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Computational Estimates of the Gas-Phase Basicity and Proton Affinity of Glutamic Acid

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Literature values for the gas-phase basicity (GB) and proton affinity (PA) of glutamic acid range from 216 to 224 kcal/mol (GB) and 218 to 241 kcal/mol (PA). In this paper, a high-level theoretical study aimed at resolving the apparent disagreement among the experimental values is presented. Hartree−Fock, MP2, and DFT calculations with large basis sets were carried out on the neutral and protonated forms of glutamic acid. Nine protonated and 21 neutral conformers were located at the HF/3-21G and B3LYP/6-31+G** levels with full geometry optimization and characterization of stationary points. The energetics were subsequently reevaluated at the MP2(full)/6-311+G(2d,p)//B3LYP/6-31+G** level. Thermodynamic data in the harmonic approximation were obtained at the B3LYP/6-31+G** level. This data was used to estimate the gas-phase distribution of conformers at 298 K. The lowest energy structures of protonated and neutral glutamic acid both exhibit cyclic structures due to the formation of intramolecular hydrogen bonds. The calculated PA and GB are 224.4 and 214.4 kcal/mol, respectively. It is shown that, when certain empirical corrections for the entropy of cyclization are omitted and appropriate adjustments are made to thermodynamic scales, the GB and gas-phase PA values reported here are in excellent agreement with a variety of previous experimental measurements.

Introduction

Gas-phase protonation/deprotonation of peptides and proteins is of considerable importance in a wide array of modern mass spectrometric techniques. Fast atom bombardment (FAB),[1] secondary ion mass spectrometry (SIMS),[2] and the more recently developed techniques of matrix-assisted laser desorption ionization (MALDI)[3] and electrospray ionization (ESI)[4] all rely on protonation of the biomolecule as the dominant mechanism for analyte ionization. Furthermore, the specific site of protonation can strongly influence peptide fragmentation,[5] thus influencing the peptide sequence fragment ions observed in a mass spectrum.[6−8] More recently, measurement of the rates of deprotonation of multiply charged peptides has been used to infer the existence of multiple gas-phase isomers.[9,10] This data, in combination with knowledge of the intrinsic basicity of the protonation site and the proximity of the charged sites, has been used to suggest various gas-phase peptide conformations.[11]

Increasingly, interpretation of experimental results of mass spectrometric studies relies on an accurate knowledge of the thermodynamic properties (gas-phase basicity (GB) and proton affinity (PA)) of amino acids and amino acid residues in small peptides. Experimentally, high-pressure mass spectrometry,[12] Fourier transform ion cyclotron resonance (FTICR) mass spectrometry,[13−16] and kinetic methods[17,18] have all been employed to measure the GB and PA values of selected amino acids and small peptides. More recently, experimental studies have been combined with theoretical investigations of the amino acid and polypeptide systems being investigated.[19−21] The information obtained in these studies has provided details regarding the lowest energy conformations of both protonated and neutral species as well as important benchmarks for the evaluation of the accuracy of various levels of theoretical treatment. Recent reviews compile this data using uniform thermodynamic scales and discuss the strengths and limitations of the various experimental methods employed to measure the PA and GB values.[22,23]

Theoretical evaluation of the GB and gas-phase PA of amino acids has relied heavily on ab initio methods. Due to the complexity of the amino acid and polypeptide systems studied and the associated computational cost, many of the studies have employed relatively modest levels of theory. Higher level calculations have been performed at the Hartree−Fock (HF) level using the 6-31G(d) and 6-31+G(d,p) basis sets on glycine and polyglycine[19b] and using the 6-31G* basis set on mixed glycine alanine dipeptides[19c] and methionine.[24] A combined experimental and theoretical study employed theory at the MP2/6-31G* level to suggest that arginine exists as a zwitterion in the gas phase.[25] However, a very recent study[26] of jet-cooled arginine employing the technique of infrared cavity ringdown laser absorption spectroscopy clearly confirmed the presence of peaks characteristic of the carbonyl stretch of a neutral carboxylic acid group and provided strong evidence that the zwitterionic form does not exist in significant amounts under these conditions. Recent calculations of the GB and gas-phase PA values for O and N protonation of glycine and alanine have employed density functional theory (DFT) and ab initio methods at different levels of theory from HF to G2 approximations.[27,28] Ab initio DZP//DZP quantum chemical computation in conjunction with semiempirical methods have been applied to the PAs of lysine and histidine to resolve the discrepancy between experimental results from the kinetic and bracketing methods.[29]

The present study focuses on the determination of the GB and gas-phase PA of glutamic acid by high-level ab initio[30] and DFT[31] methods. This amino acid was of particular interest for several reasons. First, accurate calculation of the thermodynamic and structural properties of small molecules provides an important supplement to experimental results. Second, the many possible structures resulting from intramolecular hydrogen bonding make this a challenging problem from the theoretical

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perspective. Third, experimentally determined values for the GB and, in particular, the gas-phase PA of glutamic acid vary widely. For instance, Bojesen et al. report a value of 222.3 kcal/mol for the PA, determined by kinetic methods,[17b] while Gorman et al.[14a] report a value of 240.6 kcal/mol determined by bracketing of laser-desorbed and protonated glutamic acid. Recent reviews have suggested values of 218.2[32] and 223.4 kcal/mol[23] be used for the gas-phase PA.

Computational Details

Most of the calculations were performed using the Gaussian 94[33] suite of programs. The semiempirical PM3[34] method was first used to generate various stable structures by thoroughly searching the potential energy surface starting from a large number of chemically reasonable structures. Some structures were also initially located at the HF/3-21G level. All structures were ultimately optimized at the HF/3-21G level, resulting in 21 unique neutral and 9 unique protonated structures. These geometries were then optimized at the B3LYP/6-31+G** level. Each stationary point was fully characterized as a true minimum at every theoretical level. The B3LYP/6-31+G** [35] geometries were used as input for single point MP2(full)/6-311+G(2d,p) [36] calculations. For the lowest energy protonated (Figure 1) and neutral (Figure 2) structures, the final ΔE_{elec} value (see below) was determined at the MP2(full)/6-311+G(2d,p)//MP2(full)/6-31+G** level. Basis set superposition error (BSSE) was calculated for the lowest energy protonated structure **P1** (Figure 1) by standard procedures.[37] For the purpose of BSSE computation, structure **P1** was considered to be a dimer of glutamic acid and the proton on the NH_3 group not involved in hydrogen bonding. The *relative* energies of all protonated structures were assumed to be independent of BSSE. Thermodynamic properties, including zero-point energies, were obtained at the B3LYP/6-31+G** level and were used for the calculation of the GB and gas-phase PA at 298 K and 1 atm (recommended scaling factors were taken from ref 38; as recommended in their paper, the ZPE, enthalpy, and entropy were scaled individually).

The PA and GB of glutamic acid can be calculated from eqs 1−4:

$$\Delta E^\circ = \Delta E^\circ_{\text{elec}} + \Delta E^\circ_{\text{ZPE}} + \Delta E^\circ_{\text{vib}} + \Delta E^\circ_{\text{rot}} + \Delta E^\circ_{\text{tran}} \quad (1)$$

$$\Delta H^\circ = \Delta E^\circ + RT \quad (2)$$

$$\Delta S^\circ = \Delta S^\circ_{\text{tran}} + \Delta S^\circ_{\text{vib}} + \Delta S^\circ_{\text{rot}} \quad (3)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (4)$$

Here $\Delta E^\circ_{\text{elec}}$ is the 0 K electronic energy difference, $\Delta E^\circ_{\text{ZPE}}$ is the zero-point energy difference, $\Delta E^\circ_{\text{vib}}$ is the thermal contribution to the vibrational energy difference in the harmonic approximation, and $\Delta E^\circ_{\text{rot}}$ and $\Delta E^\circ_{\text{tran}}$ are the classical contributions to the rotational and translational energy differences, respectively. Similar definitions hold for the ΔS° terms. Equations 1−4 may be Boltzmann averaged over all conformations, or may be applied to the conformer with the lowest free energy.

Cartesian coordinates for the optimized geometries and total energies at the B3LYP level are available in the Supporting Information.

Results and Discussion

Nine unique protonated structures (denoted **P1**−**P9**) and 21 neutral structures (denoted **N1**−**N21**) were found. For the lowest energy structures **P1** and **N1**, the energies obtained at the MP2(full)/6-311G+(2d,p)//B3LYP/6-31+G** level are within 0.0013 au or less of those calculated at the MP2(full)/6-311G+(2d,p)//MP2(full)/6-31+G** level. This illustrates that the B3LYP/6-31+G** method is an appropriate and efficient approach for obtaining structures of systems with significant hydrogen bonding. The effects of varying the basis set and theoretical model on the calculated energies of protonation are illustrated in Table 1 for the protonation of **N1** to yield **P1**.

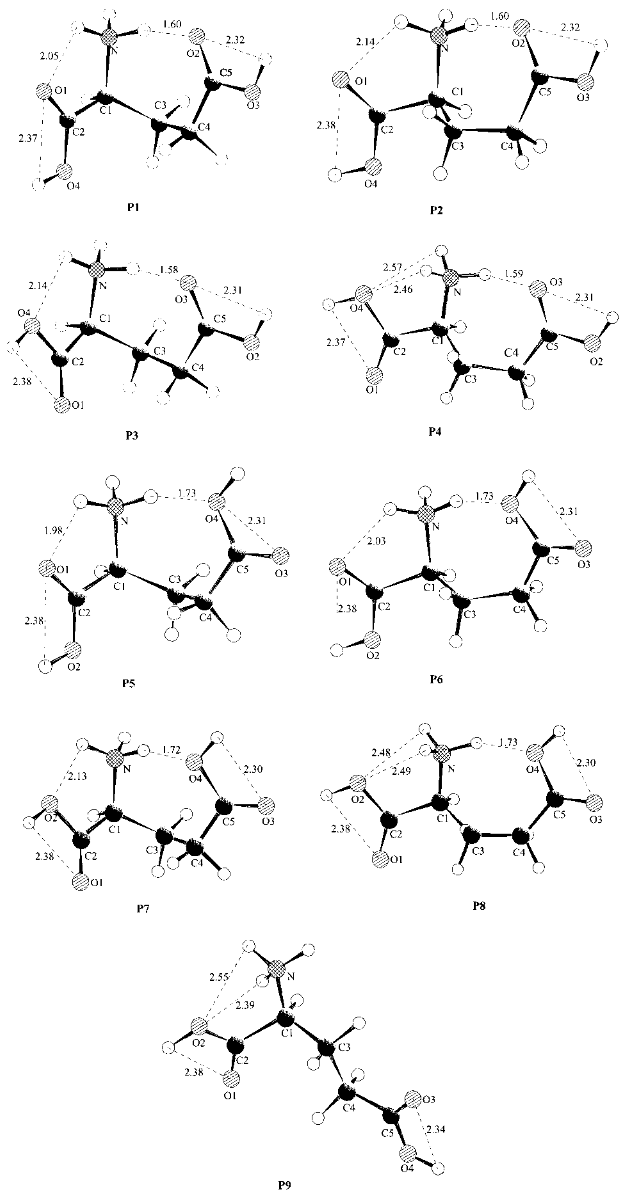

Figure 1. The nine local minima for protonated glutamic acid at the B3LYP/6-31+G** level.

$$\mathbf{N1} + \mathrm{H}^+ \rightarrow \mathbf{P1} \quad (5)$$

As expected, Hartree−Fock-based energies yield significant errors relative to higher level calculations. However, it is important to note that each minimum on the B3LYP/6-31+G** surface is also a minimum on the HF/3-21G surface. This suggests that a strategy of initially exploring these surfaces at the HF/3-21G level, followed by reoptimization at a higher theoretical level, is viable. Table 1 also suggests that DFT approaches can be remarkably accurate for proton affinities. Comparing the first two entries of Table 1, it is evident that B3LYP geometries yield a value for $\Delta E^\circ_{\text{elec}}$ within 0.5 kcal/mol of that obtained with MP2(full) geometries. Similarly, a

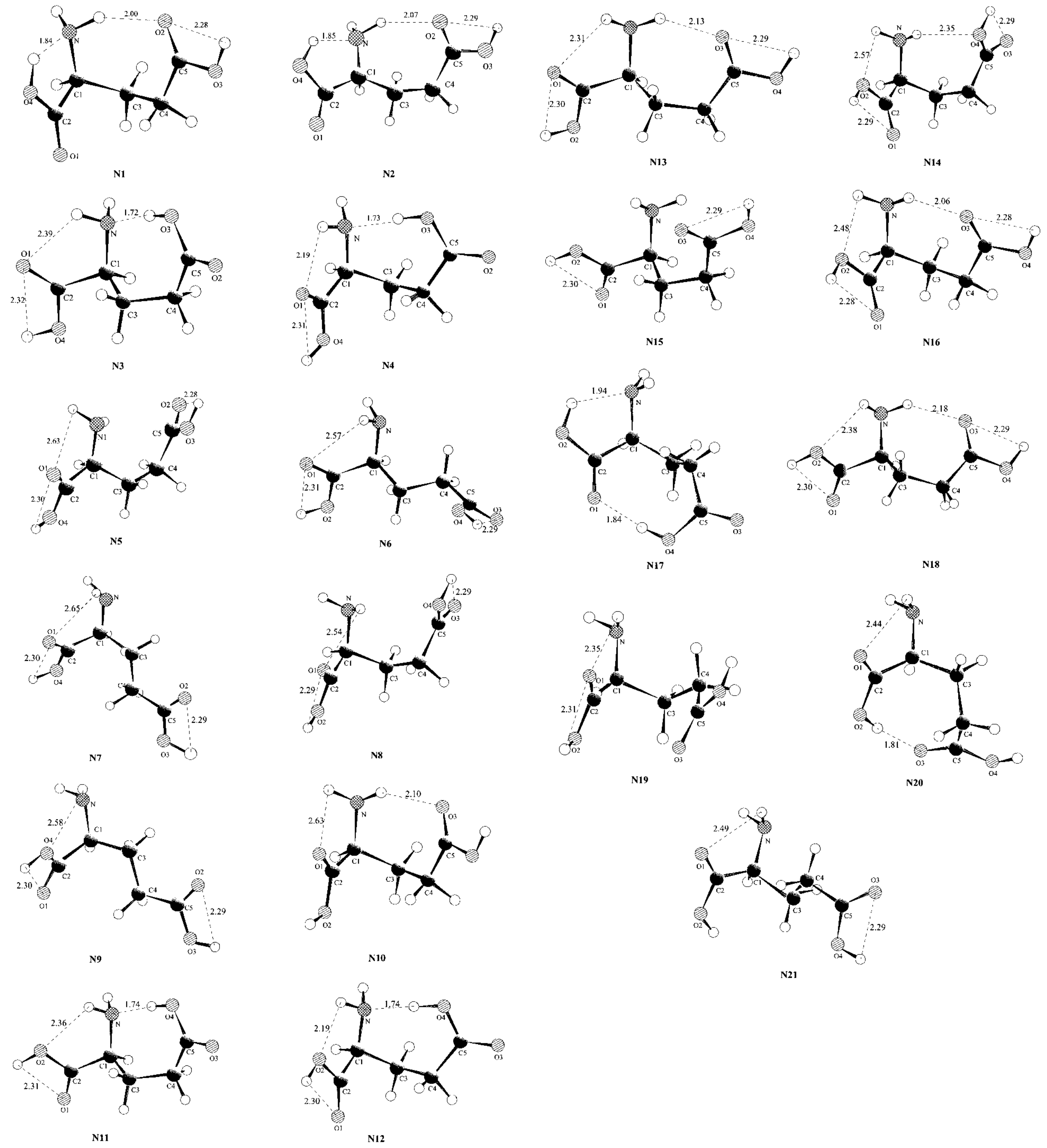

Figure 2. The 21 local minima for neutral glutamic acid at the B3LYP/6-31+G** level.

comparison of entries two and three of Table 1 illustrates that B3LYP ΔE°_{elec} values are within 1.6 kcal/mol of the corresponding MP2(full) values when using the same optimized geometry and a very large basis set.

The important intramolecular hydrogen bond distances obtained by these two methods are illustrated in Figures 1 and 2 for protonated and neutral structures, respectively. In all cases, the lowest energy structures are associated with intramolecular hydrogen bonding to carbonyl oxygens. This is clearly due to the greater basicity of carbonyl oxygens relative to hydroxylic oxygens. For instance, at the MP2(full)/6-311+G(2df,2p) level, the proton affinity of formic acid (uncorrected for thermodynamic effects) is 20 kcal/mol greater when protonation is at the carbonyl site (as opposed to the hydroxylic site).[39] For both the protonated and neutral species, the lowest energy conformers are characterized by two cyclic structures: one five-member ring and one seven-member ring. There are fewer energetically competitive structures for the protonated system than for the neutral species. This is due to the fact that the stable protonated structures are characterized by one very short (and thus strong) $N^{+}-H\cdots O=C$ hydrogen bond, which restricts the accessible conformational space. In contrast, no single type of hydrogen bond dominates the neutral structures.

Tables 2 and 3 show the relative thermodynamic data for protonated and neutral conformers. Also shown in Tables 2 and 3 are the expected relative populations of the lowest energy conformers at 298 K and 1 atm based upon a Boltzmann distribution. Because of the nature of the thermodynamic treatment (in particular the use of the harmonic approximation) the calculated Boltzmann distribution should only be considered

TABLE 1: Protonation Energies (in kcal/mol) Corresponding to Reaction 5 Uncorrected for Zero-Point Energy, BSSE, and Thermodynamic Effects

method and basis set	$\Delta E°_0$
MP2(full)/6-311+G(2d,p)//MP2(full)/6-31+G**	231.9
MP2(full)/6-311+G(2d,p)//B3LYP/6-31+G**	232.4
B3LYP/6-311+G(2d,p)//B3LYP/6-31+G**	234.0
MP2(full)/6-31+G**//MP2(full)/6-31+G**	234.1
B3LYP/6-31+G**//B3LYP/6-31+G**	234.3
HF/6-31G*//HF/6-31G*	239.0
HF/6-31G*//HF/3-21G	238.6
HF/3-21G*//HF/3-21G	247.9

TABLE 2: Relative Energies (kcal/mol) of the Protonated Glutamic Acid Conformers and Their Boltzmann Distribution

structure	$E°_0$	ZPE°	$E°_{vib}$	$E°$	$H°$	$TS°$	$G°$	%
P1[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	60.5
P2	0.39	−0.03	0.04	0.43	0.43	0.17	0.26	38.7
P3	2.90	−0.06	−0.07	2.82	2.82	0.00	2.82	0.5
P4	3.62	−0.08	0.01	3.63	3.63	0.45	3.18	0.3
P5	9.11	−0.13	0.07	9.19	9.19	0.81	8.37	0.0
P6	9.34	−0.20	0.07	9.41	9.41	1.01	8.40	0.0
P7	12.50	−0.12	0.10	12.60	12.60	0.83	11.76	0.0
P8	12.91	−0.24	0.09	13.00	13.00	1.40	11.60	0.0
P9	16.49	−0.36	0.27	16.76	16.76	2.64	14.12	0.0

[a] $E°_0 = -551.068\ 20$ au, ZPE° = 0.160 116 au, $E°_{tran} = 0.889$ kcal/mol, $E°_{rot} = 0.889$ kcal/mol, $E°_{vib} = 109.113$ kcal/mol, $E° = -550.891\ 49$ au, $H° = -550.890\ 54$ au, $S° = 99.804$ cal/mol/K, $G° = -345\ 718.81$ kcal/mol, $T = 298.15$ K. All values are at the B3LYP/6-31+G** level, except for $E°_0$, which is calculated at the MP2(full)/6-311G+(2d,p)//B3LYP/6-31+G** level. For structure **P1**, the value of $E°_0$ at the MP2/6-311G+(2d,p)//MP2/6-31+G** level is −551.068 88 au.

TABLE 3: Relative Energies (kcal/mol) of the Neutral Glutamic Acid Conformers and Their Boltzmann Distribution

structure	$E°_0$	ZPE°	$E°_{vib}$	$E°$	$H°$	$TS°$	$G°$	%
N1[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.7
N2	0.68	−0.14	−0.06	0.61	0.61	0.28	0.34	12.3
N3	1.21	0.07	0.09	1.30	1.30	−0.11	1.41	2.0
N4	1.33	0.04	−0.01	1.32	1.32	−0.28	1.61	1.4
N5	1.46	−0.53	−0.20	1.26	1.26	0.78	0.48	9.6
N6	1.80	−0.60	−0.13	1.67	1.67	1.56	0.11	18.0
N7	2.30	−0.72	−0.24	2.07	2.07	1.79	0.27	13.6
N8	2.57	−0.44	−0.07	2.50	2.50	1.05	1.45	1.9
N9	2.70	−0.73	−0.24	2.46	2.46	2.30	0.16	16.7
N10	2.85	−0.53	−0.23	2.61	2.61	0.97	1.64	1.4
N11	2.91	0.04	0.10	3.01	3.01	0.33	2.68	0.2
N12	3.48	0.05	0.00	3.48	3.48	−0.15	3.63	0.0
N13	3.93	−0.58	−0.25	3.68	3.68	1.21	2.47	0.3
N14	3.94	−0.30	−0.01	3.92	3.92	1.34	2.58	0.3
N15	4.17	−0.66	−0.26	3.91	3.91	1.44	2.47	0.3
N16	4.33	−0.20	−0.01	4.32	4.32	1.27	3.05	0.1
N17	4.62	0.24	0.12	4.74	4.74	−0.88	5.62	0.0
N18	5.19	−0.53	−0.19	5.00	5.00	1.33	3.67	0.0
N19	6.29	−0.75	−0.33	5.97	5.97	0.90	5.07	0.0
N20	6.62	0.01	0.06	6.68	6.68	−0.57	7.25	0.0
N21	7.90	−0.72	−0.22	7.68	7.68	1.55	6.12	0.0

[a] $E°_0 = -550.697\ 93$ au, ZPE° = 0.149 81 au, $E°_{tran} = 0.889$ kcal/mol, $E°_{rot} = 0.889$ kcal/mol, $E°_{vib} = 100.506$ kcal/mol, $E° = -550.5349$ au, $H° = -550.5340$ au, $S° = 101.508$ cal/mol/K, $G° = -345\ 495.6$ kcal/mol, $T = 298.15$ K. All values are at the B3LYP/6-31+G** level, except for $E°_0$, which is calculated at the MP2(full)/6-311G+(2d,p)//B3LYP/6-31+G** level. For structure **N1**, the value of $E°_0$ at the MP2/6-311G+(2d,p)//MP2/6-31+G** level is −550.699 27 au.

TABLE 4: Calculated PA and GB Values (in kcal/mol) for Glutamic Acid[a]

	PA	GB
Boltzmann distribution	224.4	214.4
lowest energy structure	223.2	215.0

[a] Includes a BSSE correction of 2.0 kcal/mol for the lowest energy protonated structure **P1**.

approximate. Despite this caveat, however, some clear trends are evident. Among the nine protonated structures (Table 2), two structures dominate. On the other hand, the neutral conformers have six structures with calculated populations of 9% or greater. This is clearly due to the weaker hydrogen bonding present in the neutral species, as described above.

The calculated Boltzmann averaged GB and gas-phase PA for glutamic acid at 298 K, and 1 atm are listed in Table 4. The largest contributions to the PA and GB arise from the electronic energy and the zero-point energy. For instance, the entropic contribution to the GB ($-T\Delta S°$, excluding translational contributions) for the reaction 5 is −0.5 kcal/mol, compared to a $\Delta E°_{elec}$ of −232.4 kcal/mol and a $\Delta E°_{ZPE}$ of 8.5 kcal/mol. This suggests that the calculated values should be quite reliable, since the electronic and zero-point energies are expected to be calculated with reasonable accuracy. Because both neutral and protonated glutamic acid forms cyclic structures, the main contribution to $\Delta S°$ is from the proton, which is 25.9 cal/mol/K.

Table 4 also reports the gas-phase PA and GB of glutamic acid as derived from the most stable structures **P1** and **N1**. These values are remarkably close to the corresponding Boltzmann-averaged results. This is gratifying, since the Boltzmann averaging can only be considered approximate and a large correction due to Boltzmann averaging would potentially introduce significant error into the theoretically derived value.

In general, the results of the present study can be reconciled with previous experimentally determined values for the PA and GB of glutamic acid. Bojesen and Breindahl[17b] reported a PA value of 222.3 kcal/mol for glutamic acid as compared with the present value of 224.4 kcal/mol. Gorman et al.[14a] reported a GB of 223.7 kcal/mol (using the Meot-Ner scale[12b]) which, if the thermodynamic scale is converted to the more recent scale of Lias,[32] corresponds to a GB value of 217 kcal/mol, in good agreement with the present value of 214.4 kcal/mol. Interestingly, Gorman et al. also report a PA value for glutamic acid of 240.6 kcal/mol which included an entropic correction based on the assumption that only protonated glutamic acid formed a cyclic structure. If the entropic correction is removed and the reported PA value adjusted to the Lias thermodynamic scale, a value of 224 kcal/mol is obtained. Our results strongly suggests that the entropic correction is unnecessary. The evaluated GB and PA values for glutamic acid of 215.6 and 223.4 kcal/mol (no cyclization entropy correction) reported by Harrison[23] are also in excellent agreement with the results of the present study.

Conclusion

We have calculated the GB and gas-phase PA of glutamic acid by using high-level ab initio and DFT methods. Nine lowest energy protonated conformers and 21 lowest energy neutral conformers were identified initially from which two protonated and six neutral conformers were identified as being significantly populated at room temperature. All lowest energy protonated conformers incorporate both five- and seven-membered intramolecular hydrogen bound rings with the protonated amine group. The four lowest energy neutral conformers also incorporate five- and seven-membered intramolecular hydrogen bound rings with the neutral amine group. When appropriate adjustments are made to thermodynamic scales and empirical entropic corrections employed in previous experimental studies are removed, the GB and gas-phase PA values reported here are in excellent agreement with previous experimental measurements.

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Supporting Information Available: Cartesian coordinates and total energies for all structures at the B3LYP level (30 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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