

Molecular mobility of trehalose in relation to its bioprotective action.

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LONG ABSTRACT

Beyond the myth and despite some mistakenly reported exceptional properties literature is plenty of, the special role of trehalose and its structural organisation at mesoscale in bioprotection seems to be a fact. This justifies the great effort in the scientific community trying to understand the molecular mechanism(s) underlying bioprotection. The comprehension of the bioprotective phenomenon is expected to have a strong impact in several fields ranging from food industry to biomedical and nanopharmaceutical applications. In front of the many different hypotheses stated in the past, the advantages of trehalose nowadays appear to come from a combination of factors and not from a single exceptional property.

The first set of experiments in this thesis work deals with the structural organisation of glassy/amorphous trehalose in the absence of water. While different crystalline polymorphs have already been recognised and characterised, in this work for the first time evidence of the existence of two different glasses is provided. Characterization of the glasses has been carried out by studying the process of physical aging with the result that different molecular mobilities and different activation energies are deduced for the two glasses. In addition to discuss the role these findings may have in bioprotection, the other heuristic result is that the existence of two amorphous forms of trehalose may explain some literature ambiguous crystallisation behaviour of the amorphous phase.

Among these results presented, the existence of two different “polyamorphic” forms of trehalose surely adds an additional complexity to the question of its role in bioprotection. Furthermore, the different dynamic and thermodynamic behaviour of the amorphous phases A and B can explain, in part, some of the controversial results reported in literature regarding pure trehalose. As long as experiments (calorimetric, spectroscopic) are carried out on dynamical properties of trehalose “amorphous phase” regardless of the existence of these two distinct phases, results will keep on being discordant. In particular, our ageing experiments led to two different activation energies for the structural relaxation of the amorphous phases, suggesting that in one of the glasses molecular mobility is slower than in the other (more homogeneous, compact structure?). This result may have a great interest also for the industrial applications (pharmaceutical, foods, ...) for getting tailored

products and optimising processes. As far as the bioprotection is concerned, however, it is necessary to highlight that whatever is the glass formed upon dehydration, the only possible species involved must derive from moderate temperature processes. According to a previously proposed mechanism and considering the conditions under which dehydration occurs in nature, the amorphous trehalose of type A seems to be involved.

In the second set of experiments, Brillouin light scattering (BLS) experiments on a wide range of concentrations of water-trehalose solutions at different temperatures were performed to explore the density fluctuations (nanoscale inhomogeneities) in the solution. A traditional acoustic analysis was carried out and the parameters describing propagating and dissipative properties were examined in the framework of two different formalisms for characterising the structural relaxation process present in this frequency range. It was found that an increase in trehalose concentration slows down the dynamics, affecting the characteristic time τ . Moreover, the activation energy of the process has a only slight dependence on temperature for diluted and semi diluted systems, that could be attributed to local hydrogen bonding. The fact that the activation energy of the main relaxation process in the frequency studied corresponds approximately to that found in pure water lets us attribute the main relaxation process to the hydrogen bond breaking and formation during density fluctuations. Nevertheless, a further analysis would validate these results, since they are based on the assumption of a single Debye relaxation process, which may not probably be the case, especially for concentrated solutions. The thriving experiments of Lelong et al. on the dynamics of semi-dilute trehalose confined solutions, supports the translation of experiments in bulk solutions to the real condition in organisms, as according to the authors confinement in the dimensions of a cell should not affect the dynamics.

The attempt of clarifying the mechanism of bioprotection is a formidable task in view of the practical absence of peculiar behavior of trehalose solution with respect to other disaccharides and the “normal behaviour” as a function of increasing concentration up to about 70% (w/w). The suggestion of a discontinuity of amorphous mixture properties upon reducing water has already been made and involves the formation of nanocrystalline dihydrate trehalose which evolves into anhydrous TRE α and amorphous TRE A. This view would encourage us to continue in the line of our experiments up to higher concentrations and enlarging the frequency range. In addition, complementary measurements are necessary for the determination of the shape of the relaxation distribution function, as with the scarce and contradictory data available in literature it was not possible to perform a full shape analysis of the spectra.