Exploring a New Role for Molecular Chaperones in Protein Folding

Bharat Jagannathan, Ph.D.
California Institute for Quantitative Biosciences, University of California, Berkeley
bharatj@berkeley.edu

Abstract: Using force spectroscopy, Mashaghi *et al.* have demonstrated that the general chaperone trigger factor (TF) promotes the correct folding of the maltose binding protein (MBP) by binding to partially folded states along the folding pathway.

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Protein folding is a remarkable biological process in which proteins are transformed from linear amino acid sequences into complex three-dimensional structures. Errors during protein folding can lead to neurodegenerative ailments such as Alzheimer's disease and Parkinson's disease. Although the precise *in vivo* mechanism of this important process remains poorly understood, a network of molecular chaperone proteins are thought to play a crucial role in guiding a protein towards its correct conformation. It has been previously reported that these chaperones assist protein folding by suppressing aggregation, as well as by rescuing misfolded states (Hartl *et al*., 2011). However, a recent study has shown that molecular chaperones directly influence a protein's conformational search to attain the native structure (Mashaghi *et al.*, 2013).

The primary technique used in this study is single-molecule force spectroscopy, in which small amounts of forces (in the piconewton range) are applied to unfold (and refold) single protein molecules, using an instrument called optical tweezers (Jagannathan and Marqusee, 2013). The magnitude of the applied force and the end-to-end extension of the protein molecule are monitored as a function of time. Rare intermediate states can be precisely identified because they have different end-to-end extensions than the native state or the completely unfolded state. Using a combination of force spectroscopy and biochemical essays, Mashaghi and co-workers explored the role of the general chaperone trigger factor (TF) in promoting the folding of maltose binding protein (MBP).

In the absence of TF, MBP unfolds predominantly via a single intermediate state, although additional rare protein states are sampled transiently (for less than 1 s). After the addition of TF, the rare, transient states were observed much more frequently and were stable for longer times, suggesting that TF promotes and stabilizes the partially folded states. Furthermore, it was shown that TF does not bind to natively folded MBP, indicating that the molecular chaperone only binds partial folds along the folding pathway, until MBP attains its native state.

Because TF association could be particularly beneficial for complex multi-domain proteins, the folding of a construct composed of four MBP repeats (MBP_4) was studied. In the absence of TF, MBP_4 folds to a compact, non-native state that does not unfold, suggesting the presence of tight misfolding interactions between the domains. The presence of TF significantly restored native folding of MBP_4, and the tightly misfolded states were only rarely observed.

The work described in this study is of importance to the protein folding field because it demonstrates that molecular chaperones can reshape the folding energy landscape by binding and protecting partially folded states. The stabilization of these states guides the folding pathway towards the correct native structure and away from misfolded conformations.

References

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