A Monte Carlo Mode of fd and Pf1 Coat Proteins in Membranes

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Condensation of the Research

Purpose of the Study

To use Monte Carlo simulations to explore the driving forces for membrane peptide insertion and to predict the tertiary structures of fd and Pf1 virus coat proteins in a lipid bilayer

Background

The transport of proteins into and across membrane-limited cell compartments plays a very important role in cell function.[1] Obviously, protein insertion into cellular membranes is a key step in such processes. However, this mechanism is not well understood. Experiments suggest that the in vivo insertion process of large proteins is driven by a complex cellular machinery.[2,3] In contrast, for small proteins in vitro insertion can be spontaneous.[4,5] These disparate results suggest that either small and large proteins insert by different mechanisms[6] or perhaps the cellular translocation machinery catalyzes what is already a spontaneous process.[7] If so, then insertion can be understood on the basis of thermodynamic principles, and one can hope to use computer simulations to provide insights into the general features of membrane protein folding and translocation.

The hydrophobic effect is believed to be the most important force that drives a membrane protein into the bilayer.[7,8] However, because a protein cannot form hydrogen bonds with the lipid tails, only intraprotein hydrogen bonds are allowed. Within a membrane, the absence of saturated intraprotein hydrogen bonds would be energetically very expensive, and the necessity of forming intraprotein hydrogen bonds is also a crucial effect that must be accounted for.[7] Thus, Engelman and Steitz have conjectured that helical hairpin structures form during the insertion process.[9] This model has been

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further refined by Jacobs and White who suggested that insertion commences by protein adsorption at the membrane interface.[7] Such adsorption partially reduces the chain configurational entropy and serves to enhance the probability of intraprotein hydrogen bond formation. Thus, at the very least, helices are conjectured to preform prior to their insertion into the membrane.

If one wishes to model this insertion process, a key question is the level of detail required to capture the essential physics. As a step toward addressing this issue, a number of investigators have undertaken simulations at full atomic detail. They take as a starting conformation a peptide–protein that is already in the bilayer.[1,10–12] Such studies have provided a variety of insights into membrane–protein interactions. However, the simulation of the insertion process would be very time consuming. Given that numerous simulations must be done to establish the reproducibility of the results, studies at atomic resolution are just beginning to become computationally tractable.

What Researchers Accomplished

In this work, the authors have adopted a different viewpoint and have developed and applied a simplified model to simulate the insertion of small proteins from the aqueous phase. The membrane itself is treated as a structureless hydrophobic medium, flanked by two interfacial regions that are contiguous to the aqueous phase. Within the "membrane," there is a large energetic cost when intraprotein hydrogen bonds are absent. The model also includes a transfer free energy of amino acids from the water to the "membrane" phase. The protein itself is modeled by a string of beads located at the backbone α–carbon positions. Unlike earlier work that confined these beads to a lattice,[13] a continuous space model was used here.

Simulations were performed on Pf1 and fd virus coat proteins. These proteins were chosen, because experimentally, in spite of their low sequence similarity, both adopt a very similar conformation in the presence of a membrane.[14,15] This structure consists of a transmembrane C-terminal helix and an interfacial, amphipathic N-terminal helix. Each protein was started as a random conformation in the aqueous phase, and a number of independent folding simulations were performed. Both proteins exhibited a very similar assembly mechanism. A typical simulation is depicted in Figure 1. Starting from a random conformation, the peptide chain was quickly adsorbed at the interface and then formed a slightly distorted, but almost fully helical, structure. In most simulations, this step was very rapid, and the system spent a large portion of the simulation time in this surface-adsorbed state. Then, the central portion of the C-terminal helix penetrated the bilayer. At this point, the C terminus itself being charged was still located at the membrane interface. The C terminus then broke loose from the interface and drifted across the bilayer, after which the final structure formed very quickly. Note that the C terminus crossed the membrane as part of an almost fully formed helical fragment. The final conformation of each peptide was very similar to that observed by NMR experiment. According to the model, the similarity of the conformations of the two proteins is due to their similar hydrophobic patterns. Finally, in other work using the identical model,[16] these authors have also been able to successfully predict the orientation and conformation of a number of other peptides including Magainin2[17] and M2δ.[17] For those peptides that form transmembrane helices, a similar picture of the insertion process emerged.

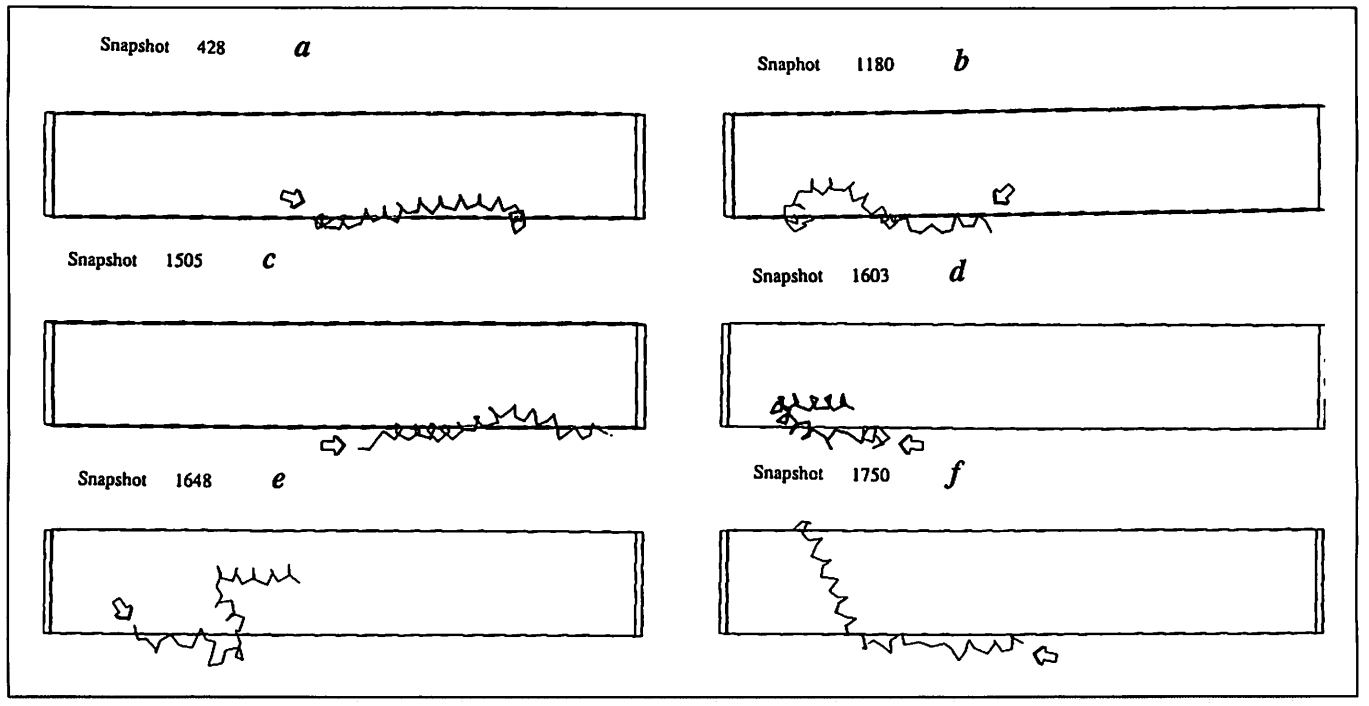

Figure 1. Snapshots from a typical simulation of the membrane insertion process of the fd filamentous bacteriophage coat protein. The arrows indicate the protein's N terminus. (Reproduced, with permission, from Milik, M., Skolnick, J. *Biophys. J.* **1995**, *69*, 1382. Copyright © 1995 by The Rockefeller University Press.)

Commentary on the Research

This research demonstrates that simplified models of membrane proteins can be used to provide a number of insights into aspects of the mechanism of membrane protein insertion. It provides substantial theoretical support for the Engelman-Steitz helical hairpin insertion process[9] as modified by Jacobs and White.[7] In this picture, spontaneous peptide insertion into a bilayer occurs in two steps. First, a helix forms at the interface. This is then followed by insertion of the fully formed helix into the bilayer. These simulations further support the idea that the pattern of hydrophobicity in the sequence dictates that location of the transmembrane helices.[7,8,18,19] Based on its success for a number of peptide fragments, it appears that a fairly robust methodology for predicting the orientation and location of membrane peptides has been developed.

There are, however, a number of crucial limitations to this kind of approach. First and foremost, it is not generally true that the membrane can be treated as a structureless medium. For example, simulations of highly curved membranes (where each lipid molecule is modeled as a dumbbell) indicate that transport from the outside to the inside is essentially irreversible.[20] This effect vanishes when the membrane is modeled as a structureless hydrophobic medium. Obviously, the details of the composition of the lipid are also important; these parameters are entirely ignored in the simplified approach described in this study.[21,22] Such simplified models will probably work reasonably well for depicting the robust features of the peptide insertion process but will inevitably fail for those cases in which the details are impor-

tant. A final and crucial limitation of this approach is that the present formulation cannot be used to predict membrane protein tertiary structure. Even though the model can describe the formation of secondary structure, it lacks terms that adequately describe tertiary interactions within a bilayer. In the case of globular proteins where there are many solved crystal structures, one can obtain an estimate of such terms.[23] The paucity of solved membrane protein structures precludes an analogous approach at present for membrane proteins. Thus, other less straightforward approaches will have to be tried.[24]

Summary

This paper demonstrates that one can use simplified models to predict both the mechanism of peptide insertion into a bilayer as well as the orientation and conformation of the peptide with respect to the membrane region.

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