Comment on “Force-Clamp Spectroscopy Monitors the Folding Trajectory of a Single Protein”

Taking advantage of major improvements in their atomic force microscopy (AFM) apparatus, Fernandez and Li were able to follow single-molecule refolding trajectories of the ubiquitin protein (1). They observed a rich variety of kinetic behavior. Using a polycistronic version of ubiquitin, lengths of three to eight tethered proteins were picked up at random locations and unfolded using a pulling force. Upon relaxation of the force, refolding occurred in continuous stages. The results were interpreted in terms of a folding scenario with no defined kinetic barrier between the unfolded and folded states.

Monomeric ubiquitin free in solution has been demonstrated to fold in a barrier-limited process (2–5), often in a two-state manner (6–15) without the multiple early collapse phases (12) seen in the AFM studies. Two-state behavior persists even when there is transition-state heterogeneity (11). The discrepancy between the ensemble and AFM measurements cannot be solely attributed to the measurement of single molecules; other single-molecule measurements, in which the proteins were monomeric and free in solution, were fully consistent with analogous solution results that show two-state folding and discrete transitions (16, 17).

One suspects that the nondiscrete folding behavior observed for tethered proteins in the AFM studies was due to the intimacy of the multiple ubiquitin chains. In free solution, detectable aggregation of refolding ubiquitin occurs at 2 μM concentration (15), which is resolved on the millisecond-to-second time scale (3, 6, 13). In the AFM measurements, the tethered ubiquitins are at relative concentration above the mM range. Therefore, the still-unfolded ubiquitin chains might be expected to associate when the pulling force is reduced, which would produce the kinds of results observed by Fernandez and Li (1).

The small number of single-protein folding events observed by Fernandez and Li appear to be barrier-limited. The trajectories [see figure 5 in (1)] have a quiescent period following a sudden collapse to the native state, the hallmark of a nucleation process. Furthermore, a histogram of the dwell times results in a zero-force extrapolated rate that is within a factor of two of the value observed for barrier-limited folding in solution.

For the single-protein events, the collapse process itself takes 0.1 s. This time scale is orders of magnitude slower than what is anticipated from the solution studies. In solution, post–transition state species do not accumu-late. Hence, their lifetimes must be less than a millisecond, the approximate time constant of the entire two-state reaction. Hopefully, further studies will clarify the nature of the slow collapse phase observed in the AFM studies.

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1 June 2004; accepted 26 August 2004

www.sciencemag.org SCIENCE VOL 306 15 OCTOBER 2004 411b
Comment on "Force-Clamp Spectroscopy Monitors the Folding Trajectory of a Single Protein"
T. R. Sosnick (October 14, 2004)
Science 306 (5695), 411. [doi: 10.1126/science.1100962]

Editor's Summary

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