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Review

# The potassium channel KcsA: A model protein in studying membrane protein oligomerization and stability of oligomeric assembly?

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ABSTRACT

Many membrane proteins are functional as stable oligomers. An understanding of the conditions that elicit and enhance oligomerization is important in many therapeutics. In this regard, protein–protein and protein–lipid interactions play crucial roles in the assembly and stability of oligomeric complexes. Recent years have seen a rapid increase in the mechanistic information on the importance of cytoplasmic termini in determining subunit assembly and stability of oligomeric complexes. In addition, the role of specific protein–lipid interaction between anionic phospholipids and "hot spots" on the protein surface has also become evident in stabilizing oligomeric assemblies. This review focuses on several contemporary developments of membrane proteins that stabilize oligomers by taking the potassium channel KcsA as an exemplary ion channel.



## Introduction

The broad category of oligomeric proteins can be classified by subunit type, strength of subunit association, and duration and avidity of subunit association. The oligomerization of proteins on cell surfaces is believed to play a fundamental role in the regulation of cellular function, including signal transduction and the immune response [1,2]. Oligomerization can increase protein stability and it can provide an additional level of regulation for those proteins that have different activities in their oligomeric and monomeric states. For hetero–oligomeric complexes, oligomerization may allow the formation of enzymatic and signaling cascades [3,4].

The specific characteristics of a soluble oligomeric protein interface depend on the nature of that interface and on the duration of the interaction defining it. Interfacial residues tend to protrude from the surface of the protein [5], and the interaction surface tends to be circular in shape [6]. Protein–protein interaction interfaces are relatively planar [7], as are many hetero–oligomer interfaces [5,8]. By contrast, many obligate homodimers and heterodimers have intertwined monomer units, and thus less planar interfaces [5,8]. As might be predicted, large oligomeric interfaces are often associated with strong interactions. The residues at an oligomeric interface may be slightly more conserved than other surface residues [9]. It has been found that certain conserved residues, or "hot spots," generally at the center of an interface, are responsible for most of the binding energy of an oligomeric interaction. These hot spots are often composed of polar residues that engage in van der Waals contact and hydrogen bonding [10,11].

On the other hand, membrane proteins (MPs) typically flank hydrophobic residues in the transmembrane (TM) domains. The interfacial interactions between lipid headgroups and the aromatic and charged residues are known to act as an organizing element [2,4]. Biological functioning is assured by the dynamics of the MPs, which largely depend on lipid–protein interactions [12]. These protein–lipid interactions are either specific [13] or non-specific, in which case they depend on more general properties of lipids, such as the extent of hydrophobic mismatch [14], the presence of non-bilayer lipids [15,16] or the charge of the lipid headgroup [17]. Negatively charged lipids constitute 20–30% of biomembrane lipids [18]. It is well established that negatively charged membranes act as a site of attraction for positively charged (basic) protein domains [19,20]. Proteins are bound to the membranes either as "intrinsic" or "peripheral" proteins. Intrinsic proteins are located within the hydrocarbon core of the membrane, whereas peripheral proteins are bound only to the membrane surface. A major driving force for the binding of peripheral proteins is electrostatic attraction between the negatively charged membrane and protein segments that accumulate positively charged amino acids (lysine,

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arginine, histidine). Such electrostatic interactions guide the membrane insertion and orientation of MPs [21]. There is also strong evidence for the existence of specific binding of small numbers of lipid molecules to some MPs. Some of these lipid molecules bind between TM α-helices, often at protein–protein interfaces in oligomeric MPs, and most of the lipid molecules identified in X-ray crystal structures of MPs correspond to lipid molecules of this type [22]. Nevertheless, X-ray crystal structures of some MPs, including bacteriorhodopsin [23], succinate dehydrogenase from *Escherichia coli* [24], and the ADP/ATP carrier from mitochondria [25], show resolved lipid molecules bound to the surface of the protein, not buried within deep clefts [26]. Although bacteriorhodopsin may represent a special case, being located in quasi-crystalline membrane patches in the membranes of *Halobacterium salinarum*, the other examples suggest that the lipid-bound surface of a MP could be heterogeneous, with some lipid molecules binding more tightly than others. Evidences are now emerging for the existence of 'hotspots' on the surface of such proteins showing marked selectivity for anionic phospholipids [27–30].

Oligomerization is central to $K^+$ channel action; individual subunits are nonfunctional and conduction, selectivity and gating involve manipulation of the common subunit interface of the tetramer. Thus, $K^+$ channel oligomerization is crucial for function in the control of potassium flow, cell volume, release of hormones and neurotransmitters, resting potential, excitability and behavior [31,32]. The potassium channel from the soil bacteria *Streptomyces lividans* named KcsA, a homotetramer made up of identical 160-amino acid subunits, was the first of such complexes to be solved [33]. The four subunits of KcsA are arranged symmetrically around the central pore. The TM segment M2, nearest to the C-terminus, contributes to the lining of the pore, while the one closest to the N-terminus, M1, is exposed to the membrane bilayer [33]. The positively charged N-terminal helix which lies at the membrane interface [34] and a large, four-helix C-terminal bundle, which is rich in charged or polar amino acid residues, is projected ≈70 Å toward the cytoplasm [35].

How do MPs assemble into oligomeric structures? What determines the stability of such complexes? How do membrane lipids affect the assembly of TM proteins and what are their roles in altering the stability of oligomeric complexes in different cell types? An appropriate protein model is required to understand these fundamental phenomena. Although, the structural information of several eukaryotic ion channels is available, however, these channels are quite difficult to deal with the fundamental understanding of MP oligomerization due to several limitations. For instance, the expression level of these channels is not very high and therefore a suitable host may be required to achieve adequate amounts of soluble and stable protein. Furthermore, an efficient membrane system may be required to avoid aggregation or degradation during proteoliposome reconstitution. Also, the intrinsic oligomeric stability is hard to determine by using simple or conventional techniques, like SDS-gel electrophoresis. In contrast, the simplicity of the protein, the ease of expression in *E. coli*, and extreme tetrameric stability make $K^+$-channel KcsA the most convenient protein to study the underlying principle of MP oligomerization [29,30,36,37]. KcsA is a versatile ion channel that deals with a number of variable conditions, i.e., different lipid composition in membrane bilayers or ionic strength. In recent years, a combination of biochemical and biophysical techniques has been applied to obtain valuable data on KcsA channel assembly and stability with special emphasis on the role of protein–protein and protein–lipid interaction. Therefore, it is the main purpose of this review. The fundamental understanding of these phenomena is important, because, according to our knowledge of molecular diversity, structure, and function, a growing number of discoveries have linked many $K^+$ channel genes with various diseases that may affect the biogenesis, assembly, stability and/or regulation of $K^+$ channel function [38].

## The $K^+$-channel KcsA as a model ion channel?

The high level of similarity in global topology, function, and the universal selectivity filter signature sequence among $K^+$-selective channels allows us to postulate that the 6-TM subunit $K^+$ channels (DRK/A, HYP, EAG, SK, and AKT), the 10-TM BK channels, and the 2-TM Kir's form permeation pathways with sequence and structural motifs similar to that of KcsA's [39]. This has led to the rather interesting finding that KcsA channel shares important structural features with "real" ion channels. Furthermore, a three-dimensional model structure for a KcsA $K^+$ channel and sequences of hundreds of voltage-gated $K^+$-selective (kV) channel proteins enable a comprehensive sequence–structure–function alignment. However, there exists a main controversy on oligomerization, function and the role of lipid regulation in kV channels with regard to voltage sensor or 4TM domains that are not present in KcsA. Nevertheless, the sequence patterns within $K^+$-selective channels found in *Bacteria*, *Archaea* and *Eukarya* [39] indicate that specific residues or clusters of residues in KcsA establish structural conservation in the areas responsible for protein–protein and protein–lipid interactions that lead to the formation and stability of oligomeric assembly in MPs.

## An association of membrane lipids with the $K^+$-channel KcsA

In recent years, an increasing number of MP structures have been determined; the majority obtained by X-ray crystallography [36,40–42]. Interestingly, some of these integral MPs contain tightly bound lipids [13,25,28,36]. In most cases, these structurally resolved lipids are endogenous and co-purified with the MPs, which are subsequently crystallized as protein–lipid complexes. These binding sites provoke discussion and stimulate research goals to elucidate their possible functions. A wealth of biochemical and biophysical studies have demonstrated the importance of protein–lipid interactions for the assembly, stability, and function of MPs [16,27,29,30,36], but careful analysis of protein–lipid interactions is required to understand the significance of specific lipid binding sites for the structure and function of MPs. A suitable model protein is required to delineate the mechanism of interaction of MPs with phospholipids. KcsA is the most convenient model in this regard. Here, the roles of anionic lipids, particularly phosphatidylglycerol (PG),[1] phosphatidic acid (PA) and cardiolipin (CL), that differ from each other with regard to their net charge and head group properties, are described to understand how these lipids may associate with MPs and that how KcsA helps us to understand the nature of oligomeric MPs that require anionic phospholipids for their structural and functional stability. It is important to mention here that this review does not discuss about the roles of bilayer and non-bilayer zwitterionic lipids. Extensive reviews [43,44] covering this subject can be consulted for additional details.

## The role of phosphatidylglycerol (PG) in KcsA $K^+$-channel assembly and stability

Phosphatidylglycerol (PG) is a ubiquitous lipid that can be the main component of some bacterial membranes, and it is found also in membranes of plants and animals where it appears to perform specific functions. The charge on the phosphate group

[1] *Abbreviations used:* PG; phosphatidylglycerol, PA; phosphatidic acid, CL; cardiolipin, SDS; sodium dodecyl sulfate, PAGE; polyacrylamide gel electrophoresis, TFE; trifluoroethanol, Trp; tryptophan, TM; transmembrane, MP; membrane protein.