

Novel force ramp AFM technique adopting single molecule events for dynamic force spectroscopy

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Abstract

Recently developed techniques such as atomic force microscopy (AFM), the biomembrane force probe (BFP) and optic/magnetic tweezers have allowed us to manipulate biomolecules at the single molecule level and have provided a wealth of information about their mechanical property. In addition, single molecule experiments with these techniques have enabled us to study the energy landscapes of conformational change or unbinding of biomolecules. It is called dynamic force spectroscopy, where the force loading rate is altered and the loading rate dependence of the force at which the reactions occur is investigated. In general, the force loading rate is altered by changing the constant retraction velocity of the scanner (piezo) or the optic/magnetic beads. However, biomolecules have a non-linear elasticity against force, giving non-linear changes of force as a function of time, while the force loading rate is usually determined by assuming that the biomolecule under study has a constant elasticity.

Recently, a real force ramp technique has been developed by the introduction of an analogue PID feedback circuit allowing the kinetic parameters (Δx , k_0) of the unfolding reaction of ubiquitin and titin to be calculated [1,2]. In the force ramp experiment, homopolymers of proteins are chosen and stretched. However, with these molecules the multiple unfolding events occur sequentially during the force ramp, which is not taken into account and the force ramp is executed “abruptly”. Consequently the values of the kinetic parameters given with this technique were much smaller than those from the constant velocity experiments.

In this study, a novel force ramp technique capable of executing a true force ramp which takes multiple unfolding events into account has been developed. This is enabled by using a software controlled PID feedback that monitors protein unfolding events during the force ramp. In the talk details of this technique will be presented. Using this technique we obtained the parameters which are almost identical to those determined by the conventional loading velocity experiments, indicating the validity of this technique and importance of consideration of the multiple unfolding events during the force ramp.

[1] M. Schlierf, H. Li and J. M. Fernandez, *Proc Natl Acad Sci USA* **2004**, 101, 7299.

[2] M. Wang, Y. Cao and H. Li, *Polymer* **2006**, 47, 2548.