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Preferred Conformers of Proteinogenic Glutamic Acid

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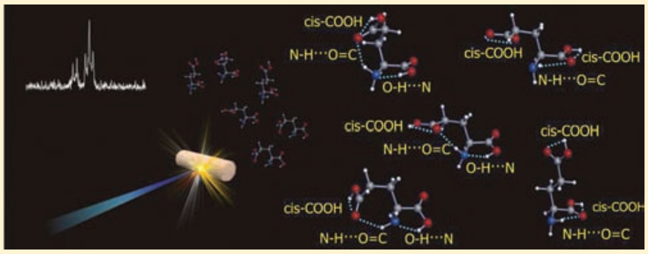
S Supporting Information

ABSTRACT: The molecular shape of proteinogenic glutamic acid has been determined for the first time. Vaporization of the solid amino acid by laser ablation in combination with Fourier transform microwave spectroscopy made possible the detection of five different structures in a supersonic jet. These structures have been identified through their rotational and ^{14}N quadrupole coupling constants. All conformers show hydrogen bonds linking the amino and alpha carboxylic group through N−H···O=C (type I) or N···H−O (type II) interactions. In three of them there are additional hydrogen bonds established between the amino group and the carboxylic group in the gamma position. Entropic effects related to the side chain have been found to be significant in determining the most populated conformations.



I. INTRODUCTION

Knowledge of the shape and conformation of molecules of biological relevance is of interest in a variety of fields, ranging from biology and chemistry to pharmacology and molecular modeling. An important subset of biologically relevant molecules is that of natural amino acids, the so-called "building blocks" of peptides and proteins. Amino acids have long been studied in solid phase[1] and in solution,[2] where they are stabilized as doubly charged species or zwitterions (R−CH(NH$_3^+$)−COO$^-$). Gas-phase studies have the advantage of providing information on the neutral forms of amino acids (R−CH(NH$_2$)−COOH, the canonical forms that are present in peptide side chains) and on their inherent molecular properties free from the intermolecular interactions occurring in the condensed media. Furthermore, gas-phase data can be easily contrasted with theoretical models and used to refine the latter. Among the techniques applied in the gas phase to investigate the structures of amino acids, rotational spectroscopy provides the high resolution necessary to distinguish without doubt between different conformers. The combination of Fourier transform microwave (FTMW) spectroscopy with laser ablation (LA) and molecular beams (MB) in the technique LA-MB-FTMW[3] has allowed our group to characterize the lowest-energy conformers of over 15 different α-amino acids,[4−6] reviving a research line that had been hampered due to the experimental difficulties in transferring solid amino acids to the gas phase.[7,8] These difficulties have been solved with the use and continuous optimization of laser ablation, which vaporizes solid amino acids as neutrals minimizing fragmentation. Our investigations of amino acids have shown that in the supersonic expansion the simplest α-amino acids present two dominant conformers stabilized by either a bifurcated N−H···O=C hydrogen bond with a cis-COOH interaction (type I) or a N···H−O hydrogen bond (type II). There is a third kind of conformers which exhibit an N−H···O−H hydrogen bond (type III). This last type has been only observed in the aliphatic α-amino acids with polar side chains investigated so far (serine,[9] cysteine,[10] threonine,[4] and aspartic acid[5]) and in the nonproteinogenic α-amino acid with a nonpolar side chain, 1-aminocyclopropanecarboxylic acid (Ac$_3$c).[11] In this work we extend our investigations on amino acids with polar side chains to glutamic acid.

Glutamic acid [COOH−CH$_2$−CH$_2$−CH(NH$_2$)−COOH] has attracted the interest of several researchers due to its role as an excitatory neurotransmitter in the brain.[12] The neutral form of glutamic acid constitutes the starting point to determine its gas-phase acidity and basicity, whose values are still being discussed in the literature.[13−15] Experimental characterization of the lower-energy conformers of glutamic acid and the intramolecular hydrogen bonds that stabilize them is essential to help elucidate the correct values of the acidity and basicity of glutamic acid in the gas phase and to understand how glutamic acid interacts with its specific receptors to function as a neurotransmitter. However, to date no experimental studies exist to determine the conformational landscape of glutamic acid in the gas phase.

The conformational study of glutamic acid poses a formidable challenge, as it has a relatively long side chain and three functional groups: an amino group and two carboxylic groups. These features increase the possibilities for intramolecular interactions and give rise to a complex conformational space with a large number of plausible conformers. It is thus not surprising that there has been some speculation about the molecular shape of glutamic acid.[13,14] From the conformational point of view, glutamic acid can be expected to show a

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behavior somehow related to γ-aminobutyric acid (GABA),[16] which has a comparable methylene chain between the amino and carboxylic groups, and to aspartic acid,[5] the other natural amino acid with two carboxylic groups. Both γ-aminobutyric acid and aspartic acid exhibit a high number of low-energy conformers. Nine and six different structures have been identified for GABA[16] and aspartic acid,[5] respectively, by LA-MB-FTMW spectroscopy. We present here the first experimental rotational study of glutamic acid using the aforementioned technique.

II. EXPERIMENTAL DETAILS

The rotational spectrum of glutamic acid was investigated using a LA-MB-FTMW spectrometer[3,4] which works in the 4−18 GHz frequency region. Samples of glutamic acid (99%, Aldrich, mp ~205 °C) were prepared by grinding the amino acid to a fine powder and adding a few drops of a commercial binder. The mixture was then introduced into an in-house designed cast to form cylindrical rods of about 2 cm long. Different binders were tried until the glutamic acid rods had the consistency and resistance required for our experiments. The rods of glutamic acid were placed in our laser ablation nozzle,[4] where they were vaporized using a picosecond Nd:YAG laser using the second (532 nm), third (355 nm), and fourth (266 nm) harmonics. The 266 nm beam provided the best signal-to-noise ratio using ca. 9 mJ per pulse. The amino acid molecules were seeded in a Ne flow at 18 bar and expanded adiabatically into a Fabry−Perot resonator to form a molecular beam. A short microwave radiation pulse (0.3 μs) was then applied to macroscopically polarize the molecules in the beam. The subsequent molecular de-excitation signal was collected and Fourier transformed to obtain the frequency spectrum. In our setup the microwave radiation travels parallel to the axis of the resonator, and consequently, all transitions appear as doublets because of the Doppler effect. The accuracy of the frequency measurements is better than 3 kHz.

III. TOOLS IN CONFORMATIONAL SEARCH AND IDENTIFICATION

Identification of the different coexisting conformers of glutamic acid is based on a thorough investigation and analysis of its rotational spectrum following a systematic procedure already employed in our studies of amino acids.[4] This involves the combined theoretical and experimental approach described below.

III.1. Theoretical Prediction of Molecular Properties.

Theory is used to predict the conformational molecular properties relevant to the investigation of the rotational spectrum and to guide the spectral searches. Only low-lying energy conformers will be sufficiently populated in the supersonic jet to be observed in the rotational spectrum. Therefore, theoretical calculations are first performed to find the lowest-energy conformations in the potential energy surface. For glutamic acid, semiempirical calculations using AM1[17] and PM3[18] were initially carried out. The 40 lowest-energy conformers obtained with each method were subsequently optimized within the Gaussian suite of programs[19] using the MP2 approach[20] and the 6-311++G(d,p) basis set. In this way 37 conformers (25 new conformers to be added to those already reported in the literature[13,14]) were predicted within 900 cm^{-1} (see Figure S1 of the Supporting Information), a large number that reflects the notable richness of the conformational landscape. Each conformer was confirmed to be a local minimum in the potential energy surface by checking that its Hessian matrix did not have any imaginary eigenvalues.

The conformers of glutamic acid have been labeled according to their intramolecular hydrogen bonds. Three possible configurations can be considered attending to the hydrogen bonds established between the α-COOH and $-NH_2$ groups, which are denoted as type **I** (N−H···O=C), type **II** (N···H−O), and type **III** (N−H···O−H). Besides, rotations around the $C_\alpha-C_\beta$ and $C_\beta-C_\gamma$ single bonds give rise to nine different "families" of conformers depending on the values of the dihedral angles $\angle C_{\alpha\text{-COOH}}-C_\alpha-C_\beta-C_\gamma$ and $\angle C_\alpha-C_\beta-C_\gamma-C_{\gamma\text{-COOH}}$. Using the labels **a**, **G**, and **g** to denote dihedral angles close to 180°, +60°, and −60°, respectively, conformers are labeled **aa**, **aG**, **ag**, **ga**, **gG**, **gg**, **Ga**, **GG**, and **Gg**, with the first label referring to the value of the $\angle C_{\alpha\text{-COOH}}-C_\alpha-C_\beta-C_\gamma$ angle and the second to that of the $\angle C_\alpha-C_\beta-C_\gamma-C_{\gamma\text{-COOH}}$ angle (see Figure 1). Finally, **c** or **t** labels indicate the cis or trans arrangement of the γ-COOH group. A number is added to provide the MP2 energy ordering within the same family. The 37 lowest-energy conformers have been grouped by family in Tables 1−4 (see Table S1 of the Supporting Information for the complete listing). No conformers belonging to the **Gg** family were predicted to be minima.

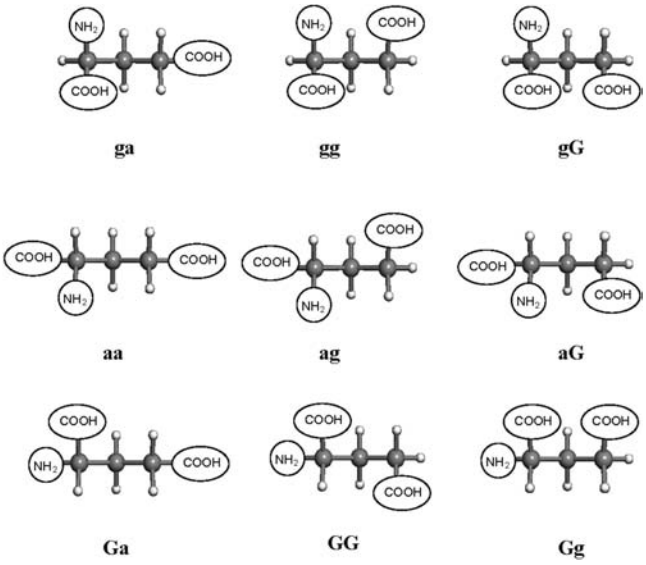

Figure 1. Families of glutamic acid classified according to the dihedral angles $\angle C_{\alpha\text{-COOH}}-C_\alpha-C_\beta-C_\gamma$ (first label) and $\angle C_\alpha-C_\beta-C_\gamma-C_{\gamma\text{-COOH}}$ (second label). The labels **a**, **G**, and **g** indicate dihedral angles close to 180°, +60°, and −60°, respectively.

Besides the relative energy of the conformers, the molecular properties relevant for our rotational studies, namely rotational constants, quadrupole coupling constants, and dipole moment components, are extracted from the theoretical calculations (see Tables 1−4 and S1). Each conformer has distinct values for these spectroscopic parameters, which means that they will give rise to different rotational spectra, enabling us to discriminate between them.

III.2. Rotational Spectrum.

Three different tools of varying importance are used for conformer assignment: rotational constants, quadrupole coupling constants, and dipole moment components. Experimental rotational and quadrupole coupling constants for each rotamer in the rotational spectrum are determined from the analysis of their transitions, once these have been identified. The rotational constants provide information on the mass distribution of each rotamer and are fundamental to obtain the conformational structures. The quadrupole coupling constants yield information on the

Table 1. Rotational Parameters of the Observed Rotamer A Compared with Those Calculated ab Initio for the Lowest-Energy Conformers of Glutamic Acid (below 900 cm^{-1}) from the aG Family^{a}

			ag				
parameter	rotamer A	IaGc1	IIaGc1	IIIaGc1	IaGc2	IaGt1	IIaGc2
A^{b} (MHz)	**3187.31(14)^{f}**	2986	**3159**	3016	2925	3027	2793
B (MHz)	**638.50517(23)**	675	**643**	674	681	650	698
C (MHz)	**568.84986(31)**	630	**573**	640	638	580	632
χ_{aa} (MHz)	**-2.071(46)**	−3.39	**−2.11**	−3.42	−4.02	−4.22	−2.16
χ_{bb} (MHz)	**1.268(98)**	2.27	**1.16**	1.72	2.44	2.70	0.31
χ_{cc} (MHz)	**0.804(98)**	1.12	**0.94**	1.71	1.58	1.52	1.85
$\Delta E_{\mathrm{MP2}}{}^{c}$(cm^{-1})		205	460	512	583	644	714
$\Delta E_{\mathrm{MP2}}{}^{d}$(cm^{-1})		29	360	364	375	623	901
$\Delta G_{298}{}^{e}$ (cm^{-1})		262	524	587	592	973	661

[a] Ab initio calculations performed at the MP2/6-311++G(d,p) level of theory. [b] A, B, and C are the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor. [c] MP2/6-311++G(d,p) electronic energies. [d] Electronic energies corrected with zero-point vibrational energies calculated at the same level. [e] Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parentheses in units of the last digit.

Table 2. Rotational Parameters of the Observed Rotamers B and D Compared with Those Calculated ab Initio for the Lowest-Energy Conformers of Glutamic Acid (below 900 cm^{-1}) from the ag Family^{a}

					ag						
parameter	rotamer B	rotamer D	Iagc1	Iagt	IIagc1	Iagc2	Iagc3	IIagc2	Iagc4	Iagc5	IIagc3
A^{b} (MHz)	**2499.347(90)^{f}**	**2760.353(71)**	**2756**	2643	**2458**	2510	2328	2442	2478	2408	2786
B (MHz)	**723.26216(44)**	**674.51485(22)**	**681**	724	**738**	736	750	736	739	806	660
C (MHz)	**650.35456(47)**	**603.91166(17)**	**612**	659	**662**	657	660	660	659	661	613
χ_{aa} (MHz)	**−2.440(21)**	**−3.488(36)**	**−4.11**	−4.22	**−2.67**	1.61	−3.49	−2.48	1.64	−1.25	−3.08
χ_{bb} (MHz)	**1.440(66)**	**1.744(18)**	**2.11**	2.53	**1.80**	3.13	1.79	1.64	3.12	−0.59	2.40
χ_{cc} (MHz)	**1.040(66)**	**1.744(18)**	**2.00**	1.69	**0.87**	−4.75	1.70	1.45	−4.77	1.84	0.68
$\Delta E_{\mathrm{MP2}}{}^{c}$ (cm^{-1})			245	380	399	549	590	714	763	825	859
$\Delta E_{\mathrm{MP2}}{}^{d}$ (cm^{-1})			0	484	427	455	387	716	648	782	733
$\Delta G_{298}{}^{e}$ (cm^{-1})			0	934	733	699	287	1050	917	1272	899

[a] Ab initio calculations performed at the MP2/6-311++G(d,p) level of theory. [b] A, B, and C are the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor. [c] MP2/6-311++G(d,p) electronic energies. [d] Electronic energies corrected with zero-point vibrational energies calculated at the same level. [e] Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parentheses in units of the last digit.

Table 3. Rotational Parameters of the Observed Rotamer C Compared with Those Calculated ab Initio for the Lowest-Energy Conformers of Glutamic Acid (below 900 cm^{-1}) from the gg Family^{a}

				gg			
parameter	rotamer C	IIggc1	Iggc1	IIggc2	Iggt1	Iggc2	Iggc3
A^{b} (MHz)	**2210.9069(19)^{f}**	**2208**	2366	2198	2433	2299	2392
B (MHz)	**807.15906(36)**	**826**	786	898	780	838	775
C (MHz)	**763.05844(29)**	**792**	767	858	697	792	750
χ_{aa} (MHz)	**1.246(29)**	**1.65/(1.36)^{g}**	−0.50	1.67	−3.23	−1.83	−0.71
χ_{bb} (MHz)	**1.779(25)**	**0.00/(1.77)**	2.73	−4.07	2.52	1.59	0.66
χ_{cc} (MHz)	**−3.025(25)**	**−1.65/(−3.14)**	−2.23	2.40	0.71	1.86	0.05
$\Delta E_{\mathrm{MP2}}{}^{c}$ (cm^{-1})		0	515	606	655	783	887
$\Delta E_{\mathrm{MP2}}{}^{d}$ (cm^{-1})		1	338	602	651	639	707
$\Delta G_{298}{}^{e}$ (cm^{-1})		342	643	1062	1074	829	866

[a] Ab initio calculations performed at the MP2/6-311++G(d,p) level of theory. [b] A, B, and C are the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor. [c] MP2/6-311++G(d,p) electronic energies. [d] Electronic energies corrected with zero-point vibrational energies calculated at the same level. [e] Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parentheses in units of the last digit. [g] The corrected values for the quadrupole constants (see text).

electronic environment of the quadrupolar nucleus (the ^{14}N for glutamic acid) and can be decisive to identify conformers with similar mass distributions but different intramolecular interactions (for example, type I vs type II hydrogen bonds). The values of the predicted dipole moment components determine the microwave power used to polarize the molecules—the higher the dipole moment component, the lower the microwave power needed—and are used in the estimation of the relative abundances of the conformers observed. Although the specific values of the dipole moment components cannot be obtained from our experiment, it is possible to roughly estimate their magnitude from the optimal microwave power used to polarize the transitions belonging to a particular rotamer and check whether they are in accordance with predictions.

Conformational identification is achieved by comparing the experimentally determined molecular properties with those predicted ab initio. Rotational constants, quadrupole coupling constants, and dipole moment components should all be consistent with ab initio values, even if only one of these tools is acting as the discriminating element.

IV. RESULTS AND ANALYSIS

According to the ab initio predictions, all the plausible lowest-energy conformers (Tables 1−4 and S1) are asymmetric rotors close to the prolate limit, and therefore their rotational spectra are characterized by groups of R-branch a-type transitions spaced approximately $B + C$. Considering this, initial scans were directed to search for ^{a}R-branch transitions arising from conformers with large values of the dipole moment component μ_a (see Table S1 of the Supporting Information) using low microwave polarization power. Three different sets of transitions belonging to three distinct rotamers were observed and measured. All observed transitions exhibited the hyperfine structure typical of a species containing a ^{14}N nucleus (see Figure S2 of the Supporting Information), which arises from the interaction between the quadrupole moment of the nitrogen atom and the electric field gradient created by the rest of the molecule at this nucleus. Initial assignments were confirmed by successively predicting additional rotational transitions and measuring them. The measured hyperfine components (see Tables S2−S4) were fit[21] using the semirigid rotor Hamiltonian of Watson in the A reduction and the I^r representation $H_R^{(A)}$[22] supplemented with a term to take into account the quadrupole interaction H_Q,[23] that is, $H = H_R^{(A)} + H_Q$. As shown in the first three columns of Tables 1−3, accurate rotational constants (A, B, C) and the diagonal elements of the quadrupole coupling tensor (χ_{aa}, χ_{bb}, χ_{cc}) were determined for the three rotamers labeled as **A**, **B**, and **C**. The complete results are given in Table S7 of the Supporting Information.

From the values of the experimental rotational constants and their comparison with those predicted by ab initio calculations, each rotamer can be assigned to a family of conformers. Hence, the rotational constants of rotamer **A** listed in the first column of Table 1 are only compatible with those predicted for the conformers of the **aG** family, which are also shown in Table 1. Identification of this rotamer (and others) as a particular conformer cannot rest on the values of the rotational constants alone because many of the predicted conformers within the **aG** family have very similar rotational constants. Fortunately, discrimination can be achieved by considering one of the other tools mentioned in section III.2: the quadrupole coupling constants, whose values depend on the electronic environment of the nitrogen nucleus and the orientation of the amino group within a conformer. By comparing the experimental quadrupole coupling constants with those predicted theoretically in Table 1, it is possible to identify rotamer **A** as conformer **IIaGc1**, which is shown in Figure 3.

The rotational constants of rotamer **B** listed in Table 2 clearly indicate that it belongs to the **ag** family. As occurred for rotamer A, discrimination among the members of the same family can be obtained from the values of the quadrupole coupling constants. Examination of the values of the experimental quadrupole coupling constants with respect to those predicted ab initio allows the identification of rotamer B as conformer **IIagc1** (see Figure 3). It should be noted that the minimum corresponding to conformer **IIagc1** has been found for the first time in this work.

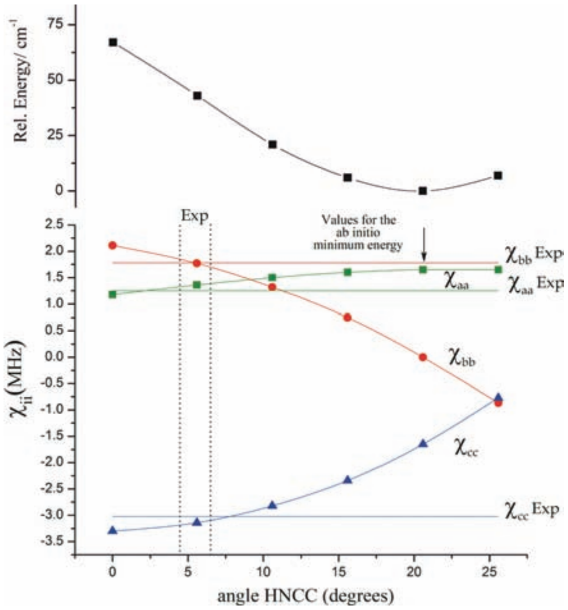

Figure 2. Variation of the quadrupole coupling constants with the dihedral angle HNCC calculated at the MP2/6-311++G(d,p) level of theory for the conformer IIggc1 of glutamic acid.

The experimental rotational constants of rotamer **C** match those predicted theoretically for members of the **gg** family (see Table 3). Again, considering only the values of the rotational constants, it is not possible to assign rotamer **C** to a particular conformer, and the other tools of section III.2 should be used for conformer assignment. Rotamer **C**, though, displays quadrupole coupling constants that are not consistent with any of those predicted for the lower-energy conformers of the **gg** family. The quadrupole coupling constants are very sensitive to the orientation of the $-NH_2$ group in the amino acid and especifically to its disposition with respect to the principal inertial axes. Considering that in the α-amino acids alanine,[7] phenylglycine,[24] and phenyalanine[6] a small change in the amino group orientation produced significant variations in their quadrupole coupling constants, the values of χ_{aa}, χ_{bb}, and χ_{cc} for rotamer C of glutamic acid were calculated for different configurations of the amino group obtained by rotating the amino group around the N−C bond. The results, displayed in Figure 2, show that when the $-NH_2$ group is rotated clockwise ~15°, an excellent agreement between the experimental quadrupole coupling constants and those predicted theoretically for conformer **IIggc1** is attained. The corrected values are given in parentheses in Table 3. Therefore, rotamer **C** is identified as conformer **IIggc1** (see Figure 3). The use of low microwave power to polarize the transitions belonging to this rotamer and the observation of a- and b-type transitions is consistent with the theoretical values of the dipole moment components predicted for conformer **IIggc1** (Table S1 of the Supporting Information), further supporting the assignment. Rotation of the amino group ~15° clockwise in conformer **IIggc1** causes the α-COOH group to move slightly from the equilibrium position of the minimum by rotating ~10° anticlockwise. The overall result is to favor the interaction between the α −COOH and $-NH_2$ groups, as will be discussed in the next section.

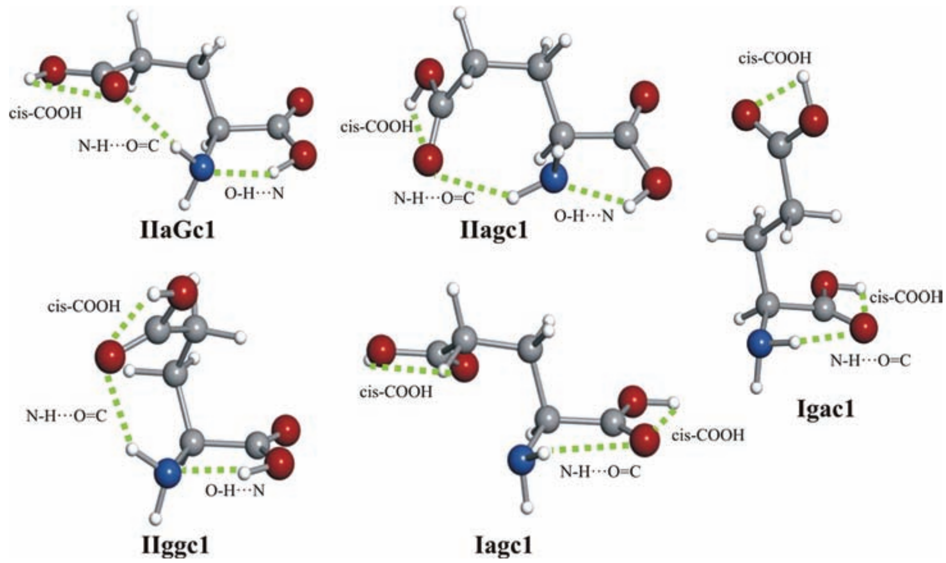

Figure 3. Five observed conformers of glutamic acid, showing their intramolecular interactions.

Subsequent sets of experiments were performed with high microwave polarization powers, aiming to find transitions from conformers with low values of μ_a. Two groups of transitions were detected, which were found to arise from two different species that were initially labeled **D** and **E**. Again, all rotational transitions show ^{14}N quadupole hyperfine structure. Following the procedure described above, the constants displayed in the second column of Table 2 and the first column of Table 4 were obtained (see measured transitions in Tables S5 and S6 and complete results in Table S7 of the Supporting Information). Unfortunately, the small number of measured hyperfine components and the small splittings of the transitions of rotamer **E** precluded an unambiguous assignment of the hyperfine components, and thus, the frequency centers were fit to determine the rotational constants of the first column in Table 4.

Table 4. Rotational Parameters of the Observed Rotamer E Compared with Those Calculated ab Initio for the Lowest-Energy Conformers of Glutamic Acid (below 900 cm^{-1}) from the ga Family[a]

		ga		
parameter	rotamer E	Igac1	IIgac1	Igac2
A^b (MHz)	**2319.1(36)**[f]	**2203**	2259	2219
B (MHz)	**663.3049(33)**	**665**	679	708
C (MHz)	**627.4408(33)**	**645**	646	625
χ_{aa} (MHz)		−0.97	1.80	−1.26
χ_{bb} (MHz)		0.38	−0.57	−0.54
χ_{cc} (MHz)		0.59	−1.23	1.80
$\Delta E_{MP2}{}^c$(cm^{-1})		712	787	862
$\Delta E_{MP2}{}^d$(cm^{-1})		438	650	667
$\Delta G_{298}{}^e$ (cm^{-1})		343	761	629

[a] Ab initio calculations performed at the MP2/6-311++G(d,p) level of theory. [b] A, B, and C are the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor. [c] MP2/6-311++G(d,p) electronic energies. [d] Electronic energies corrected with zero-point vibrational energies calculated at the same level. [e] Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parentheses in units of the last digit.

By comparison of the experimental rotational constants for rotamer **D** and those predicted ab initio, it is possible to assign rotamer D to the **ag** family (see Table 2). Joint examination of the experimental rotational and quadrupole coupling constants with respect to the theoretical ones for members of the **ag** family allow identification of rotamer **D** as conformer **Iagc1** (see Figure 3).

For rotamer E, only the rotational constants are available, and these place this rotamer within the **ga** family (see Table 4). Judging from the values of its rotational constants, rotamer E could be conformer **Igac1** or conformer **IIgac1**. Both of these conformers have a similar predicted value for the μ_a dipole moment component, but they differ in the μ_b value (see Table S1), which is significantly larger for conformer **IIgac1**. Careful searches were performed looking for *b*-type transitions for rotamer **E**, but they were not observed, which points to a low value of μ_b. Taking this into account, together with the higher relative Gibbs energy for conformer **IIgac1**, we tentatively assign rotamer **E** to conformer **Igac1** (see Figure 3). The low values of the quadrupole coupling constants predicted for this conformer are consistent with the tightly packed hyperfine structure observed for this rotamer.

Some very weak rotational transitions remained unassigned in the rotational spectrum. We tried to attribute these lines to other low-energy conformers that could be thought to be present in the supersonic jet, such as conformers **Iaac1** and **IaGc1** (see Table 1 and Table S1 of the Supporting Information). Dedicated searches were conducted to try to find transitions related to them. Unfortunately, no new sets of transitions that could arise from these or other conformers of glutamic acid were discovered, presumably because their intensity is below the detection limit of our spectrometer.

The relative abundances of the five species identified were estimated by measuring the relative intensity of several *a*-type rotational transitions. It has been assumed that the supersonic expansion brings all conformers to their lowest vibrational state[25] and that the intensity of the rotational transition is proportional to the number density of each conformer and the value of the corresponding component of the dipole moment, i.e. to $N_i \cdot \mu_{ai}$ in our case. It is thus taken into account the fact that a strong variation in the dipole moment of the different conformers affects their detectivity, and it may do so in some cases by enhancing the intensity of the lines belonging to weakly populated conformers or weakening the signals of major forms. In addition, as a consequence of the cooling in the jet, the number density, N_i, corresponds to the molecules populating all the vibrational states of a given conformer at the first stages of the expansion, just at the ablation nozzle, where the Ne gas expansion crosses the ablation plume. Evidences have been found suggesting that at this point populations of the different conformers reach a distribution close to that corresponding to the temperature of the expanding gas.[26] Considering this, and using the predicted values of the dipole moment components, the relative abundances in our supersonic jet have been found to be as follows: **Iagc1** > **IIggc1** ≈ **Igac1** > **IIaGc1** > **IIagc1**. The abundances are in very good agreement with expectations from the values of the Gibbs free energies (see Tables 1−4) at 298 K, which can be related directly to conformer populations.[5,26]

V. DISCUSSION

The identified conformers for glutamic acid all show intramolecular hydrogen bonds of type I or II between the α carboxylic group and the amino group (see Figure 3) and have the terminal COOH group in a cis configuration. The most abundant conformer **Iagc1** presents a type I hydrogen bond, i.e. a N−H···O═C hydrogen bond between the $-NH_2$ and the α-COOH that adopts a cis arrangement. Glutamic acid thus follows the behavior displayed by the overwhelming majority of the aliphatic α-amino acids studied so far,[4,5] with a type I conformer as global minimum. The γ-COOH group of **Iagc1**, also in a cis disposition, does not establish hydrogen bonds with any of the other functional groups. This is in sharp contrast with what was observed for other α-amino acids with polar side chains,[4,5,9,10] where all conformers flaunted interactions between all functional groups. For example, the most abundant conformer of aspartic acid,[5] the polar amino acid more closely related to glutamic acid, was stabilized by two N−H···O═C hydrogen bonds between the amino group and the α and β carboxylic groups. In comparison with aspartic acid, glutamic acid has a longer side chain that can adopt a larger number of dispositions. For some of these dispositions, the γ-COOH group is not able to establish any interactions with the other polar groups in the amino acid. These conformations, with less intramolecular interactions, are entropically favored.[15,13,27] This is reflected in the discrepancies between the calculated zero-point corrected energies at the MP2 level and the Gibbs energies for some of the glutamic acid conformations (see Table S1 of the Supporting Information). Extended conformers of glutamic acid with no interactions involving the γ-COOH group tend to increase their entropy and, consequently, are favored as the value of their Gibbs energies decreases, while more folded conformers with strong interactions involving the γ-COOH group decrease their entropy as some rotations around single bonds are effectively hindered. In this respect, glutamic acid shows a behavior similar to that of GABA.[16] In fact, if we substitute the α-COOH group of conformer **Iagc1** with a hydrogen atom, it shows a striking resemblance to the most abundant conformer of GABA.[16]

Conformer **Igac1** is also stabilized by a N−H···O═C hydrogen bond between the α carboxylic group (in a cis disposition) and the amino group. Like the most abundant conformer **Iagc1**, it displays an extended backbone configuration where the acid group in the side chain does not establish any additional interactions with the other polar groups in the amino acid. The other conformers identified, **IIggc1**, **IIagc1**, and **IIaGc1**, present a similar intramolecular hydrogen bond network. Each of them is stabilized by an O−H···N interaction that links the $-NH_2$ group and the α-COOH group, that has thus adopted a trans arrangement. For conformer **IIggc1**, rotation of the amino group by about 15° clockwise and the concomitant rotation of the α-COOH group of ca. 10° anticlockwise as described in the previous section diminishes slightly the distance of the hydrogen bond between these groups, thus favoring their interaction. The amino group in all three conformers **IIggc1**, **IIagc1**, and **IIaGc1** is also involved in an additional interaction: an N−H···O═C hydrogen bond with the γ-COOH group, which adopts a cis configuration. This additional hydrogen bond increases the Gibbs energies of these conformers, which are significantly less populated than conformer **Iagc1**. In the case of conformer **IIggc1**, clockwise rotation of the amino group by approximately 15° decreases the hydrogen bond distance between the hydrogen atom of the $-NH_2$ group and the carbonyl oxygen of the α-COOH group. Thus, the amino group rotation also favors the interaction between the $-NH_2$ and the γ-COOH group.

No type III conformers with an N−H···O−H hydrogen bond between the amino and α-COOH groups have been detected. Type III conformers have only been observed in those α-amino acids where rotation of the α-COOH group is hindered, either by establishment of interactions with polar groups other than the amino group, as in the aliphatic α-amino acids with polar side chains,[4,5,9,10] or by restrictions due to conjugation effects, as in the α-amino acid Ac3c.[11] In the aliphatic α-amino acids with polar side chains,[4,5,9,10] the hydrogen bonds established by the polar side chain reversed the conformational preferences (making type III conformers more stable than their type I analogues) or increased the III↔ I interconversion barriers so that type III conformers did not relax to type I forms in the supersonic expansion.[4,5,9,10] Glutamic acid has a polar side chain $-CH_2-CH_2-COOH$ with the same functional group as that of aspartic acid ($-CH_2-COOH$) but possessing just one more methylene group. However, this apparently small increase in chain length produces a strong impact on the conformational preferences. Aspartic acid has 15 conformers predicted to be within 800 cm^{-1}, of which 4 were type III conformers.[5] For glutamic acid, there are 37 predicted conformers within 900 cm^{-1}, and of these only 3 exhibit a type III hydrogen bond. The number of possible conformations has doubled in glutamic acid while the chances that these conformations are stabilized by a type III hydrogen bond have drastically diminished. This is a result of having less low-energy conformers with interactions involving the side chain, as these are not favored entropically. In addition, our ab initio calculations predict that conformers **IIIaGc1** and **IIIaac1** could relax to conformers **IaGc1** and **Iaac1**, respectively, through low interconversion barriers (see Figures S3 and S4 of the Supporting Information).[26,28−30]

VI. CONCLUSIONS

The molecular shape of glutamic acid has been investigated in the gas phase for the first time. Five different structures of this amino acid have been observed in the rotational spectrum. The most abundant conformer has been identified as a form bearing a type I hydrogen bond. This data can be of use in future studies on the gas-phase acidity and basicity of glutamic acid. In some cases, attempts have been made to explain the experimental values obtained for these properties by theoretical calculations considering a single conformer—that predicted theoretically to be most stable.[13,15,31] Given the mixture of conformers for glutamic acid established in this work, an approach that takes into account several populated conformers[14] would be more appropriate.

The role of the quadrupole coupling constants has been found to be priceless to identify without doubt the observed rotamers to specific conformers. The ^{14}N quadrupole coupling also allows knowledge of the orientation of the amino group in the amino acid and is relevant to extract conclusions about the intramolecular interactions that take place between the different functional groups.

Notable differences have been found between the behavior of glutamic acid and that shown by the other polar amino acids studied so far. In serine,[9] threonine,[4] cysteine,[10] and aspartic acid,[5] the side chain ($-CH_2-OH$, $-CH(OH)-CH_3$, $-CH_2-SH$, and $-CH_2-COOH$, respectively) has been found to change the conformational preferences with respect to aliphatic α-amino acids without polar side chains: the number of possible conformations increased, and conformers with type III hydrogen bonds between the α-COOH and $-NH_2$ groups were observed and sometimes found to be more stable than their analogues with type I hydrogen bonds. While the first effect is also—and more pronouncedly—observed in glutamic acid, the second effect is much more weakened. This is related to the longer side chain $-CH_2-CH_2-COOH$ of glutamic acid that confers larger flexibility and multiplies the conformational possibilities but also changes the influence of the interactions involving the γ-COOH group and the other polar groups in the amino acid. The increased length of the side chain in glutamic acid makes entropic contributions arising from the presence of intramolecular interactions to be more significant than those in other polar amino acids. This is reflected in the fact that the most populated conformer of glutamic acid is an extended conformer where the γ-COOH group does not interact with the $-NH_2$ or α-COOH groups.

Although the effect of the side chain in driving the conformational preferences of glutamic acid is not as spectacular as that observed for other polar α-amino acids, its influence should not be underestimated. The amino acid side chain has been found to be relevant in protein−protein binding. Some amino acid residues are much more prevalent than others in the hot spots at protein interfaces that make the major contributions to binding energy.[32] In particular, glutamic acid has been found to appear less frequently than aspartic acid in hot spots, which has been attributed to entropic effects related to side chain conformations.[33]

The investigation of individual neutral amino acids in the gas phase is revealing a complex and subtle network of forces that conditions the conformations that amino acids adopt. Building on this knowledge, interactions of amino acids with water molecules can be explored. These are relevant for the long-term attempt to describe amino acid solvation at a microscopic level and the transition from canonical structures in the gas phase to zwitterionic ones in solution.[34] Some steps have already been taken in this direction with the experimental investigation of the complexes tryptophan−$(H_2O)_{1-6}$[35,36] and phenylalanine−$(H_2O)_{1-3}$[37,38] using IR-UV double resonance spectroscopy. Complexes of the aliphatic amino acids glycine−$(H_2O)_{1,2}$[39,40] and alanine−$(H_2O)_{1,2}$[40] have been studied by LA-MB-FTMW spectroscopy, which does not need the presence of a chromophore, as do other laser spectroscopic techniques, and thus can be applied to a wider range of biological molecules. These investigations had allowed exploration of questions such as the changes induced by complexation on the balance of forces and on amino acid conformations. More investigations of intermolecular complexes involving amino acids would contribute to improving our understanding of the factors that modulate their interactions and that have implications in a huge number of biological processes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of molecular properties predicted ab initio for all conformers within 900 cm^{-1}, measured transitions, and complete set of spectroscopic constants for the five observed conformers of glutamic acid, supplementary figures, and complete ref 19. This material is available free of charge via the Internet at http://pubs.acs.org.

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