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Gas-phase acidities of aspartic acid, glutamic acid, and their amino acid amides

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Dedicated to Dr. Jean Futrell for his outstanding contributions to gas-phase ion chemistry and mass spectrometry.

Abstract

Gas-phase acidities (GA or ΔG_{acid}) for the two most acidic common amino acids, aspartic acid and glutamic acid, have been determined for the first time. Because of the amide linkage's importance in peptides and as an aid in studying side chain versus main chain deprotonation, aspartic acid amide and glutamic acid amide were also studied. Experimental GA values were measured by proton transfer reactions in an electrospray ionization/Fourier transform ion cyclotron resonance mass spectrometer. Calculated GAs were obtained by density functional and molecular orbital theory approaches. The best agreement with experiment was found at the G3MP2 level; the MP2/CBS and B3LYP/aug-cc-pVDZ results are 3–4 kcal/mol more acidic than the G3MP2 results. Experiment shows that aspartic acid is more acidic than glutamic acid by ca. 3 kcal/mol whereas the G3MP2 results show a smaller acidity difference of 0.2 kcal/mol. Similarly, aspartic acid amide is experimentally observed to be ca. 2 kcal/mol more acidic than glutamic acid amide whereas the G3MP2 results show a correspondingly smaller energy difference of 0.7 kcal/mol. The computational results clearly show that the anions are all ring-like structures with strong hydrogen bonds between the OH or NH_2 groups and the CO_2^- group from which the proton is removed. The two amino acids are main-chain deprotonated. In addition, use of the COSMO model for the prediction of the free energy differences in aqueous solution gave values in excellent agreement with the most recent experimental values for pK_a. Glutamic acid is predicted to be more acidic than aspartic acid in aqueous solution due to differential solvation effects.
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Keywords: Aspartic acid; Glutamic acid; Gas-phase acidity; Proton transfer; G3MP2 molecular orbital theory

1. Introduction

Proton transfer reactions in the gas phase can provide information on the intrinsic structural and energetic properties of amino acids and peptides. Acid–base characteristics are important because they affect properties involving protons and their transfer reactions. Changes in protonation states impact hydrogen bonding, which leads to important consequences in terms of the three-dimensional structure and biological activity of peptides and proteins. Additional properties of biomolecules such as solubility, hydrophobicity, and electrostatic interactions are also influenced by changes in the protonation state [1–3].

Proton transfer reactions are fundamental to the analysis of biomolecules by mass spectrometry because the two most commonly used ionization techniques, electrospray ionization (ESI) [4] and matrix-assisted laser desorption ionization (MALDI) [5], involve adding or removing protons. In addition, the sites of added or removed protons can affect the fragmentation patterns of peptide ions, which subsequently impact the sequence information that is obtained by mass spectrometry [6–8].

For protonated ions, gas-phase basicity (GB), which corresponds to the negative of the Gibbs free energy change ($-\Delta G$) for the protonation reaction $A + H^+ \rightarrow AH^+$, is one of the most commonly studied thermodynamic parameters. Numerous experimental [9–13] and computational [9,10,14–17] studies have focused on the GBs for amino acids and small peptides. This positive ion work has been reviewed by Harrison [18].

Although not as commonly studied as protonated peptide fragmentation, deprotonated peptide fragmentation can also be used in sequencing [19–24]. Thermodynamic values such as

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gas-phase acidity (GA or ΔG_{acid}), which is ΔG for the deprotonation reaction $AH \rightarrow A^- + H^+$, can supply valuable information to aid in understanding negative ion mode peptide fragmentation by mass spectrometry. In contrast to the numerous studies on positive ions of the amino acids, very few thermodynamic measurements have been performed for amino acids in terms of studies of anions. Only three published reports have involved measurements of amino acid GAs. Locke and McIver [25] obtained the GAs of glycine and alanine using the proton transfer equilibrium method [26]. Kebarle and co-workers [27] included glycine in their proton transfer equilibrium measurements of the GAs of 96 aliphatic carboxylic acids. Bowie and co-workers [28] determined the GAs of nineteen amino acids using the kinetic method [29] of collision-induced dissociation (CID) on a proton bound dimer. They were unable to measure the GAs of the two most acidic amino acids, aspartic acid and glutamic acid, because these compounds were too involatile to form the necessary dimer ions. Their low volatility also prevents gas-phase equilibrium measurements from being performed on these compounds.

Computational methods have advanced to the point that they can be used to predict GAs or GBs with high accuracy [30–33] using coupled cluster methods [34–36] with correlation consistent basis sets [37,38] extrapolated to the complete basis set limit and to within a few kcal/mol with other approaches such as MP2 [39,40] or density functional theory (DFT) [41,42], with gradient corrected or hybrid functionals such as B3LYP. The computational results provide an important complement to the experimental measurements as they can account for variations in the experimental results due to different approaches including different ionization techniques and instrument methodologies. In addition, they can provide insights into the bonding in the neutral molecule and the anion and how the bonding is controlling the acidity. In a number of cases, they can lower the error bars on the experimental values and provide new physical insights into the results. Several computational studies of the GBs and GAs of amino acids have been reported including the GB of glutamic acid [14], the GAs of glycine and alanine [43], and the GA and GB of serine [44]. An important conclusion from the glutamic acid GB study [14] was the presence of a substantial number of different stable conformations: 21 conformers for the neutral and 19 for the protonated form.

In the present study, the GAs of the two most acidic common amino acids, aspartic acid (Asp, D) and glutamic acid (Glu, E), are determined for the first time. When amino acids combine to form peptides, the –OH group of their C-terminus is no longer present. Instead, the linkage between residues is an amide bond, –(O=C)–NH–, the peptide bond. Therefore, amino acid amides (with a C-terminal $-NH_2$) may be better models than simple amino acids for representing the proton transfer properties of peptides. Thus, we have also determined the GAs of the amino acid amides of these two acidic amino acids. Experimental measurements employed Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry and GA values were obtained with the bracketing method, which involves a series of proton transfer reactions with reference compounds of known GA [45]. In addition, we report a computational study of these compounds at the DFT and molecular orbital theory level. The computational results allow a better understanding of the structural features affecting acidity and of the sites of deprotonation. As discussed above, aspartic acid and glutamic acid have two carboxylic acid groups (side chain and C-terminus) and therefore two potential sites of deprotonation, whereas their corresponding amino acid amides can only be deprotonated readily at the side chain carboxylic acid group.

2. Experimental methods

All experiments were performed on a Bruker (Billerica, MA, USA) BioApex 47e FT-ICR mass spectrometer with a 4.7 T superconducting magnet. Solutions of the amino acids and amino acid amides in the concentration range of $(4.5–7.0) \times 10^{-5}$ M were formed in a 50:50:1 mixture of methanol:water:ammoniun hydroxide. Using an Analytica of Branford (Branford, MA, USA) ESI source, the solutions flowed through a grounded needle at a rate of 20 μl/min and were electrosprayed across a 3.5 kV potential through a heated (225 °C) air counter and parallel current drying gas.

The resulting $[M–H]^-$ were isolated with correlated ion ejection sweeps [46] and allowed to react with constant pressures of a series of compounds of known GA. The bracketing reaction is shown in (1), where A is the acidic reference compound:

$$[M–H]^- + A \rightarrow [A–H]^- + M \quad (1)$$

Reaction rate constants were determined by observing the pseudo-first-order decay in reactant ion intensity as a function of time. All reactions were studied to greater than 80% completion. Neutral pressures were in the range of $(1–10) \times 10^{-8}$ mbar and were measured with a calibrated ionization gauge [47]. Reported reaction efficiencies are the ratio of the experimental rate constants to the collision rate constants that was obtained from the thermal capture trajectory calculations procedure of Su and Chesnavich [48,49]. Dipole moments for the reference compounds, which are needed to calculate collision rate constants, were obtained by ab initio calculations with the STO-3G basis set using the program HyperChem Version 7.0 from HyperCube, Inc. (Waterloo, Ont., Canada). All experiments were performed at room temperature (ca. 298 K).

3. Computational methods

Calculations were performed at the DFT and molecular orbital theory levels with the programs Gaussian-03 [50] and NWChem [51,52]. The geometries were optimized with the B3LYP exchange-correlation functional [53,54] with the aug-cc-pVDZ basis set. Frequencies were calculated to ensure that minima were found and to provide zero point corrections, thermal corrections to the enthalpy and entropies so that free energies could be calculated for direct comparison to experiment. We optimized the six lowest free energy structures reported by Marynick and co-workers [14] for glutamic acid as well as an additional structure found by us. Protons were removed from the main chain and side chains of these structures and the anions optimized. Aspartic acid has fewer degrees of freedom and thus should have fewer lower lying structures. We generated a num-

ber of structures based on those of glutamic with different types of hydrogen bond interactions and optimized them. The protons were removed from the aspartic acid structures and the structures of the anions optimized. Frozen core MP2 calculations were also performed on the lowest energy structures of glutamic and aspartic acid and the corresponding main chain and side chain anions with the correlation-consistent aug-cc-pVDZ, aug-cc-pVTZ, and aug-cc-pVQZ basis sets [37,38]. The MP2 energies [39,40] were extrapolated to the complete basis set (CBS) limit by using a mixed exponential/Gaussian function of the form:

$$E(n) = E_{CBS} + A\exp[-(n-1)] + B\exp[-(n-1)^2] \quad (2)$$

with $n = 2$ (DZ), 3 (TZ) and 4 (QZ), as first proposed by Peterson et al. [55]. We also calculated the GA of glycine using the same approach.

In a recent study [56], we showed that the MP2/CBS approach can be used to predict acidities of organic acids to better than 4 kcal/mol. The calculated values were more acidic than the experimental values. We also showed that the G3(MP2) method [57] improved the agreement with experiment and with CCSD(T)/CBS values for the acidities to within about 1 kcal/mol. For example, the G3MP2 value for the acidity of acetic acid, CH_3CO_2H, is 340.3 kcal/mol at 298 K, the experimental value is 341.5 ± 2.0 kcal/mol [58], and the MP2/CBS value is 337.2 kcal/mol.

All of the calculations were performed on a massively parallel HP Linux cluster with 1970 Itanium-2 processors in the Molecular Sciences Computing Facility in the William R. Wiley Environmental Molecular Sciences Laboratory or on the 144 processor Cray XD-1 computer system at the Alabama Supercomputer Center.

4. Results and discussion

4.1. Experimental GA values

Deprotonated molecular ions from L-aspartic acid, L-glutamic acid, L-aspartic acid amide, and L-glutamic acid amide were individually reacted with reference compounds of known GA. Fig. 1 is a representative series of mass spectra and shows the reaction of deprotonated aspartic acid ions, $[D–H]^-$, with 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (HP). The only product formed in abundance is the deprotonated reference compound, $[HP–H]^-$. The resulting pseudo-first-order kinetics plot yielded a proton transfer rate constant of 5.2×10^{-10} cm^3/(molecule s), which translates into a reaction efficiency of 0.34, i.e., ca. 34% of all collisions result in a reaction. The kinetics plot is linear, which indicates that there is only one dominant structure for $[D–H]^-$ or that, if multiple structures are present, their GAs are very similar.

Table 1 shows the reference compounds used in the proton transfer reactions, their GA values, and the measured reaction efficiencies. In all cases, linear pseudo-first-order kinetics was observed. It is important to note that numerical GA values are inversely proportional to the ability of the compound to transfer a proton. For example, 1,1,1,5,5,5-hexafluoro-2,4-pentanedione has the lowest numerical GA value in Table 1 (310.3 ± 2.0 kcal/mol); this means that it was the *most acidic* reference compound used in this study.

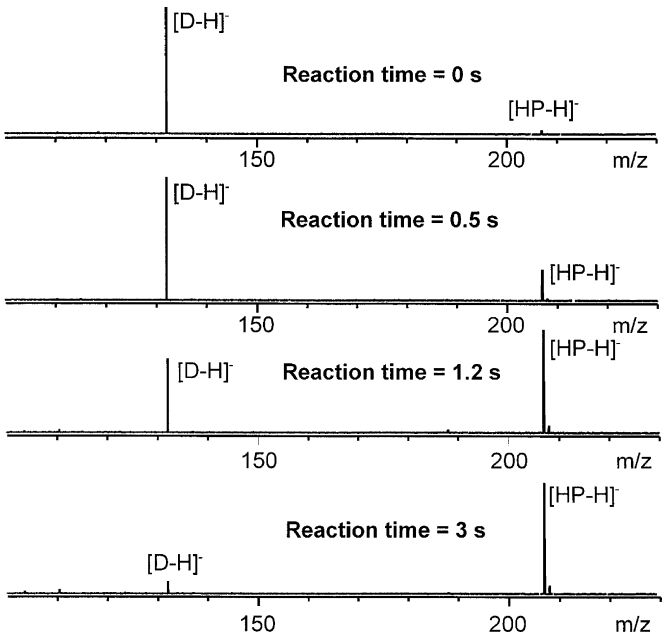

Fig. 1. Mass spectra for proton transfer reactions of deprotonated aspartic acid (D) reacting with 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (HP), which is present in the FT-ICR cell at a static pressure of 4.5×10^{-8} mbar.

In the bracketing method, the GA or GB of a compound is assigned at the point where proton transfer reactions with reference compounds go from endoergic to exoergic. Consequently, a decision must be made about the criterion to discern this point, especially given an acidity scale that is not highly populated with accurate acidities for reference compounds in the region where our molecules are found. For smaller molecules, the rise in reaction efficiency as a function of reference compound GA or GB is often steep and makes the assignment of GA or GB for the analyte unambiguous. However, for larger compounds, conformational effects, steric hindrance, and intramolecular hydrogen bonding may lead to a reaction efficiency curve with a gradual slope that does not clearly differentiate between endoergic and exoergic processes [45]. In our past studies on amino acids and small peptides, we have used a 0.10 efficiency break as the point for assigning GBs [9,12,45]. This resulted in values that agreed well with GB values obtained using the highest level of theory available at the time. However, recent re-evaluations of GB data by Bouchoux and Salpin [59,60] indicate that using a reaction efficiency break point of 0.10 results in GB values that are too low (and, therefore, GA values that are too high). Bouchoux et al. [61,62] have developed a "thermokinetic method," in which GA and GB values can be derived using the correlation between reaction efficiency and standard free energy change, ΔG°, for a series of proton transfer reactions. In a refitting of experimental proton transfer data for a wide range of organic compounds, they found that a reaction efficiency criterion of 0.269 resulted in what are thought to be the most accurate GB values [59].

Our attempts to fit the experimental data of Table 1 to the sigmoidal function used in the thermokinetic method were

Table 1
Reaction efficiencies for the proton transfer reactions of the deprotonated amino acids and amino acid amides with reference compounds

Reference compound	GA[a] (kcal/mol)	Reaction efficiency			
		Asp	Glu	Asp-amide	Glu-amide
Acetic acid	341.5 ± 2.0	NR[b]	NR	NR	NR
Formic acid	339.2 ± 1.5	–[c]	–	NR	0.01 ± 0.01[d]
3-(Trifluoromethyl)-phenol	332.4 ± 2.0	–	–	0.09 ± 0.01	0.13 ± 0.02 **BREAK**[e]
3,3,3-Trifluoropropionic acid	326.9 ± 2.0	–	–	0.22 ± 0.08 **BREAK**	0.34 ± 0.04
Difluoroacetic acid	323.8 ± 2.0	NR	0.01 ± 0.01	0.58 ± 0.02	0.55 ± 0.11
Pentafluorophenol	320.8 ± 2.0	0.03 ± 0.01	0.07 ± 0.00 **BREAK**	1.25 ± 0.08	1.29 ± 0.05
Trifluoroacetic acid	317.4 ± 2.0	0.08 ± 0.04 **BREAK**	0.33 ± 0.06	–	–
Heptafluorobutyric acid	314.9 ± 2.0	0.31 ± 0.07	0.55 ± 0.07	–	–
1,1,1,5,5,5-Hexafluoro-2,4-pentanedione	310.3 ± 2.0	0.39 ± 0.07	0.46 ± 0.06	–	–

[a] All reference compound GAs are obtained from reference [58].
[b] NR indicates that no reaction was observed.
[c] The "–" indicates that no experiment was performed.
[d] Mean (±standard deviation) for the reaction efficiency as obtained from three or more replicate measurements.
[e] "**BREAK**" signifies the point where the experimental GA value was assigned.

unsuccessful. The problem is that each amino acid or amino acid amide was only reacted with four or five reference compounds, which provided too few data points to obtain meaningful reaction efficiency curve fitting. Efforts to obtain data for additional reference compounds also provided unsuccessful. Attempts were made to use the following compounds with GA values given in parenthesis: trifluoromethanesulfonimide (291.8 ± 2.0 kcal/mol [58]), trifluoromethanesulfonic acid (299.1 ± 2.5 kcal/mol [58]), pyruvic acid (326.5 ± 2.8 kcal/mol [58]), 2,3,4,5,6-pentafluorophenylacetonitrile (327.6 ± 2.0 kcal/mol [58]), 4,4,4-trifluorobutyric acid (329.5 ± 2.8 kcal/mol [58]), 2-chloropropionic acid (329.8 ± 2.0 kcal/mol [58]), α,α,α-trifluoro-*m*-cresol (332.4 ± 2.0 kcal/mol [58]), and 3-chloropropionic acid (333.8 ± 2.0 kcal/mol [58]). All of these compounds were found to be insufficiently volatile to achieve a stable pressure in the 10^{-8} mbar range in our FT-ICR. In some cases, the high acidity of the compounds (e.g., trifluoromethanesulfonic acid is considered to be a superacid) may have caused problems in our inlet and leak valve system and contributed to the difficulty in achieving a usable pressure.

Bouchoux and Salpin [59] have suggested that in cases where a thermokinetic method sigmoidal fit cannot be applied, a simplified use of the thermokinetic method can be achieved by assigning the GB or GA value at the point where the reaction efficiency is 0.27. In order to determine if this criterion was reasonable for amino acids, we reviewed thermokinetic data that had been reported by Bouchoux and Salpin [60] for 6 amino acids and 12 dipeptides where the original bracketing reaction data had been obtained in our laboratory [9,12,45,63,64]. We found that the reactions efficiency break points for GB assignments ranged from 0.17 to 0.43, but that the average for the 18 compounds was, in fact, 0.27. Therefore, 0.27 was used as the reaction efficiency for assigning GA values in the present study. This is represented by "**BREAK**" in Table 1.

Table 2 summarizes the experimental and theoretical GAs obtained in this study. The experimental uncertainties were determined from the acidity range of the two bracketing reference compounds and from the uncertainties associated with the GAs of the reference compounds. Uncertainties for reference GAs are often not reported or are given unrealistically small values. Therefore, as a conservative measure, ±2 kcal/mol has been included in all experimental uncertainties reported here to account for uncertainties in the reference GA values.

Table 2
Experimental and theoretical GAs (ΔG_{acid}) for the amino acids and amino acid amides in kcal/mol

Compound	Experimental	G3MP2	MP2/CBS	B3LYP
Glycine[a]	334.7 ± 2.0[b]	335.3[c]	332.4[d]	333.2[e]
Aspartic acid[a]	315.3 ± 3.3	315.0[g]	311.0[h]	311.4[i]
Glutamic acid[a]	318.2 ± 3.7	315.3[j]	311.7[k]	313.0[l]
Aspartic acid amide[f]	326.5 ± 3.6	325.9[m]	321.9[n]	322.2[o]
Glutamic acid amide[f]	328.7 ± 4.8	326.4[p]	323.2[q]	323.3[r]

[a] Deprotonation site is C-terminus. Aspartic acid and glutamic acid form a cyclic structure in the anion.
[b] The experimental GA of glycine is from references [25,61].
[c] H(298 K) = 342.9 kcal/mol.
[d] H(298 K) = 339.7 kcal/mol.
[e] H(298 K) = 340.5 kcal/mol.
[f] Side chain deprotonated and form a cyclic structure in the anion.
[g] H(298 K) = 322.1 kcal/mol.
[h] H(298 K) = 317.7 kcal/mol.
[i] H(298 K) = 318.1 kcal/mol.
[j] H(298 K) = 321.7 kcal/mol.
[k] H(298 K) = 317.9 kcal/mol.
[l] H(298 K) = 319.2 kcal/mol.
[m] H(298 K) = 332.9 kcal/mol.
[n] H(298 K) = 329.2 kcal/mol.
[o] H(298 K) = 329.5 kcal/mol.
[p] H(298 K) = 334.0 kcal/mol.
[q] H(298 K) = 331.0 kcal/mol.
[r] H(298 K) = 331.1 kcal/mol.

The relatively large uncertainties in the range of 3–5 kcal/mol for the measured GA values are due to our inability to find suitable reference compounds within a narrower GA range. For example, the GA of glutamic acid amide can only be loosely bracketed between the GAs of 3-(trifluoromethyl)-phenol (GA = 332.4 ± 2.0 kcal/mol [58]) and trifluoropropionic acid (GA = 326.9 ± 2.0 kcal/mol [58]) because no other compound with a GA in this range could be found to use as a reference.

In order to confirm the GA trends shown in Table 2, two mixtures were analyzed. One mixture contained aspartic acid and glutamic acid in equimolar concentrations, while the other contained equimolar concentrations of the two amides. Both $[M–H]^-$ in a mixture were simultaneously exposed to a reference compound. In reactions of the two amino acid deprotonated ions with pentafluorophenol, glutamic acid ions reacted faster and reached 100% completion first. This indicates that glutamic acid is less acidic and has a higher GA value than aspartic acid. Likewise, glutamic acid amide ions reacted more rapidly and were the first to achieve 100% completion in reactions with trifluoropropionic acid. This means that glutamic acid amide is less acidic and has a higher GA value than aspartic acid amide. These results clearly support the experimental GA trends of Table 2 that were obtained by studying each compound individually.

The calculated and experimental values can be compared to the experimental values found for the dicarboxylic acids HCO_2H-$(CH_2)_n$-CO_2H [65]. Succinic acid with $n = 2$ can be compared directly to aspartic acid and glutaric acid with $n = 3$ can be directly compared with glutamic acid. The GA of succinic acid is 317.3 kcal/mol, similar to the value of 315.3 ± 3.3 kcal/mol obtained for aspartic acid. The experimental value for glutaric acid is 319.7 kcal/mol, quite similar to our value of 318.2 ± 3.7 kcal/mol for glutamic acid. This differs from what is found in the differences in the GAs of glycine and acetic acid (a $\Delta\Delta G$ of 6.3 kcal/mol determined from the average of the gas-phase values [25,27,66–68]) and those for alanine and propionic acid (a $\Delta\Delta G$ of 6.3 kcal/mol determined from the average of the gas-phase values [25,27,67]) with the amino acid more stable than the organic acid. The major difference in this comparison and our above diacid comparison is that the diacids form ring-like structures with a strong hydrogen bond between the CO_2^- and CO_2H groups whereas the monoacids do not. The hydrogen bonding is the dominant effect for the diacids whereas more traditional organic substituent effects are dominant in the monoacid comparison.

4.2. Theoretical GA values

Figs. 2 and 3 show the most stable neutral structures of aspartic and glutamic acids and their anions. Short hydrogen bond interactions are shown in the figures. The calculated GAs are given in Table 2 together with the values for glycine. We performed benchmark calculations on glycine as its GA is reasonably well-established and there are no conformational issues with its structure. The G3MP2 value for GA for glycine is 335.3 kcal/mol as compared to the experimental value of 334.7 ± 2 kcal/mol [27,58]. The MP2/CBS value for GA(glycine) is 332.4 kcal/mol and the B3LYP/aug-cc-pVDZ value is 333.2 kcal/mol, both latter values lower than the experimental value. The G3MP2 value of ΔH(298K) for loss of a proton from glycine is 342.9 kcal/mol in comparison with the experimental value of 341.6 ± 2.1 kcal/mol [27]. The MP2/CBS

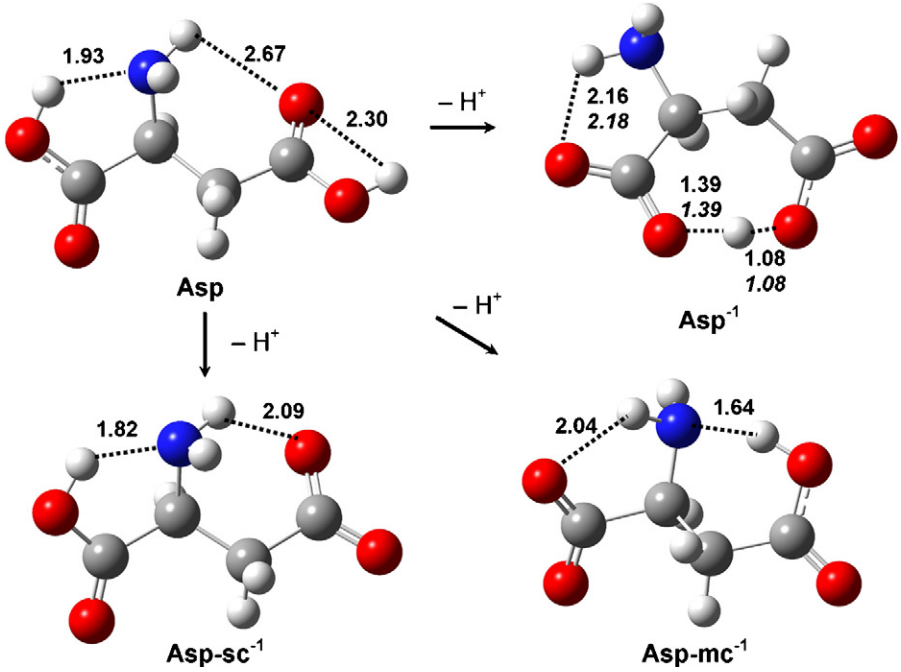

Fig. 2. Aspartic acid: **Asp** is the neutral molecule with the lowest energy; **Asp-sc^{-1}** is the anion obtained by removing a proton from the side chain; **Asp-mc^{-1}**, the anion obtained by removing a proton from the main chain; **Asp^{-1}**, the anion with the lowest energy and ring-like structure; the angles for its hydrogen bonds are ∠(N–H–O) = 113.0° (*112.5°*) and ∠(O–H–O) = 175.9° (*177.2°*), B3LYP(*MP2*) values. B3LYP hydrogen bond distances (Å) are in bold and MP2, in italic-bold.

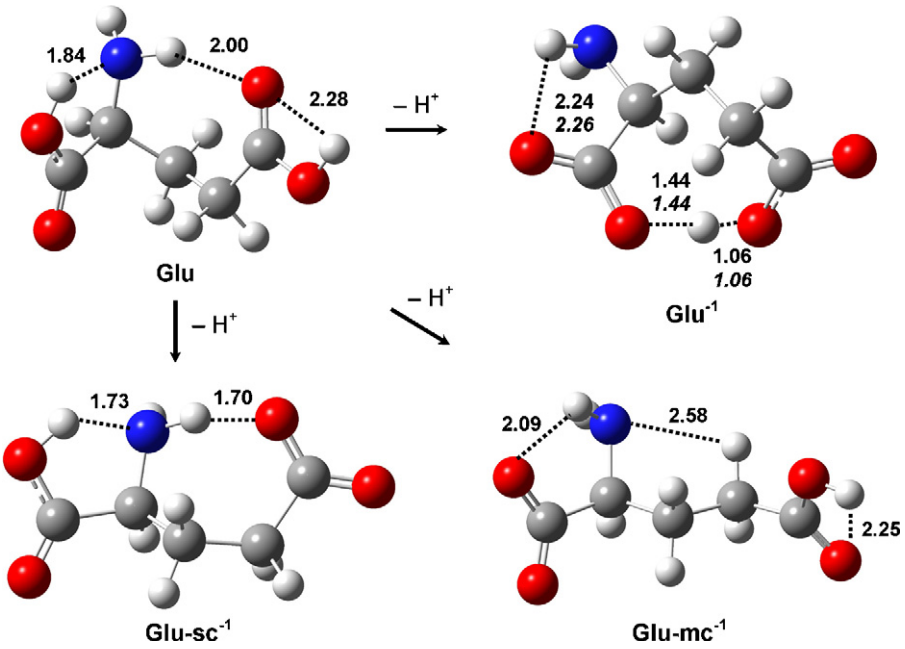

Fig. 3. Glutamic acid: **Glu** is the neutral molecule with the lowest energy; **Glu-sc^{-1}** is the anion obtained by removing a proton from the side chain; **Glu-mc^{-1}**, the anion obtained by removing a proton from the main chain; **Glu^{-1}**, the anion with the lowest energy and ring-like structure; the angles for its hydrogen bonds are ∠(N–H–O) = 109.9° (*109.6°*) and ∠(O–H–O) = 177.7° (*178.4°*), B3LYP(*MP2*) values. B3LYP hydrogen bond distances (Å) are in bold and MP2, in italic-bold.

value for ΔH(298 K) is lower at 339.7 kcal/mol at the MP2/CBS level and 340.5 kcal/mol at the B3LYP/aug-cc-pVDZ level.

The MP2 and B3LYP values for all of the calculated GAs are in good agreement with each other with only the values for glutamic acid differing by more than 1 kcal/mol (a difference of 1.3 kcal/mol). The G3MP2 values are approximately 3–4 kcal/mol more positive than the MP2/CBS values. We first discuss the calculated adiabatic acidities. Aspartic acid is predicted to have a GA(298 K) of 315.0 kcal/mol at the G3MP2 level, in excellent agreement with experiment. The MP2/CBS acidity is 311.0 kcal/mol, just below the lower limit of the experimental error bars. Glutamic acid is predicted to have essentially the same value as that for aspartic acid at the G3MP2 and MP2/CBS levels. The G3MP2 value is 315.3 kcal/mol, approximately 3 kcal/mol below the experimental value of 318.2 ± 3.7 kcal/mol. The MP2/CBS value for GA(298 K) = 311.7 kcal/mol is outside the experimental error bars and 6.5 kcal/mol below the experimental value. Aspartic acid is predicted to be a slightly stronger acid than glutamic acid consistent with experiment.

The calculations clearly show that both aspartic acid and glutamic acid form ring like structures with the proton removed from the main chain as shown in Figs. 2 and 3. There is a strong hydrogen bond between the main chain CO_2^- and the CO_2H side chain groups with the proton partially transferred to the CO_2^- group as evidenced by the long O–H bond and the short O···H–O hydrogen bond. The O–H···O hydrogen bond is almost linear with r(O–H) = 1.08 Å and r(O···H–O) = 1.39 Å for the aspartic acid anion. The corresponding distances in the glutamic acid anion are 1.06 and 1.44 Å showing slightly less transfer of the proton in the glutamic acid anion. Because of the substantial amount of proton transfer, we reoptimized the geometries of the anions at the MP2/aug-cc-pVDZ level and found essentially the same structures as predicted with the B3LYP functional. Thus, the structures of the anion have the strongest intramolecular hydrogen bond between the most acidic hydrogen, the carboxylic acid O–H, and the most basic site, the CO_2^- group as generally found. This is consistent with the results of Bowie and co-workers [28] who attributed the enhanced acidity (relatively low GA values) of arginine, asparagine, glutamine, and histidine to hydrogen bonding between the polar side chains on these amino acids with the deprotonated carboxylic acid group in the ion. There is a second hydrogen bond in both acids which corresponds to the main chain amino group stabilizing the CO_2^- group.

As shown in Figs. 2 and 3, the structures of the parent acids are not rings but are open chain molecules with, in each case, a short O–H···N(H_2) hydrogen bond of length 1.93 Å in aspartic acid and 1.84 Å in glutamic acid. Both are consistent with strongest intramolecular hydrogen bond occurring between the most acidic hydrogen and the most basic site. There are other weaker hydrogen bonds in the two acids due to the other hydroxyl group and the amine group hydrogens so a number of possible structures are present. Glutamic acid has 21 neutral local minima on its potential energy surface as shown by Marynick and co-workers [14]. Six of these minima are present in populations between 9 and 21%. The neutral molecules have three hydrogen bonds and the anions have two. Thus, there is a substantial difference between the geometries of the parent acid and lowest energy structure of the anion.

We also calculated the acidities at the MP2/CBS level for the structures where the side chain and main chain were deprotonated for both aspartic and glutamic acid but where the CO_2^-

group is involved in hydrogen bonding to the main chain NH_2 group in comparison to forming the ring. The structures for these anions are also shown in the figures. For aspartic acid, the strong hydrogen bond on the basis of the bond length is between the appropriate CO_2H and the NH_2 group for both the side-chain and main-chain deprotonated structures. The main chain deprotonated structure is 5.9 kcal/mol higher in energy than the lowest energy structure (GA(MP2/CBS) = 316.9 kcal/mol) and the side chain deprotonated structure is 11.3 kcal/mol higher in energy (GA(MP2/CBS) = 323.3 kcal/mol) for aspartic acid. For glutamic acid, the side chain deprotonated structure has two short hydrogen bond between the CO_2H and the NH_2 group and the NH_2 group with CO_2^- group. This structure is 7.9 kcal/mol higher in energy (GA(MP2/CBS) = 319.6 kcal/mol). The extended structure with the main chain deprotonated and the side chain not involved in any hydrogen bonding is significantly higher in energy than the lowest energy structure by 15.0 kcal/mol (GA(MP2/CBS) = 326.7 kcal/mol).

For glutamic acid amide and aspartic acid amide, the only feasible deprotonation site is the side chain carboxylic acid group. As shown in Figs. 4 and 5, the neutral aspartic acid amide (AspAm) has an extended side chain with hydrogen bonding between the backbone amino group and the acid group on the side chain. The anion has a ring-like structure with a strong hydrogen bond between the amide NH_2 group and the CO_2^- group. The N–H bond is elongated by 0.04–0.05 Å as compared to a normal amide N–H bond. The H···O–C(O) hydrogen bond is 1.60 Å, a short hydrogen bond but substantially longer than the O–H···O hydrogen bonds in the aspartic and glutamic acid anions. There is a weaker hydrogen bond from the other NH_2 group to the amide oxygen. There is a difference in the B3LYP and MP2 hydrogen bond distances of 0.09 Å for this latter weak hydrogen bond and this is the only example of such a difference in our calculations. The GA of aspartic acid amide is calculated to be 325.9 kcal/mol at the G3MP2 level in excellent agreement with experiment. The MP2/CBS value of GA = 321.9 kcal/mol at 298 K is slightly below the lower limits of the experimental range of 326.5 ± 3.6 kcal/mol. The structure where the shortest hydrogen bond is between the main chain NH_2 group and the CO_2^- group is higher in energy by 5.9 kcal/mol (GA(MP2/CBS) = 327.8 kcal/mol). Consistent with the energy difference is the fact that the shortest hydrogen bond length is now 2.05 Å.

The ground state structure of glutamic amide has many potential conformations. We took all 21 structures given by Marynick and co-workers [14] for neutral glutamic acid and substituted the amide functionality $CONH_2$ for the CO_2H functionality on the main chain. After optimization at the B3LYP level of all 21 structures including additional rotational conformers for some of these structures, we found the structure shown in Fig. 5 to have the lowest energy. The lowest energy structure in terms of the enthalpy at 298 K is the ring-like structure with a short hydrogen bond of 1.74 Å between the amide oxygen and the CO_2H group. The ring structure is 1.3 kcal/mol lower in energy than a structure with an extended side chain at the G3MP2 level, 1.5 kcal/mol lower in energy at the MP2/CBS level, and 0.6 kcal/mol lower in energy at the B3LYP level at 0 K. The lowest free energy structure at 298 K has the extended side chain as it has more conformational degrees of freedom than the ring structure and is lower in free energy by 0.2 kcal/mol at the G3MP2 level, 0.3 kcal/mol at the MP2/CBS level, and 1.2 kcal/mol at the B3LYP/aug-cc-

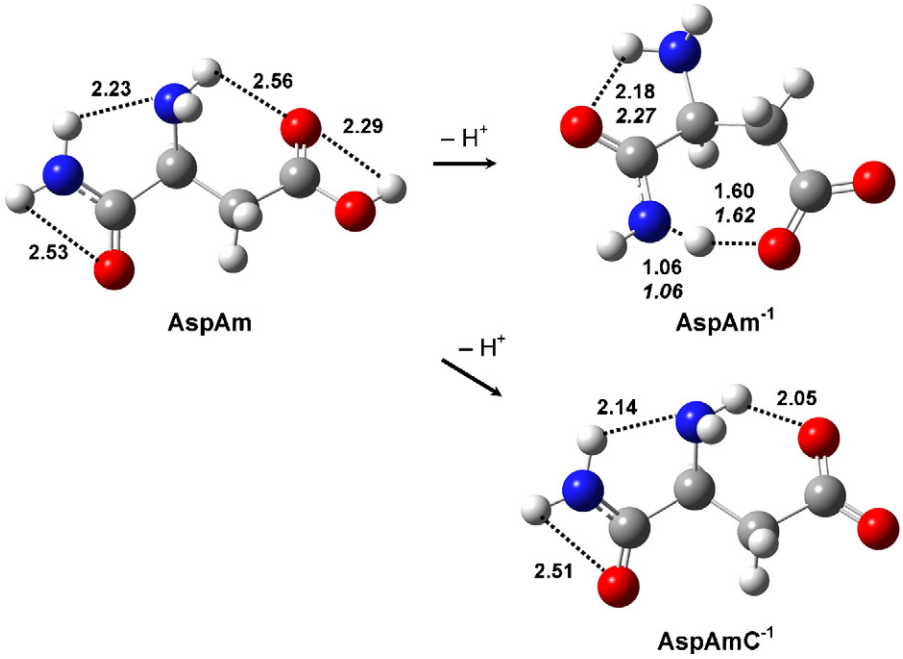

Fig. 4. Aspartic acid amide: **AspAm** is the neutral molecule with the lowest energy; **AspAmC^{-1}** is the anion obtained by removing a proton in a chain-like structure; **AspAm^{-1}**, the anion with the lowest energy and ring-like structure, the angles for its hydrogen bonds are ∠(N–H–O) = 112.3°(*107.9°*) and ∠(N–H–O) = 163.1°(*161.7°*), B3LYP(*MP2*) values, the last angle corresponds to the H-bond that forms the ring-like structure. B3LYP hydrogen bond distances (Å) are in bold and MP2, in italic-bold.

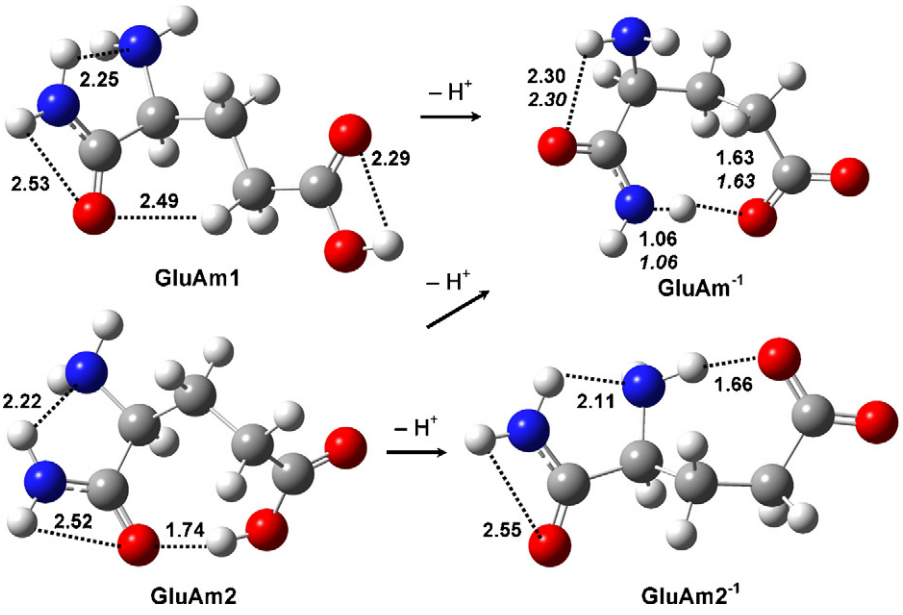

Fig. 5. Glutamic acid amide: **GluAm1** is the neutral molecule with the second lowest energy and a chain-like structure; **GluAm2** is the neutral molecule with the lowest energy and a ring-like structure; **GluAm2**$^{-1}$ is the anion obtained by removing a proton, from **GluAm2**; **GluAm**$^{-1}$, the anion with the lowest energy and a ring-like structure; the angles for its hydrogen bonds are ∠(N–H–O) = 109.3°(*109.8°*) and ∠(N–H–O) = 172.7°(*172.4°*), B3LYP(MP2) values, the last angle corresponds to the H-bond that forms the ring-like structure. B3LYP hydrogen bond distances (Å) are in bold and MP2, in italic-bold.

pVDZ level. Thus, at 298 K, there are two essentially degenerate energy structures with quite different conformations. The minimum energy structure for the anion has a ring structure with a strong hydrogen bond between the amide NH_2 and CO_2^- group with essentially the same parameters as found for the aspartic amide anion. In this case there are no real differences in the B3LYP and MP2 optimized geometries. The ring structure is similar to that in the low lying ring structure except for rotation about the C–C(amide) bond by ∼180° to enable the strong hydrogen bond between the NH_2 group and the CO_2^- group in the anion. The calculated G3MP2 GA value for glutamic amide is 326.4 kcal/mol, 2.3 kcal/mol below the experimental value of 328.7 ± 4.8 kcal/mol. The value for GA(MP2/CBS) is 323.2 kcal/mol, at the lower end of the experimental range. Again, the aspartic amide is predicted to have a lower acidity than the glutamic amide as found experimentally. Just as in the case for aspartic and glutamic acid, the calculated free energy difference of 0.7 kcal/mol is smaller than the experimental free energy difference of 2.2 kcal/mol. There is second anionic structure 1.8 kcal/mol higher in energy than the lowest energy anion at the MP2/CBS level (GA(MP2/CBS) = 325.0 kcal/mol) with the strong hydrogen bond between the between the main chain NH_2 group and the CO_2^- group.

In summary, the calculated values for the GA of all four acids are in excellent agreement with the experimental values at the G3MP2 level and at the low end of the range of the experimental values at the MP2/CBS level. The acidities for aspartic acid and aspartic amide are within 1 kcal/mol of experiment whereas those for glutamic acid and glutamic amide differ by somewhat larger amounts, 2.9 and 2.3 kcal/mol respectively. The amino acids have a lower acidity than do the acid amide compounds. This is consistent with a larger stabilization of the CO_2^- group by hydrogen bonding to a CO_2H group in the acids as compared to hydrogen bonding stabilization by an NH_2 group in the amides.

A variety of intramolecular hydrogen bonds were found (see Figs. 2–5) including O–H···O, O–H···N, N–H···O, N–H···N (only present in the amide structures) as well as C–H···O. Following the classifications of Jeffrey [69] and of Desiraju and Steiner [70], these hydrogen bonds have a substantial range from moderate (O–H···O, N–H···O) to weak (C–H···O). The weak C–H···O hydrogen bond is known to play a role in biological molecules [71–73] and its bond strength for C^{α}–H···O=C hydrogen bonds has been predicted to be in the range of 2–4 kcal/mol [74]. Only the neutral glutamic acid and glutamic acid amide conformers have C–H···O hydrogen bonds and each structure has one such bond as shown in Figs. 3 and 5.

Numerous studies have shown a correlation between a molecule's solution-phase conformation and the conformation of its ions formed by ESI, although the biomolecules involved are generally much larger than those here [75–80]. Our results show that the neutral molecules after proton transfer may be able to adopt the lowest energy conformation of the free molecule as the calculated adiabatic values and the experimental values are in excellent agreement with each other. Thus, the anion on receiving the proton from another anion can lose its constrained ring shape to form the structure of the neutral. Our results provide good evidence that the reaction is under thermodynamic control rather than kinetic control. This is consistent with other results for carboxylic acid anions [27,67].

4.3. Comparison of acidities in the gas phase and in aqueous solution

There is little agreement on the acidity order for aspartic acid and glutamic acid in aqueous solutions. An often cited

1969 data compilation lists pK_{a1} and pK_{a2} for aspartic acid as 1.99 and 3.90, respectively, and for glutamic acid as 2.10 and 4.07, respectively [81]. Some common textbooks [82,83] that use these values assume that pK_{a1} is for acid dissociation at the C-terminus and pK_{a2} is for dissociation at the side chain, but the 1969 report does not attempt to assign the sites of the dissociations. These pK_{a1} values would mean that aspartic acid is 0.15 kcal/mol more acidic than glutamic acid in solution. Others have reported that the C-terminal groups have the same acidity [84] or that aspartic acid is slightly more acidic than glutamic acid [85,86]. For the side chain, some reports list glutamic acid as slightly more acidic in solution than aspartic acid [86,87], while others list aspartic acid as more acidic [81,85]. In the most recent report on aqueous amino acid acidity, Gaš and co-workers [88] obtained pK_a values from cationic mobilities that were measured by capillary zone electrophoresis. For pK_{a1}, they found glutamic acid to be more acidic than aspartic acid (2.16 versus 2.28) by 0.17 kcal/mol. For pK_{a2}, Gaš and co-workers [88] also reported that glutamic acid is more acidic (4.324 versus 4.500) by 0.24 kcal/mol. These researchers performed no studies to determine the site of deprotonation that corresponds to each measured pK_a. In fact, we have found no evidence in the literature of any solution-phase study on aspartic acid and glutamic acid which has elucidated the site of deprotonation that corresponds to each pK_a value.

In order to estimate the solution-phase acidities of aspartic and glutamic acid, we calculated the free energy of solvation by using a self-consistent reaction field (SCRF) approach [89] with the COSMO (Conductor-like Screening Model) formalism [90] using the dielectric constant for water of 78.39. On the basis of our previous studies of acids [56], we chose to calculate the acidities relative to that of acetic acid, CH_3CO_2H, which is well-established as $pK_a = 4.76$ in aqueous solution as shown by the following reaction:

$$CH_3CO_2H + \text{amino acid anion} \rightarrow CH_3CO_2^- + \text{amino acid} \quad (3)$$

The gas-phase free energies for reaction (3) are 25.3 kcal/mol for aspartic acid and 25.0 kcal/mol for glutamic acid. The free energy of solvation contribution to the aspartic acid reaction is −22.24 kcal/mol giving a solution-phase value of $\Delta G = 3.1$ kcal/mol and pK_a (aspartic acid) = 2.5. For the glutamic acid, the free energy of solvation contribution to the reaction is −21.14 kcal/mol giving a solution-phase value for the free energy of the reaction of 3.81 kcal/mol and pK_a (glutamic acid) = 1.97. Thus, glutamic acid is predicted to be slightly more acidic than aspartic acid in aqueous solution at 298 K; this is in agreement with the most recent experimental values [88]. Both pK_a values are within <0.5 pK_a units of the most recent experimental values and show that the change in acidity from the gas-phase values is clearly due to a differential solvation effect and the fact that the predicted gas-phase values are close to each other. Both aspartic amide and glutamic amide are predicted to be less acidic than acetic acid in aqueous solution. The pK_a for aspartic amide is 6.8. In solution, the ring structure for glutamic amide is the lowest energy one and the pK_a is 9.9 forming the ring-like anion. The higher energy chain structure in solution forming the same anion has a lower pK_a of 8.5. Thus, aspartic amide is predicted to be substantially more acidic than glutamic amide in aqueous solution in contrast to the much smaller difference predicted for aspartic and glutamic acids.

5. Conclusions

The GAs of glutamic acid, aspartic acid, and their amides have been determined for the first time. The experimental and computational results at the G3MP2 level are in excellent agreement considering the experimental error bars. The computational results clearly show that the anions are all ring-like structures with strong hydrogen bonds between the OH or NH_2 groups and the CO_2 group from which the proton is removed. The two amino acids are main-chain deprotonated with a strong hydrogen bond to the side chain CO_2H group. Aspartic acid and aspartic amide are stronger gas-phase acids than the corresponding glutamic acid and amide. The two amino acids are quite strong acids being slightly weaker acids in the gas phase than CH_3SO_3H, which has a calculated value of GA = 312.4 kcal/mol at the CCSD(T)/CBS limit [56] and an experimental value of 315.0 ± 2.0 kcal/mol [91]. The two amino acid amides are also quite strong gas-phase acids, both being comparable to H_3PO_4, which has a calculated GA of 322.2 kcal/mol at the CCSD(T)/CBS limit [31] and an experimental value of 323.0 ± 4.9 kcal/mol [92]. The calculated values for the acidities at the MP2/CBS level are all too small by 3–4 kcal/mol as found previously [56]. The use of the COSMO model enabled us to predict the acidities of aspartic and glutamic acid in solution. We predict that both are main chain deprotonated and that glutamic acid is more acidic than aspartic acid in aqueous solution due to differential solvation effects.

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Appendix A. Supplementary data

Total energies at the MP2 level as a function of basis set, G3MP2 total energies (ΔH(0 K), (ΔH(298 K), (ΔG(298 K)), B3LYP total energies, zero point energies, thermal corrections and entropies, and B3LYP and appropriate MP2 Cartesian coordinates.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2007.02.009.

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