ABSTRACT

The field of membrane protein folding is relatively new compared to soluble protein folding. This thesis describes spectroscopy investigations of the refolding and dynamics of a β-barrel membrane protein. The amphiphilic, β-barrel outer membrane protein A (OmpA) refolds and inserts directly into a lipid vesicle or micelle from a denatured state in aqueous urea solution. Spectroscopic probes used to study this system are native tryptophans located at positions 7, 15, 57, 102, and 143. Steady-state and time-resolved fluorescence measurements have been performed using single tryptophan mutants of full-length OmpA (325 residues) and the truncated, transmembrane domain (176 residues). Both full-length and truncated mutants exhibit similar tryptophan emission lifetimes, suggesting that the transmembrane microenvironment is not greatly perturbed by the presence of the C-terminus.

While the microenvironments of folded full-length and truncated OmpA appear similar, the dynamics of refolding at each tryptophan position exhibit subtle differences when the C-terminus is present. Specifically, we observe that tryptophan-102, which faces the pore interior, inserts and folds the fastest while tryptophan-7, which does not cross the bilayer, is the slowest. Fluorescence anisotropy decays also indicate that tryptophan-7 is the most flexible residue compared to the other tryptophans. Temperature studies below the lipid gel-liquid transition temperature have also been performed. In the lipid gel phase, OmpA adsorbs to the surface of the vesicles but contains immediate β-sheet structure upon folding as well as very hydrophobic tryptophan environments. It is still uncertain from ensemble measurements whether this species is a true intermediate.

Fluorescence energy transfer kinetics have successfully determined the intramolecular distance between tryptophan-7 and cysteine-175 labeled with a dansyl fluorophore. These results reveal that the barrel ends of OmpA come into contact early in the refolding process and remain close together up to the final assembly of the barrel. We also have evidence that the adsorbed species at low temperatures is not an intermediate in the folding pathway since no energy transfer is observed for this species. These spectroscopic investigations have provided the foundation for further fundamental studies to dissect the molecular mechanism of the folding pathway of OmpA as well as other integral membrane proteins.