Saccharide-Structure Effect on Protein Behavior in Aqueous Media, Using PNIPA as a Model for Protein

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The stability of proteins in aqueous solutions is of great importance in numerous fields, in particular biotechnology & food science. Saccharides as co-solutes tend to provide protection to proteins against denaturation induced by heat, freezing or drying, but the mechanisms of these protective effects are still not fully understood. With the aim of shedding some new light on this intriguing problem, we studied the effects of saccharide structure in aqueous solutions on poly-N-isopropyl acrylamide (PNIPA), an isomer of poly leucin, as a simple model representing certain key behaviours of proteins. E.g., coil-to-globule transition (CGT) of PNIPA is analogous to protein cold renaturation (*1*). Using a number of systematically selected sugars, we aimed at relating structural characteristics of these co-solutes to their effect on PNIPA solution behavior. We used thermal analysis methods, including Differential Scanning Calorimetry (DSC) and Isothermal Titration Calorimetry (ITC) to study the thermodynamics of ternary solutions. Using ITC, we proved that the effect of saccharides on the polymer can not be attributed to direct binding of the sugars (*2*), although exceptions exist, e.g. cyclodextrin. Using DSC we studied the slopes ("K") of decreasing polymer CGT temperature ("cloud point") with rising sugar concentration. Beyond the relatively expected observation that steric exclusion is important (i.e, generally, the larger the molecular volume of a sugar the more effectively it reduced the CGT), we observed previously unreported significant differences between the effects of isomeric aldohexoses having identical molecular volume, on PNIPA CGT. For example, Galactose had a considerably more negative K compared to mannose. We found a good correlation between the effect of the isomer on the CGT of PNIPA, and the partial molar isentropic compressibility of this sugar, which has been suggested (*3*) as a descriptor of the effect of the sugar on the structure of its surrounding water. We suggest that stereochemically, galactose has a worse fit with water structure, and it binds the water around it more compactly than mannose and consequently the number of water molecules perturbed by galactose is larger than that of mannose. We conclude that the larger (in terms of number of water molecules) and the more compact the hydrated cluster a sugar forms, the worse a co-solvent it is for the polymer, and the stronger its lowering effect of the CGT. Such favoring of the compact globule state provides a protective effect against denaturation for globular proteins. A simulation mathematical modelling is underway to further explain the mechanism of the observed effects, and to elucidate protein behavior on the basis of the experimental results.

References

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