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Theoretical Research on Structures of γ-Aminobutyric Acid and Glutamic Acid in Aqueous Conditions

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Even though glutamic acid contains only one more carboxyl group than γ-aminobutyric acid (GABA), these neurotransmitters are recognized by their own specific receptors. To understand the ligand-recognition mechanism of the receptors, we must determine the geometric and electronic structures of GABA and glutamic acid in aqueous conditions using the *ab initio* calculation. The results of the present study showed that the stable structure of GABA was the extended form, and it attracted both cations and anions. Glutamic acid only attracted cations and was stabilized in four forms in aqueous conditions: Type 1 (an extended form), Type 2 (a rounded form), and Types 3 and 4 (twisted forms of Type 1). The former two types had low energy and the energy barrier between them was estimated to be small. These results showed that most free glutamic acid is present as Type 1, Type 2, and transient forms. The present results therefore suggest that the flexibility of the geometric structures of ligands should be taken into account when we attempt to elucidate the mechanism of recognition between ligands and receptors, in addition to the physicochemical characteristics of ligands and receptors.

Key words: GABA, geometric structure, glutamic acid, molecular orbital method, solvent effect.

Abbreviations: COSMO, the conductor-like screening model; GABA, γ-aminobutyric acid.

Simple amino acids such as γ-aminobutyric acid (GABA) and glutamic acid are involved in the vast majority of synaptic transmissions in the mammalian central nervous system (*1*). Even though it has long been difficult to fulfill all the required criteria that would give these substances a legitimate status as neurotransmitters in the mammalian brain, GABA is now considered to act as an inhibitory transmitter, and the recent rush to clone glutamate receptors and extensive research on long-term potentiation have demonstrated that glutamic acid is an excitatory transmitter in the brain (*2*).

The only difference in molecular formula between these two amino acids is an extra carboxyl group in glutamic acid, which causes it to have an entirely opposite effect on neural responses. These two molecules undoubtedly have their own geometric and electronic structures that reflect the presence or absence of this carboxyl group, resulting in their reception by specific receptors. Therefore, determination of the geometric and electronic structures of GABA and glutamic acid will help us understand the general mechanisms underlying molecular recognition between ligands and receptors.

In previous studies, we calculated the ionized forms of these two molecules using the *ab initio* method in a vacuum, and revealed their structures and physicochemical characteristics (*3*). Under these conditions, the stable structure of GABA molecules is straight and planar, and that of glutamic acid molecules is rounded. The electrostatic potential formed by GABA is separated into positive and negative parts, whereas that formed by glutamic acid is composed only of a negative part. However, a recent study involving X-ray crystallography did not show that glutamic acid bound to the glutamate receptor was of a rounded form (*4*).

In the present study, to examine the stable forms of the geometric and electronic structures of GABA and glutamic acid in aqueous conditions, we optimized them using the *ab initio* method taking into account the solvent effects with the reaction field calculation of the Onsager model. The results show that GABA has an extended form, whereas glutamic acid has four structural forms, comprising an extended form, a rounded form, and two twisted forms in aqueous conditions. The energy barrier between the extended and rounded forms of glutamic acid is so small that they are judged to be easily transient. X-ray crystallography supported our finding because the glutamate receptor (GluR2) binds to glutamic acid whose structure is a transient form between the extended and rounded forms (*4*).

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METHODS

Molecular Models—Under neutral pH aqueous conditions, the carboxyl groups and amino groups in GABA and glutamic acid are deprotonated and protonated, respectively. We thus used (NH_3^+-CH_2-CH_2-CH_2-COO^-) for GABA and [NH_3^+-CH(COO^-)-CH_2-CH_2-COO^-] for glutamic acid to obtain an accurate understanding of their intrinsic physicochemical characteristics in aqueous conditions.

Molecular Orbital Calculations in Aqueous Conditions—To determine the most stable ground states of these molecules in aqueous conditions, we first performed geometry optimization of more than 40 initial conformations of GABA and more than 60 initial conformations of glutamic acid by the MNDO-PM3 method (*5*), which is one of the most reliable semi-empirical molecular orbital methods in the MOPAC 97 program package (WinMOPAC, ver. 2, Fujitsu, Tokyo; Stewart, 1998). These initial structures were described previously (*3*). The effects of aqueous conditions on the geometry optimization were taken into account using the conductor-like screening model (COSMO) with relative dielectric constant ε = 78.3 in the MNDO-PM3 method. Note that only COSMO can be employed for the calculation of solvent effects in the MOPAC 97 program package. After confirming that the geometric structures obtained here were in the ground state by normal mode analysis (WinMOPAC), we found one candidate for the stable structure of GABA and seven candidates for that of glutamic acid.

These structures were optimized again by means of the GAUSSIAN98 program with the RHF/6–31G** basis set (ver. A.10, Gaussian, Pittsburgh, PA; Frisch *et al.*, 1998). Note that we had to perform the calculations with the 6–31G** basis set in order to shorten the calculation time for the following calculations with a large basis set, 6–311++G(3df,2pd). Throughout the *ab initio* calculations with the GAUSSIAN 98 program, the reaction field calculation of the Onsager model (ε = 78.3) was used to introduce solvent effects (*6–9*). The Onsager model for reaction field calculation has the advantage of letting us add the solvent effects directly to the geometry optimization with the GAUSSIAN 98 program. This continuum model of a solution reasonably reflects the solvent effects in the conformational equilibria when we use an adequate basis set (*7*). To properly describe anions in aqueous conditions, we should take account of diffuse functions in the basis set for *ab initio* calculation. The calculations with 6–31G** gave one stable structure of GABA and four stable structures of glutamic acid. Next, we performed geometry optimization for the most stable structure of each molecule and then calculated the normal vibration to confirm that the optimized structure was in the ground state, using the 6–311++G(3df, 2pd) basis set. The advantages of using the 6–311++G(3df, 2pd) basis set were described previously (*3*). We obtained one stable structure of GABA and four stable structures of glutamic acid. The energy barriers between these stable structures of glutamic acid were estimated by means of the MOPAC 97 program with COSMO but not with the GAUSSIAN98 program, because of the limited calculation time.

We defined the net charge of each atom as follows: net charge = (positive charge of the core) – (electron density of the same core). Based on the results obtained with the GAUSSIAN98 program, we calculated the electric dipole moments and electrostatic potentials of GABA and glutamic acid, and obtained the electrostatic force formed by each molecule on a positive point charge corresponding to a monovalent cation. The electrostatic potential and electronic force were defined as follows:

Electrostatic potential = $\sum_i [1/(4\pi\varepsilon)](Q_i/R_i)$,

Electrostatic force = $\sum_i [1/(4\pi\varepsilon)]\,(Q_i/r_i^2)$,

where ε represents the dielectric constant in aqueous conditions; Q_i the charge, which was produced to fit the electrostatic potential according to the Merz-Singh-Kollman scheme (*10*, *11*) of the i-th atom in the molecule; R_i the distance between the i-th atom and the observer; and r_i the distance between the i-th atom and the point positive charge. All the calculations for these physicochemical characteristics were based on the coordinates defined in the GAUSSIAN98 program.

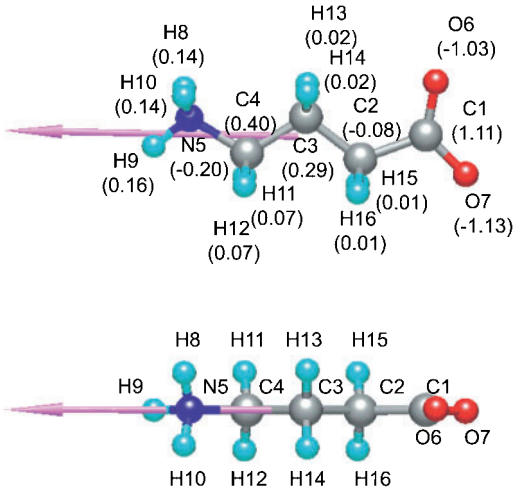

Fig. 1. **Stable form of the GABA zwitterion in aqueous conditions.** The geometric structure and dipole moment are viewed from two different directions. Light blue balls indicate hydrogen atoms, gray balls carbon ones, dark blue balls nitrogen ones, and red balls oxygen ones. Pink arrows indicate the dipole. Net charges on the atoms are shown in parentheses.

RESULTS

We found that GABA had a stable, extended form in the ground state under aqueous conditions and that glutamic acid could assume four structures in the same conditions.

Structures, Energy, and Dipole Moments—The stable structure of GABA in the ground state in aqueous conditions was the extended form (Fig. 1). The distance between the oxygen of the carboxyl group and the nitrogen of the amino group in GABA was 5.3 Å. The dipole moment was directed from the carboxyl group to the amino group, on a plane formed by the carbon backbone. The magnitude of the dipole moment was 31.3 debye.

Glutamic acid was found to form four types in the ground state in aqueous conditions (Fig. 2). The most stable structure as to energy was the extended form referred to as Type 1. The second most stable structure was the rounded form, Type 2. The difference in energy between

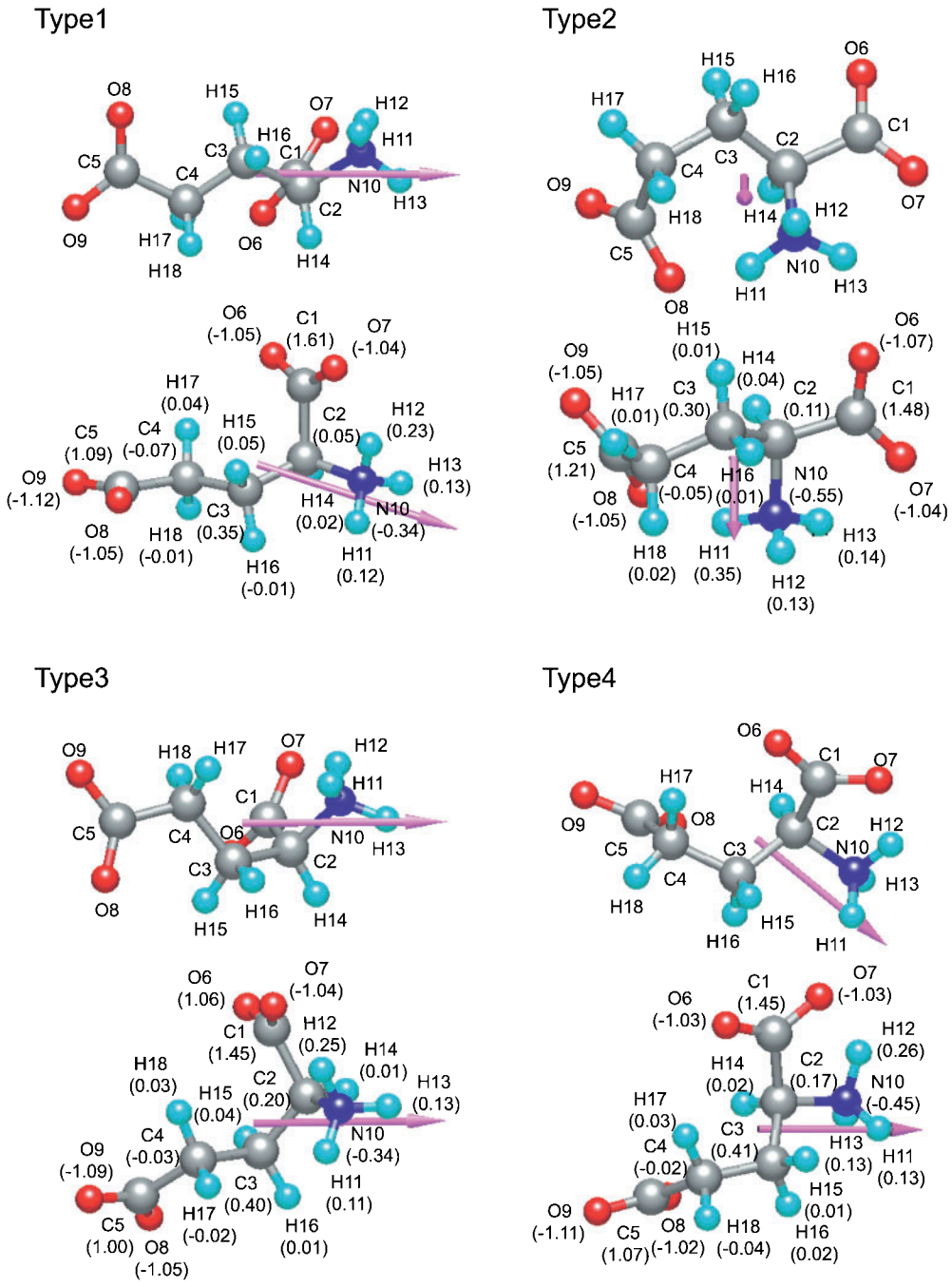

Fig. 2. **Four stable forms of glutamic acid in aqueous conditions.** The geometric structure and dipole moment for each form are viewed from two different directions. The nomenclature is the same as in Fig. 1.

these two types is 0.1 kcal/mol. To determine whether or not a transition occurs between these two types, we twisted the dihedral angle of C5-C4-C3-C2 and that of C4-C3-C2-N10 of Type 2 glutamic acid, and calculated the changes in conformation energy (Fig. 3). As mentioned under Methods, this calculation was performed by means of the MOPAC 97 program with COSMO but not with the GAUSSIAN98 program, to shorten the calculation time, so the exact values obtained here were not consistent with those obtained with the GAUSIAN98 program. Even though we had the disadvantage of these inconsistencies, we were able to address the following two important issues. (i) A structure similar to Type 1 was obtained when these two dihedral angles were each twisted through 240 degrees from in Type 2. (ii) The reaction pathway from Type 2 to Type 1 was qualitatively predicted: the dihedral angle of C5-C4-C3-C2 is first twisted and then that of C4-C3-C2-N10 is twisted. Taking this reaction pathway into account, we concluded that, in structure, glutamic acid easily changes from Type 1 to Type 2 and *vice versa* in aqueous conditions.

In Type 3, the dihedral angle of C5-C4-C3-C2 was twisted through 102.5 degrees from in Type 1. The energy of Type 3 was 2.1 kcal/mol higher than that of Type 1. There was another twisted form referred to as Type 4, which had much higher energy (8.7 kcal/mol)

than that of Type 1. These results showed that the two twisted forms (*i.e.*, Types 3 and 4) are not transient forms between Types 1 and 2 in aqueous conditions.

The dipole moments of Types 1 and 2 were 21.4 and 7.9 debye, respectively (Fig. 2). This difference was due to their structures. The dipole moment of Type 1 was directed from the carboxyl group (C5, O8, and O9) to the amino group, whereas that of Type 2 was vertical with respect to the plane formed by the carbon backbone. Types 3 and 4 had dipole moments of 19.9 and 16.4 debye, respectively.

Net Charges—The net charges, which were Mulliken charges, of GABA were calculated in aqueous conditions (Fig. 1). The carboxyl group had a negative charge of –1.0. The amino group had a small positive charge of 0.2. The positive charge of the amino group was mainly distributed on the carbon backbone. The polarization of the carboxyl group was strong, for example, C1 = 1.1, O6 = –1.0, and O7 = –1.1, whereas that of the amino group was not so strong, for example, N5 = –0.2, H8 = 0.1, H9 = 0.2, and H10 = 0.1.

The distribution of the net charges of glutamic acid varied among the four types (Fig. 2). In Types 1, 3, and 4, the carboxyl group (C5, O8, and O9) had large negative charges of –1.1. In contrast, the carboxyl group (C1, O6, and O7) and the amino group had small negative and positive charges, respectively. The total charge of C1, O6, and O7 was more than –0.7, and that of N10, H11, H12, and H13 was less than 0.2. A positive charge of about 0.5 was observed on C2 and C3 in the carbon backbone.

In Type 2, the total charge of C5, O8, and O9 was –0.9, that of C1, O6, and O7 –0.7, and that of N10, H11, H12, and H13 0.1. This was due to an intramolecular hydrogen bond between O8 and H11 at a distance of 1.7 Å. The polarization of the carboxyl group (C1, O6, and O7) near the amino group was strong in Type 1, such as C1 = 1.6, O6 = –1.1, and O7 = –1.0. The positive charge of the amino group was mainly captured by this carboxyl group. In Type 2, the polarization of the carboxyl group (C5, O8, and O9) was strong, such as C5 = 1.2, O8 = –1.1, and O9 = –1.1. The polarization of the amino group was also strong, such as N10 = –0.6, H11 = 0.4, H12 = 0.1, and H13 = 0.1. Types 3 and 4 possessed more positive charges on C2, C3 and C4, that is C^{α}, C^{β}, and C^{γ}, in comparison with Types 1 and 2 (Fig. 2). This was the same situation as found for GABA (Fig. 1).

The total net charges of C1, O6, and O7 in GABA were almost the same as those of C5, O8, and O9 in Types 1, 3, and 4 of glutamic acid (Figs. 1 and 2). This originated from the resemblance of their geometric structures (Figs. 1 and 2). In contrast, the total net charge of the amino group of GABA was more positive than those of the amino groups in Types 1, 3, and 4 of glutamic acid (Figs. 1 and 2), because glutamic acid possesses an extra carboxyl group (C1, O6, and O7) near the amino group.

Electrostatic Potentials—The electrostatic potentials of GABA were observed on six planes 5 Å from the electric charge center of the molecule (Fig. 4). Because the most notable characteristic of these electrostatic potentials was found on these planes, and the necessary distance for interaction (for example, formation of hydrogen bonds) between the GABA zwitterion and other molecules is considered to be 5 Å (see the section on Electrostatic Forces presented later), the electrostatic potentials were presented as a cube of 10 by 10 by 10 Å (Fig. 4). The carboxyl group and amino group formed negative and positive potentials, respectively. The range was from –0.07 to +0.08 V.

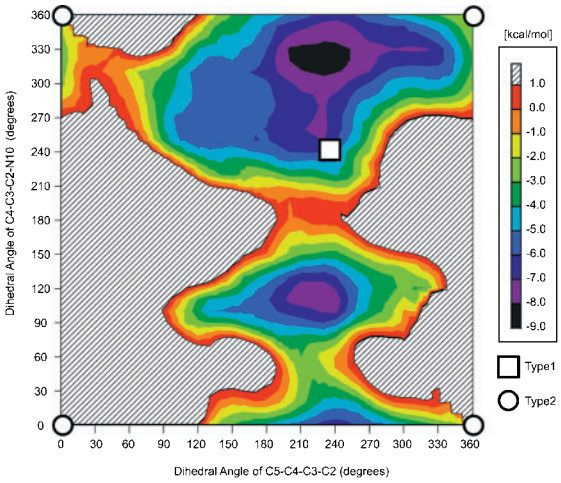

Fig. 3. **Contour map of changes in the conformation energy of glutamic acid.** The *x*- axis and *y*-axis indicate the C4-C3-C2-N10 dihedral angle and C5-C4-C3-C2 dihedral angle of the Type 2 form, respectively. The energy of Type 2 is taken as 0 kcal/mol. The shaded portion indicates energy of more than 1 kcal/mol.

We calculated the electrostatic potentials of Types 1, 2, 3, and 4 of glutamic acid (Figs. 5–8), and found that almost all the electrostatic potentials of these types were negative parts except small parts neighboring NH_3^+. Great changes in potential were found on at least one potential surface in any type of glutamic acid. This surface showed a strong negative potential that reflected the carboxyl group composed of C5, O8, and O9.

Electrostatic Forces—An electrostatic force reflects the electrostatic potential. GABA produced a strong, unidirectional electrostatic force from the carboxyl group to the amino group along the carbon backbone (Fig. 9). The other forces were small in comparison with this force. These facts indicated that GABA attracts not only cations but also anions.

The electrostatic forces of the four types of glutamic acid originate from the negative charges of the two carboxyl groups (Fig. 10). The directions of the forces were not constant because these two carboxyl groups are located oppositely in terms of the electric charge centers in all the types (*i.e.*, the origins of coordinates in Fig. 10). In particular, the carboxyl group composed of C5, O8, and O9 strongly attracted cations. These results showed that glutamic acid only attracts cations.

At about 5 Å away from the electric charge centers of GABA and all types of glutamic acid, the electrostatic forces were large (Figs. 9 and 10). This indicated that the distance of 5 Å is the onset point for interaction between the molecules calculated here and elsewhere.

Enthalpy Change on Binding—We calculated the enthalpy change on the binding between glutamic acid and the binding site of GluR2 using the MNDO-PM3 method. The model of the binding site of GluR2 used was composed of the seven amino acid residues involved

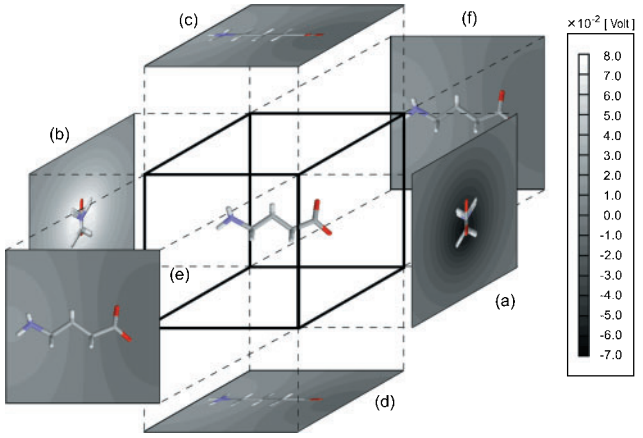

Fig. 4. **Electrostatic potentials formed by GABA in aqueous conditions.** Molecules are viewed from the following planes: (a) *y*–*z* plane at *x* = 5 Å; (b) *y*–*z* plane at *x* = –5 Å; (c) *x*–*z* plane at *y* = 5 Å; (d) *x*–*z* plane at *y* = –5 Å; (e) *x*–*y* plane at *z* = 5 Å; (f) *x*–*y* plane at *z* = –5 Å. The origin of coordinates is the electric charge center of a molecule. The numbering on the axes is in Å. The iso-electrostatic potentials are expressed in voltage.

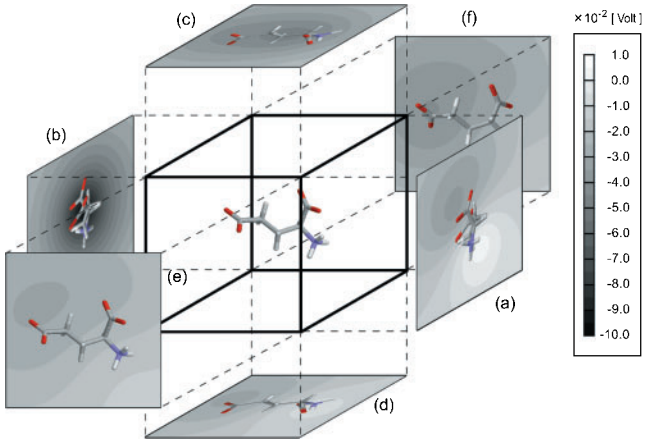

Fig. 5. **Electrostatic potentials formed by Type 1 glutamic acid in aqueous conditions.** Molecules are viewed in the same manner as in Fig. 4.

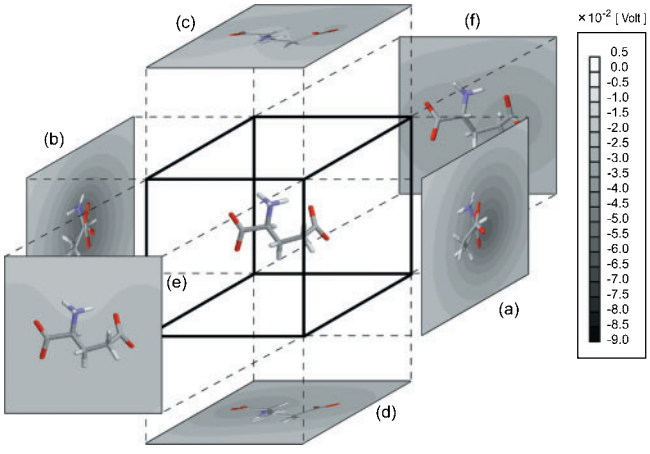

Fig. 6. **Electrostatic potentials formed by Type 2 glutamic acid in aqueous conditions.** Molecules are viewed in the same manner as in Fig. 4.

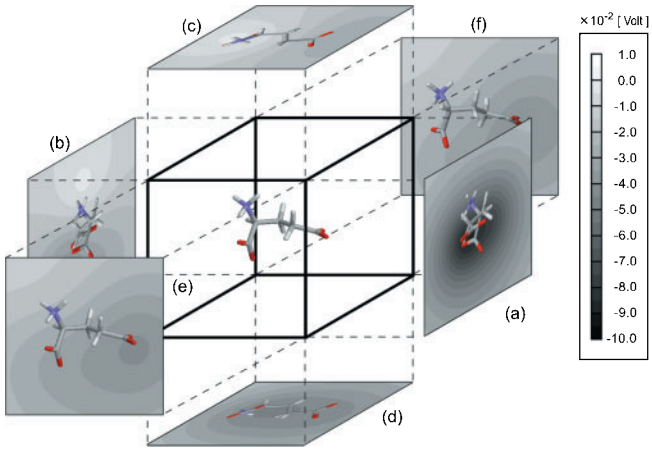

Fig. 7. **Electrostatic potentials formed by Type 3 glutamic acid in aqueous conditions.** Molecules are viewed in the same manner as in Fig. 4.

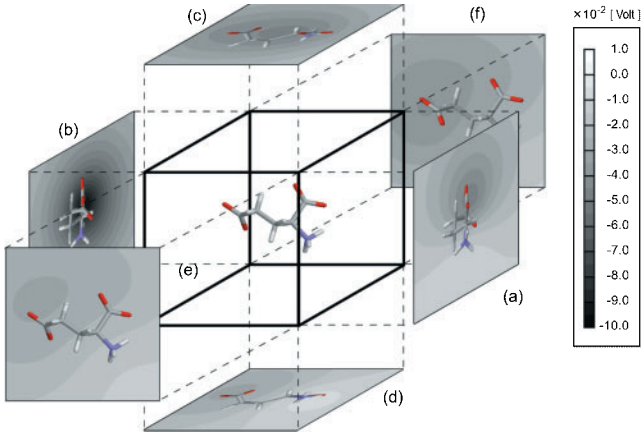

Fig. 8. **Electrostatic potentials formed by Type 4 glutamic acid in aqueous conditions.** Molecules are viewed in the same manner as in Fig. 4.

in the binding of glutamic acid (Pro478, Thr480, Arg485, Leu650, Ser654, Thre655, and Glu705) (Fig. 11) (*4*). The heavy atom coordinates of these amino acid residues were derived from the crystal structure of the ligand-binding domain of GluR2 (PDBID: 1fto for the apo state; and 1ftj for the glutamate-bound state), and the hydrogen atom coordinates were optimized with the MNDO-PM3 method. The enthalpy change caused by the binding was estimated to be –50 kcal/mol.

DISCUSSION

To elucidate the mechanism of molecular recognition of ligands by their specific receptors, we must know the geometric and electronic structures, and physicochemical characteristics of the ligands in aqueous conditions. In the present study, *ab initio* calculations with a large basis set, which has not been applied so far, were performed to determine the geometric structures, dipole moments, charge distributions, electrostatic potentials, and electrostatic forces of GABA and glutamic acid in

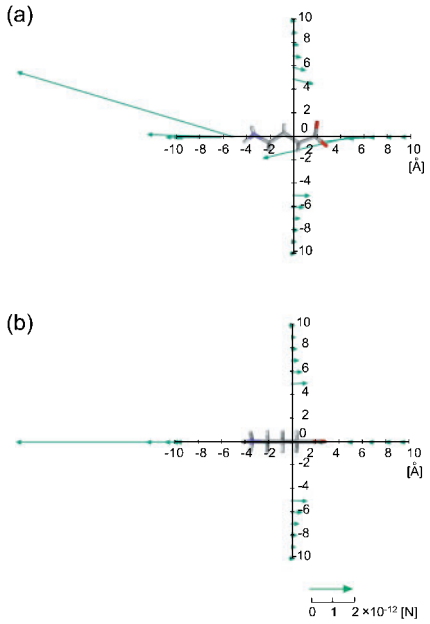

Fig. 9. **Electrostatic forces of GABA on a monovalent cation in aqueous conditions.** The cation was set on the *x*-axis and *y*-axis (a), and on the *x*-axis and *z*-axis (b). The arrows indicate the magnitude and azimuth of the electrostatically attractive force.

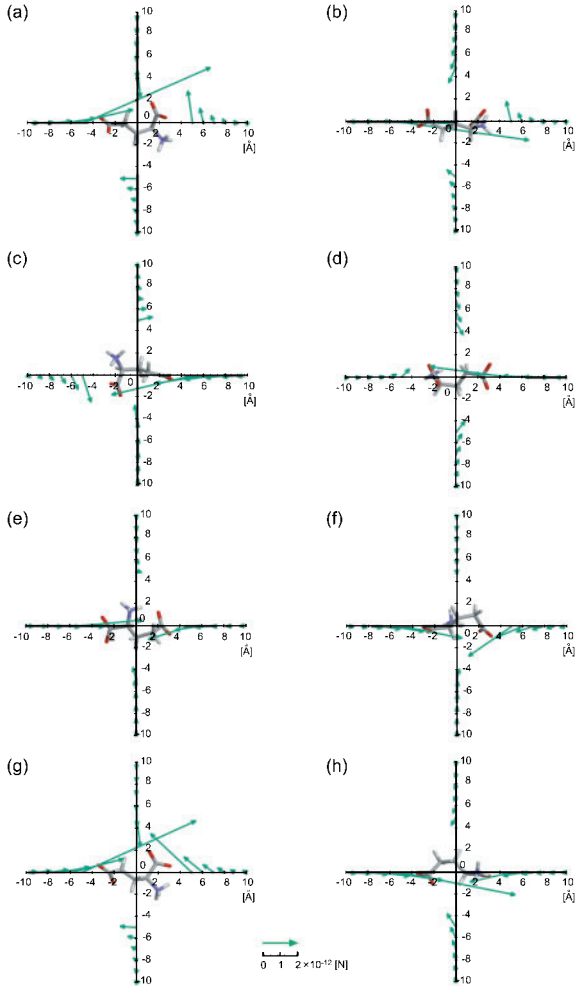

Fig. 10. **Electrostatic forces of glutamic acid on a monovalent cation in aqueous conditions.** The cation was set on the *x*-axis and *y*-axis (a), and on the *x*-axis and *z*-axis (b) for Type 1. The same situations were observed for Type 2 (c and d), Type 3 (e and f), and Type 4 (g and h). The arrows indicate the magnitude and azimuth of the electrostatically attractive force.

their ground states in aqueous conditions. When we considered ionic models, such as COO^- and NH_3^+, for these molecules under normal pH conditions, GABA exhibited the extended form, whereas glutamic acid took on four forms in structure that comprised an extended form, a rounded form, and two twisted forms. The former two structures (the extended and rounded forms) of glutamic acid were found to be reversible.

The geometric structure of GABA has been analyzed by means of various molecular orbital calculations (*3*, *12–14*). The results of these analyses suggested that the geometric structure of GABA was flexible, but they did not take into account the polarization function or solvent effects. In the present study, we used the ionic structures in aqueous conditions for our calculation models, which required us to use a large basis set with the polarization function [6-311++G(3df, 2pd)] and the Onsager reaction field for solvent effects. We consequently concluded that GABA takes on only the extended form in aqueous conditions (Fig. 1). In the previous study, we showed that GABA also takes on the extended form in a vacuum (*3*). The largest change in structure between in aqueous conditions and in a vacuum was the bond angle of C5-C4-C3. This angle increased by about 10° in aqueous conditions. Other changes observed were lengthening of the C2-C3 bond by about 0.04 Å, lengthening of the C3-C4 bond by about 0.03 Å, and an increase in the C3-C2-N1 bond angle by about 8° in aqueous conditions. The charge on C3 increased by about 0.09 in aqueous conditions. The solvent effect did not influence the structure or charge distribution of GABA, although the dipole moment of GABA in aqueous conditions was about 1.4 times greater than that in a vacuum.

Recent theoretical studies have shown that the electrostatic force plays a main role in molecular recognition (*15*). Electrostatic steering is important for effective molecular association in a particular orientation (*16*). The present results as to electrostatic potentials and electrostatic forces produced by the extended form of GABA (Figs. 1, 4, and 9) suggest that GABA approaches the entrance of the GABA receptor by adjusting its orientation to the corresponding electric field, but not by changing its conformation. So far, the binding site of the $GABA_A$ receptor has been found to have Asp62 and Arg66 as charged amino acids (*17*). Therefore, GABA can approach this receptor with an orientation matching the electrostatic potential formed by these charged amino acids. In contrast, glutamic acid cannot bind to the $GABA_A$ receptor because of the negative charge of Asp62.

Even though previous studies theoretically showed a structure of glutamic acid (*18*) and a structure of a single molecule in a vacuum (*3*), our present data show several

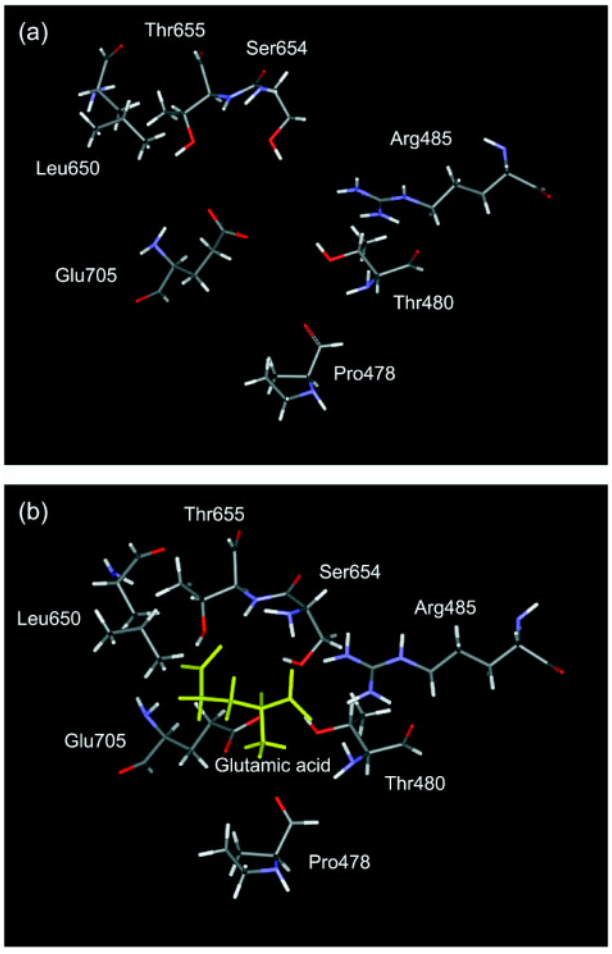

Fig. 11. **The model of the binding site of GluR2 in the apo state (a) and in the glutamate-bound state (b).** In (b), glutamic acid is shown in yellow. The heavy atom coordinates were derived from the crystal structure of the ligand-binding domain of GluR2 (PDBID: 1fto for the apo state and 1ftj for the glutamate-bound state).

structures of glutamic acid, which exists as a free molecule in aqueous conditions (Fig. 2). The most stable structure of glutamic acid in a vacuum is the rounded form (*3*). As shown by the present study, the extended form (Type 1) is the most stable one in aqueous conditions, the second most stable structure being the rounded form (Type 2). The solvent effects lead to remarkable changes in the geometry of glutamic acid from the gas to the water phase. The difference in energy between Types 1 and 2 was, however, small (about 0.1 kcal/mol). We therefore concluded that Types 1 and 2 obtained with our present calculation were flexible in structure in aqueous conditions (Figs. 2 and 3). This is consistent with the X-ray crystallographic data showing that the form bound by the glutamate receptor (GluR2) is a transient form between Types 1 and 2 (*4*).

The predicted mechanism for molecular recognition between glutamic acid and GluR2 is considered here. The ligand-binding region of GluR2 is composed of two lobe structures, S1S2 lobes (*4*). Note that the structure of the ligand-binding domain of metabotropic GluR also forms a bi-lobed construct (*19*). The X-ray crystallographic data for GluR2 suggested that glutamic acid first docks to Arg485 in the S1 lobe in an open form of the cleft between the S1S2 lobes (*4*). Furthermore, we have found that glutamic acid interacts vibrationally with Arg485 through hydrogen bonds on binding, resulting in conformational changes in glutamate receptors (*20*). In the ligand-binding cleft near Arg485, a positive electrostatic potential is considered to be formed by the following basic amino acid residues: Arg453, Arg485, Lys656, Arg660, Arg661, and Lys730. Glutamic acid, which undergoes a change in structure between Types 1 and 2, is thus attracted to Arg485 in the ligand-binding cleft because of the negative electrostatic potential of glutamic acid. On the other hand, GABA cannot remain stably in the ligand-binding cleft of GluR2. Although GABA is attracted to the ligand-binding cleft by the negative part of the electrostatic potential of GABA, GABA leaves the cleft immediately because of the positive part of the electrostatic potential of GABA (Fig. 4). The electrostatic potential of the S1S2 lobes of GluR2 will be reported elsewhere (Kubo *et al*., unpublished data).

Here, we discuss the change in free energy on the binding between glutamic acid and the binding site of GluR2. In the present study, the enthalpy change on binding was estimated to be –50 kcal/mol by the MNDO-PM3 method. A recent theoretical study revealed that the MNDO-PM3 method overestimates the charge transfer between biomolecules (*21*). Because the charge transfer between molecules contributes to the stabilization of their complex, overestimation of the charge transfer between biomolecules leads to overestimation of their binding energy. Thus, the enthalpy change on binding obtained in the present calculation by the MNDO-PM3 method may be an overestimate. Because of the entropy change on binding, the change in free energy on binding between ligands and receptors is smaller than the enthalpy change on binding. Judging from the thermodynamic data for five metabotropic receptors and four ionotropic receptors such as glycine, $GABA_A$, $5\text{-}HT_3$, and nicotinic receptors, the decrease in free energy on ligand-receptor binding was about 15 kcal/mol at most due to the enthalpy-entropy compensation (*22*). Due to the entropy decrease, therefore, the decrease in free energy on the binding between glutamic acid and the binding site of GluR2 is also considered to be smaller than the enthalpy decrease.

Both the energy difference in glutamic acid between Types 1 and 3, and that between Types 1 and 4 were more than 2 kcal/mol. The presence ratio of Type 3 or Type 4 glutamic acid to Type 1 in equilibrium at temperature T = 300 K is less than 0.035, which is the value of $\exp(-2/RT)$. Here R is the gas constant. We conclude, therefore, that Types 3 and 4 of glutamic acid are minor ones in aqueous conditions *in vivo*. However, it remains true that the effect of the electric field of glutamate receptors must be considered, so Types 3 and 4 should not be considered to be negligible in future studies.

In conclusion, we determined one stable structure of GABA and four stable structures of glutamic acid in the ground state in aqueous conditions. The most important findings were as follows. (*1*) GABA is rigid; (*2*) GABA attracts not only cations but also anions surrounding it; (*3*) the extended structure (Type 1) and rounded structure (Type 2) of glutamic acid are reversible in aqueous

conditions, which is consistent with the X-ray crystallography data; and (*4*) glutamic acid only attracts cations. The present results therefore showed that, in addition to the physicochemical characteristics of ligands and receptors, we must take the flexibility of the geometric structures of ligands into account if we wish to elucidate the mechanism of molecular recognition between ligands and receptors.

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