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Review

3D structure and allosteric modulation of the transmembrane domain of pentameric ligand-gated ion channels

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ABSTRACT

Pentameric ligand-gated ion channels mediate rapid chemo-electric signal transduction in animals. The active site of this family of proteins is their ion channel pore, which is located at the center of the transmembrane domain. The opening/closing motions of the channel pore are governed by the binding of neurotransmitter to the extracellular domain, but also by allosteric effectors acting within the transmembrane domain. Here, we review the structure of the transmembrane domain as well as its role in the allosteric modulation of pentameric ligand-gated ion channel function. We focus on two examples: the interactions of nicotinic ACh receptors with lipids, for which a novel "uncoupled" state has been proposed, and the interactions of $GABA_A$ and Glycine receptors with allosteric modulators, such as general anesthetics, ethanol and neurosteroids. We revisit these data in light of the recently solved X-ray structures of bacterial members of the family, which provide atomic-resolution insight into the structures of both the transmembrane domain and associated lipids.

1. Introduction

Pentameric ligand-gated ion channels (pLGICs) are transmembrane protein complexes that mediate chemical communication between cells in both the central and peripheral nervous systems (Taly et al., 2009; Miller and Smart, 2010). These transmembrane receptors are grouped into distinct families, named according to neurotransmitter pharmacology. In vertebrates, nicotinic acetylcholine (ACh), serotonin ($5HT_3R$), and zinc (ZAC) receptors are linked to cation-selective channels, while γ-aminobutyric acid ($GABA_A$ and $GABA_A$-ρ) and Glycine receptors are linked to an anionic chloride ion-selective channel. In invertebrates, γ-aminobutyric acid, serotonin, glutamate, histamine and proton-gated channels have also been identified. More than 40 distinct pLGIC subunits exist in humans, including numerous subunit types within each of the neurotransmitter receptor families. Within a family, these subunits combine to form a variety of both homomeric and heteromeric complexes, each with different functional and pharmacological profiles.

The primary role of most pLGICs is to convert the binding of a neurotransmitter into the opening of a transmembrane ion channel leading either to intracellular excitation or inhibition, depending on the ionic selectivity of the channel. These two primary functions, ligand-binding and ion channel conductance, are located in distinct structural domains; an extracellular domain (ECD), which carries two to five neurotransmitter binding sites per receptor, and a transmembrane domain (TMD), which carries a single ion channel along the symmetry axis of the protein (Corringer et al., 2000; Smit et al., 2001; Brejc et al., 2001; Miyazawa et al., 2003; Unwin, 2005). X-ray structural data for homologs of the nicotinic ACh receptor ECD have been collected for nearly a decade (Kalamida et al., 2007; Rucktooa et al., 2009) thus providing atomic-level insight into the nature of pLGIC-ligand interactions. In contrast, X-ray structural data for the TMD has been lacking until recently, thus precluding atomic-level investigation of the structural features responsible for both channel conductance/gating and the mechanisms by which pLGICs interact with surrounding lipids, such as cholesterol, and other hydrophobic allosteric effectors, notably general anesthetics, alcohols, and neurosteroids. This short review presents our current knowledge of the structural architecture of the TMD taking into account recent

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advances derived from the discovery (Tasneem et al., 2005; Bocquet et al., 2007) and subsequent X-ray structure determination (Hilf and Dutzler, 2008, 2009; Bocquet et al., 2009) of bacterial members of the pLGICs super-family. In light of this new structural insight, we review and discuss the role of the TMD in the allosteric modulation of pLGICs, focusing on two examples; protein–lipid interactions at the nicotinic ACh receptor and anesthetic action at anion-selective pLGICs.

2. General structure of pentameric ligand-gated ion channels

To date, structural data have been collected for 1) the *Torpedo* nicotinic ACh receptor, which is easily purified in large quantities thus allowing detailed analysis using electron microscopy and chemical labeling approaches (Unwin, 2005), 2) the acetylcholine binding proteins (AChBPs), which are water-soluble pentamers homologous to the ECD of the nicotinic ACh receptor. Structures of AChBPs have been solved by X-ray crystallography in the presence and absence of a series of nicotinic agonists and antagonists (Brejc et al., 2001; Ulens et al., 2009; Rucktooa et al., 2009), 3) the monomeric ECD of the muscle-type α1 nicotinic ACh receptor subunit bound to α-bungarotoxin (Dellisanti et al., 2007), 4) the bacterial pLGICs from the proteobacteria *Erwinia chrysanthemi* (called ELIC, Hilf and Dutzler, 2008) and the cyanobacteria *Gloeobacter violaceus* (called GLIC, Bocquet et al., 2009; Hilf and Dutzler, 2009). The structures of the latter pLGICs were solved by X-ray crystallography at 3.3 and 2.9 Å resolution, respectively, and 5) the ECD of GLIC, which crystallizes in both pentameric and hexameric forms (Nury et al., 2010a).

The collected data establish a consistent picture for the structures of all pLGICs. In each case, the five subunits of each pentamer are organized symmetrically (homo-pentamers) or pseudo-symmetric (hetero-pentameters) around a central axis that functions as the ion channel (Fig. 1). Each subunit consists of an ~200 residue long N-terminal ECD with 10 β strands (β1-β10) folded into β-sandwich, as well as an ~100 residue long transmembrane domain (TMD) consisting of four transmembrane α-helices (M1 to M4) organized into a classic four α-helix bundle. The receptor's agonist-binding site and ion channel gate reside in the ECD and TMD, respectively, and are separated from each other by more than 60 Å. The extracellular and transmembrane domains are demarked by an abrupt change in secondary structure and meet at an interface located close to the bilayer surface. Contact between the two domains is mediated by the covalent link between the C-terminus of β10 in the ECD and the N-terminus of M1 in the TMD, as well as by non-covalent connections between the β1/β2 and β6/β7 loops (in eukaryotic pLGICs the latter is referred to as the Cys-loop) of the ECD and the M2-M3 loop of the TMD. An additional cytoplasmic domain is also present between transmembrane α-helices M3 and M4 in eukaryotic pLGICs and is characterized by a high variability in sequence and in length, with only one α-helix seen by electron microscopy (Unwin, 2005).

Extensive work has focused on understanding both the mechanisms of agonist-binding to the ECD and the mechanisms by which ligand-binding leads to the opening of the ion channel (i.e. channel gating). In terms of the gating mechanism, the muscle-type nicotinic ACh receptor has been the most extensively studied. Numerous artificial and natural mutations (the latter leading to congenital myasthenic syndromes), which alter the gating mechanism, have been identified (Lee et al., 2009; Purohit et al., 2007). These mutations are spread over the entire structure of the α-subunit, particularly at the interfaces between subunits, consistent with the idea that allosteric transitions result from global motions involving all five subunits within the protein. Recent studies highlight the importance of the β1–β2 and β6–β7 loops from the ECD and the M2–M3 linker from the TMD in coupling agonist-binding site to the channel gate (Lummis et al., 2005; Lee and Sine, 2005).

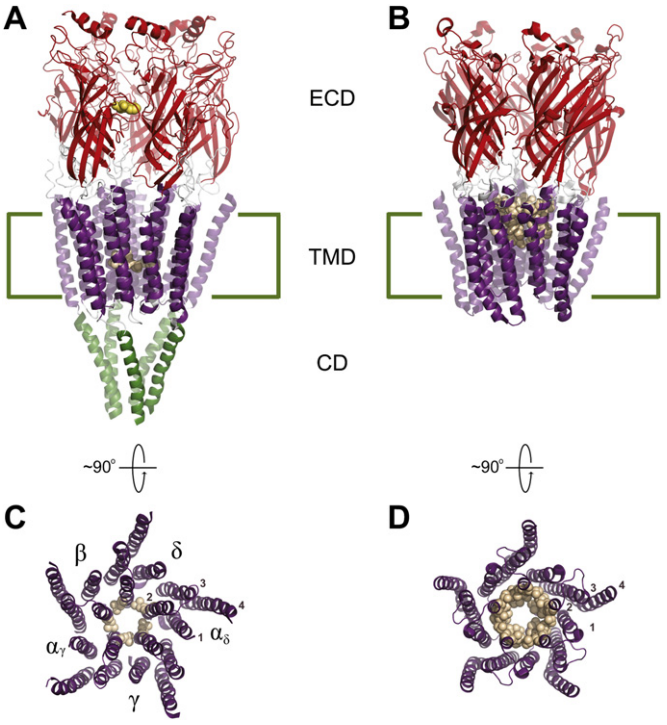

Fig. 1. 3D structure of pLGICs. Structures of A) the eukaryotic *Torpedo* nicotinic ACh receptor and B) the prokaryotic homolog, GLIC, highlighting the extracellular domain (ECD, red), the transmembrane domain (TMD, purple), and the cytoplasmic domain (CD, green). For the nicotinic ACh receptor, the agonist-binding site (αTrp-147) and the proposed channel gate (αLeu-251 and homologous residues in the β, γ, and δ subunits) are shown in orange and beige, respectively. For GLIC, residues forming the channel gate (Ile-233, Ile-236, Ala-237, Ile-240, Leu-241) are shown in beige. Views of the TMDs of the C) nicotinic ACh receptor and D) GLIC from the extracellular membrane surface highlight the M1 to M4 transmembrane α-helices in each subunit, as well as the heteromeric subunit organization of the nicotinic ACh receptor. (For the interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)

Strikingly, the systematic investigation of hundreds of muscle-type nicotinic ACh receptor mutants by patch-clamp single channel electrophysiology has demonstrated that nicotinic ACh receptor channel gating follows the Monod-Wyman-Changeux (MWC) allosteric mechanism (Purohit and Auerbach, 2010; Jha et al., 2009; Bertrand and Gopalakrishnan, 2007), in agreement with previous work on neuronal nicotinic ACh receptors (Changeux and Edelstein, 2005). The nicotinic ACh receptor spontaneously isomerizes between the basal (also called resting) and open states. Agonist-binding shifts the equilibrium between these conformations in favor of the open state, for which the ligand displays the highest affinity. In addition, prolonged agonist occupancy promotes receptor desensitization through isomerization toward one or several high affinity agonist-binding states that is(are) refractory to activation by agonist. In this context, understanding the functional architecture of pLGICs will require solution of a 3D structure for each allosteric conformation. A comprehensive mechanistic framework will also require insight into the mechanisms by which each ligand/allosteric modulator preferentially interacts with and thus preferentially stabilizes one conformation over another. The currently available structures, while each coming from a different species, offer three different conformations of the protein each endowed with a different degree of opening of the channel: ELIC is clearly closed while GLIC and nicotinic ACh receptor structure are