A Theoretical Study of Electronic and Structural States of Neurotransmitters: γ-Aminobutyric Acid and Glutamic Acid[1]

Kei Odai,* Tohru Sugimoto,[†] Dai Hatakeyama,[‡] Minoru Kubo,[‡] and Etsuro Ito[‡,2]

*Department of Electronics and Informatics, North Shore College, Atsugi 243-8501; [†]Laboratory of Biophysics, School of Engineering, Kanto Gakuin University, Kanazawa-ku, Yokohama 236-8501; and [‡]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku, Sapporo 060-0810

As a first approach to understanding the mechanism for the recognition of a ligand by its receptor, we first calculated the electronic and structural states of ionized γ-aminobutyric acid (GABA) and ionized glutamic acid using the *ab initio* method with the 6-311++G (3df, 2pd) basis set. We paid special attention to the physicochemical characteristics of these molecules, such as the electric dipole moment, electrostatic potential, and electrostatic force. Even though GABA and glutamic acid are known to exert completely opposite influences in the mammalian brain by binding their specific receptors, the only difference in their chemical structures is that glutamic acid contains one more carboxyl group than GABA. As a result, we succeeded in showing that a difference of only one carboxyl group induces significant differences in the electronic and structural states between these molecules. These differences have a crucial influence on the electric dipole moments, the electrostatic potentials, and the electrostatic forces. The most remarkable finding of the present research is that the electrostatic potential formed by glutamic acid is composed of only negative parts, while that formed by GABA is separated into positive and negative parts. These results strongly suggest that GABA can approach either positively or negatively charged amino acids by adjusting its own orientation, while glutamic acid can approach only a positively charged binding site.

Key words: electric dipole moment, electrostatic force, electrostatic potential, γ-aminobutyric acid, glutamic acid.

On the basis of neurophysiological studies, amino acids in the mammalian brain have been separated into two general classes: inhibitory amino acids [γ-aminobutyric acid (GABA), glycine, *etc.*], which hyperpolarize neurons, and excitatory amino acids (glutamic acid, aspartic acid, *etc.*), which depolarize neurons (1, 2). GABA is found in high concentrations in the mammalian brain and spinal cord. GABA acts on at least two types of receptors, $GABA_A$ and $GABA_B$. The activation of $GABA_A$ receptors leads to an increase in chloride permeability and thus to hyperpolarization. $GABA_B$ receptors usually increase potassium conductance, again causing hyperpolarization of neurons. Glutamatergic neurons are widely distributed throughout the mammalian brain. Once released, glutamate (the L-isomer) causes depolarization and thus excitation of neurons, but it does so by acting on a variety of receptors, which are best described separately. The AMPA/kainate receptor responds to the glutamic-acid analogues α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid. This response produces an increase in cation conductance, which causes depolarization. Another type of receptor, N-methyl-D-aspartate (NMDA) receptor, also belongs to the ion-chan-

nel-linked superfamily. In addition, there is a population of metabotropic receptors, so called because the initial result of their activation is a stimulation of second messenger transduction systems (1, 2).

So far, the behavior of these neurotransmitters has been mainly studied using physiological and pharmacological techniques in both vertebrate and invertebrate brains (3–10). Recent techniques in molecular biology have clarified the subunits of their receptors, and thus partially revealed the amino-acid sequences of their binding sites (11–18). However, it should be noted that even though GABA and glutamic acid exert completely opposite influences as described above, the only difference in their chemical structures is that glutamic acid contains one more carboxyl group than GABA (Fig. 1). This fact gives rise to an important question: how does the receptor recognize its matching ligand, or how does the ligand recognize its receptor? Conventional ideas in biological science maintain that molecular recognition occurs because a ligand and its receptor are geared toward each other in a relationship resembling that between a key and a keyhole, *i.e.*, their geometrical structures are determinant (see a textbook of biology, *e.g. 19*). On the other hand, physicochemical studies have pointed out that both electrostatic and hydrophobic interactions, as well as the geometrical interaction, are stabilizing factors for the formation of a ligand-receptor complex. More recent studies insist, however, that the electrostatic interaction, which is considered to operate in both a long-distance region and a short-distance region, plays a main role (20).

A

$H_2N - CH_2 - CH_2 - CH_2 - COOH$

B

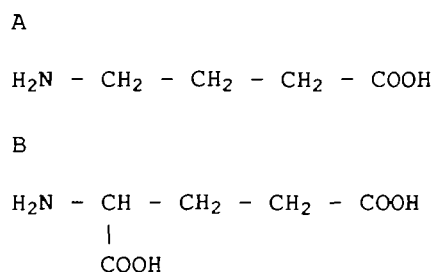$H_2N - CH - CH_2 - CH_2 - COOH$
  |
  COOH

Fig. 1. **Chemical structures of GABA (A) and glutamic acid (B).**

This latter finding is plausible for the following two reasons. First, the influence of the electrostatic interaction decays only in proportion to the first-order term of distance, so that the influence is exerted sufficiently over a long distance; second, because the electrostatic interaction acts to create a hydrogen bond, it also exerts its influence sufficiently over a short distance.

In the present study, we calculated the electronic and structural states of GABA and glutamic acid as an approach to understanding the mechanism of the recognition of a ligand by its receptor. In particular, we examined the differences in the physicochemical characteristics of these molecules, including the electric dipole moment, electrostatic potential, and electrostatic force.

METHODS

Under neutral pH liquid conditions, the carboxyl groups and amino groups in GABA and glutamic acid are deprotonated and protonated, respectively. We thus used the ionized models and calculated them in a vacuum to obtain an accurate understanding of their intrinsic physicochemical characters. The results under liquid conditions will be reported elsewhere. Briefly, we add a comment concerning the effects of solution on the molecular structures. For example, to obtain the ionized model for GABA in solution, the structure of GABA was calculated from its various initial geometrical parameters by the MNDO-PM3 method, one of the most reliable semi-empirical molecular orbital methods, in the MOPAC2000 program package (ver. 1.08 and ver. 1.11, Fujitsu, Tokyo). As a result, we found that the structure of GABA in solution is very similar to that in a vacuum.

The electronic and structural states of GABA and glutamic acid in a vacuum were calculated using the *ab initio* method [GAUSSIAN94 (revision D.3), Gaussian, Pittsburgh, PA] in order to optimize the charge distributions and structures. To determine the most stable ground state, geometry optimizations of about 40 initial conformations of GABA and about 60 initial conformations of glutamic acid were first performed by the MNDO-PM3 method. These initial structures of GABA and glutamic acid were made by changing the NH_3^+-C-C-C dihedral angle and the C-C-C-COO$^-$ dihedral angle in the 30-degree step, and both of these dihedral angles in the 60-degree step (Fig. 1). For glutamic acid, some conformations produced by changing the combination between the C-C-C-O dihedral angle and these dihedral angles in the 60-degree step were added in the initial structures. As a result, one stable structure of GABA and four stable structures of glutamic acid were

found. These structures were optimized again by the GAUSSIAN94 program using the RHF/6-31G**. Geometry optimization was further performed for the most stable structure of each molecule and the frequency was then calculated to confirm that the optimized structure was the ground state geometry, using the 6-311++G(3df,2pd) basis set. Here, the 6-311++G(3df,2pd) basis set places three sets of valence functions, diffuse functions (5s and 5p functions), and polarization functions (three sets of d functions and a set of f functions) on heavy atoms; it places three sets of valence functions, diffuse function (4s function), and polarization functions (two sets of p functions and a set of d functions) on hydrogen atoms. Polarization and diffuse functions are useful for describing the electric dipole moment and the electronic structure of the negatively ionized molecule, respectively. We defined the net charge of each atom as follows: net charge = (positive charge of the core) − (electron density of the same core).

From the results obtained by the GAUSSIAN94 program, the electric dipole moment and electrostatic potential of GABA and glutamic acid were calculated, and the electrostatic force formed by each molecule on a positive point charge corresponding to a monovalent cation was obtained. Here, the electrostatic potential and the electronic force are given as

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

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

where ε represents a dielectric constant in a vacuum; Q_i the charge, which was produced to fit the electrostatic potential according to the Merz-Singh-Kollman scheme, 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 of the calculations were based on the coordinates defined in the GAUSSIAN94 program. Because the electric dipole moment reflects the net-charge distribution of a calculated molecule when the molecule is observed from far away, the electric dipole moment is important when a ligand and its receptor are separated over a long distance. On the other hand, the fine structures of the electrostatic potential and electrostatic force are important when a receptor binds its ligand (*21, 22*).

Recent point-mutation experiments utilizing techniques such as the substituted cysteine accessibility method have produced interesting findings concerning the binding sites of GABA and glutamate receptors (*12, 15*). On the basis of those findings, we will discuss the following four sets of interactions: (i) interactions between GABA and the binding site of the $GABA_A$ receptor, (ii) between glutamic acid and the binding site of the $GABA_A$ receptor, (iii) between glutamic acid and the binding site of the AMPA/kainate receptor, and (iv) between GABA and the binding site of the AMPA/kainate receptor.

RESULTS

Molecular Structures, Electric Charge Distributions, and Electric Dipole Moments—The most stable structure for GABA is the straight and planar structure (Fig. 2A). No hydrogen bond is formed between the positive electric charge (+0.147) of the amino group and the negative electric charge (−0.838) of the carboxyl group within GABA,

because the amount of energy produced by the electric interaction between the two ends of the molecule is suffi-

N1	-0.246
C2	0.462
C3	0.203
C4	-0.148
C5	1.196
O6	-1.017
O7	-1.017
H8	0.144
H9	0.144
H10	0.105
H11	0.056
H12	0.056
H13	0.033
H14	0.033
H15	-0.002
H16	-0.002

N1	-0.602
C2	0.065
C3	1.479
O4	-1.048
O5	-1.042
C6	0.398
C7	-0.110
C8	1.214
O9	-1.069
O10	-1.008
H11	0.132
H12	0.411
H13	0.025
H14	0.011
H15	0.000
H16	0.019
H17	0.002
H18	0.123

Fig. 2. **Optimized structures and net charges of GABA (A) and glutamic acid (B).** Light blue balls indicate hydrogen atoms, gray balls indicate carbon, dark blue balls indicate nitrogen, and red balls indicate oxygen. The summation of the net charges is zero for GABA and minus one for glutamic acid.

Fig. 3. **Electric dipole moments of GABA (A) and glutamic acid (B).** The values are 21.98 debye and 4.97 debye, respectively. Two figures in (A) and (B) are views from two different directions.

ciently small (0.296 eV) that it fails to round out the amino-acid backbone. The distance between the oxygen of the carboxyl group and the nitrogen of the amino group in GABA is 4.999 Å.

Glutamic acid is most stable when rounded out (Fig. 2B). In contrast to GABA, the positive electric charge (+0.064) of the amino group in glutamic acid was found to interact with the negative electric charge (-0.863) of the carboxyl group in the side chain. We found that a hydrogen bond is made between H12 and O9 in glutamic acid. The electric charges of these atoms are 0.411 and -1.069, respectively, and the length between them is 1.551 Å. This hydrogen bond results in a round conformation. The distance between the oxygen atoms of the two carboxyl groups in glutamic acid is 4.644 Å. In other words, the size of glutamic acid is almost the same as that of GABA.

The electric dipole moments are shown in Fig. 3. As we described above, we found that because GABA forms a straight structure containing the positive electric charge of the amino group and the negative electric charge of the car-
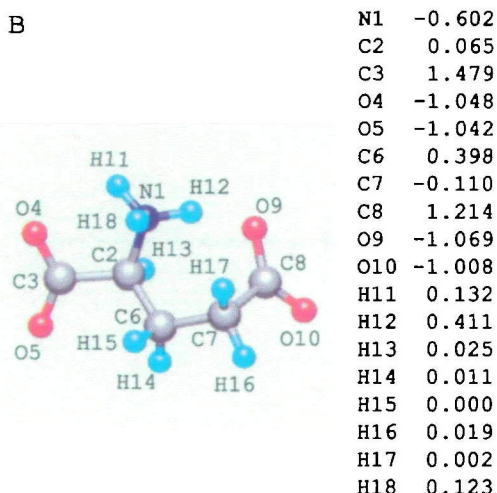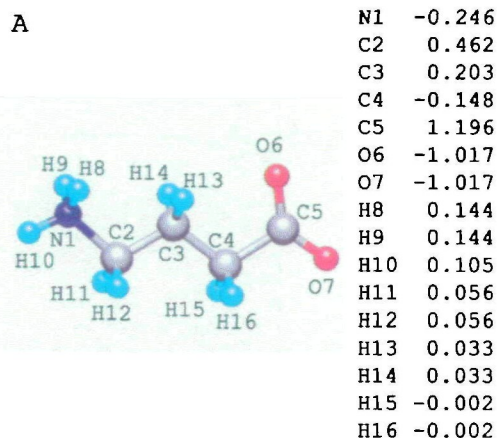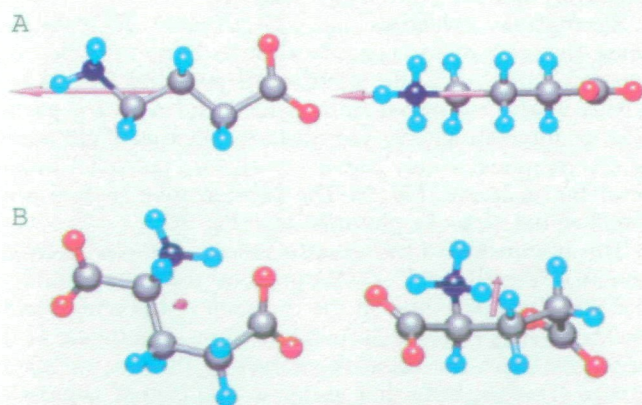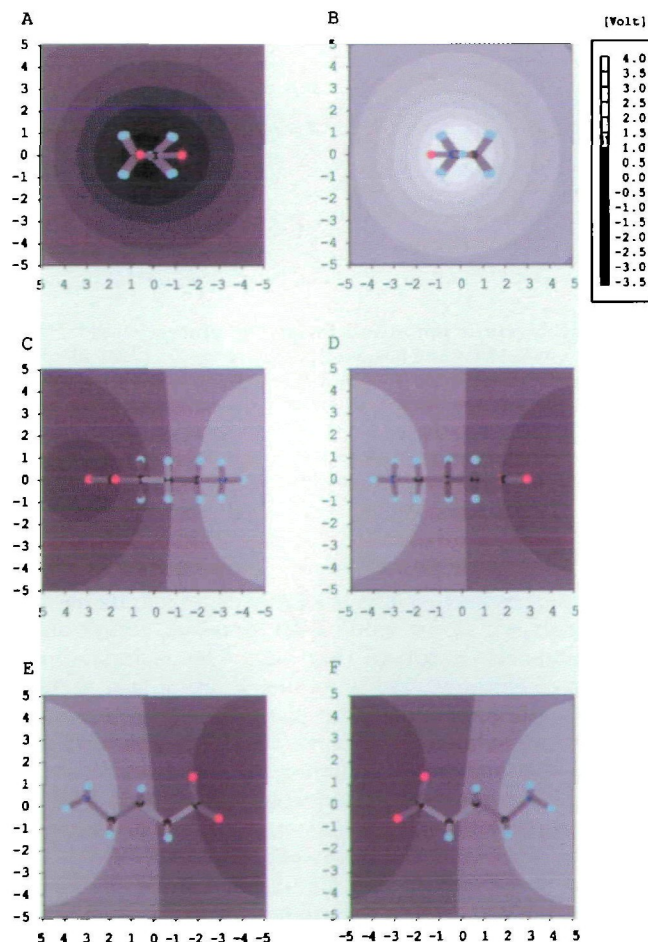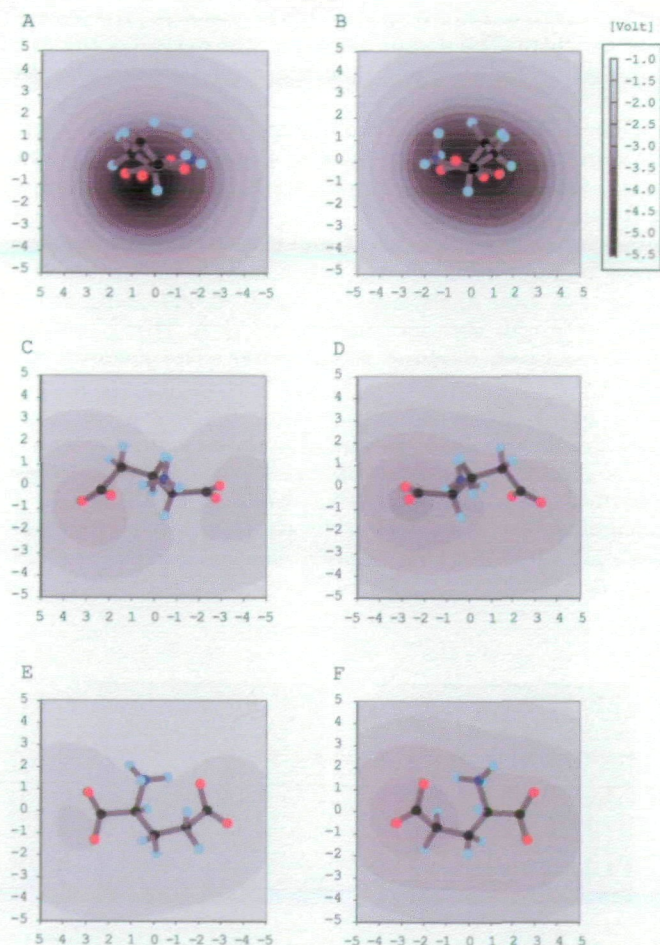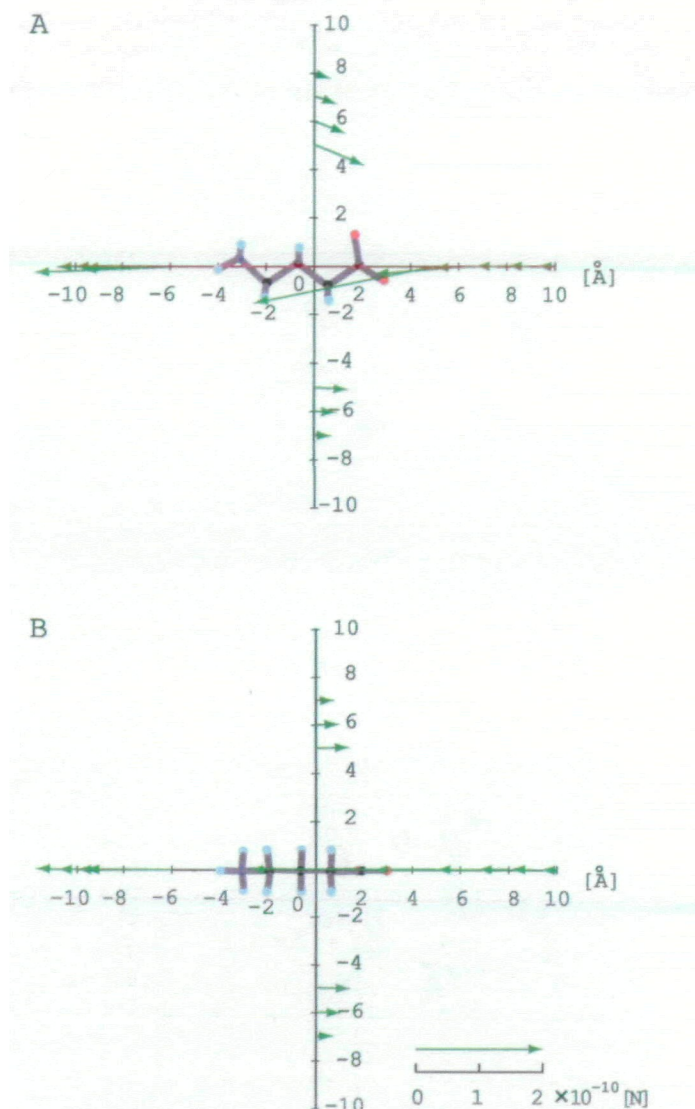

Fig. 4. **Electrostatic potentials formed by GABA.** Molecules are viewed from the following planes: on the *y-z* plane at *x* = 5 Å (A); on the *y-z* plane at *x* = -5 Å (B); on the *x-z* plane at *y* = 5 Å (C); on the *x-z* plane at *y* = -5 Å (D); on the *x-y* plane at *z* = 5 Å (E); and on the *x-y* plane at *z* = -5 Å (F). The origin of the coordinates is the electric charge center of a molecule. The numberings on the axes are expressed in Å. The iso-electrostatic potentials are expressed in voltage.

Fig. 5. **Electrostatic potentials formed by glutamic acid.** Molecules are viewed from the following planes: on the y-z plane at $x = 5$ Å (A); on the y-z plane at $x = -5$ Å (B); on the x-z plane at $y = 5$ Å (C); on the x-z plane at $y = -5$ Å (D); on the x-y plane at $z = 5$ Å (E); and on the x-y plane at $z = -5$ Å (F). The origin of the coordinates is the electric charge center of a molecule. The numberings on the axes are expressed in Å. The iso-electrostatic potentials are expressed in voltage.

Fig. 6. **Electrostatic force of GABA on a monovalent cation.** The cation was set on the x-axis and the y-axis (A) and on the x-axis and the z-axis (B). The arrows indicate the magnitude and the azimuth of the electrostatically attractive force.

boxyl group on each end, it is largely polarized. Its electric dipole moment, whose value is 21.98 debye, occurs along the amino-acid backbone (Fig. 3A). The electric dipole moment of glutamic acid is formed in an almost vertical direction against the molecular plane, and its value is 4.97 debye, which is about one-fourth that of GABA (Fig. 3B). These results suggest the following two points. (i) When the molecules are away from their receptors, GABA can be expected to approach the binding site of the GABA receptor along the direction of the amino-acid backbone, while glutamic acid may approach the binding site of the glutamate receptor in the vertical direction against the molecular plane. (ii) If the GABA receptor and glutamate receptor have similar electric fields, and the solvent surrounding each ligand is in a similar condition, GABA can interact with the entrance of the GABA receptor from a long distance because of its larger electric dipole moment in comparison with the case of glutamic acid and the glutamate receptor. In other words, GABA at low concentration may

effectively approach the GABA receptor.

Electrostatic Potentials and Electrostatic Forces—Because the positive and negative electric charges are clearly separated in GABA, the electrostatic potential formed by GABA is also separated into positive and negative parts (Fig. 4). Interestingly, the electrostatic potential of glutamic acid is composed of only negative parts on a plane 5 Å away from the molecule (Fig. 5). The two negative centers are found on the x-axis for glutamic acid (Fig. 5C).

The electrostatic force greatly reflects the electrostatic potential (Figs. 6 and 7). GABA produces a strong, unidirectional electrostatic force in the direction of the amino-acid backbone (Fig. 6). The electrostatic force of glutamic acid originates from the negative charges of the two carboxyl groups. This suggests that glutamic acid cannot approach any negatively charged molecules, but it can easily approach positively charged molecules.
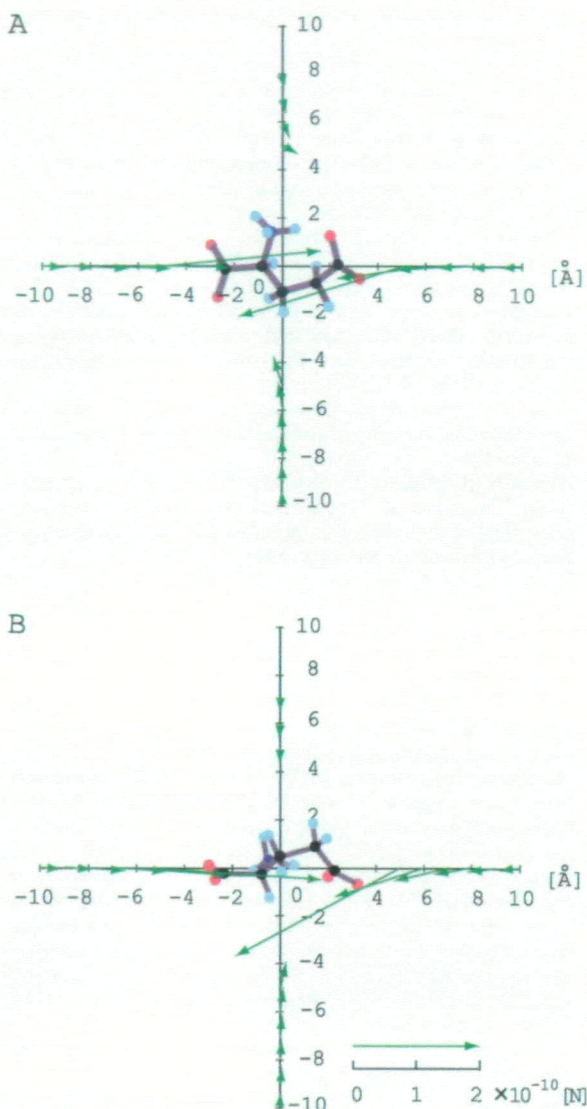
Fig. 7. **Electrostatic force of glutamic acid on a monovalent cation.** The cation was set on the x-axis and the y-axis (A) and on the x-axis and the z-axis (B). The arrows indicate the magnitude and the azimuth of the electrostatically attractive force.

DISCUSSION

In the present study, we calculated the electronic and structural states of ionized GABA and ionized glutamic acid using the *ab initio* method with the 6-311++G (3df, 2pd) basis set. We succeeded in showing that a difference of only one carboxyl group induces significant differences in the electronic and structural states between GABA and glutamic acid (Fig. 2). In turn, these significant differences have a crucial influence on the electric dipole moments, the electrostatic potentials, and the electrostatic forces (Figs. 3–7). The most remarkable finding is that the electrostatic potential formed by glutamic acid is composed only of negative parts, while that formed by GABA is separated into positive and negative parts (Figs. 4 and 5).

Previous studies have examined the structure of GABA using the extended Hückel method (23) and the CNDO/2 method (24). Majumdar and Guha calculated the structures of GABA and glutamic acid using the CNDO/2 meth-

od (25). However, these semi-empirical calculations do not take into account the effects of hydrogen bonds. The *ab initio* calculation with the STO-3G basis set was first employed in a study of the optimization of the dihedral angle in GABA (26). Recently, Fugler-Domenico *et al.* (27) and Tsuda *et al.* (28) used the 6-31G basis set and the STO-3G basis set, respectively, to calculate GABA. Unfortunately, the former study calculated only the non-ionized molecule, so that the behavior of GABA under liquid conditions was unknown; the latter study, because it used the STO-3G basis set, also did not deal sufficiently with ionized molecules, even though the researchers attempted to consider them. These studies (23–28) were achieved when the *ab initio* method was underdeveloped or when the performance of a computer was limited. At present, a large basis set, such as the 6-311++G(3df,2pd), is practical for many workstation computers. This issue of basis set dependency will be expatiated in future work.

The flexibility of GABA was suggested in a previous study (29). However, we could not find GABA flexibility according to the geometry optimization of various initial structures using the 6-311++G(3df,2pd) basis set. GABA and glutamic acid in Fig. 2 are the unique structures of the ground state.

Recently, Kunishima *et al.* reported the structure of glutamic acid bonded in the binding site of the glutamate receptor (30), revealing that glutamic acid forms an extended structure. This structure is different from the folded structure of Fig. 2B in which the hydrogen bond between NH_3^+ and COO^- can be observed. Glutamic acid in the glutamate receptor forms some hydrogen bonds with amino acid residues, Ser165, Thr188, Asp318, and Lys409, in the binding site and with water molecules, HOH11, HOH17, and HOH46, in the binding site. We confirmed that the energy of the folded glutamic acid obtained in the present study is lower than that of the extended structure by the RHF/6-311++G(3df,2pd) calculation. The folded structure is the unique, most stable conformation. This result suggests that the folded, negatively charged glutamic acid enters the binding site of the glutamate receptor by losing its own hydrogen bond and changing its structure to the extended conformation. Then, the extended glutamate is energetically stabilized by forming hydrogen bonds with amino acid residues and water molecules in the binding site.

GABA forms an extended structure with the positive and negative parts at either end, whereas glutamic acid is a folded, negatively charged molecule. This structural difference indicates that GABA approaches the entrance of the GABA receptor by adjusting its orientation to the corresponding electric field; if the entrance is negatively or positively charged, GABA approaches the entrance with its own NH_3^+ or COO^-, respectively. On the other hand, the entrance of the glutamate receptor has many positive amino acid residues. This indicates that glutamic acid easily approaches the entrance by folding its carbon chain and forming a hydrogen bond, thus masking the positive charge on NH_3^+. These considerations support the experimental data that GABA at high concentration can bind to the glutamate receptor in *Aplysia* neurons (31).

Finally, the results of the present study can help illuminate the interactions between the following four pairs of ligands and receptors: (i) GABA and the binding site of the

GABA$_A$ receptor, (ii) glutamic acid and the binding site of the GABA$_A$ receptor, (iii) glutamic acid and the binding site of the AMPA/kainate receptor, and (iv) GABA and the binding site of the AMPA/kainate receptor. With regard to the first two pairs, Boileau et al. showed that the binding site of the GABA$_A$ receptor has Asp62 (negative charge) and Arg66 (positive charge) as its charged amino acids (15). Therefore, when GABA approaches this receptor, our present results predict that GABA will be oriented to match the electrostatic potential formed by these charged amino acids. As a result, GABA will be more attracted to the binding site. In contrast, glutamic acid will be prevented from entering the GABA$_A$ receptor binding site because of the negative charge on Asp62.

Next, we consider the interactions between glutamic acid and the AMPA/kainate receptor and between GABA and the AMPA/kainate receptor. The charged amino acids at the binding site of the AMPA/kainate receptor are Arg507 (positive charge), Lys678 (positive charge), and Glu727 (negative charge) (12). The summation of charges in the binding site is therefore plus one. This suggests that when glutamic acid approaches the AMPA/kainate receptor, for example at a distance of about 5 Å, it will adjust its orientation so that it is attracted to the binding site. As noted earlier, we have confirmed that even when the electrostatic potential produced by glutamic acid is observed within 5 Å, it is still negative (data not shown). When GABA approaches the AMPA/kainate receptor, GABA may be oriented to match the electrostatic potential formed by Arg507, Lys678, and Glu727. Whether GABA can approach the AMPA/kainate receptor or not will be carefully examined again in future work, as the changes in the net charges of the ligands and receptors when a receptor binds to a ligand must also be considered.

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