Gas-Phase Protonation Thermochemistry of Glutamic Acid

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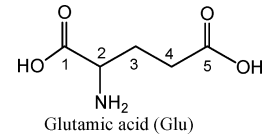
Proton affinity, PA(Glu), and protonation entropy (i.e., the difference $\Delta_p S°(\text{Glu}) = S°(\text{GluH}^+) - S°(\text{Glu})$) of glutamic acid have been experimentally determined by the extended kinetic method using electrospray ionization triple quadrupole-time-of-flight (ESI-Q-TOF) tandem mass spectrometry. The values deduced from these experiments are PA(Glu) = 945.3 ± 2.8(5.8) kJ·mol^{-1} and $\Delta_p S°(\text{Glu}) = -28 \pm 4(9)$ J·mol^{-1}·K^{-1} thus leading to a gas-phase basicity, GB(Glu), of 904.4 ± 3.0(6.4) kJ·mol^{-1} (uncertainties are standard deviation and, in parentheses, 95% confidence limit). Theoretical calculations performed at the G3MP2B3 level provide information on the structures, conformations, and energetics of the neutral and protonated species. Thermochemical data are calculated at this level and include a correction to the computation of the entropy associated with hindered rotation. When the lowest energy conformers of protonated and neutral glutamic acid are considered the following values are calculated: PA(Glu) = 948.1 kJ·mol^{-1} and $\Delta_p S°(\text{Glu}) = -31.3$ J·mol^{-1}·K^{-1}. Using G3MP2B3 data to estimate the gas-phase distribution of conformers at 298 K, the averaged molar quantities becomes PA(Glu) = 949.8 kJ·mol^{-1} and $\Delta_p S°(\text{Glu}) = -36.0$ J·mol^{-1}·K^{-1}. Both computations give comparable GB(Glu) = 906.4−906.7 kJ·mol^{-1}.

1. Introduction

Analysis of peptides and proteins by mass spectrometry techniques invariably relies on protonation as the major mode of formation of ionized analyte. Therefore, the knowledge of the sites of proton attachment and of the related protonation thermochemistry is essential in the understanding of mass spectrometry results. As building blocks of peptides, but also for their participation to a number of physiological processes, amino acids are of considerable importance. As a consequence, gas-phase protonation thermochemistry of the 20 naturally occurring α-amino acids has attracted the interest of the researchers for several decades.[1,2] However, detailed examination of the presently available data reveals serious discrepancies. The reasons of this situation are multiple. First, the low volatility and the thermal lability of the amino acid molecules considerably limit the use of methods of measurement of thermochemical parameters based on equilibrium or bimolecular rate constants determinations where the determination of vapor pressure is essential.[3] Owing to this limitation, the "kinetic method", either in its "simple" formulation or in its "extended" form, is generally utilized since it does not involve the measurement of the amino acid partial pressure. The second reason is related to the limitations of the "simple" kinetic method when significant entropy change is associated with the protonation process (as measured by the "protonation entropy" $\Delta_p S(\text{M}) = S°(\text{MH}^+) - S°(\text{M})$). Accordingly, in such situation the "simple" kinetic method provides an apparent proton affinity (PA_{app}) at variance from the true value by deviations which may attain tenths of kilojoules. Amino acids, and particularly those bearing a basic residue on their side chain, are expected to lead to substantial entropy losses during protonation at least because of the possibility of internal chelation of the incoming proton which freezes internal rotations and thus lessens the corresponding entropy term. This has been demonstrated for methionine,[4] aspartic acid,[5] asparagine,[5] glutamine,[5] arginine,[6] lysine,[5,7,8] phenylalanine,[9] tyrosine,[9] histidine,[5,7] and suspected for glutamic acid.[1,5]

SCHEME 1



Glutamic acid (Glu)

Glutamic acid (Glu, $CO_2HCH_2CH_2CH(NH_2)COOH$, Scheme 1) is not only one of the amino acids entering into the composition of the living peptides, but it also plays an important role in the central nervous system since it is a molecule which presents neuroexcitatory properties and acts as a neurotransmitter. Its tasting properties are also extensively used in the agro alimentary industry where its anionic form is known as the E620 additive and is responsible of the "umami tasting".[10] Returning to structural and acido−basic properties, glutamic acid offers also a clear example of a molecule expected to present a change in entropy during protonation and for which the determination of protonation thermochemistry should be cautiously considered.

The difficulties to obtain the gas-phase protonation thermochemical information for glutamic acid are confirmed by a brief survey of the presently published data. The first tentative of determination of the gas-phase basicity (GB) of glutamic acid is due to Locke[11] who used the equilibrium method in an ion cyclotron mass spectrometer and derive the value GB(Glu) = 879 kJ·mol^{-1}. Ten years later, Gorman et al.[12] used the occurrence or nonoccurrence of proton transfer between amine reference bases and laser-desorbed neutral amino acids in a Fourier transform ion cyclotron resonance mass spectrometer. This bracketing technique led the authors to place the gas-phase basicity of glutamic acid between that of trimethylamine and diethylamine. Considering the presently accepted GB values of the two latter bases,[13] this leads to GB(Glu) ∼918−919

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kJ·mol^{-1}. Competitive dissociations of proton-bound clusters GluHB$^+$ were also used to bracket the gas-phase basicity of glutamic acid with respect to other amino acids B (with B = Tyr, Asn, Pro, Trp).[14−16] From these experiments, and considering the tabulated GB values of the amino acids B, it may be concluded that GB(Glu) should be in the range of 885−915 kJ·mol^{-1} (with a probable uncertainty of ±10 kJ·mol^{-1} on the limits of this range). Thus, altogether these previously published data lead to the conclusion that the gas-phase basicity of glutamic acid is situated between 879 and 920 kJ·mol^{-1}. A 40 kJ·mol^{-1} range of uncertainty on a gas-phase basicity determination is clearly unsatisfactory. In his review, Harrison[1] pointed out the large difference between the Locke's and Gorman's results recalled above and considered the former data as suspect, because of a probably incorrect determination of the partial pressure of the neutral amino acid. On the other hand, Gorman et al.[12] noted that the basicity of glutamic acid they determined is anomalously high.

Similar uncertainties arise on the determination of the proton affinity of glutamic acid. The simple kinetic method has been used to obtain "apparent" proton affinity of glutamic acid by Bojesen and Breindahl[17] with reference to several monoamines. Using the presently tabulated proton affinities values of these later molecules, we obtain a corrected PA_{app}(Glu) equal to 938 kJ·mol^{-1}. In 2000, Afonso et al.[18] used also the simple kinetic method to determine the apparent proton affinity of a set of amino acids. The proton affinity order was determined from protonated pairs of amino acids, and five of them were chosen as reference bases. The apparent proton affinity of glutamic acid has been determined to be larger than that of methionine by 7.0 kJ·mol^{-1} and lower than that of tryptophan by 2.1 kJ·mol^{-1}. Using the tabulated PA values of methionine and tryptophan,[13] these experiments lead to PA_{app}(Glu) = 942−947 kJ·mol^{-1}. Thus, apparent proton affinity of glutamic acid is predicted to be situated between 938 and 947 kJ·mol^{-1}. The range of values is consequently less expanded than for the gas-phase basicity, but as recalled above, the significance of the apparent proton affinity, as determined by the simple kinetic method, is highly questionable when an entropy change occurs during protonation, a situation which may be expected for glutamic acid. A tentative determination of the true proton affinity and protonation entropy of glutamic acid has been undertaken 5 years ago by the "extended" kinetic method.[5] Unfortunately, the experimental data accessible at that time were limited to a restricted domain of basicity thus rendering delicate the use of the method. These limitations lead to considerable overestimate of the derived thermochemical quantities and to unacceptably large error limits. For example, a GB(Glu) value of 920.6 kJ·mol^{-1} was obtained with 11.0 kJ·mol^{-1} of standard deviation and ∼24 kJ·mol^{-1} of 95% confidence limit.

Finally, quantum chemical calculations were also used to determine the proton affinity of glutamic acid. From MP2/6-311+G(2d,p)//B3LYP/6-31+G(d,p) calculations, Sun et al.[19] proposed a PA(Glu) value of 933.9 kJ·mol^{-1}, shifted to 938.9 kJ·mol^{-1} if a 298 K Boltzmann distribution of conformers is considered. In 2004, on the basis of B3LYP/6-31G(d) calculations and isodesmic correction, an estimate of PA(Glu) = 949.9 kJ·mol^{-1} was proposed.[5] More recently, Bleiholder et al.[20] used B3LYP/6-31+G(d,p) and G2(MP2) methods and obtained PA(Glu) = 950.9 kJ·mol^{-1} and PA(Glu) = 946.3 kJ·mol^{-1}, respectively. Again, the comparison of the literature data reveals a large panel of values since theory places PA(Glu) in the 934−951 kJ·mol^{-1} range.

Clearly, in view of these observations, a re-examination of the protonation thermochemistry of glutamic acid is of interest. The present study presents new experimental data obtained using the extended version of the kinetic method in order to determine both the proton affinity and the protonation entropy with a more sensitive apparatus than previously used.[5] Quantum chemical computations complete this study by including estimate of the protonation entropy due to the hindrance of internal rotations.

2. Methods

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) experiments were carried out in a Waters Q-TOF Premier mass spectrometer working in the MassLynx 4.1 environment. Cone voltage was set at ∼10 V while capillary voltage was varied between 3.0 and 3.7 kV to optimize the conditions for obtaining maximum intensity of the protonated dimers. Typical values for the other source parameters were sampling cone −90 V, extraction cone −5 V, ion guide −4 V. The pulse velocity in the T-Wave apparatus was 300 m/s, and the source temperature was set to 80 °C. Collision-induced dissociation MS/MS (CID-MS/MS) spectra were obtained using argon as the collision gas at a pressure of 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 acquired spectra were summed for interpretation. Sample solutions were prepared in a 50/50 methanol/water mixture acidified by 0.1% formic acid and dissolved to achieve typically a concentration of 10^{-4} M for both the amino acid and the reference bases. All solutions were infused at a flow rate of 0.1−1.0 μL·min^{-1} with a CIL Cluzeau (Courbevoie, France) syringe. Six reference bases B_i have been used to produce the relevant proton-bound heterodimer $[MHB_i]^+$ (where M represents the molecule of interest, glutamic acid in the present case): *n*-hexylamine, pyridine, *t*-butylamine, pyrrolidine, isopropyl-methylamine, di-*n*-propylamine. 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 CIDs of the mass selected $[MHB_i]^+$ ions were examined by the kinetic method, i.e., the natural logarithm of the fragment ions 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 (i.e., in particular, *m*/*z* 148, 130, 102, and 84 for glutamic acid). This procedure is essentially correct if no further excitation energy is given to the produced fragments 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 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 four typical E_{cm} values of 1, 2, 3, and 4 eV. The data were analyzed by using the ODRPACK program for weighted orthogonal distance regression.[21,22]

Molecular orbital calculations have been conducted using the Gaussian suite of programs.[23] Search for local minima has been first conducted at the HF/6-31+G(d,p) levels of theory by scanning various dihedral angles using the "relaxed rotation" approach, i.e., by optimization of all geometrical parameters except the explored dihedral angle. A 10° step has been used during these explorations of the conformational space of neutral and protonated glutamic acid. Geometries of the most relevant local minima were then optimized at the B3LYP/6-31+G(d,p) levels in order to identify the most stable conformers. Although geometrical parameters are well reproduced at this level of theory, limitations of B3LYP/6-31+G(d,p) computations to evaluate precise conformer energies were documented in recent review.[24] Means to correct for these deficiencies consist to perform single-point energy calculations with enlarged basis set or with different correlated methods. A popular procedure, which may be applied to systems containing a reasonably large number of non-hydrogen atoms (i.e., up to ~25−30), uses a simple extension of the basis set size such as, for example, B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) single-point calculation.[25] The method of choice to obtain accurate thermochemical parameters when the system contains a limited number of heavy atoms (i.e., less than 10−15) is a composite recipe such as the Gn or CBSn procedures.[25] In the present study, the various conformers identified at the B3LYP/6-31+G(d,p) level were explored using the G3MP2B3 method.[26] This procedure is a composite technique that employs a sequence of ab initio molecular orbital calculations to derive an accurate total energy of the considered system. Briefly, the G3MP2B3 method uses B3LYP/6-31G(d)-optimized geometry in a sequence of single-point energies calculations at the QCISD(T)(FC)/6-31G(d) and MP2(FC) levels. The latter calculation uses a very large basis set, the so-called GTMP2Large basis set, which involves multiple valence shells, polarization through f orbitals, and diffuse functions. Higher level correction and 298 K thermal correction, based on B3LYP/6-31G(d) vibrational frequencies, are further included. The zero-point energy and energy correction for a finite temperature are dependent on the precise values of the frequency vibrations. A scaling factor of 0.96 is uniformly applied to account for the slight overestimate of computed B3LYP/6-31G(d) vibrational frequencies with respect to experiment. Since gas-phase basicities will be also discussed, computation of third-law entropies $S°$ is obviously needed to derive Gibbs free energies. In order to account for the vibrational term included in $S°$, the B3LYP/6-31G(d) vibrational frequencies is scaled by a factor 1.013.[27] Finally, bearing in mind the Gaussian's limitations due to the difficulties to estimate low vibrational frequencies and to treat anharmonic effects, special account of the entropy terms associated with hindered rotations has been considered to derive corrected protonation entropy and gas-phase basicity. This point will be discussed in the text.

Unless otherwise indicated, only G3MP2B3 results will be presented here; geometries optimized at the B3LYP/6-31+G(d,p) level, and additional data, in particular comparison of 298 K enthalpies calculated at the 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 G3MP2B3, are gathered in Tables S1−S3 of the Supporting Information.

3. Results and Discussion

3.1. Experimental Protonation Thermochemistry of Glutamic Acid. ***3.1.1. Extended Kinetic Method.*** Proton affinity, PA(M), and gas-phase basicity, GB(M), of a molecule M are defined as the standard enthalpy, $\Delta_1H°$, and standard Gibbs free energy, $\Delta_1G°$, of reaction 1, respectively:

$$\underset{(\text{gas})}{MH^+} \rightarrow \underset{(\text{gas})}{M} + \underset{(\text{gas})}{H^+} \quad (1)$$

These quantities are generally given at the temperature of 298 K. Introducing the entropy of reaction 1, $\Delta_1S°_T$, the thermodynamic relationship $\Delta G°_T = \Delta H°_T - T\Delta S°_T$ obviously leads to eq 2 at 298 K:

$$GB_{298}(M) = PA_{298}(M) - 298\Delta_1S°_{298} \quad (2)$$

If we now define the "protonation entropy" for a species M,[3,13] at temperature T, by the difference

$$\Delta_pS°_T(M) = S°_T(MH^+) - S°_T(M) \quad (3)$$

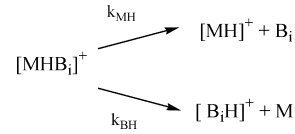
then, the entropy of reaction 1, $\Delta_1S°_{298}$, which is obviously equal to $S°_{298}(M) + S°_{298}(H^+) - S°_{298}(MH^+)$, reduces to

$$\Delta_1S°_{298} = S°_{298}(H^+) - \Delta_pS°_{298}(M) \quad (4)$$

Using these notations, the gas-phase basicity and proton affinities are interrelated following eq 5:

$$GB_{298}(M) = PA_{298}(M) - 298[S°_{298}(H^+) - \Delta_pS°_{298}(M)] \quad (5)$$

The kinetic method[28] considers the competitive dissociations of a series of proton-bound dimers $[MHB_i]^+$, involving the molecule of interest M and a reference base B_i:



The starting point of the method is to assume that the ratio of measured peak intensities $[MH]^+/[B_iH]^+$ is equal to the ratio of rate constants k_{MH}/k_{BH}. Then, using the canonical transition state theory to express k and considering several simplifying assumptions, the natural logarithm of the ratio of peak intensities may be expressed by

$$\begin{aligned} y_i &= \ln([MH]^+/[B_iH]^+) \\ &\sim \ln(k_{MH}/k_{BH})_j \\ &= [G°_T(M) + G°_T(B_iH^+) - G°_T(MH^+) - \\ &\qquad G°_T(B_i)]/RT \\ &= [PA_{298}(M) - PA_{298}(B_i) + T\Delta S°_i + \\ &\qquad \Delta H°_{298\rightarrow T} + T\Delta S°_{298\rightarrow T}]/RT \end{aligned} \quad (6)$$

where T is an "effective temperature" related to the excitation energy of the dissociating $[MHB_i]^+$ species and $\Delta S°_i = \Delta_pS°_{298}(M) - \Delta_pS°_{298}(B_i)$. 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 $MH^+ + B_i$ in one hand and $M + B_iH^+$ in the other, is generally assumed to cancel to zero.[3,13] In this hypothesis, eq 6 reduces to

$$y_i = [PA_{298}(M) - PA_{298}(B_i) + T\Delta S°_i]/RT \quad (7)$$

and thus, for a series of experiments using several bases B_i at a temperature T, y_i versus $PA_{298}(B_i)$ follows a linear relationship characterized by a slope equal to $1/RT$ and an intercept with the PA_{298} scale given by $PA_{app} = PA_{298}(M) + T\langle\Delta S°_i\rangle$ (where $\langle\Delta S°_i\rangle$ is the mean value of the $\Delta S°_i$ terms, i.e., $\Delta_pS°_{298}(M) - \langle\Delta_pS°_{298}(B_i)\rangle$).

The "simple kinetic method" considers that the "apparent" proton affinities PA_{app} may be equated to $PA_{298}(M)$. This is obviously only possible if $\langle\Delta S°_i\rangle$ is equal to zero, i.e., if $\Delta_pS°(M) = \langle\Delta_pS°(B_i)\rangle$. If the $\langle\Delta S°_i\rangle$ term cannot be neglected, eq 7 cannot be simplified, and several experiments at different effective temperatures T are necessary in order to obtain a proton affinity estimate. In the "extended kinetic method", both $PA_{298}(M)$ and $\Delta_pS°(M)$ are determined from several sets of experiments realized under different conditions of activation of the $[MHB_i]^+$ ions and thus corresponding to different effective temperatures T_j (eq 8).

$$y_{ij} = \Delta S°_i/R + [PA_{298}(M) - PA_{298}(B_i)]/RT_j \quad (8)$$

The y_{ij} versus $PA_{298}(B_i)$ points may be fitted by a set of regression lines $(y_{ij})_{calc} = y_0 + b_j(x_0 - x_i)$ intersecting in a common point of coordinate $x_0 = PA_{iso}(M)$ and $y_0 = \Delta S°_{iso}/R$, called the "isothermal"[29,30] or "isoequilibrium"[21] point. A statistical treatment of eq 8, leading to $PA_{iso}(M)$, $\Delta S°_{iso}/R$ and the values of the n_j effective temperatures T_j, has been proposed by Ervin and Armentrout.[21] The method is based on the orthogonal distance regression (ODR) method,[22] a least-squares regression analysis which takes into account simultaneously all the $[n_i, n_j]$ data points. In the present study, the ODR method has been applied to several sets of y_{ij} (eq 8) values obtained at variable collision energies. The coordinates of the isothermal point are thus expected to provide $PA_{298}(M)$ and $\Delta_pS°(M)/R$.

3.1.2. Results. A first tentative of application of the extended kinetic method to the protonation energetic of glutamic acid has been done by us 5 years ago using a triple quadrupole mass spectrometer equipped with an electrospray source.[5] Unfortunately, difficulties to measure accurate peak intensity ratios above 10^2 (or below 10^{-2}) limited the accuracy of the method since the correct location of the isothermal point was not possible within the explored proton affinity domain. Consequently, incorrect estimates of PA(Glu) and Δ_pS(Glu) were obtained and considerable uncertainties resulted. The present investigation does not exhibit comparable deficiencies since the experimental data were obtained using a more sensitive device.

The experimental data obtained here using glutamic acid as unknown base M and a set of six monofunctional references bases B_i are summarized in Table 1. The corresponding plot of y_{ij} (eq 8) versus $PA(B_i)$ is presented in Figure 1. The correlation lines show a correct location of the isothermal point leading to an accurate determination of both PA(Glu) and $\Delta_pS°$(Glu). The ODRFIT procedure[22] allows the assignment of the following values: PA(Glu) = 945.3 ± 2.8(5.8) kJ·mol^{-1} and $\Delta_pS°$(Glu) = −28 ± 4(9) J·mol^{-1}·K^{-1} (uncertainties are standard deviation and, in parentheses, 95% confidence limit). Combining these two terms we deduce a gas-phase basicity value, GB(Glu), equal to 904.4 ± 3.0(6.4) kJ·mol^{-1}. These results are indicated in bold in Table 2 which gathers also previous experimental and theoretical thermochemical data presently available in the literature.

As recalled in the Introduction, estimates of the gas-phase basicity of glutamic acid have been previously deduced from measurements of proton-transfer equilibrium constants using an ion cyclotron resonance mass spectrometry device[11] and various bracketing experiments,[12] leading to a very large range of values situated between 879 and 919 kJ·mol^{-1}. Our experimental determination, 904 kJ·mol^{-1}, appears to fall into the range delimited by these previous estimates. It may be emphasized that the GB(Glu) obtained here is 25 kJ·mol^{-1} above that given in ref 11. It is consequently confirmed that, as suggested by Harrison,[1] the Locke's result[11] is by far underestimated, probably because of an incorrect estimate of the neutral glutamic acid pressure due to an imperfect correction of the gauge reading and(or) thermal decomposition of the sample. This conclusion may be underlined since the GB(Glu) value which has been retained in the Hunter and Lias compilation[13] is precisely Locke's value.[11]

An essential finding, evidenced here by the use of the extended kinetic method, is the occurrence of a significant negative protonation entropy, $\Delta_pS°(Glu) = -28$ J·mol^{-1}·K^{-1}. It should be emphasized that the absolute value of the protonation entropy given by the extended kinetic method is generally an underestimate of the true $\Delta_pS°$ value.[3,31] For example, similar $\Delta_pS°$ values of −20/−30 J·mol^{-1}·K^{-1} were obtained using the extended kinetic method for 1,3-, 1,4-, 1,5-diaminoalkanes and 1,3-, 1,4-aminoalkanols, whereas the values obtained using equilibrium methods (expected to provide the "true" values) are in the −45/−75 J·mol^{-1}·K^{-1} range.[31] Thus, the absolute value of the protonation entropy of glutamic acid is possibly higher than 28 J·mol^{-1}·K^{-1}. It is clear that the assignment of $\Delta_pS°(Glu) = -5$ J·mol^{-1}·K^{-1} used by default in the Hunter and Lias tabulation[13] should be corrected.

A comparison of the proton affinity determined here with the values derived from the use of the simple kinetic method is done in Table 2.[13−15] As recalled in the preceding section (see eq 7 and the related discussion), the simple kinetic method provides an apparent proton affinity given by $PA_{app} = PA_{298} + T\langle\Delta S°_i\rangle$ where T is the effective temperature of the experiment. If B_i are monofunctional reference bases, $\Delta_pS°(B_i)$ are close to zero, and the term $\langle\Delta S°_i\rangle = \Delta_pS°(M) - \langle\Delta_pS°(B_i)\rangle$ is consequently close to $\Delta_pS°(M)$ (for the six reference bases presented in Table 2, $\langle\Delta_pS°(B_i)\rangle = -2$ J·mol^{-1}·K^{-1}). It results that $T\langle\Delta S°_i\rangle$ is a negative term and the apparent proton affinity deduced from the simple kinetic method is an underestimate of the true PA_{298}. This is exactly what is observed in Table 2 when considering the Bojesen and Breindahl[17] result. The PA_{app} value (938.0 kJ·mol^{-1}) is less than the 945.3 kJ·mol^{-1} obtained by the extended kinetic method. We note that, using our data obtained at 1 eV of the center-of-mass collision energy, the apparent proton affinity is equal to 932.0 kJ·mol^{-1}. The difference in PA_{app} value presented in ref 17 and that obtained in the present study originates from the difference in "effective temperature" T. Accordingly, Bojesen and Breindahl[17] follow the metastable dissociations of the proton-bound clusters between glutamic acid and reference bases, whereas we investigated collisionally activated species which correspond to higher T values (at least 510 K, see Table 1). If the reference bases are not monofunctional molecules, $\Delta_pS°(B_i)$ may be different from zero and cannot be neglected beside $\Delta_pS°(M)$. A particular case, however, is when, fortunately, $\Delta_pS°(M) \sim \langle\Delta_pS°(B_i)\rangle$ leading thus to $PA_{app} \sim PA_{298}$. From this point of view it is interesting to recall the result of Afonso et al.[18] who determined PA_{app}(Glu) with reference to methionine and tryptophan. In fact these two amino acids present non-negligible protonation entropies. Values of

TABLE 1: Kinetic Method Data Relevant to the Protonation of Glutamic Acid

reference base B	PA(B) kJ·mol^{-1} [a]	Δ_pS(B) J·K^{-1}·mol^{-1} [a]	GB(B) kJ·mol^{-1} [a]	y(1) [510][b]	y(2) [607][b]	y(3) [730][b]	y(4) [760][b]
n-hexylamine	926.2	−5	892.3	1.2	0.35	0.1	−0.25
pyridine	930.0	2	898.2	0.6	0.25	0.0	−0.2
t-butylamine	934.1	−5	900.2	−0.2	−0.9	−1.40	−1.60
pyrrolidine	948.3	−2	915.3	−4.0	−3.9	−3.8	−3.7
i-propylmethylamine	952.4	−2	919.4	−5.1	−4.9	−4.8	−4.6
di-*n*-propylamine	962.3	−2	929.3	−6.8	−6.2	−5.5	−5.25

[a] From ref 13. [b] $y_i = \ln([MH]^+/[BH]^+)$; in parentheses are indicated the E_{cm} values in eV and in brackets the effective temperatures in K; uncertainty on $y = 0.2$.

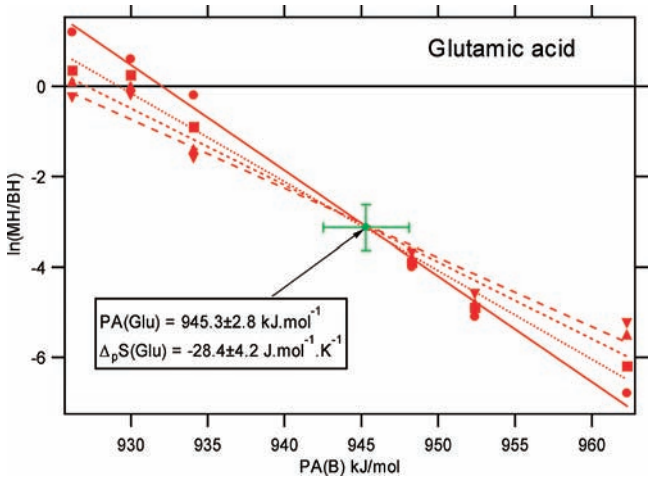


Figure 1. Extended kinetic plot of glutamic acid.

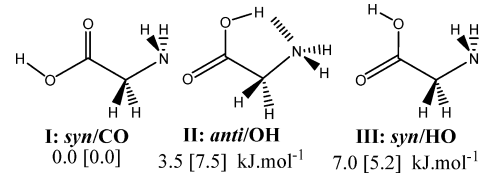
TABLE 2: Summary of the Protonation Thermochemistry of Glutamic Acid (in Bold, This Work)

method	GB(M) kJ·mol^{-1}	PA(M) kJ·mol^{-1}	$\Delta_pS°$(M) J·K^{-1}·mol^{-1}
equilibrium bracketing	878.9[a]		
	918−919[b]		
	885−915[c]		
simple kinetic		938.0[d]	
		942−947[e]	
extended kinetic	*920* ± 11(24)[f]		
	904.4 ± 3.0(6.4)	**945.3 ± 2.8(5.8)**	**−28 ± 4(9)**
theoretical "monoconformer"		949.9[f]	
	899.6[e]	933.9[g]	
		950.9[h]	
		946.3[i]	
	906.4	**948.1**	**−31**
"average"	897.0[g]	938.9[g]	
	906.7	**949.8**	**−36**
evaluated	879.1[j]	913.0[j]	−5[j]
	902[k]	934.7(947.3)[k]	(−42)[k]
	904	**947**	**−35**

[a] Ref 11 as adapted by Hunter and Lias in ref 13. [b] Ref 12. [c] Refs 14−16. [d] Ref 17. [e] Ref 18. [f] Ref 5. [g] Ref 19. [h] Ref 20, B3LYP/6-31+G(d,p). [i] Ref 20, G2(MP2). [j] Ref 13. [k] Ref 1 (in parentheses, PA deduced from GB = 902 and an arbitrarily assumed Δ_pS(Glu) of −42 J·K^{-1}·mol^{-1}).

$\Delta_pS°$(methionine) = −20 J·mol^{-1}·K^{-1} and $\Delta_pS°$(tryptophan) = −25 J·mol^{-1}·K^{-1} have been determined using the extended kinetic method.[4,10] It is noteworthy that these $\Delta_pS°$ values are very close to that obtained here for glutamic acid. Consequently, the apparent proton affinity determined for glutamic acid with reference to methionine and tryptophan should fall close to the true PA_{298} value because of the cancellation of the entropic terms. Indeed, the range of PA_{app} values deduced from the Afonso et al.[18] results, 942−947 kJ·mol^{-1}, is in agreement with the proton affinity of 945.3 kJ·mol^{-1} obtained by the extended kinetic method.

SCHEME 2

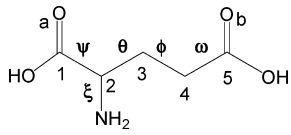


3.2. Computed Protonation Thermochemistry of Glutamic Acid. ***3.2.1. Conformations of Neutral Glutamic Acid.*** More than 10^3 possible conformations, resulting from rotations around the seven σ bonds, may be a priori envisaged for glutamic acid. Simplifying considerations may, however, be taken into account to limit the domain of investigation. Concerning the amino acid part, important information may be deduced from examination of the glycine case. Extensive theoretical examination of neutral glycine has revealed a number of stable conformers arising from internal rotations.[32,33] From a structural point of view, conformers possessing a *syn*-HOCO arrangement present an intrinsic electrostatic attraction between the acidic hydrogen and the basic carbonyl oxygen and are expected to be more stable than their anti counterpart (a difference in energy of ca. 20 kJ·mol^{-1} is associated with the syn/anti pair of conformers in acetic acid).

The most stable conformer of glycine, **I** (Scheme 2), presents such a *syn*-HOCO arrangement and a bifurcated $NH_2\cdots O{=}C$ hydrogen-bonding type interaction. A close rotamer, which is distinguishable by the existence of only one hydrogen bond between one amino hydrogen and the oxygen of the carbonyl group, is 5.0 kJ·mol^{-1} higher in a 298 K enthalpy scale (G3 calculations[33]). The second glycine conformer, **II**, is an exception of the aforementioned rule since it presents an *anti*-HOCO arrangement. However, this destabilizing situation is efficiently counterbalanced by the existence of a strong $OH\cdots NH_2$ hydrogen bond.[34] As a consequence, **II** is almost as stable as **I** since it is destabilized by only 3.5 kJ·mol^{-1} ($\Delta H°_{298}$ calculated at the G3 level[33]). Conformer **III** is structurally comparable to **I** in that sense that it is also characterized by a *syn*-HOCO arrangement and a bifurcated hydrogen bonding. However, **III** involves as H-bond acceptor the hydroxylic oxygen rather than the carbonyl one (Scheme 2) and is predicted to be situated 7.1 kJ·mol^{-1} above **I** on the 298 K enthalpy scale (G3 calculations[33]). It may be noted that the order of decreasing stability, **I** > **II** > **III**, given by the 298 K enthalpies is changed to **I** > **III** > **II** when the Gibbs free energies $G°_{298}$ are considered (values in brackets in Scheme 1). This is due to the fact that **II** is entropically disfavored because of the strong intramolecular hydrogen bond $OH\cdots NH_2$ which froze the C−C and C−N rotations.[33−35]

In line with these observations, glutamic acid conformers will be classified as type **I**, **II**, or **III** depending on the arrangement of the amino acid part of the molecule as described in Scheme 2. A second important structural characteristic is the syn or anti conformation of the second acidic function. It will be denoted

SCHEME 3



s or ***a*** after the relevant conformational type, e.g., **I*s*** or **I*a***. Additional information, in particular the dihedral angles θ, ϕ, ω (Scheme 3) are given as [θ, ϕ, ω] in Figures 2 and 3.

Fourteen local minima were identified on the conformational energy surface of glutamic acid at the G3MP2B3 level in an ~10 kJ·mol^{-1} enthalpy range. The geometries of these conformers are presented in Figure 2 while Table 3 gathers their G3MP2B3 enthalpies and Gibbs free energies at 298 K.

Seven type **I** (*syn*-CO) conformers with a syn arrangement of the COOH of the acidic function of the side chain were identified. They are denoted **I*s*A**−**I*s*G** by order of decreasing stabilities (Figure 2, Table 3). These structures present clearly one hydrogen-bond type NH···O$_a$C interaction, the interatomic distance being in the range of 2.50−2.65 Å. No interaction seems to occur between the amino acid part and the second acidic function for the five most stable conformers **I*s*A**−**I*s*E**. The two low-lying structures **I*s*A** and **I*s*B** are very close in energy (3.0 kJ·mol^{-1}). They differ only by the C2C3−C4C5 (ϕ) dihedral angle (~60 and 180° for **I*s*A** and **I*s*B**, respectively). The five other conformers, **I*s*C**−**I*s*G**, are situated 6−11 kJ·mol^{-1} above **I*s*A**. It may be noted that conformers **I*s*A** and **I*s*D** were previously unidentified and that **I*s*B**, **I*s*C**, **I*s*E**, **I*s*F**, and **I*s*G** correspond to the structures N6, N5, N7, N8, and N10, respectively, located by Sun et al.[19] As underlined above, the anti conformation of a COOH function is less stable by ca. 20 kJ·mol^{-1}. However, if this atom arrangement allows the formation of a hydrogen bond the corresponding conformation may be significantly stabilized. This phenomenon clearly occurs for conformers **I*a*A** and **I*a*B** where the COOH of the side chain is indeed in its anti conformation. In both cases the H$_2$N···HOCO$_b$ distance is equal to 1.73 Å, i.e., shorter than the O$_a$COH···NH$_2$ distance in structures of type **II** (Scheme 2). This additional stabilization explains why the relative enthalpies of **I*a*A** and **I*a*B** are only ~7 kJ·mol^{-1} with respect to **I*s*A** and **I*s*B**.

Turning now to the type **II** (*anti*-OH) conformers, four of them are situated in the ~10 kJ·mol^{-1} enthalpy range. All are belonging to the **II*s*** type, i.e., the COOH of the side chain is in its syn conformation. The two most stable, **II*s*A** and **II*s*B**, are stabilized by two simultaneous, cooperative, intramolecular hydrogen bonds: the specific interaction O$_a$COH···NH$_2$ (distance 1.85 Å) of the type II conformers, and a new interaction involving the side-chain acidic function NH···O$_b$COH (distance 2.05 Å). The enthalpies of these conformers are very close to that of conformers **I*s*A** and **I*s*B** depicted above. Note that structures **II*s*A** and **II*s*B** were identified as N1 and N2 by Sun et al.[19] Conformational exploration around **II*s*A** and **II*s*B** reveals the existence of several other conformers, two of them, namely, **II*s*C** and **II*s*D**, precedingly unidentified, are situated ~10 kJ·mol^{-1} above **II*s*A**. Their main characteristic is that they found their stability exclusively in the O$_a$COH···NH$_2$ intramolecular hydrogen bonding (distance 1.90 Å) since the side chain is elongated thus preventing the occurrence of a favorable interaction of the side-chain acid function with the amino acid moiety. It may be noted that conformers of type **II*a*** identified in the present work were located more than 20 kJ·mol^{-1} above **I*s*A** and **I*s*B** and were consequently not further considered.

Finally, only one conformation pertaining to the type **III** was found close to 10 kJ·mol^{-1} of relative 298 K enthalpy. This structure, **III*s*A**, may be seen as a 180° C3C2−C1O$_a$ (dihedral angle Ψ) rotamer of **I*s*A** and **I*s*B**. The enthalpy difference between **III*s*A** and **I*s*A**, ~11 kJ·mol^{-1}, is indeed close to the difference in 298 K enthalpy between **I** and **III** (Scheme 3). For information, the most stable conformer of type **III*a*** is situated 15 kJ·mol^{-1} above **I*s*A** (**III*a*A**, Table 3).

The $\Delta H°_{298}$ provided by the G3MP2B3 calculation lead to the following order of decreasing stabilities of the first conformers of glutamic acid: **I*s*A**, **II*s*A**, **I*s*B**, **II*s*B**, **I*s*C**, **I*a*A**, **I*s*D**, **I*a*B**, **I*s*E**, **II*s*C**, **I*s*F**, **I*s*G**, **II*s*D**, and **III*s*A**. The first four conformers are situated in the ~3 kJ·mol^{-1}, whereas the remaining are lying 6−11 kJ·mol^{-1} above. This order is significantly changed when considering the computed $\Delta G°_{298}$. As evident from examination of Table 3, the crude results obtained using the G3MP2B3 estimates of the 298 K Gibbs free energies lead to the order of decreasing stabilities: **I*s*A**, **I*s*B**, **I*s*D**, **I*s*E**, **II*s*A**, **II*s*B**, **I*s*C**, **III*s*A**, **II*s*C**, **II*s*D**, **I*a*A**, **I*a*B** (the four latter being above 10 kJ·mol^{-1}). In fact, considering the entropies calculated at the G3MP2B3 level (Table 3), the following observations can be made:

- Low third-law entropies ($S°$ ~ 430 J·mol^{-1}·K^{-1}) are calculated for **II*s*A**, **II*s*B**, **I*a*A**, and **I*a*B**. It clearly corresponds to conformers supporting strong intramolecular hydrogen bonds since for the four conformers two hydrogen-bonding types are operating.
- High third-law entropies ($S°$ ~ 445 J·mol^{-1}·K^{-1}) are obtained for **I*s*A**−**I*s*D**, **II*s*D**, and **III*s*A**. A common feature of these six conformers is the lack of interaction between the side-chain COOH and the amino acid part thus allowing facile internal rotations inside the side chain.

As a consequence the latter conformers are entropically promoted, and in particular, conformers **I*s*A** and **I*s*B** become significantly more stable in a Gibbs free energy scale than conformers **II*s*A** and **II*s*B**. It may thus be concluded that **I*s*A** and **I*s*B** are predominantly populated at room temperature at the detriment of **II*s*A** and **II*s*B**. This point may be quantified by assuming a Boltzmann distribution of N conformers in thermal equilibrium at temperature T. Accordingly, under this hypothesis, the individual populations x_i of each conformer are given by

$$x_i = \exp(-G_i/RT)/\sum_1^N \exp(-G_i/RT) \qquad (9)$$

with G_i representing the individual Gibbs free energies. The x_i values obtained using the 298 K Gibbs free energies provided by the G3MP2B3 are listed in the last column of Table 3. It appears that the mixture of neutral glutamic acid conformers present in thermal equilibrium at 298 K contains essentially two major components: **I*s*A**, 50%, and **I*s*B**, 28%. Each remaining noticeable conformer (i.e., **II*s*A**, **II*s*B**, **I*s*C**, **I*s*D**, **I*s*E**) represents only 3−5% of individual population.

It should be noted that two recent papers dealing with the gas-phase acidity of glutamic acid considered **II*s*A**[36] and **I*s*B**[37] as the lowest energy structure rather than the presently identified conformer **I*s*A**; slight change in the corresponding theoretical value would consequently result.

3.2.2. Conformations of Protonated Glutamic Acid. Protonation of glycine unambiguously occurs preferentially on the nitrogen atom, the most stable conformation is clearly of type **I** (*syn*-CO) since the conformers of type **II** and **III** are situated more than 20 kJ·mol^{-1} above (Scheme 4).[33] Protonation on the

TABLE 3: Enthalpies and Free Energies of Neutral Conformers of Glutamic Acid Calculated at the G3MP2B3 Level[a]

	H_0	ΔH_0	H_{298}	ΔH_{298}	S_{298}	G_{298}	ΔG_{298}	x_i (%)
Is*A*	−550.918038	0.0	−550.906049	0.0	444.5	−550.956498	0.0	50.38
Is*B*	−550.917038	2.6	−550.904925	3.0	449.5	−550.955944	1.5	28.16
Is*C*	−550.915756	6.0	−550.903855	5.8	438.8	−550.953661	7.4	2.56
Is*D*	−550.915375	7.0	−550.903286	7.3	449.2	−550.954267	5.9	4.84
Is*E*	−550.914841	8.4	−550.902701	8.8	452.9	−550.954101	6.3	4.06
Is*F*	−550.913942	10.8	−550.902106	10.4	437.6	−550.951770	12.4	0.35
Is*G*	−550.913610	11.6	−550.901823	11.1	439.8	−550.951745	12.5	0.34
I*a*A	−550.914835	8.4	−550.903413	6.9	426.7	−550.951847	12.2	0.38
I*a*B	−550.914527	9.2	−550.90317	7.6	428.0	−550.951749	12.5	0.34
II*s*A	−550.91683	3.2	−550.90554	1.3	425.4	−550.953824	7.0	3.04
II*s*B	−550.916200	4.8	−550.904736	3.4	432.4	−550.953815	7.0	3.01
II*s*C	−550.914317	9.8	−550.902631	9.0	438.8	−550.952433	10.7	0.71
II*s*D	−550.91364	11.6	−550.901833	11.1	443.6	−550.952177	11.3	0.54
III*s*A	−550.913874	10.9	−550.901814	11.1	450.8	−550.952981	9.2	1.25
III*a*A	−550.911708	16.6	−550.900241	15.2	434.3	−550.949532	18.3	0.03

[a] Values in hartree (1 hartree = 2625.5 kJ·mol^{-1}) and, for the ΔX values, in kJ·mol^{-1}.

carbonyl oxygen leads to structures situated more than 100 kJ·mol^{-1} above **IH**. The most stable of them presents a anti/syn arrangement of the $C(OH)_2$ moiety, whereas the syn/syn conformer is situated 25 kJ·mol^{-1} above.

As expected from the above-mentioned glycine case, the most stable protonated forms of glutamic acid are of type **I** and bring a syn conformation of the side-chain acid function (Figure 3, Table 4). Conformers **IH*s*A** and **IH*s*B** are characterized by two concurrent hydrogen bondings between the protonated amino group and the two carbonyls. It is noteworthy that the stronger interaction occurs with the acidic group of the side chain. The $NH^+\cdots O_bCOH$ distance (1.60 Å) is indeed shorter than its $NH^+\cdots O_aCOH$ homologue (2.05 and 2.14 Å for **IH*s*A** and **IH*s*B**, respectively). A comparable situation arises for the two *syn*-HO conformers **IIIH*s*A** and **III*s*B** where the $NH^+\cdots O_bCOH$ distance is still close to 1.60 Å, whereas the $NH^+\cdots O_aCOH$ distances are equal to 2.14 and 2.46 Å, respectively. The energy gap separating the two sets of conformers **IH*s*A/IH*s*B** and **IIIH*s*A/III*s*B** is equal to ca. 13 kJ·mol^{-1}, a difference lower than that observed between **IH** and **IIH** in the glycine case (22 kJ·mol^{-1}, Scheme 4).

The enthalpy order of the four conformers **IH*s*A**, **IH*s*B**, **IIIH*s*A**, and **III*s*B** is not altered when passing to Gibbs free energy. In fact, practically identical third-law entropies of ∼425 J·mol^{-1}·K^{-1} are calculated for these four species in agreement with comparable intramolecular interactions, in particular the strong hydrogen-bonding interaction $NH^+\cdots O_bCOH$. According to G3MP2B3 calculations, through the use of eq 9, nearly identical amounts of conformers **IH*s*A** (53%) and **IH*s*B** (46%) are expected to be present in the mixture of conformers of protonated glutamic acid in thermal equilibrium at 298 K.

3.2.3. Correction of Entropies for Hindered Rotation and Mixing. The Gaussian codes[23] use the harmonic oscillator approximation to calculate the vibrational contributions to thermodynamic parameters. On the other hand, it is well-known that the vibrational contribution to entropy is particularly sensitive to low frequencies, i.e., to frequencies associated with degrees of freedom presenting large amplitude or rotational motions, where the classical harmonic approximation does not hold.[3] In particular, the harmonic oscillator approximation may be not adapted to the computation of entropy on species containing hindered rotations. A means to more correctly estimate the entropy in such situation is to treat separately each internal rotation by using a hindered rotor model such as that developed by Pitzer and Gwinn.[38] This approach has been successfully applied to monofunctional molecules containing one, two, or three internal rotations[39] and to the protonation of several bifunctional bases.[40] Briefly, this procedure involves calculation of the rotational energy barrier, V_0, appearing in the variation of the potential energy with the dihedral angle ϕ, $V_0(\phi) = (V_0/2)(1 - \cos n\phi)$, where n is the symmetry of the rotation. Rotational energy levels are obtained by solving the corresponding Schrödinger equation. Then, the hindered rotor partition function is calculated, and the corresponding thermochemical functions are deduced from the usual statistical thermodynamic relationships.[3] In the present study, the contributions to entropy, $S°_{hind}$, have been calculated for each individual rotation Ψ, θ, ϕ, ω, and ξ (see Scheme 3 for the symbol conventions) in neutral and protonated glutamic acid. The V_0 values were assigned to the difference in total energy, obtained at the HF/6-31+G(d,p) level, between the global minima **Is*A*** or **IH*s*A** and the maximum of the relaxed rotational scan for each dihedral angle Ψ, θ, ϕ, ω, and ξ. The results are quoted in Table 5. The uncertainty on the computed $S°_{hind}$ values is essentially related to the uncertainty on the V_0 barriers. Assuming a relative error of 25% on the HF/6-31+G(d,p) rotational barrier estimates, an error of ∼1.0 J·mol^{-1}·K^{-1} per hindered rotation results in the V_0 range explored here. The expected error on the computed contribution to entropy of the hindered rotation, $S°_{hind}$, reported in Table 5 is thus probably ∼5 J·mol^{-1}·K^{-1}.

In order to fully estimate the entropy of neutral and protonated glutamic acid, the second point to consider is the entropy of mixing since several conformers are expected to be populated at 298 K. For a mixture of N distinguishable conformers, the entropy of mixing is given by the expression:

$$\Delta S°_{mix} = -R\sum_{1}^{N} x_i \ln x_i \qquad (10)$$

where x_i is the molar fractions of each component of the mixture (see eq 9). Using the x_i values presented in Tables 3 and 4, eqs 10 gives $\Delta S°_{mix}$ terms equal to 12.2 and 6.1 J·mol^{-1}·K^{-1} for neutral and protonated glutamic acid, respectively.

3.2.4. Theoretical Protonation Thermochemistry. Most of the time, the thermochemical parameters associated with the concept of gas-phase basicity, and defined from reaction 1, are computed by considering only the most stable conformer for both the neutral and the protonated molecule. This "monoconformer" procedure may be used here assuming that protonated and neutral glutamic acid are exclusively the pure structures **IH*s**a*** and **Is*A*I**, i.e., the most stable species in term of enthalpy or Gibbs free energy at the G3MP2B3 level. Proton affinity

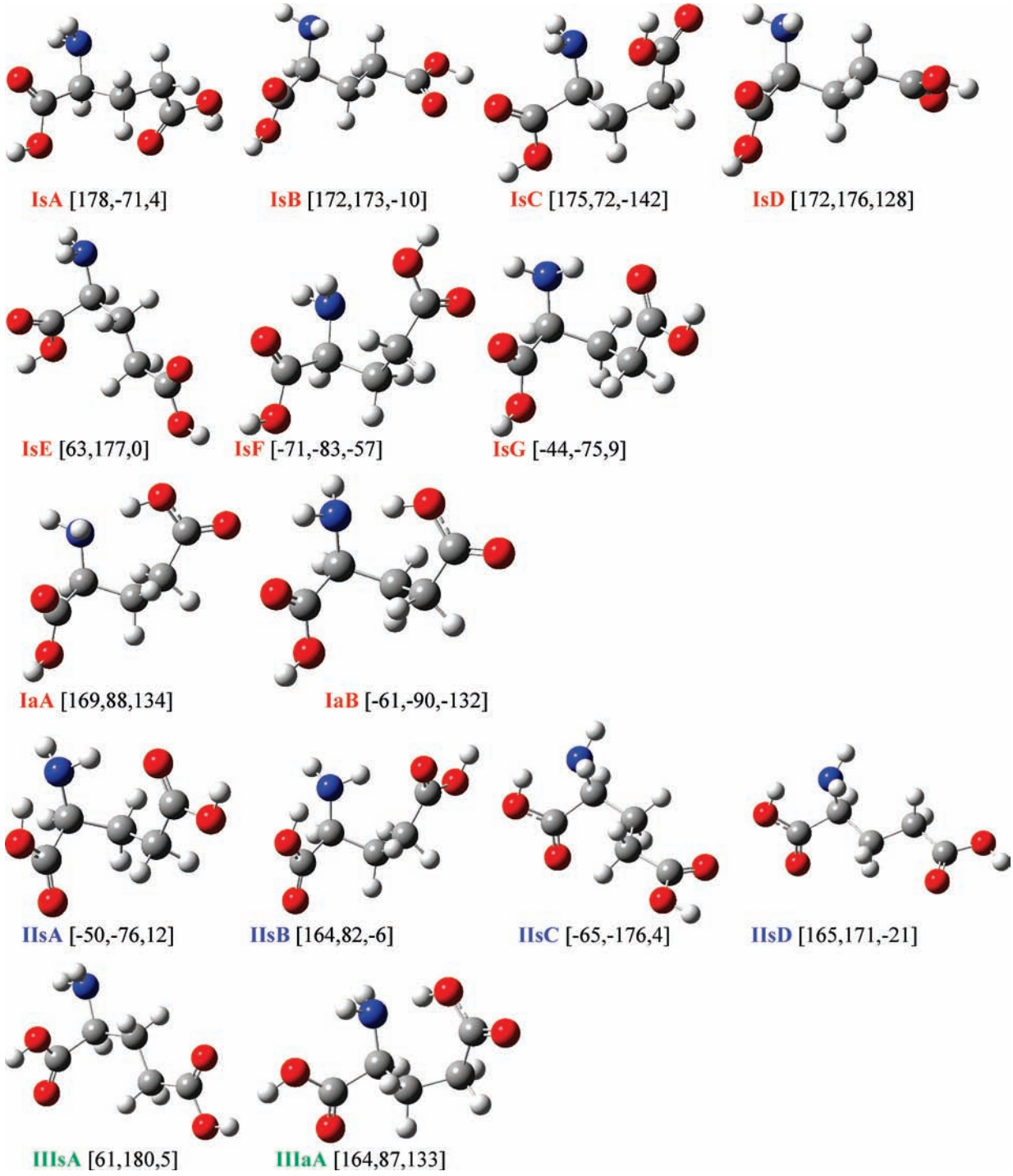


Figure 2. B3LYP/6-31+G(d,p)-optimized geometries of the most stable conformers of neutral glutamic acid (dihedral angles θ, ϕ, ω in deg, see Scheme 3, are indicated in brackets).

computed in this manner is equal to $PA_{mono}(Glu) = 948.1$ kJ·mol^{-1} (Table 2). In their estimation, Bleiholder et al.[20] proposed a 0 K proton affinity of glutamic acid equal to 944.7 or 940.1 kJ·mol^{-1} based on B3LYP/6-31+G(d,p) or G2MP2 calculations, respectively. A 298 K estimate based on the $\Delta H°_{0\rightarrow 298K}$ correction presented in Tables 3 and 4 leads to PA_{298} values of 950.9 or 946.3 kJ·mol^{-1}, in excellent agreement with our G3MP2B3 calculation. It is also noteworthy that our 2004 estimate, based on B3LYP/6-31G(d) calculations and isodesmic correction anchoring the computation to the experimental proton affinity of glycine, PA(Glycine) = 886.5 kJ·mol^{-1}, falls also in the same range of values (PA(Glu) = 949.9 kJ·mol^{-1} [5]). It may be noted that, at the B3LYP/6-31G(d) level, **IIsA** is predicted to be the most stable conformer of glutamic acid; this structure has been consequently considered in this study. The same remark applies to the investigation by Sun et al.;[19] however, the proton affinity value they proposed (933.9 kJ·mol^{-1}) from MP2/6-311+G(2d,p)//B3LYP/6-31+G(d,p) calculations shows a deviation of ca. 15 kJ·mol^{-1} with the preceding mentioned estimates. The reason of this discrepancy is not clear, even if we consider that conformer **IIsA** (N1 in their nomenclature), rather than **IsA**, is the most stable at this level of theory. Indeed the difference in G3MP2B3 enthalpy between these two conformers (Table 3) is equal to only 1.3 kJ·mol^{-1} and cannot consequently account for the difference observed.

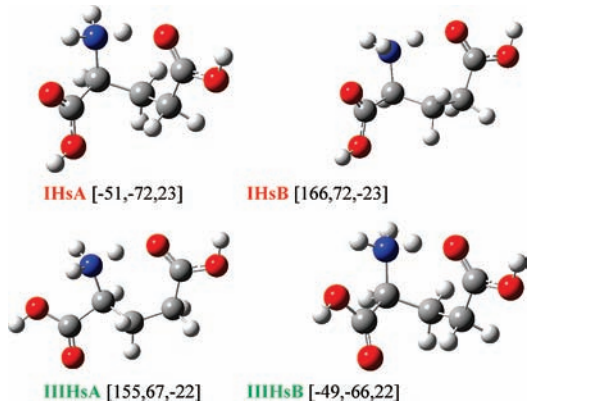
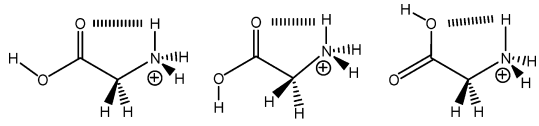


Figure 3. B3LYP/6-31+G(d,p)-optimized geometries of the most stable conformers of protonated glutamic acid (dihedral angles θ, ϕ, ω in deg, see Scheme 3, are indicated in brackets).

SCHEME 4



The protonation entropy of glutamic acid calculated by considering the entropies of conformers **IH*sa*** and **IsA** corrected for the hindered rotation terms (Table 5) is equal to $\Delta_p S°_{mono}(Glu) = -31.3$ J·mol^{-1}·K^{-1} (Table 2). It may be noted that using the crude entropy values provided by Gaussian at the B3LYP/6-31G(d) level (G3MP2B3 calculations, Tables 3 and 4), the theoretical $\Delta_p S°_{mono}(Glu)$ becomes -21.5 J·mol^{-1}·K^{-1}; this figure is consequently slightly lower than the corrected value but still points to a significant entropy loss during protonation of glutamic acid. This observation comes obviously from the fact that the most stable conformers of protonated glutamic acid, **IHsA** and **IHsB**, exhibit significant stabilizing interaction between the carbonyl oxygen of the acid function of the lateral chain (CO_bOH, Scheme 3) and one hydrogen brought by the protonated amino group, whereas the situation is exactly opposite for the most stables neutral forms **IsA** and **IsB** where the lateral chain is fully extended and does not allow any favorable interaction with the amino group. The internal hydrogen bond $CO_b\cdots HN^+$ present in **IHsA** and **IHsB** considerably hinders all the related internal rotations and consequently lowers the corresponding vibrational entropy.

Finally, a "monoconformer" gas-phase basicity value, corresponding to the **IH*sa*/IsA** system, of $GB_{mono}(Glu) = 906.4$ kJ·mol^{-1} is deduced from the above-mentioned proton affinity and protonation entropy values (Table 2).

Ideally, thermochemical quantities relevant to reaction 1 should correspond to molar species in thermal equilibrium at a given temperature *T*. Computation of these quantities is possible assuming a Boltzmann distribution of the MH^+ and M populations of conformers at this temperature.[3] In order to calculate an averaged proton affinity over *N* conformers of molar fractions x_i, the summed molar enthalpy, given by 11

$$\langle H°_T\rangle = \sum_1^N x_i (H°_T)_i \qquad (11)$$

has to be considered. Using the data summarized in Tables 3 and 4 for neutral and protonated glutamic acid, a 298 K averaged proton affinity $\langle PA(Glu)\rangle$ equal to 949.8 kJ·mol^{-1} is computed (Table 2).

Following this reasoning, the populations of neutral and protonated conformers may be also considered to estimate the averaged entropy terms via eq 12:

$$\langle S°_T\rangle = \sum_1^N x_i (S°_T)_i - R\sum_1^N x_i \ln x_i \qquad (12)$$

where the second component corresponds to the entropy of mixing (see eq 10). Using (i) the relative entropies given in Tables 3 and 4 and anchoring these data to the corrected entropies of **IsA** and **IH*s*A** given in Table 5 and (ii) including the entropies of mixing, eq 12 leads to averaged entropies $\langle S°_{298}(Glu)\rangle = 499.0$ J·mol^{-1}·K^{-1} and $\langle S°_{298}(GluH^+)\rangle = 463.2$ J·mol^{-1}·K^{-1}. A theoretical averaged protonation entropy of $\langle \Delta_p S°(Glu)\rangle = -35.8$ J·mol^{-1}·K^{-1} is consequently predicted. It may be observed that $\langle \Delta_p S°(Glu)\rangle$ can be decomposed into two terms corresponding to the two components of eq 12: one due to the difference in averaged molar entropies (-29.7 J·mol^{-1}·K^{-1}) and the second due to the difference in entropy of mixing (-6.1 J·mol^{-1}·K^{-1}). It is evident that the first contribution is dominant in the present case. The first reason, the increased hindrance of internal rotation in the protonated forms, has been commented in the preceding paragraph. The second reason is that a similar restricted number of conformers is sufficient to represent the population of both the neutral and the protonated glutamic acid, the difference of entropy of mixing is consequently limited. Combining the averaged proton affinity and protonation entropy we deduce an averaged theoretical gas-phase basicity at 298 K $\langle GB(Glu)\rangle$ of 906.7 kJ·mol^{-1} (Table 2).

A brief comparison between thermochemical parameters calculated by considering either the most stable conformers or the 298 K mixture of conformers reveals only slight differences. This is related to the fact that neutral and protonated glutamic acid are essentially represented by two conformers at 298 K; moreover, each couple of conformers presents similar thermochemical properties. Differences in proton affinities and entropy of mixing are consequently limited.

Comparison of these theoretical estimates with experiment shows generally good agreement. Proton affinity obtained using the extended kinetic method (945.3 kJ·mol^{-1}, Table 2) is very close to the theoretical value calculated by considering the equilibrium populations of neutral and protonated conformers of glutamic acid at 298 K, $\langle PA(Glu)\rangle = 949.8$ kJ·mol^{-1} or the monoconformer approximation $PA_{mono}(Glu) = 948.1$ kJ·mol^{-1}. The slight deviation observed (experimental PA(Glu) is ~4 kJ·mol^{-1} lower than theory) and is within the cumulated errors. It clearly appears that the theoretical averaged protonation entropy, $\langle \Delta_p S°(Glu)\rangle = -36$ J·mol^{-1}·K^{-1}, or its monoconformer approximation, $\Delta_p S°(Glu)_{mono} = -31$ J·mol^{-1}·K^{-1}, is in good agreement with the experimental value (-28 J·mol^{-1}·K^{-1}, Table 2). This observation should, however, be pondered by the fact that (i) the experimentally determined $\Delta_p S°(Glu)$ value probably represents only a lower limit of the true protonation entropy and that (ii) the uncertainty on the averaged $\langle \Delta_p S°(Glu)\rangle$ or monoconformer $\Delta_p S°(Glu)_{mono}$ estimates are unknown but may probably attain 5−10 J·mol^{-1}·K^{-1}. Finally, averaged and monoconformer gas-phase basicities, $\langle GB(Glu)\rangle = 906.7$ kJ·mol^{-1}, and $GB_{mono}(Glu) = 906.4$

TABLE 4: Enthalpies and Free Energies of Neutral Conformers of Protonated Glutamic Acid Calculated at the G3MP2B3 Level[a]

	H_0	ΔH_0	H_{298}	ΔH_{298}	S_{298}	G_{298}	ΔG_{298}	x_i (%)
IH*s*A	−551.276078	0.0	−551.264764	0.0	422.9	−551.312769	0.0	53.3
IH*s*B	−551.275815	0.7	−551.264390	1.0	425.0	−551.312627	0.4	46.0
IIIH*s*A	−551.271460	12.1	−551.260117	12.2	423.3	−551.308168	12.1	0.4
IIIH*s*B	−551.270659	14.2	−551.259167	14.7	428.8	−551.307841	12.9	0.3

[a] Values in hartree (1 hartree = 2625.5 kJ·mol^{-1}) and, for the ΔX values, in kJ·mol^{-1}.

TABLE 5: Summary of Entropy Calculation for the Most Stable Conformers of Neutral and Protonated Glutamic Acid

		hindered rotations[b]			
species	$S°_{transl}$ $S°_{rot}$ $S°_{vib}$[a]	dihedral angle (n)	V_0	$S°_{hind}$ (Pitzer)	$S°_{total}$[c]
	171.0	Ψ(1)	12	28.5	
	125.9	θ(1)	27	30.8	
I*s*A	56.1	ϕ(1)	23	29.1	487.4
		ω(1)	8	31.5	
		ξ(1)	16	14.9	
	171.1	Ψ(1)	37	21.8	
IH*s*A	125.1	θ(1)	66	26.2	456.1
	54.7	ϕ(1)	60	25.5	
		ω(1)	52	21.6	
		ξ(3)	10	10.2	

[a] Translational, rotational and vibrational contributions to entropies calculated at the B3LYP/6-31G(d) level without scaling. Hindered rotations are not included in the $S°_{vib}$ term. [b] Dihedral angles are defined starting at the carbonyl carbon of the amino acid moiety (Scheme 3). Potential energy barrier V_0 (in kJ·mol^{-1}) of the internal rotation calculated at the HF/6-3+G(d,p) level. Contribution to the entropy of the torsional modes $S°_{hind}$ is calculated using the Pitzer's procedure (see text). In parentheses, n is the symmetry of the corresponding rotation. [c] Total calculated entropy (J·K^{-1}·mol^{-1}) of the species considered.

kJ·mol^{-1}, demonstrate an excellent agreement with the experimental value of 904.4 kJ·mol^{-1} obtained by the extended kinetic method (Table 2).

4. Concluding Remarks

The present study provides new determinations of the thermochemical parameters associated with the gas-phase protonation of glutamic acid. The experimental method used, namely, the "extended kinetic method", allows the measurement of proton affinity, PA(Glu) = 945.3 ± 2.8 kJ·mol^{-1}, and protonation entropy, $\Delta_p S°(Glu) = S°(GluH^+) - S°(Glu) = -28 \pm 4$ J·mol^{-1}·K^{-1}, of this important amino acid. The occurrence of a significant and negative protonation entropy is evidenced here for the first time.

Quantum chemical investigation of a large set of conformers has been conducted up to the G3MP2B3 level. Proton affinities appear to be correctly reproduced using G3MP2B3 computation either by considering only the most stable conformers **I*s*A** and **IH*s*A** of neutral and protonated glutamic acid (948.1 kJ·mol^{-1}) or a 298 K equilibrium mixture of conformers (949.8 kJ·mol^{-1}). Calculation of third-law entropies has been done for a subset of conformers by including explicit treatment of hindered rotations. Using these data, and considering the entropy of mixing, protonation entropy of −36 J·mol^{-1}·K^{-1} was computed. This entropy loss originates mainly from the formation of a strong interaction between the NH_3^+ group and the carbonyl oxygen of the acid function of the side chain in the conformers **IH*s*A** and **IH*s*B**, whereas no particular interaction seems to exist in the neutral conformers **I*s*A** and **I*s*B**.

It emerges from these experimental and computational data that new evaluated thermochemical parameters should be proposed: GB(Glu) = 904 kJ·mol^{-1}, PA(Glu) = 947 kJ·mol^{-1}, $\Delta_p S°(Glu) = -35$ J·mol^{-1}·K^{-1} (with probable uncertainties of ± 2 kJ·mol^{-1} for the energetic quantities and ±10 J·mol^{-1}·K^{-1} for the protonation entropy). These new values compare favorably with the critical estimates given by Harrison.[1] It may be underlined that they are significantly at variance from the Hunter and Lias evaluation[13] because the latter authors used erroneous gas-phase basicity and protonation entropy. Accordingly, (i) the GB(Glu) value of 879.1 kJ·mol^{-1} given by Locke[11] and used in ref 13 appears to be significantly underestimated and (ii) the $\Delta_p S°(Glu)$ value assumed to be equal to −5 J·mol^{-1}·K^{-1} in ref 13 (i.e., a value comparable to that of a primary amine) is clearly underestimated by comparison with the experimental and computational values discussed in the present work.

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Supporting Information Available: B3LYP/6-31+G(d,p)-optimized structures and total energies calculated at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) and B3LYP/6-31+G(3df,2p)//B3LYP/6-31+G(d,p) levels on neutral and protonated glutamic acid (Tables S1−S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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