Structure and Stability of γ -Aminobutyric acid-(H₂O)_n (n = 0-5) Clusters: Zwitterionic vs. Canonical forms

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Calculations are presented for the γ -aminobutyric acid-(H₂O)_n (n = 0-5) clusters in both canonical and zwitterionic forms. We examine the effects of microsolvation on the structures and transformation between the canonical and zwitterionic forms. The canonical forms are predicted to be more stable for n = 0-4. With five microsolvating water molecules, the two forms of γ -aminobutyric acid become quasidegenerate, with the energies of zwitterionic forms slightly (by 1 - 3 kcal/mol) higher. The lowest energy zwitterionic conformer of γ -aminobutyric acid-(H₂O)₅ cluster is calculated to isomerize to canonical form through a barrier-less proton transfer process and is thus predicted to be kinetically unstable. Therefore, we predict that the canonical conformers of γ -aminobutyric acid should be observed predominantly in the gas phase at low temperature in presence of up to five water molecules.

Key Words: γ -Aminobutyric acid, Microsolvation, Zwitterion

Introduction

Structures and stability of canonical and zwitterionic amino acids¹⁻²⁰ in the presence of solvent molecules are of fundamental interest. It is well known that amino acids exist in canonical (non-zwitterionic) form in the gas phase, whereas zwitterionic conformer is the predominant form in aqueous solution.^{19,21-24} Studies of amino acid-water clusters have proved very useful to unravel the influence of water molecules on the structure of amino acids, both canonical and zwitterionic, in which the structures of the amino acid-water clusters were monitored in the gas phase as a function of the number of microsolvating water molecules. There was, however, some disagreement between theoretical calculations, and experimental observations. Paizs, Oomens and co-workers³ found that the zwitterionic form of phenylalanine and tryptophan may be stabilized by four water molecules, whereas Bowen and co-workers⁴ reported that five molecules of water are required for glycine (Gly). On the other hand, the theoretical work by Aikens and Gordon⁵ predicted that the zwitterionic conformers of glycine become energetically stable only with 7 - 8 microsolvating water molecules. This apparent discrepancy was attributed to kinetic stability,^{8g} that is, long lifetime of the thermodynamically less stable zwitterionic amino acids due to significant barrier for transformation from zwitterionic to canonical form.

In this work, we study the structures and stability of zwitterionic to canonical γ -aminobutyric acid (GABA)²⁵⁻²⁹ whose role in regulating neuronal inhibition processes in the mammalian central nervous system is well-known. GABA is structurally different from α -amino acids because the amino and carboxyl groups are separated by three carbon atoms. In the gas-phase²⁵ GABA has been predicted to exist in various forms of similar energy in the absence of solvent with the two functional groups either close to each other, or located at the far sides of GABA. We examine how the flexible structure of GABA is

affected by the microsolvating water molecules, focusing on the thermodynamic and kinetic stability of the zwitterionic form. We monitor the energy and Gibbs free energy of canonical and zwitterionic forms of GABA as a function of the number of interacting water molecules, and calculate the magnitude of barrier and the paths of transformation from zwitterionic to canonical forms of GABA.

Computational Methods

We employ the density functional theory methods (B3-LYP)^{30,31} with the 6-311++G** basis set. Gibbs energies are computed at 5 K. The intrinsic reaction coordinate (IRC) analysis is carried out to obtain the reaction paths and to identify the transition state for a given dynamic path. All calculations are carried out with Gaussian03 set of programs.³² Default criteria (10^{-5} for SCF energies, 10^{-5} for SCF gradients, and 10^{-7} for second derivatives and correlated wave functions) are employed for all optimizations.

Results

Fig. 1 and Table 1 present the lowest energy conformers of GABA. We find that (ca-1), (ca-2) and (ca-3) are of similar energy and Gibbs free energy to within 1 kcal/mol. The amino and carboxyl groups in conformers in (ca-1) and (ca-3) are at opposite sides of the amino acid, whereas they are close enough in (ca-2) to allow direct transfer of proton from the carboxyl to the amino group. These positions of the two functional groups should make a significant difference in the kinetic stability of the zwitterionic forms of GABA-water clusters, because a direct proton transfer between the adjacent carboxyl and amino groups is expected to occur via a very small barrier, whereas the canonical $\leftrightarrow$ zwitterionic isomerization should proceed *via* a significant barrier through the assistance of water molecules when

Table 1. Energy E (hartree), ZPE (kcal/mol), relative energy ΔE (kcal/mol), Gibbs energy G (hartree) at 5 K, and relative Gibbs energy ΔG of GABA-(H₂O)_n ($n = 0-5$) (B3LYP/6-311++G(d,p)).

	Conformer	E	ZPE	G_{5K}	ΔE	ΔG
GABA	(canonical)					
	ca-1	-363.17929	85.6	-362.90665	0.0	0.0
	ca-2	-363.18004	86.4	-362.90498	0.3	1.0
	ca-3	-363.17803	85.4	-362.90597	0.6	0.4
	ca-4	-363.17862	86.7	-362.90265	1.5	2.5
	(zwitterionic)					
	zw-1	-363.09513	85.6	-362.82238	52.8	52.9
GABA-(H ₂ O) ₁	(canonical)					
	ca-1-1	-439.65388	101.4	-439.33102	0.0	0.0
	ca-1-2	-439.65121	101.7	-439.32719	2.0	2.4
	ca-1-3	-439.65062	101.4	-439.32753	2.1	2.2
	ca-1-4	-439.64933	101.4	-439.32635	2.9	2.9
	ca-1-5	-439.64987	102.3	-439.32391	3.5	4.5
GABA-(H ₂ O) ₂	(canonical)					
	ca-2-1	-516.12967	117.5	-515.75531	0.0	0.0
	ca-2-2	-516.12892	117.4	-515.75505	0.3	0.2
	ca-2-3	-516.12920	117.8	-515.75415	0.5	0.7
	ca-2-4	-516.12745	117.1	-515.75461	0.9	0.4
	ca-2-5	-516.12824	118.2	-515.75184	1.5	2.2
	ca-2-6	-516.12680	117.3	-515.75322	1.6	1.3
	ca-2-7	-516.12684	117.8	-515.75162	2.0	2.3
	ca-2-8	-516.12559	117.1	-515.75276	2.1	1.6
GABA-(H ₂ O) ₃	(canonical)					
	ca-3-1	-592.60640	133.2	-592.18210	0.0	0.0
	ca-3-2	-592.60428	132.7	-592.18144	0.9	0.4
	ca-3-3	-592.60370	133.4	-592.17865	1.9	2.2
	ca-3-4	-592.60205	133.3	-592.17733	2.9	3.0
	ca-3-5	-592.60001	133.1	-592.17598	3.9	3.8
	ca-3-6	-592.60069	133.6	-592.17511	4.0	4.4
	ca-3-7	-592.59753	132.1	-592.17672	4.5	3.4
	ca-3-8	-592.59852	132.8	-592.17565	4.5	4.0
GABA-(H ₂ O) ₄	(canonical)					
	ca-4-1	-669.08078	148.8	-668.60683	0.0	0.0
	ca-4-2	-669.07966	148.4	-668.60678	0.4	0.0
	ca-4-3	-669.08028	148.9	-668.60599	0.4	0.5
	ca-4-4	-669.07948	148.5	-668.60659	0.5	0.2
	ca-4-5	-669.08029	149.2	-668.60496	0.7	1.2
	ca-4-6	-669.07861	148.3	-668.60605	0.9	0.5
	ca-4-7	-669.07932	148.9	-668.60506	1.0	1.1
	ca-4-8	-669.07853	148.4	-668.60568	1.1	0.7
	(zwitterionic)					
	zw-4-1	-669.07992	149.5	-668.60384	1.2	1.9
	zw-4-2	-669.07974	149.7	-668.60277	1.6	2.6
	zw-4-3	-669.07867	149.4	-668.60282	1.9	2.5
	zw-4-4	-669.07847	149.4	-668.60254	2.1	2.7
	zw-4-5	-669.07871	149.9	-668.60136	2.4	3.4
	zw-4-6	-669.07786	149.5	-668.60160	2.6	3.3
GABA-(H ₂ O) ₅	(canonical)					
	ca-5-1	-745.55692	164.3	-745.03355	0.0	0.0
	ca-5-2	-745.55604	165.1	-745.03006	1.4	2.2
	ca-5-3	-745.55612	165.0	-745.02981	1.4	2.3
	ca-5-4	-745.55332	163.4	-745.03241	1.5	0.7
	ca-5-5	-745.55421	164.2	-745.03073	1.7	1.8
	ca-5-6	-745.55293	164.2	-745.02941	2.6	2.6
	ca-5-7	-745.54616	162.7	-745.02718	5.4	4.0
	(zwitterionic)					
	zw-5-1	-745.55875	165.8	-745.03044	0.4	2.0
	zw-5-2	-745.55934	166.7	-745.02842	0.8	3.2
	zw-5-3	-745.55758	166.3	-745.02793	1.6	3.5
	zw-5-4	-745.55480	165.0	-745.02914	2.0	2.8
	zw-5-5	-745.55515	166.3	-745.02551	3.1	5.0

Figure 1. Structures of the lowest energy conformers of GABA (relative energy and Gibbs free energy at 5 K in kcal/mol). **Figure 2.** Structures of the lowest energy conformers of GABA-(H₂O)₁ (relative energy Gibbs free energy at 5 K in kcal/mol).

the carboxyl and amino groups are far apart. The lowest energy zwitterionic form (zw-1) is calculated to be much higher in energy (by ~53 kcal/mol) than (ca-1), and thus cannot be observed in the gas phase.

The low energy canonical complexes GABA-(H₂O) are shown in Fig. 2. We find that the lowest energy conformer (ca-1-1) derives from the (ca-1) conformer of bare GABA. The amino and carboxyl groups are, however, not in proximity to each other in this configuration, and the proton transfer to zwitterionic form is not feasible. The energies and Gibbs free energies of the other conformers of the canonical GABA-(H₂O) complex resulting from a water molecule microsolvating (ca-2) conformer of bare GABA are 2 - 4.5 kcal/mol above (ca-1-1), and are very similar to within 2 - 3 kcal/mol, as also presented in Table 1. In these latter conformers, a single water molecule bridges the amino and carboxyl groups to stabilize the cluster.

These structural and energetic features are also seen in GABA-(H₂O)_n ($n = 2,3$) clusters presented in Table 1, Fig. 3 and Fig. 4, indicating that the amino and carboxyl groups in GABA, although separated by three carbon atoms, behaves much like those in “normal” amino acids such as Gly or Ala. The two func-

Figure 3. Structures of the lowest energy conformers of GABA-(H₂O)₂ (relative energy Gibbs free energy at 5 K in kcal/mol).

Figure 4. Structures of the lowest energy conformers of GABA-(H₂O)₃ (relative energy Gibbs free energy at 5 K in kcal/mol).

Figure 5. Structures of the lowest energy conformers of GABA-(H₂O)₄ (relative energy Gibbs free energy at 5 K in kcal/mol).

tional groups get close to each other by the influence of micro-solvating water molecules. Since the energies of zwitterionic forms of GABA-(H₂O)_n ($n = 2, 3$) clusters are calculated to be significantly higher (by ~11, and 4.5 kcal/mol, respectively), we do not present them. The GABA-(H₂O)₄ and GABA-(H₂O)₅ clusters would be very interesting to study, because several studies have suggested that 4 - 5 water molecules may be sufficient to stabilize the zwitterionic forms of amino acids. Our calculated conformers of the GABA-(H₂O)₄ and GABA-(H₂O)₅ clusters are presented in Fig. 5 and Fig. 6, respectively. In these

Figure 6. Structures of the lowest energy conformers of GABA-(H₂O)₅ (relative energy Gibbs free energy at 5 K in kcal/mol).

structures, five water molecules largely connect the amino and carboxyl groups, constituting a “proton channel” to facilitate the transformation between the canonical and zwitterionic forms. The energies of the most stable canonical conformers are again calculated to be very similar, within 1 kcal/mol to each other, suggesting that experimentally assigning these structures may be quite difficult. The zwitterionic conformers are predicted to be thermodynamically unstable, because their energies and Gibbs free energies are calculated to be slightly higher (1 - 3 kcal/mol) than the low energy canonical ones. One interesting issue concerns the kinetic stability of the zwitterionic GABA-(H₂O)_n ($n = 4, 5$) clusters because they may survive long enough for experimental detection in a low-temperature gas-phase environment if there exists significant barriers ($> \sim 10$ kcal/mol) between the zwitterionic and canonical forms. We find that, although some zwitterions are at energy minima of the potential energy surface, their energy is above that of the canonical forms when the zero point energy is included, thus transforming without barrier into the latter forms. The lowest energy zwitterionic conformers (zw-4-1) and (zw-5-1) are thus predicted to trans-

Figure 7. Proton transfer from the zwitterionic to canonical form of GABA-(H₂O)₄ and GABA-(H₂O)₅ (relative energy, Gibbs free energy at 5 K and barrier in kcal/mol. B3LYP/6-311++G(d,p)).

form spontaneously into the canonical conformers (ca-4-8) and (ca-5-1), respectively.

This observation may be easily understood by noting that a proton is transferred directly from the ammonium to the carboxylate group without intervening water molecules, as depicted in Fig. 7. Therefore, it seems that five water molecules are not sufficient to stabilize the zwitterionic conformers of GABA, and that only the canonical forms should be observed in gas phase, even at ultra-low temperature.²⁹

Conclusion

Due to the presence of the three-carbon chain, GABA is a very flexible molecule. The lack of constraint allows the distance between the amino and carboxyl terminal groups to become shorter than in α -amino acids. We have observed that proton transfer between these groups is then not influenced by the presence of even up to 5 water molecules. The canonical form thus remains in that case the most stable in contrast with the situation encountered in the case of α -amino acids where the zwitterionic form becomes stabilized. The question of the minimum number of water molecules for achieving the transition between the canonical and the zwitterionic form thus remains open.

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Supporting Information. Coordinates of GABA-(H₂O)_n ($n = 0-5$) presented in this work are available on request from the correspondence author (SL: sylee@khu.ac.kr, (fax) +82-31-202-7337).

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