

Ultra-rapid folding of the B domain of *Staphylococcal* protein A

George Dimitriadis, Graham Spence, Jennifer Clark, Daniel Lund,
Sheena Radford and Alastair Smith

The three-helix bundle B domain of protein A (BdpA, Fig. 1) has been the protein of choice for a number of theoretical, and more recently, experimental protein folding studies because of its small size and simple topology. Although theoretical studies suggest the fast folding of BdpA follows a diffusion-collision-like mechanism, in which pre-existing structural elements that persist in the denatured state coalesce, developing cooperative interactions that stabilise the native state, differences exist in different models in the importance of individual structural elements in the folding process.

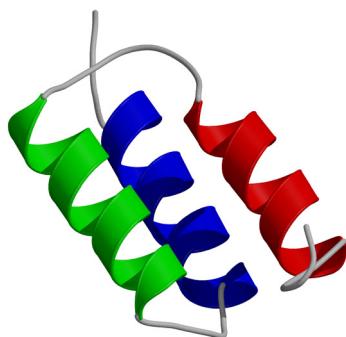


Fig. 1. The structure of the B domain of *Staphylococcal* protein A. Helix 1 is shown in red, helix 2 in green and helix 3 in blue. Drawn using Molscript and Raster3D.

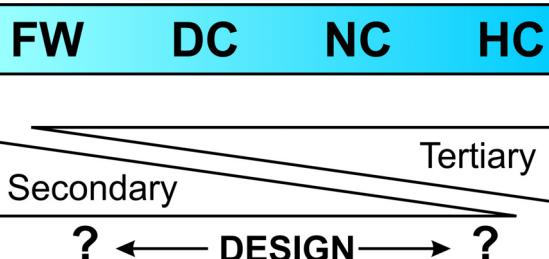


Fig. 2. A continuum of folding mechanisms, from framework (FW), through diffusion-collision (DC) and nucleation-condensation (NC), to hydrophobic collapse (HC), and the relative importance of secondary and tertiary interactions for these models.

The diffusion-collision model is supported by work from the Oas and Weaver groups, which has shown that the experimentally determined folding rate constant is consistent with that predicted by the diffusion-collision model (Myers, 2001; Islam, 2002). However, a further study of BdpA using phi-value analysis by the Fersht group (Sato, 2004) suggests that folding occurs *via* a nucleation-condensation-like mechanism, capturing further aspects of the theoretical studies, but leaving open important questions about how BdpA folds to its native state. These findings highlight the fact that proteins do not fold by discrete mechanisms, but rather through an infinite variety of mechanisms determined by the relative importance of secondary and tertiary interactions (Fig. 2).

Development of a state-of-the-art temperature jump apparatus has led to a fully computer controlled instrument capable of producing temperature jumps of up to 25°C within 8 ns, able to detect changes in the sample fluorescence with a dead time of better than 130 ns, and capable of directly measuring rate constants on the order of 150000 s⁻¹. In combination with the measurement of equilibrium free energy surfaces, which follow the population of species with respect to denaturant concentration and temperature, the microscopic rate constants for folding and unfolding can be determined over a wide range of final conditions. The combination of this instrument and protein model allow exciting and fundamental questions about protein folding to be addressed.

Current Work

Using a combination of the laser induced temperature jump apparatus and 10 selected single amino acid variants, the predictive ability of a general diffusion-collision model has been investigated. Seven of the ten mutants were designed to alter the propensity of the three

helices of BdpA by either mutating residues within the helices to alanine (increasing helical propensity, but decreasing the burial of hydrophobic surface area) or glycine (decreasing helical propensity and burial of hydrophobic surface area), or by replacing residues that form an N-terminal hydrophobic helix cap. The three remaining mutants each removed specific tertiary contacts from the hydrophobic core of the protein. The results showed that unless the change in helical propensity is dramatic, *i.e.* mutations to glycine residues, diffusion-collision is unable to predict the observed rate constants, necessitating the development of a refined model.

Analysis of theoretical and experimental evidence suggests that BdpA folds *via* a mechanism that lies somewhere between diffusion-collision and nucleation-condensation. We are currently investigating whether it is possible to use rational design to alter the relative importance of secondary and tertiary interactions during BdpA folding, therefore altering the mechanism by which this protein folds (Fig. 2). Two variants have been designed with the aid of AGADIR (Lacroix, 1998), an algorithm that predicts the helical propensity of a sequence. The first of these has been designed to have as little intrinsic helical propensity as possible, whilst not disrupting the native helical interfaces, the second having been designed to have a high degree of secondary structure present in the denatured ensemble. Characterisation of these variants is underway.

BdpA is also being developed as a system to study protein folding and dynamics at the single molecule level, using fluorescence resonance energy transfer (FRET) between the dyes AlexaFluor 488 and AlexaFluor 594. Variants have been designed and purified that contain a pair of cysteine residues at sites predicted to give a large change in FRET as the protein folds and unfolds. Currently, the labelling strategy is being optimised to give the largest yield of protein labelled with both dyes.

Future Work

Perhaps the most exciting possibility is to try and engineer BdpA to fold in the “downhill” regime, where the rate of folding is under diffusion control. This would, for the first time, provide an experimental measure of the pre-exponential factor, which defines the fastest rate at which a protein can fold. This could be achieved by using knowledge from phi-value analysis to specifically stabilise the rate-limiting transition state. Other projects include using a diffusion-collision model to predict the changes in rate constants caused by increasing linker lengths between helices, or by making changes to the propensity of individual helices.

Publications

Dimitridis, G., Drysdale, A., Myers, J. K., Arora, P., Radford, S. E., Oas, T. G. and Smith, D. A. (2004) Microsecond folding dynamics of the F13W G29A mutant of the B domain of Staphylococcal protein A by laser induced temperature jump. *Proc. Nat. Acad. Sciences USA.* **101**, 3809 – 3814.

Collaboration

The study of the B domain mutants is in collaboration with Terrance Oas and Pooja Arora at the Department of Biochemistry, Duke University, North Carolina.

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