Review

Size matters in activation/inhibition of ligand-gated ion channels

Juan Du, Hao Dong and Huan-Xiang Zhou

Department of Physics and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA

Cys loop, glutamate, and P2X receptors are ligand-gated ion channels (LGICs) with 5, 4, and 3 protomers, respectively. There is now growing atomic level understanding of their gating mechanisms. Although each family is unique in the architecture of the ligand-binding pocket, the pathway for motions to propagate from ligand-binding domain to transmembrane domain, and the gating motions of the transmembrane domain, there are common features among the LGICs, which are the focus of the present review. In particular, agonists and competitive antagonists apparently induce opposite motions of the binding pocket. A simple way to control the motional direction is ligand size. Agonists, usually small, induce closure of the binding pocket, leading to opening of the channel pore, whereas antagonists, usually large, induce opening of the binding pocket, thereby stabilizing the closed pore. A cross-family comparison of the gating mechanisms of the LGICs, focusing in particular on the role played by ligand size, provides new insight on channel activation/inhibition and design of pharmacological compounds.

Common threads of LGICs

As with change in transmembrane voltage, ligand binding is a common stimulus for ion channels. The term LGICs often specifically refers to three families of ionotropic receptors: Cys loop receptors [in particular, nicotinic acetylcholine receptors (nAChRs)], ionotropic glutamate receptors (iGluRs), and P2X receptors (P2XRs). The functional units of these receptors are all oligomers comprising identical or homologous protomers, but the numbers of protomers differ (Figure 1) [1–3]. Cys loop receptors, iGluRs, and P2XRs have 5, 4, and 3 protomers, respectively. In each family, the minimum construct for channel function consists of an extracellular domain (to be referred to as the ligand-binding domain, or LBD) that harbors the ligand-binding sites, and a transmembrane domain (TMD) that contains the pore for ion permeation, with additional domains that play other functional roles such as regulation of channel activity and trafficking. The ligand-binding sites are located in the interprotomer interfaces for Cys loop receptors and P2XRs, but in the cleft between two lobes of each LBD protomer for iGluRs (Figure 1). With a significant amount of structural and mechanistic knowledge accumulated for each family of LGICs, it now seems appropriate to look for common lessons.

A common set of questions that define the gating mechanisms of all the LGICs is: What rearrangement of the binding sites does ligand binding induce? How are the motions of the LBD propagated to the TMD? And what are the motions of the pore-lining helices that are responsible for pore opening/closing? Given their distinct molecular architectures, the three families of LGICs are expected to have different solutions to these questions. However, as suggested recently [4], the different LGICs could have some common elements in their gating mechanisms. The gating mechanisms provide the basis for understanding ligand actions and designing pharmacological compounds. This review focuses on ligand size as a common factor in affecting ligand actions on the three families of LGICs.

Significant advance in the understanding of the gating mechanisms of Cys loop receptors and iGluRs was made by comparing the structures of the LBDs bound with various agonists and antagonists. For Cys loop receptors, this was made possible by the fact that the LBD is homologous to a water-soluble, homopentameric acetylcholine-binding protein (AChBP) [5]. For iGluRs, this was made possible by constructs in which the TMD is removed, and the open

Glossary

Mutant cycle analysis: a way to obtain information on the contribution of the interaction between two neighboring residues to channel gating, by studying the effects of mutating these residues. The perturbations of the two single mutations and the double mutation on the free energy difference between open and closed states of the channel (from measuring single channel dwell times) are obtained. A significant deviation of the effect of the double mutation from the sum of the effects of the two single mutations indicates the importance of the interaction between the two residues in channel gating.

Normal mode analysis: a modeling approach to obtain functionally important conformational changes of a protein. The motions of the protein are modeled as harmonic. By solving an eigenvalue problem, harmonic modes are obtained. Typically, some of the low frequency modes, which involve collective motions of a large fraction of the atoms, are assumed to be associated with biological function.

Rate equilibrium free energy relation: a way to assess whether the environment of a residue in the transition state for channel opening is more similar to that in the closed state or that in the open state. Mutations on the residue are made and their effects on the activation energy for channel opening and on the free energy difference between open and closed states are obtained. The former are then expressed as a fraction of the latter. A fraction close to 0 indicates that the environment of the residue in the transition state is similar to that in the closed state; a fraction close to 1 indicates that the environment of the residue in the transition state is similar to that in the open state.

Substituted cysteine accessibility method: a given residue in a channel protein is mutated into cysteine and a cysteine modifying agent is then applied in the absence and presence of an agonist. The change in modification rates is interpreted as indicating whether the residue becomes more or less accessible upon channel activation.

Targeted molecular dynamics simulation: a part of a protein is forced to move in a given direction, and the goal is to see how the rest of the protein responds in the simulation. In particular, a part of the ligand-binding domain may be moved to mimic the motion induced by an agonist, and the response of the transmembrane domain may suggest a gating mechanism.

Corresponding author: Zhou, H-X. (hzhou4@fsu.edu).

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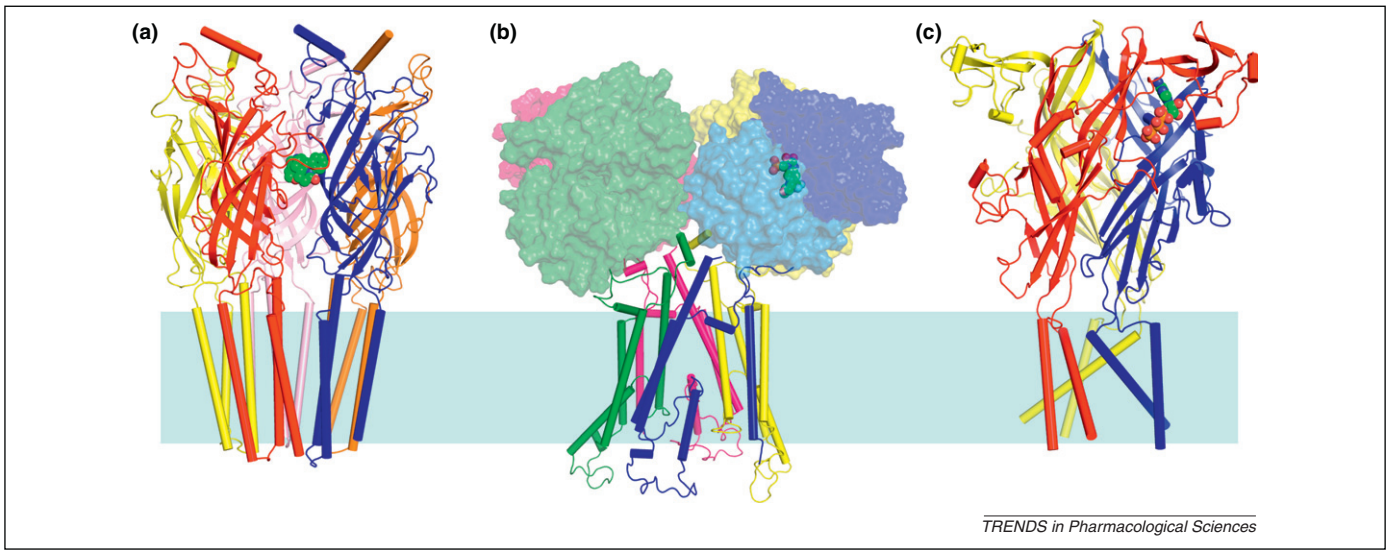

Figure 1. Structures of three families of ligand-gated ion channels. The protomers in each receptor are shown in different colors; ligands are shown as spheres. **(a)** Torpedo acetylcholine receptor in the apo form [Protein Data Bank (PDB) entry 2BG9] [1]. The M3–M4 linker and M4 are not shown. Strychnine, an antagonist, is built into the binding site by superimposing against PDB entry 2XYS [9]. (**b**) GluR2 α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid (AMPA) receptor bound with antagonist ZK 200775 (PDB entry 3KG2) [2]. In chain A (front right), the two lobes of the ligand-binding domain are shown as dark (D1) and light (D2) blue surfaces. Chain B (front left) is in green. **(c)** Zebra fish P2X4 receptor in the apo form (PDB entry 3H9V) [3]. ATP in the 'distal' orientation, which was suggested to stabilize the closed state by molecular dynamics simulations [4], is built into the putative binding site.

ends are then connected by a short peptide linker [6,7]. By comparing the iGluR LBD structures bound with agonists, partial agonists, and competitive antagonists, Armstrong and Gouaux [7] observed that agonists induce significant cleft closure, partial agonists do so to a lesser extent, and antagonists keep the LBD in the open cleft conformation. Hansen *et al*. [8] similarly observed that the AChBP C loop closes down on a nAChR agonist but opens up when an antagonist is bound. This observation was confirmed recently by a comparison of AChBP structures bound with various agonists and antagonists [9].

In developing a model for nAChR activation and inhibition, it was further observed that small ligands can fit better into a closed-down binding pocket, whereas larger ligands can fit better into an opened-up binding pocket, leading to the conclusion that agonists tend to be small and antagonists tend to be large [10]. As shown below, a systematic analysis of the available AChBP and iGluR LBD structures generally supports this conclusion. Although no antagonist-bound structure is yet available for any P2XR, structural models obtained from molecular dynamics simulations [4] suggest that the same conclusion may extend to P2XRs as well. It seems that ligand size is a key determinant in the activation/inhibition of all the three families of LGICs. Agonists, usually small, induce closure of the binding pocket, leading to opening of the channel pore, whereas competitive antagonists, usually large, induce opening of the binding pocket, thereby stabilizing the closed channel pore. This review pays only passing attention to inverse agonists, which close spontaneously open channels, and allosteric modulators, which bind the receptors at sites distinct from the agonist-binding sites.

Cys loop receptors

In addition to nAChRs, the Cys loop family also includes 5-hydroxytryptamine (5-HT), γ-aminobutyric acid, and glycine receptors, and glutamate-gated chloride channels (GluCl); the first two are cation-selective, whereas the last three are anion-selective. In the LBD, each protomer comprises two β-sheets (Box 1). The C loop, connecting β9 and β10 in the outer sheet, moves a considerable distance to accommodate the bound ligand (Figure 2a). At the apex of the C loop is a pair of adjacent Cys residues (at positions 189 and 190 in the homomeric neuronal α7 nAChR) that form a disulfide bond. Among 31 AChBP structures in the Protein Data Bank (PDB), the distances between the first C loop Cys residues of two neighboring protomers ($d_{Cys–Cys}$) span a wide range, from 35.5 to 48.4 Å (Table 1). A shorter interprotomer $d_{Cys–Cys}$ signifies closing down of the C loop, whereas a longer $d_{Cys–Cys}$ signifies opening up of the C loop. Confirming previous observations [8,9], histograms in $d_{Cys–Cys}$ (Figure 2b) show that agonist-bound structures tend to have the C loop closed down, antagonist-bound structures tend to have the C loop opened up, and partial agonist-bound structures have intermediate opening of the C loop.

The information in Table 1 also provides support to the notion that small ligands fit better into a closed-down binding pocket, whereas larger ligands fit better into an opened-up binding pocket [10]. Among 27 structures bound with nonpeptide ligands, there is a moderate correlation (R^2 = 0.48) between $d_{Cys–Cys}$ and ligand molecular weight (Figure 2c). The peptide ligands have much higher molecular weights (although in each case much of the molecular weight may be positioned outside the ligand-binding pocket) and correspond to larger $d_{Cys–Cys}$ values, further buttressing the trend.

The outliers in the correlation plot of Figure 2c are interesting. In PDB 2XNV, the ligand is 2-[2-(4-phenylpiperidin-1-yl)ethyl]-1H-indole (compound 6 from an *in silico* screening) (Figure 2d), an α7 nAChR antagonist [11]. The bound ligand adopts a wedge-shaped conformation, with the indole and phenyl rings forming the two adjoining faces. The indole ring is stacked against the C loop of