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Theoretical Investigation of the Neutral/Zwitterionic Equilibrium of γ-Aminobutyric Acid (GABA) Conformers in Aqueous Solution

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Since γ-aminobutyric acid does not form a stable zwitterionic species in the gas phase, as calculated at the HF/6-311++G** level, the GABA·$2H_2O$ system was optimized for several neutral and zwitterionic GABA tautomers/conformers. The obtained molecular geometries and vibrational frequencies determined for the dihydrates reflect structural changes for GABA due to close and strongly bound water molecules. By use of GABA geometries optimized in the dihydrates, relative free energies of different species in aqueous solution were calculated. MP2/6-311++G**//HF/6-311++G** energy values show that the neutral form is strongly preferred over the zwitterionic one for the isolated GABA species. The neutral tautomer, which is most stable in the gas phase, is only marginally changed by hydration; it is without an intramolecular hydrogen bond and has nearly gauche−gauche arrangements, 54 and −83°, for the NCCC and the CCCC torsion angles, respectively, as determined in the dihydrate. In aqueous solution the zwitterionic structure is dominant. Comparison of cyclic gauche−gauche forms and a partially extended, gauche−trans structure indicates the preference of the more extended form. This structure differs from the trans−gauche zwitterionic conformer found for GABA by X-ray experiments in the crystalline phase. The experimental GABA conformer is not stable either in the isolated form or in the gas-phase dihydrate. It is, however, more stable by about 6.5 kcal/mol than the gas-phase gauche−trans form, as turned out in a restricted geometry optimization. Such a large internal stabilization may allow the existence (even preference) of the experimental zwitterionic GABA structure in aqueous solution if solvent effects are preferable. Partition of GABA between water and chloroform is not favored. At least 7.5 kcal/mol free energy increase is required if the zwitterion either directly or after transformation to a neutral form would leave the aqueous phase and enter chloroform. This result supports the experimental finding that GABA does not cross the blood−brain barrier.

Introduction

Organic compounds having both protonating and deprotonating groups in a single molecule can exist, depending on the environment, in structural isomers of the regular neutral and/or zwitterionic forms. Proper determination of their equilibrium presents a special challenge to theoretical and computational chemistry. Usual quantum chemical procedures treat molecules as isolated entities, i.e., without any intermolecular interactions. For amino acids this means that the isolated neutral form corresponds to an energy minimum on the potential energy surface. The questions may be raised, however, whether the zwitterion also forms a local energy minimum and, if yes, is it higher or lower in energy than the neutral isomer.

Many zwitterionic systems are expected to be stable in condensed phases. Sulfamic acid,[1] pyridinecarboxylic acids,[2] ectoine, a tetrahydropyrimidine carboxylic acid derivative,[3] and the methylamine−formaldehyde dimer[4] were studied in aqueous solution and in solutions of organic solvents with considerably different dielectric constants. Zwitterionic structures of amino acids, such as alanine and serine,[5] the alanine dipeptide,[6] glutamine,[7] the pentapeptide bis(penicillamine) enkephalin,[8] and the tautomeric zwitterions of aspartic acid,[9] have also been extensively studied in aqueous solution. Most of these studies applied a continuum solvent approach[1,3,5,7] or considered a large number of explicit water molecules.[2,8,9]

So far, however, the glycine zwitterion[10] has drawn the largest interest. Performing a combination of ab initio calculations for obtaining the internal energy and Monte Carlo simulations for estimating the solvent effects, Alagona et al.[10e,f] calculated the relative energy of two zwitterionic glycine conformers in aqueous solution, without optimizing, however, the solute geometries. The intramolecular proton transfer of glycine in aqueous solution was recently investigated by applying the DFT and the empirical valance bond methods.[11,12]

Relative stability of the neutral vs zwitterionic glycine has been explored in detail in several ab initio investigations. Geometry optimization at the HF/6-31G* level located a stable, isolated zwitterionic structure on the energy surface.[13] This structure, however, collapsed to the neutral form with basis sets HF/6-31+G* or larger[14a] or at the MP2/6-31+G* level.[14b] The common conclusion of these investigations was that the zwitterionic form is unstable in vacuo. In a different attempt, complexes of glycine with one or more water molecules were considered by independent research groups. Although already one water molecule turned out to stabilize the zwitterionic structure,[15] two water molecules “give rise to a potential energy minimum with at least one vibrational level”.[13b]

The purpose of the present study is to explore the conformational/tautomeric equilibrium of neutral and zwitterionic

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γ-aminobutyric acid (GABA) in aqueous solution. GABA is a substance of outstanding interest in physiology and molecular biology, since it is the major inhibitory neurotransmitter in the mammalian central nervous system.[16] Most of its role is due to the fact that GABA forms a zwitterion, $^{+}H_3N(CH_2)_3COO^{-}$ in the solid state[17-19] or in polar environment, whereas the neutral form, $H_2N(CH_2)_3COOH$, is more stable in the gas phase or in matrix isolation. Thus the zwitterionic form is the one involved in neuronal activity, and virtually all structural features are needed to perform this function: the negatively charged $-COO^{-}$ group allows a sensitive reaction to Na^+, K^+, or Ca^{2+}, which are known to be important in nervous signal processing. The positively charged $-NH_3^+$ group allows a docking to phosphate groups. Furthermore, the zwitterionic form can form bent structures, in which the charged terminal groups partly neutralize each other. This partial neutralization seems to be a prerequisite for the ability of GABA to pass nonpolar membranes.[20]

Previous quantum chemical ab initio calculations of GABA therefore focused either on the neutral form[21-23] or on extended conformers of the zwitterion,[24,25] which could serve as a model for GABA analogues that fix the extended form by incorporation into a ring system or a space-filling substituent at C_γ.[26] The biochemical importance of GABA, however, calls for a proper treatment of the zwitterionic form.

The present study is a calculation of the internal free energy using ab initio methods, and utilization of the free energy perturbation method as implemented in Monte Carlo simulations[27] for calculating the relative solvation free energy in aqueous solution. This combination for studying the neutral form/zwitterion equilibrium was first applied for the pyridinecarboxylic acids.[2] Since the gas-phase zwitterion is not stable either for α- and β-amino acids[9] or, as turned out in the present study, for the γ-amino acids, the relative internal free energy was calculated upon considering the dihydrate of the zwitterion. This approach when applied to the α- and β-zwitterions of aspartic acid produced relative protonation constants in good agreement with experimental values.[9] Thus the approach was also used in the present theoretical investigation of the corformational/tautomeric equilibrium for the neutral and zwitterionic GABA species in aqueous solution. This study is a part of our long lasting interest in the structure analysis of biologically important small molecules including γ-hydroxybutyric acid,[28,29] histamine,[29,30] and dopamine.[31]

Methods and Calculations

The total relative free energy, ΔG_{tot}, of the neutral and zwitterionic GABA conformers in aqueous solution is a sum of two terms:

$$\Delta G_{tot} = \Delta G_{int} + \Delta G_{sol} \tag{1}$$

Here ΔG_{int} and ΔG_{sol} are the relative internal and solvation free energy contributions, respectively, to the total relative free energy.

Calculation of the ΔG_{sol} term for a zwitterion needs no special effort as compared to that for a neutral molecule. Calculation of the ΔG_{int} term for a zwitterion needs, however, special considerations. As discussed above, the zwitterionic form does not correspond to a local energy minimum on the PES for an isolated amino acid. In our all geometry optimizations, an isolated zwitterionic GABA species produced a proton jump and converged to some neutral conformers. The zwitterionic structure can be, however, stabilized by adding two water molecules at proper locations, in analogy to glycine.[13b] For comparison of the zwitterionic and neutral conformers on an equal foot, the neutral conformers were also optimized in the presence of two water molecules. In the starting arrangements the water molecules were placed in positions where favorable hydrogen bonds could be formed with the amino and/or the carboxylic groups.

The energy minima reached for the zwitterion−dihydrate enable the calculation of vibrational frequencies and subsequently zero point energy and thermal corrections. This is a new approach introduced recently.[9] The isolated zwitterion does not form an energy minimum on the PES; thus no vibrational analysis can be performed for the isolated species in the gas phase. Wong et al.[1] carried out geometry optimization in solution, and pointed out that zero point energy corrections may be 1−2 kcal/mol for the neutral form relative to the zwitterion. This is a considerably large value if a subtle equilibrium is to be calculated. Geometry optimization in solution using the self-consistent reaction field method[32] based on the Onsager reaction field theory[33] requires the selection of the sensitive parameter a_o for the cavity radius. There is no solid theoretical basis for selecting this value, and calculations for the dopamine cation indicate results depending on the selected radius.[31] Using a recent method, Hsu et al.[34] determined the optimized cavity radii for alkali-metal and alkaline-earth-metal cations, but the method fails for simple anions. The radius may be calculated on the basis of the molar volume in the crystalline phase.[1] Although the crystal structure for GABA is known,[17-19] the derivable molar volume does not suffice for different zwitterionic conformers and for the neutral form also investigated in this study. Our trial calculations indicated geometry changes with different cavity radii. Use of the isodensity polarizable continuum method[35] for geometry optimization in solution could be very time consuming for a GABA-size molecule, due to convergence problems recently met for the zwitterionc aspartic acid.[9] Thus, in the absence of a technically affordable and for *all* tautomers/conformers usable clear-cut principle, the gas-phase optimized dihydrates were considered as references.

As discussed in ref 9, optimization for the dihydrate could reflect geometric changes for the zwitterion in the presence of strongly bound water molecules. The determined frequencies reflect the effect of hydrogen bonds not only for the solute but for two water molecules in a strong hydrogen bond to it. This effect is not considered in Monte Carlo simulations with rigid solvent molecules, while the frequency shift in calculating internal free energy corrections may be important. Consideration of further water molecules beyond the necessary minimum of two makes the frequency-dependent free energy contributions (zero-point energy and thermal correction) less justified. There may be water molecules forming water clusters, mainly in the case of the neutral species. Thus, in some conformers or tautomers there would be water units hydrogen bonded only to other water molecule(s), leading to comparison of complexes with different principles of organization.

The frequency-dependent corrections were calculated for the dihydrates due to the strong coupling of the low-frequency intra- and intermolecular vibration modes. Internal energies, however, were calculated in single-point calculations for the isolated solute (zw or n) using the geometry optimized in the dihydrate. Relative energies of the different hydrated structures contain a fraction of the total relative solvation energy in solution. Consideration of the optimized dihydrate as a rigid solute in Monte Carlo simulations would emphasize an ordered structure, which is, however, not so stable if thermal motion is also considered. Thus, the frequency-dependent corrections were

TABLE 1: Geometric Parameters for the GABA Tautomers/Conformers As Calculated in Dihydrates[a]

	n1	n2	n3	n4[b]	n5	zw1	zw2	zw3	exp[c]
$NC_4C_3C_2$	64.4	71.0	58.2	54.4 (53.1)	59.5	43.0	72.0	54.2	175.3
$C_4C_3C_2C_1$	−91.0	−83.2	−87.1	−82.9 (−81.7)	−80.3	39.7	−76.6	172.0	−72.6
$O-C_1C_2C_3$	63.8	38.5	23.6	156.4 (155.2)	162.2	−76.6	39.3	175.3	170.9
$O{=}C_1C_2C_3$	−116.7	−144.4	−157.3	−26.4 (−28.4)	−19.3	101.7	−142.3	−4.5	−9.4
HOC_1C_2	−11.8	−2.7	14.9	175.4 (177.9)	−172.6				
N−C	1.462	1.463	1.465	1.461 (1.455)	1.464	1.488	1.483	1.512	1.486
O−C	1.323	1.321	1.311	1.319 (1.328)	1.314	1.275	1.281	1.241	1.265
O═C	1.185	1.184	1.192	1.192 (1.185)	1.189	1.202	1.198	1.215	1.246
N−H						1.077	1.083	1.027	1.039[d] 1.053 1.058
O−H	0.965	0.971	0.962	0.955 (0.946)	0.958				
N···HO	1.83	1.75	1.85						
NH···O						1.50	1.44		

[a] Geometric data in Å and deg. [b] Values in parentheses were obtained in optimization for the isolated n4 GABA species. [c] Reference 17. Values without thermal correction. [d] All three N−H are involved in intermolecular hydrogen bonds.

TABLE 2: Relative Energies for the Isolated Species with Geometries Optimized in Dihydrates and Frequency−Dependent Corrections from the Dihydrates[a]

	ΔE/HF	ΔE/MP2	ΔZPE	$\Delta H(T)$	$T\Delta S(T)$	$T\Delta S_{vibr}(T)$	$\Delta G_{th}(T)$	$\Delta G_{int}(T)$
n1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
n2	0.98	0.62	−0.53	0.19	0.97	0.89	−1.31	−0.69
n3	2.66	2.44	−0.14	−0.02	−0.45	−0.41	0.28	2.72
n4	−2.83	−0.36	−0.53	0.20	0.54	0.51	−0.87	−1.23
n5	−1.78	0.73	0.05	−0.12	−0.65	−0.63	0.58	1.32
zw1	24.93	19.24	0.42	−0.63	−1.78	−1.76	1.57	20.81
zw2	21.66	17.40	−0.49	−0.22	−0.42	−0.39	−0.29	17.12
zw3	59.19	57.64	0.74	−0.44	−1.19	−1.26	1.49	59.13

[a] Energy terms in kcal/mol. ΔE energies calculated by using the 6-311++G** basis set. Frequencies obtained at the HF/6-311++G** optimization of the dihydrates. Correction terms are calculated with unscaled frequencies at $T = 310$ K and $p = 1$ atm. $\Delta G_{th}(T) = \Delta ZPE + \Delta H(T) - T\Delta S(T)$. $\Delta G_{int}(T) = \Delta E/MP2 + \Delta G_{th}(T)$.

calculated for the dihydrate, while the quantum-chemical internal energy was calculated for the isolated species as follows:

$$\Delta G_{int} = \Delta E(0)_i + \Delta ZPE_{dh} + \Delta H_{dh}(T) - T\Delta S_{dh}(T) \quad (2)$$

Subscript i stands here for calculations with the isolated GABA species, while dh refers to calculations using the optimized dihydrate. The terms $\Delta H_{dh}(T)$ and $\Delta S_{dh}(T)$ denote the differences of the enthalpy and entropy changes for the tautomers/conformers, respectively, when the temperature T changes from 0 to 310 K at $p = 1$ atm. The relative solvation free energies were determined by taking the isolated GABA isomers with geometries optimized in the dihydrates.

Ab initio geometry optimizations and frequency analysis for the dihydrated species were carried out at the HF/6-311++G** level[36a] and utilizing the GAMESS software[37a] running at the Technische Universität Graz. Single-point MP2/6-311++G**//HF/6-311++G** calculations were performed using the Gaussian 94 package[37b] running on a T90 machine at the Ohio Supercomputer Center. Optimized geometric parameters are summarized in Table 1. Single-point energies and frequency-dependent corrections to the internal free energy are shown in Table 2. Energy results from restricted geometry optimizations are shown in Table 3, and comparison of X−H frequencies (X = N, O) without and with H-bond involvement has been made in Table 4.

Monte Carlo simulations[38] for the zwitterionic and neutral GABA conformers were performed by using the BOSS 3.6 software[39] running on a Silicon Graphics Indigo 2 workstation at the University of Toledo. Calculations were performed in the NpT (isobaric−isothermal ensembles) at $p = 1$ atm and $T = 310$ K. In the study of the aqueous solution, the 25 × 25 × 25 Å^3 periodic box contained one solute and 504 TIP4P water molecules.[40] Preferential sampling proportional to $1/(R^2 + c)$ was applied, with a value of 120 for the constant c. R is the distance between the atom C_β of the solute (reference atom) and the oxygen atom of a water molecule to be displaced in a water move. In generation of a new configuration upon a solute move (attempted every 50 steps), the maximum allowed translation was set to 0.1 Å, and a rigid rotation about a randomly defined axis through the reference atom was limited to 10°. Corresponding values for the solvent were 0.15 Å and 15°. Volume changes were allowed in every 1000 steps with a maximum change of 250 Å^3 at a time. With these simulation parameters, the acceptance ratio of a new configuration was 40−45%. Totals of 3500 and 5000 K configurations were generated in the equilibrium and averaging phases, respectively.

TABLE 3: Relative HF/6-311++G Energies for the Zwitterion Obtained with Restricted Geometry Optimizations**[a]

	restriction	ΔE
zw1′	fixed N−H distances[b]	2.06
	geometry from the dihydrate[c]	3.25
zw2′	fixed N−H distances[b]	−1.28
	geometry from the dihydrate[c]	0.00
zw3′	fixed N−H distances[b]	36.02
	fixed NCCC and CCCC torsion angles[d]	35.86
	geometry from the dihydrate[c]	37.48
zw4′	fixed N−H distances[b]	−1.28
	exptl NCCC and CCCC torsion angles[e]	29.37

[a] Relative energies in kcal/mol. Primes after the codes zw1, zw2, ... have been used to indicate that no water was considered in any calculations. All geometric parameters except those subject to "restriction" were optimized. [b] Distances taken from the optimized zw2 species in the dihydrate: N−H = 1.0039, 1.0060, 1.0826 Å. [c] Single point calculation with geometry taken from the dihydrate. [d] Fixed NCCC and CCCC torsion angles taken from the optimized dihydrated zwitterion. [e] The NCCC and CCCC torsion angles were set to 175.3 and −72.6°, based on an X-ray experiment for GABA.[17]

TABLE 4: Calculated X−H Frequencies (X = N, O) without and with Involvement in H-Bond[a]

	ν_{XH} (cm^{-1})	R(XH···Y) (Å)		ν_{XH} (cm^{-1})	R(XH···Y) (Å)
zw1			zw3		
NH	3760		NH	3752, 3686	
	3598	1.95 (O_w)		*3335*	1.79 (O_w)
	2461	1.50 (O^-)	O_wH_w	4200, 4213	
O_wH_w	4207, 4212			*3746*	1.79 (O_w)
	3904	1.87 (O_w)		*3434*	1.68 (O^-)
	3812	1.82 (O^-)	n2		
zw2			NH	3806	
NH	*3774*	2.46 (O_w)		*3722*	2.20 (O_w)
	3691	2.29 (O_w)	OH	*3500*	1.75 (N)
	2307	1.44 (O^-)	O_wH_w	4214, 4215	
O_wH_w	4216, 4217			*4067*	2.06 (O^-)
	4005	2.02 (O^-)		*4025*	2.00 (O_w)
	3988	2.01 (O^-)			

[a] Italicized values indicate the X−H stretching frequencies involved in a X−H···Y bond. R(XH···Y) stands for the H···Y distance with the symbol in parentheses for the atom type Y.

TABLE 5: Solvation Free Energy Terms and Total Relative Free Energies in Aqueous Solution[a]

	ΔG_{sol}	DM[b]	ΔG_{Ons}[c]	ΔG_{int}[d]	ΔG_{tot}[e]
n1	0.00	7.35 (7.10)	0.00	0.00	0.00
n2	−0.86 ± 0.26	7.76 (7.48)	−0.02	−0.69	−1.57
n3	−0.13 ± 0.24	7.26 (7.07)	−0.01	2.72	2.58
n4	0.60 ± 0.29	1.67 (1.31)	0.13	−1.23	−0.50
n5	−0.59 ± 0.30	1.70 (1.27)	0.13	1.32	0.86
zw1	−23.77 ± 0.37	11.64 (11.32)	−0.24	20.81	−3.20
zw2	−25.12 ± 0.19	11.83 (11.78)	−0.25	17.12	−8.25
zw3	−68.42 ± 0.34	20.59 (20.49)	−1.00	59.13	−10.29

[a] Energies in kcal/mol. [b] Dipole moments in D, calculated from net atomic charges used in Monte Carlo simulations. Values in parentheses are the quantum chemically calculated values using the HF/6-31G* basis set. [c] Correction for the long-range electrostatic, using the Onsager formula.[34] Cavity radii in the range of 13.6−14.0 Å. [d] Values from Table 2. [e] $\Delta G_{tot} = \Delta G_{sol} + \Delta G_{Ons} + \Delta G_{int}$.

TABLE 6: Coordination Numbers and Numbers of Solute−Water Hydrogen Bonds

	n2	n4	zw2	zw3
N/O_w	3.5	2.7	2.3	4.6
(N)H/O_w	1.2[a]	0.4[b]	2.3	3.1
N/H_w	nc[c]	1.0		
(O)H/O_w	nc[c]	1.0		
=O[d]/H_w	1.6	1.7	2.6	2.9
−O[e]/H_w	1.0	0.7	1.4	3.0
N_{HB} (E_{min})[f]	2.3 (−5)	3.6 (−4)	6.5 (−5)	8.5 (−8)

[a] Sum of the NH/O_w coordination numbers for the two bonds. [b] Only one of the NH/O_w rdfs showed a minimum below 3 Å. Coordination number calculated for this bond. [c] Not calculated because of the intramolecular N···H−O bond. [d] =O corresponds to the carboxylate oxygen in the shorter C−O bond in zwitterions. [e] −O corresponds to the carboxylate oxygen in the longer C−O bond in zwitterions. [f] Number of solute−water hydrogen bonds. E_{min} in parentheses indicates the upper limit when integrating the pair-energy distribution function.

For calculation of intermolecular interactions, the 12−6−1 OPLS potential function[41] was employed. The united atom model was used for the CH_2 groups of GABA; all other atoms were considered explicitly. Steric parameters were taken from the program library. Atomic charges were derived by using the CHELPG procedure[42] upon fitting charges to the HF/6-31G* electrostatic potential of the *isolated* solute, since, in comparison with experimental values, atomic charges derived upon this potential turned out to be useful in free energy calculations.[43] The solvent−solvent (RCUT) and solute−solvent (SCUT) cutoff radii were set to 8.5 and 12 Å, respectively. The ICUT = 2 option was used in calculating the solute−solvent interactions. This option allows for a solvent molecule to be seen by the solute, if any distances between an explicit solute atom and the water oxygen is not larger than SCUT = 12 Å.

$CHCl_3$ parameters for simulations in the organic solvent were taken from Jorgensen et al.[38d] One GABA solute and 263 $CHCl_3$ molecules were considered in a 33 × 33 × 33 Å^3 periodic box. The constant c was set to 250 in the factor for the preferential sampling. Solvent translational and rotational parameters (see above) were set to 0.2 Å and 20°, respectively. The volume change was maximized at 600 Å^3. The RCUT and SCUT values were taken at 14 and 16 Å, respectively. Other parameters are equal to those for the aqueous solution simulations.

Relative solvation free energies were calculated by using the free-energy perturbation method as implemented in Monte Carlo simulations.[27] Geometric and OPLS potential parameters for the perturbed systems were calculated as a linear function of the perturbation step parameter, λ. Using double-wide sampling, conformers of a given tautomer were transformed along real physical pathways, meaning changes basically in the torsion angles. (Transformation of the n3 neutral form to the zw2 zwitterionic structure was carried out along a nonphysical pathway in aqueous solution: a hydrogen atom was annihilated at the carboxylic group and an additional proton was developed at the amino group.) Solvation results are given in Table 5. Long-range electrostatic effects were calculated by using the Onsager correction.[33] The radius of the average-sphere cavity was calculated from the union volume of the (overlapping) spheres taken around all explicit atoms of the solute. The radius around the solute atoms was set to 12 Å, in accordance with the SCUT parameter in aqueous solution and the ICUT = 2 sampling option. Solution structure and energetics have been characterized on the basis of integration of radial distribution and solute−solvent pair-energy distribution functions. Relevant data are collected in Table 6.

Results and Discussion

Geometry. Table 1 contains important geometric parameters for the GABA species optimized in the dihydrate. Both the neutral and the zwitterionic structures can be divided in groups with or without intramolecular hydrogen bonds. Three neutral structures, n1, n2, and n3 (Figure 1a−c), form a $H_2N\cdots HO-C{=}O$ bond that becomes possible by the anti conformation of the carboxylic group. When the −COOH moiety is of syn conformation, there is no internal hydrogen bond with structures n4 and n5 (Figure 1d,e). The bonding is clear for the zw1 and zw2 zwitterions allowing only the $NH_3^+\cdots OCO^-$ interaction (Figure 2a,b).

In principle, hydrogen bonds of NH···O types (with O− and O=) are also possible in the neutral form. Such hydrogen bond formation would not require the anti position of the carboxylic group, which conformation is higher in energy than the syn −COOH by about 5 kcal/mol.[44] Since the carbonyl oxygen is a better proton acceptor than the carboxylic OH (at least in the −OH···O=C bonds[28b]), a HNH···O=C bond still could be preferable in GABA. However, the NH_2 group, which is a strong proton acceptor turned out to be a weak proton donor when studying the intramolecular hydrogen bonds e.g. for histamine.[30,45] Thus no neutral structures with NH···O bonds have been studied here for GABA.

Formation of an intramolecular hydrogen bond in the neutral structure requires nearly gauche $NC_4C_3C_2$ and $C_4C_3C_2C_1$

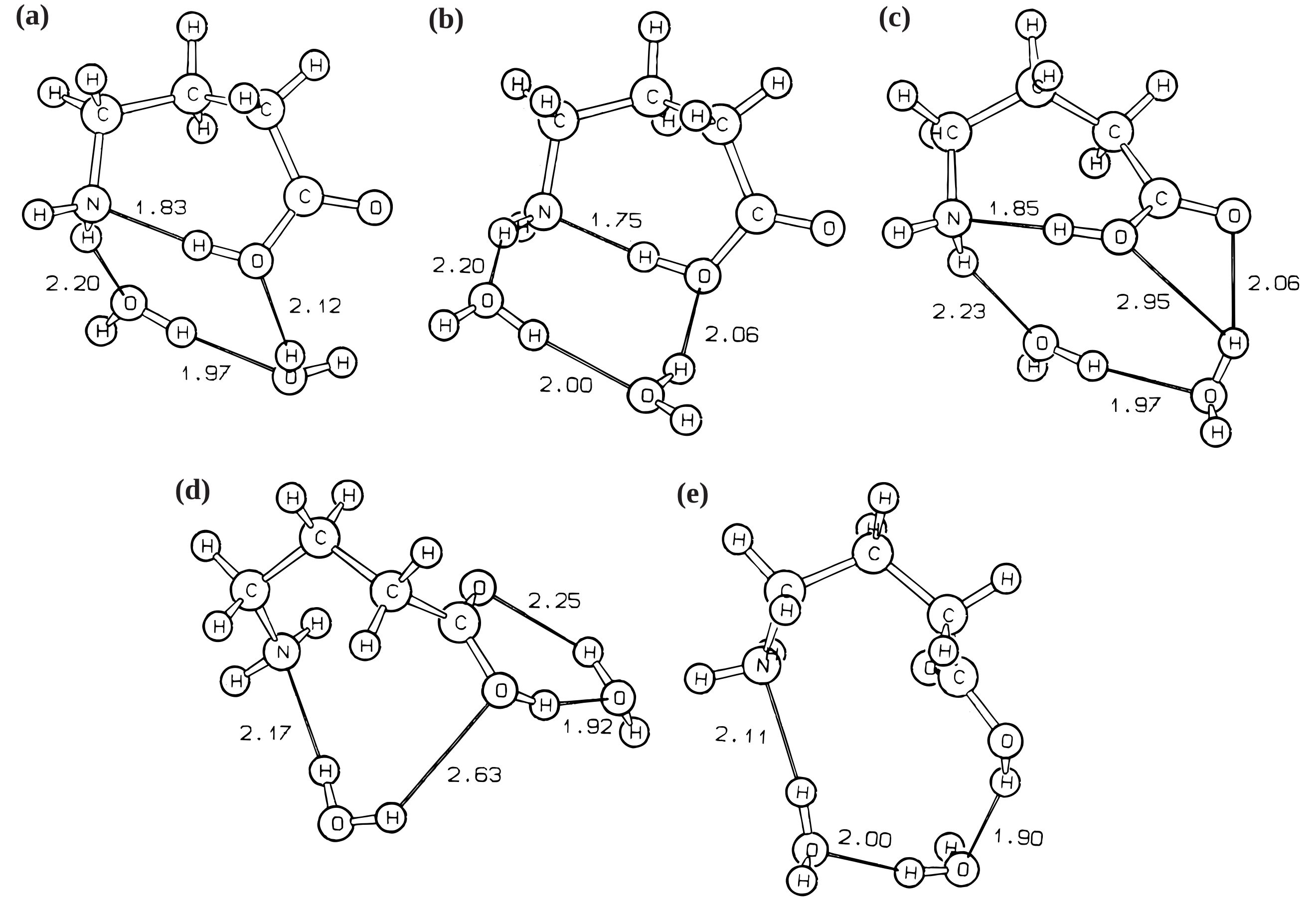

Figure 1. (a) Neutral (n1) GABA dihydrate with an intramolecular hydrogen bond of 1.83 Å. The lengths of intermolecular hydrogen bonds are also indicated. (b) Neutral (n2) GABA dihydrate with an intramolecular hydrogen bond of 1.75 Å. (c) Neutral (n3) GABA dihydrate with an intramolecular hydrogen bond of 1.85 Å. (d) Neutral (n4) GABA dihydrate without an intramolecular hydrogen bond. (e) Neutral (n5) GABA dihydrate without an intramolecular hydrogen bond.

arrangements. The latter value may be close to 90° as found for n1, n2, and n3 in Table 1. The $OC_1C_2C_3$ torsion angle changes within a 40° range, with anti $-COOH$ (HOC_1C_2 torsion angles are not far from 0°). The bond lengths show small changes upon the more expressed conformational change of the heavy atom chain. The N···HO hydrogen bond lengths, 1.85, 1.83, and 1.75 Å, become shorter in the n3, n1, and n2 series, in parallel with the increase of the O−H bond length.

The above N···HO bond lengths clearly indicate intramolecular hydrogen bonds for these structures; nonetheless the effect is not sufficient for an overall stabilization of the n1, n2, and n3 conformers. Previous 4-31G geometry optimization for the neutral form[23] resulted in a global energy minimum with a structure close to n4 in the present study (Figure 1d). Although geometric parameters for GABA in Table 1 were obtained by optimizing the dihydrates, consideration also of values in parentheses for the isolated n4 structure indicates only small changes of the optimized torsion angles in the presence of two close water molecules. As a result, single-point calculations for the isolated neutral species (Table 2), at geometries optimized in the dihydrates, show that n4 is still the lowest energy form of the n1, n2, ..., n5 conformers. Since there is no internal hydrogen bond for n4 and n5, the "uneffected" H−O, O−C, and N−C bond lengths at the HF/6-311++G** level can be assessed when considering these parameters for n4 and n5. When H−O bond lengths are compared for n4 and n1−n3, the increase up to almost 0.02 Å emphasizes the effect of the hydrogen bond in enhancing the H−O length. No similar effect was observed, however, for the O−C and N−C bond lengths.

Intermolecular hydrogen bonds to the water molecules are also indicated in Figure 1a−e. When there is an internal bond as well (Figure 1a−c), the water molecules comprise a N−H···O−H···O−H···O−H···(N) eight-member ring network of hydrogen bonds. All distances are below 2.25 Å for n1, n2, and n3, with one exception. There is a "long" hydrogen bond of 2.95 Å for n3 within the ring. In fact, the water molecule is moved toward the carbonyl oxygen and forms a regular O−H···O═ hydrogen bond of 2.06 Å. The role of the water molecules is bridge formation between the NH_2 and COOH groups for n4 and n5. It is remarkable that while the torsion angles are close for these two GABA conformers, the GABA−water interaction is geometrically different. The different geometry is partially attributed to the change of the sign for the HOOC torsion angle. There is one bridging water for n4, and the second water molecule simply hydrates the carboxylic group as was found earlier for carboxylic acid monohydrates.[44] In contrast, both water molecules are involved in the bridge formation for n5, not allowing development of bifurcated hydrogen bonds to the −COOH group as in n4. The dihydrate energies are more negative for n5 as compared to n4 by 2.12 and 3.20 kcal/mol at the HF and MP2 levels, respectively. The relative energies for the isolated GABA conformers are, however, of opposite sign: n5 is higher in energy by 1.05 and 1.10 kcal/mol at the HF and MP2 levels, respectively (Table 2). The better hydration of n5 relative to n4 is maintained when many water molecules are considered in Monte Carlo simulations (see next section).

Cyclic zwitterionic conformers, zw1 and zw2 (Figure 2a,b), have basically gauche $NC_4C_3C_2$ and $C_4C_3C_2C_1$ moieties with

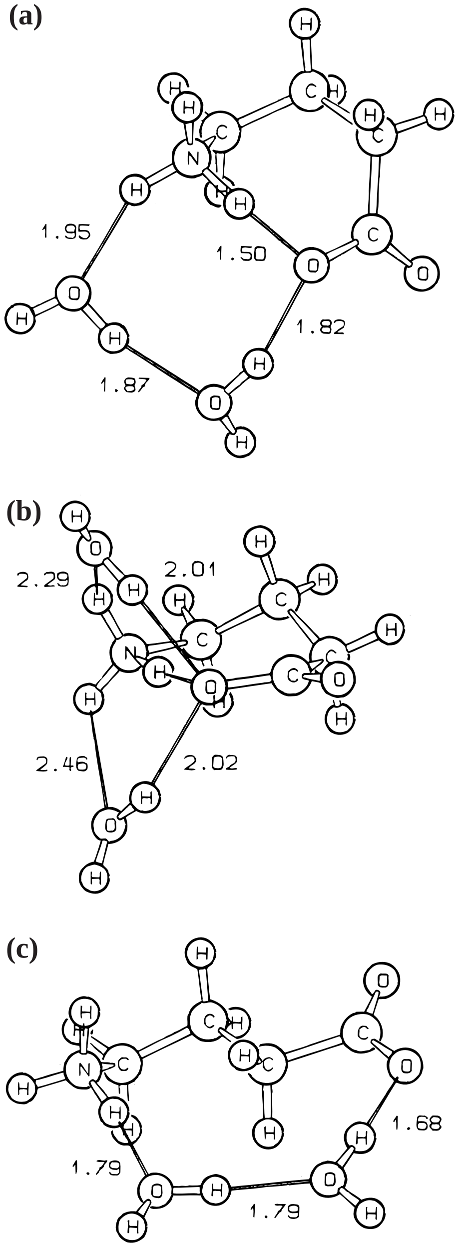

Figure 2. (a) Zwitterionic (zw1) GABA dihydrate with an intramolecular hydrogen bond of 1.50 Å. (b) Zwitterionic (zw2) GABA dihydrate with an intramolecular hydrogen bond of 1.44 Å (not indicated; see Table 1). (c) Zwitterionic (zw3) GABA dihydrate without an intramolecular hydrogen bond.

different sign for the latter torsion angle. Signs of the $OC_1C_2C_3$ torsion angles are also opposite. Although the N−C−C−C−C−O folding is quite different, the N−H···O hydrogen bond distance is still similar: 1.50 and 1.44 Å for zw1 and zw2, respectively. The shorter H···O is accompanied by a more stretched N−H bond length of 1.083 Å for zw2. (For comparison, the HF/6-311++G** optimized N−H bond lengths are 0.998−1.005 Å for the neutral form and are in the range of 1.003−1.012 Å for the zwitterion if not involved in internal bond formation.) For internally bound zwitterions the two O−C bonds show large deviations. The shorter is about 1.20 Å while the O−C bond length in N−H···O−C bonds are 1.275 and 1.281 Å (Table 1). Although there is no internal hydrogen bond for zw3 with the trans $C_4C_3C_2C_1$ moiety (Figure 2c), the N−H bond length is still longer than the "regular" value above. Data in Table 1 were taken from the dihydrates, where zw3 also forms hydrogen bonds to water molecules. As a result, one N−H and one of the O−C bonds are stretched: the hydrogen bonded O−C of 1.241 Å is longer than the other O−C of 1.215 Å with no hydrogen bond involvement.

Geometric data from X-ray structure determination for the zwitterionic GABA are also included in Table 1. When calculated and experimental torsion angles are compared, it may be most conspicuous that all studied structures have a nearly gauche $NC_4C_3C_2$ arrangement in contrast to the experimental trans conformation. In fact, we performed a geometry optimization for the dihydrated zwitterion, starting from experimental torsion angles for GABA. The water location allowed a $^+H_2N{-}H\cdots O{-}H\cdots O{-}H\cdots OCO^-$ bridge formation between the ionic sites. After the optimization process was started, the torsion angles changed rapidly, and following a proton jump, the system converged to the n1 dihydrate structure. Since our optimization was started from the only dihydrate structure in logical accord with arrangements used for the other species, we concluded that the zwitterionic dihydrate at near experimental torsion angles does not correspond to a stable trimer, at least when requiring two water molecules bridging the ionic heads. To have, however, some estimate of the relative energy of GABA with nearly experimental geometry, we have performed restricted geometry optimizations for some *nonhydrated* zwitterionic conformers (see next section).

Gas-Phase Results. Relative internal free energies of the *isolated* GABA species (Table 2) utilizing geometries optimized in dihydrates were calculated at the MP2/6-311++G**//HF/6-311++G** level. The MP2 and HF relative energies, ΔE/MP2 and ΔE/HF, respectively, agree in finding n4 as the most stable neutral conformer. This structure was found as the global energy minimum at the 4-31G level considering 62 conformers.[23] Second-lowest-energy conformers are n5 and n1 at the HF and MP2 levels, respectively. n5 is a structure close to n4 (Table 1) while n1 is a conformer with an internal hydrogen bond and having, correspondingly, an anti carboxylic group. Formation of the internal bond is favored at the MP2 level even at the expense of the anti conformation for the −COOH group. The $E(\mathrm{n5}) - E(\mathrm{n1})$ energy difference changes by 2.51 kcal/mol when considering correlation effects. In the chemically similar γ-hydroxybutyric acid[28] the relative energy change for the conformers XVIII and XI, with and without an internal hydrogen bond by an anti carboxylic group, respectively, is 1.82 kca/mol. Favor of the internal hydrogen bond with an anti carboxylic group may be, however, due to a delicate balance of the structural parameters. While structure n2 is still of lower energy than n5, n3 is of large relative energy even at the MP2 level.

The zw3 zwitterion without an intramolecular hydrogen bond is the highest internal energy species in the whole series studied. This structure is higher in energy than the second-highest-energy group with an intramolecular H-bond within the zwitterion (zw1, zw2) by 35−40 kcal/mol. Despite the large energy difference, the separation of the energy terms must be precisely determined because the solvation term is also very large for zw3, and the final relative free energy is basically determined by the sum of two main contributions with opposite signs.

$\Delta G_{\mathrm{th}}(T)$ values, defined by the last three terms in eq 2, are within a 3 kcal/mol range. The ΔZPE values define a range of 1.27 kcal/mol, close to the values by Wong et al.[1] In the present case, however, there is not a clear trend for the neutral and zwitterionic ZPE values. The lowest values were found for n2 and zw2 with and for n4 without an intramolecular hydrogen bond. ΔZPE values in Table 2 stand for unscaled frequencies. For HF/6-31G* frequencies 0.9 is a useful overall scaling factor.[36] Considering MP2/6-31G* optimization for pyrrole and imidazole,[46] a factor of 0.97 was derived. The larger basis set and consideration of electron correlation leads to a better agreement with the experimental values; thus, one may expect a scaling factor of 0.9−1.0 with the HF/6-31++G** basis set. Accordingly, the ΔZPE values may be reduced by up to 10%.

Ranges for $\Delta H(T)$ and $T\Delta S(T)$ are 0.83 and 2.75 kcal/mol, respectively. In general, $-T\Delta S(T)$ provides the largest contribution of the three terms defining $\Delta G_{\text{th}}(T)$. As far as the breakdown of $T\Delta S(T)$ is concerned, the $T\Delta S_{\text{vibr}}(T)$ term is the decisive one (Table 2). This finding is in accord with our previous results.[28b,47] The translational entropy is constant with constant mass, and the rotational entropy may be assessed from the $(\Delta S - \Delta S_{\text{vibr}})/\Delta S$ fraction, which is no more than 10%.

The total internal ΔG was calculated by using the ΔE/MP2 values (see the footnote in Table 2). The n4 tautomer/conformer is the most stable neutral one followed by n2. For the zwitterionic form zw2 is more stable than zw1 by more than 3 kcal/mol. The extended structure is still very high in relative internal free energy.

Table 3 shows the relative HF/6-311++G** energies calculated in restricted geometry optimizations. Restrictions meant fixed N−H distances or fixed NCCC and CCCC torsion angles with full optimization of the remaining internal coordinates. When the "geometry from the dihydrate" was taken, the energy corresponds to a single-point energy calculation with the indicated geometry.

The relative energies of the partially optimized zwitterions (zw1′, zw2′, and zw3′) are always more negative than the corresponding value from the single-point calculation. This is reasonable and indicates that dihydration triggers geometry distortion that increases the internal energy of the zwitterion by 1−2 kcal/mol. (Of course, this must be compensated by the zwitterion···$2H_2O$ interaction when the whole dihydrate takes its locally minimum-energy geometry.) Geometries of the "free" zwitterions differ from their counterparts in the dihydrates by changes in some torsional angles up to 10°.

The fixed N−H distance calculation for zw4′ was started from a GABA geometry where the NCCC and CCCC torsion angles were set to their experimental values of 175.3 and −72.6°, respectively, from X-ray experiments.[17] The N−H distances both in this calculation and in that for zw2′ were taken from the optimized zw2 dihydrate. Restricted geometry optimizations for these conformers led to identical results. The values of the torsion angles in the optimized structures are close to that obtained for zw2 in the dihydrate. The new values are 74.0 and −69.7° for the NCCC and CCCC torsion angles, respectively, as compared to 72.0 and −76.6° for zw2 in Table 1. The relative zw2′ and zw4′ energy is −1.28 kcal/mol.

The most remarkable result of the restricted geometry-optimization calculations was obtained when the torsion angles were kept at their experimental value for zw4′. Within our series, this calculation should provide the best estimate of the relative energy for the trans−gauche GABA zwitterion with nearly experimental geometry. The relative energy is 29.37 kcal/mol, as compared to the lowest energy gauche−trans zw3′ structure with relative energy of 35.86 kcal/mol. The energy decrease of 6.49 kcal/mol in favor of the experimental zw4′ GABA structure is very remarkable, mainly in the light of the results of the relative hydration-free-energy calculations.

Vibrational Frequencies. Calculated X−H frequencies (X = N, O) are compared for some optimized dihydrates in Table 4. The italicized ν frequency values refer to vibrations where the X−H bond is involved in a X−H···Y hydrogen bond. Data in Table 4 indicate the general experience: X−H frequencies are shifted toward lower values if the H atom is weakly bound to an electronegative Y atom.[48] Extended hydrogen-bond patterns in Figures 1 and 2 provide an opportunity for comparing the X−H stretching frequencies in dihydrates at different H···Y distances.

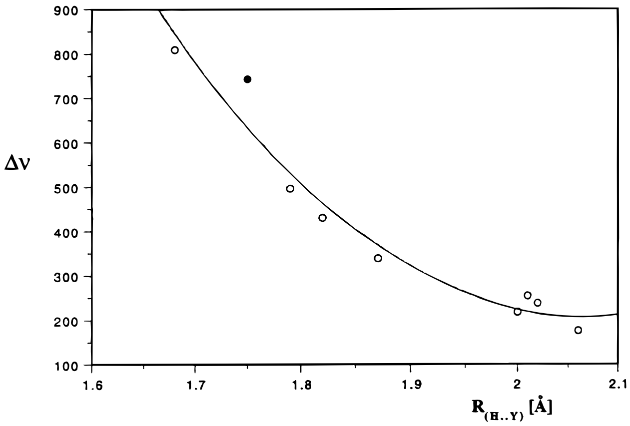

Figure 3. Correlation of the frequency shift, $\Delta\nu$ (cm^{-1}) = 4243 − ν (cm^{-1}) and the R (Å) = (O)H···Y distance (Y = N, O). $\Delta\nu = 4354(R - 2.063)^2 + 205$ ($n = 9$; $r = 0.977$). Empty circles stand for O_wH_w stretching frequencies, and the filled circle refers to the O−H stretching frequency in the anti COOH group involved in a N···H−O intramolecular hydrogen bond.

The largest value for the N−H stretching frequency in Table 4 is 3806 cm^{-1} calculated for a free N−H in the dihydrate n2. In the zwitterionc species the free N−H values are in the range of 3686−3760 cm^{-1}. It is interesting that the largest N−H value of 3774 cm^{-1} for zw2 refers to a N−H bond involved in a hydrogen bond to a water oxygen, O_w. The large H···O_w distance, $R = 2.46$ Å, suggests, however, a very weak hydrogen bond that does not result in remarkable decrease in the N−H frequency value. Other N−H frequencies decrease also only moderately, if the H···O_w distance is at least 2.20 Å. The values are, however, 3598 and 3335 cm^{-1} with H···O_w distances of 1.95 and 1.79 Å, respectively. When the N−H is in a intramolecular hydrogen bond to the carboxylate oxygen (O^-) atom, an extremely large red shift was calculated. Frequencies are 2461 and 2307 cm^{-1} at $R = 1.50$ and 1.44 Å, respectively.

Stretching frequencies for free O−H bonds are within the narrow range of 4200−4217 cm^{-1}, irrespective of whether the O−H bond was found in a neutral or a zwitterionic dihydrate. A reference value of 4243 cm^{-1} was calculated for the asymmetric O−H stretching frequency of water at the HF/6-311++G** level. The dependence of the $\Delta\nu$ (cm^{-1}) = 4243 − ν values (italicized in Table 4) on the R (OH···Y) distances is shown in Figure 3. Regression analysis produced a good fit of the points to a quadratic curve. Considering all nine points ($n = 9$) in Figure 3, a regression polynomial of $\Delta\nu = 4353(R - 2.063)^2 + 205$ was obtained. The empty circles in the figure refer to O_wH_w bonds, while the obvious, filled-circle outlier refers to the anti carboxylic O−H bound to the N atom in the neutral n2 dihydrate. Although if considering also this point in the regression analysis the correlation is still good, $r = 0.977$, but performing the regression analysis only for the O_wH_w frequencies ($n = 8$) the correlation coefficient increases to $r = 0.994$, while the minimum site of the parabola is shifted from $R = 2.063$ Å only to $R = 2.048$ Å. Thus $\Delta\nu$, according to its above definition, is in strong quadratic correlation with the (O)H···Y distance, and statistical results show only a slight dependence on the chemical character of parent molecules containing the O−H bond under investigation.

Equilibrium in Solution. The solvent effects produce a dramatic change of the relative total free energies of the isomers as compared to the gas-phase values (Table 5). The relative hydration free energies for the zwitterionic conformers, ΔG_{sol}, are within a range of −23 to −69 kcal/mol. While the solvent stabilization effect for zwitterions with intramolecular hydrogen bond is only 24−25 kcal/mol, the extended conformer is stabilized by about 68 kcal/mol. This is such a large stabilization that this structure becomes the most stable one in aqueous solution despite its outstandingly high relative internal free energy, ΔG_{int}.

The relative Onsager correction for the long-range electrostatics is almost negligible as compared to ΔG_{sol}. Even the absolute corrections,[33] using the formula $(-\mu^2/a^3)(\epsilon - 1)/(2\epsilon + 1)$, are no more than −1.14 kcal/mol. Cavity radii, a, were calculated as 13.6−14.0 Å, taking the cutoff radius of 12 Å for the solute−solvent interactions. The long-range electrostatic solvent effects in the continuum dielectrics approximation were small even for a 20 D dipole placed in the center of such a large cavity.

Table 5 contains the dipole moment values used in the calculation of the Onsager corrections. Dipole moments in the table were calculated from atom-centered net charges. Values in parentheses are the exact HF/6-31G* calculated dipole moments, which were very well reproduced (within 0.01−0.02 units) by the CHELPG fitting procedure.[42] The consistently larger dipole moments from atomic charges are the consequence of the united atom model applied. The CHELPG charges for hydrogens were added to the charge of the connecting carbon atom, and the sum was assumed as the charge for the united CH_2 atom located at the place of the C atom from ab initio geometry optimization.

ΔG_{tot} was calculated as the sum of the ΔG_{sol}, ΔG_{Ons}, and ΔG_{int} terms. For zwitterions the extended form (zw3) is more stable than the cyclic zw2 by about 2 kcal/mol. All zwitterionic conformers are more stable in aqueous solution than any neutral forms studied. Thus, this theoretical result is in conformity with the experiment that amino acids exist in zwitterionic rather than neutral form in aqueous solution. Similar results were recently reached by Tuñón et al.[11] and Nagaoka et al.[12] using a combination of quantum-mechanical and molecular dynamics methods for studying the proton transfer from neutral to zwitterionic glycine in aqueous solution. As to our best knowledge, however, the present study is the first theoretical investigation of the neutral−zwitterion equilibrium in aqueous solution for an amino acid with longer aliphatic chain, thus allowing large conformational freedom.

Although we have not considered all possible conformers, the two most important neutral types, the extended structure and the cyclic one with intramolecular hydrogen bond, were included in the tested series. All of them have turned out to be remarkably less stable than the zwitterions. Even the most stable neutral conformer, n2, is less stable than zw1, the least stable zwitterion considered here, and n2 is less stable by about 9 kcal/mol than the most stable zwitterion considered in the present study. Since only a limited number of zwitterionic conformers has been considered here, no theoretical equilibrium composition can be given. The values, however, suggest a preference for a partially extended GABA zwitterion over the cyclic ones in aqueous solution. The zw4′ experimental GABA structure (Table 3) is lower in internal energy by 6.49 kcal/mol than zw3′ at the HF/6-311++G** level. This is a large stabilization and may allow the appearance of a zw4′ type conformer in the equilibrium mixture with nonnegligible fraction even if its hydration free energy is less favorable by about 5 kcal/mol than that of zw3. In the present methodology, however, it has been unable to estimate the frequency-dependent, thermal correction for zw4′ (its dihydrate does not save the experimental torsion angles). Since this term may even be 1−2 kcal/mol (Table 2), no MP2 single point and relative hydration free energy calculations have been performed for zw4′.

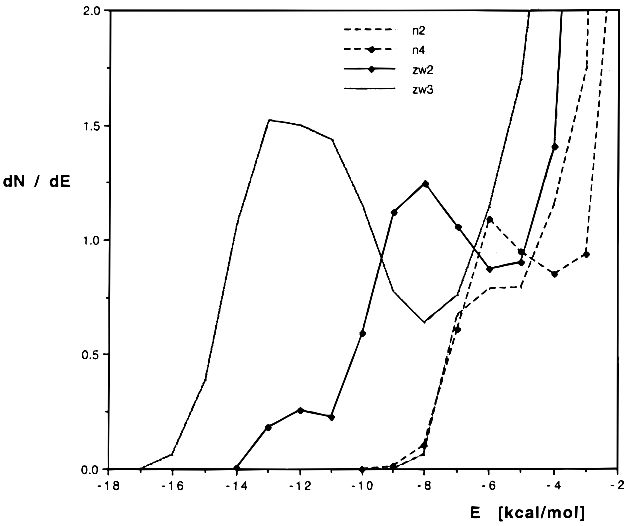

Figure 4. Solute−water pair-energy distribution functions for the n2, n4, zw2, and zw3 species. For the line code see insert at the figure top.

Unless a very favorable neutral form has been overlooked, results are informative enough for predicting that the free energy gap between the neutral and zwitterionic forms is large in aqueous solution. Using pK_a values for isoguvacine, a conformationally restrained GABA analogue, the zwitterion/neutral form equilibrium constant was calculated at 2×10^5.[49] The ratio corresponds to 7.5 kcal/mol relative free energy in favor of the zwitterion at 310 K. This value is close to 8.7 kcal/mol calculated here for the free energy difference of the most stable zw3 and n2 isomers. Thus, only a very small fraction of the neutral form can be experimentally observed, if at all, in the equilibrium mixture.

This latter conclusion may be valid for other saturated amino acids, as well, although it is far from generality. For instance, both theoretical calculations and experiments showed about 5% for the neutral form of the pyridinecarboxylic acids in aqueous solution.[2] Recent experimental results for oxoquinazoline carboxylic derivatives[50] also found remarkable neutral fraction in the mixture at the isolectric pH. Thus neutral and zwitterionic forms can be in experimentally observable equilibrium, and shift toward the exclusive existence of one of the isomers depends on the chemical system.

Solute Hydration. Table 6 is the summary of the radial distribution function (rdf) analysis. Integration of the rdf until its first minimum gives the O_w and H_w coordination numbers; i.e., the number of these water atoms in the first hydration sphere of the reference solute atom. The sum of the H/O_w, N/H_w, and O/H_w values provides an upper limit for the possible number of hydrogen bonds to the solute. Because of rounding errors and numerical integration, the N_{HB} value—number of the strong solute−water hydrogen bonds—may slightly exceed the above sum. In general, however, the sum is obviously larger than N_{HB}, indicating that only a fraction of the water molecules in the first hydration shell forms strong hydrogen bonds with the solute. N_{HB} is obtained upon integration of the pair-energy distribution function (pedf) until its first minimum or until the end of a plateau (Figure 4). For polar solvents forming strong interaction with the solute the upper integration limit is well in the negative interaction energy range. The weaker hydrogen bonds with interaction energy less negative than $E_{\min}$ are not counted in N_{HB}. For example, energies of the N−H···O$_w$ bonds of a *neutral* NH$_2$ group were calculated as more positive than −4 kcal/mol,[51] thus providing contribution from the energy range above the upper limits in the present study.

Values in Table 6 show typical differences between structures with (n2, zw2) and without (n4, zw3) an intramolecular hydrogen bond, or when comparing the neutral and zwitterionic solutes. There are 2.3 solute−water hydrogen bonds to the n2 species. The intramolecular hydrogen bond between N and the anti carboxylic HO occupies the space where intermolecular hydrogen bonds to the basic nitrogen and/or the acidic OH could be formed. When this intramolecular bond is disrupted, as in conformer n4, N_{HB} increases to 3.6. The increase is, however, less than 2 units, indicating that even with formally open N and OH in the n4 conformer, no 2 further stable hydrogen bonds have been formed to the new sites. The value of 1 for the N/H$_w$ coordination number suggests no steric hindrance when hydrating this site, as shown in Figure 1d. The sum of the N/H$_w$ and (O)H/O$_w$ coordination numbers is 2, allowing one strong N···H$_w$O$_w$ and one O−H···O$_w$ hydrogen bond. The increase from 2.3 to only 3.6 can be interpreted by assuming partial hydration of the intramolecular hydrogen bond in n2, which effect results in some contributions to its total of 2.3 hydrogen bonds. As far as the hydration of the carboxylic group in n4 is concerned, Figure 1d is not informative any longer. The bifurcated hydration of the −COOH group is a consequence of the small number of water molecules considered in the quantum-chemical calculations. Such bifurcation has also been found for carboxylic acid monohydrates in the gas phase[44] but never either in the snapshots of the present Monte Carlo simulations or in those showing hydration of the γ-hydroxy butyric acid[28b] and benzoic acid derivatives.[52] In summary, most of the hydrogen bonds, up to 1.6, are expected to be of =O···H$_w$O$_w$ type for the n2 species. For n4 about one N···H$_w$O$_w$, one O−H···O$_w$ and 1.6 =O···H$_w$O$_w$ strong hydrogen bonds are likely.

For zwitterionic conformers almost all water molecules in the first hydration shell form hydrogen bonds. There is an internal N−H···O bond in zw2, and water oxygens around the nitrogen atom form N−H···O$_w$ bonds with the two free N−H bonds, as concluded from the equal value of 2.3 for the N/O$_w$ and (N)H/O$_w$ coordination numbers. (These N−H bonds belong to a charged $-NH_3^+$ group; thus, the N−H···O$_w$ hydrogen bonds were counted in N_{HB}.) The carboxylic oxygen in the internal bond (referred to as −O in the table) is still surrounded by 1.4 water hydrogens. Thus this oxygen may be involved altogether in $1 + 1.4 = 2.4$ hydrogen bonds. The value is only slightly less than the O/H$_w$ coordination number of 2.6 for the free carboxylate oxygen (assigned as =O in the table). Assuming all close water molecules to form strong hydrogen bonds to zw2, the number of the intermolecular hydrogen bonds calculated from the sum of the coordination numbers, $2.3 + 2.6 + 1.4 = 6.3$, is nearly the same as calculated by integration of the pedf (Figure 4). For zw4 all three N−H bonds are expected to form hydrogen bonds to neighboring water oxygens (N/O$_w$ coordination number is 3.1). The oxygens of the practically free carboxylate group are surrounded by 2.9 + 3.0 H$_w$ atoms. The sum of the coordination numbers would allow 9 strong hydrogen bonds to zw4, as compared to 8.5 found by the pair-energy analysis. This number is larger by 2 units than N_{HB} for zw2. The increase corresponds to formation of two new hydrogen bonds at the opened NH and O$^-$ sites.

Comparison of these results with the coordination number analyses of the zwitterionic glycine conformers in aqueous solution by Mezei et al.[10c] and Alagona et al.[10e,f] shows both similarities and differences. The carboxylate group of the glycine conformer with an intramolecular N−H···O bond was hydrated by 5.1−5.3 water molecules in the above studies. This number is remarkably larger than $1.4 + 2.6 = 4.0$ obtained here for the zw2 conformer. The deviation is attributed to the large difference in the geometry of the intramolecular bond in glycine and GABA. The H···O distance and the N−H···O angle are 2.03 Å and 113.7° for glycine,[10e] while the corresponding parameters for GABA zw2 are 1.44 Å and 170.3°. Thus the intramolecular hydrogen bond in zw2 is much shorter and more linear than that for glycine. At this geometry one O$_w$H$_w$ bond to be connected to the GABA carboxylate oxygene can be entirely replaced by the N−H bond, leading to a smaller coordination number for the carboxylate group in the internally bound zw2 as compared to the corresponding glycine conformer. The (N)H/O$_w$ coordination number is 2.3 for zw2, in very good agreement with the value 2.4 for glycine in ref 10e. The agreement indicates no remarkable difference in the hydration of the NH$_3$ group on its *outer* surface.

For the glycine conformer without intramolecular hydrogen bond, Alagona and Ghio[10f] found coordination numbers of 5.2 and about 3 for the carboxylate and the NH$_3$ group, respectively. The corresponding values for zw3 GABA are 2.9+3.0=5.9 and 3.1. We would interpret the differences between the two studies that the smaller values for glycine are due to the larger proximity of the ionic site in glycine than in GABA. The first hydration shells overlap for glycine (see Figure 1 in ref 10f); thus the total numbers of the water molecules are smaller in these shells than in the separated ones for zw3 GABA. Overall, different coordination numbers are due to intrinsic structural differences between α- and γ-amino acids.

Figure 4 shows considerable difference in the most negative solute−solvent interaction energies for the neutral and the zwitterionic species. The onset value for the pedfs of the neutral solutes is about −10 kcal/mol. Solute−water interactions can be as strong as −14 kcal/mol for zw2 or −17 kcal/mol for zw3. The neutral solutes produce a plateau (n2) or a clear maximum−minimum shape (n4) for the pedfs in the −10 to −4 kcal/mol energy range. A shoulder and a maximum have been produced by zw2 and a well-defined maximum−minimum structure for pedf by zw3. While the maximum and the plateau for the neutral solutes are in the same energy range, maxima for the zwitterionic pedfs are at remarkably different places. Shift of the zw3 pedf toward lower energies is in accord with the more negative hydration free energy for this conformer as compared to zw2.

GABA Partition in Chloroform/Water. Relative solvation free energy was calculated for the n3−zw2 pair in chloroform solvent, following the proton jump from the anti carboxylic group (Figure 1c) to the amino group (Figure 2b). The geometries were not optimized in the intermediate reference positions. In fact, the internal coordinates were obtained from a linear interpolation of the corresponding values for n3 and zw2, with a pertubation step-size parameter of $\lambda = 0.02-0.06$. Thus the course of the curve can be expected to be qualitatively correct at most, but the calculated relative solvation free energy, being independent of the perturbation pathway, is correct for the n3−zw2 isomer pair. The curve decreases monotonically and with moderate steepness from n3 to zw2. The zwitteionic structure is more stable by 5.60 ± 0.04 kcal/mol than the neutral n3 species in chloroform at $T = 310$ K.

Taking relative internal free energies from Table 2, the stabilization free energy of the n3 form relative to the zw2 zwitterion in chloroform is (2.72 − 17.12) + 5.60 = −8.8 kcal/mol. This is a large free energy difference and indicates the preference of the neutral form in a nonpolar (slightly polar) phase. The n3−zw2 free energy difference is 10.8 kcal/mol in aqueous solution; thus, the following equations apply (indicating the phase in parentheses):

$$\Delta G_1 = \text{zw(wat)} - \text{n(wat)} = -10.8\ \text{kcal/mol} \qquad (2a)$$

$$\Delta G_2 = \text{zw(chl)} - \text{n(chl)} = +8.8\ \text{kcal/mol} \qquad (2b)$$

$$\Delta G_3 = \text{zw(chl)} - \text{zw(wat)} \qquad (3a)$$

$$\Delta G_4 = \text{n(chl)} - \text{n(wat)} \qquad (3b)$$

Using the definition of the ΔG terms in eqs 2 and 3 and using the calculated values for ΔG_1 and ΔG_2, eqs 4 and 5 follow:

$$\Delta G_1 + \Delta G_3 = \Delta G_2 + \Delta G_4 \qquad (4)$$

$$\Delta G_3 - \Delta G_4 = \Delta G_2 - \Delta G_1 = 8.8 - (-10.8) = 19.6\ \text{kcal/mol} \qquad (5)$$

Equation 5 indicates that the free energy change related to the move of the zw2 zwitterion from water to chloroform, ΔG_3, is more positive by 19.6 kcal/mol than that for the neutral (n3) form. If the 8.7 kcal/mol free energy difference is used for ΔG_1 (relative values of the most stable neutral (n2) and zwitterionic (zw3) forms in aqueous solution), ΔG_3 is still as large as 17.5 kcal/mol. Since the neutral GABA has two polar sites, it is expected to preferably stay in the aqueous phase. But even in the opposite case, and allowing as much as 10 kcal/mol free energy preference of the neutral form in chloroform, thus setting ΔG_4 to −10 kcal/mol, the calculated $\Delta G_3 = 17.5 + \Delta G_4 = 17.5 - 10 = 7.5$ kcal/mol value still corresponds to a large free energy increase needed for the entrance of the zwitterion in the organic phase. If $K = c(\text{chloroform})/c(\text{water})$ is the equilibrium constant for the partitioning GABA species, the calculated K value is 5.1×10^{-6} by using the relationship $\ln K = -\Delta G/RT$ at $T = 310$ and with $\Delta G = 7.5$ kcal/mol.

Since the zwitterionic form is the dominant form in the aqueous solution, neither mechanism of the GABA partitioning from water to chloroform, namely either the zw to n transformation in water and entering the chloroform phase as the neutral form or the direct partition of the zwitterion, is energetically favored. All together, the calculations support the experimental finding[49] that GABA does not cross the blood−brain barrier and, thus, does not enter the lipophilic phase from the hydrophilic one.

Summary

The zwitterionic γ-aminobutyric acid structure does not correspond to a local energy minimum on the gas-phase potential energy surface. To calculate relative internal free energies for the neutral and zwitterionic GABA tautomers/conformers in aqueous solution, the geometries of the dihydrated GABA structures were determined. Geometries for the isolated GABA species were taken from these structures. Molecular geometries and vibrational frequencies determined for the dihydrates reflect structural changes for GABA due to close and strongly bound water molecules. Vibrational frequency-dependent relative free energy corrections determined for the dihydrates turned out to be essential in order to reproduce the experimental difference of the protonation constants for isomeric aspartic acid zwitterions.[9] The X−H (X = N, O) stretching frequencies show remarkable red shift if involved in a hydrogen bond. Regression analysis provides a good quadratic correlation between the $\Delta\nu$ OH frequency shift and the OH···Y (Y = O, N) distance ($n = 9$, $r = 0.977$).

MP2/6-311++G**//HF/6-311++G** relative energies for the *isolated* GABA species show strong preference of the neutral over the zwitterionic forms. The most stable (gas-phase) neutral structure is without an intramolecular hydrogen bond and has nearly gauche−gauche arrangements, 54 and −83°, for the NCCC and the CCCC torsion angles, respectively, as determined in the dihydrate. In aqueous solution the zwitterionic structure exists. Although calculations could consider only some selected conformers, comparison of cyclic gauche−gauche forms and a partially extended, gauche−trans structure indicated the preference of the more extended form. This structure is different from the trans−gauche conformer found for GABA by X-ray experiments in the crystalline phase. The experimental conformer is not stable in a dihydrate where a $-NH_3^+\cdots OH_2\cdots OH_2\cdots {}^-OCO-$ arrangement was required in the starting geometry. When the NCCC and CCCC torsion angles were kept frozen at their experimental values, the otherwise optimized experimental trans−gauche GABA zwitterionic conformer is more stable by 6.5 kcal/mol than the gauche−trans form. Depending on the solvent effects (not calculated in the present study; see the text) the existence (even preference) of the experimental zwitterionic GABA structure may not be ruled out in aqueous solution. The GABA zwitterions, due to their large stabilization in aqueous solution, are not expected to form dimers or larger associations.

Calculation of the relative solvation free energy for a neutral and a zwitterionic tautomer in chloroform pointed out that the neutral form is the prevailing tautomer in a slightly polar solvent. Partition of GABA between aqueous and chloroform solutions is not favored either in the zwitterionic form or after transformation of the zwitterion to the neutral form in aqueous solution.

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