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Quantum Chemical Study of Agonist–Receptor Vibrational Interactions for Activation of the Glutamate Receptor[1]

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To understand the mechanism of activation of a receptor by its agonist, the excitation and relaxation processes of the vibrational states of the receptor should be examined. As a first approach to this problem, we calculated the normal vibrational modes of agonists (glutamate and kainate) and an antagonist (6-cyano-7-nitroquinoxaline-2,3-dione: CNQX) of the glutamate receptor, and then investigated the vibrational interactions between kainate and the binding site of glutamate receptor subunit GluR2 by use of a semiempirical molecular orbital method (MOPAC2000-PM3). We found that two local vibrational modes of kainate, which were also observed in glutamate but not in CNQX, interacted through hydrogen bonds with the vibrational modes of GluR2: (i) the bending vibration of the amine group of kainate, interacting with the stretching vibration of the carboxyl group of Glu705 of GluR2, and (ii) the symmetric stretching vibration of the carboxyl group of kainate, interacting with the bending vibration of the guanidinium group of Arg485. We also found collective modes with low frequency at the binding site of GluR2 in the kainate-bound state. The vibrational energy supplied by an agonist may flow from the high-frequency local modes to the low-frequency collective modes in a receptor, resulting in receptor activation.

Key words: agonist–receptor vibrational interaction, glutamate receptor, kainate, normal vibrational mode analysis, PM3 method.

After a receptor binds an agonist, the receptor conformation changes and the signal transduction systems are induced. This activation mechanism for receptors, however, has not yet been elucidated. The dynamics of conformational changes of proteins are generally dominated by the excitation and relaxation processes of their vibrational states as well as their electronic states (*1*, *2*). Taking into account that a receptor can electrostatically bind both an agonist and an antagonist (*3*), but that only the agonist can activate the receptor, some specific vibrational modes of agonists are considered to play special roles in receptor activation. The energy transferred through agonist-receptor vibrational couplings probably originates from the kinetic energy of the agonist. This kinetic energy increases with the electrostatic attraction between the agonist and the receptor. Analyses of the vibrational modes of agonists, and of the vibrational interactions between agonists and receptors are therefore indispensable for clarifying receptor activation mechanisms.

Recently, the X-ray crystal structure of the agonist-binding region of glutamate receptor (GluR) subunit GluR2 in a complex with kainate was determined (*4*). Ionotropic GluRs including the kainate receptor are agonist-gated cation channels that mediate the vast majority of fast excitatory neurotransmission in the brain (*5–7*). Calcium entry through GluR channels or through calcium channels opened due to membrane depolarization caused by sodium influx plays important roles in the development and in forms of synaptic plasticity that may underlie higher-order processes such as learning and memory (*8*, *9*). The agonist-binding region of GluR2 is composed of two globular subdomains (S1 and S2 lobes), and kainate is bound in the cleft between them. In the present study, as a first approach for understanding the mechanism of activation of receptors, we compared the normal vibrational modes of two agonists (glutamate and kainate) and an antagonist (6-cyano-7-nitroquinoxaline-2,3-dione: CNQX) of the glutamate receptor, and then investigated the vibrational interactions between kainate and the binding site of GluR2. Some normal vibrational modes that interact with GluR2 were found in glutamate and kainate, but not in CNQX.

METHODS

Models of Glutamate, Kainate, and CNQX—The geometrical and electronic structures of glutamate, kainate, and CNQX were optimized by the PM3 method (*10*), one of the most reliable semiempirical molecular orbital methods,

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Abbreviations: GluR, glutamate receptor; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; IVR, intramolecular vibrational-energy redistribution.

using the MOPAC2000 program package (ver. 1.08 and ver. 1.11; Fujitsu, Tokyo). Under neutral pH conditions, the carboxyl groups and amine group in glutamate are deprotonated and protonated, respectively. The carboxyl groups and amine group in kainate are also deprotonated and protonated, respectively. We thus used the ionized models for these molecules in a physiological condition. The calculated geometrical and electronic structures of molecules were found to depend on their initial geometrical parameters, such as bond lengths, bond angles, and dihedral angles. In the present study, the stable structure of kainate was determined as follows: (i) the heavy-atom coordinates of kainate were taken from the crystal structure of the kainate-GluR2 complex registered in the Protein Data Bank (ID: 1gr2; The Research Collaboratory for Structural Bioinformatics, NJ); (ii) hydrogen atoms were added to complete the valence shells of the heavy atoms; (iii) two carboxyl groups were deprotonated and an amine group was protonated; and (iv) the positions of all atoms were optimized. The stable structure of glutamate was determined as follows: (i) 60 initial structures, particularly with the dihedral angles varying every 30° in the flexible carbon chain, were constructed; (ii) two carboxyl groups were deprotonated and an amine group was protonated; (iii) the positions of all atoms were optimized with these initial structures, and then four stable structures were obtained; (iv) the positions of all atoms were optimized again with these four structures using GAUSSIAN94, an *ab initio* molecular orbital method program (revision D.3; Gaussian Inc., Pittsburgh, PA), at the 6-31G** level; (v) three of these four structures converged on non-zwitterionic forms through intramolecular proton transfer; and (vi) the rest of the structure was thus determined to be the stable structure. The stable structure of CNQX was determined as follows: (i) the initial structure was constructed with reference to the ring structure of naphthalene, in which all the atoms were not on one plane. The structure of naphthalene was taken from its template in Winmopac (ver. 3.0; Fujitsu); (ii) the positions of all atoms were optimized, and then all the atoms except for the oxygen atoms in the nitro group were found to form a plane. Because the ring and the cyano group were rigid, this planar structure is stable; and (iii) the positions of all atoms were optimized with the initial structures with the nitro group rotated every 30° from the molecular plane to determine the direction of the nitro group.

Model of the Binding Site of GluR2 in the Kainate-Bound State—The structure of the binding site of GluR2 in the kainate-bound state was determined as follows. Our calculation model included kainate, ten amino acid residues packing kainate, and two water molecules near kainate. These ten amino acid residues were Tyr450, Pro478, Leu479, Thr480, Arg485, Ser652, Gly653, Ser654, Thr655, and Glu705. One of the two water molecules was hydrogen bonded to Ser652 and the other to Glu705. The α-carbons next to the peptide bonds at the N-terminus of Pro478, the C-terminus of Thr480, the N-terminus of Ser652, the C-terminus of Thr655, and both termini of Tyr450, Arg485, and Glu705 were substituted by methyl groups. We have confirmed the lack of an effect of this capping by such methyl groups on the charge distributions of the amino acid residues. The heavy-atom coordinates of these molecules were taken from 1gr2. Hydrogen atoms were added to complete the valence shells of the heavy atoms. The two carboxyl groups of kainate and the carboxyl group of Glu705 were deprotonated. The amine group of kainate and the guanidinium group of Arg485 were protonated. The positions of all atoms were optimized by the MOPAC2000-PM3 method. As a result, the atoms in Ser652–Thr655 moved approximately 4 Å from the crystal structure, and the hydrogen bond between kainate and the water molecule bonded to Ser652 disappeared. To avoid these problems, after the coordinates of Ser652–Thr655 and those of the water molecule hydrogen bonded to Ser652 had been reorganized to fit those of the crystal structure, the positions of all atoms were optimized again. Furthermore, the optimization was repeated after the coordinates of the other amino acid residues had also been reorganized to fit those of the crystal structure. The stable structure nearly reproduces the crystal structure and at least maintains the hydrogen bonds expected for the crystal structure.

Analyses of Normal Vibrational Modes—The normal vibrational modes of our models of glutamate, kainate, CNQX, and the binding site of GluR2 in the kainate-bound state were calculated using MOPAC2000 (*11*). All the internal degrees of freedom ($3n$-6) were taken into account in our calculation, where n is the number of atoms in the

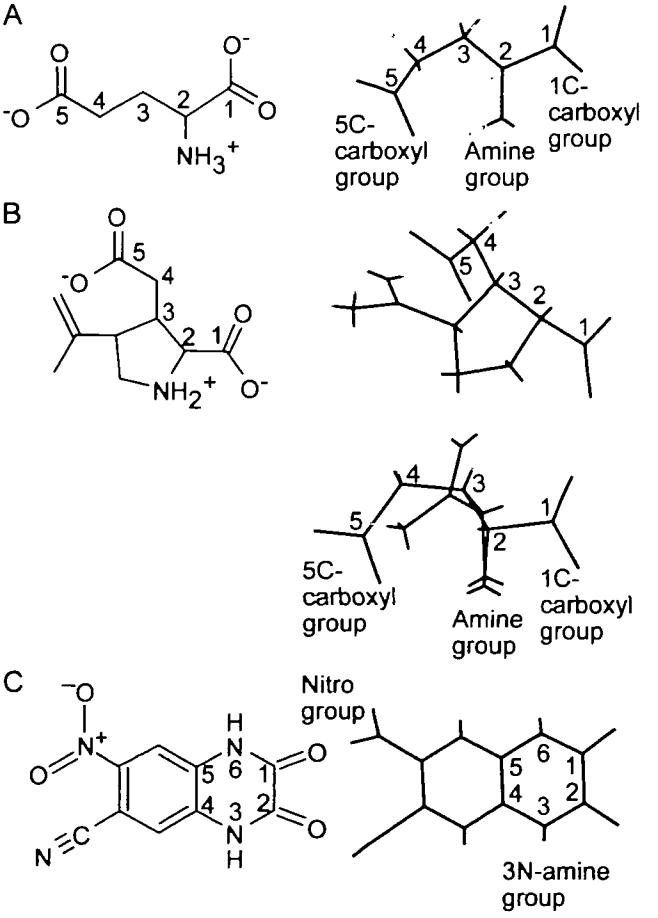

Fig. 1. Chemical (left panels) and calculated structures (right panels) of (A) glutamate, (B) kainate, and (C) CNQX. In (B), the second figure for the calculated structure is viewed from the direction parallel to the pyrrolidine ring. The carbon, nitrogen, oxygen, and hydrogen atoms are shown in green, purple, red, and yellow, respectively.

model. Glutamate, kainate, CNQX, and the binding site of GluR2 in the kainate-bound state have 48, 81, 57, and 729 internal degrees of freedom, respectively.

RESULTS

Molecular Structures of Glutamate, Kainate, and CNQX—The stable structures of glutamate, kainate, and CNQX are shown in Fig. 1. Glutamate and kainate were folded because the 5C-carboxyl group was hydrogen bonded to the amine group (Fig. 1, A and B). The 3C-4C bond of kainate was rotated by 132° from the crystal structure in GluR2. CNQX was planar except for the nitro group, which was rotated from the plane by approximately 50° (Fig. 1C). Between an agonist and an antagonist, the following three moieties were found to be correspondent in their structures and charge distribution: (i) the 1C-carboxyl group of glutamate or kainate and the part consisting of the two carbonyl groups of CNQX; (ii) the 5C-carboxyl group and the nitro group; and (iii) the amine group and the 3N-amine group.

Molecular Structure of the Binding Site of GluR2 in the Kainate-Bound State—A stable structure was obtained having the same formation of hydrogen bonds as that expected for the crystal structure (*4*), except for a hydrogen bond between the amine group of kainate and the carbonyl oxygen of Pro478 of GluR2 (Fig. 2). This hydrogen bond was neglected in the present study because the infrared spectroscopy data suggested that it is so weak that the environment of this site is the same in the unliganded and kainate-bound states (*12*). In our model structure, the 1C-carboxyl group of kainate was hydrogen bonded to the guanidinium group of Arg485 as well as to the backbone amine of Thr480. The 5C-carboxyl group of kainate was hydrogen bonded to the following five molecules: the backbone amine of Ser654; the backbone amine of Thr655; the hydroxyl group of Thr655; and the two water molecules. The amine group of kainate was hydrogen bonded to the carboxyl group of Glu705.

Normal Vibrational Modes of Glutamate, Kainate, and CNQX—Glutamate, kainate, and CNQX have 48, 81, and

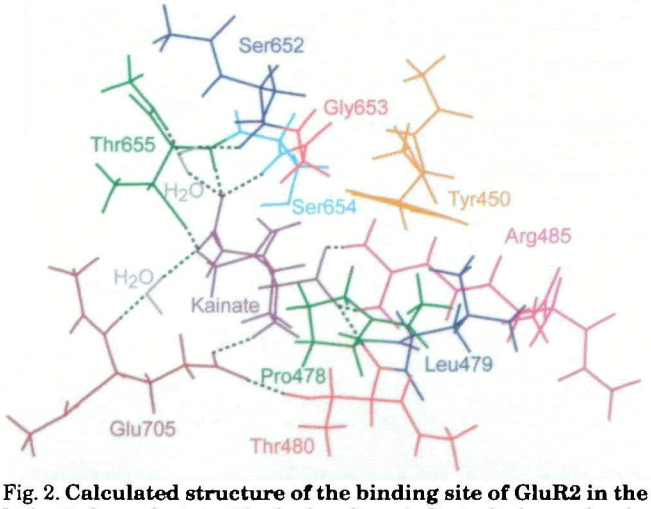

Fig. 2. **Calculated structure of the binding site of GluR2 in the kainate-bound state.** The broken lines indicate hydrogen bonds. Every amino acid residue is in a distinct color.

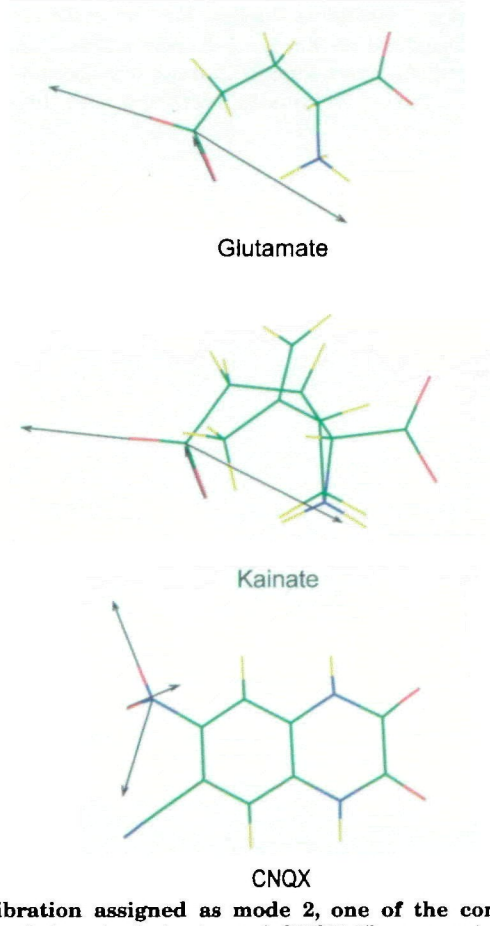

Fig. 3. **Vibration assigned as mode 2, one of the common modes in glutamate, kainate, and CNQX.** The arrows indicate the atomic motions with large displacement, that is, the asymmetric stretching of the 5C-carboxyl group. See Fig. 1 for the colors.

TABLE I. **Common modes in glutamate, kainate, and CNQX.**

Mode #	Vibrations of kainate	Glutamate (cm^{-1})	Kainate (cm^{-1})	CNQX (cm^{-1})
1	Asymmetric stretching of the 1C-carboxyl group	1,938	1,936	1,917
2	Asymmetric stretching of the 5C-carboxyl group	1,886	1,856	1,911
3	Symmetric stretching of the 5C-carboxyl group	1,527	1,555	1,599

TABLE II. **Common modes in glutamate and kainate, but not in CNQX.**

Mode #	Vibrations of kainate	Glutamate (cm^{-1})	Kainate (cm^{-1})
4	Stretching of the amine group	2,942	2,975
5	Bending of the amine group	1,561	1,602
6	Symmetric stretching of the 1C-carboxyl group	1,484	1,472

57 normal vibrational modes, respectively. By comparing these modes of glutamate, kainate, and CNQX, we found three common modes in glutamate, kainate, and CNQX (Table I and Fig. 3). Furthermore, we found three other common modes in glutamate and kainate that were not observed in CNQX (Table II and Fig. 4). Here, the normal vibrational modes of the same motions with almost the same frequencies (<5%) between molecules were judged as being the common modes. These six modes were the local vibrations of the charged groups that were expected to be bound to GluR2. The frequencies of these vibrational modes ranged from 1,000 to 3,000 cm^{-1}.

Normal Vibrational Modes of the Binding Site of GluR2 in the Kainate-Bound State—Our binding site model has 729 normal vibrational modes. We found the motions of kainate assigned as modes 1–6 (see Tables I and II, and Figs. 3 and 4), even though kainate was bound to GluR2 (Table III). Mode 5 of kainate interacted with the stretching vibration of the carboxyl group of Glu705 of GluR2 through a hydrogen bond (Fig. 5A). Mode 6 of kainate interacted with the bending vibration of the guanidinium group of Arg485 through two hydrogen bonds (Fig. 5B). Mode 2 of kainate interacted with the bending vibrations of the two water molecules (data not shown). No motions correlated with modes 1, 3, and 4 of kainate were observed in the receptor atoms hydrogen bonded to kainate. Modes 1, 3, and 4 did not contribute to the vibrational interaction between kainate and the receptor.

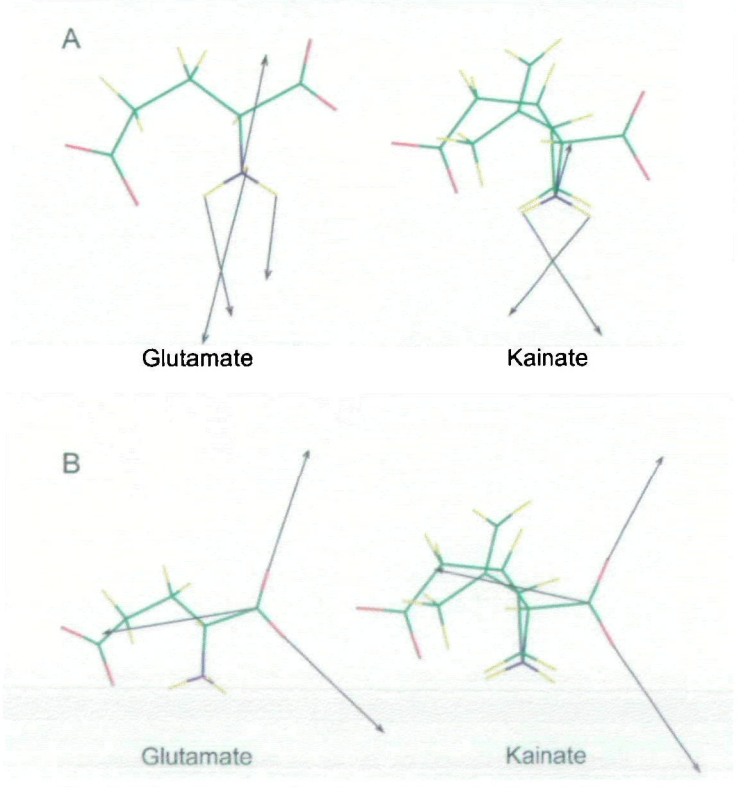

Fig. 4. **Vibrations assigned as (A) mode 5 and (B) mode 6.** These modes are the common modes in glutamate and kainate, but not in CNQX. The arrows indicate the atomic motions with large displacement, that is, the bending of the amine group (mode 5) and the symmetric stretching of the 1C-carboxyl group (mode 6). See Fig. 1 for the colors.

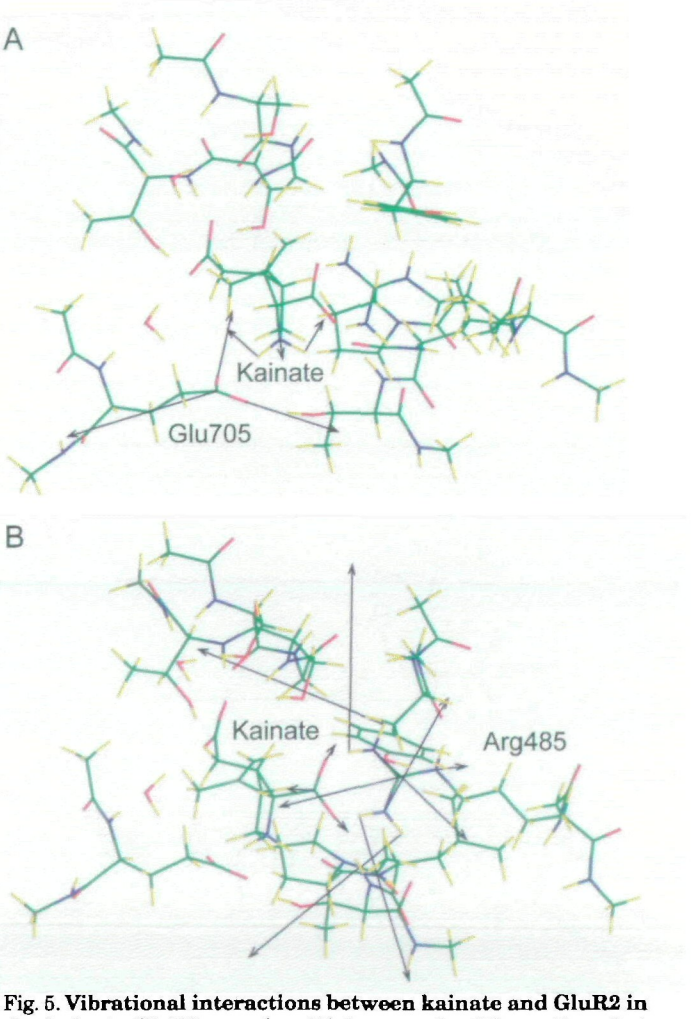

Fig. 5. **Vibrational interactions between kainate and GluR2 in the kainate-GluR2 complex.** (A) An example of the motions that show the interaction between mode 5 of kainate and the stretching vibration of the carboxyl group of Glu705 (the 1627 cm^{-1} mode). (B) An example of the motions that show the interaction between mode 6 of kainate and the bending vibration of the guanidinium group of Arg485 (the 1653 cm^{-1} mode). See Fig. 1 for the colors.

TABLE III. **Normal vibrational modes of the binding site of GluR2 in the kainate-bound state.**

Mode #	Frequencies of the modes of the binding site in the kainate-bound state (cm^{-1})	Vibrational interactions between kainate and the binding site through hydrogen bonds
1	1866, 1852, 1818	No vibrational interactions
2	1812, 1754	The 5C-carboxyl group of kainate and the two water molecules
3	1614, 1527	No vibrational interactions
4	2903	No vibrational interactions
5	1627, 1584	The amine group of kainate and the carboxyl group of Glu705
6	1653, 1589, 1512	The 1C-carboxyl group of kainate and the guanidinium group of Arg485

Kainate bound to GluR2 still maintained modes 1–6 of kainate alone.

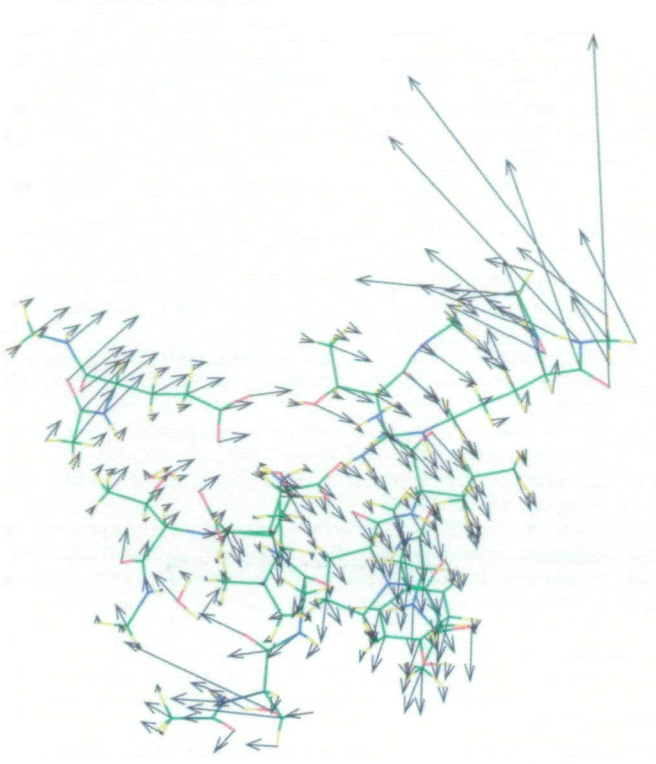

Fig. 6. **An example of the collective modes in the binding site of GluR2 in the kainate-bound state (the 6 cm^{-1} mode).** See Fig. 1 for the colors.

We also found collective modes of the binding site of GluR2 in the kainate-bound state (Fig. 6). The frequencies of these modes were below 50 cm^{-1}.

DISCUSSION

In the present study, we analyzed the normal vibrational modes of glutamate, kainate, and CNQX. The calculated frequencies of the normal vibrational modes of glutamate and kainate were approximately 20% higher than those assigned in an infrared spectroscopic study (see Table I and Ref. *12*), primarily because molecular orbital methods often overestimate the frequencies of molecular vibrations. In addition, D_2O used as the solvent in spectroscopic experiments may make this difference larger, as D_2O can depress the molecular vibrations. These differences in the frequencies of normal vibrational modes between the spectroscopic experiments and our calculations, however, did not prevent us from constructing a working hypothesis regarding the mechanism of activation of receptors in relation to agonist–receptor vibrational interactions. The calculated vibrational spectra assigned as the asymmetric stretching vibrations of the 1C- and 5C-carboxyl groups of glutamate and kainate reproduced the bands in the infrared spectra (*12*), showing the validity of our calculation of the normal vibrational modes.

We further investigated the vibrational interactions between kainate and the binding site of GluR2. With regard to the calculation model of GluR2, we considered only the amino acid residues around kainate. The kinetic energy of an agonist cannot be directly propagated to a whole receptor but is released only around the binding site of the receptor on its binding, as the mass of the agonist is much smaller than that of the receptor. Our model is thus suitable as a first approach for understanding the energy transfer between agonist and receptor. The frequency of the asymmetric stretching vibration of the 1C-carboxyl group of kainate decreases in response to binding to the S1S2 lobes in spectroscopic experiments (*12*). Our calculation was able to mimic this decrease due to the formation of hydrogen bonds, showing the validity of our GluR2 model (see mode 1 in Tables I and III).

We found three common normal vibrational modes in glutamate, kainate, and CNQX, and three other common modes in glutamate and kainate, but not in CNQX. These six common modes of kainate alone were maintained in kainate bound to GluR2. These six modes of kainate were assigned to local vibrations (see Tables I and II), and thus they were not affected by a conformational change, such as the rotation of the 3C-4C bond, of kainate on its binding to GluR2. Two of these six modes of kainate, which were also observed in glutamate but not in CNQX, interacted through the hydrogen bonds with the vibrational modes of GluR2: (i) the bending vibration of the amine group of kainate (assigned as mode 5 in Fig. 4A), interacting with the stretching vibration of the carboxyl group of Glu705 of GluR2, and (ii) the symmetric stretching vibration of the carboxyl group of kainate (mode 6 in Fig. 4B), interacting with the bending vibration of the guanidinium group of Arg485. The frequencies of modes 5 and 6 were found to be approximately 1,500 cm^{-1}. The amplitudes of such high-frequency modes have been reported to be small (*13*); the motions themselves are thus not important in relation to the large conformational changes of the receptor. Modes 5 and 6 of the agonists are possibly involved in the energy transfer from the agonist to the receptor. On the other hand, because CNQX did not have these modes, it cannot transfer the energy for receptor activation through these modes. The energy transfer in the agonist-binding reaction will be calculated in our next study.

Although the asymmetric stretching vibration of the 5C-carboxyl group of glutamate and kainate (mode 2 in Fig. 3) was found to interact with the bending vibrations of the two water molecules, this mode is not thought to be important as to activation of the receptor for the following two reasons. First, our calculation has shown that the oscillator strength of the bending vibrations of water molecules is less than unity, and the energy of an agonist cannot be transferred to these water molecules through mode 2. Second, CNQX, an antagonist, also has this mode.

We found collective modes with low frequency at the binding site of GluR2 in the kainate-bound state. Various computer simulations and X-ray diffraction techniques have suggested that slow, collective motions of a protein contribute to the transition from one equilibrium conformation to another (*13–16*). The closure of the S1S2 lobes has been suggested to be directly induced by agonist binding (*17*). In contrast, the unliganded bilobate domain has also been suggested to be in dynamic equilibrium between an open form and a closed one, and the role of the agonist may be stabilization of the closed form (*18–20*). To clarify the role of the agonist in the domain closure, the correlation between agonist binding and the excitation of collective modes of the S1S2 lobes should be examined. We should calculate the intramolecular vibrational-energy redistribu-

tion (IVR, see Refs. *21* and *22* for the IVR) of the energy supplied by the agonist in future studies. More recently, the crystal structures of the S1S2 lobes of GluR2 were determined in the apo state and in the presence of agonists [glutamate, kainate, and α-amino-3-hydroxy-5-metyl-4-isoxazole propionic acid (AMPA)], and an antagonist [6,7-dinitro-2,3-quinoxalinedione (DNQX)] by Armstrong and Gouaux (*17*). These data will now enable us to calculate the changes in the normal vibrational modes between unliganded and liganded structures.

In conclusion, we have analyzed the normal vibrational modes of agonists from the perspective of the agonist–receptor vibrational interactions for receptor activation. Glutamate and kainate were found to interact vibrationally with a GluR subunit through two local modes with high frequency. These modes may transfer the vibrational energy of the agonist to the receptor and excite collective modes with low frequency through the IVR in the receptor, resulting in receptor activation.

The calculations were performed using the super server HP Exemplar V2500 at the Hokkaido University Computing Center. We would like to thank the staff of the Hokkaido University Computing Center for the introduction of the MOPAC2000 program package to this super server.

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