Is Protein Stabilization by Compatible Solutes a Consequence of Induced Rigidification?

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The ability of osmolytes to increase the stability of proteins has long been known and several thermodynamic theories have been proposed to explain the phenomenon [1, 2]. However, the molecular mechanisms underlying the stabilizing effect still elude us. Do the solutes induce structural changes in the protein? Are alterations in solvation the key factors? Do specific solute/protein interactions play a role? Why are some solutes used in vivo and not others? What are the chemical features of the solute that are relevant to protection?

Previous studies have strongly suggested that the stabilising effect is associated with a reduction in protein internal mobility rather than with alterations of protein structure, without discarding the possibility of protein/solute preferred interactions [3].

We tried to shed some light on this issue by using NMR to study the effect of a stabilizing solute isolated from hyperthermophiles, mannosylglycerate (MG), upon a well characterised protein, staphylococcal nuclease (SNase). MG at 500 mM is able to increase the melting temperature of SNase by 6.7^{o}C.

We used the model free formalism [4] to build structurally detailed dynamic models of SNase from 15N relaxation data in several conditions: a range of MG concentration (0-350 mM), temperature variation, and the effect of other osmolytes i. e., urea (a destabiliser), glycerol (to mimic viscosity enhancement), and KCl (to simulate the increase in ionic strength but with no stabilizing effect).

These experiments showed a consistent reduction of protein mobility with increasing MG concentrations, not observed in the presence of glycerol or KCl. This reduction was even stronger when we looked at wider, lower frequency motions by amide proton-deuterium exchange rate measurements.

To probe possible preferential solute/ protein interactions we followed chemical shift variation with increasing MG concentrations. Most of these variations proved to be very small with the exception of some amide protons that may be establishing stronger hydrogen bonds as a consequence of solute addition.

Structural compaction and changes in dynamical regimes will be discussed as possible sources of protein stabilization by compatible solutes.

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