Peptide Partitioning Properties from Direct Insertion Studies

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ABSTRACT Partitioning properties of polypeptides are at the heart of biological membrane phenomena and their precise quantification is vital for ab-initio structure prediction and the accurate simulation of membrane protein folding and function. Recently the cellular translocon machinery has been employed to determine membrane insertion propensities and transfer energetics for a series of polyleucine segments embedded in a carrier sequence. We show here that the insertion propensity, pathway, and transfer energetics into synthetic POPC bilayers can be fully described by direct atomistic peptide partitioning simulations. The insertion probability as a function of peptide length follows two-state Boltzmann statistics, in agreement with the experiments. The simulations expose a systematic offset between translocon-mediated and direct insertion free energies. Compared to the experiment the insertion threshold is shifted toward shorter peptides by ~2 leucine residues. The simulations reveal many hitherto unknown atomic-resolution details about the partitioning process and promise to provide a powerful tool for urgently needed calibration of lipid parameters to match experimentally observed peptide transfer energies.

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The transfer of hydrophobic peptide segments into lipid bilayers to form independently stable transmembrane (TM) helices represents the crucial first step in membrane protein folding and assembly. However, the mechanisms and energetics underlying this process are currently not fully understood. Recently, a remarkable set of experiments has utilized the cellular translocon machinery to measure the insertion propensity of systematically designed peptides (1). This has provided the first quantitative estimates of the insertion threshold and partitioning free energies (ΔG) of short TM helix forming polyleucine peptides. Segments of the form GGPG-L_n-GPGG (n = 4–12) were engineered into the *Escherichia coli* leader peptidase carrier sequence, to find the minimum length required for TM insertion. The GGPG flanks serve as helix breakers insulating the guest segment from the host sequence. Stable TM insertion was found to require at least 10 leucine residues ($n \geq 10$), whereas peptides with $n \leq 8$ did not insert. Circular dichroism (CD) experiments of identical synthetic peptide constructs in oriented POPC bilayers gave qualitatively similar results, with $n \geq 10$ required for spectra consistent with TM helices (2). Others have reported minimum polyleucine lengths in a similar range ($n > 9$–12) for incorporation into synthetic bilayers, or translocon-mediated insertion into microsomal membranes (3).

Although a picture about the overall insertion propensities and translocon mediated transfer energetics is emerging, the conformational equilibria, insertion pathways and kinetics remain unknown, as experimental methods cannot currently resolve the peptides at sufficient spatial and temporal resolution. Molecular dynamics offers a powerful tool that can provide both atomic detail thermodynamic and kinetic data, provided the equilibrium insertion properties can be captured in their entirety within the timeframe of the simulations. In addition, simulations provide detailed insights into the peptide-bilayer interactions driving the partitioning process. We therefore used all-atom simulations to study the spontaneous partitioning of polyleucine peptides of the form ace-$(L)_n$-ame (n = 5–12) into POPC bilayers. The peptides were acetylated (ace) and amidated (ame) and initially placed 10 Å from the bilayer surface with fully extended backbones. Simulations were performed with gromacs 4.0 (www.gromacs.org), using the OPLS all-atom force field (4) in combination with united-atom lipids recently parameterized by us (5), and analyzed using hippo beta (www.biowerkzeug.com). See supporting information for more details.

To achieve converged partitioning trajectories within the currently feasible 1 μs simulation timeframe the temperature has to be elevated. Heating the system to 120°C increases sampling sufficiently for full convergence. This approach requires that upon heating 1), the insertion propensity does not change and 2), the peptides should be sufficiently thermo-stable that they retain their native helical structure.

The first issue was addressed by determining from the simulations the change in insertion propensity of the L_8 peptide upon heating. This was found to be invariant (Fig. 1 *B*), suggesting that elevated temperatures do not alter the partitioning process.

The second issue was addressed by a set of heating simulations for L_8 peptides. No significant loss of TM helicity was observed upon raising the temperature from 30 to

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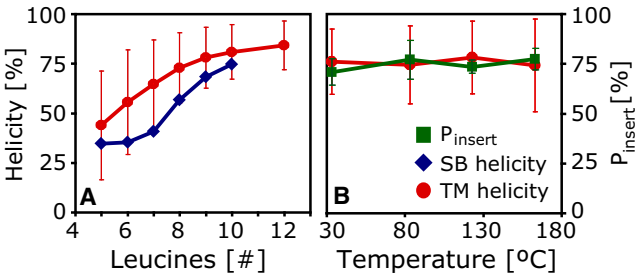

FIGURE 1 (*A*) Helicity versus number of leucines. Secondary structure is more pronounced for TM than SB as the peptide is more deeply buried in the hydrophobic phase, compared to the polar interface. Shorter peptides are less stable. (*B*) The insertion propensity of L_8 does not vary, suggesting the partitioning thermodynamics depends little on temperature.

160°C (Fig. 1 *B*). This can be attributed to the deep burial of the peptides in the hydrophobic membrane core, where the penalty of exposing unmatched backbone hydrogen bonds is too severe to allow unfolding even at highly elevated temperatures. These results are consistent with recent experimental findings that hydrophobic membrane-inserting model peptides display exceptional thermostability (6). CD spectra of tryptophan flanked hydrophobic core peptides (WALP: ace-AWW-$(LA)_n$-WWA-ame) incorporated into synthetic (DMPC, DPPC & DOPC) bilayers showed that the peptides remained fully helical even at 90°C. It is therefore reasonable to assume polyleucine peptides to be similarly thermo-stable.

Having established the feasibility of high temperature simulations, we proceeded to simulate spontaneous peptide insertion. Fig. 2 summarizes the partitioning behavior of L_n peptides with n = 6–10, which generally follows the three-stage thermodynamic model of White (7) and Engelman (8). Rapid absorption ($t < 10$ ns), consistent with insoluble hydrophobic peptides is followed by interfacial folding, which precedes insertion. For short peptides ($n \leq 6$) surface bound (SB) configurations dominate, whereas longer polyleucine segments ($n \geq 10$) insert to form stable transmembrane (TM) helices.

Peptides of intermediate lengths (n = 7–9) display an equilibrium alternating between SB and TM configurations. SB states generally have lower helicity than TM states as the peptide backbone can also form hydrogen bonds with interfacial water or the lipid headgroups (Fig. 1 *A*). In contrast, the TM state is in a completely apolar environment where helix formation is essential to lower the energetic cost of peptide backbone burial.

Two-state behavior is apparent in projections of ΔG as a function of peptide tilt angle and center of mass position along the membrane normal (Fig. 3). There is a continuous shift from SB to TM free energy minima as the peptide length is increased from 5 to 10 leucines. For very short peptides ($n \leq 7$) strong negative mismatch leads to widening of the TM minima because the helix can diffuse along the membrane normal, frequently residing in just one leaflet of the bilayer (Fig. 2). This phenomenon is less prominent for longer peptides.

The barrier separating the TM and SB minima is relatively low for all L_n peptides: L_6 and L_7 have $\Delta G^{\ddagger}_{SB\rightarrow TM}$ ~1 kcal/mol, while $\Delta G^{\ddagger}_{TM\rightarrow SB} = 2.2$ kcal/mol for L_8 and 3.4 kcal/mol for L_9, with a steady increase for longer peptides, for which insertion becomes dominant. Multiple transition events are observed for all peptides, demonstrating that ~1 μs is sufficient for full convergence of the trajectories at 120°C. Control simulations of L_8 at lower temperatures of 80°C and

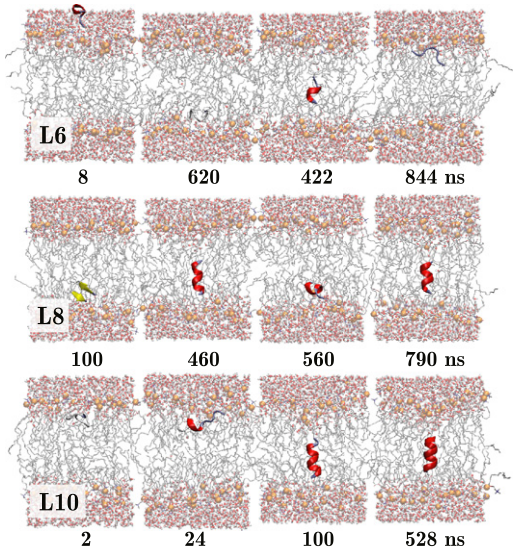

FIGURE 2 Peptide partitioning behavior of L_n peptides with n = 6–10. All peptides absorb rapidly. Shorter peptides largely remain at the interface, while longer peptides ($n \geq 8$) predominantly insert to form TM helices.

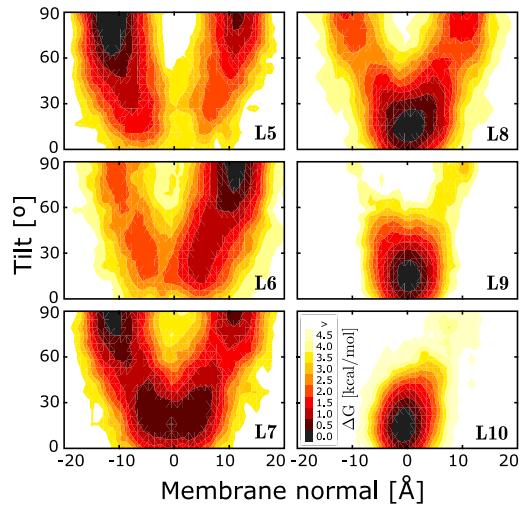

FIGURE 3 Free energy profile for L_n peptides (n = 5–10), as a function of position along the membrane normal z and tilt angle α. Smaller peptides ($n \leq 7$) have interfacial minima (z = ~12 Å, α = ~90°C), while for longer sequences ($n \geq 8$) the TM inserted minima dominate (z = ~0 Å, α = ~10°).

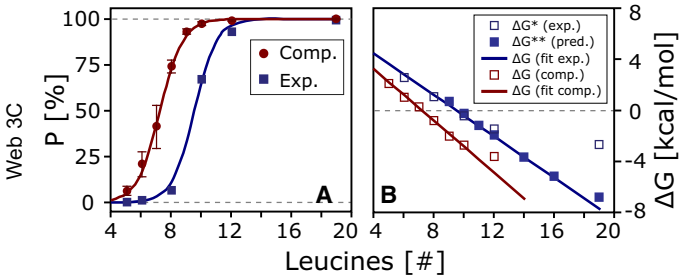

FIGURE 4 (*A*) Bilayer insertion efficiency as a function of peptide length *n*. The experimental values are for translocon mediated insertion into dog pancreas rough microsomes of GGPG-(L)$_n$-GPGG constructs embedded into the leader peptidase carrier sequence, as determined by Jaud et al. (2). The computed values are for spontaneous partitioning of ace-(L)$_n$-ame peptides into POPC lipid bilayers. Both systems display perfect two-state Boltzmann behavior ($R^2 > 0.99$). (*B*) Free energy of insertion as a function of peptide length. The straight lines indicate the two-state Boltzmann fit, while the data points show the computed (*red*) and experimental (*blue*) values for the individual peptides (*measured ΔG (1); **predicted ΔG (http://syrah.cbr.su.se/DGpred/).

30°C show identical behavior, but at greatly reduced rates (Fig. 1 *B*). For example, the average lifetime of the TM state is increased from 44 ns at 120°C to 97 ns at 80°C and 193 ns at 30°C. However, the population of the TM state (~76 ± 5%) does not vary, indicating that the effect of temperature on the partitioning thermodynamics is small (Fig. 1 *B*). This suggests that the hydrophobicity profile of the bilayer is the key force driving peptide partitioning.

The converged thermodynamics allow the direct quantification of the overall insertion free energy $\Delta G_n = -RT \ln(f_{TM}/f_{SB})$, and probability $p_n = f_{TM}/(f_{TM} + f_{SB})$ from the relative surface-bound f_{SB} and transmembrane-inserted f_{TM} populations. Jaud et al. (2) have previously shown that the experimental insertion propensity as a function of the number of leucine residues n can be fitted perfectly by a sigmoidal function $p_n = [1 + \exp(-\beta\Delta G_n)]^{-1}$, characteristic of a two-state Boltzmann system, where $\beta = 1/kT$. Fig. 4 *A* shows the experimental and computed insertion propensities together with the best-fit models ($R^2 > 0.99$). Both curves display two-state Boltzmann behavior, with a transition to TM-inserted configurations for longer peptides. Fig. 4 *B* shows that ΔG_n increases perfectly linearly with n in both simulations and experiment. However, the offset and slope varies slightly, reflecting a shift of the computed insertion probability curve toward shorter peptides by 2.3 leucine residues. This corresponds to a $\Delta\Delta G = 0.25 \pm 0.02$ kcal/mol per leucine offset between the experimental and computational insertion free energies.

The origin of this offset is not entirely clear and could have several reasons. Firstly, the experimental peptides contain GGPG flanks, which cannot form secondary structure and therefore might increase the penalty of insertion compared to the simulated constructs. Secondly, the noninserted state is probably different in both systems. In the simulations this is a surface bound helix, but the noninserted state from the translocon experiments is currently not known. Thirdly, the high temperature used in the simulations might alter the strength of the peptide-bilayer interactions, thus shifting the TM/SB equilibrium, although this was not observed for L_8. Finally, the results might simply reflect that the lipid parameters used may be too hydrophobic and need tuning. Determining which of the above factors contribute significantly will require more experiments and simulations.

The results are remarkable since they demonstrate that all-atom computer simulations can now be used to directly determine peptide partitioning properties, with excellent qualitative agreement with experimental observations. The approach outlined above suggests a new avenue for measuring and calibrating atomic detail water-to-bilayer transfer properties of arbitrary polypeptides. Since these properties are critical to the structural stability of membrane proteins, and consequently their function, their accurate theoretical description and precise quantification is of the utmost importance.

SUPPORTING MATERIAL

Methods and references are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00408-X.

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