Brief communication

Inhibition of trehalose crystallization by cytoplasmic yeast components

Luis Espinosa a, Carolina Schebor a,*,1, Pilar Buera a,1, Silvia Moreno b,1, Jorge Chirife c

a Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina
b Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina
c Facultad de Ciencias Agrarias, Universidad Católica, Argentina

Received 29 June 2005; accepted 19 October 2005
Available online 5 December 2005

Abstract

The influence of different yeast (Saccharomyces cerevisiae) cellular fractions was studied in an attempt to gain knowledge on the feasibility of trehalose crystallization in yeast cells. Certain constituents of S. cerevisiae cells inhibited/delayed trehalose crystallization upon humidification at high relative humidities.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Trehalose; Crystallization; Yeast; Glass transition

It is well known that anhydrobiotic organisms are capable to store certain saccharides in their cells under stress conditions. The nonreducing disaccharide trehalose (1-α-D-glucopyranosyl-α-D-glucopyranoside) is considered to play an important physiological function in a variety of organisms, among them, bacteria, fungi, seeds, resurrection plants, and fungal spores [5]. Trehalose levels greater than 10% on dry weight were observed in some commercial strains of the yeast Saccharomyces cerevisiae. However, in laboratory strains trehalose levels are usually lower [1]. High levels of endogenous trehalose in yeast are associated with the tolerance to stress conditions such as heat, freezing, desiccation, and exposure to toxic chemicals [6]. A possible explanation for the protective action of trehalose on the dehydration of living organisms is known as the water-replacement hypothesis and is derived from model membrane studies [4]. According to this hypothesis the OH groups of the trehalose generate hydrogen bonds with the phosphate groups of the phospholipids and with polar residues in the proteins. This interaction results in the maintenance of dry proteins and membranes in a physical state similar to that observed in the presence of hydration water. Another hypothesis establishes that the sugar

* The authors are thankful for financial support from CONICET (PIP2734) and UBACyT (X226).
* Corresponding author. Fax: +54 11 4576 3366.
E-mail address: cschebor@di.fcen.uba.ar (C. Schebor).
1 Member of CONICET, Argentina.
provides a glassy environment in which the immobilization minimizes the stress damage on the cellular structures [10]. These two hypotheses are not mutually exclusive; indeed, the consensus of workers in this field seems to be that both the direct interaction and formation of the glassy state are required. A glass is a kinetically metastable, time-dependent and nonequilibrium physical state, characterized by almost an absence of molecular movement. The most important parameter describing the glassy state is the glass transition temperature \( T_g \), below which materials exhibit extremely high viscosity that delays crystallization and gives them “solid-like” properties. Above the glass transition range, viscosity drops significantly and the molecular mobility of the system increases accordingly, which in turn may result in solute crystallization [10]. Many authors have related the efficacy of trehalose as a dehydrating agent to certain characteristics of the sugar. An important characteristic of trehalose is that being a nonreducing sugar it does not participate in Maillard reaction which often severely affects the activity of the proteins. Also, trehalose is one of the most stable sugars and does not caramelize unless it is hydrolyzed, which occurs under extreme heating. Trehalose is known to form quite stable glasses compared to other sugars, and this fact has also supported a special role of trehalose for protecting dry biomaterials [1]. It is known that sugar crystallization is detrimental to preservation of biological activity, and the stabilizing effect is related to the delay/inhibition of matrix crystallization [3]. Trehalose crystallizes as a dihydrate, which could be an advantage as it cannot crystallize at relative humidities below 43% [7].

It is the purpose of the present work to study the influence of different yeast cellular fractions on crystallization of trehalose in supercooled systems, to gain knowledge on the feasibility of trehalose crystallization in yeast cells.

Materials and methods

Strain and growth conditions

Saccharomyces cerevisiae YSH 312 (tpsΔ::TRP1, hxx2Δ::LEU2, isogenic to W303-1A) mutant strain (a generous gift from J. Thevelein) which has no ability to synthesize trehalose (disrupted in trehalose 6P synthase gene) was employed. This strain was chosen in order to control the amount of trehalose present in the model systems. Cells were grown in YPD medium (2% peptone, 1% yeast extract, and 2% glucose) in a rotatory shaker at 30°C during 30 h (corresponding to cultures in stationary phase, \( 3 \times 10^8 \) cells/ml).

Preparation of samples

Cells from a stationary growing culture were collected by centrifugation at 5000g for 5 min and the pellet was washed twice with distilled water. The pellet was distributed in 1.5 ml plastic vials and further purification was performed to obtain “yeast extracts” (YE, cell constituents without membranes and nucleic acids) or “purified yeast proteins” (YP). The pellet (≈80% moisture content) was suspended in distilled water and homogenized by vortexing with glass beads, 1:1 ratio pellet:beads (five times 1 min shaking interspersed with 30 s ice-cooling intervals). Nucleic acids were precipitated by the addition of a streptomycin sulfate solution (7.5% w/v) in a 1:10 ratio in an ice bath during 40 min, followed by 10 min centrifugation at 10,000 rpm. The supernatant was used as YE. The proteins present in the yeast extract were precipitated with ammonium sulfate (Merck) 80% (w/v) followed by 20 min centrifugation at 10,000 rpm. The pellet was desalted by dialysis during 12 h against distilled water. The dialyzed extract was used as YP. Model systems (YET and YPT) were prepared making mixtures of YE and YP with trehalose (50% w/w). The samples were frozen at −26°C during 12 h, immersed in liquid nitrogen and freeze-dried. The freeze-drying process lasted 48 h. A Heto–Holten A/S, cooling trap model CT110 freeze-dryer (Heto Lab Equipment, Denmark) was used which operated at −110°C and at a chamber pressure of \( 4 \times 10^{-4} \) mbar. The freeze-dried samples were transferred into desiccators and kept for 14 days at 26°C over saturated salt solutions that provided constant relative humidities (RH) between 11 and 90%.

Determination of water content

The water content of the humidified samples was determined (in duplicate samples) by difference in weight before and after drying in a vacuum oven at 96°C during 48 h in the presence of desiccant (magnesium perchlorate).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to determine glass transition temperatures \( T_g \) and
endothermal melting peaks (related to the amount of crystalline sugar present in the sample). Glass transitions were recorded as the onset temperature of the discontinuities in the curves of heat flow versus temperature. Crystallization was evaluated from the integration of the endothermal peak of melting. The instrument used was a Mettler DSC 822 with a STARE thermal analysis software. The instrument was calibrated using indium. All measurements were made at 10 °C/min, using hermetically sealed aluminum pans (Mettler, 40 µL capacity), and an empty pan was used as a reference. An average value of at least two replicate samples is reported and the standard deviation for the thermal transitions is lower than 1 °C.

Results and discussion

To study the influence of different yeast cellular fractions on the crystallization of trehalose in supercooled samples, the DSC thermal behavior of yeast extract: trehalose (YET) and purified yeast protein: trehalose (YPT) models, is analyzed. Both model systems contain 50% (w/w) trehalose. Fig. 1 shows the thermal events present in YET (Fig. 1A) and YPT (Fig. 1B) samples previously humidified at various relative humidities. The glass transition temperature \( T_g \) values and the relative humidities are indicated over each curve on both figures. Only one \( T_g \) value is observed in the thermograms which can be an indication of miscibility between the sugar and the yeast components. The \( T_g \) values for YET samples (Fig. 1A) are lower than those for YPT samples (Fig. 1B) at all the relative humidities analyzed, which is expected because YET samples contain low molecular weight compounds.

An endothermic peak near 100 °C (melting of crystalline trehalose) is observed in the thermograms for YPT samples (Fig. 1B), indicating that sugar crystallization has occurred at 58 and 75% RH. On the contrary, the melting peak for trehalose is absent in the thermograms for any of the YET samples analyzed (Fig. 1A).

![Fig. 1. Glass transition temperature \( T_g \) and the melting peak (trehalose), for YET (A) and YPT (B) samples equilibrated at various relative humidities.](image-url)
Table 1 shows water sorption data at 26 °C for YPT and YET model systems, and for pure amorphous trehalose. Trehalose crystallization (as determined by DSC) is also indicated in Table 1. Amorphous trehalose adsorbs water when relative humidity increases reaching a plateau at about 43% RH; at this RH the amount of water is the required to form the dihydrate crystal [3]. In YPT samples, trehalose crystallization is observed at 58% RH and above, showing an increase in the degree of trehalose crystallization when the relative humidity is increased. The extent of crystallization is 13% at 58% RH and 37% at 75% RH (as determined by DSC). YET samples do not show trehalose crystallization at any of the relative humidities analyzed. Even at 75% RH, at which YET samples contain 36.2% water content (three times higher than the amount of water required for trehalose crystallization) and a T−Tg value of 98 °C (at which instantaneous crystallization could be expected for a pure sugar), no evidence of crystallinity is observed. These results are confirmed by microscopic observations under polarized light. The lack of trehalose crystallization suggests that the yeast extract components, which are absent in the YPT samples, inhibited/delayed trehalose crystallization.

Although crystallization of sugars in the cytoplasm has been considered as a cause of viability loss, no evidence of sugar crystallization in cells or tissues was ever reported [8]. It has been shown that the molecular mobility in seeds increases about one order in magnitude at approximately 50 °C above Tg [2]. Our samples did not show crystallization even at 98 °C above Tg (Fig. 1A), conditions at which molecular mobility should not be an impediment for the formation of crystals. Moreover, the samples in which trehalose crystallization is not observed are those of lower Tg values. The cytoplasmic glass of seeds is formed by sugars (sucrose and oligosaccharides comprise over 10–20% of the dry weight), high molecular weight oligosaccharides, and proteins. Mixtures of sucrose and oligosaccharides were found to inhibit crystallization, and it was suggested that this could be the reason for the lack of crystallization in seeds [9]. In accordance to these previous observations, present work shows that the delay/inhibition of sugar crystallization is attributable to the presence of some cytoplasmatic component/s and not to molecular mobility restrictions.

It is noteworthy that ice crystallization is inhibited in many biological systems by different mechanisms, thus preserving life under extreme conditions. It can be postulated that cells of anhydrobiotic organisms may have also mechanisms/compounds to avoid sugar crystallization in order to protect them. These mechanisms operate in the supercooled state, well above the Tg, and are not thus related to solute crystallization in the cytoplasm.

References