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Rotamers Matter for Ion Conductance

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Abstract

A major determinant of the ion flux rate through acetylcholine receptors is a ring of five residues, four glutamates and a glutamine, at the channel's cytoplasmic mouth. The glutamates adopt alternate rotamer conformations so that only two directly affect channel conductance.

The single channel conductance, a measure of the ease of ion translocation through a channel, is an important characteristic of an ion channel. The conductance is largely determined by electrostatic and steric interactions of ions with the residues lining the ion translocation pathway. This includes residues lining the transmembrane channel and those lining the vestibules at the extracellular and cytoplasmic ends of the channel. Nicotinic acetylcholine receptors (AChR), members of the Cys loop receptor superfamily of neurotransmitter-gated channels, are formed by the pseudo-symmetrical assembly of five homologous or identical subunits around the central axis (Fig. 1A and B)^{1, 2}. The subunits have similar transmembrane topologies with an ~200 amino acid extracellular N-terminal domain, four membrane-spanning segments (M1, M2, M3, M4) and a large intracellular loop connecting M3 and M4 (Fig. 1B)^{1, 3–5}. The transmembrane channel is lined by rings of aligned residues, one from each subunit's M2 segment (Fig. 1C and D)⁶. In the cation-selective AChR, previous work identified three rings of mostly negatively charged residues in and flanking M2, named the extracellular, intermediate and cytoplasmic rings⁷. Altering the number of charged residues in the intermediate ring had the greatest impact on conductance and raised questions about the ionization state of the four intermediate ring glutamates⁷. In an elegant set of experiments using mutagenesis and single channel patch clamp recordings, Cymes and Grosman demonstrate that all four glutamates are ionized but that only two appear to face into the channel lumen and have a significant effect on channel conductance⁸. They speculate that the side chains of the other two adopt an alternate rotamer conformation pointing away from the channel axis and have minimal electrostatic interactions with permeating ions.

Using voltage clamped patch recordings, the current amplitude through individual channels was measured at several voltages and the single channel conductance for wild type AChRs was calculated to be ~140 pS. Mutating all intermediate ring residues to alanine reduced the conductance to 30 pS⁸. The conductance increased in ~50 pS steps as the number of intermediate ring glutamates increased. With one glutamate and four alanines the conductance was ~80 pS, with two glutamates it was 140 pS. Surprisingly, with two, three or four glutamates the conductance remained at 140 pS, similar to wild type. However, with five glutamates the conductance rose to 185 pS. Cymes and Grosman inferred that the ~50

pS steps arose due to increased numbers of ionized glutamates facing into the channel; 30 pS (0 glutamates), 80 pS (1 Glu), 140 pS (2 Glu), 185 pS (3 Glu). This raised the question, with three, four and five glutamates in the ring, are all of the glutamates ionized?

Wild type AChR has a single conductance state. However, a mutant with two glutamates and at least one glutamine had an ~80 pS subconductance state as well as the main current level that provided insight into the glutamates' ionization state. The subconductance state could be due to protonation of one of the glutamates that would reduce the negative potential at the channel mouth. Strikingly, neither symmetrical changes in bath and pipette pH between 6.0 and 8.5 nor replacement of H₂O with D₂O altered the rate of entry into or exit from the subconductance state. This implies that the subconductance state was not due to protonation of an intermediate ring glutamate. The authors inferred that the subconductance state arose due to changes in side chain conformation relative to the pore, i.e., moving the negatively charged glutamate into and out of the channel. They hypothesize that in the wild type channel two of the four glutamates must adopt a rotamer conformation that orients the negatively charged carboxylates towards the central channel axis where their electrostatic potential could facilitate cation permeation (Fig. 1C and D). In contrast, the other two glutamates, although ionized and negatively charged, must face away from the channel axis so that they do not have significant electrostatic interactions with permeating cations. Tight packing of the cytoplasmic ends of M2⁹ must prevent interconversion of the rotamer orientations.

X-ray crystal structures exist for two homopentameric AChR homologues, the cation-selective GLIC channel^{3, 4} and the anion-selective GluCl channel⁵. Neither of these channels has a glutamate at the position aligned with the intermediate ring. In both cases the intermediate ring residue faces towards the adjacent subunit M2 segment and not directly towards either the channel lumen or the cytoplasmic vestibule (Fig. 1C). In GLIC, the residue adjacent to the intermediate ring is a glutamate. Two groups have crystallized GLIC^{3, 4}. In one structure all five glutamates face the channel axis⁴. In contrast, in the other they all face the cytoplasmic vestibule³. Cymes and Grosman suggest that the AChR intermediate ring glutamates may likewise adopt different two distinct rotamer conformations with the β and δ glutamates facing the channel lumen and the two α glutamates facing away from the lumen (Fig. 1C and D). Finally, due to the heteromeric subunit composition of the AChR, the structure of each subunit must be slightly different in this region. With only two glutamates in the intermediate ring, the functional effects of those glutamates depended on the specific subunits in which they were located⁸.

The extent to which these results can be generalized to other Cys-loop receptor superfamily members is uncertain. In the homologous, cation-selective, serotonin 5-HT_{3A} receptor, M3-M4 loop residues that line the portals into the cytoplasmic vestibule are the main determinants of channel conductance¹⁰. In the anion selective Cys-loop superfamily members, alanine is almost always present in the intermediate ring. This will have no electrostatic effect on anion permeation or channel conductance. One final observation, x-ray crystal structures of ion channels have significantly advanced our understanding of the structural basis of the functional properties of ion channels. However, the functional state of the crystallized protein is uncertain, limiting their utility. Thus, detailed molecular functional

studies, such as those in the current paper⁸, will be essential to achieve a comprehensive understanding of the structural basis of ion channel function.

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Figure.

Cys-loop receptor channel structure illustrated using the GluCl channel structure (PDB: 3RIF)⁵. (A) Cartoon representation of the GluCl channel subunit secondary structure. The channel lumen would be on the left. The M2 membrane-spanning segment is in dark orange. The position of the intermediate ring residue is indicated in gray near the cytoplasmic end of the M2 segment. (B) View from the cytoplasmic channel mouth up the channel. Channel lumen is indicated by the pale blue circle in the center. Muscle-type AChRs contain four subunits, two α (light orange) and one each of β (green), δ (blue) and ϵ (cyan). The channel is lined by the five M2 segments. The position of the other membrane-spanning segments is indicated on one of the α subunits. (C) Close up view of the cytoplasmic end of the channel surrounded by the five M2 segments. The intermediate ring residues in the AChR have been inserted into the GluCl structure in stick representation using Pymol software: α , β and δ have a glutamate and ϵ has a glutamine. For illustrative purposes the glutamate rotamer conformation in β and δ faces the carboxylates towards the channel, whereas in the α subunits the carboxylates face away from the channel lumen. (D) Side view of the cytoplasmic end of the channel. The front two subunits, α and ϵ , have been removed to visualize the channel. Intermediate ring side chains are shown in stick representation. Figure generated using Pymol. Subunit color scheme is maintained throughout the figure.