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Assembly of GABA<sub>A</sub> receptors (Review)

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Assembly of GABA\textsubscript{A} receptors (Review)

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Abstract

GABA\textsubscript{A} receptors are the major inhibitory transmitter receptors in the central nervous system. They are chloride ion channels that can be opened by \(\gamma\)-aminobutyric acid (GABA) and are the targets of action of a variety of pharmacologically and clinically important drugs. GABA\textsubscript{A} receptors are composed of five subunits that can belong to different subunit classes. The existence of 19 different subunits gives rise to the formation of a large variety of distinct GABA\textsubscript{A} receptor subtypes in the brain. The majority of GABA\textsubscript{A} receptors seems to be composed of two \(\alpha\), two \(\beta\) and one \(\gamma\) subunit and the occurrence of a defined subunit stoichiometry and arrangement in \(\alpha\beta\gamma\) receptors strongly indicates that assembly of GABA\textsubscript{A} receptors proceeds via defined pathways. Based on the differential ability of subunits to interact with each other, a variety of studies have been performed to identify amino acid sequences or residues important for assembly. Such residues might be involved in direct protein-protein interactions, or in stabilizing direct contact sites in other regions of the subunit. Several homo-oligomeric or hetero-oligomeric assembly intermediates could be the starting point of GABA\textsubscript{A} receptor assembly but so far no unequivocal assembly mechanism has been identified. Possible mechanisms of assembly of GABA\textsubscript{A} receptors are discussed in the light of recent publications.

Keywords: GABA\textsubscript{A} receptor, heterogeneity, assembly, intermediates, mechanism

GABA\textsubscript{A} receptor heterogeneity

GABA\textsubscript{A} receptors are the major inhibitory transmitter receptors in the central nervous system. They are chloride ion channels that can be opened by \(\gamma\)-aminobutyric acid (GABA) and are the targets of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants [1]. From the effects of these drugs it can be concluded that these receptors regulate the excitability of the brain, anxiety, vigilance, and learning and memory.

GABA\textsubscript{A} receptors are composed of five subunits that can belong to different subunit classes. Six \(\alpha\), three \(\beta\), three \(\gamma\), one \(\delta\), one \(\epsilon\), one \(\pi\), one \(\theta\)- and three \(\rho\)-subunits as well as splice variants of some of these subunits have been cloned from the mammalian nervous system [2], and depending on their subunit composition, receptors exhibit distinct pharmacological and electrophysiological properties [1,3]. Individual subunits exhibit a distinct but overlapping and often widespread distribution throughout the nervous system [4] resulting in a large variety of GABA\textsubscript{A} receptor subtypes in the brain. Immunoprecipitation studies indicated that \(\alpha\) and \(\beta\) subunits can assemble with all the other GABA\textsubscript{A} receptor subunits investigated, but that \(\gamma\) and \(\delta\) subunits seem not to co-exist in the same receptor subtype [5]. The possible existence of receptors containing two different \(\alpha\) and/or two different \(\beta\) subunits as well as a \(\gamma\), \(\delta\), \(\epsilon\), or \(\pi\) subunit or receptors containing \(\theta\) or \(\epsilon\) subunits in unusual combinations with other subunits contributes to the extreme heterogeneity of GABA\textsubscript{A} receptors [5–9].

This heterogeneity was supported by recombinant receptor studies. After transfection of MDCK or HEK cells with single subunits, only \(\beta\) subunits and less efficiently \(\beta\) subunits could be detected on the cell surface to a significant extent [10,11]. In addition, a robust expression of GABA-activated homo-oligomeric chloride channels was observed with \(\rho\) subunits and possibly \(\beta\) and \(\rho\) subunits [12,13] but different \(\rho\) subunits might also co-assemble to form hetero-oligomeric receptors. Channels composed of \(\alpha\) and \(\beta\) subunits formed more efficiently than homo-oligomeric channels [5] and co-expression of \(\alpha\), \(\beta\), and \(\gamma\) subunits resulted in a robust formation of
GABA-gated chloride channels and properties resembling those of the majority of native receptors [1]. As suspected from the co-precipitation data of subunits from the brain, these receptors seem to be composed of two α, two β and one γ subunit [5,14] where alternating α and β subunits are connected by the γ subunit. Concatenated GABA<sub>A</sub> receptor subunits [15] as well as homology modeling studies [16] allowed to distinguish between two possible mirror image arrangements of these subunits and to identify the absolute subunit arrangement of αβγ receptors (Figure 1). The occurrence of a defined subunit stoichiometry and arrangement in αβγ receptors strongly indicates that assembly of GABA<sub>A</sub> receptors proceeds via defined pathways.

Recombinant receptor studies also indicated that receptors containing two different α or β subunits are able to assemble and to exhibit properties different from receptors that contain only a single α or β subunit type. However, on heterologous co-expression of more than three subunits probably a variety of different receptors with one, two, three, four, and if present, even five different subunits are assembled and complicate interpretation of data [5]. Only a few studies are available on recombinant receptors containing ε, π, or θ subunits and assembly of these receptors so far was not investigated.

**Receptor assembly**

Receptor assembly occurs in the endoplasmic reticulum and is supported by chaperones

The assembly of hetero-oligomeric receptors of the ligand-gated ion channel superfamily (Cys-loop receptors) comprising the nicotinic acetylcholine receptors, GABA<sub>A</sub> receptors, glycine receptors, and 5-hydroxytryptamine, type 3 receptors, is a complex, multi-step process [17]. The assembly of subunits seems to occur in the endoplasmic reticulum (ER) and to involve interaction with chaperone molecules [10,18]. For the assembly of GABA<sub>A</sub> receptors, the chaperones calnexin, immunoglobulin heavy-chain-binding protein (BiP), and protein disulphide isomerase seem to be required. Calnexin recognizes immature glycans (mono-glucosylated proteins), BiP recognizes exposed hydrophobic regions and protein disulphide isomerase catalyses the formation of disulphide bridges between appropriate cysteine residues. Together, these chaperone proteins monitor the fidelity of protein folding and assembly. Unassembled GABA<sub>A</sub> receptor subunits are bound efficiently to BiP and calnexin and are retained in the ER (by virtue of luminal KDEL ER retention signals within the chaperone proteins) until degraded [19]. On the other side of the membrane, the masking of ER retention signals during assembly ensures the cytoplasmic fidelity and transport competence of receptors. Correctly assembled receptors are released and traffic to the cell surface [20].

**Amino acid residues important for assembly**

Based on the differential ability of subunits to interact with each other, a variety of studies have been performed to identify amino acid sequences or residues important for assembly. Two different approaches were applied. A ‘positive approach’ aimed to enhance interaction of subunits or subunit fragments by replacing sequences of assembly-incompetent with homologous sequences of assembly-competent subunits (‘knock-in’). If the chimera could then be co-precipitated with the other subunit, it could be assumed that the incorporated sequence was important for assembly. In a ‘negative approach’, a ‘knock-out’ strategy aimed to reduce interaction of subunits by replacing amino acid sequences or residues of assembly-competent with homologous sequences of assembly-incompetent subunits. With this strategy, loss of binding of a truncated, chimeric or a mutated subunit to another subunit suggested that the sequence or residue was important for assembly.

Subunits or subunit fragments that can initially interact with another subunit but are unable to
participate in the formation of completely assembled receptors can also be identified by their interfering with receptor assembly [21]. Their interaction with assembly competent subunits generates non-functional assembly intermediates that usually are degraded. This result in a deprivation of subunits needed for assembly and thus, in a reduced receptor expression at the cell surface. The absence of an effect, however, does not necessarily imply that a construct does not contain assembly signals. The mutant subunit may not be folded, processed or trafficked in a way that permits interaction with the target protein.

Experimental evidence as well as information from structural models of GABA_A receptors [16] generated using the acetylcholine binding protein (AChBP) [22] as a template indicate, however, that GABA_A receptor subunits contain more than one N-terminal sequence making direct contacts with the same neighbouring subunit [23]. This adds an additional complication for experimental studies. Whereas the very N-terminally located sequences important for assembly can be easily studied by investigating assembly between N-terminal fragments and full length subunits, using a positive as well as a negative approach, this no longer is possible for the identification of further putative binding sequences located more C-terminally. If the shorter N-terminal fragment already contains a sequence important for assembly, it will assemble with the other subunit whether or not an additional sequence important for assembly is present or incorporated in the longer N-terminal fragments. In such a case only knockout approaches are possible that usually result in weak effects due to the presence of additional binding sites.

It has to be kept in mind, however, that on identifying residues as being important for assembly, no information is gained on their function. Such residues might be involved in direct protein-protein interactions, but they might also have stabilized regions elsewhere in the protein that then could have interacted with the adjacent subunit. And replacement of these residues by homologous residues of assembly incompetent subunits might have caused a disturbance in protein packing or subunit folding by incorporating the residues into a new environment, leading to artefacts and wrong conclusions. Similarly, when interactions between truncated and full length subunits are investigated, contact regions within the truncated fragments could be exposed that normally would be buried and thus not be available for interaction in full-length subunits. Overall, however, the methods used are complementary to each other and applied together provide good information on residues important for assembly. Following this discussion, the term ‘assembly signals’ seems inappropriate: the presence of these residues just only directly or indirectly allows a contact or confers the necessary flexibility to the protein for direct contact between subunits.

To identify single amino acid residues that are directly involved in protein-protein interactions, residues forming direct contacts with each other in homology models of GABA_A receptors were replaced by cysteines in the hope that they might form spontaneous disulfide bridges between subunits that then could be identified by Western blot analysis under suitable conditions. Absence of cross-link between subunits, however, does not exclude a direct interaction between residues because formation of a disulfide bond between cysteines requires an appropriate distance and angle between interacting —SH groups that not necessarily can be achieved even if the two residues are sitting close together. In addition, it has to be kept in mind that contact sites between subunits in the fully assembled receptors not necessarily are identical with the sites making primary contacts between subunits, because such primary contact sites might become abandoned after subsequent conformational changes of the proteins needed for further assembly of subunits.

Residues important for homo-oligomeric assembly of GABA_A receptors. As mentioned above, only ρ1 or β3 subunits seem to be able to robustly form homo-oligomeric receptors [11,12]. When co-expressed in Xenopus oocytes, the N-terminus of ρ1 interfered with ρ1 but also with with p2 receptor formation indicating that the N-terminal regions of p subunits contain the initial signals for both homo-oligomeric and hetero-oligomeric assembly [24]. Using a series of GABA_A receptor β1 subunit chimeras containing different regions of the p1 N-terminus for interference with ρ1 and p2 subunit assembly into functional GABA_A receptors, it was demonstrated that transfer of 70 residues of ρ1 (p1(137–206)) to the β1 subunit created a chimera that disrupted ρ1 but not p2 assembly into functional receptors. These observations suggest that different sequences are important for the formation of ρ1 homo- and ρ1/p2 hetero-oligomeric assembly [13].

Interestingly, the chimera β1p1(137–206) did not confer the same level of dominant-negative effect on ρ1 subunit assembly as the chimera β1p1(137–264). In addition, chimera β1p1(137–182) containing the first half, or chimera β1p1(183–206) containing the second half of the 70 amino acid region were less effective at reducing p1 currents than the chimera β1p1(137–206) with the entire 70 amino acid region. This could be interpreted by the assumption
that several N-terminal ρ1 sequences are important for assembly.

The presence of different domains ('assembly boxes') important for homo- and hetero-oligomeric assembly has also been demonstrated for glycine receptors [25]. However, only a few sequences important for glycine receptor assembly are located within the 70 amino acid region identified as important for the homo-oligomeric assembly of ρ1. In addition, two amino acid residues of the nACh receptor ε subunit, ε106 and ε115 important for assembly with the nACh receptor α subunit are located within the ρ1 sequence of the chimera β1p(137–182) [26].

Some of the β3 subunit residues important for homo-oligomeric assembly have been identified by exploiting the differing capabilities of the β2 and β3 subunits to form functional homomeric receptors at the cell surface. Substitution of four homologous residues within the β2 subunit by β3 residues (β2D171G, β2N173K, β2T179E, β2K180R; β2GKER) in a region homologous to contact forming residues located at the (-) side of the AChBP, was sufficient to enable homo-oligomerization of β2 subunits in HEK or COS cells. The reciprocal mutation (β2 residues to replace those of β3; β3DNTK) abolished the ability of β3 to assemble with itself. These residues, however, were not critical for the formation of hetero-oligomeric receptors composed of α1β2 or α1β3 subunits [27].

In another study it was demonstrated that by replacing amino acid residues 76–89 in β3 subunits by the homologous α1 residues the formation of α1β3 receptors was drastically reduced [28]. This β3 sequence, however, was not important for the formation of homo-oligomeric β3 receptors (Sarto-Jackson and Sieghart, unpublished work). So homo-oligomeric assembly possibly might be guided by sequences promoting homo-oligomeric and others preventing hetero-oligomeric assembly.

**Residues important for hetero-oligomeric assembly of GABA<sub>A</sub> receptors.** Sequences important for a selective assembly of different subunits are responsible for the formation of a distinct subunit stoichiometry and arrangement within the receptors. They might form direct contact sites or ensure an adequate subunit structure. Although homologous sequences might be important for selective assembly in different subunit types the individual contact forming residues are

<table>
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<tr>
<th>Residues important for assembly</th>
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<th>(+)/(-) side experimental evidence</th>
<th>Reference</th>
<th>Homologous residues important for assembly</th>
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Table I. Sequences important for GABA<sub>A</sub> receptor assembly.
most likely different (i.e., not conserved – [22]) and are possibly also not located at homologous positions in different subunits (Table I).

**Residues important for the selective assembly of the α–β interface.** The discovery of an α6 splice variant (α6S) lacking 10 amino acids within the N-terminal extracellular domain, which was unable to form ligand-binding sites upon co-expression with β2 and γ2 subunits [29], prompted investigation of its assembly properties. It was demonstrated that only the α6L splice variant containing the 10 amino acids 58–67 was able to form α6β2 or α6β3 surface receptors, whereas the α6S subunit was retained within the ER. Interestingly, this sequence located at the α (−) side, is highly conserved in all GABA<sub>A</sub> receptor subunits and might thus be essential also for the assembly of other subunits. Consistent with this possibility, immunoprecipitation experiments using either the short version of α1 (with deleted residues 58–67), or a version in which these α1 residues were replaced by the corresponding residues of p1, revealed that these residues are also important for oligomerization of α1 with β3 subunits probably forming the α1(−)β3(+) interface [30]. Not surprisingly, however, they were without effect on the production of αγ complexes that probably form a contact between the α1(+) and γ2(−) side as in intact GABA<sub>A</sub> receptors.

The importance of amino acid residues within the region α1(54–69) for assembly with β subunits was independently confirmed by several other studies [30–33]. Within this sequence, α1Q67 [30] and α1W69 [31] were conserved widely in the superfAMILY. These residues, thus, might rather have a structural role in stabilizing an adequate conformation of the subunit than representing a direct contact site.

The sequence β3(52–66), that is homologous to the above mentioned sequence α1(54–68), was demonstrated to be important for assembly with α1 subunits [32]. In addition, the sequence β3(76–89), and here especially β3(85–89) seemed also to be important for assembly with α1 subunits [28]. Interestingly, residues β3N85 and β3V87 are homologous to residues S75/P77 in the AChBP that are involved in direct inter-subunit contacts. This region might thus represent a direct assembly site in GABA<sub>A</sub> receptor subunits.

**Residues important for the selective assembly of the α–γ interface.** In other studies it was demonstrated that the construct γ2(1–234) consisting of the complete extracellular N-terminal domain was able to reduce the expression of α1β3γ2 receptors on the cell surface by 50% [21]. A subsequent reduction in the size of the truncated subunit indicated that fragments γ2(1–130), γ2(1–117), and γ2(1–113) also were able to interfere with assembly of these receptors, but to an increasingly smaller extent. The observation that the longest N-terminal fragment exhibited the strongest interference with GABA<sub>A</sub> receptor assembly, can be explained by the possibility that longer fragments are more able to stabilize their binding sites for α1 or β3 subunits. Alternatively, additional contact sites for these subunits might be located between γ2(113–234).

γ2(1–113) was the smallest N-terminal fragment that could reduce the expression of α1β3γ2 receptors on the cell surface [21]. This N-terminal γ2 subunit fragment was able to assemble with α1 subunits by forming a high affinity [3H]flunitrazepam binding site indicating that the respective assembly site must be located at the γ2(−) side. By incorporating amino acid sequences from this γ2 fragment into a homologous α1(1–100) fragment that could not bind to α1 subunits, the sequence γ2(91–104) was identified to be important for the assembly of GABA<sub>A</sub> receptor γ2 with α1 subunits [21].

Using a similar approach, the sequence γ2(67–81) [32] and the homologous sequence γ3(70–84) [23], here especially the sequence γ3(70–78) were also found to induce assembly with α1 subunits. The importance of these sequences was verified by demonstrating that chimeric subunits in which the respective γ2 or γ3 sequences were substituted by the homologous p1 sequence drastically reduced the formation of receptors containing γ2 or γ3 subunits at the cell surface [23,32].

Finally, the putative contact site of the sequence γ2(91–104) was identified on the neighbouring α1 subunit (α1(80–100)) by incorporating various α1 sequences into the N-terminal fragment β3(1–115) that was unable to assemble with γ2 subunits. So far, these are the only sequences identified that might form direct contacts with each other [34]. Single residues that actually make the contacts have not been identified yet, but the residues γ2S100 and γ2M102 (as well as their counterparts α1N87 and α1M89), are homologous to residues S75/P77 in the AChBP that actually contribute to direct interaction of a given monomer. In addition, residue α1D97 is homologous to residue D85 in the AChBP that forms direct contacts with the neighbouring subunit and, as expected, is located at the (+) side of the respective monomer.

**Residues important for the selective assembly of the β–γ interface.** Using comparative models of the extracellular domains of GABA<sub>A</sub> receptor subunits [16] several residues were predicted to be exposed for inter-subunit contacts. These residues were individually substituted by cysteines and a possible
formation of receptors at the cell surface by interfering with the formation of \( \gamma 2 \beta 3 \), but not of \( \gamma 2 \alpha 1 \) intermediates. Thus, amino acid residues \( \gamma 2 T125 \) and \( \gamma 2 P127 \) seem to be important for assembly with \( \beta 3 \) subunits. If these residues form direct and final contacts between subunits, they should thus be located at the \( \gamma 2(+) \) side [35]. The homologous residues of the AChBP, however, are located at the \( (--) \) side. Since in this particular region, the \( (+) \) and the \( (--) \) side of the protein are only separated by 3 amino acid residues, it is possible that a slightly different protein fold shifts the position of these residues. Alternatively, the residues investigated here are structurally important or represent only initial contact sites that are no longer present in the completely assembled pentameric receptor.

Finally, the point mutation \( \gamma 2 R43Q \) recently demonstrated to be associated with epilepsy [36] is located at the \( \gamma 2(+) \) side and has been reported to prevent binding to \( \beta 2 \) subunits, suggesting a failure of \( \beta 2 - \gamma 2 \) interface formation during receptor assembly [37]. Similar results were observed when the homologous mutation was present in either the \( \alpha 1 \) or \( \beta 2 \) subunits [37]. Given the complete conservation of the residue R43 in all members of the cys-loop superfamily, this implies a more structural role for this amino acid residue in receptor assembly.

Residues important for non-selective assembly. The sequences \( \gamma 2(83-90) \) [21] or \( \gamma 3(70-84) \) [23] identified in a ‘knock-in’ approach in assembly incompetent truncated subunits induced association with full-length \( \alpha 1 \) as well as \( \beta 3 \) subunits. These sequences thus might either have stabilized or exposed the binding sites to \( \alpha 1 \) and \( \beta 3 \) subunits or might be important for association of subunit intermediates at an early stage of assembly, where discrimination between neighbouring subunits might not be necessary.

Mechanism of \( GABA_A \) receptor assembly

Homo- and hetero-oligomeric assembly intermediates could represent different starting points of assembly. The occurrence of assembly intermediates was investigated in the brain of adult and young rats by sucrose density gradient centrifugation [38]. Whereas in the brain of adult rats \( GABA_A \) receptor \( \alpha 1, \beta 3, \) and \( \gamma 2 \) subunits sedimented as a single peak corresponding to completely assembled pentameric receptors, in the brain of young rats the sedimentation pattern was much more heterogeneous. Thus, \( \alpha 1, \beta 3, \) and \( \gamma 2 \) subunits could be identified in the subunit dimer, trimer, tetramer and pentamer peaks. These results indicate that different hetero- and/or homo-dimers are formed in the brain.

When HEK cells transfected with single \( GABA_A \) receptor subunits were investigated by sucrose gradient centrifugation, it was demonstrated that only a small amount of subunit monomers, and unexpectedly, higher oligomeric complexes were present within the cell. Thus \( \alpha 1 \) subunits formed homo-di-, tri- and tetramers, \( \beta 3 \) subunits formed all different homo-oligomers up to pentamers, while \( \gamma 2 \) subunits formed homo-di- and trimers (Ehya and Sieghart, unpublished work). This indicates that in each of these subunits contact sites for homooligomerization are present. The failure to form homo-pentameric \( \alpha 1 \) or \( \gamma 2 \) receptors then might indicate that on homo-oligomerization possible conformational changes of subunits necessary for accommodating five identical subunits into completely assembled receptors are not induced. In addition, the fact that in fully assembled hetero-pentameric \( \alpha 3 \gamma \) receptors no direct contacts between two \( \alpha 2, \) two \( \beta 2, \) or two \( \gamma 2 \) subunits do occur, suggests that homo-oligomeric interaction might either be used as a default mechanism in the absence of other subunits, or the sites for homo-oligomeric interaction only serve as primary contact sites for initial stabilization of subunits until another more competent assembly partner arrives.

When \( \alpha 1 \) and \( \gamma 2 \) subunits, or \( \beta 3 \) and \( \gamma 2 \) subunits were transfected together, in both cases the majority of assembly intermediates are found in the dimer peak [14], thus reducing the heterogeneity of assembly intermediates. These data also indicated that once formed, the hetero-dimers cannot form higher oligomers in the absence of the missing third subunit. When \( \alpha 1 \) and \( \beta 3 \) subunits were transfected together, predominantly subunit tetramers and pentamers were formed, whereas co-transfection of \( \alpha 1, \beta 3 \) and \( \gamma 2 \) subunits resulted in a single peak of pentameric receptors. These results supported previous conclusions [10] that only \( \alpha 1 \beta 3 \gamma 2 \) and \( \alpha 1 \beta 3 \), but not \( \alpha 1 \gamma 2 \) or \( \beta 3 \gamma 2 \) subunits, give rise to completely assembled pentameric \( GABA_A \) receptors that then are transported to the cell surface. The formation of \( \alpha 1 \beta 3, \alpha 1 \gamma 2, \) and \( \beta 3 \gamma 2 \) dimers on heterologous co-expression might indicate that each one of these dimers could become the starting point of receptor assembly. The formation of tetrameric and pentameric receptors in cells transfected with \( \alpha 1 \beta 3 \) subunits, only, and the exclusive formation of
pentameric receptors in cells transfected with α1, β3, and γ2 subunits, seem to indicate that the speed of incorporation of a γ2 subunit into tetrameric α1β3 assembly intermediates is faster than that of a β3 or α1 subunit. These data do not necessarily indicate that the γ2 subunit is the last subunit incorporated into GABA<sub>A</sub> receptor intermediates during assembly, but they suggest a hierarchical assembly mechanism, in which some preferred subunit combinations dominate receptor assembly.

Spontaneous cross-link of mutated α1 subunits provides hints on a possible mechanism of GABA<sub>A</sub> receptor assembly. Using comparative models of GABA<sub>A</sub> receptors for predicting amino acid residues in pentameric receptors in cells transfected with 308 I. Sarto-Jackson & W. Sieghart, mRNA and thus, probably have a higher chance to interact with each other than with β3 or γ2 subunits that might form direct contacts with each other [16] it was demonstrated that on cotransfection of α1H109C with wild-type β3 and γ2 subunits, mutated α1H109C subunits spontaneously cross-linked with each other forming subunit dimers that were then transported to the cell surface [39]. In addition, completely assembled and functional GABA<sub>A</sub> receptors composed of α1H109C and wild-type β3 and γ2 subunits were observed at the cell surface, exhibiting wild-type stoichiometry and subunit arrangement and containing two opposing α1H109C subunits cross-linked by a disulfide bond bridging the channel mouth. The segment around residue α1H109 protrudes furthest toward the channel lumen and in contrast to the sequence of the AChBP, GABA<sub>A</sub> receptor subunits contain an insertion of three amino acid residues in this region that are most likely also located luminally. The position of α1H109 might be relatively flexible resulting in a fairly mild distortion of GABA<sub>A</sub> receptors containing cross-linked α1 subunits.

The formation of completely assembled receptors containing cross-linked α1H109C subunits most directly can be explained by an interaction of two α1 subunits being at least one starting point of wild-type receptor formation. This possibility is supported by the fact that the α1 subunits are synthesized side by side by different ribosomes on the same mRNA and thus, probably have a higher chance to interact with each other than with β3 or γ2 subunits that are synthesized via a different mRNA-ribosome complex. In addition, α1H109C homo-dimer formation and cross-link seemed to occur more rapidly than the assembly between α1H109C and β3 or γ2 subunits as indicated by the large surplus of cross-linked α1H109C dimers over completely assembled receptors at the cell surface. Dimerization of α1 subunits via their respective (+) and (−) sides would have caused a dead end of assembly. But interaction of α1 subunits via the side forming the channel mouth might have been weakly stabilized, by the interaction of the two sterically exposed α1H109 residues, thus enhancing the probability of interaction with a β3 subunit that can approach from the one or the other side (Figure 2A).

Formation of two correct α/β interfaces via the (+) or (−) sides of the two α1 subunits then probably causes opening of the weak luminal interactions between the two α1 subunits, thus allowing the fourth and fifth subunits to enter and complete GABA<sub>A</sub> receptor assembly. To finally result in receptors with the correct subunit stoichiometry and arrangement, it is mandatory, however, that subunits assembling with the trimer preferentially bind either to the accessible α1(+)/γ2 subunits or the α1(−)/β3 subunits. Overall, such a mechanism is consistent with the finding that even in the presence of a covalent cross-link of two α1H109C subunits completely assembled receptors with a wild-type stoichiometry and arrangement are formed presumably before the cross-linked dimers could be transported to the cell surface. Covalent cross-linking with subsequent transport to the cell surface could thus have trapped a labile assembly intermediate that could not have been detected without the use of mutated subunits [39]. Alternatively, the assembly of GABA<sub>A</sub> receptors could have started with the formation of a hetero-dimer, either by a direct interaction of α1 and β3 (or γ2 subunits via their respective (+) and (−) sides, or again via an initial contact with a subsequent rearrangement forming the final (+)/(−) interaction. As soon as the correct (+)/(−) interface has been established in the dimer, each additional subunit then has to assemble in the correct order.

Figure 2. Schematic drawing of possible mechanisms of GABA<sub>A</sub> receptor assembly. The mutated and sterically exposed residues α1H109C are indicated by a black line at the luminal side of the subunit. + and − indicate the principal and complementary side of a subunit, respectively. (A) In one mechanism the two α1H109C subunits interact via their luminal side and offer the possibility that β3 subunits encountering the dimer from the one or the other side could assemble by forming two correct interfaces with two different α1 subunits. (B) In the second mechanism GABA<sub>A</sub> receptor assembly starts with the formation of a hetero-dimer of α1 and β3 (or γ2) subunits via their respective (+) and (−) sides. As soon as the correct (+)/(−) interface has been established, each additional subunit then has to assemble in the correct order. Taken from [39] with permission.
(Figure 2B) to give rise to a receptor with correct subunit stoichiometry and arrangement. In this case, cross-linking of the non-neighboring α1H109C subunits could have occurred at the hetero-trimeric assembly stage via luminal contacts of the two α1H109C subunits before correct formation of all interfaces of the α1-β3-α1 trimer. If assembly of GABAA receptors started with the formation of hetero-dimers, then the simultaneous formation of a large surplus of cross-linked α1H109C homo-dimers represented an independent and competitive event that reduced the amounts of α1 subunits available for receptor assembly. Cross-linked homo-dimers could then have been a dead end of assembly or could have led to completely assembled receptors containing cross-linked α1H109C subunits before the homo-dimers were transported to the cell surface. The described sequential assembly mechanism is similar to that proposed for the nACh receptor [18]. For the nACh receptor an additional assembly mechanism has been discussed in which two subunit dimers could combine with a fifth subunit to form the completely assembled pentameric receptor [40]. Further experiments will have to decide between all these possibilities.

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References


