

Mechanically unfolding the small, topologically simple protein L.

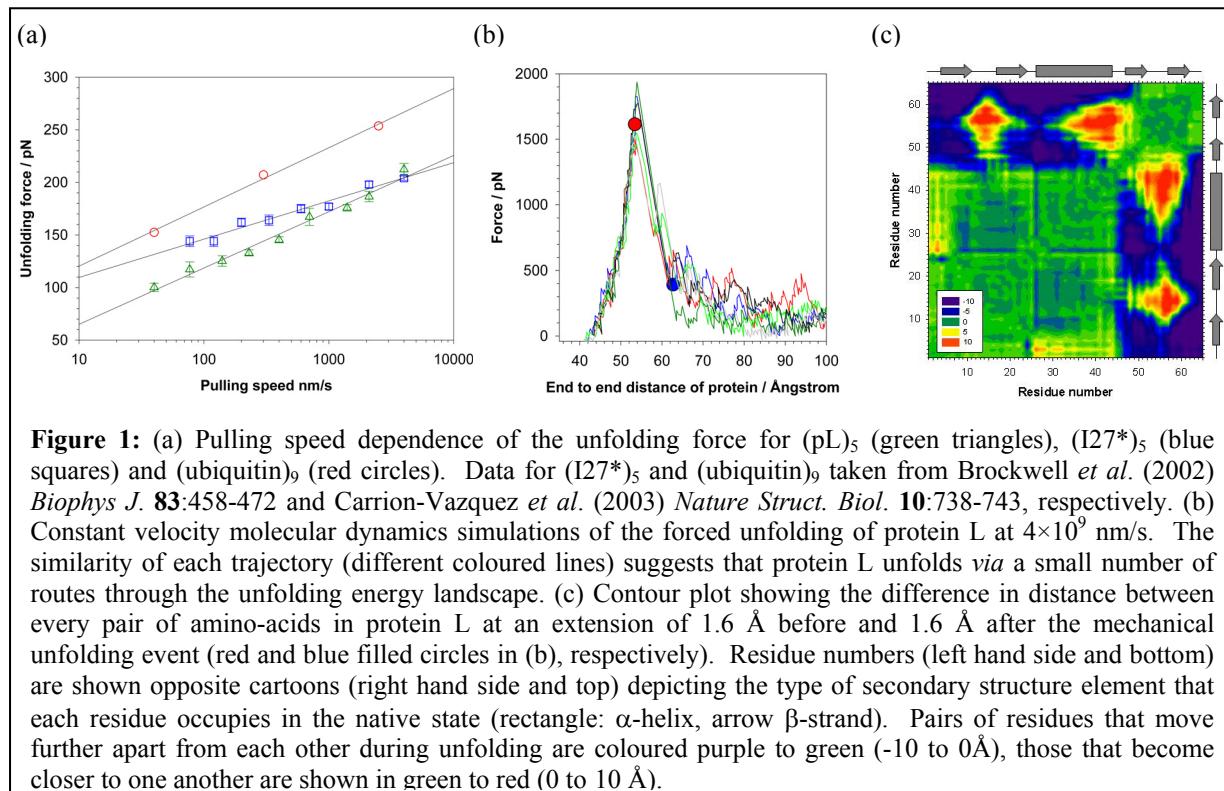
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Introduction

Mechanical force is ubiquitous in biology. The role that some proteins or their complexes play in resisting or reacting to a mechanical extension on the macroscopic scale is well understood. Some proteins resist or respond to force at the sub-cellular level, providing scaffolds or acting as force sensitive triggers. Mechanically unfolding proteins using the AFM now allows the mechanical properties of these proteins to be characterised at the single molecule level. Our current understanding of the unfolding process suggests that the geometry of extension and protein topology are important factors which define the mechanical resistance of proteins. The greatest mechanical strength is found in proteins that have parallel and directly hydrogen bonded β -strands at their termini. However, such proteins display a broad range of unfolding forces under similar extension rates that are difficult to rationalize.

Protein L is mechanically resistant

In order to quantify the factors which modulate protein mechanical resistance, it is necessary to perform a systematic study of a large number of proteins with different folds. Most mechanical studies to date have focused on immunoglobulin and fibronectin type domains. To further test the hypothesis that the extension of parallel, directly hydrogen bonded terminal β -strands correlates with high unfolding forces and to identify any other features which endow mechanical resistance, we commenced a study of protein L, a small 62 amino-acid protein with a simple $\alpha+\beta$ topology.



In accord with the above hypothesis, analysis of the mechanical unfolding parameters of a pentameric homopolymer of protein L ($pL)_5$ showed that the protein is significantly resistant

to extension at all speeds tested (Fig. 1(a)). This finding provides strong evidence that mechanical strength is determined predominantly by topology and not by evolved function, in agreement with earlier experiments on non-force bearing proteins such as the E2lip3 domain from *E.coli* studied previously in this laboratory. The gradient of a force *versus* the logarithm of the pulling speed plot (Fig. 1(a)) gives information on the distance between the native and transition states. Comparing the force *versus* log pulling speed plots for (pL)₅ and (I27*)₅ shows that the unfolding transition state for these proteins occur at different extensions from their native states, as expected for proteins with completely different folds. Interestingly, ubiquitin, a protein within the same fold family as protein L, shows an identical pulling speed dependency, but unfolds at a significantly higher force, even though this protein has fewer hydrogen bonds between its N- and C-terminal strands.

Simulations reveal a simple unfolding mechanism for protein L

Molecular dynamics simulations can be used to gain insight into the structural origin of the mechanical resistance of proteins in atomistic detail. The force-extension profiles of replicate trajectories of protein L are highly reproducible suggesting that this protein unfolds *via* an unusually well defined transition state that is represented by a narrow structural ensemble (Fig. 1(b)). A distance difference map was calculated to highlight the contacts which are broken when the protein traverses the unfolding transition state barrier. The resulting diagram (Fig. 1(c)) is striking, showing that protein L unfolds by the shearing apart of two distinct structural units. This mechanism is similar to that proposed for ubiquitin. Importantly, the number of long-range contacts that span the two unfolding units in protein L is both significantly smaller (22 and 38, respectively) and in fewer clusters than those for ubiquitin. Thus, although each protein has to be extended to a similar extent to reach the transition state to unfolding, a significantly greater force may be required for ubiquitin to reach the transition point as this protein shows greater co-operativity across the surfaces which are to be sheared.

These data suggest that the mechanism of mechanical unfolding is conserved in proteins within the same fold family and demonstrate that whilst the topology and presence of a hydrogen bonded clamp are of central importance in determining mechanical strength, hydrophobic interactions also play an important role in modulating the mechanical resistance of these similar proteins. Further experiments using protein L, its homologues and other proteins with related folds, combined with site-directed mutagenesis studies, are now underway to determine and quantify the balance of these effects in determining the mechanical stability of proteins.

Publications

Brockwell, D.J., Beddard, G.S., Paci, E., West, D.K., Olmsted, P.D., Smith, D.A. and Radford, S.E. Mechanically unfolding the small, topologically simple protein L. Submitted *Biophys. J.* (2005).

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