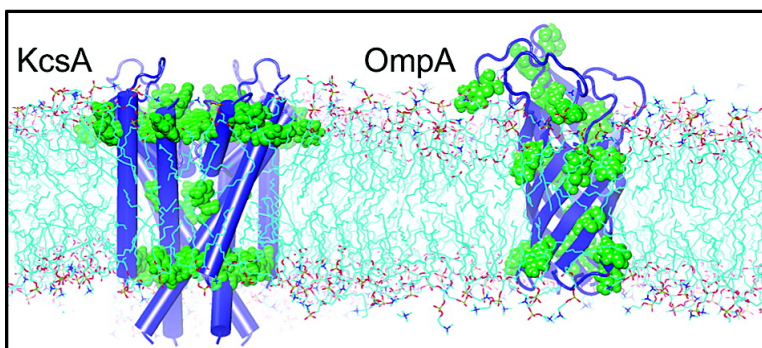Communication

Lipid/Protein Interactions and the Membrane/Water Interfacial Region

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Lipid/Protein Interactions and the Membrane/Water Interfacial Region

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Received June 3, 2003; E-mail: mark@biop.ox.ac.uk

Despite the importance of lipid/protein interactions in the folding, assembly, stability, and function of membrane proteins, information at an atomic level on how such proteins interact with the lipids that surround them remains sparse. The dynamic and flexible nature of the protein/bilayer interaction make it difficult to study, for example, by crystallographic means. However, based on recent progress in molecular simulations of membranes it is possible to address this problem computationally. This communication reports one of the first attempts to use multiple nanosecond molecular simulations to establish a qualitative picture of the intermolecular interactions between the lipids of a bilayer and two topologically different membrane proteins for which a high resolution (2 Å or better) X-ray structure is available (Figure 1).

The environment experienced by membrane proteins is complex, ranging from a hydrophobic bilayer core, to aqueous solutions on either side of the membrane. Furthermore, as emphasized by for example White [2], the "interface" between lipid and water is actually a region of thickness ~10 Å, composed of lipid headgroups and water molecules. Thus, to be stably inserted in a bilayer, the surface of a membrane protein must possess a region which interacts favorably with the interfacial region of the bilayer.

Traditionally, ionic or hydrophobic interactions have been thought to be the main interactions between the protein and the lipid. More recently, a combination of crystallographic [3] and less direct experimental approaches has been used to identify a range of associations of proteins and lipids. Attention has been paid to the interactions between amphipathic aromatic side chain "belts" on the surface of the protein and interfacial region of the bilayer.[4] These aromatic belts, which are constituted mainly by Trp and Tyr amino acids, are suggested to anchor the protein within the mobile and flexible membrane. Several other roles have been attributed to these aromatics residues.[5–7]

Molecular dynamics simulations of a bacterial potassium channel, KcsA,[8] and an outer membrane protein, OmpA,[9] were performed in a model membrane environment, i.e, a POPC and a DMPC lipid bilayer, respectively. DMPC was used for OmpA as it has a thinner transmembrane zone (~27 Å in KcsA vs ~21 Å in OmpA, as indicated by the spacing between the aromatic belts). The starting structures corresponded to PDB files 1k4c (KcsA) and 1bxw (OmpA). Both simulations were performed using the GROMACS MD simulation package. Resulting system sizes were of ~50 000 atoms. Further details concerning the simulation protocols can be found elsewhere.[10,11]

On the basis of these simulation results we have analyzed the nature of the protein/lipid interactions. The principal side chains in close (≤3.5 Å) proximity to the lipids are aliphatic hydrophobic and aromatic. The number of atomic contacts as a function of time (Figure 2A) shows time-dependent changes and differences between the two simulations. For KcsA the number of contacts rises during the first ~3 ns of the simulation and then remains constant at ~700. This represents a buried surface accessible area of ~5000 Å[2]. The
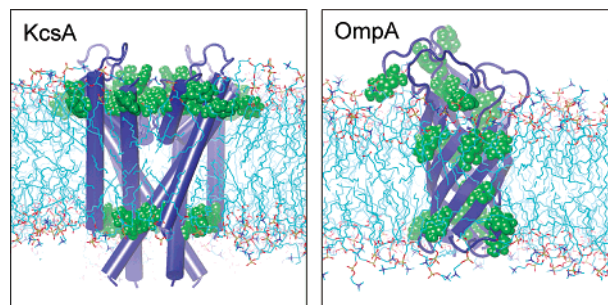

Figure 1. Schematic diagrams (drawn using VMD [1]) of KcsA in a POPC bilayer and OmpA N-terminal domain in a DMPC bilayer. The aromatic amino acid side chains on the protein surface are shown in space-filling format in green.
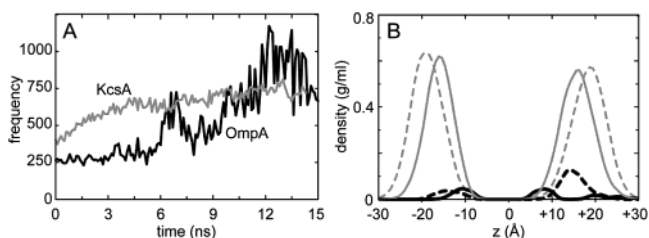

Figure 2. (A) Number of atomic contacts (≤3.5 Å) between protein side chains and lipids as a function of time for KcsA (gray line) and OmpA (black line). (B) Density profile along bilayer normal for lipid headgroups (gray lines) and for protein aromatic residues (black lines). The profiles are for OmpA (solid lines) and for KcsA (broken lines).

number of protein/lipid contacts for the OmpA simulation stays at ~250 for the first 5 ns but then rises and fluctuates between ~500 and ~1000 (these fluctuations continue when the simulation is extended beyond 15 ns—data not shown). This is reflected in the buried surface accessible area, which increases from ~5000 to ~6000 Å[2] over the last 10 ns. For reference, the total solvent-accessible surfaces areas of the X-ray structures of KcsA and OmpA are ~13 000 and ~10 000 Å[2] respectively. The ~5 ns time scale fluctuations in the total number of protein/lipid atomic contacts are of interest. They reveal the complex dynamic behavior of these systems and the relatively slow (~5 ns) time scale of fluctuations in lipid/protein interactions. We have analyzed the interactions further in terms of H-bonding interactions between lipid headgroups and protein. Both of these analyses (data not shown) show fluctuations, in magnitude and time scale, similar to those seen for the total atomic contacts.

The aromatic belts of membrane proteins generally lie close to the membrane/water interface of the bilayer. As can be seen from Figure 1, both KcsA and OmpA possess a clear aromatic belt at each end of the molecule. In KcsA there are 28 and 8 (Tyr + Trp) residues in the upper (periplasmic) and lower (cytoplasmic) belts, respectively. However, in the case of KcsA the lower, intracellular aromatic belt is not significantly solvent exposed, at least not in
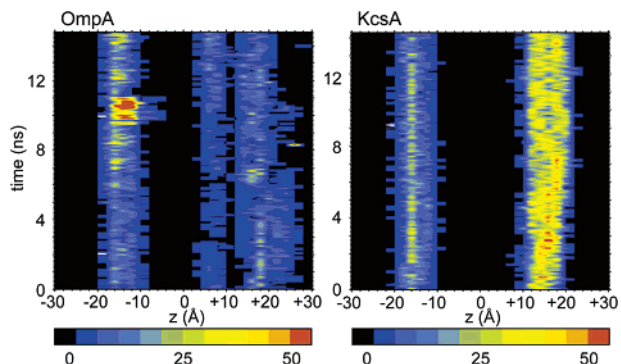
Figure 3. Interactions of the aromatic belts of OmpA and KcsA with lipid polar headgroups. The number of interactions (≤ 3.5 Å) are shown as a function of position along the bilayer normal (z) and time.

the closed state of the channel. In contrast, in OmpA there are five (Tyr + Trp) residues in the upper (extracellular) and six in the lower (periplasmic) belts, respectively. However, there is an effective third aromatic belt in OmpA provided by four Tyr residues of the extracellular loops, above the transmembrane β-barrel region. Note that Trp and Tyr residues are able to form H-bonds with interfacial water molecules and polar headgroups of lipids, while also forming hydrophobic interactions with the bilayer core. Density profiles along the bilayer normal (Figure 2B) reveal that in both simulations the aromatic belts clearly overlap with the locations of the lipid headgroups.

Previous simulation studies, for example on model systems such as single TM α-helices, have provided some evidence for interactions between Trp side chains and the glycerol ester oxygens of phospholipid molecules.[12] The current, more extended, simulation results enable us to probe the interactions of the aromatic belts with lipid headgroups more quantitatively.

In KcsA there two distinct bands of contacts between the aromatic side chains and the lipid headgroups (Figure 3). The majority of these polar contacts are in the upper (periplasmic) band, reflecting the greater solvent exposure of aromatics in this region. There are rather fewer such interactions involving the lower (cytoplasmic) band. This may be significant as gating of the KcsA channel (i.e. the conformational transition from closed to open) is thought to primarily involve an expansion of the lower (cytoplasmic) half of the protein, while the upper (periplasmic) half remains largely unchanged. It is conceivable that in the intact KcsA protein additional interactions with the headgroups of the cytoplasmic leaflet are provided by the amphipathic N-terminal helix (not present in the X-ray structure of KcsA and thus not included in the current simulation). It should be noted that the aromatic side chain/polar headgroup interactions fluctuate on a ~ 2 ns time scale.

For OmpA, there are three distinct interaction regions, corresponding to the three aromatic belts (lower = periplasmic, middle = extracellular, upper = extracellular loops). The most extensive interactions originate from the aromatic side chains of the lower belt. Previously, on the basis of somewhat shorter simulations, it was reported[11] that the OmpA barrel tends to tilt (by $\sim 5-10°$) relative to the bilayer normal. This was suggested to enable the protein to optimize aromatic belt positioning and to maximize the buried hydrophobic surface area.

The broad upper band of interactions corresponds to the aromatic residues of the extracellular loops that are revealed to interact significantly with the polar heads of the lipids. This may relieve a potential bilayer/protein mismatch and thus explain how OmpA, with a somewhat narrower central hydrophobic region (see above), can be accommodated stably within, for example, a DMPC bilayer. In fact, the mobility of the extracellular loops leads to conformational changes resulting in a narrower, more "uniform" aromatic belt, matching the regular bilayer interface. Moreover, all the tyrosine rings become more perpendicular to the membrane plane, optimizing interfacial interactions; this may result in tighter lipid/protein packing. In the bacterial outer membrane the extracellular loops may be expected to form multiple interactions with the rather more complex lipopolysaccharide (LPS) headgroups of the outer leaflet of this membrane. It is possible that, while the LPS lipid acyl chain lengths (normally 12 or 14 carbons long[13]) present a fairly constant hydrophobic environment to the protein, mobile extracellular loops may enable tyrosine residues to hydrogen-bond and flexibly partake in ring stacking with a variety of heterogeneously composed[13] and irregularly arranged LPS sugar residues. This is important because the resultant tight linkage between OmpA and the membrane is essential for maintaining bacterial cell integrity.[14]

In summary, these studies have contributed to an improved understanding of the how integral membrane proteins are stably embedded within a lipid bilayer. In particular, the aromatic belts seem to facilitate the anchoring of the protein within the biomembrane through their interactions with the polar heads of membrane lipids. Having established the value of extended (>10 ns) MD simulations to the analysis of such interactions, it will be of interest to extend such studies to probe selective interactions of membrane proteins with specific lipid species.

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JA0364874