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Review

Conformational stability and folding mechanisms of dimeric proteins

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ABSTRACT

The folding of multisubunit proteins is of tremendous biological significance since the large majority of proteins exist as protein–protein complexes. Extensive experimental and computational studies have provided fundamental insights into the principles of folding of small monomeric proteins. Recently, important advances have been made in extending folding studies to multisubunit proteins, in particular homodimeric proteins. This review summarizes the equilibrium and kinetic theory and models underlying the quantitative analysis of dimeric protein folding using chemical denaturation, as well as the experimental results that have been obtained. Although various principles identified for monomer folding also apply to the folding of dimeric proteins, the effects of subunit association can manifest in complex ways, and are frequently overlooked. Changes in molecularity typically give rise to very different overall folding behaviour than is observed for monomeric proteins. The results obtained for dimers have provided key insights pertinent to understanding biological assembly and regulation of multisubunit proteins. These advances have set the stage for future advances in folding involving protein–protein interactions for natural multisubunit proteins and unnatural assemblies involved in disease.

Contents

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1. Introduction

Protein folding can be considered as the final step in the central dogma of life i.e. DNA encodes RNA encodes proteins, which must then fold in order to achieve their functional native state. Since the early 1980s, there have been tremendous advances in experimental and theoretical studies of protein folding that have revolutionized our understanding of the processes and principles of protein folding, as well as the misfolding of proteins, which often leads to disease. The majority of these studies have focused on monomeric proteins, which have been extensively characterized at atomic resolution using numerous experimental and theoretical methods (for reviews see Dill et al., 2007; Dinner et al., 2000; Gianni et al., 2003; Han et al., 2007; Lindorff-Larsen et al., 2005; Wolynes, 2005). Due to their relatively small size and simplicity, monomeric proteins were particularly tractable subjects for initial in depth analyses. However, monomeric proteins represent only ~19% of proteins in *Escherichia coli*, and the percentage is even lower in higher organisms (Goodsell and Olson, 2000). Among oligomeric proteins, including homomers and heteromers, the most common assemblies are homodimers, which constitute about 38% of proteins in *E. coli* (Goodsell and Olson, 2000). In recent years, many folding studies have been conducted on oligomeric proteins, in particular on homodimers, for which there is now a considerable body of data (Bjelic et al., 2006; Blond and Goldberg, 1985; Gittelman and Matthews, 1990; Guthe et al., 2004; Luke et al., 2006; Mateu et al., 1999; Milla and Sauer, 1994). Given the preponderance and biological importance of oligomeric proteins, it is essential to extend our understanding of monomeric proteins to higher order complexes.

A dimeric protein can be considered as a stable complex composed of two identical or nonidentical subunits. An increased complexity is introduced, however, if one extends the definition of dimers to incorporate a continuum of states which at one end includes weak and/or transient complexes as well as crystal structure dimers, and at the other end highly intertwined dimers that are only folded when part of a complex (Levy et al., 2004). The details of the protein–protein interactions that lead to strong, weak and transient association have been examined in several studies and reviews (Lukatsky et al., 2007, 2006; Nooren and Thornton, 2003b; Levy et al., 2004). We consider here the study of proteins which, under non-denaturing conditions, have a dissociation constant on the order of 10^{-6} or lower so that the majority of their monomeric subunits are in the associated dimeric state at a concentration of 10 μM dimer. Previous studies on the folding of dimeric proteins (Mei et al., 2005; Neet and Timm, 1994; Tiana and Broglia, 2002) and oligomeric proteins (Jaenicke and Lilie, 2000; Ragone, 2000) have been reviewed; the focus here is on recent quantitative analyses of denaturant induced folding transitions of dimeric proteins, as these have been studied the most extensively and trends are becoming clear. In addition, the folding behaviour of dimers illustrates principles that are pertinent to the folding of other oligomers. We will describe first the common models used to analyze dimeric protein folding, emphasizing key differences from monomer folding (Figs. 1–8, Tables 1 and 2). The critical fundamental difference lies in the change in molecularity that occurs for dimeric proteins, which has ramifications for many aspects of data analysis and interpretation that have frequently been overlooked. Next is a summary of equilibrium and kinetic experimental results obtained for dimeric proteins (Tables 3 and 4). The majority of these proteins exist as homodimers under moderate solution conditions (20–25 °C, pH 7–8). Noted exceptions are R67 DHFR which is studied at pH 5.0 since it is tetrameric at pH 8.0, and luciferase which is a heterodimer. Due to space restrictions, it is not possible to review all papers on dimers and so common folding behaviours are highlighted using illustrative examples. We conclude with a discussion of general trends and biological implications of the observed folding mechanisms, including regulation of function and protein misfolding in disease.

2. Overview of folding models of dimeric proteins

In principle, the folding of dimeric proteins can occur via many different mechanisms, ranging from the simplest mechanism of a two-state transition involving only native dimer (N_2) and unfolded monomers (U), to also forming various numbers of monomeric (M) or dimeric intermediates (I_2). We will focus on the most commonly observed folding mechanisms: two-state, and three-state with monomeric or dimeric intermediates (Fig. 1, Schemes 1–3).

The stability and folding mechanisms of proteins are commonly analyzed using equilibrium chemical (guanidinium chloride or urea) denaturation and renaturation curves combined with measurements of the unfolding and refolding kinetics (Fersht, 1999b,d). Complementary thermodynamic data can also be obtained by calorimetry, which have been reviewed (Privalov and Potekhin, 1986; Sturtevant, 1987); our focus here is on chemically induced structural transitions. The measured experimental quantities are the Gibbs free energy change for a structural transition (ΔG) which is the difference in the Gibbs free energy between two states (i.e. folded, intermediate, unfolded) and the denaturant dependency of ΔG, or *m*-value, which is proportional to the change in solvent accessible surface area for the structural transition (ΔASA) (Myers et al., 1995). In order for experimental results to be valid, it is essential that folding be reversible, that equilibrium is reached prior to measurement of denaturation and renaturation curves, and that these curves are analyzed using the appropriate model. The equations governing the equilibrium and kinetic transitions for dimers (Fig. 1) have been described in detail (Mallam and Jackson, 2005; Nölting, 2006; Park and Bedouelle, 1998) and are summarized in Tables 1 and 2. These equations were used to model equilibrium curves and input into the program Kinsim (Barshop et al., 1983) to illustrate by simulation some principles of dimeric protein folding transition and how these differ from those of monomeric proteins (Figs. 2–8). Note that for proteins that bind ligands, related but different equations are used (Rumfeldt et al., 2006); these are not discussed further here.