Protein folding mechanisms

Victoria Morton, Claire Friel, Graham Spence, Stuart Knowling, Eva Sanchez Cobos, Daniel Lund, Inigo Rodriguez-Mendieta, Alastair Smith and Sheena Radford

Introduction

One of the greatest challenges in modern structural biology is to understand how a newly formed polypeptide sequence finds its correct and unique fold. Real progress has been made towards establishing a fundamental and universal mechanism by which protein folding takes place, and such advances have come from the combination of experimental and theoretical techniques. In our laboratory we are studying the folding of three all α-helical proteins. The four-helix bacterial immunity proteins Im7 and Im9 have been the focus of our studies for 8 years. These proteins are structural homologues with high sequence identity, however they fold with mechanisms of different complexity. Im7 has been shown to fold *via* a compact, helical, on-pathway intermediate, while under the same conditions Im9 folds directly from the unfolded to the native state. We have shown the folding landscape of these closely homologous proteins to be finely balanced, such that small changes in sequence, or minor alterations in the folding conditions, can switch the kinetic mechanism of folding from two- to three-state. More recently we have extended our studies to include the ultra-fast folding three-helix bundle B domain of staphylococcal protein A (BdpA). We are using an array of biophysical methods to explore the folding landscapes of these three proteins, including laser temperature jump, ultra-rapid mixing, stopped flow and NMR.

Ultraviolet resonance raman studies of Im7

Understanding the nature of partially folded proteins is challenging and best accomplished by combining several techniques. Ultraviolet resonance raman (UVRR) spectroscopy studies have been used to study the environments of the tryptophan and tyrosine residues in Im7, Im9 and variants of Im7 specifically designed to trap partially folded states at equilibrium. Results show that the environments of the tryptophan and tyrosine residues in the native state of wild-type Im7 and Im9 are indistinguishable, whereas these aromatic residues experience a more hydrophobic environment in the partially folded intermediate of Im7. These data suggest, therefore, that non-native interactions involving aromatic and aliphatic side chains play a role in the folding of Im7.

Sulphate-induced effects in the on-pathway intermediate of Im7

Im7 folds through an on-pathway intermediate which has been shown to contain three of the four native helices. The structural properties of this intermediate were previously determined in the presence of 0.4M Na_2SO_4. To determine the effect of Na_2SO_4 on the properties of the Im7 intermediate, the folding of Im7 has been studied as a function of the concentration of Na_2SO_4 (0-0.4M) (Fig. 1). Detailed kinetic analysis, including Φ-value analysis at 0.2M Na_2SO_4, has shown that the structural properties of the intermediate are not significantly altered by the addition of Na_2SO_4. Therefore, whilst sulphate stabilises compact species on the Im7 folding landscape it does not alter their structural properties, confirming that the non-native interactions observed during Im7 folding are a generic feature of the folding of this polypeptide sequence.

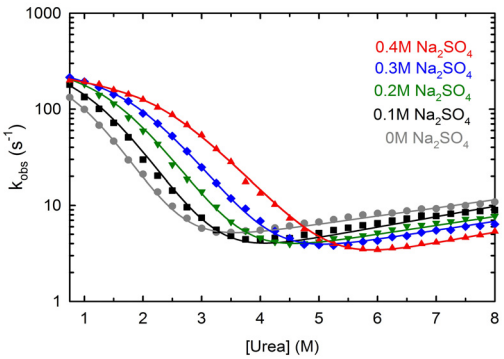

Fig. 1. Folding and unfolding kinetics of Im7 at different concentrations of Na_2SO_4. Continuous lines show the best fit to the three-state on-pathway model.

Semisynthesis of a glycosylated analogue of Im7

To establish a system to address questions concerning the influence of glycosylation on protein folding pathways, we have set up a new project to create *N*-linked glycosylated variants of Im7 using a semisynthetic approach. The strategy involves the native ligation of a glycosylated synthetic peptide with a bacterially expressed fragment (Fig. 2). An initial study of one such variant has shown that semi-synthetic Im7 analogues are well suited for protein folding studies, and that methods are available which will allow the complete characterisation of the role of individual sugar moieties on the folding free energy landscape of a protein. Further studies are currently underway using other glycoprotein analogues of Im7.

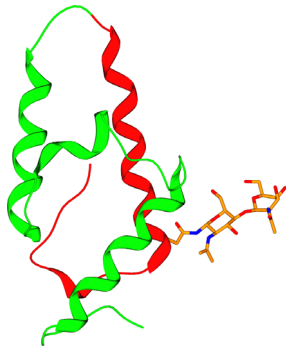

Fig. 2. Semisynthesis of Im7 involves the native ligation of a glycosylated synthetic peptide (red) with a bacterially expressed fragment (green), to create a glycoprotein with a disaccharide placed uniquely in the polypeptide chain.

Helix stability and hydrophobicity in the folding mechanism of Im9

The mechanism of protein folding has been proposed to be determined by the balance between the stability of secondary structural elements and the hydrophobicity of the sequence. To decipher the role of these factors in the folding of Im9, the secondary structural propensity or hydrophobicity of helices I, II and IV in Im9 was altered by substitution of residues at solvent exposed sites. The results of this study support a diffusion-collision model for immunity protein folding, in which intermediates are predicted to be stabilised both by increased helical propensity and by increasing stabilising contacts between helices by optimisation of either native or non-native contacts.

Outlook

In the next stages of this work, we are focusing on the fastest, earliest events in folding, using a combination of site-directed mutagenesis, Φ-value analysis, laser-induced temperature jump, ultra-rapid mixing techniques, and also a newly developed version of diffusion collision theory to analyse the folding of the four helix immunity proteins and the three-helix bundle B domain of staphylococcal protein A.

Collaborators Barbara Imperiali, Massachusetts Institute of Technology.

Publications

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