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Free energy of WALP23 dimer association in DMPC, DPPC, and DOPC bilayers

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ABSTRACT

The MARTINI coarse-grained model is used to gain insight into the association of WALP23 helices in three different lipid membranes: DMPC, DPPC and DOPC. Potentials of mean force describing the association of two WALP23 helices embedded in different lipid bilayers indicate no barrier of association and a stabilization of more than 20 kJ mol^{-1} of the associated state relative to the fully dissociated state. Association is strongest in DMPC, followed by DPPC and DOPC. Helix–helix association appears to be enthalpically favorable in all lipid bilayers, while the entropic contribution appears favorable only in the presence of significant positive hydrophobic mismatch, in DMPC lipids. The interpretation of this requires care given the coarse-grained nature of the simulations, but the sign of the thermodynamic quantities agrees with experimental measurements on dimerization of $(AALALAA)_3$ peptides and the observed association free energies are within the experimental range. Both protein–protein and lipid–lipid interactions appear to strongly favor protein dimerization, while the interactions between a dimer and lipid are unfavorable relative to the interactions between two separated monomers and lipids. Dimers with antiparallel orientation appear to be thermodynamically favored over parallel dimers, particularly in conditions of greater hydrophobic mismatch, but elucidating the detailed origin of this likely requires simulations of helices for which there is structural data on the dimer. We analyze 3D density, membrane order, and membrane thickness maps using new freely available analysis programs. Although these properties differ somewhat for each lipid, perturbations extend to about 1 nm for lipid density, ~2 nm for ordering and ~2.5 nm for thickness. A striking feature is the appearance and extent of systematic density fluctuations around the helices.

1. Introduction

Cellular membranes consist of a phospholipid bilayer and a variety of proteins that are involved in a wide range of essential physiological processes. Membrane proteins are the primary connection between the inside and the outside of cells, for example acting as cell surface receptors (He and Hristova, 2012), as transporters of nutrients, metabolites (Giacomini et al., 2010) and other molecules including entire proteins (Driessen and Nouwen, 2008), as conduits for a variety of signals (Rosenbaum et al., 2009), and as key player in cellular processes such as endocytosis (Doherty and McMahon, 2009) and fusion (Sudhof and Rothman, 2009). In many cases, these processes involve changes in structure of transmembrane domains, including domain motions, dimerization of transmembrane helices, and larger-scale conformational changes. Therefore, understanding the relationships between sequence, structure, dynamics and functions of membrane proteins is of wide interest in many fields of medicine, biology, biochemistry, and physical chemistry.Functional proteins can be formed in the membrane via the association of domains with well-defined structure – for instance, in helical proteins, the association of previously inserted transmembrane helices. This is a key event in membrane protein folding and assembly (MacCallum and Tieleman, 2011; White and von Heijne, 2004; Wimley, 2012; Woolhead et al., 2004). Significant effort has been devoted to understand the driving forces and thermodynamics of this process in detail (Cymer et al., 2012; Fink et al., 2012; Langosch and Arkin, 2009; MacKenzie and Fleming, 2008; Strandberg et al., 2012). The association of proteins in membranes depends both on specific protein–protein interactions and on less specific protein–lipid interactions. For instance, it is well known that the transmembrane GxxxG motif of Glycophorin A (GpA) contributes to the formation of a stable right-handed helix dimer in membranes (Fleming and Engelman, 2001) – a clear example of specific protein–protein interaction, although this motif only occurs in a relatively small number of proteins and does not explain the binding affinity of a larger set of GpA mutants, thus encouraging

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the search for a broader explanation of helix–helix aggregation (Li et al., 2012). Protein association also depends on protein–lipid interactions. For example, a recent simulation study by Janosi et al. highlighted the importance of hydrophobic mismatch (i.e., the difference between protein hydrophobic length and the thickness of the hydrophobic region in the membrane, defined as positive when the protein hydrophobic length is longer than the hydrophobic thickness of the membrane) in determining the stability of GpA dimers in lipid membranes (Janosi et al., 2010a,b). In addition to direct protein–protein and protein–lipid interactions it is also quite plausible that indirect interactions such as lipid-mediated helix–helix interactions play an important role. Experimental studies have confirmed the key role played by membrane thickness on the association process of membrane proteins (Orzaez et al., 2005).

Specific and aspecific contributions are difficult to distinguish when studying biologically relevant proteins. Model systems are therefore a useful tool both in experiments and computational studies. Computational models range in complexity from two-dimensional idealized cylinders (Lague et al., 2001) and simplified particle-based simulations (Benjamini and Smit, 2012; de Meyer et al., 2008) to detailed atomistic models of proteins (Psachoulia et al., 2010). The use of small membrane proteins and model peptides with tunable features for experimental studies has been a particularly powerful approach that many research groups have followed to elucidate basic principles of transmembrane helix interactions (Killian and Nyholm, 2006; Langosch and Arkin, 2009; Vostrikov et al., 2010).

WALP23 (GWW(LA)$_8$LWWA) is one such model peptide that has been widely used as a model for more complex membrane proteins. WALP23 was designed in part to minimize protein–protein interaction to probe the effects of helix-bilayer hydrophobic mismatch on peptide behavior (de Planque and Killian, 2003; de Planque et al., 1998, 2001). Yet, fluorescence spectroscopy experiments showed a weak attractive peptide–peptide interaction between WALP23 helices (Sparr et al., 2005a). For these reasons, WALP23 appears to be an ideal system to study generic aspects of protein association in membranes.

Ash et al. carried out detailed free energy calculations on WALP23 and polyleucine, with excellent agreement in thermodynamic properties between coarse-grained MARTINI simulations and experimental measurements (Ash, 2009). Schafer et al. (2011) recently reported a computer simulation study combined with optical microscopy on WALP peptides (WALP23 and WALP31) in lipid membrane mixtures, proving that peptide association depends on the hydrophobic mismatch and determines partitioning of WALP between liquid crystalline and gel phases. Kim and Im (2010) performed molecular dynamics simulations on single WALP helices of different lengths (WALP16, WALP19, WALP23, and WALP27) in different lipid membranes. Even though the aim of this study was not directly related to the association event of WALP helices and the time scale was shorter than is likely required for full sampling (Monticelli et al., 2010; Schafer et al., 2011; Sengupta and Marrink, 2010), it did provide significant insight into helix-bilayer hydrophobic mismatch effects. Free energy profiles using as reaction coordinate the tilt angle of the helix with respect to the normal axis of the membrane suggested that the tilting of the helix is the major response to a hydrophobic mismatch. Similar qualitative results have been also observed in KALP peptides (GKK(LA)$_n$LKKA)(de Planque et al., 2001; Kandasamy and Larson, 2006; Killian and Nyholm, 2006). We also note that PMF calculations with MARTINI have recently been published for the interaction between two rhodopsins, a 7-helix G-protein coupled receptor (Periole et al., 2012).

WALP23 has been also used as a model peptide to determine helix orientation in the association process. Pyrene fluorescence experiments have shown that association between WALP23 peptides under conditions of hydrophobic mismatch primarily occurs when the helices are oriented antiparallel with respect to each other (Monticelli et al., 2010; Sparr et al., 2005b). Dithionite quenching experiments on Ac-(LALAAAA)$_3$-amide and fluorescence resonance energy transfer experiments on NBD-(AALALAA)$_3$-NH$_2$ yielded similar conclusions (Yano et al., 2002, 2006, 2011).

In this manuscript we present a detailed study of the antiparallel and parallel association of WALP23 helices in three different lipid membranes (DPPC, DMPC, and DOPC) at the coarse-grained level using the MARTINI force field (Marrink et al., 2007; Monticelli et al., 2008). We calculate potentials of mean force as a function of the lateral separation between the peptides in three different lipid membranes: DMPC, DPPC and DOPC, with different hydrophobic thicknesses and area per lipid. We are interested in features of the potential of mean force and membrane structure, in particular at a distance where one might imagine a single lipid between the two helices. In the association profile of two hydrophobic helices in aqueous solution there is a clear barrier in the enthalpy component when water no longer fits between the two helices, but this is compensated by an entropic well because water is no longer trapped in between helices (MacCallum et al., 2007). Similarly, in lipid bilayers a single lipid in between two helices might cause an entropic barrier, but it is also possible that lipids are too flexible to be an important factor. We analyze the lipid structure at different points along the PMF and make an attempt to elucidate the microscopic driving forces for helix aggregation, including the role of hydrophobic mismatch, helix orientation, and lipid properties.

2. Methods

2.1. System set up

The coarse-grained structures of antiparallel and parallel WALP23 dimers were generated using a four-to-one mapping from previous atomistic structures obtained by Sparr et al. (2005a). The minimum distance between the centers of mass of both helices was fixed at 0.75 nm by aligning the axes of both helices with the bilayer normal and translating one helix along the *X* axis. Additional structures were created with a larger separation (see below). The structures were then embedded in pre-equilibrated coarse-grained lipid bilayers (DMPC, DPPC, and DOPC) followed by a water solvation step using a procedure described by (Kandt et al., 2007). A typical snapshot is shown in Fig. 1. In MARTINI, DMPC has three tail beads per acyl chain, DPPC four tail beads per acyl chain, and DOPC five tail beads per chain, with the middle slightly less apolar (MARTINI type C3 instead of C1) and the angle between that bead and the two beads adjacent to it is 120° instead of 180° in saturated hydrocarbon chains. The three bilayers consisted of 272 lipids (136 per leaflet) and the total number of water molecules was 4872.

2.2. Molecular dynamics simulations and potential of mean force calculations

All molecular dynamics (MD) simulations were carried out using the GROMACS 4.0.5 software package (Hess et al., 2008) and the MARTINI force field (Marrink et al., 2007; Monticelli et al., 2008). Non-bonded interactions were calculated within a cutoff of 1.2 nm using shift functions (shift between 0.9 and 1.2 nm for Lennard–Jones interactions, between 0 and 1.2 nm for electrostatics). These parameters are standard for the MARTINI force field. Prior to potential of mean force calculations, energy minimization of the system was carried out with the steepest descent algorithm. For each system, minimization was followed by a short equilibration run (2.5 ns). The temperature of the systems was set to 325 K

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