Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7394–7398, October 1988
Neurobiology

Conserved quaternary structure of ligand-gated ion channels: The postsynaptic glycine receptor is a pentamer

(postsynaptic membrane/chloride channel/crosslinking/protein structure/evolution)

DIETER LANGOSCH, LEO THOMAS, AND HEINRICH BETZ*

Center for Molecular Biology, University of Heidelberg, 6900 Heidelberg, Federal Republic of Germany

Communicated by Solomon H. Snyder, June 16, 1988

ABSTRACT **The postsynaptic glycine receptor of rat spinal cord is a glycosylated membrane protein that, after affinity purification, contains membrane-spanning subunits of M_r 48,000 and 58,000 and an associated peripheral polypeptide of M_r 93,000. Here, the quaternary structure of the transmembrane core of the receptor was investigated by chemically crosslinking its subunits. Upon treatment with crosslinking reagents of different side-chain specificities and lengths, a consistent set of adducts up to M_r 260,000 was detected after separation by NaDodSO$_4$/PAGE. The observed pattern of adducts was similar irrespective of whether purified receptor protein or synaptosomal membranes were crosslinked. Compositional analysis revealed that the crosslinked adducts contained the M_r 48,000 and 58,000 subunits in varying ratios but not the peripheral M_r 93,000 polypeptide. Thus adducts of intermediate molecular weight represent dimers, trimers, and tetramers of the transmembrane subunits, whereas the major adduct of M_r 260,000 corresponds to a pentameric assembly of subunits forming the ion channel of the glycine receptor. This subunit arrangement is similar to that reported for the nicotinic acetylcholine receptor of fish electric organ and skeletal muscle. Hence, we suggest that the different ligand-gated ion channels of excitable membranes share a similar quaternary structure.**

Recent results obtained by cDNA cloning of subunits of the nicotinic acetylcholine (1–3), the glycine (4), and the γ-aminobutyric acid (5) receptors revealed significant homology in primary structure and predicted transmembrane topology between these chemically gated ion channel proteins. In particular, four hydrophobic segments (M1–M4) presumed to span the lipid bilayer are conserved in the subunits of the different receptors. These data established the existence of a previously postulated superfamily of phylogenetically related ion channel-forming receptor polypeptides (6, 7).

Electron microscopy (8, 9) and crosslinking experiments (10) have demonstrated that the four subunits of *Torpedo* and muscle nicotinic acetylcholine receptor (nAcChoR) are arranged in a quasisymmetrical pentameric ($\alpha_2\beta\gamma\delta$) structure around a central pit presumed to represent the transmembrane channel. Little, however, is known about stoichiometry, spatial arrangement, and total number of subunits constituting neuronal ligand-gated ion channels. The postsynaptic γ-aminobutyric acid receptor (GABA$_A$R) has been proposed to be a tetrameric transmembrane protein (11). For neuronal nAcChoR, tetrameric and pentameric structures are discussed (12, 13).

Another member of the chemically gated ion channel family, the glycine receptor (GlyR), mediates postsynaptic inhibition in spinal cord and other parts of the central nervous system (14). Binding of the agonist glycine induces an increase in chloride permeability of the postsynaptic membrane and thus antagonizes depolarization—i.e., reduces neuronal firing rate (15). The convulsive alkaloid strychnine antagonizes glycine-mediated inhibition, and glycine-displaceable [^{3}H]strychnine binding has been used to investigate and localize the GlyR in the nervous system of different species (16–19). The GlyR has been purified from spinal cord of different mammals by affinity chromatography on aminostrychnine-agarose and shown to be a large glycoprotein containing polypeptides of M_r 48,000 (α), 58,000 (β), and 93,000 (γ) (20–22). The M_r 48,000 subunit can be covalently labeled with [^{3}H]strychnine by UV illumination and thus contains the antagonist-binding site of the GlyR (23). The M_r 48,000 and 58,000 polypeptides are glycosylated (21, 24) and assumed to be homologous on the basis of immunological crossreactivity and related peptide maps (6). These subunits are thought to constitute the channel-containing transmembrane structure of the GlyR. The M_r 93,000 subunit, in contrast, can be extracted from spinal cord membranes (24) and was localized on the cytoplasmic face of the postsynaptic membrane by immunoelectron microscopy (25, 26). Hence, this subunit is believed to be a peripheral component of the receptor and may relate to its postsynaptic anchoring (14, 24).

In this report, we describe the investigation of GlyR quaternary structure by use of chemical crosslinking in combination with molecular weight determination. Chemical crosslinking has been widely applied for the investigation of subunit organization and nearest-neighbor analysis of membrane proteins (for review, see refs. 27 and 28). Our results indicate that the different ligand-gated ion channel proteins are not only related in terms of amino acid sequence and transmembrane topology but also share a similar arrangement of subunits.

METHODS

Purification of GlyR. GlyR was purified from rat spinal cord by affinity chromatography on aminostrychnine-agarose as described (20, 29). For removal of lipids, washing and elution of the affinity column were performed by using buffers without phosphatidylcholine.

Preparation of Synaptosomes. Synaptosomes were prepared from freshly dissected rat spinal cord tissue (3 g) based on the method of Gray and Whittaker (30). All solutions contained 5 mM MgCl$_2$, 2 mM EGTA, 2 mM dithiothreitol, 1 milliunit of aprotinin per ml (Sigma), and 10 μg of soybean trypsin inhibitor per ml (Serva, Heidelberg) and α_2-macro-

Abbreviations: nAcChoR, nicotinic acetylcholine receptor; GABA$_A$R, γ-aminobutyric acid receptor; GlyR, glycine receptor; DTSP, 3,3′-dithiobis(sulfosuccinimidylpropionate); SSADP, sulfo-*N*-succinimidyl(4-azidophenyl)-1,3′-dithiopropionate; APTP, *N*-(4-azidophenylthio)phthalimide; APAB, *p*-azidophenacyl bromide; DMSI, dimethyl suberimidate; SSANPAH, sulfo-*N*-succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate; mAb, monoclonal antibody.

*To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

globulin (Boehringer), each. After sucrose gradient centrifugation, 4 ml of the interphase between the sucrose layers was diluted with 5 vol of 25 mM sodium phosphate buffer (pH 7.4) containing 7 mM iodoacetamide and centrifuged at 48,000 × *g* for 20 min at 4°C. Pellets were resuspended in the same buffer (250 ml) before crosslinking.

Crosslinking. Aliquots of purified GlyR (29) were incubated with 100 mM dithiothreitol for 15 min at room temperature before dialysis against 2000 vol of 25 mM potassium phosphate buffer, pH 7.4/1 M KCl, 1% (wt/vol) sodium cholate/ 0.15% (wt/vol) egg yolk phosphatidylcholine/0.05% (wt/vol) soybean phosphatidylcholine and protease inhibitors as described (20). Prior to reaction with 3,3′-dithiobis(sulfosuccinimidylpropionate) (DTSP, Serva) and sulfo-*N*-succinimidyl(4-azidophenyl)-1,3′-dithiopropionate (SSADP, Pierce), the dialyzed receptor preparation was incubated with 10 mM iodoacetamide for 10 min at room temperature. For crosslinking, aliquots containing ≈3 pmol of GlyR in 100 μl were treated at room temperature with the different crosslinking reagents (dissolved in dried dimethyl sulfoxide) as described in the figure legends. Reactions were stopped by the addition of 100 mM Tris·HCl (pH 7.4), when DTSP or SSADP, and by the addition of 10 mM iodoacetamide, when *N*-(4-azidophenylthio)phthalimide (APTP, Sigma) or *p*-azidophenacyl bromide (APAB, Sigma) was used. When using the photoactivatable reagents SSADP, APTP, and APAB, crosslinking was subsequently initiated by photolysis with 10–30 flashes (31) from a Xenon flash lamp (Revue Tron 34T). Crosslinked samples were dialyzed for 5 hr at 4°C against 1000 vol of 20 mM Tris·HCl, pH 7.4/200 mM NaCl/1% (wt/vol) sodium cholate and protease inhibitors. Subsequently they were equilibrated overnight at room temperature with 200 vol of electrophoresis sample buffer.

For compositional analysis of individual adducts, ≈30 pmol of GlyR was crosslinked with DTSP or SSADP as described in the legend to Fig. 1. After separation by 3.5–10% $NaDodSO_4$/PAGE, gel pieces containing adducts were excised with reference to adduct bands detected in a separate lane. For cleavage of crosslinks, the gel pieces were immersed in reducing electrophoresis sample buffer for 1 hr at 37°C and heated to 56°C for 15 min. Electrophoresis in the second dimension was performed after loading the reduced gel pieces on top of a 10% $NaDodSO_4$/polyacrylamide gel.

For crosslinking in membranes, aliquots of diluted synaptosomes were treated with dimethyl suberimidate (DMSI, Pierce) or sulfo-*N*-succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate (SSANPAH, Pierce) as described in the legend to Fig. 3. Crosslinked membranes were centrifuged for 10 min in an Eppendorf centrifuge, and the pellets were boiled in reducing sample buffer prior to $NaDodSO_4$/PAGE and immunoblotting.

Gel Electrophoresis and Immunoblotting. $NaDodSO_4$/PAGE (10% or 3.5–10% slab gels) was performed according to Laemmli (32). The sample buffer used consisted of 0.02 M sodium borate/acetate buffer (pH 6.8), 5% (wt/vol) $NaDodSO_4$, and 6 M urea for nonreducing gels. For reducing gels 10% (vol/vol) 2-mercaptoethanol was included. Samples were prepared either by dialysis or dissolution of trichloroacetic acid-precipitated receptor preparation (20) in sample buffer and heating to 56°C for 15 min. Protein bands were detected by staining with silver (33) or Coomassie blue R-250. Where indicated, the relative intensities of GlyR-specific bands were determined by scanning with a Hirschmann Elscript 400 densitometer and computer calculation of respective area increments. Molecular weights were calculated with reference to marker proteins: carbonic anhydrase (M_r 31,000), ovalbumin (M_r 45,000), bovine serum albumin (M_r 66,000), phosphorylase b (M_r 93,000), β-galactosidase (M_r 116,000), myosin (M_r 205,000), and hemocyanin (M_r 285,000). For immunoblotting after $NaDodSO_4$/PAGE, proteins were transferred to nitrocellulose (Schleicher & Schuell) in a horizontal semidry electroblotting apparatus (34). For efficient transfer, 0.01% (wt/vol) $NaDodSO_4$ was included in the transfer buffer, and a current of 1 mA/cm^2 was applied for 3 hr. Immunodetection of M_r 48,000 polypeptide was accomplished (C.-M. Becker, W. Hoch, and H.B., unpublished data) by using a signal-amplification technique (35) with immunocomplexed alkaline phosphatase (Dianova, Hamburg, F.R.G.). Monoclonal antibodies (mAbs) were used at the following dilutions: mAb 4a at 1:1000 or mAb 2b at 1:20 with blotted purified GlyR, and mAb 4a at 1:100 with blotted synaptosomal proteins (6).

Density Gradient Centrifugation. Sedimentation of GlyR protein in linear sucrose gradients prepared with H_2O or ^2H_2O and data analysis were performed essentially as described (20, 24). Gradient fractions were analyzed by an immunochemical dot receptor assay employing mAb 4a at a dilution of 1:100 (C.-M. Becker, W. Hoch, and H.B., unpublished data) and silver staining after $NaDodSO_4$/PAGE.

RESULTS

Relative Subunit Stoichiometry. The polypeptides present in purified preparations of GlyR were separated by $NaDodSO_4$/PAGE after trichloroacetic acid precipitation and stained with Coomassie blue, or silver, and their relative intensities were determined densitometrically (data not shown). Thus mean ratios of 2.2 ± 0.9:1 (Coomassie staining, 4 preparations) and 3.3 ± 1:1 (silver staining, 10 other preparations) of M_r 48,000 (α) to M_r 58,000 (β) subunits were obtained. The relative amount of M_r 93,000 polypeptide found in the different preparations was even more variable. Ratios of M_r 48,000 to M_r 93,000 polypeptide between 3:1 and 1:6 were found. These variations may result from proteolytic degradation and/or nonquantitative precipitation of receptor polypeptides prior to electrophoresis.

Crosslinking of Purified GlyR. Bifunctional reagents of varying side-chain specificities and chain lengths were employed to crosslink the subunits of the GlyR. With increasing concentrations of lysine-specific crosslinkers, $NaDodSO_4$/PAGE revealed the appearance of a major adduct concomitantly with the gradual loss of individual GlyR subunits. This major adduct had an apparent M_r of 240,000 when the homobifunctional reagent DTSP was used and of 260,000 after crosslinking with the heterobifunctional photoactivatable reagent SSADP (Fig. 1). In addition, high molecular weight material at the top of the running gel (M_r >800,000) was detected in both cases. With SSADP, minor bands of M_r

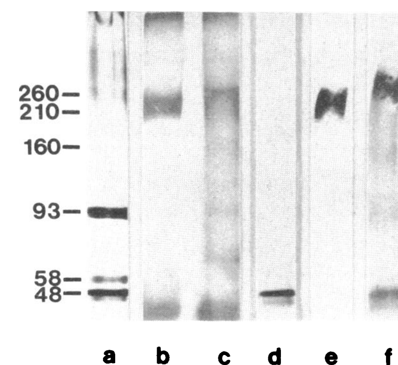

FIG. 1. $NaDodSO_4$/PAGE (3.5–10%) of purified GlyR after extensive crosslinking. Adduct bands were visualized by silver staining (lanes a–c) or immunoblotting with mAb 4a (lanes d–f). Lanes: a and d, GlyR uncrosslinked; b and e, GlyR crosslinked with 1 mM DTSP for 30 min; c and f, GlyR incubated with 1 mM SSADP for 15 min and crosslinked by flash-photolysis. Molecular weights are given as $M_r \times 10^{-3}$.

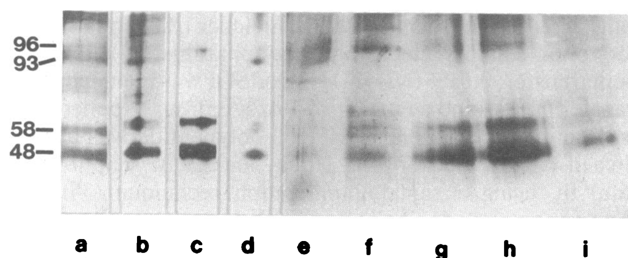

FIG. 2. Compositional analysis of adducts produced by crosslinking of purified GlyR. After electrophoretic separation, adduct bands were excised from the gel and cleaved. Resultant cleavage products were separated by 10% $NaDodSO_4$/PAGE and detected by silver staining. (The occasional appearance of the M_r 58,000 polypeptide as a doublet is attributed to exoproteolytic degradation. For the data given in Table 1, the staining intensities of both bands have been added.) Lanes: a, GlyR uncrosslinked; b, GlyR crosslinked with 1 mM DTSP and cleaved prior to electrophoresis; c and d, polypeptide composition of GlyR adducts (c, M_r 240,000; d, M_r >800,000) produced upon crosslinking with 1 mM DTSP; e–i, polypeptide composition of GlyR adducts (e, M_r 95,000; f, M_r 160,000; g, M_r 210,000; h, M_r 260,000; i, M_r >800,000) obtained upon crosslinking with 1 mM SSADP. Molecular weights are given as $M_r \times 10^{-3}$.

95,000, 160,000, and 210,000 were also seen; their intensity decreased with increasing crosslinker concentrations, whereas the position of the major high molecular weight adduct remained unchanged. The formation of adduct bands of intermediate size was favored when using the shorter sulfhydryl-specific heterobifunctional reagents APTP and APAB (see Fig. 3, lanes a and b).

For compositional analysis, adducts obtained upon crosslinking with DTSP, or SSADP, were excised from the gel, cleaved by incubation with 2-mercaptoethanol, and separated on a second $NaDodSO_4$/polyacrylamide gel. For control, aliquots of crosslinked GlyR were reduced without prior electrophoretic separation. The following observations were made. (*i*) After crosslinking and cleavage, the apparent molecular weights of M_r 48,000 and 58,000 subunits, but not of M_r 93,000 polypeptide, appeared slightly elevated (Fig. 2, lane b), presumably due to molecular weight increments by attached crosslinkers. (*ii*) Cleavage of the band at M_r 240,000 produced upon crosslinking with DTSP yielded only M_r 48,000 and 58,000 subunits, whereas after cleavage of the M_r >800,000 material, bands corresponding to the M_r 48,000, 58,000 and 93,000 polypeptides were detected (Fig. 2, lanes c and d). (*iii*) Cleavage of adducts obtained upon crosslinking with SSADP generated polypeptide patterns as shown in Fig. 2 (lanes e–i). Adducts of M_r 160,000, 210,000, and 260,000 all gave M_r 48,000 and 58,000, but not the M_r 93,000, polypeptides. The adduct at M_r 95,000 contained M_r 48,000 and unreacted M_r 93,000 polypeptides. Similar to crosslinking with DTSP, cleavage of the M_r >800,000 material yielded M_r 48,000, 58,000, and 93,000 polypeptides. Bands at M_r 96,000 and above are attributed to incompletely cleaved adducts.

We interpret these data in that all adducts observed up to M_r 260,000 resulted from crosslinking of components of the transmembrane core of the GlyR—i.e., of M_r 48,000 and 58,000 subunits. Densitometric analysis of the gel shown in Fig. 2 (lanes e–i) yielded relative intensities of bands corresponding to M_r 48,000 and 58,000 subunits, which suggested adduct compositions as listed in Table 1.

Given these findings, the lower molecular weight intermediates are thought to represent dimeric, trimeric, and tetrameric assemblies of crosslinked α and β subunits. The major adduct at M_r 260,000 is assumed to correspond to the completely crosslinked core structure. Consequently, we propose that the transmembrane core of the GlyR exists as a pentamer.

The appearance of the M_r >800,000 adducts may be attributed to chance crosslinking of randomly colliding but independent receptor oligomers. In light of the low protein concentration used here (≈10 μg/ml), the probability of random collisions between receptor molecules during the crosslinking reaction appears, however, low (36). Furthermore, the relative proportion between the amount of the M_r >800,000 material and the major M_r ≈250,000 adduct was comparable after chemical (DTSP) and photoactivated (SSADP) crosslinking. A significant number of collisions cannot be expected to occur within the reaction time of flash photolysis, which is in the range of milliseconds (31). Thus the resultant adducts are likely to represent stable structures of complexed polypeptides as discussed below.

Crosslinking of Synaptic Membranes. To ascertain that the subunit arrangement as deduced from crosslinking of purified GlyR is not an artefact of solubilization and/or purification, we also crosslinked the receptor in synaptosomal membranes. One problem involved with crosslinking protein-rich membranes is the possible formation of random crosslinks between unrelated protein components due to lateral diffusion and random collisions (31, 37). Coomassie blue staining of electrophoresed synaptosomal protein revealed that crosslinking with the homobifunctional reagent DMSI indeed had resulted in considerable alteration of the overall polypeptide pattern (Fig. 3). Only minor changes were seen, however, when the photoactivatable reagent SSANPAH was used, consistent with the general advantages of flash-initiated crosslinking as discussed above. Fig. 3 also shows immunoblots of crosslinked synaptosomes after $NaDodSO_4$/PAGE. Irrespective of whether DMSI or SSANPAH was used as a crosslinker, the resulting pattern of adducts reacting with mAb 4a was similar to those obtained upon crosslinking of purified GlyR. It thus appears that the subunit composition of membrane-bound GlyR is preserved upon purification.

Table 1. Polypeptide adducts obtained upon crosslinking GlyR and deduced subunit compositions

	Adduct $M_r \times 10^{-3}$*						
Crosslinker							
DTSP	—	—	—	—	—	238 (4)	>800 (4)
SSADP	95 (3)	106 (2)	—	160 (4)	211 (4)	264 (8)	>800 (8)
APTP or APAB	—	116 (3)	132 (3)	165 (5)	217 (5)	268 (5)	>800 (5)
Polypeptides found upon cleavage	48 + 93	ND	ND	48 + 58	48 + 58	48 + 58	48 + 58 + 93
	M_r 48,000/M_r 58,000 ratio†						
	1:0.1	ND	ND	2:1.8	3:1	3:1.3	3:1.3
	Suggested subunit composition						
	α_2	(β_2)	(α_3)	$\alpha_2\beta/\alpha\beta_2$	$\alpha_3\beta_1$	$\alpha_3\beta_2$	$\alpha_3\beta_2\gamma_n$

*Values are means of molecular weights calculated with reference to marker proteins. The number of experiments in which the respective adduct was observed is given in parentheses. ND, not determined.

†Determined by densitometry of silver-stained $NaDodSO_4$/polyacrylamide gels.

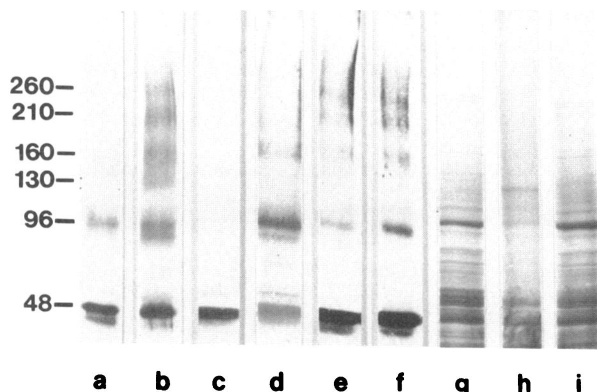

FIG. 3. NaDodSO$_4$/PAGE (3.5–10%) of intermediate-sized adducts generated by crosslinking purified and membrane-bound GlyR. Lanes a and b, purified GlyR was crosslinked and electrophoresed, and adduct bands were detected by immunoblotting with mAb 2b; a, GlyR uncrosslinked; b, GlyR incubated with 0.2 mM APAB for 60 min and crosslinked by flash-photolysis. (The band at $M_r \approx 90,000$ probably corresponds to a dimer of degraded M_r 48,000 polypeptide visible in lane a.) Lanes c–i, crosslinked synaptosomal proteins were electrophoresed and GlyR specific bands were visualized by immunoblotting with mAb 4a (c–f); corresponding samples were stained for protein with Coomassie blue (g–i); c and g, uncrosslinked synaptosomes; d, synaptosomes crosslinked with DMSI at 0.1 mg/ml for 60 min; e and h, synaptosomes crosslinked with DMSI at 1.5 mg/ml for 20 min; f and i, synaptosomes incubated with 0.05 mM SSANPAH for 15 min and flash-photolyzed. Molecular weights are given as $M_r \times 10^{-3}$.

Density Gradient Centrifugation. Previously, the M_r of the GlyR solubilized in the presence of Triton X-100 and phosphatidylcholine has been determined as 246,000 using density gradient centrifugation in H_2O or ^2H_2O in combination with gel-permeation chromatography (20). Under the conditions employed, the strychnine-binding core of M_r 48,000 and 58,000 subunits migrates separately from a rapidly sedimenting structure, which predominantly contains the M_r 93,000 polypeptide (24).

The hydrodynamic properties of detergent micelles have been reported to change upon mixing with lipids (38). To examine a possible influence of lipid binding on the sedimentation behavior of the GlyR—i.e., to validate the previously determined molecular weight—density gradient centrifugation was repeated using GlyR purified without exogeneous lipids. Gradient fractions were analyzed for the presence of GlyR core polypeptides immunochemically and by NaDodSO$_4$/PAGE with silver staining (not shown). From the data obtained, a $s_{20,w}$ value of 8.08 S was calculated (mean of two determinations), which is very similar to that reported previously (8.2 S). Under the conditions used, the GlyR peak fractions did not contain significant amounts of M_r 93,000 polypeptide. Hence, the determined sedimentation coefficient and thus the calculated M_r of 246,000 (20) are not affected by lipid binding and apparently representative of the membrane-spanning core structure of the GlyR.

DISCUSSION

In this study we provide evidence that the postsynaptic GlyR has a pentameric transmembrane structure. Upon chemically crosslinking its subunits with reagents of varying side-chain specificities and lengths, a major adduct of apparent M_r 240,000–260,000 was formed together with lower molecular weight intermediates. This adduct pattern was similar for purified receptor preparations and GlyR embedded in its natural membrane environment. Compositional analysis revealed that the adduct of M_r 95,000 contained M_r 48,000 (and unreacted M_r 93,000) and that adducts of M_r 160,000, 210,000, and 260,000 contained the M_r 48,000 and 58,000, but not the M_r 93,000 polypeptides. We therefore conclude that the lower molecular weight intermediates correspond to dimers, trimers, and tetramers, respectively, whereas the largest adduct of M_r 260,000 represents a pentameric structure of the membrane-spanning subunits. Upon density gradient centrifugation and gel-permeation chromatography the same subunits migrate as a core complex distinct from high molecular weight forms of the GlyR, which contain the peripheral M_r 93,000 polypeptide. The M_r of this core complex of 246,000 as calculated from its hydrodynamic properties (20) correlates well with the M_r 260,000 determined by NaDodSO$_4$/PAGE after exhaustive crosslinking.

Data obtained by densitometry of Coomassie blue-stained NaDodSO$_4$/polyacrylamide gels are equally compatible with a subunit stoichiometry of either $\alpha_4\beta_1$ or $\alpha_3\beta_2$, corresponding to calculated M_rs of 250,000 and 260,000, respectively. Albeit our present molecular weight and crosslinking data do not allow us to definitely distinguish between these stoichiometries, we currently favor a $\alpha_3\beta_2$ model of the GlyR core for the following reasons. (*i*) In some of our crosslinking experiments with purified GlyR, a M_r 116,00 adduct was seen that stained with silver and, on immunoblots, weakly reacted with mAb 4a but not mAb 2a (D.L., unpublished data). At the dilutions used here, mAb 4a, but not mAb 2b, crossreacts with the M_r 58,000 chain of the GlyR (see also ref. 6). We therefore tentatively identify the M_r 116,000 adduct as a β_2 dimer. (*ii*) The glycine response produced in *Xenopus* oocytes upon injection of poly(A)$^+$ RNA from rat brain or spinal cord exhibits a Hill coefficient for glycine of 2.7–3.3 (ref. 39; V. Schmieden, personal communication). As the glycine-binding site of the GlyR is assigned to the M_r 48,000 subunit (ref. 4; V. Schmieden, P. Schofield, D. Pritchett, H. Sontheimer, H. Kettenmann, C. M. Becker, G. Grenningloh, P. Seeburg, and H.B., unpublished data), these data also are consistent with an $\alpha_3\beta_2$ structure. Consequently, we propose that a quasisymmetrical pore-forming arrangement of five transmembrane subunits is conserved between at least two ligand-gated ion channels, the GlyR and the nAcChoR.

Indirect support for a pentameric structure also arises from electrophysiological data on cultured neurons. The diameter of the ion channels intrinsic to GlyR and GABA$_A$R was estimated from ion-permeation studies to be between 5 Å and 6 Å (40). This value is consistent with a model in which the channel is formed by a pentameric assembly of α-helices. For the GABA$_A$R, all hydroxy-rich transmembrane segments (M1–M3) of its subunits have been proposed to line the channel interior (5, 41). On the other hand, in nAcChoR subunits, the putative transmembrane helix M2 appears to be that segment most essential in forming the pore structure. In photoaffinity labeling experiments using noncompetitive channel blockers (42, 43), residues of segment M2 reacted. Furthermore, patch clamp studies on *Xenopus* oocytes translating nAcChoR hybrid mRNAs engineered from different species revealed that segment M2 constitutes the major determinant of channel characteristics (44). Also, segment M2 exhibits the highest degree of homology between GABA$_A$R subunits and the GlyR M_r 48,000 polypeptide (4, 5, 7), suggesting that it is indeed crucial for chloride channel function. These data support a general model in which each of five receptor subunits contributes its M2 region to the inner wall of the respective ion channel (45).

Based on these considerations we suggest that, by analogy to the conservation of primary structure and transmembrane topology within the family of ligand-gated ion channel proteins, its members also share a similar pentameric structure. Incompatible with this hypothesis is the tetrameric structure proposed for the GABA$_A$R (11). This model, however, was solely based on determinations of molecular weight and subunit stoichiometry using methods with considerable in-

trinsic inaccuracies. Specifically, calculation of molecular weight based on hydrodynamic measurements involves a number of general theoretical assumptions (46). Further, densitometric evaluation of Coomassie blue-stained $NaDodSO_4$/polyacrylamide gels does not necessarily yield unambiguous subunit stoichiometries. Variation of staining intensity with amino acid composition of polypeptides, selective proteolysis of subunits, and incomplete protein precipitation are some possible artefacts involved. It, therefore, does not seem inappropriate to tentatively propose that a pentameric structure is also shared by the $GABA_AR$.

A relevant question concerning GlyR structure addresses the function of the peripheral M_r 93,000 polypeptide. As previously discussed (24), this subunit may be a functional analog of the M_r 43,000 polypeptide that has been implicated in postsynaptic anchoring of the nAcChoR (47). Several observations support this speculation. Upon crosslinking of purified GlyR, high molecular weight adducts of M_r >800,000 are formed that contain M_r 93,000 polypeptide in addition to the core-forming subunits. At the same time, a high molecular weight form of GlyR consisting predominantly of M_r 93,000 polypeptide can be separated from the core complex by hydrodynamic methods (24). The existence of a cytoplasmic network of M_r 93,000 polypeptide immobilizing the GlyR may explain these data assuming that two subpopulations of GlyR—with and without M_r 93,000 polypeptide—arise from incomplete dissociation of the M_r 93,000 polypeptide from the transmembrane core upon solubilization. Elucidation of the function of the M_r 93,000 polypeptide in postsynaptic receptor anchoring and/or synapse formation may provide further evidence for common structural principles governing architecture and synaptic organization of ligand-gated ion channel proteins.

We thank W. Hoch for many helpful discussions, Drs. C.-M. Becker and B. Schmitt for communicating biochemical and immunoreceptor assay techniques prior to publication, C. Schröder for production of antibodies, Dr. R. Schwarting for a gift of immunocomplexed alkaline phosphatase and related technical advice, Drs. D. Zopf, B. Shivers, and E. Gundelfinger for critically reading the manuscript, and I. Baro for preparation of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 317) and the Fonds der Chemischen Industrie. D.L. is recipient of a predoctoral fellowship from the Fonds der Chemischen Industrie.

1. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Furutani, Y., Hirose, T., Takashima, H., Inayama, S., Miyata, T. & Numa, S. (1983) *Nature (London)* **302**, 528–532.
2. Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S. & Patrick, J. (1986) *Nature (London)* **319**, 368–374.
3. Hermans-Borgmeyer, I., Zopf, D., Ryseck, R. P., Hovemann, B., Betz, H. & Gundelfinger, E. D. (1986) *EMBO J.* **5**, 1503–1508.
4. Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D. & Betz, H. (1987) *Nature (London)* **328**, 215–220.
5. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H. & Barnard, E. A. (1987) *Nature (London)* **328**, 221–227.
6. Pfeiffer, F., Simler, R., Grenningloh, G. & Betz, H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7224–7227.
7. Grenningloh, G., Gundelfinger, E. D., Schmitt, B., Betz, H., Darlison, M. G., Barnard, E. A., Schofield, P. & Seeburg, P. H. (1987) *Nature (London)* **330**, 25–26.
8. Brisson, A. & Unwin, P. N. T. (1985) *Nature (London)* **315**, 474–477.
9. Kubalek, E., Ralston, S., Lindstrom, J. & Unwin, N. (1987) *J. Cell Biol.* **105**, 9–18.
10. Hucho, F., Bandini, G. & Suarez-Isla, B. A. (1978) *Eur. J. Biochem.* **83**, 335–340.
11. Mamalaki, C., Stephenson, F. A. & Barnard, E. A. (1987) *EMBO J.* **6**, 561–565.
12. Whiting, P. J. & Lindstrom, J. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 595–599.
13. Whiting, P., Esch, F., Shimasaki, S. & Lindstrom, J. (1987) *FEBS Lett.* **219**, 459–463.
14. Betz, H. (1987) *Trends Neurosci.* **10**, 113–117.
15. Coombs, J. S., Eccles, J. C. & Fatt, P. (1955) *J. Physiol. (London)* **130**, 326–373.
16. Young, A. B. & Snyder, S. H. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2832–2836.
17. Young, A. B. & Snyder, S. H. (1974) *Mol. Pharmacol.* **10**, 790–809.
18. Young, A. B. & Snyder, S. H. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4002–4005.
19. Snyder, S. H. & Bennet, J. P. (1976) *Annu. Rev. Physiol.* **38**, 153–175.
20. Pfeiffer, F., Graham, D. & Betz, H. (1982) *J. Biol. Chem.* **257**, 9389–9393.
21. Graham, D., Pfeiffer, F. & Betz, H. (1985) *Biochemistry* **24**, 990–994.
22. Becker, C. M., Hermans-Borgmeyer, I., Schmitt, B. & Betz, H. (1986) *J. Neurosci.* **6**, 1358–1364.
23. Graham, D., Pfeiffer, F. & Betz, H. (1983) *Eur. J. Biochem.* **131**, 519–525.
24. Schmitt, B., Knaus, P., Becker, C.-M. & Betz, H. (1987) *Biochemistry* **26**, 805–811.
25. Triller, A., Cluzeaud, F., Pfeiffer, F., Betz, H. & Korn, H. (1985) *J. Cell Biol.* **101**, 683–688.
26. Altschuler, R. A., Betz, H., Parakkal, M. H., Reeks, K. A. & Wenthold, R. J. (1986) *Brain Res.* **369**, 316–320.
27. Peters, K. & Richards, F. M. (1977) *Annu. Rev. Biochem.* **46**, 523–551.
28. Gaffney, B. J. (1985) *Biochim. Biophys. Acta* **822**, 289–317.
29. Rienitz, A., Becker, C. M., Betz, H. & Schmitt, B. (1987) *Neurosci. Lett.* **76**, 91–95.
30. Gray, E. G. & Whittacker, V. P. (1962) *J. Anat.* **96**, 79–88.
31. Kiehm, D. J. & Ji, T. H. (1977) *J. Biol. Chem.* **252**, 8524–8531.
32. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
33. Merril, C., Goldman, D. & Van Keuren, M. (1982) *Electrophoresis (Weinheim, Fed. Repub. Ger.)* **3**, 17–20.
34. Kyhse-Andersen, J. (1984) *J. Biophys. Biochem. Methods* **10**, 203–209.
35. Cordell, J. L., Falini, B., Erber, W. N., Ghosh, K. A., Abdaluziz, Z., MacDonald, S., Pulford, K. A. F., Stein, H. & Mason, D. Y. (1984) *J. Histochem. Cytochem.* **32**, 219–224.
36. Hajdu, J., Bartha, F. & Friedrich, P. (1976) *Eur. J. Biochem.* **68**, 373–383.
37. Frye, L. D. & Edidin, M. (1970) *J. Cell Sci.* **7**, 319–335.
38. Furth, A. J., Bolton, H., Potter, J. & Priddle, J. D. (1984) *Methods Enzymol.* **104**, 318–328.
39. Gundersen, C. B., Miledi, R. & Parker, I. (1984) *Proc. R. Soc. London Ser. B* **221**, 235–244.
40. Bormann, J., Hamill, O. P. & Sakmann, B. (1987) *J. Physiol. (London)* **385**, 243–286.
41. Barnard, E. A., Darlison, M. G. & Seeburg, P. H. (1987) *Trends Neurosci.* **10**, 502–509.
42. Hucho, F. L., Oberthür, W. & Lottspeich, F. (1986) *FEBS Lett.* **205**, 137–142.
43. Giraudat, J., Dennis, M., Heidmann, T., Haumont, P. T., Lederer, F. & Changeux, J. P. (1987) *Biochemistry* **26**, 2410–2418.
44. Imoto, K., Methfessel, C., Sakmann, B., Mishina, M., Mori, Y., Konno, T., Fukuda, K., Kurasaki, M., Bujo, H., Fujita, Y. & Numa, S. (1986) *Nature (London)* **324**, 670–674.
45. Changeux, J. P., Giraudat, J. & Dennis, M. (1987) *Trends Pharmacol. Sci.* **8**, 459–465.
46. Clarke, S. (1975) *J. Biol. Chem.* **250**, 5459–5469.
47. Froehner, S. C. (1986) *Trends Neurosci.* **9**, 37–41.