Mechanical unfolding of proteins using dynamic force spectroscopy

Kirstine Anderson, Eleanore Hann, Gerard Huysmans, David Sadler, David Brockwell, Alastair Smith and Sheena Radford

Determining the factors that confer mechanical resistance on globular proteins

There are many cellular functions that require proteins to exhibit a resistance to applied force. For example, scaffold proteins such as the giant muscle protein titin and extracellular matrix proteins such as fibronectin are constantly subject to changing forces. Proteins are also thought to be subject to force during membrane translocation and protein degradation, hence their mechanical resistance may affect the efficiency of these processes. Mechanical unfolding of proteins can be readily studied using techniques such as force mode AFM and optical tweezer instruments. The role that the type of secondary structure plays in force resistance is understood (helical proteins, in general, are force labile relative to beta-sheet containing proteins), but current knowledge is not sufficient to accurately predict the mechanical properties of a protein.

Protein L is a 62 amino-acid protein with no mechanical function. It has a simple $\beta$-grasp topology and demonstrates a surprisingly high resistance to force. These observations, together with experiments on other small proteins have led to the dogma that parallel and directly hydrogen bonded terminal $\beta$-strands confer mechanical resistance to a protein. In accord with this, other small proteins with this topology (e.g. protein G and ubiquitin) also show mechanical strength, irrespective of their biological function. Interestingly, however, despite their shared topology, ubiquitin is more highly resistant to force than protein L, suggesting that differences in the details of their sidechain packing also contributes to mechanical stability. To test this hypothesis, mechanical phi-value analysis is being carried out on a number of protein L variants that have been designed to alter different sidechain contacts in the native protein. Mechanical unfolding of one of these mutants, L10A, reveals that it shows a similar force resistance to wild-type, despite being significantly less thermodynamically stable.

Another factor which has been shown to affect the mechanical resistance of a protein is the geometry of the applied force. Most experimental data are collected by extending proteins between their N- and C-termini, although a limited number of studies have mechanically unfolded proteins by applying force in alternative directions. I27 is a model protein that has been characterised extensively by application of force from its N- and C-termini. However, a method is being developed by which I27, or indeed any model protein, can be extended between its N-terminus and any other position in the domain. This method can be used to characterise the whole mechanical unfolding landscape of a protein and importantly, will unveil further clues about the factors that determine mechanical resistance and serve as a powerful benchmark for simulations of the mechanical unfolding of proteins using different approaches (see report by West et al).

As well as the intrinsic properties of a protein, external factors can affect the extent to which a protein can resist force. These include interactions with a ligand (see below) and the external environment. Membrane proteins are often inaccessible to protein folding studies because of their large size and insolubility. One way in which membrane proteins can be studied is AFM, which can be used to directly unfold proteins out of membranes. To date these studies have been carried out on $\alpha$-helical inner membrane proteins. In a new project we have initiated studies of the mechanical properties of outer membrane proteins with all $\beta$-sheet structures.
Mechanical consequences of the interaction between Im9 and E9

The interaction between the colicin E9 and its associated immunity protein Im9 is amongst the strongest protein-protein interactions characterised to date, with a $K_d$ of the order of $10^{-16}$ M. An interesting and readily used application of force-mode AFM is the investigation of mechanical strength of interactions between proteins and their natural ligands. For example, both antibody-antigen and avidin-biotin complexes have been dissociated by force spectroscopy. Since the Im9-E9 interaction is stronger than either of these interactions, we are using this system to determine both the mechanical strength of the interaction and the effect that tight and specific binding has on the mechanical strength of one of the protein in the complex, Im9 (see Fig. 1).

![Fig. 1. The difference in mechanical stability of (a) Im9 alone and (b) Im9 in complex with E9. Top: Ribbon representations of Im9 (yellow) with and without E9 (red). Middle: Force-extension curves of Im9-protein L concatamers. Peaks at around 150 pN correspond to protein L unfolding whereas peaks at around 40 pN (right hand plot only) correspond to Im9 unfolding. A worm-like chain model has been fitted to each unfolding event and is shown in red. The final peak in each trace corresponds to the protein detaching from the AFM tip. Bottom: Representation of the concatamer in which Im9 is being pulled. Rectangles represent protein L, circles Im9 and pentagons E9. Green arrows: direction of the applied force.](image)

Collaborators

Colin Kleanthous, University of York; Godfrey Beddard, School of Chemistry, University of Leeds; Peter Olmsted, Emanuele Paci, and Dan West, School of Physics and Astronomy, University of Leeds.

Publications


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