Available online at www.sciencedirect.com

ScienceDirect

Chemical Physics 345 (2008) 250–258

Chemical Physics

www.elsevier.com/locate/chemphys

Neutron scattering studies on dUTPase complex in the presence of bioprotectant systems

B. Varga [a], F. Migliardo [b], E. Takacs [a], B. Vertessy [a], S. Magazù [b,*], C. Mondelli [c]

[a] *Institute of Enzymology, Hungarian Academy of Sciences, POB 7, H-1518, Budapest, Hungary*
[b] *Dipartimento di Fisica, Università di Messina, P.O. Box 55, I-98166 Messina, Italy*
[c] *CNR-INFM OGG & CRS-SOFT, c/o ILL, BP 156, 38042 Grenoble Cedex 9, France*

Received 29 March 2007; accepted 3 July 2007
Available online 27 July 2007

Abstract

The aim of the present work is to investigate the chemical physics mechanisms of protein stabilization by homologous disaccharides (trehalose, maltose and sucrose). On this purpose the structural and dynamical properties of dUTPase-inhibitor candidate and dUTPase-inhibitor candidate/disaccharide mixtures have been investigated by elastic neutron scattering (ENS), quasi-elastic neutron scattering (QENS) and photon correlation spectroscopy (PCS).

The decrease in the ENS intensity profiles vs temperature for the disaccharide–water mixtures is less marked in the case of trehalose/water mixture. This indicates that trehalose shows a larger structural resistance to temperature changes and a higher "rigidity" in comparison with maltose/H_2O and sucrose/H_2O mixtures. In addition the protein/hydrated–disaccharide mixtures show a linear dependence between the solvent viscosity and the local mean-square displacement of hydrated dUTpase/disaccharide systems. This result shows that the protein dynamics is coupled with that of the surrounding matrix. Furthermore, QENS results on the binary disaccharide–H_2O/D_2O mixtures indicate that the water dynamics is affected by all the disaccharides and particularly by trehalose. Finally, PCS findings indicate that the protein hydrodynamic radius in solution does not change at low disaccharide concentrations, while reveal, at high disaccharide concentration, a breakdown of the Stokes–Einstein law.

The experimental findings are discussed and interpreted in the frame of the current theories.

Keywords: Disaccharides; Protein; Stabilization; Structural properties; Dynamical properties; Neutron scattering

1. Introduction

In recent years a lot of attention has been addressed to the understanding of the mechanisms responsible for the cryptobiosis (from Greek *κριπτοσ*, "hidden" and "coated" and *βιοσ*, "life") referring to a particular state of organisms inactivating when prohibitive environmental conditions occur [1–3].

A class of homologues disaccharides, e.g. trehalose, maltose, sucrose ($C_{12}H_{22}O_{11}$), with trehalose to a greater extent, are cryptobiotic activating substances.

Fig. 1 shows the structure of the three disaccharides. α,α-trehalose is a disaccharide of glucose (α-D-glucopyranosil α-D-glucopyranoside) constituted by two pyranose (six-membered) rings in the same α configuration, linked by a glycosidic bond between the chiral carbon atoms C1 of the two rings. Maltose (4-*O*-α-D-glucopyranosil-D-glucose) is also constituted by two pyranose rings of glucose in the α configuration, but the oxygen bridge links the two carbon atoms C1 and C4 of the two rings. Finally, sucrose (α-D-glucopyranosil β-D-fructofuranoside) is constituted by a glucose ring (pyranose) in the α configuration and a fructose ring (furanose) in the β configuration; the α and β structures of the same monosaccharide differ only in the orientation of the OH groups at some carbon atom in the ring itself (mutarotation equilibria). Contrarily, to

---

[*] Corresponding author. Tel.: +39 0906765025; fax: +39 090395004.
E-mail address: smagazu@unime.it (S. Magazù).

doi:10.1016/j.chemphys.2007.07.004

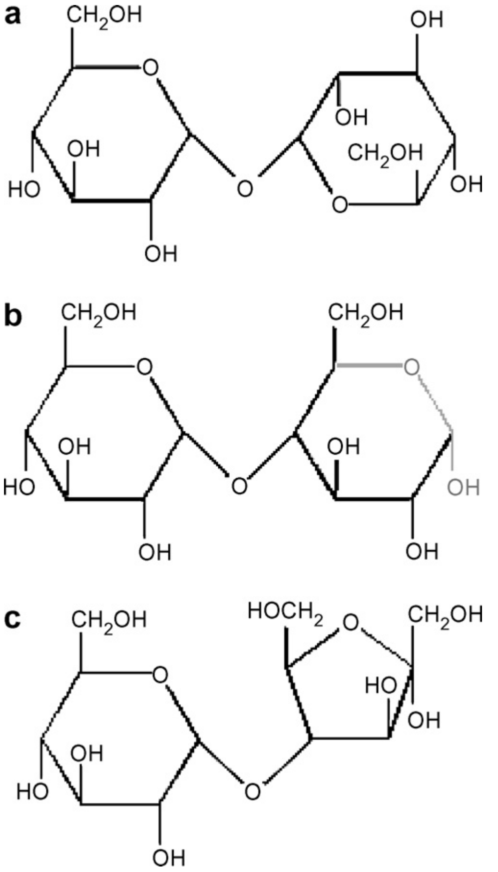

Fig. 1. Structure of: (a) trehalose, (b) maltose and (c) sucrose. The grey colour points out the hemiacetal part of maltose, which makes it non-reducing.

trehalose and sucrose, maltose is a reducing sugar because the anomeric carbons on the right-hand sugar are part of a hemiacetal and, therefore, is capable to rise Maillard reactions, a type of non-enzymatic browning which involves the reaction of reducing sugars, mainly D-glucose, and a free amino acid or a free amino group of an amino acid that is part of a protein chain [4]. However, although a negligible stabilization effect can be attributed to this disaccharide, its comparative study with the other homologous disaccharides reveals to be significant for clarifying the protein–water–cosolute interaction mechanisms.

In spite of the several bioprotection hypotheses formulated, the effectiveness mechanisms remain still partially cryptic.

Green and Angell [5] suggest that the higher value of the glass transition temperature of trehalose and its mixtures with water, in comparison with that of the other disaccharides, is the only reason for its superior bioprotectant effectiveness: this would imply that, at a given temperature, the glass transition for trehalose mixtures always occurs at a higher water content. As a matter of fact such a hypothesis alone is not entirely satisfactory because of the many counter-examples. For example, dextran [6], a linear polysaccharide with α(1–6) glycosidic links, presents even a higher T_g value but does not show a comparable bioprotective action.

Crowe and Reid [7] formulated the hypothesis that a direct interaction between the sugars and the object of protection occurs. More specifically their ‘‘water replacement hypothesis’’ justifies the trehalose protective function with the existence of direct hydrogen bonding of trehalose with the polar head groups of the lipids as water does. This hypothesis was supported by some simulation studies [8], which argue that the structure of trehalose is perfectly adaptable to the tetrahedral coordination of pure water, whose structural and dynamical properties are not significantly affected by trehalose.

As a matter of fact many experimental findings [9–14] have clearly shown that the structural and dynamical properties of water are perturbed by disaccharides. In particular, neutron diffraction results [9] showed for all disaccharides, and for trehalose to a large extent, a strong distortion of the peaks linked to the hydrogen bonded network in the partial radial distribution functions which can be attributed to the destroying of the tetrahedral coordination of pure water. Further neutron diffraction studies [15], performed in combination with a Monte Carlo simulation by the EPSR code, have provided high quality structural information revealing that the water structure is strongly perturbed by the presence of trehalose, with an effect on the water second hydration shell strongly resembling that produced by high pressure.

Raman spectroscopy [10], by the analysis of the intramolecular OH stretching vibration band, shows that the addition of trehalose, in respect to the other disaccharides, more deeply destroys the tetrahedral intermolecular network of water, which by lowering temperature would give rise to ice. In particular the decomposition of the isotropic spectrum of the three disaccharide aqueous solutions into an ‘‘open’’ and a ‘‘closed’’ contribution, shows that the integrated area of the ‘‘open’’ band is smaller in the trehalose aqueous solution, revealing that a more marked destructuring effect occurs in the presence of trehalose than in the presence of sucrose or maltose [10].

Uchida and co-workers [16], detecting freeze-fractured replica images of disaccharide (trehalose, sucrose, and maltose) solutions, using a field-emission type transmission electron microscope (FE-TEM), have recently confirmed that trehalose molecules have a greater inhibitory effect of sucrose on the growth of ice crystals.

As far as dynamics is concerned, preliminary QENS results on disaccharides solutions [11] indicate that also the water dynamics is strongly affected by the presence of disaccharides and in particular by trehalose. Confirmation has been furnished by molecular dynamics simulation studies performed by Descamps and co-workers [17]. In their work the orientational order parameter and the dynamical structure factor have been combined to differentiate the actions of trehalose, sucrose and maltose. They showed for trehalose a higher distortion of the hydrogen bonded network of water from its tetrahedrality and that the relaxation times of water in the presence of disaccharides result 1.2–10 times longer than those of pure water; in addition