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The determinants of hydrophobic mismatch response for transmembrane helices

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ABSTRACT

Hydrophobic mismatch arises from a difference in the hydrophobic thickness of a lipid membrane and a transmembrane protein segment, and is thought to play an important role in the folding, stability and function of membrane proteins. We have investigated the possible adaptations that lipid bilayers and transmembrane α-helices undergo in response to mismatch, using fully-atomistic molecular dynamics simulations totaling 1.4 μs. We have created 25 different tryptophan-alanine-leucine transmembrane α-helical peptide systems, each composed of a hydrophobic alanine–leucine stretch, flanked by 1–4 tryptophan side chains, as well as the β-helical peptide dimer, gramicidin A. Membrane responses to mismatch include changes in local bilayer thickness and lipid order, varying systematically with peptide length. Adding more flanking tryptophan side chains led to an increase in bilayer thinning for negatively mismatched peptides, though it was also associated with a spreading of the bilayer interface. Peptide tilting, bending and stretching were systematic, with tilting dominating the responses, with values of up to ~45° for the most positively mismatched peptides. Peptide responses were modulated by the number of tryptophan side chains due to their anchoring roles and distributions around the helices. Potential of mean force calculations for local membrane thickness changes, helix tilting, bending and stretching revealed that membrane deformation is the least energetically costly of all mismatch responses, except for positively mismatched peptides where helix tilting also contributes substantially. This comparison of energetic driving forces of mismatch responses allows for deeper insight into protein stability and conformational changes in lipid membranes.

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1. Introduction

Comprising nearly a third of the human genome [1], membrane proteins are a major component of biological membranes and perform a variety of essential cellular functions, making them chief drug targets [2–4]. Membrane proteins function as enzymes [5–7], receptors [8–10] and transport proteins that catalyze the transport of ions [6,7,11,12] and other small molecules (e.g. [13,14]) for cellular communication [15]. Atomic-level molecular dynamics (MD) simulations can provide fundamental descriptions of the interplay between proteins and their host membranes that governs structure and function, enabling future developments of novel pharmacological and therapeutic agents.

Hydrophobic mismatch is known to be an important factor controlling membrane protein insertion and folding [16], protein activity [17] and aggregation [18]. Protein transmembrane (TM) segments attempt to match their hydrophobic segments to the host lipid membrane to avoid significant energy costs of dehydrating polar or hydrating nonpolar moieties of the protein or lipids. When a mismatch in hydrophobic lengths exists, whether positive (peptide longer than the bilayer) or negative (peptide shorter than the bilayer), the membrane and peptide will undergo structural changes [19], including peptide tilting, bending or stretching (as well as conformational and secondary structural changes) and membrane deformations. Here we explore the extents, origins and relative importance of each possible adjustment to the structure and stability of the protein segment.

TM peptides, characterized by having helical structures composed of hydrophobic residues, have been designed to study hydrophobic mismatch experimentally [19–21]. These peptides are usually composed of a poly-leucine (Leu) stretch [22], or pairs of alternating Leu and alanine (Ala) residues [23], and are flanked at each end by one or more aromatic or charged residues, thought to anchor the peptide across the lipid membrane [22–24]. One of the most frequently used models [24] includes two tryptophan (Trp) side chains at each end of the hydrophobic stretch, inspired by the small Trp-flanked ion channel, gramicidin A (gA), and an abundance of aromatic-flanked TM segments in membrane proteins [25–27]. These α-helical, Trp-flanked, poly-Ala-Leu peptides, or WALPs, with the general sequence GWW(LA)$_n$LWWA, have been used here to systematically explore the effects of hydrophobic mismatch on the TM segment and the surrounding bilayer.

Responses of the surrounding lipid bilayer include the thickening or thinning of the bilayer accompanied by an increase or decrease in the acyl chain order, respectively [19]. ^{2}H NMR measurements of acyl chain ordering of perdeuterated phosphatidylcholines (PC) point to increases and decreases in mean bilayer thickness (ranging from −0.4 to 1.4 Å) as a response to positive and negative mismatches, respectively [28].

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X-ray diffraction studies of WALPs incorporated into di-C_{12}-PC, di-C_{13}-PC and di-C_{14}-PC bilayers, on the other hand, do not show any effect on the bilayer thickness [29]. However, the mean bilayer thickness does not fully reflect the effect of mismatch on the lipids immediately adjacent to the peptide, with persistence lengths for the perturbation suggested to be as small as just a single shell of lipids [30]. Interestingly, the same studies have revealed that the β-helical gA peptide can affect the mean thickness of PC bilayers (e.g. by nearly 3 Å in dimyristoylphosphatidylcholine), and has been shown to clamp the bilayer thickness in bilayers of varying hydration [31]. The authors ascribed this difference to the increased size of the inclusion, compared to the lipid-sized membrane spanning α-helix (~10 Å diameter), leading to a marked difference in lipid packing, as has also been suggested based on coarse-grained simulations of membrane-spanning nanotubes [30]. However, gA possesses double the number of Trp side chains compared to the WALP peptides and this could also have a perturbative effect on the host membrane. In this study we explore the role of the number and distribution of anchoring Trp side chains in modulating mismatch response. In the accompanying study [32], we investigate the role of Trp in controlling mismatch phenomena in greater detail by producing quantitative analysis of the energetics associated with Trp–membrane interactions.

The peptides themselves respond by tilting, bending and stretching, or undergo structural isomerizations of flanking side chains. In extreme cases the peptides may experience changes in secondary structure (though Fourier transform infrared spectroscopy (FTIR) has suggested that an α-helical structure is maintained for a wide range of mismatch [23,33,34]), undergo oligomerization, or even do not partition into the bilayer at all [33,35–38]. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) experiments reveal mismatch-induced tilting of Trp-flanked peptides that are embedded in thin bilayers [33,39], as confirmed by NMR measurements of ^{15}N-labeled peptides [35] and ^{2}H NMR studies of peptides with deuterated alanine side chains [40]. However, the angles inferred from these methods are smaller (~5°) [33,41,42] than that observed in multiple simulation studies (~32°) [42–44]. Experiments performed on WALPs using geometric analysis of labeled alanines (GALA) also indicated relatively small tilting angles [45,46]. These small angles come from a neglect of peptide degrees of freedom in the analysis of NMR observables [47–49]. To remedy this, complementary anisotropic constraints have been introduced when analyzing the dynamics of peptide motion, making use of triple-isotope-labeled WALP23 (^{2}H, ^{13}C and ^{15}N), to find tilt angles that are larger and nearer to simulations (~21°) [48]. The difficulty in incorporating the full extent of protein fluctuations when interpreting experimental data highlights the role fully-atomistic simulations can play in studying the structural responses to mismatch phenomena.

Using all-atom MD simulations, we investigate 25 membrane protein model systems, employing models of classical WALPs and WALP-like peptides of varying hydrophobic lengths to examine the structural changes brought about by hydrophobic mismatch. Prompted by the gA structure, which has four Trp residues at the interfacial ends of each monomer, we have varied the number of Trp residues from 1 to 4, to observe the effects on mismatch response (with a more detailed examination of Trp–lipid interactions in the accompanying study [32]). The structural adaptations and underlying energetics of mismatch response will be studied here to better understand the role of hydrophobic mismatch in membrane protein structure and stability, with implications for the activities of all integral and peripheral membrane proteins and membrane-active peptides.

2. Computational methods

2.1. Model systems

We have investigated a series of WALP and WALP-like peptides embedded in dipalmitoylphosphatidylcholine (DPPC) bilayers, chosen as it is the best characterized model membrane in MD simulations and a reasonable model for a biological membrane [50–54]. We acknowledge, however, that biological membranes may contain hundreds of lipid species and exhibit a range of topologies, mechanical properties, chemistries and heterogeneities [55,56]. Lipids of varying chemistry can lead to specific lipid–protein interactions that can determine hydrophobic mismatch response (e.g. phosphatidylethanolamine (PE) may exhibit stronger cation–π interactions with Trp-flanked peptides [56], discussed further in the accompanying study). However, in this study we investigate homogeneous DPPC bilayers as an illustration of the different peptide and membrane degrees of freedom responsible for mismatch phenomena, leaving investigation of the subtleties of compositional dependence for future studies.

The 25 independent model systems were built using previous membrane building methods [47,57,58], and include 20 smaller 48 DPPC lipid patches and five larger 96-lipid membrane patches. Each bilayer was hydrated with ~44 waters/lipid, with the total number of atoms being ~13,000 for the small bilayers and ~25,000 for the large bilayers. The WALP-like peptides span a wide range of hydrophobic lengths from 10.5 to 31.5 Å and have one of the two general sequences: acetyl-G$(W)_n$-$(LA)_m$-L-$(W)_n$-A-amide, referred to as $(LA)_mL(W_n)_2$, or acetyl-G$(W)_n$-$(LA)_m$-$(W)_n$-A-amide, referred to as $(LA)_m(W_n)_2$, depending on whether they have an even or odd number of residues in the hydrophobic L–A stretch. Those that have two flanking Trp residues ($n=2$) are the classical WALPs of Killian and co-workers [24], which we have simulated in large membrane patches for comparison and validation of our models. The peptide sequences are summarized in Table 1 where the approximate hydrophobic lengths have been calculated from the length of the inner L–A stretch assuming an ideal α-helical pitch of 1.5 Å/residue [59]. This choice of hydrophobic length measure is based on previous experimental observations that mismatch effects appear to correlate with the length of the hydrophobic stretch between flanking side chains, and not on the total peptide length itself [19,60]. Based on this, the $(LA)_8L$ series of peptides would provide the closest match to a DPPC bilayer, whose hydrophobic thickness is 26.5 Å [19], but will be explored below. The sample shown in Fig. 1a is that of a negatively mismatched peptide, $(LA)_5(W_1)_2$.

Hexagonal periodic boundary conditions were imposed, with the simulation box having a variable height of ~75 Å and xy-translation lengths of 44–45 Å. The latter dimension was based on a 64-Å^2 area/lipid ratio [61] and peptide cross-sectional areas ranging from 170 to 220 Å^2, calculated by probing the surface with a methylene-sized sphere. For each system, 50 ns of simulation was carried out for analysis, following an initial 6 ns of equilibration, representing a total of 1.4 μs simulation time.

For each of the five hydrophobic lengths studied, the number of Trp residues at each end of the helix varied from one to four. This produces an asymmetry in the arrangement of the Trp side chains around the helix (Fig. 1b) that will influence how the peptide resides in the membrane. The Trp side chains were initially built in a rotamer in which the indole N–H bond was directed toward the lipid–water interface, assuming that the hydrogen (H)-bonding between the N–H moiety and the lipid carbonyls is an important interaction [56,62]. The chosen orientation was among the possible rotamers of a Trp side chain within a poly-Leu-Ala α-helix (Fig. S1, specifically rotamers 1 and 3, for the top and bottom Trp side chains, respectively) and among those indicated by Dunbrack and Karplus [63]. However, as will be seen below, other allowed rotamers were visited during the simulations.

2.2. Molecular dynamics

The CHemistry at HARvard Macromolecular Mechanics (CHARMM) program [64] was used for this study, employing the PARAM27 force field [65–67]. Waters were modeled using the TIP3P model [68] and