Lipid Composition Influences the Insertion and Folding of pHLIP Peptides

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The study of polypeptide insertion into biological membranes can inform our understanding of membrane protein stability and folding, and has potential practical applications. This has been hard to investigate, since peptides hydrophobic enough to form transmembrane helices are likely to be insoluble in aqueous buffers, and soluble peptides are unlikely to insert. pH-Low Insertion Peptides (pHLIPs) provide an opportunity to study insertion since they are soluble monomers that bind to bilayer surfaces at neutral pH, and can be triggered to form monomeric transmembrane helices in acidic conditions. This set of unusual properties allows the study of spontaneous insertion and exit of a transmembrane polypeptide by changing the pH of its microenvironment. Previous studies showed that pHILP inserts into a POPC liposomes through rapid formation of an interfacial helix (~ 0.1s), followed by a slow insertion pathway. The time-course of pHILP insertion can be changed by varying the number of protonatable groups at the inserting end of the peptide, which need to be moved across the bilayer membrane.

We have employed various biophysical methods: fluorescence spectroscopy, anisotropy, CD, OCD, and stopped-flow fluorescence, to study the pHILP insertion into bilayers composed of different monounsaturated lipids (dC(14:1)PC, dC(16:1)PC, dC(18:1)PC, dC(20:1)PC, and dC(22:1)PC). We found that pHILP can form a TM helix in bilayers of different thickness. The kinetics of pHILP insertion vary, and correlate with bilayer thickness and fluidity. Further, pHLIP association with bilayer surfaces also depends on the lipid composition. The activation energy of pHILP insertion increases with the membrane thickness but the process of inserted helix formation does not significantly depend on the membrane type. A model for membrane-associated insertion/folding is discussed. This work was supported by grants from National Institute of Health, GM073857, and CA133890.