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Folding Peptides into Lipid Bilayer Membranes

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Abstract: The adsorption, insertion, and folding of a synthetic 16-residue WALP peptide was studied at physiological time scales (>μs) by atomic detail molecular dynamics simulation using a fully explicit DPPC/DMPC lipid bilayer setup. The temperature was elevated to 80 °C/44 °C respectively to increase sampling. After spontaneous adsorption the peptide crosses the polar interfaces to locate at the hydrophobic bilayer core. Remarkably, insertion occurs before folding, and the dominant configurations are inserted beta-hairpins. For the DPPC simulation a trans-membrane helix formed but was not stable. Unfolded membrane insertion of WALP was first observed by Nymeyer and co-workers using a replica exchange method. However, both results are in stark contrast to current theory and simulations with implicit membrane models, which rule out unfolded insertion into the hydrophobic core. At present the exact reasons for this unexpected behavior cannot be unambiguously determined, due to the lack suitable experimental and simulation data to compare to. Nevertheless, the results demonstrate that simulation studies can now in principle provide atomic detail insights into complex biophysical phenomena at physiologically relevant time scales. Future effort must now concentrate on suitable ways to verify current force fields and methodologies for such simulations.

Folding and integration of peptides into lipid bilayer membranes remains one of the most intriguing processes in biophysics, as it cannot be directly observed at sufficient temporal and spatial resolutions. Recent experiments applying the translocon machinery to insert designed peptides[1,2] as well as statistical analyses of membrane protein structures[3] revealed that the distributions of individual amino acid types correlate strongly with their expected solvation energies along the membrane normal.[4] In addition, the Sec translocon structure revealed that nascent peptides are threaded into a narrow water filled channel, which opens laterally allowing hydrophobic segments to partition into the bilayer.[5] Many details of this process, including how much folding actually occurs inside the channel are currently unclear, but direct peptide-bilayer interactions (i.e., the solvation free energy) seem to be the key determining the partitioning and folding properties of a particular sequence. From a physical chemistry perspective transfer of solvated peptides into a hydrocarbon phase should follow a two-stage pathway,[6] where helical segments fold at the phase boundary before inserting, due to the high cost (estimated at ~4 kcal/mol) of desolvating exposed peptide bonds.[7,8]

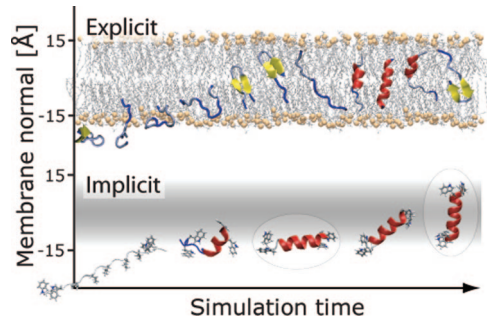

Figure 1. Folding pathways of the WALP peptide observed in an explicit lipid bilayer and the implicit membrane at 80 °C. The pathways is markedly different. In the implicit membrane, based on generalized Born theory, the folding pathway follows the two-state model as expected from current partitioning theory (adsorption, interfacial folding and folded insertion). In the explicit system there is no interfacial state, and insertion occurs in an unfolded conformation.

Computer simulations using implicit solvent models,[9,10] which treat the membrane as a low-dielectric slab, have confirmed this folding pathway for simple hydrophobic peptides,[11,12] including the synthetic WALP peptide (see Figure 1).[13] The peptide was found to quickly adsorb to the membrane surface. Stable insertion was only observed after interfacial folding into helical conformers. However, these models lack the complex polar bilayer interfaces and neglect entropic effects due to the liquid crystalline order of the lipids and water molecules and therefore do not represent a realistic membrane.

We therefore repeated the simulation in a fully explicit setup using the GROMOS96 force field (methods are given in the Supporting Information). As in the implicit system WALP was placed into bulk water and positioned parallel to the surface of a DPPC lipid bilayer in a completely extended conformation. The temperature was elevated to 80 °C to increase sampling

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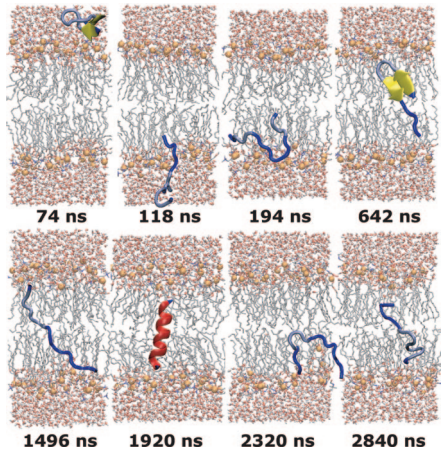

Figure 2. Adsorption, insertion, and folding of the WALP peptide in an explicit DPPC/SPC bilayer system. The peptide passes the lipid head-groups in an unfolded conformation and inserts into the hydrophobic core before folding. A large number of beta-structures are sampled, and several water molecules are dragged into the hydrocarbon core of the membrane.

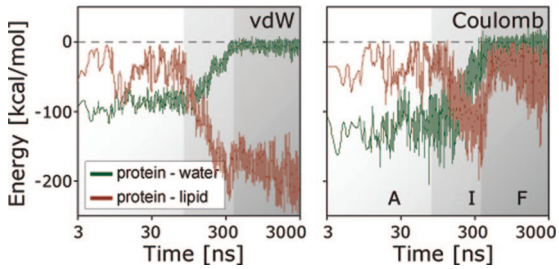

Figure 3. Interaction energies of the peptide with the water and lipid bilayer. Favorable van der Waals interactions of the peptide with the lipids are the main driving force for insertion, which occurs in the first 500 ns. Time is plotted logarithmically indicating the three distinctive phases: adsorption (**A**), insertion (**I**), and folding (**F**).

and ensure the lipids remain in the fluid phase. Below this temperature stably inserted WALP helices were observed to be the dominant configuration in the implicit membrane.[12]

The simulation was run for 3 μs and shows strikingly unexpected behavior (see Figure 2). First, adsorption is slow, and the peptide remains fully solvated for ~100 ns. After precipitating onto the membrane surface the peptide immediately starts to cross the phosphocholine head-groups. This process is driven by favorable van der Waals interactions and takes ~300 ns. During this time the peptide remains virtually completely unfolded, and lost electrostatic interactions with the water molecules are compensated by interactions with the polar head-groups, which are of the same order of magnitude (see Figure 3). Subsequently the peptide inserts into the hydrophobic membrane core in an unfolded configuration, and for the remaining ~2 μs the peptide oscillates between deeply inserted completely unfolded and misfolded conformations (beta-hairpins). Both the absence of a stable interfacial state and the unfolded insertion are remarkable, as is the persistence of fully inserted beta-hairpins, which are the dominant configuration (Figure 4). Such conformations should be less favorable than helices since only half the backbone hydrogen bonds can be

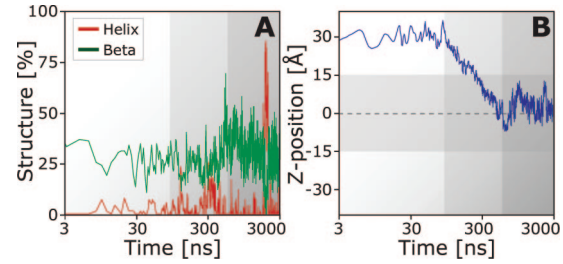

Figure 4. Peptide secondary structure (**A**) and insertion depth (**B**). The membrane center is at $z = 0$, and the hydrophobic core is shaded.

satisfied. Eventually, after ~1.9 μs rapid formation of a helical conformer is observed from a completely extended membrane spanning configuration. Surprisingly, however, this is not concomitant with further energetic stabilization as would be expected, and the helix remains stable for only ~200 ns before unfolding again.

An unfolded insertion pathway for WALP was first observed in explicit bilayer replica exchange simulations by Nymeyer and co-workers, on a time scale of ~4 ns, with the CHARMM22 force field.[14] Similar to the results presented here, insertion of unfolded conformations preceded the formation of helical structure. However, contrary to our results the trans-membrane helix was found to be stable at 350 K, the lowest temperature replica. Instead, we observe only marginal stability at roughly the same temperature (353 K), with several folding/unfolding events and no clear energetic stabilization of helical conformations over unfolded ones. In a control simulation an inserted helix also unfolded after ~300 ns, indicating that it is not stable at 80 °C.

We performed several other control simulations. A 1 μs simulation of WALP in a DMPC bilayer, which has a lower chain melting temperature of ~25 °C also showed unfolded insertion. However, unlike the DPPC case the peptide inserts only in one leaflet of the bilayer, and insertion is generally less deep than in the DPPC case. Due to the lower simulation temperature of 44 °C sampling was found to be significantly slower, which might explain the absence of trans-membrane configurations. The insertion process took ~400 ns, and no helical folding was observed.

We also performed a 1 μs simulation of an unfolded, inserted conformer in DPPC at a reduced temperature of 50 °C. The peptide was found to remain stably inserted in an unfolded configuration. Sampling is severely reduced, and some ordering of the lipids is observed as expected for temperatures close to the DPPC chain melting temperature.

At room temperature WALP generally forms stable membrane spanning helices. Unfortunately, no structural data are currently available for WALP at elevated temperatures. Furthermore, the insertion process is very difficult to study experimentally since few methods can provide the necessary submicrosecond time-resolution. Gai and co-workers have recently reported a promising method to explore the coil to helix transition associated with membrane binding via fluorescence resonance energy transfer.[15] This method has been applied to a range of peptides, and while they generally find folding to occur before insertion, they do not rule out unfolded insertion.

It should be noted that the experimental internalization of WALP into bilayers might differ from the present simulations. Like most hydrophobic sequences WALP is not soluble and aggregates in water. Insertion is therefore achieved by hydration of a WALP-lipid suspension, resulting in vesicles containing the peptide in a membrane spanning conformation, generally assumed to be helical.[16] Simulation of membrane peptides generally describe 'infinite' dilution scenarios (i.e., one WALP per bilayer), which is a reasonable approximation at low peptide-lipid ratios.

The above results can be interpreted in three ways: i. Unfolded insertion is an artifact and highlights major force field issues (water, lipid, peptide). This could be due to overestimation of the van der Waals interactions and entropic effects and/or an underestimation of the Coulomb terms and hence the solvation energies. This interpretation is supported by currently established theory, which dictates that only helical conformers can insert and reside stably in the membrane, due to the huge ~4 kcal/mol penalty to break a backbone hydrogen bond in the membrane.[8] ii. Folding and insertion of membrane bound peptides varies strongly with subtle temperature differences. iii. The results are genuine and suggest that entropic terms can compensate for the huge desolvation penalties associated with unfolded inserted conformers. This view can be supported by previous simulations of Nymeyer et al. who also observe unfolded insertion and subsequent folding for WALP using a different force field.[14]

Recent computational studies have revealed that the contributions of alanine and leucine side chain analogs, which make up the core of WALP residues, are favorable,[17−19] as expected from statistical analyses of membrane proteins and translocon mediated peptide insertion experiments.[1−3,20] However, burial of the peptide backbone as well as the formation of internal hydrogen bonds greatly contributes to the overall partitioning properties of peptides. In the absence of solid experimental evidence it is very difficult to conclude which of the many contributions is the dominant one since peptide partitioning into lipid bilayers is a complex process that requires very long time scale simulations with a large number of adjustable parameters (choice of lipids, temperature, force field parameters, simulation algorithms, etc.). More computational and experimental studies are needed to ultimately clarify these issues.

Since the simulation protocol and force field used are being very extensively employed by a large number of groups,[21] the results raise several important issues regarding long time scale simulations. The most important of which are the fundamental questions of how such simulations are to be verified and if current force field parameters and simulation methods are suited to allow accurate predictions of complex systems at physiological time scales.

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Supporting Information Available: Methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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