CHAPTER 1

Introduction to membrane protein folding

1.1 INTRODUCTION

The protein folding problem

A challenging and fundamental question in biology is determining how biomolecules fold from a highly disordered polymer to their biologically active and unique native structures during biologically relevant timescales. Protein misfolding has been implicated in diseases such as Alzheimer's, Parkinson's, and type II diabetes (Dobson, 2001; Dobson, 2001). Therefore, a deeper understanding of the structures, energetics, and dynamics of unfolded, partially folded, and folded proteins can provide clues to understanding these diseases.

The field of protein folding has grown significantly since Anfinsen's early folding studies of ribonuclease. These classic studies led to the hypothesis that the protein sequence contains all the necessary information for formation of the biologically active three-dimensional structure (Anfinsen, 1973). The amino acid sequence determines the folding mechanism of the protein and thus, its native structure. If proteins were to fold by searching all possible conformations, the folding time for a protein, even as small as ~100 amino acids, would not take place on biological timescales (Levintha.C, 1968; Levinthal, 1969). Instead of a single folding pathway, a revised model suggested that multiple folding pathways in a funnel-like energy landscape are occurring. Individual protein molecules fold on their own pathway in this energy landscape to obtain the lowest energy native state. Proteins that collapse to the native topology will fold faster than those that collapse to the non-native topologies.

In the years following these early works, there has been tremendous progress in the overall understanding of protein folding due to advances in experimental methods and

computational concepts (Dill & Chan, 1997; Onuchic et al., 1997; Daggett & Fersht, 2003; Winkler, 2004). Although there is no theoretical agreement on the folding process, the kinetic partitioning model (KPM) summarizes the key concepts in this folding problem (Guo & Thirumalai, 1995; Dill & Chan, 1997). These concepts describe the free energy landscape as complex and containing both a global minimum that corresponds to the native state and other low energy minima where misfolded conformations are adopted. Therefore, the folding process includes both direct and indirect pathways to the native state. A fraction of the unfolded molecules directly reach the native state without formation of intermediates while the remainder of the population undergoes a more complex multistep folding mechanism.

Membrane protein folding

Approximately a third of cellular proteins are composed of integral membrane proteins serving important functions such as enzymes, gates, pumps, and receptors (Wallin & von Heijne, 1998; Bowie, 2000). With only about 0.2% of structures solved, membrane proteins have been labeled as the "last frontier" of structural biology. Membrane protein misfolding is implicated in diseases such as cystic fibrosis, Charcot-Marie-Tooth disease, diabetes, and retinitis pigmentosa (Naef & Suter, 1998; Garriga & Manyosa, 2002; Stojanovic & Hwa, 2002; Sanders & Myers, 2004) and approximately 60-70% of therapeutic drugs target membrane associated proteins. Many bacterial pore-forming toxins, such as α-hemolysin and the anthrax protective antigen, are also membrane associated (Song et al., 1996; Nguyen & Kamio, 2004; Bayley et al., 2005). By studying *in vitro* membrane protein folding, we can gain insight into the complex molecular mechanisms of this problem *in vivo*. Application of the established folding

concepts and models would allow the development of methods to target folding intermediates and assembly stages of membrane proteins in hopes of finding new therapeutics.

Difficulty in obtaining high-resolution structures for membrane proteins has caused a lag in our understanding of membrane proteins compared to the extensively studied soluble proteins (Bowie, 2005). However, since the first structure of bacteriorhodopsin was resolved in 1975 by electron microscopy (Henderson & Unwin, 1975), our understanding of how these membrane proteins fold has increased as new structures have been reported in recent years. Today, approximately 120 unique membrane protein structures have been solved (White).

Despite significant progress towards understanding the structure and function of membrane proteins, complex fundamental questions still remain. The molecular mechanisms of folding, insertion, and assembly of integral membrane proteins that give rise to functional structures are still unknown (White & Wimley, 1999). There are two major structural classes of integral membrane proteins, the transmembrane α-helical bundles and the transmembrane β-barrels (Bowie, 2005). Membrane protein folding is expected to differ from soluble protein folding because membrane proteins reside in variable and anisotropic environments (White & Wimley, 1999). These proteins are present in three different environments; the aqueous phase, the water-membrane interface, and the intramembrane space. Interactions with lipids or detergents are important to the folding and stability of membrane proteins (Lee, 2004). Membrane protein folding must somehow involve binding to the membrane interface, insertion into the membrane, and the final assembly. Similar to the protein folding problem for soluble

proteins, membrane protein folding likely occurs down a funnel-like energy landscape to some minimum representing the native state (Dill & Chan, 1997). Indeed, experimental evidence shows that the folding of bacteriorhodopsin exhibits multiple pathways (Lu & Booth, 2000).

1.2 THESIS OVERVIEW

The experiments presented in this thesis utilize spectroscopic techniques to investigate membrane protein folding and dynamics using the outer membrane protein A (OmpA), a β-barrel membrane protein. The specific aims of this research are to (1) investigate the role of the C-terminal domain in the refolding mechanism, (2) its effects on the transmembrane structure, and (3) to further characterize folding intermediates by elucidation of site-specific intramolecular distances as the protein evolves from an unfolded state to the folded, native structure. Chapter 2 describes protocols in molecular biology used for construction, expression, and purification of OmpA mutants as well as preparation and characterization of phospholipid vesicles. Chapter 3 discusses circular dichroism and fluorescence spectroscopic data that probe the structural features of the unfolded and folded states of single tryptophan OmpA mutants in micelles and vesicles. Chapter 4 describes experiments using brominated lipids to quench tryptophan fluorescence in the folded OmpA to further characterize the transmembrane structure. Chapter 5 addresses the role of the C-terminal domain in the refolding kinetics of OmpA into the lipid bilayer by monitoring the development of secondary structure and changes in tryptophan environment and solvation. Finally, in Chapter 6, fluorescence energy transfer kinetics are utilized to investigate site-specific intramolecular distances of

OmpA. Specifically, the formation of the β-barrel ends is addressed. These fluorescence energy transfer kinetic experiments are the first to explore the refolding of an integral membrane protein and lay the foundation for further advanced fluorescence studies of this "last frontier" of structural biology.

1.3 REFERENCES

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