Gas-Phase Conformations

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Conformations of γ-Aminobutyric Acid (GABA): The Role of the n→π* Interaction**

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γ-Aminobutyric acid (GABA) is arguably the most important inhibitory neurotransmitter in the brain and brainstem/spinal cord.[1] Owing to the high torsional flexibility of its heavy-atom backbone, this molecular system has a large number of low-energy conformers (see Figure 1). Identifying the stable conformers of GABA can be relevant to understanding the selectivity of the biological processes in which the neurotransmitter participates. This can be done by placing GABA on a supersonic jet such that conformers are cooled down and trapped in their energy minima. In such an isolated environment the conformers with sufficient population can be detected and studied by spectroscopic methods. In this context, several methods have made great contributions to the elucidation of the structures of biomolecules in the gas phase.[2] In the present work we have observed and characterized nine conformers of GABA using Fourier transform microwave spectroscopy in supersonic jets combined with laser ablation.

Microwave spectroscopy, considered the most definitive gas-phase structural probe, can distinguish between different conformational structures since they have unique moments of inertia and give separate rotational spectra. In general, large molecules, in particular those of biological importance, have low vapor pressures and tend to degrade upon heating, making them unsuited for structural studies in the gas phase. Recently, rotational studies of biomolecules have entered in a new stage with the LA-MB-FTMW experiment, which combines laser ablation (LA) with molecular beam Fourier transform microwave spectroscopy (MB-FTMW),[3] an approach that overcomes the problems of thermal decomposition associated with conventional heating methods. To date, different α- and β-amino acids[4] have been studied using this technique, making it possible to characterize their preferred conformations. Even in conformationally challenging systems these can be identified by rotational spectroscopy, as has been illustrated with the assignment of seven low-energy conformers of serine[5] and threonine,[4] six of cysteine,[6] and four of β-alanine[7] and proline.[8]

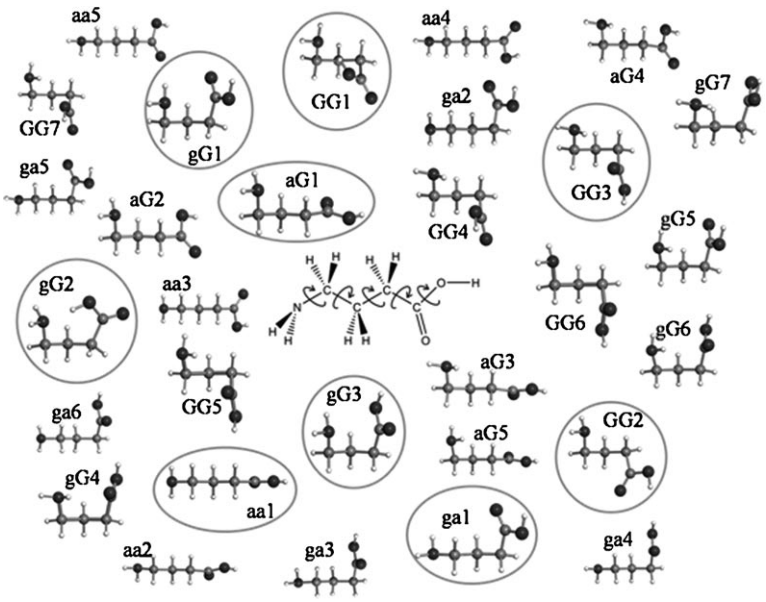

Figure 1. Predicted low-energy conformers of GABA. The nine observed conformers are circled.

In the present work, we have examined the conformations of GABA. In this system the separation of the polar amino and carboxylic groups, characteristic of many families of neurotransmitters,[9] opens new conformational possibilities with respect to α-amino acids[4] if one considers the balance of intramolecular forces that contribute to stabilizing the different conformations.

The five hindered rotations around the single bonds generate a plethora of conformational species (Figure 1). An overall picture of the conformational landscape obtained from theoretical predictions at the MP2/6-311++G(d,p) level[10a] confirms the conformational richness of GABA: the 30 feasible conformers shown in Figure 1 were localized with relative energies below 900 cm^{-1}. These conformers are labeled by two letters (**a**, **G**, or **g**) followed by a number. The first letter refers to the configuration at C_α and the second one to the configuration at C_γ: **a** means *anti* conformers, **G** *gauche* conformers with positive value of the torsional angle C_{COOH}-C_α-C_β-$C\gamma$ or N-$C\gamma$-C_β-C_α, and **g** *gauche* conformers

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with negative values of this angle. Using these labels, five families of conformers, **gG**, **GG**, **ga**, **aG**, and **aa**, can be distinguished. The number that completes the designation corresponds to the order within each family by increasing calculated MP2 energy. The **aa**, **ga**, and **aG** conformers correspond to extended configurations of the amino acid backbone, while the **gG** and **GG** conformers correspond to folded forms (Figure 2). In **GG** conformers the amino and carboxylic acid polar groups are on different sides of the plane C_α-C_β-C_γ, while in **gG** forms the amino and carboxylic acid polar groups lie on the same side. The predicted rotational constants, electric dipole moment components, nuclear quadrupole coupling constants, and Gibbs free energies are listed in Table 1 (see also Tables S10 and S11 in the Supporting Information).

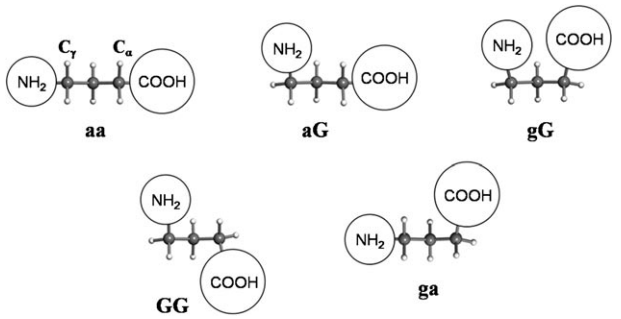


Figure 2. The five families of conformers of γ-aminobutyric acid arising from the *anti* (**a**) or *gauche* (**g** or **G**, see text) configurations at C_α and C_γ atoms.

Using the experimental setup described below, wide-frequency scans were conducted to search for the spectral signatures of the most stable conformers of GABA. A crowded and complex rotational spectrum was observed in the range 4 to 12 GHz. Several sets of μ_a-type R-branch transitions belonging to rotamers close to the prolate symmetric top limit were identified. For each set an iterative process of fittings and predictions led to the observation of additional μ_b- and/or μ_c-type R-branch transitions. All transitions were split into several hyperfine components arising from the presence in GABA of ^{14}N (I = 1). This nucleus has a non-zero quadrupole moment which interacts with the electric field gradient created at the nitrogen atom position by the rest of charges in the molecule. This interaction couples the nuclear spin and the rotation angular momentum which results in a nuclear hyperfine structure in the rotational spectrum.[11] Analysis of this structure gives the elements of the quadrupole coupling tensor $\chi_{\alpha\beta}$, which is related to the electric field gradient tensor elements by $\chi_{\alpha\beta} = eQq_{\alpha\beta}$, where eQ is the nuclear quadrupole moment. MB-FTMW spectroscopy provides the high resolution needed to fully resolve this hyperfine structure (see Figure S1 in the Supporting Information). Thorough analysis of the rotational spectra finally led to the assignment of nine different rotamers of GABA labeled *a*–*i*. All measured transition sets (see Tables S1–S9 in the Supporting Information) were analyzed[12] using the Hamiltonian $H = H_R^{(A)} + H_Q$, where $H_R^{(A)}$ represents Watson's A-reduced semirigid rotor Hamiltonian[13] in the I^r representation, and H_Q is the nuclear quadrupole coupling interaction term.[14] The rotational and quadrupole coupling constants determined for the nine rotamers are summarized in Table 2. (A complete set of parameters is provided in Table S10 of the Supporting Information).

The spectroscopic parameters listed in Table 2 provide unequivocal evidence of the existence of nine conformers of GABA which differ in the folding and orientation of their functional groups in the molecular frame. A distinctive attribute of these spectroscopic constants is that they can be directly compared with those predicted in vacuo from ab initio methods (see Table 1 and Figure 1). The rotational constants, which depend directly on the mass distribution and geometry, can then be used in a first step to identify the conformers.[4] The constants for rotamers *a*–*f* (see Table 2) are compatible with those predicted for several members of the **gG** and **GG** families, while those of rotamers *g* and *h* are comparable to those of the **ga** and **aG** families (see Table 1). The values of rotamer *i* match only those of the **aa** family. For conformers showing a similar amino acid backbone the difference in the rotational constants between conformers is not large enough to distinguish them. Fortunately in these cases an independent approach to identify the conformers, based on the ^{14}N quadrupole coupling effects, can be used. These effects depend strongly on the orientation of the NH_2 group with respect to the principal inertial axis system which is different for each conformer. This is reflected in the values of the ^{14}N nuclear quadrupole coupling constants χ_{aa}, χ_{bb}, and χ_{cc} listed in Table 2, which can be used to discriminate the conformers. Thus, a detailed comparison between the experimental and theoretical values of the rotational and quadrupole coupling constants in Tables 1 and 2 lead to the following identification: rotamer *a* corresponds to conformer **gG1**, *b* to **gG2**, *c* to **gG3**, *d* to **GG1**, *e* to **GG2**, and *f* to **GG3**. The rotational constants of rotamer *g* are consistent with those predicted for conformers **ga1**, **ga2**, and **ga5** but are assigned to **ga1** because of the quadrupole coupling constants. In the same way, the rotational constants of rotamer *h* agree with those predicted for the entire family of conformers **aG**, and rotamer *i* with those of family **aa**, but the quadrupole coupling constants match only with those predicted for conformers **aG1** and **aa1**, respectively. The presence of nitrogen in GABA thus makes the ^{14}N quadrupole coupling an exceptional tool for conformer identification. These assignments are further confirmed by the consistency of the microwave power applied to polarize each rotational transition and the intensities of the observed lines with the predicted electric dipole moments (see Table 1).

All of the above considerations conclusively support the existence of the nine conformers of GABA in the gas phase circled in Figure 1. The good agreement between the observed and predicted values of rotational and quadrupole coupling constants also indicate that their actual geometries should be close to those theoretically predicted (see Table S12 in the Supporting Information). Thus, the intramolecular interactions in GABA can be analyzed through the configurations adopted by the detected conformers (see structures

Table 1: Predicted spectroscopic parameters for the low-energy conformers of γ-aminobutyric acid (GABA) from ab initio MP2/6-311++G(d,p) calculations.

Parameter	**gG1**(*a*)[a]	**gG2**(*b*)	**gG3**(*c*)	**gG4**	**gG5**	**gG6**	**gG7**	**GG1**(*d*)	**GG2**(*e*)	**GG3**(*f*)
A [MHz]	4129	4806	3937	3824	3940	3785	3886	4320	4705	4232
B [MHz]	1835	1759	1898	1943	1817	1893	1908	1955	1492	1859
C [MHz]	1671	1380	1717	1649	1646	1668	1624	1583	1426	1563
χ_{aa} [MHz][b]	−2.64	−3.63	−3.54	0.81	−1.49	−1.78	0.43	−2.78	−1.65	1.12
χ_{bb} [MHz]	2.61	2.35	2.64	2.50	−0.46	−0.32	2.41	1.30	0.38	1.07
χ_{cc} [MHz]	0.03	1.28	0.90	−3.30	1.95	2.10	−2.84	1.48	2.03	−2.18
$\mu_a/\mu_b/\mu_c$/[D]	1.4/0.2/0.7	7.0/0.9/0.5	2.4/0.3/1.5	1.2/1.0/0.2	0.7/1.2/1.1	0.3/2.1/1.1	1.8/0.7/2.5	6.2/1.1/1.3	1.3/0.4/0.4	0.3/0.9/1.2
ΔE_{MP2} [cm^{-1}][c]	0	216	423	544	677	756	839	30	173	129
ΔG [cm^{-1}][d]	136	559	513	615	622	732	892	667	0	270
$\Delta E_{MP2+ZPC}$ [cm^{-1}][e]	0	370	385	563	622	732	827	329	94	217
Parameter	**GG4**	**GG5**	**GG6**	**GG7**	**ga1**(*g*)	**ga2**	**ga3**	**ga4**	**ga5**	**ga6**
A [MHz]	4163	4099	4296	4085	5772	5776	4884	4830	5994	4856
B [MHz]	1859	1847	1657	1850	1292	1289	1347	1368	1280	1347
C [MHz]	1556	1567	1480	1556	1209	1202	1345	1342	1191	1339
χ_{aa} [MHz][b]	1.05	−0.11	1.68	0.11	1.13	1.58	0.94	2.76	2.83	1.89
χ_{bb} [MHz]	0.40	0.58	0.87	0.32	−2.02	1.23	−2.93	0.36	0.36	−0.58
χ_{cc} [MHz]	−2.20	−0.47	−0.29	−0.42	0.89	−2.81	1.99	−3.13	−3.19	−1.31
$\mu_a/\mu_b/\mu_c$/[D]	0.8/1.2/0.4	0.7/2.3/0.7	1.7/2.0/0.7	0.7/0.2/1.9	0.8/0.2/0.9	0.8/1.8/0.8	0.3/0.5/1.3	1.0/0.6/0.6	0.1/1.9/1.5	0.4/1.2/2.3
ΔE_{MP2} [cm^{-1}][c]	295	456	493	528	475	543	568	638	663	665
ΔG [cm^{-1}][d]	440	499	378	607	255	319	456	459	404	531
$\Delta E_{MP2+ZPC}$ [cm^{-1}][e]	366	485	459	557	387	439	532	592	547	612
Parameter	**aG1**(*h*)	**aG2**	**aG3**	**aG4**	**aG5**	**aa1**(*i*)	**aa2**	**aa3**	**aa4**	**aa5**
A [MHz]	6240	6268	6339	6313	6334	8107	8101	6900	7015	6846
B [MHz]	1196	1210	1212	1196	1177	1044	1054	1064	1075	1071
C [MHz]	1108	1136	1069	1136	1070	945	953	1011	1013	1025
χ_{aa} [MHz][b]	−0.72	−0.58	0.80	1.55	−4.33	−1.92	2.53	−2.46	2.37	2.36
χ_{bb} [MHz]	−1.18	2.41	2.38	−3.87	2.15	−0.11	1.37	1.84	1.44	−3.37
χ_{cc} [MHz]	2.31	−1.83	−3.18	2.32	2.18	2.03	−3.90	0.62	−3.81	1.00
$\mu_a/\mu_b/\mu_c$/[D]	1.7/0.4/0.6	2.5/1.1/1.1	1.1/1.4/1.5	1.8.0.1/0.0	0.9/0.9/0.0	1.0/0.5/0.0	0.4/1.8/1.2	0.1/1.4/0.3	1.5/1.5/1.8	1.3/0.0/0.9
ΔE_{MP2} [cm^{-1}][c]	365	507	711	801	820	709	729	778	784	793
ΔG [cm^{-1}][d]	72	250	348	552	387	235	341	422	493	703
$\Delta E_{MP2+ZPC}$ [cm^{-1}][e]	240	435	566	746	651	523	567	679	670	373

[a] The designation for the experimentally observed conformers is given in parentheses (see text). [b] The ^{14}N quadrupole coupling constants were obtained using the conversion: $\chi_{\alpha\alpha}/MHz = 2.34965\ (Q/fm^2)\ (q_{\alpha\alpha}/au)$, where $q_{\alpha\alpha}$ is the corresponding element of the electric field gradient at the N nucleus and the quadrupole coupling moment for ^{14}N is taken to be $Q = 2.09\ fm^2$, after Ref. [10b]. Only diagonal elements are shown. [c] Electronic energies relative to conformer **GG1** have been calculated at the MP2/6-311++G(d,p) level. [d] Free energies relative to conformer **GG1** have been calculated at 298.15 K at the MP2/6-311++G(d,p) level with a harmonic model and analytical frequency computations. [e] Electronic energies relative to conformer **GG1** have been corrected by zero-point harmonic energies.

Table 2: Experimental spectroscopic constants for the nine conformers observed for γ-aminobutyric acid (GABA).[a,b]

Constants	*a* (**gG1**) Exptl	*b* (**gG2**) Exptl
A [MHz]	4194.9642(12)	4812.370(26)
B [MHz]	1792.83869(43)	1744.79613(28)
C [MHz]	1630.69973(40)	1366.92136(31)
χ_{aa} [MHz]	−2.2730(22)	−3.5199(27)
χ_{bb} [MHz]	2.5531(33)	2.2356(44)
χ_{cc} [MHz]	−0.2801(33)	1.2844(44)

Constants	*c* (**gG3**) Exptl	*d* (**GG1**) Exptl
A [MHz]	3945.5756(29)	4353.071(31)
B [MHz]	1870.42013(50)	1921.21022(50)
C [MHz]	1692.91852(47)	1556.84937(65)
χ_{aa} [MHz]	−3.3400(27)	−2.6881(57)
χ_{bb} [MHz]	2.5410(54)	1.204(14)
χ_{cc} [MHz]	0.7990(54)	1.484(14)

Constants	*e* (**GG2**) Exptl	*f* (**GG3**) Exptl
A [MHz]	4900.06532(81)	4262.004(29)
B [MHz]	1443.40600(26)	1819.53105(45)
C [MHz]	1383.35361(26)	1538.74556(60)
χ_{aa} [MHz]	−1.1416(20)	0.9642(37)
χ_{bb} [MHz]	−0.6623(30)	1.0724(61)
χ_{cc} [MHz]	1.8039(30)	−2.0366(61)

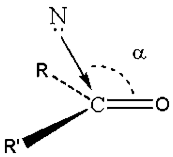
Constants	*g* (**ga1**) Exptl	*h* (**aG1**) Exptl
A [MHz]	6114.0(1.6)	6264.18514(70)
B [MHz]	1274.39025(91)	1198.21581(17)
C [MHz]	1174.57435(95)	1094.52623(17)
χ_{aa} [MHz]	1.197(19)	−1.0180(17)
χ_{bb} [MHz]	−2.063(77)	−1.2396(24)
χ_{cc} [MHz]	0.865(77)	2.2576(24)

Constants	*i* (**aa1**) Exptl
A [MHz]	8135.3(1.1)
B [MHz]	1043.37754(40)
C [MHz]	946.32993(40)
χ_{aa} [MHz]	−1.696(18)
χ_{bb} [MHz]	−0.004(75)
χ_{cc} [MHz]	1.700(75)

[a] A, B, and C are the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are the diagonal elements of the ^{14}N nuclear quadrupole coupling tensor. [b] Standard error in parentheses in units of the last digit.

in Table 2). In conformers **GG2** and **aG1**, and in the extended forms **aa1** and **ga1**, the two polar groups are far apart and no intramolecular interactions are apparent, apart from a stabilizing *cis*-COOH functional group interaction. In folded configurations noncovalent interactions can be established between the two polar groups. The most obvious interaction to be expected is hydrogen bonding, which plays a crucial role in the conformational equilibria of α-amino acids.[4] Conformers **gG2** and **GG1** are stabilized by intramolecular hydrogen bonds O–H···N that force a *trans*-COOH arrangement, while conformer **GG3** is stabilized by a N–H···O=C hydrogen bond. These hydrogen-bond interactions are similar to those observed in nonpolar aliphatic α-amino acids[13] except for the nonbifurcated character of N–H···O=C hydrogen bond.

Conformers **gG1** and **gG3** show an arrangement of the amino and carboxyl groups that does not correspond to the existence of hydrogen bonds between them (see Table 2). The N atom of the amino group approaches the the C=O group in a way that resembles the Bürgi–Dunitz trajectory[16] for the most favorable approach of a nucleophilic N atom to the C atom of a carbonyl group in an addition reaction (see Scheme 1). A n→π* interaction is established from the hyperconjugative delocalization of the nonbonding electron pair of the nitrogen atom into the π* orbital at the carbonyl group (see Figure 3).



Scheme 1. Geometry of the Bürgi–Dunitz trajectory.

In the Bürgi–Dunitz trajectory[16] (see Scheme 1) the approach path of the nucleophile (N:) lies in the plane bisecting the R-C-R′ angle with α values of about (105 ± 5)° for shotr N to C distances. The ab initio N to C distance for conformers **gG1** and **gG3**, predicted in both cases to be 2.86 Å, is within the range of distances observed in crystals (1.5 to 3 Å).[16] For conformer **gG1** the calculated N-C-O angle is 88°, not far from the range of best values of α, while for conformer **gG3** the predicted value, 108.7°, is optimal. We have performed a nonbonding orbitals (NBO) analysis[17] of the different forms of GABA at the B3LYP/6-311++G(d,p) level that further supports the existence of this n→π* interaction for conformers **gG1** and **gG3**, with calculated stabilization energies of 10.2 and

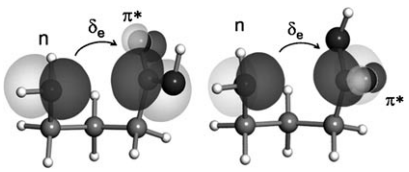


Figure 3. Representation of the n→π* interaction in conformers **gG1**(*a*) and **gG3**(*c*) of GABA.

14.8 kJ mol^{-1}, respectively. The higher stabilization energy for conformer **gG3** is in good agreement with the most favorable Bürgi–Dunitz arrangement in this conformer. The fact that conformer **gG1** is more stable could be explained by the existence of a N–H to C=O dipole–dipole interaction, which is more stabilizing than the possible N–H to C–OH interaction in conformer **gG3**. We have observed this n→π* interaction in β-alanine.[7] Interactions in which the donor atom is oxygen, reported to be relevant in proteins,[18] have been detected in (4*S*)-hydroxyproline[19] between the oxygen atom of the 4-hydroxy group and the carbonyl group.

The relative populations of the conformers in the supersonic jet provided by relative intensity measurements carried out on μ_a-type selection rules transitions could be correlated qualitatively with the predicted relative ΔE and ΔG electronic and Gibbs energies of Table 1.[15a] By assuming that the expansion cools down all molecules to the low vibrational energy state of each conformer, the intensity of a transition belonging to conformer *i*, with number density N_i in the jet, has been assumed to be proportional to $\mu_{ai}\ N_i$. Taking into account the predicted μ_a electric dipole moment values (see Table 2), the relative populations in the supersonic jet follow the order *GG2* > *aG1* > *gG1* > *aa1* > *ga1* in concordance with the theoretical Gibbs energies ΔG calculated for GABA (see Table 2) as could be expected. Conformers *GG1*, *GG3*, *gG2* and *gG3* have weaker lines so that relative intensity measurements on them are not reliable. In previous investigations on the rotational spectra of amino acids[15] the relative populations of conformers in the supersonic jet could be correlated qualitatively with the predicted electronic energies. For GABA the relative population ordering strongly disagrees with the predicted MP2 relative energies: *gG*1 < GG1 < GG3 < *GG*2 < *gG*2 < aG1 < gG3 < ga1 < aa1. This disagreement can be taken as a proof for the coexistence in GABA of conformers having intramolecular interactions (folded) with those free of them (extended).[20] The later are less rigid and would have an increase in entropy related to a higher density of accessible vibrational states. In other words, intramolecular interactions contribute to decrease entropy and to increase the Gibbs energy thus diminishing number density. In this way conformers of GABA free of intermolecular interactions are the most abundant. The fact that conformer gG1 is still one of those highly populated in the supersonic expansion and the concordance between relative intensities and predicted Gibbs energies can be taken as an indication that the conformational stability order could be close to the theoretical one that predicts conformer *gG1* as the global minimum. These results also show the importance of using Gibbs energies instead of electronic energies to correctly predict relative population of conformers when forms with and without intramolecular interactions coexist.[20]

Notice the fact that for the families of conformers *ga*, *aG* and *aa*, that do not show any intramolecular interaction, only the most stable conformer within each family has been observed. This could be attributed to conformation relaxation processes occurring in the supersonic jet that bring molecules from high energy to low-energy conformers when the path between them involves low energy barriers.[21] The investigation of the potential energy surface for interconversion between the different conformers of GABA shows that conformational relaxation is possible (see figures S2 of supplementary information).

The present experimental observations of GABA contribute o improve our understanding of the role of intramolecular forces in the conformational behaviour of amino acids in gas phase. The results indicate that: (i) the prevalence of O–H···N and N–H···O=C hydrogen bonds as main stabilizing contributions in α-amino acids disappears as the length of the carbon chain between the -NH_2 and -COOH groups increases; (ii) The intramolecular n→π* interaction, of different nature, appears to be relevant in the stabilization of folded configurations and in GABA contributes to stabilize the conformer predicted at the global minimum; (iii) Non-folded configurations with no intramolecular interactions appear to have high concentrations relative to folded configurations due to entropy effects. GABA thus constitutes a reference molecule to probe the coexistence of a variety of intramolecular interactions stabilizing biomolecules.

Experimental Section

The rotational spectrum of GABA was investigated by using a LAMB-FTMW spectrometer[3d] working in the 5–18 GHz frequency region. Solid rod samples were made from powdered GABA (99%, Aldrich) and vaporized by ablation with the second and third harmonics of a Nd:YAG laser (ca. 50 mJ and 18 mJ per pulse, 5 ns and 150 ps width pulse, respectively). The vaporized molecules were seeded in a Ne (5 bar) stream expanding supersonically between the mirrors of an evacuated Fabry-Pérot microwave resonator. A microwave radiation pulse (0.3 ms), was subsequently applied to cause the macroscopical polarization of the molecules in the beam. The immediate molecular de-excitation signal, which containing the resonance frequencies corresponding to the rotational transitions, was collected and transfer to the frequency domain spectrum by a Fourier transform process. The spectrometer has a collinear arrangement of the supersonic jet and resonator axis, for this reason each line in the spectrum appeared as a Doppler doublet. The transition frequencies are calculated as the arithmetic mean of the Doppler components. Different experiments, at the same frequency polarization, can be phase coherently co-added, so thousand of cycles can be accumulated to measure very weak transitions. The estimated accuracy of the frequency measurements is better than 3 kHz.

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