**Figure 1-3** An arrow represents a transmembrane strand and a circular column represents a transmembrane helix. Regions before the first arrow represent extracellular domains. Extracellular regions in a box and in the membrane contain fragments that partition in aqueous and membrane-associated fractions and are interpreted as being involved in protein-protein interactions with regions of the receptor that are membrane associated. The agonist/antagonist binding sites are indicated by a boxed "L ". The glycosylation site at N38 is marked with a branched symbol (Adapted from Leite and Cascio, 2001).
Figure 1-4 Pie chart model of the subunit composition of the major GABA<sub>A</sub> receptor subtypes in rat brain. \( \beta \) subtypes indicate \( \beta_{1,2} \) or \( \beta_3 \) (Whiting et al., 1999).
Figure 1-6 Diagrammatic representation of a prototypic GABA\textsubscript{A} receptor showing the putative localization of the binding sites of the agonist and various modulatory agents. BZ, benzodiazepine; PTX, picrotoxin. The locations of binding sites for barbiturates, steroids, avermectins, and propofol remain unclear. The P in a circle represents the phosphorylation sites located on the cytoplasmic domain between TM3 and TM4 of the $\alpha$, $\beta$, $\gamma$ subunits (Whiting et al., 1999).
Figure 1-7 Alignments of the ligand-binding domain of the LGICS family. The human GABA<sub>A</sub> receptor (G-A) β<sub>2</sub>, α<sub>1</sub> and γ<sub>2</sub> subunits, and Torpedonicotinic acetylcholine (ACh) receptor α1- and γ subunits, with ACh-binding protein (AChBP) were aligned. Secondary structure elements of the AChBP structure are shown below its sequence (α-helix: column; β strands: arrow). The conserver 15-residue Cys-loop is highlighted in a light gray box. Also highlighted are some of the residues implicated experimentally in agonist binding. These residues fall into six regions, known in the six-loop agonist-binding-site model as loops A, B and C (black boxes) from the principal subunit, and loops D, E and F (grey shadow) from the complementary subunit. Residues within 5 Å of HEPES molecules in the AChBP crystal structure also fall within these regions of the subunit, corresponding to loops A, B and C, or loops D, E and F, respectively. (Note: GABAA receptor residues α1Trp95 and γ2Thr142 were originally sequenced as Arg and Ser, respectively. Subsequent sequencing, including that of the human genome, indicates that these were probably errors in the original sequence determination.) (Cromer et al, 2002)
Figure 2-2 The strategy of PCR-based site-directed mutagenesis. The protocol was modified from that of ExSite™ PCR-based site-directed mutagenesis kit (Stratagene). Both oligonucleotide primers were phosphorylated. The linear amplification product was purified with agarose gel electrophoresis prior to ligation.
Figure 3-1 SDS-PAGE analysis of GABA<sub>A</sub> receptor α<sub>1</sub> subunit fragments. Expression and purification of receptor segments were as described in Materials and Methods. (A) 1 day at room temperature and (B) 100 days at room temperature after purification. Lane 1: marker; lane 2: Q28-L296; lane 3: Q28-R248; lane 4: Q28-E165; lane 5: C166-L296.
Figure 3-2 Western Blotting analysis of controlled trypsin lyzed Q28-L296. The polyclonal antibody against fragment Q28-E165 was used. Lanes 1-9, Q28-L296 samples were digested for 0 s, 30 s, 5 min, 10 min, 20 min, 30 min, 40 min, 60 min and 24 hours respectively.
Figure 3-9 The Molecular mass of the fragment Q28-E165 in presence or absence of 0.1% SDS. The molecular mass was calculated using laser scattering spectroscopy as described by Xue et al (2001). The elution profile of Q28-E165 using 10 mM Gly, pH 9.6 as elution buffer, in the absence (thin line) and presence of 0.05% SDS (thick line) monitored with both UV (dashed line) and laser scattering (solid line) spectroscopy. The corresponding average molecular mass under the experimental conditions was calculated to be 3,100 kD and 58 kD, in the absence and presence of 0.05% SDS respectively.
Figure 3-10 The molecular mass of the fragment C166-L296 in presence or absence of 0.1% SDS. The molecular mass was calculated using laser scattering spectroscopy as described by Xue et al (2001). The elution profile of C166-L296 using 10 mM Gly, pH 9.6 as elution buffer, in the absence (thin line) and presence of 0.05% SDS (thick line) monitored with both UV (dashed line) and laser scattering (solid line) spectroscopy. The corresponding average molecular mass under the experimental conditions was calculated to be 250 kD and 225 kD, in the absence and presence of 0.05% SDS respectively.
Figure 4-1 SDS-PAGE analysis of purified Cys- mutants of fragment Cys166-Leu296. Lane 1: Markers; lane 2 wild type fragment; lane 3: C166A; lane 3: C180A; lane 4: C261A; lane 5: C166AC180A; lane 6: C180AC261A
Figure 4-8 Schematic diagrams of ‘pins’ in Ig-like domains. (A) The four-member ‘pin’-like structure formed in N9 neuraminidase, chain H formed by 2 Cys and 2 Trp residues (34). The residues C22, W36, C92 and W103 have separations of 14, 56 and 11 residues respectively. The distance from W36 (indole moiety, C5) or W103 (indole moiety, C4) to C92 (S) is 4.61 or 7.79 Å, respectively. The relative surface accessibilities for the Trp residues are 0.3 % and 30.4 % for W36 and W103 respectively (Guex and Peitsch, 1997). (B) The proposed five-member ‘pin’-like model for C166-L296. The circles represent Cys and the hexagons represent Trp. The larger the overlap of the shapes, the closer the residues and the more severe the structural change should the residue pair be mutated. Mutations of residue pairs on the same side of the line tend to cause more structural change than single mutations and vice versa for mutations of residue pairs on opposite sides of the line. Considering residues C180, W198, C261 and W273, the separations are 18, 63 and 12 respectively.
Figure 5-2 Purification and SDS-PAGE analysis (a) FPLC purification profile. The major peak corresponds to the recombinant protein fragment. Only protein from the peak fractions was used for further analysis. (b) SDS-PAGE analysis: lane 1, molecular mass marker; lane 2, total cell lysate of non-induced E. coli; lane 3, total cell lysate of E. coli expressing the GlyR fragment; lane 4, 4 M urea extracts of E. coli expressing the GlyR fragment; lane 5, purified Ala165-Met291 fragment.
Figure 5-7 Quaternary structure analyzed by electron microscopy. (a) a typical electron micrograph of negatively stained recombinant GlyR fragment Ala165-Met291 is shown. Examples of single trimeric molecules are marked by arrows (↑) in the main panel and enlarged representative single molecules are shown in the inset. Note that smaller particles (possible dimers or side-on projections of trimers, boxed (□) as well as larger particles (higher order oligomers/aggregates, arrowheads (↑) can also be discerned in the preparation. The only consistently observed projection to occur with the highest frequency and to converge in the analysis corresponds to a face-on projection of a trimer. The scale bar corresponds to 50 nm in the main panel and 10 nm in the inset. (b) A 3-fold symmetry revealed by rotational symmetry analysis. (c) Final contoured average after enhancing the 3-fold symmetry. Scale bar corresponds to 5 nm.
Figure 6-1 Recursive PCR product. (a) Product of first PCR with six oligonucleotides. Lane 1: 100 bp DNA ladder; lane 2: β2A24-A159, lane 3: γ2Q41-G176. (b) Product of second PCR with a pair of amplifying primers. Lane 1: 100 bp DNA ladder; lane 2: β2A24-A159, lane 3: γ2Q41-G176
Figur 6-4 SDS-PAGE analysis of GABA<sub>A</sub> receptor fragments of β<sub>2</sub> and γ<sub>2</sub> subunits. A-D: fragments of β<sub>2</sub> subunit; E-H: fragments of γ<sub>2</sub> subunit. A: β<sub>2</sub>A24-A159; B: β<sub>2</sub>A159-G243; C: β<sub>2</sub> A24-G243; D: β<sub>2</sub> A24-A270; E: γ<sub>2</sub>Q41-G176; F: γ<sub>2</sub>C190-D299; G: γ<sub>2</sub>R183-M315; H: γ<sub>2</sub>Q41-G273
Figure 6-7 SDS-PAGE analysis of co-expressed C166-L296 of α₁ subunit and C190-D299 of γ₂ subunit. A,B,C: three different colonies. Cells were cultured at 37°C. Lane 3, 6, 9: supernatant of cell lysates; lane 2, 5, 8: pellet of cell lysates; lane 1, 4, 7: urea washed pellets.