Expression and Purification of the Extracellular Ligand Binding Region of Metabotropic Glutamate Receptor Subtype 1*

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Tomoyuki Okamoto‡, Naohiro Sekiyama‡, Mieko Otsu‡, Yoshimi Shimada‡, Atsushi Sato‡, Shigetada Nakanishi, and Hisato Jingami‡**

From the ‡Department of Molecular Biology, Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, Japan and the ‡Department of Biological Sciences, Kyoto University Faculty of Medicine, Yoshida, Sakyo-Ku, Kyoto 606, Japan

Each metabotropic glutamate receptor possesses a large extracellular domain that consists of a sequence homologous to the bacterial periplasmic binding proteins and a cysteine-rich region. Previous experiments have proposed that the extracellular domain is responsible for ligand binding. However, it is currently unknown whether the extracellular ligand binding site can bind ligands without other domains of the receptor. We began by obtaining a sufficient amount of receptor protein on a baculovirus expression system. In addition to the transfer vector that encodes the entire coding region, transfer vectors that encode portions of the extracellular domain were designed. Here, we report a soluble metabotropic glutamate receptor that encodes only the extracellular domain and retains a ligand binding characteristic similar to that of the full-length receptor. The soluble receptor secreted into culture medium showed a dimerized form. Furthermore, we have succeeded in purifying it to homogeneity. Dose-response curves of agonists for the purified soluble receptor were examined. The effective concentration for half-maximal inhibition (IC50) of quisqualate for the soluble receptor was 3.8 × 10^-8 M, which was comparable to that for the full-length receptor. The rank order of inhibition of the agonists was quisqualate ≫ ibotenate ≫ L-glutamate ≈ (1S,3R)-1-aminothiophen-1,3-dicarboxylic acid. These data demonstrate that a ligand binding event in metabotropic glutamate receptors can be dissociated from the membrane domain.

Glutamate receptors are divided into two distinct classes: ionotropic glutamate receptors (iGluRs)1 and metabotropic glutamate receptors (mGluRs) (1, 2). The iGluRs consist of N-methyl-d-aspartate receptors and non-N-methyl-d-aspartate receptors. Non-N-methyl-d-aspartate receptors are further subdivided into two groups: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and kainate receptors. iGluRs are ligand-gated ion channels that transduce glutamate binding into cation influx. mGluRs that have been discovered most recently comprise eight subtypes, which are divided into three groups according to agonist selectivity, coupling to different effector systems, and sequence homology (3–6). Group I includes mGluR1 and mGluR5, which are coupled to inositol phospholipid metabolism. Group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) are negatively coupled to adenylate cyclase activity. Functional analyses of these mGluRs are now avidly being performed. The evidence is accumulating that mGluRs modulate excitatory synaptic transmission (7) through various neural transduction pathways, such as regulation of neurotransmitter release (8), influences on ion channel activity (9), and modulation of synaptic plasticity (10).

mGluRs have a remarkably large extracellular domain that has no homology with the other G protein-coupled receptors (GPCRs) except Ca2+-sensing receptors (11). Previous experiments (12, 13) have proposed that the ligand binding site resides mainly in the extracellular domain. Thus, the mode of ligand binding of mGluRs is different from that of other GPCRs for small molecule transmitters, such as adrenaline and acetylcholine. Thus, it is a subject of great interest how extracellular signals are transmitted into cells and what roles other parts of the receptor protein perform in mGluRs. Recently, various data of mGluRs on the brain function have been obtained, and the more specific and stronger antagonists and agonists have been urgently requested both in neurobiology and clinical fields (6, 14). For designing the antagonists, the information on the three-dimensional structure of the receptor protein is very valuable. In order to determine a tertiary structure, a sufficient amount of pure receptor protein is needed. However, at present, it is extremely difficult to solubilize the receptor protein from membrane preparations. Therefore, we tried to produce a ligand binding region in a soluble form. If a ligand binding region can be produced independently of the membrane domain, it will be also a good tool for understanding activation mechanism of this intriguing receptor. In an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, one of glutamate-gated ion channels, Kuoisinen et al. (15) have shown that an agonist binding site can be reconstituted by making a fusion protein of two discontinuous segments: one is proximal to the first transmembrane segment, and the other is located between the third and fourth transmembrane segments.

In the current study, we have expressed a mGluR subtype 1α (described as mGluR1 from now on) in insect cells on a baculovirus system. We have isolated an extracellular ligand bind-
ing region that has a biochemical characteristic comparable to that of the full-length receptor. Purification and characterization of the soluble mGlUR are presented. This is the first demonstration among GPCRs for small molecule transmitters that a lipid binding region is produced in a soluble form.

**EXPERIMENTAL PROCEDURES**

**Materials**—Quisqualic acid, (1S,3R)-1-aminoacycloptene-1,3-dicarboxylic acid (ACPD), (2S,1R,2R,3R,4R)-2,3-dicarboxycyclopropylglycine, t-2-amino-4-phosphonobutyrte, (S),3,5-dihydroxyphenylglycine, (R)-1-a-minoindan-1,5-dicarboxylic acid, (S)-o-methyl-4-carboxyphenylglycine, hydroxymiminocyclopropyl glycine, N-phenyl-7-(hydroxymimino)cyclopropylbromen-1-axarboxamide, (S)-o-ethyl-4-carboxyphenylglycine, (R)-2-a-amin o-4-(3-hy droy-5-methylisoxazol-4-yl)butyric acid, (2S)-o-ethylglutamic acid, (R)-1-cycloproplyl-4-phosphonophenylglycine, and (R)-o-methylserine-O-phosphate were purchased from Tocris Cookson Inc. (St. Louis, MO). t-Glutamic acid was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ibotenic acid was purchased from Sigma. All other reagents were of analytical grade.

**Cell Culture—Spodoptera frugiperda cells** (Sf9 cells) were propagated in a monolayer at 27 °C in TNNM-FH (Grace's powder medium, 0.4% yeastolate, 0.4% l-actalbumin hydrolysate, and 0.1% pluronic F-68 from Boehringer-Mannheim Biochemicals, Inc.), supplemented with 10% fetal bovine serum (Irvine Science, Santa Ana, CA) or in suspension at 27 °C in IPI-41 (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 1.0% transplote phosphate (Life Technologies, Inc.). 100 units/ml of penicillin, 100 μg/ml streptomycin, and 25 ng/ml amphotericin B were used. *Trichoplusia ni* BTI-TN-5B1–4 (High Five cells) were cultured in a monolayer at 27 °C in Express Five serum-free medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 1% L-proline, and 10% dialyzed fetal bovine serum (Life Technologies, Inc.). Penicillin (100 units/ml) and streptomycin (100 μg/ml) were used.

**Construction of Transfer Vectors for Expression of mGluRs in Insect Cells**—A SacII fragment that contains the full coding region of mGlUR1 was excised from pmGR1 (3) and was ligated into SacII-digested pBluescriptII SK (+) (Stratagene, La Jolla, CA). A Not1-XbaI fragment of the intermediate construct was ligated into XbaI-Not1-digested pVL1392 (PharMingen, San Diego, CA) to generate pmGluR102.

**Cell Membrane Preparation**—Sf9 cells were then blocked in TBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20) with 1% bovine serum albumin and centrifuged at 100,000 × g for 30 min to pellet down the membranes. The membranes were divided into aliquots, frozen in liquid nitrogen, and stored at −80 °C until use.

**Western Blot Analyses**—Cell membranes and culture medium were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and electroblotted onto a nitrocellulose membrane (Schleicher & Schuell). The membranes were divided into aliquots, frozen in liquid nitrogen, and stored at −80 °C until use.

**Immunoblotting**—TBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20) with 1% bovine serum albumin and incubated overnight at room temperature with a 1:5000 dilution of anti-mGlUR1 monoclonal antibody (MAb) mG1Na-1 and a 1:5000 dilution of polyclonal antibody A52 in TBS2. After being washed, the membrane was incubated with a goat anti-mouse IgG or a goat anti-rabbit IgG conjugated with alkaline phosphatase. Color development was done with chromogenic reagent kit (Promega). Anti-mGlUR1 was made by Dr. Akio Neki as follows. The spleen cells from Balb/c mice immunized with glutathione S-transferase (GST)-fused mGlUR1 peptide (amino acid residues 104–154) were fused with X63-Ag8.653 cells as described (17). The character of MAb mG1Na-1 was verified using CHO cells, which produce mGlUR1, in comparison with polyclonal antibodies G18 and A12 (18). Polyclonal antibody A52 was raised against an mGlUR1 C-terminal peptide (amino acid residues 859–1199) (19).

**Immunonficiency Column Chromatography Using Mab mG1Na-1**—About 2 liters of supernatant of hybridoma mG1Na-1 cells was concentrated to 30 ml using XM50 (Millipore Corp., Bedford, MA). After addition of 150 ml of PBSSC (8 mM Na2HPO4, pH 7.3, 1.5 mM KH2PO4, 138.9 mM NaCl, and 3.8 mM KC1) and concentration again to 45 ml, the concentrate was applied onto a HiTrap protein G column (Amersham Pharmacia Biotech). The bound material was eluted with 0.1 mM glycine-HCl, pH 2.7. Peak fractions were pooled and dialyzed against PBSC at 4 °C. MAb mG1Na-1 (11.2 mg) was coupled with 1 ml of Hitrap NHS-activated column (Amersham Pharmacia Biotech) in Buffer L (200 mM NaHCO3, pH 8.3, and 500 mM NaCl) at 4 °C overnight. The column was washed for several times with Buffer L (0.5 mM ethylamine, pH 8.3, 5.0 mM NaHCO3, and 500 mM NaCl) and Buffer W2 (100 mM Tris-HCl, pH 4.0, and 500 mM NaCl). After neutralization with 100 mM Heps, pH 7.5, the supernatant of Five High Five cells prepared below was loaded.

**Ligand Binding Assay**—Membrane fractions (20–100 μg) of baclofen—-infected cells were incubated with [3H]Hqisqualate (20 mM) in a total volume of 200 μl of Buffer B (40 mM Heps, pH 7.5, and 2.5 mM CaCl2) for 1 h at room temperature. The reaction mixture was aspirated...
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RESULTS

Fig. 1 shows a schematic drawing of the transfer vectors used in the baculovirus infection experiments. pmGluR102 and pmGluR104 encode the membrane-bound forms of mGluR1, pmGluR103, pmGluR107, and pmGluR108 are designed to produce the truncated forms of mGluR1, which are expected to be secreted into the culture medium.

Recombinant baculoviruses for the expression of full-length mGluR1 were prepared and used to infect Sf9 insect cells in monolayer cultures. Immunoblotting analysis of expressed mGluR1 is shown in Fig. 2A. Both the polyclonal antibody A52, which was raised against a C-terminal peptide of mGluR1, and the MAb mG1Na-1 yielded a 155-kDa band in the membrane preparation from Sf9 cells infected with recombinant viruses but not in the control membrane preparation from Sf9 cells infected with wild-type virus, Autographa california nuclear polyhedrosis virus (AcNPV). mGluR1 expressed in CHO cells showed a more slowly migrating band. This size difference may reflect less extensive glycosylation in the insect cells. A ligand binding assay was performed using membrane fractions of Sf9 cells infected with their respective recombinant viruses. \(^{3}H\)-Labeled quisqualate specifically bound the membranes, and the binding was displaced by unlabeled glutamate (Fig. 2B). The control membranes prepared in parallel from wild-type virus (AcNPV)-infected cells did not show any specific binding. Both levels of expression of the receptor protein and the ligand binding ability seemed not to show a significant difference between the cells infected with recombinant viruses for mGluR102 and for mGluR104. The inhibition of \(^{3}H\)-quisqualate binding with several agonists was examined as shown in Fig. 2C. The effective concentration for half-maximal inhibition (IC\(_{50}\)) of quisqualate was \(3.0 \times 10^{-8}\) M, which is comparable to the effective concentration for half-maximal response (EC\(_{50}\)) in inducing the stimulation of phosphatidylinositol hydrolysis in CHO cells permanently expressing mGluR1 (16). The rank order of inhibition was quisqualate \(\gg\) L-glutamate \(\sim\) ibotenate \(\geq\) (1S,3R)-ACPD. Ohashi et al. (20) have obtained comparable data using the membrane fractions of Sf9 cells infected with recombinant baculovirus for the human full-length mGluR1. They reported that \(^{3}H\)-labeled quisqualate binds the receptor in a saturable manner, with a Kd value of 5.26 \(\times 10^{-8}\) M. Their IC\(_{50}\) value, 2 \(\times 10^{-8}\) M, of quisqualate for the human full-length mGluR1 agrees with ours.

Next, we tried to dissect the ligand binding region of mGluR1. We infected High Five cells with recombinant viruses for mGluR103, mGluR107, and mGluR108, which were expected to produce the extracellular portions of mGluR1. Fig. 3 shows the Western blotting analyses of culture medium of cells infected with the recombinant baculoviruses. mGluR103 and mGluR107 produced a 66-kDa band, and mGluR108 produced a 74-kDa band under the reducing conditions. The level of expression of mGluR108 was much higher than those of mGluR103 and mGluR107. Under the nonreducing conditions, both bands shifted to the bands of approximately twice the size of the bands observed under the reducing conditions, suggesting a dimer formation. A minor faster migrating band observed in mGluR108 under the nonreducing conditions seemed to be a monomer. The 74-kDa band under the reducing conditions looked broad and may reflect heterogeneity of the glycosylation pattern. mGluR107, which differs from mGluR103 only in the flag tag sequence added to the N-terminal end, did not show any obvious band under the nonreducing conditions. Although the cause of the anomalous electrophoresis of the mGluR107 protein was not clear, it suggested an aggregated or an unfolded protein formation, which may be brought about by the disruption of the tertiary structure due to the peptide tag.

To study ligand binding properties of soluble receptors, we developed a new binding assay using Ni\(^{2+}\)-conjugated beads, which can trap the histidine tag added to the soluble mGluRs. Concentrated culture medium (100–200 \(\mu\)l) was incubated with Ni\(^{2+}\)-conjugated beads. Although mGluR103 and mGluR107 showed no appreciable binding, mGluR108 did reveal a significant binding ability. This binding was inhibited in the presence of 1 mM L-glutamate. Neither concentrated control culture medium nor separately prepared His-tagged soluble receptor (soluble low density lipoprotein receptor; data not shown) showed any significant binding. We examined the agonist selectivity of mGluR108 as shown in Fig. 4. Inhibition of labeled quisqualate binding by several agonists was analyzed. The rank order of inhibition was quisqualate \(\gg\) L-glutamate \(\sim\) ibotenate. (2S,1R,2R,3R)-2,2-(3-Dicarbonylcyclopropyl)-glycine and (1S,3S)-ACPD inhibited the quisqualate binding by 37 and 56% at 1 \(\times 10^{-8}\) M, respectively; however, L-2-amino-4-phosphonobutyrate, which is a group III-specific agonist, hardly inhibited it (data not shown). The iGluR ago-
nists N-methyl-d-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate did not show any significant inhibition at 1 × 10^{-4} M (data not shown). These binding characteristics clearly indicate that the expressed soluble receptor, mGluR108, is capable of binding ligands in the same manner as the native group I mGluR.

Because newer ligands have recently been used in physiological and pharmacological experiments on mGluRs, we examined their reactivities with the soluble mGluR108. Fig. 5A shows the dose-response curves of the newer ligands. (S)-3,5-Dihydroxyphenylglycine, which is a group I-specific agonist (21–23) and (R,S)-1-aminooindan-1,5-dicarboxylic acid, which is a group I-specific antagonist (24), inhibited the [3H]quisqualate binding at IC_{50} values of 0.23 × 10^{-4} and 0.50 × 10^{-4} M, respectively. (S)-α-Methyl-4-carboxypheynylglycine, which is a nonselective group I/group II antagonist (25, 26, 23), also inhibited the quisqualate binding at an IC_{50} value of 1.2 × 10^{-4} M. 7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester and N-phenyl-7-(hydroxyimino)cyclopropab[b]chromen-1a-carboxamide, which are structurally novel group I antagonists (27), did not show any inhibition. Results of other producing mGluR1 and membrane fractions (30 μg) of Sf9 cells infected with recombinant viruses for mGluR102, mGluR104, and wild-type virus (AcNPV) were loaded onto a 7.5% SDS-polyacrylamide gel. Proteins were transferred onto a nitrocellulose membrane and probed with MAb mG1Na-1, as described in the legend to Fig. 2A.

FIG. 2. Biochemical and pharmacological characterization of full-length mGluR1 in insect cells infected with recombinant baculoviruses. A, expression of full-length mGluR1 in insect cells was examined by immunoblotting. Membrane fraction (60 μg) of CHO cells producing mGluR1 and membrane fractions (30 μg) of Sf9 cells infected with recombinant viruses for mGluR102, mGluR104, and wild-type virus (AcNPV) were loaded onto a 7.5% SDS-polyacrylamide gel. Proteins were transferred onto a nitrocellulose membrane and probed with MAb mG1Na-1 and polyclonal antibody A52. Immunostained proteins were visualized by a goat anti-mouse IgG or a goat anti-rabbit IgG coupled to alkaline phosphatase as described under “Experimental Procedures.” Marker proteins are myosin heavy chain (203 kDa), phosphorylase B (110 kDa), bovine serum albumin (70.8 kDa), and ovalbumin (43.6 kDa). B, ligand binding of full-length mGluRs was measured. Membrane fractions (100 μg) of Sf9 cells infected with recombinant viruses for mGluR102, mGluR104, and wild-type virus (AcNPV) and of CHO cells producing mGluR1 were incubated in a binding buffer (40 mM Hepes, pH 7.5, and 2.5 mM CaCl_{2}) with [3H]quisqualate (20 nM) at room temperature for 1 h. The reaction mixtures were aspirated onto GF/C filters, and the material remaining on the filters were counted with a scintillation counter. Nonspecific binding was determined with 1 mM L-glutamate. Each binding was performed in triplicate and is shown as mean ± S.D. A representative of two experiments is shown. C, dose-response curves of various agonists in inhibiting [3H]quisqualate binding to full-length mGluR104 were determined. Indicated concentrations of quisqualate (open circles), L-glutamate (closed circles), (1S,3R)-ACPD (open squares), and ibotenate (closed squares), each with the addition of [3H]quisqualate (20 nM), were incubated with 100 μg of membrane fraction of Sf9 cells infected with recombinant virus for mGluR104 prepared as described under “Experimental Procedures.” Binding counts obtained without any agonist were 7507 ± 624 dpm. Each point shows the mean ± S.E. of three experiments done in triplicate.
mGluR subtype 6-specific agonist (29), showed a slight inhibition of Amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid, which is an point

![Graph](image)

**Fig. 4.** Dose-response curves of various agonists in inhibiting [3H]quisqualate binding to the soluble mGluR108 secreted into culture medium. The supernatant of High Five cells infected with recombinant virus for mGluR108 was concentrated 40-fold. 200 µl of it was incubated with Ni2+-conjugated beads. Then, the indicated concentrations of quisqualate (open circles), L-glutamate (closed circles), (1S,3R)-ACP (open squares), and ibotenate (closed squares), together with [3H]quisqualate, were added into the reaction mixture. Ligand binding was assayed as described under “Experimental Procedures.” Binding counts obtained without any agonist were 9268 ± 1387 dpm. Each point shows the mean ± S.E. of two experiments done in triplicate.

ligands are shown in Fig. 5B. (R,S)-o-Ethyl-4-carboxyphenylglycine (23, 28) showed an inhibition of 37% at 1 × 10^-4 M. (R,S)-2-Amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid, which is an mGluR subtype 6-specific agonist (29), showed a slight inhibition of 10% at 1 × 10^-4 M. Other compounds, including (2S)-o-ethylglutamate acid, (R,S)-o-cyclopropyl-4-phosphonophenylglycine, and (R,S)-o-methylerine-O-phosphate, which are classified as group II or group III antagonists, did not show significant inhibition.

Next, we started the purification of the soluble receptor mGluR108 from the culture medium. Approximately 2.5 liters of the culture medium was concentrated, dialyzed, and loaded on an immunoaffinity column as described under “Experimental Procedures.” After being washed with the binding buffer, the bound material was eluted. Aliquots of the flow-through and the eluates were analyzed by silver staining (Fig. 6A) and immunoblotting (Fig. 6B). Fractions that were positively stained by the immunoblot completely agreed with the ligand binding activity, and these fractions were pooled and purified by size exclusion column chromatography (Superdex 200) as shown in Fig. 7. A single peak was obtained. Peak fractions were collected and analyzed by SDS-PAGE under reducing and nonreducing conditions, followed by silver staining. On the basis of the result of an overloaded gel electrophoresis of the purified soluble receptor protein, we estimated that the purity of our final material is 99% (data not shown). Calibration with the molecular size markers showed the eluting position of the purified soluble receptor protein to be around 190 kDa. The purified material may reflect the difference between the crude membrane preparation and the purified receptor protein.

**DISCUSSION**

mGluRs have occupied a unique place in the sense that they have a very large extracellular domain and no amino acid sequence homology in the transmembrane helix with the other conventional GPCRs. Takahashi et al. (12) have made chimeric receptors between mGluR1 and mGluR2 and have shown that the large extracellular domain plays a crucial role in determining agonist selectivity. O’Hara et al. (13) have discovered the homology between the N-terminal extracellular region (residues 1–496) (which we tentatively call region I) and the bac-
They have constructed the molecular model of the glutamate binding site. The model has led them to point out the two critical amino acid residues, substitutions of which abolished [3H]glutamate binding. In the extracellular domain of mGluR, there is also a short stretch of the cysteine-rich sequence (region II) just proximal to the membrane-spanning domain. However, the ligand binding site has not yet been isolated as a soluble molecule. In this investigation, we have succeeded in producing a soluble metabotropic glutamate receptor without the membrane-anchored domain and have provided compelling evidence that the soluble receptor consisting of the extracellular regions I and II is sufficient for conferring the affinity and selectivity of ligand binding characteristic of the mGluR.

Extensive studies of structure-function relationships have been done in GPCRs, and different modes of agonist binding are known (30, 31). Monoamines and other small ligands, such as catecholamine and acetylcholine, are bound to the pockets formed within the transmembrane segments. In the receptors for neuropeptides or chemokines, a major binding site is located in the N-terminal segments of the receptors, with some contribution of the extracellular loops or the outer portions around the transmembrane segments. In receptors for large glycoprotein hormones, such as luteinizing hormone and thyrotropin, a large extracellular domain is responsible for high affinity ligand binding (32–35), but a low affinity site is located in the extracellular loops in luteinizing hormone receptor (36, 37). Although the role(s) of the extracellular loops and transmembrane segments of mGluR remains to be determined in the present study, it should be pointed that the soluble form of mGluR1 has a ligand affinity and specificity comparable to those of the full-size mGluR1. Thus, our results indicate that the ligand binding event is really dissociable from transmembrane signaling in this receptor system. Pharmacological analyses with the conventional agonists and the newer ligands suggest that the soluble mGluR would be a reliable method for binding studies of future ligands that are expected to be developed.

We have made and analyzed several truncated forms of soluble mGluR1, which encode parts of the extracellular domain of mGluR1. The IC50 value of quisqualate for mGluR108 is remarkably similar to those of the full-length membrane-anchored form of the receptor. The agonist selectivity examined also constitutes a typical feature of mGluR1. These results indicate that the secreted extracellular domain forms a correct conformation for ligand binding. The soluble form of mGluR1 shows a dimerized form. A similar dimerization has been reported for several members of the conventional GPCRs, and this dimerization is ascribed to the molecular association in the transmembrane domains of these receptors (38, 39). In addi-

![Fig. 6. Immunoaffinity chromatography of the soluble mGluR108. Culture medium (2.5 liters) of High Five cells infected with recombinant virus for mGluR108 was concentrated 50-fold and dialyzed against Buffer C. The dialyze was loaded on a HiTrap Affinity column (1 ml) that had been coupled with mGluR1-specific MAb mG1Na-1 and equilibrated with Buffer C. The column was washed with 30 ml of Buffer C, and the bound material was eluted with 500 mM NaCl containing 500 mM sodium thiocyanate and 10 mM Hepes, pH 7.5. A, indicated aliquots of the eluate were subjected to 7.5% SDS-PAGE and silver stained. B, identical samples were analyzed by Western blot with MAb mG1Na-1 as described in the legend to Fig. 2A.](image1)

![Fig. 7. Gel filtration chromatography of the soluble mGluR108. Soluble mGluR108 (64 µg) from the immunoaffinity column (Fig. 6) was loaded onto a HiLoad 16/60 Superdex 200 gel filtration column (Amersham Pharmacia Biotech). The column was equilibrated and eluted with 10 mM Hepes, pH 7.5, containing 200 mM NaCl and 10% glycerol. 2.5 ml of each fraction was collected. The molecular masses of the marker proteins were blue dextran (>2000 kDa), bovine thyroglobulin (669 kDa), horse spleen apoferritin (443 kDa), sweet potato β-amylase (200 kDa), yeast alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), and bovine erythrocytes carbonic anhydrase (29 kDa). Aliquots (20 µl) of the indicated gel filtration fractions were subjected to 7.5% SDS-PAGE under the reducing conditions with 2% β-mercaptoethanol and under the nonreducing conditions, followed by silver staining.](image2)
tion, Romano et al. (40) have reported that the truncated form of mGluR5 containing the first transmembrane segment forms a dimer. They demonstrated that an N-terminal 17-kDa region is required for dimerization. Our observation that mGluR103 dimerized is consistent with theirs. However, the truncated forms of the receptor, mGluR103 and mGluR107, which contain only region I, do not express well, and they show no significant ligand binding. Furthermore, mGluR108, which contains both region I and region II, expresses well and binds the ligand. This cysteine-rich region II of mGluR may thus impose a structural constraint on the receptor protein. A less possibility is that the ligand binding site might be composed of the two regions. In this context, the GABA$_A$ receptor (41), although belonging to the mGluR family with a large extracellular domain and a low but significant sequence homology to region I of mGluR, does not possess a region homologous to the cysteine-rich region II of mGluR. Furthermore, such a cysteine-rich region is absent in bacterial periplasmic amino acid-binding proteins. Thus, the real role of the cysteine-rich region II of mGluR remains an open question.

The bacterial periplasmic binding proteins serve as initial receptors of active transport for a variety of amino acids, sugars, peptides, oxyanions, and other nutrients. Although the binding proteins have different sizes (20–60 kDa) and share little sequence homology, they all fold a similar two-lobed tertiary structure. Atomic structures of the binding proteins have been analyzed by Quiocho and co-workers (42) and other groups (43) using crystallography. On the basis of the comparison between ligand-bound and ligand-free forms, the “Venus flytrap” model has been proposed (44): the ligand binds preferentially to one lobe of the open, ligand-unloaded form. A binding motion at the hinge region between the two lobes in turn causes the other lobe to participate in binding and completely entraps the ligand. The closed, ligand-loaded form of the binding protein then interacts with membrane-bound components, thereby initiating nutrient translocation or flagella motion.

Therefore, it should give us insights into the receptor activation mechanism of mGluR to reveal the three-dimensional structure of the mammalian glutamate receptor and determine whether a domain movement similar to that of the bacterial binding protein is produced upon ligand binding in mGluR. We speculate that glutamate induces a conformational change of the preexisting dimer or oligomer of glutamate receptor and triggers the signal transmission to the cytoplasmic signaling domain through the cysteine-rich region and the seven-transmembrane domain. The soluble form of mGluR1 produced in a sufficient amount will make it possible to conduct the biophysical analysis of mGluR1.

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