mol^{-1}·K^{-1}. It is noteworthy that, on the basis of the x_i values proposed by Ling et al.[27] for neutral arginine at 298 K, we derive a value of $\Delta S_{mix}° = 15$ J·mol^{-1}·K^{-1}. We can thus retain a contribution *of* -15 ± 5 J·mol^{-1}·K^{-1} to the overall protonation entropy of arginine, $\Delta_p S$(Arg), due to the entropy of mixing of its neutral forms.

This contribution added to the loss of entropy due to the increase of V_0 after protonation leads to an estimate of −45 J·mol^{-1}·K^{-1} for the protonation entropy of arginine, $\Delta_p S°$(Arg) (with a probable error of 12 J·mol^{-1}·K^{-1}). This may be compared with the experimental value of −23 J·mol^{-1}·K^{-1} (or −31 J·mol^{-1}·K^{-1} if the Wu and Fenselau[14] data are included in the computation). The discrepancy observed between theory and experiment is in keeping with the fact that the extended kinetic method used for the experimental determination is known to underestimate the absolute value of the protonation entropy.[20−23]

IV. Conclusion

The present study brings two kinds of information on the gas-phase basicity of arginine: experimental, by the use of the extended kinetic method in its isothermal point version, and theoretical, by density functional theory at the 6-311++G(3df,-2p)//B3LYP/6-31++G(d,p) level including 298 K and isodesmic corrections completed by a semiempirical estimate of the protonation entropy.

The first major finding is the evidence for a significant loss of entropy during protonation of arginine, as expected from its bidentate character. This point is evidenced by the influence of the excitation energy on the branching ratio of the competing dissociations of MHB_i^+ adducts. A negative limit of ln(MH/B_iH) is attained at high excitation energy with M = arginine and B_i = TMG, DBN, and DBU. The extended kinetic method confirms this trend in providing a lower limit of 27 ± 7 (15) J·mol^{-1}·K^{-1} to the absolute value of the so-called "protonation entropy" $\Delta_p S°(\text{Arg}) = S(\text{ArgH}^+) - S°(\text{Arg})$. Consideration of the rotational barriers and of the conformational populations in both the neutral and protonated forms of arginine allows a theoretical estimate of the protonation entropy of $\Delta_p S°(\text{Arg}) = -45 \pm 12$ J·mol^{-1}·K^{-1}. This may be compared to 1,3-propanediamine and 1,3-aminopropanol for which the value deduced from the extended kinetic method was equal to −23 J·mol^{-1}·K^{-1}, while the literature values are −49 and −43 J·mol^{-1}·K^{-1}, respectively.[23]

The second important information is the experimental gas-phase basicity value obtained by the extended kinetic method. The use of our data coming from ESI-MS−MS experiments leads to GB(Arg) = 1004.3 ± 2.2 (4.9) kJ·mol^{-1}; if we include in the statistical treatment the previous data obtained by Wu and Fenselau,[14] the result is shifted to GB(Arg) = 1005.9 ± 3.1 (6.6) kJ·mol^{-1}. Thus, a conservative value of GB(Arg) = 1005 ± 4 (8) kJ·mol^{-1} may be safely deduced from the experiments.

The proton affinity value deduced from the extended kinetic method experiments is situated between 1043.9 ± 1.9 (4.0) and 1047.7 ± 2.6 (5.5) kJ·mol^{-1}. It should be emphasized again that the proton affinity value obtained by this method represents a lower limit as observed in similar systems. For example, the proton affinities of 1,3-propanediamine and 1,3-aminopropanol given by the extended kinetic method were underestimated by 7 and 9 kJ·mol^{-1}, respectively.[23] Indeed, the present study points to a theoretical proton affinity of arginine close to 1053 kJ·mol^{-1} and consequently to an underestimate of 5−9 kJ·mol^{-1} by the extended kinetic method.

To summarize, and considering the discussed limitations of the extended kinetic method (mainly the systematic errors on the proton affinity and the protonation entropy), the following evaluated thermochemical parameters may be proposed: GB(Arg) = 1005 ± 4 kJ·mol^{-1}, PA(Arg) = 1051 ± 5 kJ·mol^{-1}, and $\Delta_p S°(\text{Arg}) = -45 \pm 12$ J·mol^{-1}·K^{-1}.

Acknowledgment. We thank Kaheina Aïzel for her participation in the experiments and Prof. K. Ervin and P. Armentrout for providing us with the ODR program.

References and Notes

(1) Guillen-Schilppe, Y. V.; Hedstrom, L. *Arch. Biochem. Biophys.* **2005**, *433*, 266−278.
(2) Das, C. *Principles and practice of biological mass spectrometry*; Wiley: New York, 2001.
(3) Paizs, B.; Suhai, S. *Mass Spectrom. Rev.* **2005**, *24*, 508−548.
(4) Zwinselman, J. J.; Nibbering, N. M. M.; van der Greef, J.; Ten Noever de Brauw, M. C. *Org. Mass Spectrom.* **1983**, *18*, 525−529.
(5) Dookeran, N. N.; Yalcin, T.; Harrison, A. G. *J. Mass Spectrom.* **1996**, *31*, 500−508.
(6) Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2000**, *195/196*, 565−590.
(7) Geller, O.; Lifshitz, C. *J. Phys. Chem. A* **2003**, *107*, 5654−5659.
(8) Csonka, I. P.; Paizs, B.; Suhai, S. *J. Mass Spectrom.* **2004**, *39*, 1025−1035.
(9) Shek, P. Y. I.; Zhao, J.; Ke, Y.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2006**, *110*, 8282−8296.
(10) Bouchoux, G. *Mass Spectrom. Rev.* **2007**, *26*, 775−835.
(11) (a) Bojesen, G. *J. Am. Chem. Soc.* **1987**, *109*, 5557−5558. (b) Isa, K.; Omote, T.; Amaya, M. *Org. Mass Spectrom.* **1990**, *25*, 620−628.
(12) Gorman, G. S.; Speir, J. P.; Turner, C. A.; Amster, I. J. *J. Am. Chem. Soc.* **1992**, *114*, 3986−3988.
(13) Bojesen, G.; Breindahl, T. *J. Chem. Soc., Perkin Trans.* **1994**, *2*, 1029−1037.
(14) Wu, Z.; Fenselau, C. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 403−405.
(15) Hunter, E. P.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998**, *27*, 413−656.
(16) Hunter, E. P.; Lias, S. G. Standard reference database no. 69. *NIST Chemistry Webbook*; National Institute of Standards and Technology: Gaithersburg, MD, 2005; http//webbook.nist.gov/chemistry/.
(17) (a) Cooks, R. G.; Kruger, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1279−1281. (b) Cooks, R. G.; Patrik, J. S.; Kotaho, T.; McLuckey, S. A. *Mass Spectrom. Rev.* **1994**, *13*, 287−339. (c) Cooks, R. G.; Wong, P. S. H. *Acc. Chem. Res.* **1998**, *31*, 379−386. (d) Cooks, R. G.; Koskinen, J. T.; Thomas, P. D. *J. Mass Spectrom.* **1999**, *34*, 85−92.

(18) Bouchoux, G.; Djazi, F.; Gaillard, F.; Vierezet, D. *Int. J. Mass Spectrom.* **2003**, *227*, 479−496.
(19) Bouchoux, G.; Buisson, D. A.; Bourcier, S.; Sablier, M. *Int. J. Mass Spectrom.* **2003**, *228*, 1035−1054.
(20) Drahos, L.; Vekey, K. *J. Mass Spectrom.* **2003**, *38*, 1025−1042.
(21) Ervin, K.; Armentrout, P. B. *J. Mass Spectrom.* **2004**, *39*, 1004−1015.
(22) Drahos, L.; Peltz, C.; Vekey, K. *J. Mass Spectrom.* **2004**, *39*, 1016−1024.
(23) Bouchoux, G. *J. Mass Spectrom.* **2006**, *41*, 1006−1013.
(24) Maksik, Z.; Kovacevic, B. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2623−2629
(25) Rak, J.; Skurski, P.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11695−11707.
(26) Bleiholder, C.; Suhai, S.; Paizs, B. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1275−1281.
(27) Ling, S.; Yu, W.; Huang, Z.; Lin, Z.; Haranczyk, M.; Gutowsky, M. *J. Phys. Chem. A* **2006**, *110*, 12282−12291.
(28) Zhang, D.; Wu, L.; Koch, K. J.; RCooks, R. G. *Eur. J. Mass Spectrom.* **1999**, *5*, 353−361.
(29) Giles, K.; Pringle, S. D.; Worthington, K. R.; Little, D.; Wildgoose, J. L.; Bateman, R. H. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2401−2414.
(30) Boggs, P. T.; Byrd, R. H.; Rogers, J. E.; Schnabel, R. B. *ODRPACK version 2.01 Software for weighted orthogonal distance regression*; Report NISTIR 92-4834; National Institute of Standards and Technology: Gaithersburg, MD, 1992.
(31) (a) *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998. (b) *Gaussian 03*, revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003.
(32) Bouchoux, G. *J. Phys. Chem. A* **2006**, *110*, 8259−8265.
(33) Ervin, K. M. *Int. J. Mass Spectrom.* **2000**, *195/196*, 271−284.
(34) Decouzon, M.; Gal, J. F.; Maria, P. C.; Raczynska, E. D. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 599−602.
(35) Raczinska, E. D.; Decouzon, M.; Gal, J. F.; Maria, P. C.; Gelbard, G.; Vielfaure-Joly, F. *J. Phys. Org. Chem.* **2001**, *14*, 25−34.
(36) Harrison, A. G. *Mass Spectrom. Rev.* **1997**, *16*, 201−217.
(37) Green, M. K.; Lebrilla, C. B. *Mass Spectrom. Rev.* **1997**, *16*, 53−71.
(38) Price, W. D.; Jockush, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1997**, *119*, 11988−11989.
(39) Gdanitz, R. J.; Cardoen, W.; Windus, T. L.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 515−518.
(40) Julian, R. R.; Beauchamp, J. L.; Goddard, W. A., III. *J. Phys. Chem. A* **2002**, *106*, 32−34.
(41) Chapo, C. J.; Paul, J. B.; Provencal, R. A.; Roth, K.; Saykally, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 12956−12957.
(42) Miller, T. F.; Clary, D. C. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2563−2571.
(43) Shek, P. Y. I.; Zhao, J.; Ke, Y.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2006**, *106*, 32−34.
(44) Bush, M. F.; O'Brien, J. T.; Prell, J. S.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 1612−1622.
(45) Pitzer, K. S.; Gwinn, W. D. *J. Chem. Phys.* **1942**, *10*, 428−440.
(46) Curtiss, L. A.; Blander, M. *Chem. Rev.* **1988**, *88*, 827−841.
(47) Jeffrey, G. A. *An introduction to hydrogen bonding*; Oxford University Press: New York, 1997.
(48) Mautner, M. *J. Am. Chem. Soc.* **1984**, *106*, 1257−1264.
(49) Bouchoux, G.; Buisson, D.A. *Int. J. Mass Spectrom.* **2006**, *249/250*, 412−419.
(50) Bouchoux, G.; Choret, N.; Berruyer-Penaud, F.; Flammang, R. *Int. J. Mass Spectrom.* **2002**, *217*, 195−230.
(51) Amekraz, B.; Tortajada, J.; Morizur, J-P.; Gonzalez, A. I.; Mo, O.; Yanez, M.; Leito, I.; Maria, P-C.; Gal, J-F. *New J. Chem.* **1996**, *20*, 1011−1021. The gas-phase basicity reported in this paper (950.5 kJ·mol^{-1}) has been determined from proton transfer equilibrium constant measurement at 338 K with dimethylcyclohexylamine as the reference base. Using for the latter a gas-phase basicity[15,16] of 952.7 kJ·mol^{-1} and $\Delta_p S$(guanidine) $= -RT \ln 6 = -14.9$ J·mol^{-1}·K^{-1}, a proton affinity of 987.5 kJ·mol^{-1} is obtained for guanidine.
(52) Scheiner, S. *J. Phys. Chem. B* **2007**, *111*, 11312−11317.
(53) Hao, C.; Seymour, J. L.; Turecek, F. *J. Phys. Chem. B* **2007**, *111*, 8829−8843.
(54) Forbes, M. W.; Bush, M. F.; Polfer, N. C.; Oomens, J.; Dunbar, R. C.; Williams, E. R.; Jockusch, R. A. *J. Phys. Chem. A* **2007**, *111*, 11759−11770.