Stochastic emergence of multiple intermediates detected by single-molecule quasi-static mechanical unfolding of protein

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Experimental probing of a protein-folding energy landscape can be challenging, and energy landscapes comprising multiple intermediates have not yet been defined. Here, we quasi-statically unfolded single molecules of staphylococcal nuclease by constant-rate mechanical stretching with a feedback positioning system. Multiple discrete transition states were detected as force peaks, and only some of the multiple transition states emerged stochastically in each trial. This finding was confirmed by molecular dynamics simulations, and agreed with another result of the simulations which showed that individual trajectories took highly heterogeneous pathways. The presence of Ca2+ did not change the location of the transition states, but changed the frequency of the emergence. Transition states emerged more frequently in stabilized domains. The simulations also confirmed this feature, and showed that the stabilized domains had rugged energy surfaces. The mean energy required per residue to disrupt secondary structures was a few times the thermal energy (1–3 kBT), which agreed with the stochastic feature. Thus, single-molecule quasi-static measurement has achieved notable success in detecting stochastic features of a huge number of possible conformations of a protein.

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