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Trehalose effects on α-crystallin aggregates

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Abstract

α-Crystallin in its native state is a large, heterogeneous, low-molecular weight (LMW) aggregate that under certain conditions may progressively became part of insoluble high-molecular weight (HMW) systems. These systems are supposed to play a relevant role in eye lens opacification and vision impairment. In this paper, we report the effects of trehalose on α-crystallin aggregates. The role of trehalose in α-crystallin stress tolerance, chaperone activity and thermal stability is studied. The results show that trehalose stabilizes the α-crystallin native structure, inhibits α-crystallin aggregation, and disaggregates preformed LMW systems not affecting its chaperone activity.

Keywords: α-Crystallin; Trehalose; Aggregates; Chaperone activity; Thermal stability

Crystallins are the predominant proteins within the eye lens [1]. In mammals, the crystallins family is composed of three classes denoted α, β and γ. α-Crystallin, the principal lens protein, belongs to the heat shock proteins family; it acts as a molecular chaperone, being thus crucial to prevent the aggregation and/or precipitation of other partially denatured proteins [2], itself included due to a self-chaperone property [3]. In its native state α-crystallin consists of two relatively homologous subunits αA and αB, each of about 20 kDa and is present under the form of a large, heterogeneous, water-soluble low-molecular weight (LMW) aggregate (M_W about 800 kDa) [4]. In aged lenses, the appearance of high-molecular weight (HMW) aggregates (more than 1000 kDa) and insoluble proteins has been observed [5,6]. These changes in molecular mass and heterogeneity of aggregates can even be due to modification of experimental conditions for protein manipulation [4–9]. In particular it has been demonstrated that there is a direct correlation of increasing temperature with size and shape of α-crystallin aggregates [10,11].

A number of studies have been focused on the involvement of α-crystallin in the process of eye lens opacification known as cataractogenesis [12,13]. Two relevant mechanisms have been proposed: the first is related to conformational changes [14,15] with the loss of α-crystallin molecular chaperone function due to post-translational modifications, unfolding or destabilization; the second refers to the formation of high-molecular weight (HMW) aggregates of sufficient size to scatter incident light [12].

Cataract is a relevant cause of vision impairment in the elderly and actually the surgical lens replacement represents the only available cure; therefore, it is of great pharmacological interest the identification of new molecules that are able to counteract this conformational protein disease. To stabilize protein folding, inhibit and prevent protein aggregation and modification, several small molecules have been studied [16–20]. Among these, trehalose, a non-reducing disaccharide composed of two glucose molecules linked by an α,α-1,1 bond, has been used as a protector

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against various stresses, such as heat, freezing, osmotic shock and desiccation [21–23].

It has been shown, for example, that trehalose inhibits the formation of fibrillar aggregates of insulin [17] and the aggregation of the peptide beta-amyloid (Aβ) [24]. More recently, a reduction of aggregates formation and symptoms delay has been observed in a transgenic mouse model of oculopharyngeal muscular dystrophy after treatment with trehalose [25]; moreover, it has been reported that trehalose reduces the polymerization rate of mutants of neuroserpin and α_1-antitrypsin responsible of dementia and cirrhosis [26].

In this work, we studied the effects of trehalose on the α-crystallin aggregates and demonstrated its protective effect in heat shock tolerance by means of ThT fluorescence and CD measurements. The effect of trehalose on chaperone activity and thermal stability of α-crystallin was also studied by turbidity measurements and differential scanning calorimetry (DSC). Scanning force microscopy was used to determine the influence of the disaccharide on the aggregate morphology.

Materials and methods

Chemicals. All chemicals and reagents were purchased from Sigma–Aldrich (Milan, Italy).

Circular dichroism (CD) measurements. Bovine α-crystallin was dissolved at 0.5 mg/ml in 0.0025 M phosphate buffer solutions, pH 7, containing different trehalose concentrations (0.1–1 M). Aliquots of these samples were incubated at 25 and 60 °C for 3 h.

CD spectra of α-crystallin samples were recorded at room temperature on a Jasco (Tokyo, Japan) J-810 spectropolarimeter and processed by Jasco Spectra Manager 1.5 software. The CD spectra were corrected by subtraction of the background solvent spectrum obtained under identical experimental conditions and smoothed for clarity of display. The CD spectra were made in duplicate and were acquired using a quartz cell with 1 mm path length. All the data were converted to give specific ellipticity values [Ψ] based upon the sample concentration and expressed in deg cm^2 decagram^{-1} units. α-Crystallin concentration was determined spectrophotometrically by measuring the absorbance at 280 nm using an extinction coefficient $\varepsilon = 0.83$.

Thioflavin T (ThT) fluorescence measurements. Fluorescence emission spectra of ThT are shifted towards the long-wave region upon incorporation into β-sheet aggregates structures [27,28]. The change in the extent of α-crystallin aggregation was measured by mixing α-crystallin (125 μl) from incubated samples with ThT solution (2.35 ml) in 0.0025 M phosphate buffer (pH 7.00). Final solutions resulted 50 μM ThT and contained 0.05 mg/ml protein. The measurements were carried out using a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter.

Fluorescence emission spectra were monitored from 450 to 600 nm in a 1 cm light path quartz cell. An excitation wavelength of 442 nm was used. Both excitation and emission bandwidths were set to 5 nm. The ThT spectra were corrected by subtraction of the background solvent spectrum obtained under identical experimental conditions. The experiments were performed in triplicate.

Scanning force microscopy (SFM). SFM imaging was performed using a Multimode/Nanoscope IIIA (Digital Instrument, Santa Barbara, CA). Bovine α-crystallin (0.5 mg/ml) was dissolved in 0.0025 M phosphate buffer, pH 7, alone or in the presence of 1 M trehalose. The samples were incubated at 25 and 60 °C for 3 h. α-Crystallin was also dissolved at 5 mg/ml in 0.0025 M phosphate buffer, pH 7, alone or in the presence of 1 M trehalose and incubated at 60 °C for 5 days. Prior to SFM measurements, the samples were diluted 1:1 with 0.0025 M phosphate buffer, pH 7, and with 1 M trehalose, respectively. Each sample (10 μl) was applied to freshly cleaved mica, left for 5 s and then rinsed five times with double distilled water. Finally, the samples were dried by a nitrogen stream for one minute and imaged immediately.

In order to avoid protein damage, the instrument was set in dynamic mode working in the net tip-sample attractive regime, as previously described [29]. Commercially available etched silicon probes (Digital) were used and 512×512 points were collected for each image by maintaining the scan rate at about 1 Hz. Statistical analysis was carried out by using Nanoscope III software. Note that the lateral dimension of nanoscopic objects imaged by SFM is affected by the well-known tip-sample convolution effect, resulting in a broadening artefact [30] which in our case was in the range of 10–20 nm; thus, the height values better represent the dimension of the structures.

Chaperone activity experiments. Chaperone activity assay was used to measure the ability of α-crystallin to protect β_L-crystallin against heat-induced aggregation. Bovine β_L-crystallin (1 mg/ml) was dissolved in 1 M trehalose in the absence or presence of α-crystallin with a β_L- to α-crystallin molar ratio of 2.2:1. Aggregation of β_L-crystallin was monitored by absorption due to light scattering at a wavelength of 360 nm in a diode-array Agilent 8453 spectrophotometer (Santa Clara, CA, USA) equipped with a ThermoHaake C40P programmable refrigerated circulating bath.

Assays were performed at the temperature of 60 °C using a quartz cell with 1 mm path length.

Calorimetric studies. Calorimetric measurements were carried out on a VP-DSC Microcalorimeter (MicroCal Inc., Northampton, MA). All measurements were performed in 0.0025 M phosphate buffer, pH 7. Protein and trehalose concentration was 0.5 mg/ml and 1 M, respectively. The scan rate was 1 K/min for all experiments. To estimate the reversibility of the unfolding transition, samples were scanned for a second time after cooling. Buffer baselines were measured under identical conditions and subtracted from the corresponding scans of protein and protein–trehalose samples. All protein solutions were degassed at 20 °C before calorimetric measurements. Data analysis was performed using Origin software (MicroCal).

Results and discussion

Lens α-crystallin in solution appears as a large, heterogeneous, low-molecular weight (LMW) aggregate that progressively becomes part of a high-molecular weight (HMW) aggregate and insoluble proteins; in aging and cataractous lenses, a large quantity of HMW aggregates is known to occur [31,32]. These aggregates can be also formed *in vitro* under different conditions [10,11]. Previous works have shown that some small molecules are able to stabilize protein folding, inhibit protein aggregation and preserve enzymatic activities against heat shock and freeze drying [16–19]. In particular, trehalose has been observed to provide different protective ability against aggregation of various proteins [17,23–26].

To study the trehalose ability to dissolve preformed LMW α-crystallin aggregates, the protein (0.5 mg/ml) was incubated alone and in the presence of increasing trehalose concentration (0.1–1 M) at 25 °C for 3 h. Trehalose dissolved the LMW α-crystallin aggregates in a concentration-dependent manner as indicated by the decrease of the ThT fluorescence intensity (Fig. 1A). The CD spectra (Fig. 1C) showed a minimum at around 218 nm, which indicates the prevalence of a high proportion of β-sheet conformation [33]. According to the fluorescence measurements, a small progressive decrease in the negative signal at