Observing the osmophobic effect in action at the single molecule level

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Protecting osmolytes are widespread small organic molecules able to stabilize the folded state of most proteins against various denaturing stresses in vivo. The osmophobic model explains thermodynamically their action through a preferential exclusion of the osmolyte molecules from the protein surface, thus favoring the formation of intrapeptide hydrogen bonds.

By using a combination of experimental strategies based on the Atomic Force Microscopy (AFM)-Single Molecule Force Spectroscopy, evidence was obtained of a protecting osmolyte slowing down the unfolding kinetics of a globular protein while concurrently accelerating its folding rate without any complexation in the unfolding transition state. This observation, to the best of our knowledge, has provided the first single-molecule evidence for a mechanism of protein fold stabilization by protecting osmolytes by a purely indirect, backbone-based mechanism in full accord with the osmophobic model.

Few works addressed the influence of protecting osmolytes on the protein unfolding transition state and kinetics. Among those, previous single molecule force spectroscopy experiments evidenced a complexation of the protecting osmolyte molecules at the unfolding transition state of the protein, in apparent contradiction with the osmophobic nature of the protein backbone. We present single-molecule evidence that glycerol, which is a ubiquitous protecting osmolyte, stabilizes a globular protein against mechanical unfolding without binding into its unfolding transition state structure. We show experimentally that glycerol does not change the position of the unfolding transition state as projected onto the mechanical reaction coordinate. We have also shown via single-molecule mechanical unfolding experiments that the osmolyte glycerol stabilizes the native state of the human cardiac I27 titin module against unfolding without shifting its unfolding transition state on the mechanical reaction coordinate. Notably, our force-clamp and velocity-clamp simulations exhibit no shift in the position of the unfolding transition state of GB1 and I27 under the effect of various osmolytes.

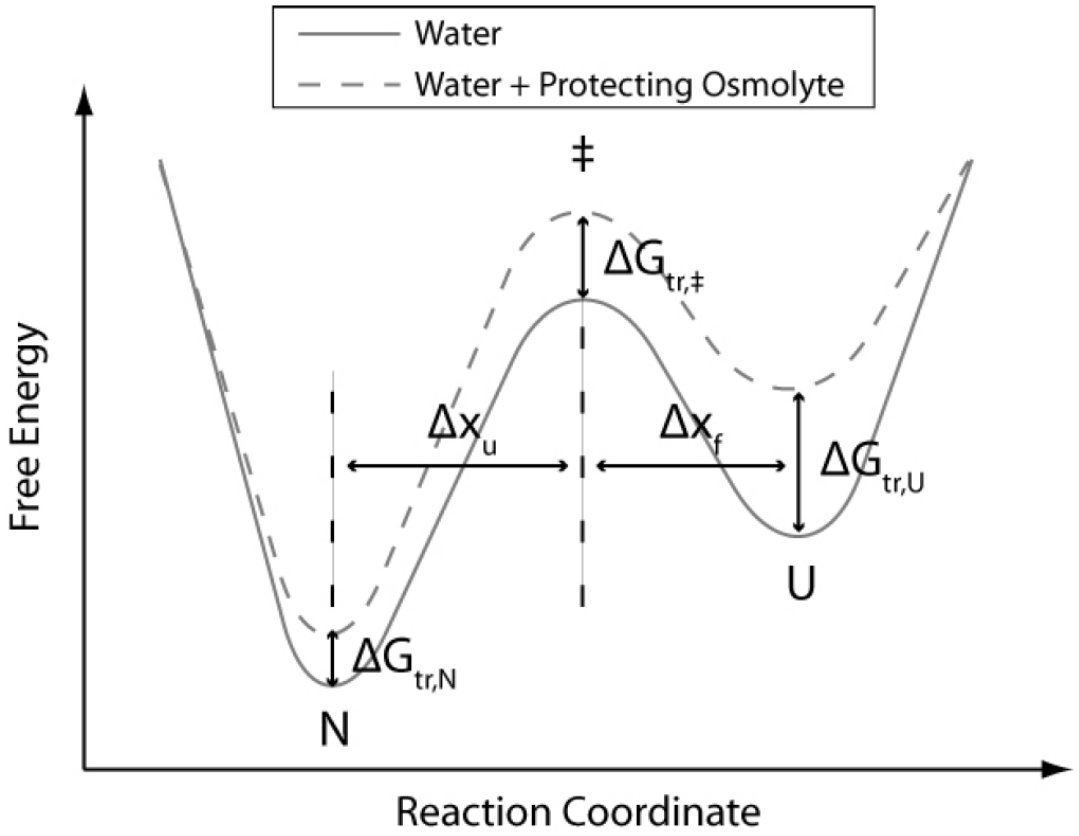
We have investigated the mechanism also on a theoretical basis via an Ising-like model for protein mechanical unfolding that adds worm-like-chain behavior to a recent generalization of the Wako-Saitoˆ-Munˇoz-Eaton model with support for group-transfer free energies. The thermodynamics of the model are exactly solvable, while protein kinetics under mechanical tension can be simulated via Monte Carlo algorithms, the projection of the unfolding transition state onto two other common reaction coordinates, that is, the number of native peptide bonds and the weighted number of native contacts. Using this model, we found again that the position of the unfolding transition state does not change in the presence of glycerol, giving further support to the conclusions based on the single-molecule experiments.

The excellent agreement between experiment and simulation strongly suggests that osmolytes do not assume a structural role at the mechanical unfolding transition state of proteins, acting instead by adjusting the solvent quality for the protein chain analyte, in full accord with the osmophobic model, i.e. with a purely indirect, backbone-based mechanism..

References

[1] Daniel Aioanei, Shanshan Lv, Isabella Tessari, Aldo Rampioni, Luigi Bubacco, Hongbin Li, Bruno Samorì, Marco Brucale , Single Molecule Level Proof of the Osmophobic Effect
ANGEWANDTE CHEM INT. ED. (DOI: 10.1002/anie.200) (IF 11.829) 2011, 50 (19) 4394–4397
[2] Daniel Aioanei, Isabella Tessari, Luigi Bubacco, Bruno Samorì, Marco Brucale, Observing the osmophobic effect in action at the single molecule level, PROTEINS: 2011; 79:2214–2223

[3] Daniel Aioanei, Bruno Samorì, Marco Brucale Worm-like Ising model for protein mechanical unfolding under the effect of osmolytes BIOPHYS. J. (in pubbl.)



Schematic representation of the unfolding-refolding energy landscape of a two-state protein under the effect of a protecting osmolyte. Kinetically, a protecting osmolyte accelerates protein folding by increasing the height of the unfolding activation barrier (ΔΔGu = ΔGtr,‡ – ΔGtr,N > 0) and decreasing the height of the folding activation barrier (ΔΔGf = ΔGtr,U – ΔGtr,‡ > 0), where ΔGtr,‡, ΔGtr,N, and ΔGtr,U represent the free energy of transfer from water to the water-osmolyte mixture of the transition (‡), native (N) and unfolded (U) state, respectively. It follows as a thermodynamical consequence that ΔΔG = ΔGtr,U – ΔGtr,‡ = ΔΔGf + ΔΔGu > 0, where ΔG is the free energy difference between the unfolded and native states, therefore recovering the inequality commonly referred to as the osmophobic effect.