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Review

Structural insights into functional lipid–protein interactions in secondary transporters☆

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ABSTRACT

Background: Structural evidences with functional corroborations have revealed distinct features of lipid–protein interactions especially in channels and receptors. Many membrane embedded transporters are also known to require specific lipids for their functions and for some of them cellular and biochemical data suggest tight regulation by the lipid bilayer. However, molecular details on lipid–protein interactions in transporters are sparse since lipids are either depleted from the detergent solubilized transporters in three-dimensional crystals or not readily resolved in crystal structures. Nevertheless the steady increase in the progress of transporter structure determination contributed more examples of structures with resolved lipids.
Scope of review: This review gives an overview on transporter structures in complex with lipids reported to date and discusses commonly encountered difficulties in the identification of functionally significant lipid–protein interactions based on those structures and functional in vitro data. Recent structures provided molecular details into regulation mechanism of transporters by specific lipids. The review highlights common findings and conserved patterns for distantly related transporter families to draw a more general picture on the regulatory role of lipid–protein interactions.
Major conclusions: Several common themes of the manner in which lipids directly influence membrane-mediated folding, oligomerization and structure stability can be found. Especially for LeuT-like fold transporters similarities in structurally resolved lipid–protein interactions suggest a common way in which transporter conformations are affected by lipids even in evolutionarily distinct transporters. Lipids appear to play an additional role as joints mechanically reinforcing the inverted repeat topology, which is a major determinant in the alternating access mechanism of secondary transporters.
General significance: This review brings together and adds to the repertoire of knowledge on lipid–protein interactions of functional significance presented in structures of membrane transporters. Knowledge of specific lipid-binding sites and modes of lipid influence on these proteins not only accomplishes the molecular description of transport cycle further, but also sheds light into localization dependent differences of transporter function. This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins.

1. Introduction

Biological membranes are essential cellular components central to all life processes. They provide a selective and electrochemically sealed permeability barrier for cells and allow compartmentalizing cellular organelles. Proteins embedded in these lipid bilayers mediate transport and communication between the two sides delineated by the membrane. These integral membrane proteins are involved in many crucial life-sustaining processes like respiration, transport and photosynthesis. Not surprisingly, they typically comprise almost 20–30% of the annotated genes of known organisms [1]. It has long been understood that the lipid bilayer surrounding membrane proteins is not just a passive environment but actively contributes to membrane protein properties. For instance, lipids are known to confer structural stability and mediate oligomerization as seen in aquaporins and bacteriorhodopsin [2,3]. They help in the assembly of supercomplexes like cytochrome bc1 [4]. Some membrane proteins require specific lipids as chaperons in topogenesis, e.g., lipids assist in folding and correct insertion as documented in the potassium channel KcsA and lactose transporter LacY [5,6]. In fact, LacY can adopt altered topologies by simply changing the lipid composition of the membrane. Lipids also directly affect and modulate protein function as seen in mechanosensitive channels MscL [7] responding to hypoosmotic

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stress. Bilayer adjustments to alleviate hydrophobic mismatch gate the opening of inward rectifying potassium channels K_{ir} where binding of specific signaling lipid phosphatidylinositol-4,5-bisphosphate (PIP2) controls channel opening (reviewed in detail in [8]). Identification of lipid-binding sites in membrane proteins that are involved in human diseases has led to the development of membrane–lipid therapies with specific lipid–protein interactions being increasingly used as therapeutic targets in molecular medicine [9].

Lipid–protein interactions can be either of chemical nature when individual amino acids in proteins coordinate individual lipids, thereby forming a specific binding site or of physical nature when properties of the bulk lipids, e.g., fluidity, membrane tension, curvature or polarity, affect the protein collectively (Fig. 1a). These chemical and physical properties of lipids are well described, although mostly in artificial systems, it is still unclear how lipid interactions specifically affect protein function at a molecular level. Ultimately it remains difficult to distinguish experimentally between the effect of the lipid bulk and the action of an individual lipid in biological membranes [10].

The complexity of these interactions makes probing using standard structural biology methods non-trivial. Notwithstanding their tremendous importance, molecular details of these interactions are known only for few membrane proteins. Roughly 11% of known membrane protein structures revealed lipid densities (derived from the Membrane Protein Structure Database http://blanco.biomol.uci.edu/mpstruc/, Stephen White). The major holdups in identifying functional lipid interactions in protein structures are the dynamic nature of these interactions, coupled with the modest resolutions usually obtained for membrane proteins. Purification and crystallization procedures also deplete weakly bound lipid moieties. Sometimes even when structural evidences for lipid interactions are present in structures, deducing their functional effects is challenging. On the other hand their small dimensions make membranes and the embedded proteins impossible to image using standard fluorescence microscopy approaches. One bottleneck is therefore to bridge the gap between cellular processes; biochemical/biophysical data on recombinant, often heterologously expressed membrane proteins and structural data.

The understanding of lipid–protein interactions and how they control cellular locations, conformations and the activity of membrane proteins was the motivation to develop new tools for lipid research. Improved imaging techniques such as structured illumination microscopy (SIM), stimulated emission depletion microscopy (STED) and photo-activated-localization microscopy (PALM) emerged to break the diffraction barrier and allow imaging of cellular structures far below the conventional 200 nm limit [11]. Structural information on lipid–protein and protein–protein interactions observed in membrane mimicking environments like two-dimensional crystals was exploited also by spectroscopy, e.g., FT-IR. Techniques that specifically include lipids into the 3D crystallization process [12] have been introduced and successfully applied for receptors, channels and recently also for transporters. Hereby, membrane proteins were either maintained in a lipidic environment during extraction and purification or re-lipidated in bicelles, in lipid cubic phase (LCP) or crystallized in the presence of high concentrations of lipids and detergents (HiLiDe) [13]. The number of structures solved by LCP or derivative techniques like lipid sponge phase (in which the cubic phase is modified by hydrophobic additives) is constantly increasing since the high-resolution structure of bacteriorhodopsin [14]. According to (http://cherezov.scripps.edu/structures.htm) structures of 47 membrane proteins were solved in lipidic phases, 5 of them being transporters (see Section 3). However it is important to note that crystallization in the presence of lipids has not necessarily resulted in the observation of lipids in those structures. Often lipid sites are occupied by detergent molecules (Fig. 1b and c), which although

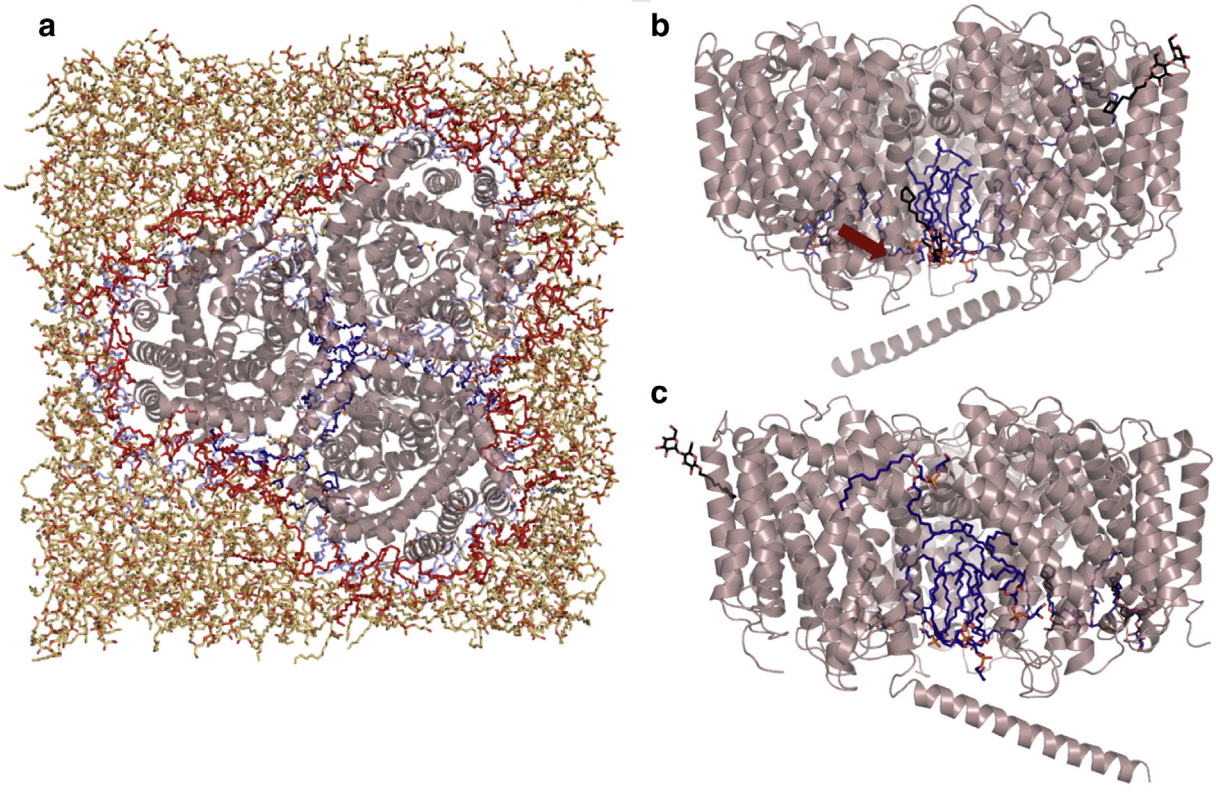

Fig. 1. a) Top view of the X-ray structure of the betaine transporter BetP (PDB: 4C7R) embedded in a hydrated, POPG bilayer. Bulk lipids are depicted in yellow, lipids in direct contact with the transporter trimer are colored in red and specifically bound POPG lipids observed in the crystallographic data are colored in blue. Bilayer water molecules are omitted for clarity. b) and c) Side views of two BetP protomers within the trimer in complex with anionic POPG lipids. The crystallization detergent CYMAL-5 is depicted in black. The red arrow highlights a detergent position, which is next to a lipid observed in another structure of BetP. The detergent positions also align well with the membrane limits like the resolved head groups indicating a possible lipid-binding site position in vivo.

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