Biopreservation and homogeneity of sugar/water matrices

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Disaccharides such as trehalose, sucrose or maltose have received a huge interest in the last decades for their preservation capabilities of biosystems such as cells, vaccines or therapeutic proteins employed in the food and pharmaceutical industries. Indeed, disaccharides can be added to biologically active solutions to overcome the limited stability range of proteins (in pH, in temperature, in salt concentration, etc.). These additives prevent the partial or even total degradation of biomolecules due to the lethal thermal or dehydration stresses encountered during industrial conservation methods (lyophilization), where trehalose has been found the most effective.

The molecular mechanisms at the origin of the superior capabilities of trehalose and, more generally, of the biopreservation phenomenon itself still remain unclear, despite various experimental and numerical works. Several hypotheses have been proposed but none of them can be considered as fully accepted:

Green and Angell suggested that dehydrated sugar solutions convert to a glassy state which has been pictured as acting like amber, encaging molecules and membranes in the same way that amber traps insects. The higher glass transition temperature T_g of trehalose could explain its greater preservation efficiency compared to other protectants (sucrose, maltose, sorbitol, ...). Nevertheless, it is now well accepted that biopreservative efficiency does not necessarily scale with the glass transition temperature, as was shown for trehalose-glycerol mixtures.

Crowe *et al.* suggested that sugar molecules were able to directly interact with the polar groups of membranes or proteins via HBs, by substituting to the hydration water shell, essential for proteins structure, dynamics and activity. This would preserve the three-dimensional structure of biomolecules even at low water content. The water replacement hypothesis seems reasonable for water binding sites at the protein surface but it is less plausible for internal water molecules.

Alternatively, an approach based on the *destructuring effect* of sugars on the water hydrogen bond network (HBN) has been proposed by Magazù*et al.*. They particularly demonstrated that trehalose promotes a more extended hydration than other disaccharides and binds more strongly to water molecules, thus preventing more efficiently the crystallization of ice, which causes lethal damages to biosystems. This hypothesis seems well suited to explain the enhanced cryoprotective efficiency of trehalose, but not its lyoprotective one.

In order to get a deeper understanding of sugar solutions properties in the framework of the biopreservation problem, we have carried out a careful comparative study of trehalose, maltose and sucrose in aqueous solutions by molecular dynamics (MD) simulations. The H-bonding capabilities of these three sugars are directly comparable since they possess the same chemical formula $C_{12}H_{22}O_{11}$ and the same number of OH groups. Water-water, water-sugar and both intra and inter sugar-sugar properties and their relationships have been probed in order to measure the homogeneity of the different mixtures. Different relevant structural parameters have been used: partial static structure factors, two-dimensional radial distribution functions, probability of HB formation, water clusters size, hydration number, molecular flexibility and sugar clusters size. A schematic model of the structure of sugar-water mixtures showing the destructuring effects of the different sugars on the water HBN is proposed.

Results show that maltose-water systems are less homogeneous owing the tendency of maltose molecules to form sugar clusters and thus reducing their possibility to destructure the water HB network. For sucrose-water solutions, the higher probability of sucrose molecules to form intra-molecular HBs strongly reduces their interaction with both water or other sugar molecules. Unlikely to maltose or sucrose, trehalose molecules possess similar capabilities to interact with both sugar and water molecules which make trehalose/water a more homogeneous system. This homogeneity of the disaccharide/water matrices may rule their biopreservative efficiency [1]. This point will be illustrated from results on lysozyme-disaccharides-water solutions.

References

[1] A. Lerbret, P. Bordat, F. Affouard, M. Descamps and F. Migliardo, J. Phys. Chem B, 109 (2005) 11046-11057