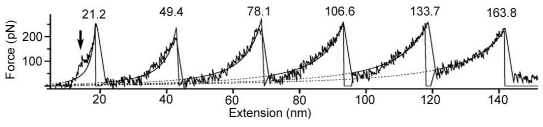
Unfolding Single Titin (Muscle) Protein Molecules using Frequency Modulation AFM.

M. Higgins[1] and S. P. Jarvis[1]

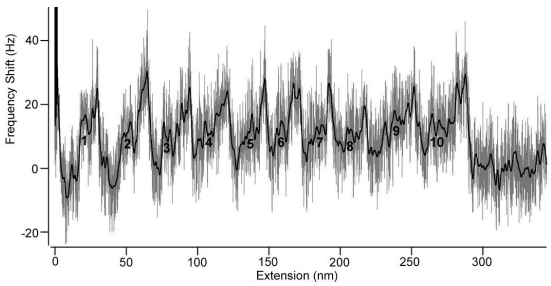
[1]Centre for Research on Adaptive Nanodevices and Nanostructures (CRANN), University of Dublin, Trinity College, Dublin 2, Ireland
michael.higgins@tcd.ie

The reversible unfolding of Immunoglobulin (Ig) domains in the I-band region of the modular protein titin is believed to function for the passive elasticity of muscle. The mechanical unfolding of the well-defined tertiary structure of the wild-type titin Ig 27 domain (I27) has been studied using Atomic Force Microscopy (AFM) in the static mode [1]. By stretching recombinant proteins consisting of multiple repeats of a single domain using AFM, a suite of information on protein folding has been resolved, including the mechanical unfolding forces of individual domains and kinetic parameters for both unfolding and refolding pathways [2]. Related to this study, an unfolding intermediate due to the disruption of hydrogen bonds between A and B ß-strands of the I27 domain was initially observed using Steered Molecular Dynamic (SMD) simulations [3] and later shown in AFM measurements [4].
In this study, we use Frequency Modulation (FM) AFM to investigate the unfolding of tandem repeats of the I27 domain and make comparisons to static measurements that are also performed in the study. For static measurements, force-extension curves for the stretched protein revealed a saw-tooth pattern with periodic spacing between adjacent peaks (Fig. A), indicating the sequential unfolding of modular domains (6 domains in this case) within the polymer construct. The unfolding intermediate, as described above, was observed as a prominent 'hump' or force transition in the first unfolding peak occurring at ≈ 100-120 pN (arrow in Fig. A). However, the force transition was not clear, or even present, in subsequent unfolding events. In contrast, FM–AFM was able to detect individual force transitions (numbered peaks in Fig. B) associated with each main unfolded peak regardless of the domain number observed in the frequency shift curves. Thus, FM-AFM was more sensitive to the force gradient in the unfolded polypeptide region compared to the static mode, and confirmed the reversibility of the unfolding intermediate process for each domain. This work highlights the potential of dynamic techniques for future studies on protein folding, including the detection of novel unfolding intermediates.

A



B



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