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Review

Dynamic regulation of lipid–protein interactions☆

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ABSTRACT

We review the importance of helix motions for the function of several important categories of membrane proteins and for the properties of several model molecular systems. For voltage-gated potassium or sodium channels, sliding, tilting and/or rotational movements of the S4 helix accompanied by a swapping of cognate side-chain ion-pair interactions regulate the channel gating. In the seven-helix G protein-coupled receptors, exemplified by the rhodopsins, collective helix motions serve to activate the functional signaling. Peptides which initially associate with lipid-bilayer membrane surfaces may undergo dynamic transitions from surface-bound to tilted-transmembrane orientations, sometimes accompanied by changes in the molecularity, formation of a pore or, more generally, the activation of biological function. For single-span membrane proteins, such as the tyrosine kinases, an interplay between juxtamembrane and transmembrane domains is likely to be crucial for the regulation of dimer assembly that in turn is associated with the functional responses to external signals. Additionally, we note that experiments with designed single-span transmembrane helices offer fundamental insights into the molecular features that govern protein–lipid interactions.
This article is part of a Special Issue entitled: Lipid–protein interactions.

Contents

1. Overview

Dynamic properties as well as time-averaged structural features are crucial for the functions performed by membrane proteins. In this review article, we address the nature and importance of transmembrane helix motions for selected functional membrane proteins and model single-span helical peptides. The collected results reveal that subtle molecular interactions can govern the stabilities of model systems, the resting states of membrane proteins, and the activation of biological function.

2. Helix motions in voltage-gated channels

Although the overall ionic concentration is similar on both sides of a cell membrane, the concentrations of individual ion species vary. A membrane potential on the order of −100 mV can be generated by the charge separation resulting from the pumping of specific ions

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against their concentration gradients. The cell membrane acts as an insulating barrier separating the charges, with the low dielectric inner hydrophobic core sandwiched on both sides by the high dielectric media of the inside and outside of the cell. The thinness of the hydrophobic section (~27 Å) causes the membrane potential to form a robust electric field reaching values up to 107 V/m, which many membrane proteins use to regulate cell functions. A change in the electric field causes reorientation of electric charges or dipoles within a protein, triggering conformational changes that may regulate protein function. The movement of charges or dipoles induces a "gating current" that can be measured experimentally to obtain direct indications of the conformational changes.

Proteins sense voltage by multiple methods: 1) Polar residues such as Asp, Glu, Arg, Lys and His can reorient in an electric field, a mechanism found to be important in voltage-gated ion channels (Fig. 1A). 2) Side chains with an intrinsic dipole moment such as Tyr may also orientate in the electric field (Fig. 1B). 3) Though not recognized yet as a voltage sensor, the α-helix with its intrinsic dipole can also be a voltage-sensing structure, with the ability to reorient in the field (Fig. 1C). 4) Some proteins contain cavities within which free ions can associate (Fig. 1D). A change in the electric field may move free ions, initiating a conformational change, a mechanism found in Na^+–K^+ pump. The complex molecular structure of the channel causes the field strength near the voltage center to be different from the field strength near the lipid bilayer [1].

Voltage-gated Na^+, K^+ and Ca^{+2} channels play crucial roles in excitable cells. Each of these channels contains voltage sensors and a selective ion-conduction pore. Their general structure comprises either four independent protein subunits (K^+ channels) or one long polypeptide containing four homologous domains (eukaryotic Na^+ and Ca^{+2} channels). Each domain contains six transmembrane segments (S1–S6) and a pore loop between segments S5 and S6. The voltage sensor is made up of the first four transmembrane segments, with the conduction pore formed by the last two segments and the pore loop. The channels are arranged symmetrically around a central conduction pore, surrounded by four copies of the voltage sensor. The S4 segment was early recognized as a potential candidate for voltage sensing owing to the arrangement of basic residues (Arg and Lys) at every third position [2]. The first four most extracellular basic residues of S4 and the most intracellular acidic residue of S2 are involved in gating currents [3,4].

Two models of channel function, the sliding helix [5,6] and the helical screw model [7], predict that the S4 segments have a transmembrane orientation and that the positively charged residues within S4 serve as gating charges. The charges would move outward across the membrane in response to depolarization, thereby initiating the activation process. Based on thermodynamic and structural considerations, along with extensive structure-function studies and molecular modeling, the sliding helix and helical screw models propose that the positively charged residues in S4 form ion pairs with negatively charged residues in the neighboring S1, S2 and/or S3 segments [8]. The negative resting membrane potential draws the positively charged residues of S4 inward; upon depolarization, this electrostatic force is relieved, allowing each of the S4 segments to move outward spirally such that each positively charged side chain can form a new ion pair with a negatively charged residue (Fig. 2). This configuration would provide the thermodynamic stability required to allow the gating charges of S4 segment to move within the hydrophobic environment of membrane. Studies on the effects of mutating these charges on the gating current of K^+ channels have directly confirmed the importance of the S4 positive charges and also the highly conserved second negatively charged residue on S2 [3,4] for channel opening.

One of the most important tenets of the sliding helix model is that S4 remains in a transmembrane position as it translocates the gating charges. The intracellular end of S4 is linked covalently to the N-terminal of S5, which forms the outer circumference of the pore. Site specific mutagenesis and gating charge measurements reveal that the S3–S4 linker is on the extracellular side of the membrane in the resting state [9,10]. Real-time fluorescence quenching and energy transfer measurements have confirmed S4 motions. Experiments in which selected Arg-to-Cys mutants within S4 were labeled with methanethiosulfonate (MTS) revealed that the inner Arg/Cys replacements are susceptible to MTS from inside the cell in resting state, and accessible to MTS from outside the cell after depolarization [11,12]. Fluorescent labeling studies indicate that several residues on S4 spiral outward toward a hydrophilic environment during activation. Detailed studies of channels labeled at multiple S4 positions provided evidence for both outward translocation and rotation of S4 up to 180° during activation [13,14].

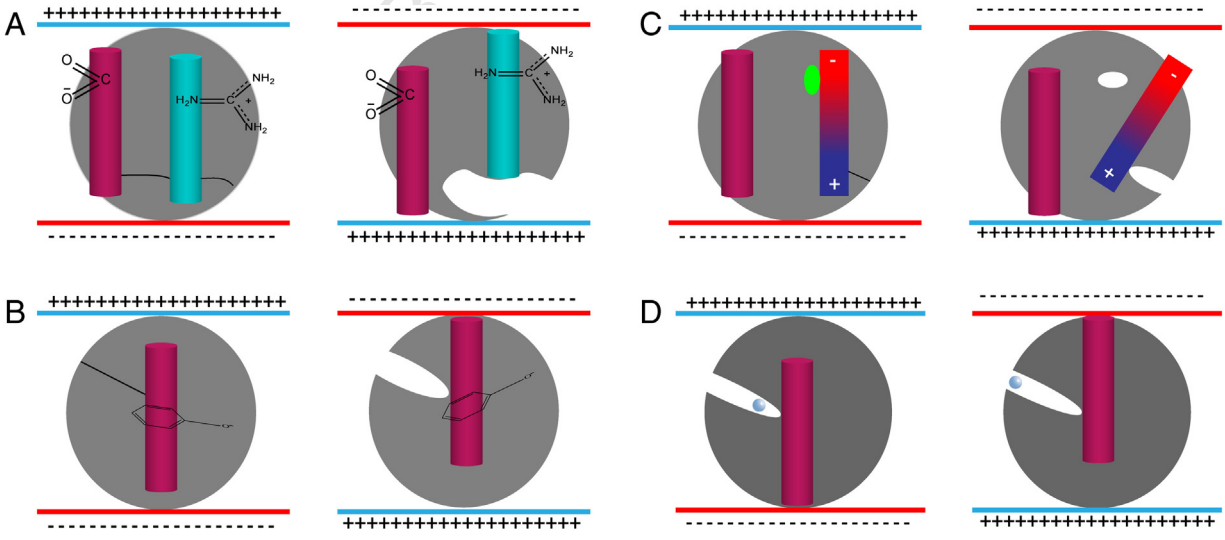

Fig. 1. Possible structures of voltage sensors. The diagram shows a hypothetical protein (gray circle) and a formation of an active site resulting from voltage-induced conformational change that is regulated by specific regions of the protein (magenta and cyan cylinders). A. Charged amino acids may move within membrane as a response to changes in membrane voltage. The carboxyl and guanidinium groups of Asp and Arg are shown. B. Changes in the field may result in reorientation of an intrinsic dipole such as Tyr. C. An α-helical protein the length of the membrane (red to blue gradient) has a dipole moment that can reorientate when field is changed. The oval attached to the helix represents a fluorophore that may be quenched as a consequence of the conformational change. D. A channel within the protein can redistribute ions (light blue circle) in the direction of the field to initiate a conformational change. Figure redrawn from [1].

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