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Effect of α-crystallin on thermal denaturation and aggregation of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase

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Abstract

The study of thermal denaturation of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the presence of α-crystallin by differential scanning calorimetry (DSC) showed that the position of the maximum on the DSC profile (T_{max}) was shifted toward lower temperatures with increasing α-crystallin concentration. The diminishing GAPDH stability in the presence of α-crystallin has been explained assuming that heating of GAPDH induces dissociation of the tetrameric form of the enzyme into dimers interacting with α-crystallin. The dissociation of the enzyme tetramer was shown by sedimentation velocity at 45 °C. Suppression of thermal aggregation of GAPDH by α-crystallin was studied by dynamic light scattering under the conditions wherein temperature was elevated at a constant rate. The construction of the light scattering intensity versus the hydrodynamic radius (R_h) plots enabled estimating the hydrodynamic radius of the start aggregates ($R_{h,0}$). When aggregation of GAPDH was studied in the presence of α-crystallin, the start aggregates of lesser size were observed.

Keywords: α-Crystallin; Glyceraldehyde-3-phosphate dehydrogenase; Thermal aggregation; Thermal denaturation

1. Introduction

Thermal treatment of proteins results in destabilization of the protein compact structure which may be followed by protein aggregation and precipitation. α-crystallin, a member of a small heat shock protein (sHSP) superfamily, exhibits the chaperone-like activity and prevents thermally induced aggregation of proteins [1,2]. α-crystallin can recognize and bind unfolded proteins over a range of temperatures, and a direct correlation between temperature, monomer exchange and chaperone-like activity of α-crystallin has been demonstrated [3–5]. The ability of α-crystallin to suppress heat-induced aggregation of denatured proteins increases when α-crystallin is heated [6–8]. At 25 °C α-crystallin exhibits relatively poor chaperone-like activity [3,8–10], and a structural transition at 30–40 °C leads to enhanced chaperone activity by increasing or favorably reorganizing the hydrophobic substrate-binding surfaces [4,5,11–13]. The increased activity of aggregates of α-crystallin suggests that its chaperone-like activity depends in part on the packing parameters of the aggregate and on conformation of the subunit within the aggregate. α-Crystallin undergoes two structural transitions as a function of temperature: one around 30 °C and the other around 55 °C, resulting in the exposure of hydrophobic surfaces. The first transition involves subtle tertiary structural changes and quaternary structural reorganization in the heteroaggregates. [4,7,10]. The structure and the thermal stability of bovine α-crystallin studied by Fourier-transform infrared spectroscopy, circular dichroism, and

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differential scanning calorimetry show that α-crystallin undergoes a major thermotropic transition with a midpoint at 60–62 °C [7]. That results in partial unfolding of the protein, which then doubles in molecular weight and increases in size [7,8,10], but does not aggregate further [12]. Infrared spectroscopic data show that the secondary structure of α-crystallin is highly ordered and consists predominantly of beta-sheets [7] and Fourier-transform infrared spectra provide evidence that conformational transition at 60 °C is associated with a massive loss of the native beta-sheet structure [7]. Under physiological conditions the quaternary assembly of α-crystallin is characterized by a relatively fast subunit-exchange, indicating a certain degree of conformational flexibility of the α-crystallin protomers [8,11,14–17]. With increase in temperature the number of α-crystallin monomers per oligomer changes. This proceeds by temperature-dependent monomer exchange among individual oligomers [14].

Two main questions regarding the interaction of α-crystallin with target protein are in the focus of intense research: (1) what structural particularities of the target protein are important in the chaperone function of α-crystallin and (2) what are the protein structures which interact with α-crystallin [2,18,19]. It is known that a higher number of target protein binding sites become available at elevated temperatures [2]. The sites available at low temperatures are a subset of the total sites available at elevated temperatures.

Studies of thermal denaturation of carbonic anhydrase and α-lactalbumin show that only the aggregation-prone proteins in the molten globule state bind to α-crystallin [20]. Study of interaction of human recombinant αA- and αB-crystallin with early and late unfolding intermediates of citrate synthase at its thermal denaturation demonstrate that the chaperone activity of α-crystallin involves both transient and stable interactions depending on the nature of intermediates on the unfolding pathway; one type of interaction leads to reactivation of the enzyme activity while the other prevents aggregation [18].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12), a tetrameric enzyme of 144 kDa, has been extensively studied for structural changes during denaturation [21–23]. It has been shown that the secondary structure of GAPDH is relatively heat stable, showing no appreciable change at 38.5 °C [23]. At this temperature at pH 8.5, the enzyme first dissociates within several minutes – probably into dimers – and with prolonged heating it becomes irreversibly aggregated. The dissociative mechanism of thermal denaturation of GAPDH was established in Ref. [24] using differential scanning calorimetry (DSC) and analytical ultracentrifugation. Aggregation and precipitation of rabbit muscle GAPDH at 55 °C is effectively inhibited in the presence of αB-crystallin [25].

The goal of the present work was to study thermal denaturation and aggregation of rabbit muscle GAPDH in the presence of α-crystallin. The results obtained by differential scanning calorimetry (DSC) showed that stability of the enzyme was reduced in the presence of α-crystallin. The study of kinetics of GAPDH aggregation using dynamic light scattering (DLS) allowed us to demonstrate that in the presence of α-crystallin the transition from the regime of diffusion-limited cluster–cluster aggregation (DLCA) to the regime of reaction-limited cluster–cluster aggregation (RLCA) takes place.

2. Materials and methods

2.1. Isolation and purification of GAPDH

GAPDH was isolated from rabbit muscles as described by Scopes and Stoter [26] with an additional purification step using gel-filtration on Sephadex G-100 (superfine). GAPDH concentration was determined spectrophotometrically at 280 nm using the absorption coefficient $A_{cm}^{1\%}$ of 10.6 [27].

2.2. Isolation and purification of α-crystallin

Purification of α-crystallin from freshly excised lenses of 2-year-old steers was performed according to the procedure described earlier [28,29]. α-Crystallin concentration was determined spectrophotometrically at 280 nm using the absorption coefficient $A_{cm}^{1\%}$ of 8.5 [8].

2.3. Calorimetric studies

Thermal denaturation of GAPDH and α-crystallin was studied by DSC. DSC experiments were performed using a DASM-4M differential scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia). All measurements were performed in 10 mM Na-phosphate buffer, pH 7.5. The protein solution was heated at a constant rate of 1 K min^{-1} from 5 to 90 °C, and at a constant pressure of 2.2 atm. The reversibility of the thermal transition of GAPDH and α-crystallin was tested by checking the reproducibility of the calorimetric trace in a second heating of the sample immediately after cooling. Calorimetric traces of irreversibly denaturing proteins were corrected for instrumental background and for possible aggregation artifacts by subtracting the scans obtained from the second heating of the samples. The temperature dependence of the excess heat capacity was further analyzed and plotted using Origin software (OriginLab Corporation, USA).

2.4. DLS studies

For light scattering measurements a commercial instrument Photocor Complex was used (Photocor Instruments Inc., USA; www.photocor.com). This instrument allows measuring both dynamic and static light scattering at various scattering angles with stepper-motor controlled turntable [30]. An He–Ne laser (Coherent, USA, Model 31-2082, 632.8 nm, 10 mW) has been used as a light source. The temperature of sample cell was controlled by the proportional integral derivative (PID) temperature controller to within ±0.1 °C. The quasi-cross correlation photon counting system with two photomultiplier tubes (PMT) was used to increase the accuracy of particle sizing in the range of 0.5–10 nm. DLS data have been accumulated and analyzed with multifunctional real-time correlator Photocor-FC that has both logarithmic multiple-tau and linear time-scale modes.