Dielectric Features of Neurotransmitters, γ-Aminobutyric Acid and L-Glutamate, for Molecular Recognition by Receptors

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The neurotransmitters γ-aminiobutyric acid (GABA) and L-glutamate (Glu) have confined plate and disk geometries caused by 2 and 3 tightly hydrated water molecules in aqueous solution, respectively. They also have large dipole moments ($\boldsymbol{\mu}$): ca. 14 and 7.1 D, respectively. The direction of $\boldsymbol{\mu}$ for both GABA and Glu is included in planes of the plate and disk geometries. Moreover, $\boldsymbol{\mu}$ is parallel to the long molecular axis for GABA, whereas it is perpendicular to the long axis for Glu. Receptors for these neurotransmitters bear a "clamshell-like" bi-lobate structure in their ligand binding regions and maintain an open−close motion of the two lobes at a rate of $\sim 10^8$ s^{-1} in aqueous solution. These receptors detect differences in magnitude and direction of $\boldsymbol{\mu}$ for GABA and Glu, allowing correct molecular recognition. As the first step of the molecular recognition process, the receptors effectively control the orientation of GABA and Glu via dipole−dipole interaction between their $\boldsymbol{\mu}$ and a time-dependent dipole moment, i.e., an electric field is generated between the binding sites on the moving lobes.

Introduction

The neurotransmitters γ-aminobutyric acid (GABA; inhibitory) and L-glutamate (Glu; excitatory) have opposite effects on neurons in the central nervous system. Neurons are hyperpolarized by activation of GABA receptors caused by GABA binding. In contrast, neurons are depolarized by activation of glutamate receptors caused by Glu binding. The single difference in chemical structure between these two neurotransmitters is that Glu bears one more carboxyl group than GABA, and their receptors must distinguish this small difference to achieve correct molecular recognition.[1−5] Little is known about molecular recognition mechanisms that function with such extremely precise discrimination in biological systems. Consequently, most parts of the molecular recognition process are still unclear. The conventional idea[6] is that recognition occurs when a ligand and its receptor are related to each other like a key and keyhole. This implies that recognition is determined by the geometries of the ligand and its receptor. However, this idea does not explain how the ligand is oriented in the correct direction, relative to the binding sites on its receptor, to start the recognition process.

Glu carries an electric charge and is attracted to its receptor via electrostatic interaction, whereas GABA has no total electric charge. GABA is a neutral zwitterion that maintains a well-defined dipole moment ($\boldsymbol{\mu}$) with a relatively large magnitude in aqueous solution. In contrast, the magnitude of $\boldsymbol{\mu}$ for Glu must be treated carefully because Glu is an anionic species. Recently, it has been confirmed that some anionic species, such as *p*-toluenesulfonate, possess finite dipole moments in aqueous solution.[7] Thus, Glu likely bears both an electric charge and $\boldsymbol{\mu}$. In the present study, dielectric features of Glu and GABA in aqueous solution were investigated in detail, and the importance of dipole−dipole interaction of GABA and Glu with their receptors for molecular recognition is discussed. Also, we propose an explanation of how GABA and Glu are oriented in the correct direction, relative to their binding sites on their receptor, for the initial stage of the molecular recognition process.

Experimental Section

Materials. GABA (purity, >98%) was purchased from Wako Pure Chemicals Ltd. (Osaka) and used as received. Sodium L-glutamate monohydrate ($NaGlu \cdot H_2O$; purity, 99.99%) was kindly supplied by Ajinomoto Co. Inc. (Tokyo). Highly deionized water (specific resistance, >16 MΩ cm) obtained using a Milli Q system (Nippon MilliPore, Tokyo) was used as the solvent of sample solutions for dielectric relaxation measurements. The concentrations (*c*) of GABA and NaGlu in sample solutions ranged from 100 to 1000 mM.

Methods. The dielectric relaxation behavior of aqueous solutions of GABA and Glu was investigated using two measuring systems. A system consisting of an LCR meter (4287A, Agilent Technologies) equipped with a homemade electrode cell was operated in a frequency (*f*) range from 10^6 to 10^9 Hz. A dielectric material probe system (HP85070B; includes an 8720ES network analyzer; Hewlett-Packard) equipped with a coaxial probing cable and controlled by a program supplied by Hewlett-Packard was operated in an *f* range of 10^8 to 2×10^{10} Hz.[7−9] The temperature of sample liquids was maintained at 25 °C with circulating thermostated water. Detailed descriptions of the measurement procedure are given elsewhere.[7−9]

Densities of the aqueous GABA and $NaGlu \cdot H_2O$ solutions were measured as a function of *c* using a digital density meter (DMA5000, Anton Paar, Graz) at 25 °C to evaluate the partial molar volume of GABA and NaGlu in aqueous solution.

Ab initio quantum chemical calculations to determine the optimized geometries and $\boldsymbol{\mu}$ for GABA dihydrate and Glu trihydrate were performed using Gaussian 98[10] on a computer system in Professor Ueyama's laboratory, Department of

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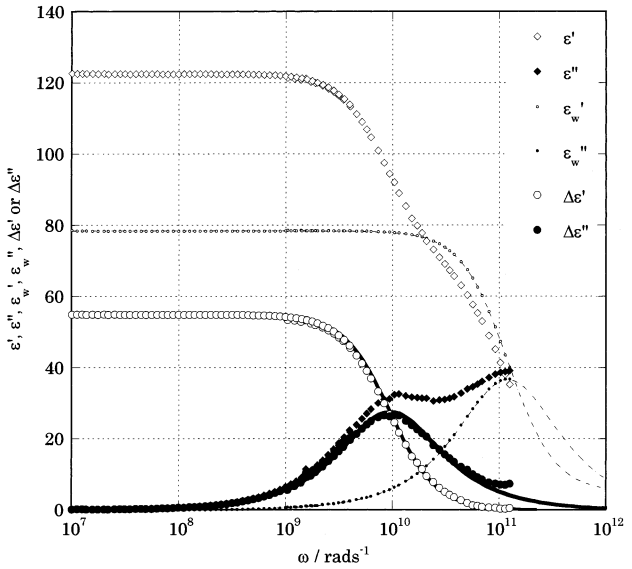

Figure 1. Frequency ω dependencies of the real and imaginary part, ϵ' and ϵ'', of electric permittivity for an aqueous solution of GABA at 1000 mM; ϵ_w' and ϵ_w'' for pure water and $\Delta\epsilon'$ and $\Delta\epsilon''$ for GABA calculated by equations in the text are also plotted. Thick solid lines represent the best fitting Debye-type relaxation functions, eq 1, for $\Delta\epsilon'$ and $\Delta\epsilon''$ with $\tau = 1.0 \times 10^{-10}$ s and $\Delta\epsilon = 55$, respectively.

Macromolecular Science, Osaka University. A conventional Hartree−Fock theory was applied at the level of a 6-31G** basis set widely used for precise quantum chemical calculations.[11]

Results and Discussion

Dielectric Relaxation. Typical dielectric relaxation spectra (real and imaginary part of complex permittivity; ϵ' and ϵ'') for aqueous solutions of GABA at a concentration of $c = 1000$ mM are shown in Figure 1 as a function of angular frequency ($\omega = 2\pi f$). Thin broken lines reproducing data for pure water, ϵ_w' and ϵ_w'', represent the ω dependencies of published data[12] used as calibration curves for the measuring systems used in this study: $\epsilon_w' = (\epsilon_w - \epsilon_{w\infty})(1 + \omega^2\tau_w^2)^{-1} + \epsilon_{w\infty}$, $\epsilon_w'' = (\epsilon_w - \epsilon_{w\infty})\omega\tau_w(1 + \omega^2\tau_w^2)^{-1}$, $\epsilon_w = 78.4$, $\epsilon_{w\infty} = 5.1$, and $\tau_w = 8.3 \times 10^{-12}$ s at 25 °C (cf. eq 1). It is obvious that the relaxation strength of free water molecules found around $\omega = 1.2 \times 10^{11}$ rad s^{-1} decreases dramatically in the presence of GABA at $c = 1000$ mM, whereas a new relaxation mode is found around $\omega = 10^{10}$ rad s^{-1}. As a result, the value of ϵ' in the low-ω region increases to 122, which is higher by 44 than the dielectric constant of pure water ($\epsilon_w = 78.4$).

Two relaxation modes were observed in the ϵ' and ϵ'' spectra at $\omega_f = 1.2 \times 10^{11}$ and $\omega_s = 1.0 \times 10^{10}$ rad s^{-1} (Figure 1). The fast mode, in which strength decreases with increasing c, was assigned to the rotational relaxation mode of water molecules because the relaxation time of the most preferential relaxation mode for pure water ($\tau_w = 8.3 \times 10^{-12}$ s at 25 °C[12]) is identical to the reciprocal of ω_f. The other mode, found at ω_s, in which relaxation time (τ) is essentially independent of c and relaxation strength ($\Delta\epsilon$) is proportional to c (described below), was assigned to a relaxation mode caused by the presence of GABA.

If the distribution of relaxation time for bulk aqueous phase is not affected by the presence of GABA but is well expressed by Debye-type single-relaxation formulas for pure water (described above), adequate subtraction of the dielectric contribution of pure water and ionic components from ϵ' and ϵ'' provides the dielectric spectra, $\Delta\epsilon'$ and $\Delta\epsilon''$, for GABA, as follows: $\Delta\epsilon' = \epsilon' - \phi\epsilon_w$ and $\Delta\epsilon'' = \epsilon'' - \phi\epsilon_w - G_{dc}C_{0\omega}^{-1}$, where ϕ represents the relative contribution of water to the total dielectric behavior, G_{dc} represents direct current conductance for samples due to the presence of ionic components, and C_0 represents the cell constant for a used electrode cell. Because the ω dependence of the obtained $\Delta\epsilon'$ and $\Delta\epsilon''$ ($\phi = 0.865$) is well described by the Debye-type single-relaxation formulas (eq 1) indicated by thick solid lines in Figure 1, GABA possesses a simple dielectric feature in aqueous solution with only one set of c-independent relaxation time, $\tau = 1.0 \times 10^{-1}$ s, and strength, $\Delta\epsilon$, proportional to c (Figure 2).

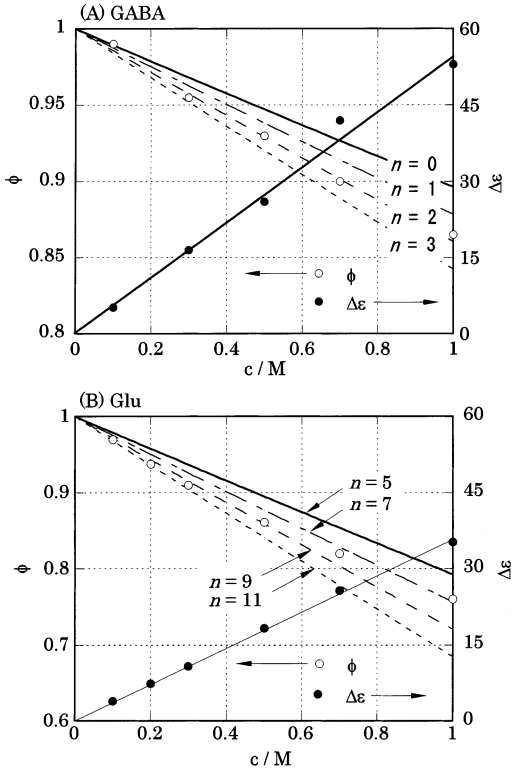

Figure 2. Relationship between ϕ and $\Delta\epsilon$ and c for aqueous solutions of GABA (A) and NaGlu (B).

$$\Delta\epsilon' = \frac{\Delta\epsilon}{1 + \omega^2\tau^2}$$

$$\Delta\epsilon'' = \frac{\Delta\epsilon\omega\tau}{1 + \omega^2\tau^2} \qquad (1)$$

If the dielectric spectra for bulk aqueous phase are affected by the presence of GABA and are different from those of pure water, the $\Delta\epsilon'$ and $\Delta\epsilon''$ obtained by subtraction of the contribution of pure water from ϵ' and ϵ'' do not show Debye-type formulas (Figure 1). Although the dielectric spectra for bulk aqueous phase are identical to those of pure water, the $\Delta\epsilon'$ and $\Delta\epsilon''$ obtained by subtraction do not show the types of ω dependencies seen in Figure 1 if the relaxation mode of GABA in aqueous solution is not described by the Debye-type formulas (eq 1). These findings indicate that the ω dependencies of dielectric spectra for the aqueous GABA system are well decomposed into two types of Debye-type relaxation modes, as observed in some other aqueous solutions of small solutes.[7−9] The value of ϕ was determined rather precisely with experimental errors of <3%.

Because dielectric relaxation spectra similar to those in Figure 1 were obtained in aqueous solutions of sodium L-glutamate, NaGlu·H_2O, Glu also has the simple dielectric features described

by eq 1. The value of τ for Glu in aqueous solution was determined to be 1.0×10^{-1} s and was independent of c. The value of $\Delta\epsilon$ for Glu is proportional to c, as is the case with GABA (Figure 2), despite its negative charge. The fact that the τ values we obtained for GABA and Glu are identical to each other indicates that the hydrodynamic sizes of these neurotransmitters in aqueous solution are quite similar. On the other hand, the difference in slope between $\Delta\epsilon$ and c, for both GABA and Glu (Figure 2), suggests a difference in magnitude of their dipole moments, $\boldsymbol{\mu}$, as discussed below.

The dependence of ϕ on c for GABA and Glu in aqueous solution (Figure 2) is relevant to the total number (n) of water molecules tightly hydrated to them, the rotational relaxation mode of which is highly restricted and is detected at the same relaxation frequency as that of the tightly hydrated GABA and Glu in aqueous solution.[7−9] Solid lines in the plots for ϕ represent a proposed function which has been widely used to evaluate n in aqueous solution:[7−9,12] $\phi = (1-\upsilon_m c)/(1 + 0.5\upsilon_m c) - nc/55.6$, where υ_m represents the partial molar volume of a solute (73.0 $cm^3\ mol^{-1}$ for GABA and 81.5 $cm^3\ mol^{-1}$ for NaGlu in aqueous solution at 25 °C). The fact that a line predicted for GABA assuming $n = 2$ shows perfect agreement with the present data implies that GABA is tightly hydrated by two water molecules. Although the value of n for NaGlu solutions is estimated to be ∼9 for low values of c, Glu is tightly hydrated by three water molecules, because the counterion, Na^+, is tightly hydrated by six water molecules.[7,13]

If interaction between a solute and water molecules is not as strong as in mixtures of alcohol and water, $\Delta\epsilon c^{-1}$ is directly related to the magnitude of $\boldsymbol{\mu}$ for the solute, which is tightly hydrated by some water molecules in aqueous solution in many cases, as indicated by eqs 2 and 3.[7−9] The expression of eq 2 is identical to that of Oncley's model,[14,15] which has been used for estimation of the magnitude of dipole moments of biological molecules such as proteins in aqueous solution. The observation that the value of $\Delta\epsilon c^{-1}$ for GABA (ca. 55 M^{-1}) is greater than that for Glu (ca. 35 M^{-1}) suggests that the magnitude of $\boldsymbol{\mu}$ for GABA is greater than that for Glu in bulk aqueous phase

$$\frac{\Delta\epsilon_s}{c} = \frac{AN_A\boldsymbol{\mu}^2}{2\epsilon_v kT} \tag{2}$$

$$A = 1 + \frac{|\boldsymbol{\mu}_w| z \cos\gamma}{|\boldsymbol{\mu}|} \tag{3}$$

where $\boldsymbol{\mu}_w$, z, and γ represent the dipole moment of a water molecule (ca. 1.85 D), the number of the first neighbor water molecules surrounding a tightly hydrated solute, and the average angle between $\boldsymbol{\mu}$ of the tightly hydrated solute and $\boldsymbol{\mu}_w$ of the first neighbor water molecules, respectively. N_A, ϵ_v, and kT represent Avogadoro's number, the permittivity of a vacuum, and the product between Boltzmann's constant and the absolute temperature, respectively. The values of z for GABA and Glu in aqueous solution were estimated as 25 and 30, respectively, from their υ_m values, using a method proposed previously;[7−9,13] $\upsilon_m = 87.8\ cm^3\ mol^{-1}$ for Glu. Because the value of cos γ ranges from 0.6 to 0.8 for small dipolar molecules and ions in aqueous solution, such as trimethylamine oxide,[9] glycine betaine [8] and *p*-toluenesulfonate,[7] the magnitude of $\boldsymbol{\mu}$ for GABA and Glu in aqueous solution was calculated to be 13 ± 1.0 and 8.0 ± 1.0 D, respectively.

Ab Initio Calculations. Because GABA and Glu are tightly hydrated by two and three water molecules in aqueous solution, respectively, as described above, molecular geometries for GABA and Glu in the bulk aqueous phase are estimated as the optimized geometries of GABA dihydrate and Glu trihydrate obtained by ab initio quantum chemical calculations using Hartree−Fock theory at the level of a 6-31G** basis set. The obtained optimized geometries for these hydrates are shown in Figure 3A and B, and some important atomic distances (λ_{i-ii}) and triangular ($\alpha_{i-ii-iii}$) and dihedral ($\beta_{i-ii-iii-iv}$) angles are summarized in Table 1; subscripts i, ii, iii, and iv represent related atoms, as shown in Figure 3A and B. It is interesting to note that a zwitterionic structure is not obtained but an electrically neutral molecular-type structure (e.g., $NH_2(CH_2)_3$-COOH) is always obtained when no tightly hydrated water molecules are introduced in the optimized geometry calculations for GABA by ab initio quantum calculations using Gaussian 98. The presence of the two tightly hydrated water molecules on GABA is likely to be essential for maintenance of the zwitterionic structure of GABA in aqueous solution.

These hydrates have relatively flat 3/4-moon-like plate and disk geometries including two and three water molecules, respectively, as depicted schematically in Figure 3A and B. Directions of the calculated $\boldsymbol{\mu}$ for the hydrates, represented by arrows, are included in planes of the plate and disk geometries. Moreover, $\boldsymbol{\mu}$ for GABA dihydrate is parallel to the long axis of a GABA molecule, whereas $\boldsymbol{\mu}$ for the Glu trihydrate is almost perpendicular to the long axis of Glu.

The calculated magnitude of $\boldsymbol{\mu}$ for GABA dihydrate (ca. 14 D) is almost twice as large as that of Glu trihydrate, as expected

TABLE 1: Some Important Parameters, Atomic Distances, Triangular and Dihedral Angles, and the Values of Dipole Moments at the Optimized Geometries of GABA dihydrate (Figure 3A) and Glu Trihydrate (Figure 3B)

parameters[a]	GABA dihydrate	Glu trihydrate
λ_{O1-C1}/nm	0.122	0.123
λ_{O2-C1}/nm	0.125	0.125
λ_{C1-C2}/nm	0.155	0.154
λ_{C2-C3}/nm	0.154	0.154
λ_{C3-C4}/nm	0.152	0.152
λ_{O4-N}/nm	0.151	0.151
λ_{C4-C5}/nm		0.156
λ_{O5-O3}/nm		0.124
λ_{O5-O4}/nm		0.122
$\beta_{O1-C1-C2-C3}$/deg	105.0	91.6
$\beta_{C1-C2-C3-C4}$/deg	149.5	160.9
$\beta_{C2-C3-C4-N}$/deg	−58.5	−72.2
$\beta_{C3-C4-N-H1}$/deg	−35.6	−2.6
$\beta_{C3-C4-C5-O3}$/deg		119.4
$\beta_{C4-C5-O3-H2}$/deg		0.4
$\lambda_{Hw11-O1}$/nm	0.189	0.183
$\alpha_{Ow1-Hw11-O1}$/deg	166.5	168.7
$\alpha_{Ow1-O1-C1}$/deg	107.1	116.6
$\beta_{Ow1-O1-C1-C2}$/deg	−34.7	−18.5
$\beta_{Hw12-Ow1-O1-C1}$/deg	−135.2	−106.1
$\lambda_{Hw21-H1}$/nm	0.162	0.186
$\lambda_{Hw22-O2}$/nm	0.157	0.168
$\alpha_{Ow2-Hw22-O2}$/deg	173.8	171.8
$\alpha_{Ow2-H1-N}$/deg	162.7	176.4
$\alpha_{Ow2-O2-C1}$/deg	123.6	116.9
$\beta_{Ow2-O2-C1-C2}$/deg	8.3	13.1
$\beta_{Ow2-N-C4-C3}$/deg	−24.2	−0.2
$\beta_{Hw21-Ow2-O2-C1}$/deg	148.4	128.6
$\lambda_{Hw31-Ow1}$/nm		0.22
$\lambda_{Hw32-O4}$/nm		0.21
$\alpha_{Ow3-Hw31-Ow1}$/deg		172.7
$\alpha_{Ow3-Hw32-O4}$/deg		162.6
$\beta_{Ow3-Ow1-O1-C1}$/deg		12.1
$\beta_{Ow3-O4-C5-C4}$/deg		−21.2
$\lvert\boldsymbol{\mu}\rvert$/Debye	14 (13 ± 1.0)[b]	7.1 (8.0 ± 1.0)[b]

[a] Description for atoms and coordinates is presented in Figure 3. [b] Determined by dielectric relaxation measurements.

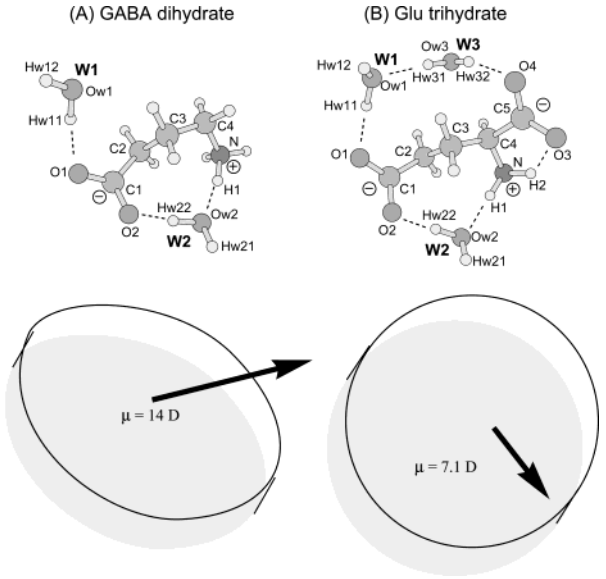

Figure 3. Optimized geometries for a GABA dihydrate (A) and Glu trihydrate (B) obtained by ab initio calculations. Lower pictures schematically represent rough contours, a 3/4-moon-like plate and disk, for the two hydrates and magnitudes and direction of the calculated dipole moments, μ, with arrows. Dotted lines show hydrogen bonding.

from the difference in the values of $\Delta\epsilon c^{-1}$. Although the magnitude of $\boldsymbol{\mu}$ for Glu trihydrate is calculated to be 6.9 D by Gaussian 98, the value is slightly corrected (to 7.1 D) by a previously proposed method,[7] because the Glu trihydrate is not a neutral molecule but an ionic species. In the method for precise calculation of dipole moment for a monoanionic species such as Glu, an unnecessary term, $-e_0(\mathbf{r}_- - \mathbf{r}_G)$, is subtracted from the $\boldsymbol{\mu}$ value obtained for neutral molecules using ordinary calculations and Gaussian 98, where e_0, $\mathbf{r}_-$, and $\mathbf{r}_G$ represent the elementary charge, the position vector for the center of negative charges, and the position vector for the center of gravity for the monoanionic species, respectively.[7] In practice, correction of $\boldsymbol{\mu}$ for Glu is slight and of relatively low importance, because the contribution of the calculated $\mathbf{r}_- - \mathbf{r}_G$ value is small.

The fact that the experimentally determined values of $\boldsymbol{\mu}$ for GABA and Glu in aqueous solution agree well with those obtained by calculations for GABA dihydrate and Glu trihydrate indicates that the optimized geometries of GABA dihydrate and Glu trihydrate (Figure 3A and B) provide a reasonably good description of the dissolved states of GABA and Glu in aqueous solution. The tightly hydrated water molecules, especially W2, which connects a carboxylate and ammonium group by hydrogen bonding in both GABA dihydrate and Glu trihydrate (Figure 3A and B), likely confine the plate and disk geometries.

Molecular Recognition by Receptors. The three-dimensional crystal structure of the extracellular ligand-binding region of a metabotropic glutamate receptor (mGluR1) has been determined in a complex form with Glu.[16] Interestingly, mGluR1 binds Glu in its binding sites in a conformation quite similar to that of Figure 3B, with three water molecules in positions not very different from those of the Glu trihydrate.[16] This strongly suggests that Glu is bound by mGluR1 in a geometry resembling that of the bulk aqueous phase, maintaining the number of tightly hydrated water molecules. mGluR1 has a "clamshell-like" bi-lobate architecture in which two lobes move quickly to form open and closed conformations, due to Brownian motion.[16] The binding of Glu to mGluR1 stabilizes the closed conformation in dynamic equilibrium between open and closed conformations. The rate of (random) open−close (O−C) motion for bi-lobate type receptors in the presence and absence of a ligand has been roughly estimated to be ca. 2×10^8 s^{-1}.[17,18] Thus, the rate of the O−C motion for the two lobes of mGluR1 would be on the same order.

The free rotational rate of Glu tightly hydrated by three water molecules in the bulk aqueous phase is estimated to be 10^{10} s^{-1} from its dielectric relaxation time, τ ($= 1.0 \times 10^{-10}$ s). Thus, the motion of the two lobes of mGluR1 is much slower than the free rotation of Glu in the bulk aqueous phase. A binding site on a portion of a lobe (LB1) bears positive charges at amino acids R78 and K409, while a binding site on a portion of the other lobe (LB2) bears negative charges at amino acids E292, D208, and D318 and a positive charge at amino acid R323; the total charge of LB2 is negative.[16] Thus, the O−C motion of the lobes creates a time-dependent dipole moment or electric field that can effectively harmonize the orientational motion of the $\boldsymbol{\mu}$ of Glu in the cleft of the lobes due to dipole−dipole interaction. Because the rotational relaxation time of Glu is much shorter than the time necessary for the O−C motion, which is estimated to be longer than 5×10^{-9} s, Glu experiences strong torque during orientation of its $\boldsymbol{\mu}$ to the electric field. Consequently, the orientation of Glu to the electric field generated between the lobes completely follows the O−C motion if the electric field is strong enough. Moreover, the strength of the electric field becomes greater as closing of the cleft of the lobes (and, therefore, the orientation of Glu to the electric field) achieves the optimum time course (see Figure 4).

Glu with $\boldsymbol{\mu}$ highly orientated to the electric field between the lobes would be first bound to LB1 due to the electrostatic interaction between the total negative charge of Glu and a positive portion of LB1, through several time matching or gearing tests harmonizing to the O−C motion of the lobes in the direction of its disk geometry with the shape of the depression of the binding site. Once Glu is bound to LB1, Glu easily finds another binding site on LB2, because the two lobes continue the O−C motion irrespective of the presence of the bound Glu. The total negative charge and a phenyl ring of Tyr236 of the binding site on LB2 attract an ammonium group of the bound Glu due to the electrostatic and cation−π interaction.[16,19] The sequence of molecular recognition and binding for a system of Glu and mGluR1 is depicted schematically in Figure 4.

The amino acid sequence of the metabotropic GABA receptor $GABA_B$ is similar to that of mGluR1.[20] At present, the three-dimensional crystal structure of $GABA_B$ with or without bound GABA has not been determined; however, $GABA_B$ may have a bi-lobate architecture like that of mGluR1. If $GABA_B$ has such a bi-lobate architecture, it is likely that dielectric features of GABA tightly hydrated by two water molecules in the bulk aqueous phase, which likely maintains geometry similar to that shown in Figure 3A, play roles in molecular recognition by $GABA_B$. This suggests a mechanism for recognition of GABA by $GABA_B$, assuming $GABA_B$ has a ligand-binding region containing two moving lobes. Two binding sites on each lobe of $GABA_B$ would bear total electric charges opposite as those of mGluR1; the O−C motion of the lobes would generate a time-dependent dipole moment or electric field that can harmonize the orientation of $\boldsymbol{\mu}$ of GABA in the cleft due to the dipole−dipole interaction. The electric field generated between the lobes would also pull GABA into the biding sites. Because GABA has no total electric charge, the question of which binding site on the lobes binds GABA first is statistical. The mechanism for binding of GABA by $GABA_B$ would be

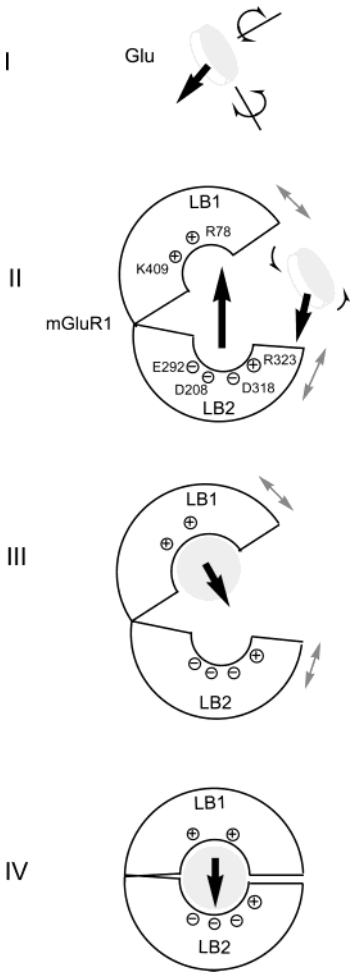

Figure 4. Schematic depiction of the sequence for molecular recognition and binding for Glu by mGluR1. (I) free rotation of Glu far from mGluR1, (II) harmonized rotation of Glu by the open−close motion of lobes of mGluR1 owing to dipole−dipole interaction, (III) recognition of Glu by a binding site on LB1, (IV) binding of Glu to both binding sites on LB1 and LB2. Thick arrows represent the directions of $\boldsymbol{\mu}$.

essentially the same as that for binding of Glu by mGluR1 described above (Figure 4).

The three-dimensional crystal structure of bacterial periplasmic lysine/arginine/ornithine binding protein (LAO; an amino acid binding protein) has been determined for forms with and without lysine.[18] LAO has a "clamshell-like" bi-lobate architecture in its ligand-binding region, similar to that of mGluR1. The two ligand-binding sites of LAO (R77 on LB1 and D161 on LB2) bear opposite electric charges, allowing it to catch a carboxylate and α-ammonium group belonging to lysine, which has another ϵ-ammonium group and is a dipolar cation in aqueous solution.[18] LAO has another negative charge, at D11 on LB1, which allows it to catch the ϵ-ammonium group of lysine. The O−C motion of the lobes of LAO generates a time-dependent electric field that helps orient lysine in the correct direction to start the molecular recognition process, gearing test.

In summary, interaction between the dipole moment of a ligand and the time-dependent electric field generated by the O−C motion of the lobes on its receptor bearing opposite electric charges is apparently essential for orientation of the ligand in the correct direction, relative to the binding sites, to start the molecular recognition process in many kinds of receptors possessing a "clamshell-like" bi-lobate architecture in the ligand-binding region.

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