

Probing the determinants of mechanical resistance in proteins.

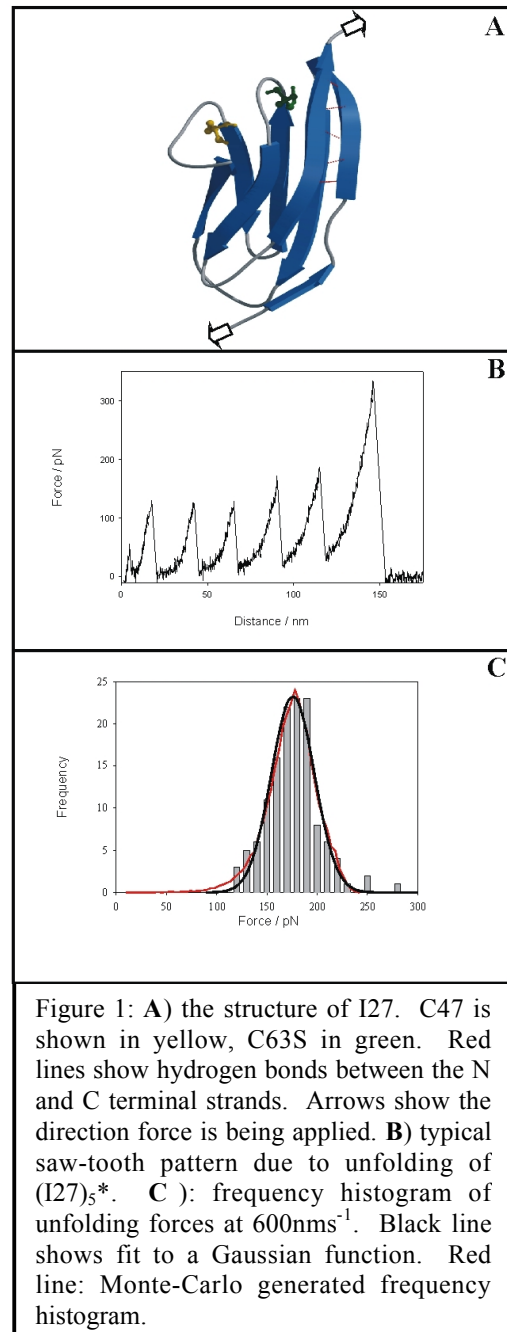
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Introduction

The use of force to denature single molecules was first reported in 1996. The force was applied by pulling the protein's N and C termini in opposite directions *via* an atomic force microscope or laser tweezers (see Figure 1A). The giant muscle protein titin was used in initial experiments due to its size and modular 'beads-on-a-string' structure (each bead being 1 of ~300 immunoglobulin and fibronectin type domains each comprising ~90 amino-acids). Pulling on the ends of this molecule revealed that the protein domains of titin can resist an applied force of the order of hundreds of piconewtons after which one domain unfolds causing a reduction in the applied force. Further extension gradually increases the applied force until another domain unfolds. This stepwise single domain unfolding results in a so called 'saw-tooth' pattern (similar to Fig. 1B). To allow quantitative measurements of the thermodynamics and kinetics of unfolding of individual domains, concatamers consisting of multiple copies of a single domain (the 27th Ig domain of titin (I27) have been constructed (Fig 1A). This work has led to the hypotheses that denaturation by chemical means probes the same transition state as that for mechanical unfolding and that the hydrogen bonds between the N and C strands of I27 (see Fig. 1A) play a major role in providing mechanical strength.

Construction of a generic protein scaffold

We have constructed a plasmid vector which contains five cassettes bordered by pairs of unique restriction sites. This allows rapid construction of homo- or heteropolymers with domains linked by the same amino-acids. Our construct consists of four copies of the I27 mutant C47S C63S, together with a single copy of C63S I27 as the central domain. This construct is denoted (I27)₅*. However we have also constructed concatamers containing different domains in either the central module, or at the N- and C-domains, as well as concatamers consisting entirely of different non-Ig proteins. By measuring the mechanical properties of these proteins with differing topologies we hope to elucidate the features which make some proteins mechanically resistant whilst others are not.



The effect of core destabilisation upon the mechanical resistance of (I27)₅*.

The mutations C47S and C63S in I27 maintain the hydrogen network between its N and C-terminal strands that have been proposed as the origin of its mechanical resistance. To test whether this is the case, and whether chemical and mechanical unfolding of this domain occur by similar mechanisms, we have used AFM to unfold the concatamer (I27)₅* at different speeds, from which data the intrinsic unfolding rate constant at zero force can be determined and the height of the unfolding energy barrier inferred. Our data (Fig. 1) demonstrated that the mutations do not effect the mechanical resistance of the domains, yet they increase the rate of denaturation measured by chemical denaturation significantly. The data demonstrate, for these mutations at least, that mechanical and chemical unfolding probe different pathways. Moreover, the reduction in unfolding barrier height upon application of force is the same for wild-type and core destabilised concatamers, and much greater than domains containing mutation in the terminal regions of the chain, supporting the view that the hydrogen bonds between the N- and C-terminal strands are uniquely important in determining the mechanical resistance of this domain.

Collaborators

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References

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