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Effects of small heat shock proteins on the thermal denaturation and aggregation of F-actin ☆

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Abstract

Effect of recombinant chicken small heat shock protein with molecular mass 24 kDa (Hsp24) and recombinant human small heat shock protein with molecular mass 27 kDa (Hsp27) on the heat-induced denaturation and aggregation of skeletal F-actin was analyzed by means of differential scanning calorimetry and light scattering. All small heat shock proteins did not affect thermal unfolding of F-actin measured by differential scanning calorimetry, but effectively prevented aggregation of thermally denatured actin. Small heat shock protein formed stable complexes with denatured (but not with intact) F-actin. The size of these highly soluble complexes was smaller than the size of intact F-actin filaments. It is supposed that protective effect of small heat shock proteins on the cytoskeleton is at least partly due to prevention of aggregation of denatured actin.

Keywords: Cytoskeleton; Actin; Small heat shock proteins; Thermal denaturation; Aggregation; Chaperone activity

Small heat shock proteins with apparent molecular mass 24/27 (Hsp24/27) and αB-crystallin are closely related members of a large family of small heat shock proteins (sHsp) that are highly expressed in different muscles [1–3]. Many investigations (reviewed in [4,5]) were devoted to analyses of interaction of sHsp with cytoskeleton and especially with actin. There are two main lines of investigation.

Hsp25 (another name for Hsp24/27) was claimed to be an actin-capping protein that depending on oligomeric state and extent of phosphorylation may inhibit actin polymerization [6,7]. Two peptides derived from Hsp25 and αB-crystallin effectively inhibited actin polymerization [8]. However, these peptides are located close to the subunit contacts inside of oligomers formed by Hsp24/27 and αB-crystallin [8], and therefore are not easily available for interaction with actin. Moreover, intact αB-crystallin slightly activated actin polymerization [9], whereas unphosphorylated Hsp25 had no effect on actin polymerization [10,11]. Phosphorylated Hsp25 or Hsp25 mutants mimicking phosphorylation only weakly activated [10] or inhibited actin polymerization [11]. Thus, the data concerning direct effect of Hsp24/27 or αB-crystallin on actin polymerization are very contradictory.

The second line of investigation dealt with protective effect of sHsp on cytoskeleton. Multiple publications

☆ *Abbreviations:* DSC, differential scanning calorimetry; Hsp24, recombinant chicken small heat shock protein with apparent molecular mass 24 kDa; Hsp27, recombinant human heat shock protein with apparent molecular mass 27 kDa; 3D mutants of Hsp24 or Hsp27, mutant with replacing SI5, S77, S81 (Hsp24) or SI5, S78, S82 (Hsp27) with aspartic acid; sHsp, small heat shock proteins; WT, wild type.

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indicate that the small heat shock proteins protect actin filaments from fragmentation induced by oxidative stress [12], heat shock [13,14], energy depletion [15,16], repeated stretches [17] or cytochalasin D [9,13,14]. It has been shown that different types of injury induced translocation of sHsp from cytosol to cytoskeleton and it was followed by stabilization of actin filaments and their contacts with membrane and membrane scaffolding proteins [13,14,16,17]. The detailed molecular mechanism of protective action of small heat shock proteins is unknown, but one can suppose that sHsp either protect actin from different types of damages or prevent aggregation of unfolded actin. It is worthwhile to mention that actin concentration in muscle is very high and is of the order of 1000 μM [18]. Aggregation of even a small part of actin will be dangerous for the cell and therefore actin seems to be a very important intracellular substrate for different chaperones. However, to our knowledge actin was only rarely used as a substrate for analyzing the chaperone activity of sHsp [9,11]. In order to understand the protective effect of small heat shock proteins on cytoskeleton, we analyzed the effect of sHsp on the heat-induced denaturation and aggregation of F-actin.

Materials and methods

Proteins. Recombinant chicken Hsp24 and human Hsp27 and their 3D mutants with replacing Ser15, Ser77/78, and Ser81/82 by Asp residues were obtained as described earlier [11,19]. Rabbit skeletal actin was obtained according to Spudich and Watt [20]. All proteins were homogeneous according to SDS-gel electrophoresis [21].

Protein aggregation. Heat-induced F-actin aggregation was measured by light scattering on a Cary Eclipse fluorescence spectrophotometer (Varian) equipped with temperature controller and thermoprobes. F-actin in the absence or in the presence of different quantities of sHsp was heated with constant rate (1 or 2 °C/min) from 30 up to 75–90 °C. The light scattering at 350 nm was measured with excitation and emission slits equal to 2.5 and 1.5 nm, respectively. At fixed temperatures aliquots of the sample were withdrawn, cooled down, and subjected to ultracentrifugation (140,000*g* for 30 min). The protein composition of the supernatant and pellet was determined by SDS-gel electrophoresis [21].

Differential scanning calorimetry. Differential scanning calorimetry (DSC) experiments were performed on a DASM-4M differential scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia) as described earlier [22,23]. All measurements were performed in 30 mM Hepes/KOH buffer (pH 7.3) containing 2 mM $MgCl_2$ and 100 mM KCl. Protein solution (1.0–1.5 mg/ml) was heated with constant rate of 1 K/min from 5 to 100 °C at the constant pressure of 2.2 atmospheres. The reversibility of the thermal transitions was tested by checking the reproducibility of the calorimetric trace in a second heating of the sample immediately after cooling. The thermal denaturation of F-actin was fully irreversible while the denaturation of sHsp was completely reversible. Calorimetric traces of F-actin 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 (MicroCal Inc.). Transition temperatures (T_m) were determined from the maximum of the thermal transition.

Analytical centrifugation. Sedimentation velocity experiments with unheated or heated F-actin in the absence or in the presence of sHsp were carried out on Beckman model E analytical ultracentrifuge equipped with a photoelectric scanner. All the experiments were performed in a standard four-hole rotor An-F Ti at 40,000 rpm for F-actin or 56,000 rpm for isolated 3D mutant of Hsp27. Sedimentation was followed by UV absorption at 280 nm. Distribution of sedimentation coefficients was determined by SEDFIT program [24].

Results

Effect of small heat shock proteins on thermal denaturation of F-actin

Differential scanning calorimetry (DSC) was used to analyze thermal denaturation of F-actin, small heat shock proteins, and their complexes. In good agreement with earlier published results [22,23], we found that the heat sorption peak for isolated F-actin was at 62 °C (Fig. 1A) and denaturation of F-actin was irreversible. Thermal transition of isolated 3D mutant of Hsp27

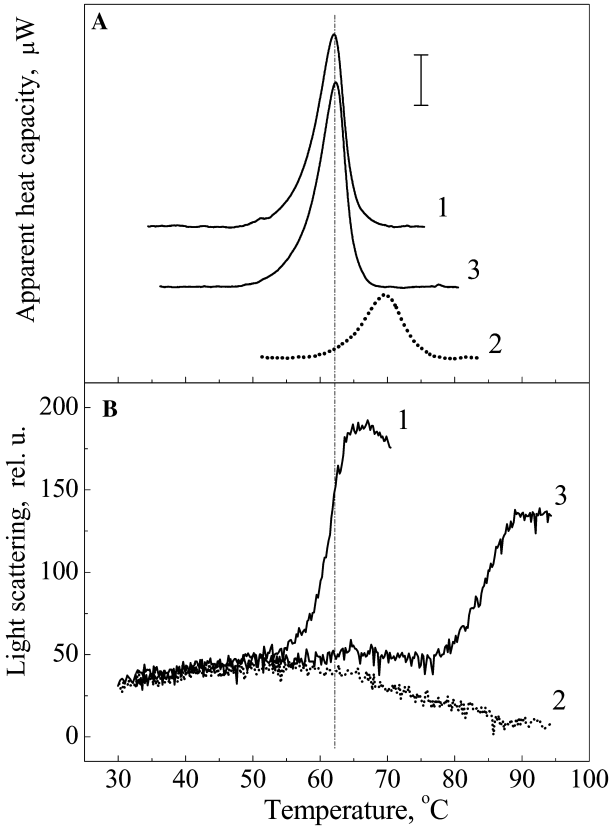

Fig. 1. Effect of 3D mutant of Hsp27 on the thermally induced denaturation (A) and aggregation (B) of F-actin. Isolated F-actin (1 mg/ml) (1), isolated 3D mutant of Hps27 (1 mg/ml) (2) or F-actin (1 mg/ml) in the presence of 3D mutant of Hsp27 (0.33 mg/ml) (3) were heated with the constant rate of 1 °C/min and thermal denaturation or aggregation were measured by differential scanning calorimetry (A) or light scattering (B). The vertical bar in (A) corresponds to 5 μW.