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Interaction of the disaccharides trehalose and gentiobiose with lipid bilayers: A comparative molecular dynamics study

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ABSTRACT

The disaccharide α,α-trehalose (TRH) is known for its bioprotective action in organisms subject to stressful environmental conditions. However, the mechanisms whereby TRH stabilizes biomolecules remains matter of debate, the five main hypotheses being the water replacement (WRH), headgroup bridging (HBH), vitrification (VIH), water entrapment (WEH) and hydration forces (HFH) hypotheses. Four hypotheses (all except HFH) are in principle compatible with a preferential affinity of the sugar molecules (compared to water) for the biomolecular surface. According to the recently proposed sugar-like mechanism (Pereira and Hünenberger [29]), preferential affinity would result from the entropy gain upon releasing many water molecules from the surface region to the bulk, at the cost of immobilizing and rigidifying fewer sugar molecules. Thus, a more flexible disaccharide such as gentiobiose (GNT) should evidence a weaker preferential affinity, limiting its bioprotective ability. In this work, molecular dynamics (MD) simulations of a dipalmitoyl-phosphatidylcholine (DPPC) bilayer patch in the presence of either pure water or aqueous solutions of GNT or TRH are performed in order to assess the validity of this suggestion. At 475 K and 1.6 m (molal), TRH indeed preserves the bilayer structure to a larger extent compared to GNT. However, the present investigation does not unambiguously indicate which of the above mechanism takes place, since the simulations reveal characteristic features of all of them. This suggests either that multiple mechanisms may be simultaneously active or that their definitions are not precise enough.

1. Introduction

Nature has evolved numerous strategies for the long-term survival of organisms exposed to potentially damaging conditions like extreme dryness, cold, heat, pressure, salt concentration, acidity or oxygen deprivation [1,2]. One of these strategies, cryptobiosis, involves a reversible suspension of the metabolism and a temporary effective isolation from the environmental stress [3,4]. Probably the best studied case of cryptobiosis concerns anhydrobiosis, namely the resistance to nearly complete dehydration [1,4–8]. A characteristic feature of the anhydrobiotic process is the accumulation of large amounts of saccharides in the cell [9,10], and in particular of the disaccharide trehalose [5,11] (α,α-trehalose, TRH; Fig. 1).

It is generally accepted that bioprotection involves the stabilization of biomolecules such as proteins [12–18] (against irreversible denaturation) and lipid membranes [7,9,19–26] (against mechanical disruption). A possible stabilization of nucleic acids [27] has also been recently proposed. Five possible mechanisms have been put forward to tentatively explain the bioprotective action of sugars, principally concerning the best documented example of anhydrobiosis [28,29]:

1. The *water replacement hypothesis* (WRH) suggests that during dehydration, sugar molecules can substitute water molecules in forming hydrogen bonds (H-bonds) with the polar and charged groups present at the biomolecular surface, thereby inhibiting the denaturation (proteins) or a transition to the gel phase (membranes) [5,30–40].
2. The *headgroup-bridging hypothesis* (HBH) is an extension of the WRH formulated based on simulations in the context of membrane bioprotection, which proposes that sugar molecules form a scaffold of H-bonds bridging multiple headgroups, thereby inhibiting a transition to the gel phase upon dehydration [29] (this scaffold is labile in the dilute regime [29,41–43] but expected to strengthen upon dehydration, as a result of the removal of the water molecules competing for the headgroup H-bonding sites and of the reduction of dielectric screening effects).

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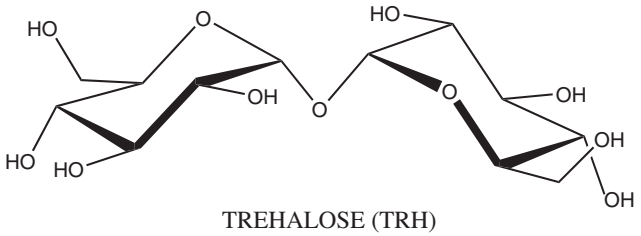
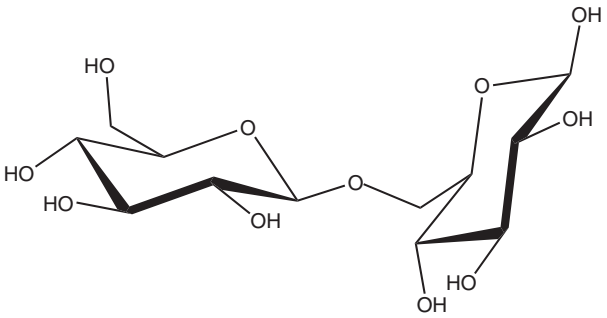


TREHALOSE (TRH)



GENTIOBIOSE (GNT)

Fig. 1. Structures of the two disaccharides considered in the present study. Trehalose (α,α-trehalose, TRH; Glcα (1→1)αGlc) is the non-reducing disaccharide consisting of two D-glucopyranose (Glc) residues in a α (1→1)α (two-bond, axial–axial) glycosidic linkage. Gentiobiose (GNT; represented here in its β-anomeric form at the reducing residue; Glcβ(1→6)Glcβ) is the reducing disaccharide consisting of two Glc residues in a β(1→6) (three-bond, equatorial–equatorial) glycosidic linkage.

3. The *vitrification hypothesis* (VIH) suggests that sugars found in anhydrobiotic systems, known to be good vitrifying agents, protect biomolecules through the formation of amorphous glasses, thereby reducing structural fluctuations and preventing denaturation (proteins) or mechanical disruption (membranes) [44–50].
4. The *water-entrapment hypothesis* (WEH) proposes that sugars concentrate residual water molecules close to the biomolecular surface, thereby preserving to a large extent its solvation and native properties in the quasi-dry state [51–57].
5. The *hydration forces hypothesis* (HFH), introduced more recently in the context of membrane bioprotection, proposes that sugar molecules are preferentially excluded from the biomolecular surface [58,59], and indirectly reduce the compressive stress in the membrane upon dehydration ("hydration repulsion" between bilayers brought in contact), thereby preventing a transition to the gel phase [58,60].

Note that these mechanisms are not necessarily all mutually exclusive. For example, the WRH and the VIH are often invoked simultaneously to explain the bioprotective effect of sugars against dehydration [4,9,10,34,49,61]. Since there is experimental or/and theoretical evidence supporting each of the five above hypotheses in specific situations, it is not impossible that the bioprotection mechanism varies depending on the nature of the environmental stress and its intensity, as well as on the type of protected biomolecule.

Recent molecular dynamics (MD) simulation studies indeed suggested that there is a qualitative difference in the way sugars interact with membranes and with proteins [62]. In the case of membranes, the simulations [29,41–43,63–72] systematically revealed a preferential affinity of the sugar molecules for the bilayer surface and a direct interaction (through H-bonds) with the lipid headgroups, even in dilute solutions, providing support for the WRH (possibly along with [29,41–43] the HBH). In contrast, in the case of proteins, the simulations [51,53,57,54,56,73–77] rather suggested a preferential exclusion of the sugar molecules from the biomolecular surface (trapped water layer), even in concentrated solutions, providing support for the WEH (along with the VIH for concentrated sugar solutions [74]).

Closely related to the question of the bioprotection mechanism is that of the particular efficiency of TRH (compared to, e.g. other disaccharides or oligosaccharides) in terms of bioprotective action. TRH is the non-reducing disaccharide composed of two D-glucopyranose units in an (axial–axial) $\alpha(1 \rightarrow 1)\alpha$-linkage. Recent MD simulation studies of the 11 glucose-based disaccharides in water [78] (see also Refs. [79,80]) have revealed two peculiar features of TRH which, in combination, confer to this compound rather unique properties in the series, namely a high conformational rigidity (low conformational entropy) and the absence of significant intramolecular H-bonding (suggesting a high propensity to intermolecular H-bonded interactions). In pictorial terms, TRH could thus be viewed as a kind of "hydroxyl porcupine", presenting a high density of molecular hydroxyl groups in essentially fixed relative positions and orientations, and exempt of intramolecular H-bonding interactions (i.e. available for intermolecular interactions with the solvent, with other sugar molecules or with biomolecules).

In a recent study, Pereira and Hünenberger [29] proposed (based on MD simulations and available experimental data) two mechanisms for the interaction of polyhydroxylated cosolutes with lipid bilayers. The *alcohol-like mechanism* (active for small alcohols and polyols) involves preferential affinity of the cosolute (compared to water) for the superficial region of the bilayer interior, and is driven by the hydrophobic effect. It results in a lateral expansion of the membrane, a disorder increase within the bilayer, and a partial substitution of water by cosolute molecules at the H-bonding sites provided by the membrane (predominantly at the level of the ester groups). The *sugar-like mechanism* (active for larger polyols including saccharides; see also Ref. [62]) involves preferential affinity of the cosolute (compared to water) for the bilayer surface and is mainly driven by entropic effects. It results in the absence of lateral expansion and change in disorder within the bilayer, and in a partial substitution of water by cosolute molecules at the H-bonding sites provided by the membrane (predominantly at the level of the phosphate groups). It also involves the clustering of the cosolute molecules at the membrane surface (formation of a coating layer) and the bridging of lipid molecules *via* H-bonded cosolute molecules. H-bonding itself is not viewed as a driving force for these two mechanisms, which only involve the (partial) substitution of water-lipid by cosolute-lipid H-bonds (the sum of the two remaining essentially constant, irrespective of the nature and concentration of the cosolute).

If one accepts the sugar-like mechanism, which is compatible with the WRH, HBH, VIH and possibly WEH (but not with the HFH), as providing an essentially correct description of sugar–membrane interactions, the main driving force for the clustering of sugar molecules at the bilayer surface is the release of many water molecules from the surface region to the bulk (large entropy increase) at the cost of immobilizing and rigidifying much fewer (already inherently rather rigid) sugar molecules in this same region (limited entropy cost), the process being enthalpically essentially neutral (exchange of H-bonding partners for the lipid headgroups). In this case, the particularly strong bioprotective capacity of TRH could be in large part a consequence of its very limited conformational flexibility in solution (low entropy cost of rigidification), secondary factors [19,20,81] being a favorable hydroxyl group disposition [82,83] (to interact with a planar surface), a strong propensity to self-aggregate [84,85], strong kosmotropic properties [86–94], an elevated glass transition tem-