Folding and misfolding of the four-helix proteins Im7 and Im9

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Introduction

Most single domain proteins can fold spontaneously from a random coil into a precise three-dimensional structure within a few seconds. Understanding how this transition occurs would not only help us to uncover the way in which an amino acid sequence codes for a protein structure, but is likely to provide significant insight into the misfolding processes that underlie a variety of diseases. In order to shed light on the mechanism of protein folding we are studying the folding of two homologous four-helix proteins, Im7 and Im9 (see Fig. 1). Although these proteins have the same native structure, and 50% sequence identity, our previous work has shown that Im7 folds through a rapidly populated on-pathway intermediate whereas, under identical conditions, the more stable homologue, Im9, apparently folds without populating a partially folded intermediate state.

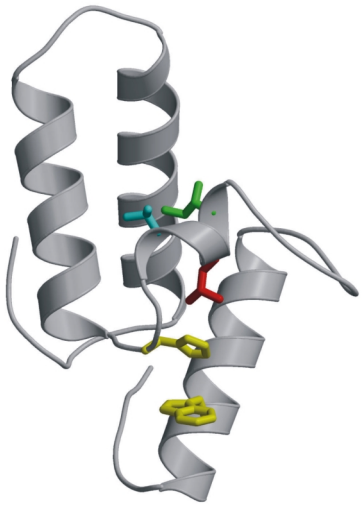

Fig. 1 Ribbon diagram of the structure of Im7. The side-chains Thr 51 (cyan), Leu 53 (red) and Ile 54 (green), all located in helix III, are shown. This helix docks very late in the folding process and is responsible for locking the protein in the native conformation. Refolding kinetics (Fig. 2) are monitored by following the fluorescence of the single Trp (W75, shown in yellow). In the native state the fluorescence is highly quenched by His 47 (yellow).

High energy intermediates

To explore the folding mechanisms of Im7 and Im9 in more detail we have now analysed the folding and unfolding kinetics of both proteins across a wide range of pH values. For Im7 it was found that the kinetic intermediate is stabilised by acidic conditions, while the native state is destabilised. As a consequence, the intermediate becomes a major species at equilibrium at pH 5.5. Excitingly, these results provide a direct link between a kinetically important partially folded state and equilibrium denatured states that are often observed at low pH. NMR studies are currently underway to analyse the structure of Im7 under acidic conditions in more detail. Importantly, a parallel analysis of the pH dependence of the folding of Im9 has shown that this protein also populates an intermediate during folding at low pH, but this species it is too unstable to detect at pH 7 and above. These results suggest that the folding mechanisms of Im7 and Im9 might be more similar than suggested by their folding kinetics at neutral pH. More importantly, they suggest that other small proteins might also fold through partially folded intermediates but, since they are less stable than the unfolded state, they are difficult to detect. This has profound implications for distinguishing between potential folding mechanisms since the related diffusion collision and hierarchical mechanisms of folding both predict the formation of on-pathway intermediates, whilst nucleation mechanisms do not.

Structure of the Im7 intermediate and transition state ensembles

A major project in the laboratory this year has focused on determining the structure of the on-pathway intermediate of Im7 using a combination of mutagenesis and kinetic analysis (phi-value analysis). By global analysis of the folding/unfolding kinetics of more than 30 point mutants of Im7 we have now mapped the structure of this on-pathway intermediate, together with the rearrangements that this species undergoes as it converts to the native structure. Sample data for the folding/unfolding kinetics of the mutant I54V are shown in Fig. 2. For

all 30 mutants the rate data was fitted simultaneously with the corresponding kinetic amplitudes to allow the effect of the mutation on the stability of the intermediate as well as the folding and unfolding rate constants for each mutant to be determined. From these data, the folding pathway of the protein could be mapped in detail. The data showed that the on-pathway intermediate that forms in the first millisecond of folding is highly structured, containing three stable helices (I, II and IV) packed around a specific hydrophobic core. Most remarkably, however, the intermediate contains many non-native interactions, especially involving residues that interact with helix III in the native state. These non-native interactions are partially disrupted in the rate limiting transition state, initiating the exposure of the binding site for helix III, and allowing the intermediate to fold to the native state. These data provide one of the most detailed views of the folding of a protein that populates an intermediate. Moreover, they suggest that misfolding may be a natural consequence of hierarchical folding when secondary structural elements form rapidly but have unpartnered hydrophobic residues. The observation that substantial misfolding can occur, even in a small single domain protein like Im7, demonstrates that misfolding may be a more common feature of protein folding than previously suspected and suggest that non-native interactions will have to be included in computational models in order to fully describe the folding process.

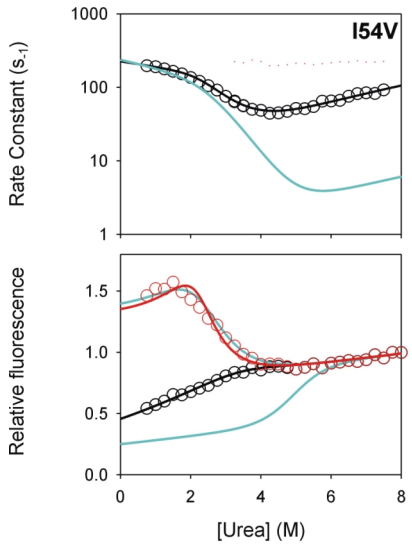

Fig. 2 Observed rate constants and fluorescence signals for the folding and unfolding of the Im7 mutant I54V. The open circles in the lower panel show the initial fluorescence signals extrapolated to time=0 (red) and final fluorescence signals (black) from the folding kinetics. The solid red and black lines show the best simultaneous fit to an on-pathway three-state kinetic model. The blue lines show the best fit to the wild type data for comparison.

Collaborators

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Acknowledgements

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