FEBS Letters 582 (2008) 1407–1412

Small heat shock protein Hsp27 protects myosin S1 from heat-induced aggregation, but not from thermal denaturation and ATPase inactivation

Denis I. Markov[a], Anastasia V. Pivovarova[a,b], Ivan S. Chernik[c], Nikolai B. Gusev[c], Dmitrii I. Levitsky[a,d,*]

[a] *A. N. Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky prosp. 33, Moscow 119071, Russia*
[b] *School of Bioengeneering and Bioinformatics, Moscow State University, Russia*
[c] *Department of Biochemistry, School of Biology, Moscow State University, Russia*
[d] *A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia*

Received 28 December 2007; revised 11 February 2008; accepted 8 March 2008

Available online 1 April 2008

Edited by Felix Wieland

Abstract **We applied different methods, such as turbidity measurements, dynamic light scattering, differential scanning calorimetry and co-sedimentation assay, to analyze the interaction of small heat shock protein Hsp27 with isolated myosin head (myosin subfragment 1, S1) under heat-stress conditions. Upon heating at 43 °C, Hsp27 effectively suppresses S1 aggregation, and this effect is enhanced by mutations mimicking Hsp27 phosphorylation. However, Hsp27 was unable to prevent thermal unfolding of myosin heads and to maintain their ATPase activity under heat-shock conditions.**

Structured summary:
MINT-6490863, MINT-6490872:
LC1 (S1) (uniprotkb:P02602), *Myosin subfragment 1 (S1)* (uniprotkb:P02562) and *Hsp27* (uniprotkb:P04792) *physically interact* (MI:0218) by *dynamic light scattering* (MI:0038)
MINT-6490833:
LC1 (S1) (uniprotkb:P02602), *Myosin subfragment 1 (S1)* (uniprotkb:P02562) and *Hsp27* (uniprotkb:P04792) *physically interact* (MI:0218) by *cosedimentation* (MI:0027)
MINT-6490770, MINT-6490782:
LC1 (S1) (uniprotkb:P02602), *Myosin subfragment 1 (S1)* (uniprotkb:P02562) and *Hsp27* (uniprotkb:P04792) *physically interact* (MI:0218) by *light scattering* (MI:0067)

Keywords: Myosin subfragment 1; Small heat shock proteins; Thermal denaturation; Protein aggregation; Differential scanning calorimetry; Dynamic light scattering

1. Introduction

Mammalian small heat shock protein with apparent molecular mass 27 kDa (Hsp27) and αB-crystallin are closely related members of a large family of small heat shock proteins (sHSPs) [1]. Almost all sHSPs assemble into large oligomeric complexes that vary in structure and number of monomers [2]. The dynamic organization of sHSPs oligomers seems to be important for regulation of their activity. *In vitro*, sHSPs act as molecular chaperones in preventing partially or fully unfolded proteins from irreversible aggregation and insolubilization [2–5], and chaperone activity of sHSPs is dependent on their quaternary structure [6,7]. Different protein kinases phosphorylate sHSPs, and by this means might affect their oligomeric structure and chaperone activity [2,8,9]. Hsp27 is effectively phosphorylated by MAPKAP2 kinase [9], and this phosphorylation modulates its activity in response to different stimuli and stress conditions [10,11]. Hsp27 phosphorylation (or mutations mimicking phosphorylation) was demonstrated to induce dissociation of large Hsp27 oligomers and formation of dimers and tetramers [6,7,12–14].

Both Hsp27 and αB-crystallin are highly produced in muscle tissues and their content is increased upon different types of injury, such as heat shock and ischemia. It seems very likely that one of the main functions of these sHSPs in muscles is their interaction with actin and myosin under unfavorable conditions. Myosin and actin are the most abundant muscle proteins and their interaction coupled with ATP hydrolysis by myosin heads is the molecular basis of muscle contraction. Many investigations have been devoted to analyses of the interaction of sHSPs with actin [8,15–20]. In particular, we have shown that Hsp27 mutant S15D, S78D, S82D mimicking phosphorylation (denoted as Hsp27-3D) does not interact with intact actin filaments, but prevents heat-induced aggregation of F-actin by forming relatively small, stable and highly soluble complexes with denatured actin [18,20].

Much less is known about interaction of sHSPs with myosin. Up until now, the only publication by Melkani et al. [21] has been devoted to the interaction of αB-crystallin with skeletal muscle myosin. The authors showed that, upon incubation at heat-shock temperature (43 °C), αB-crystallin not only prevents the aggregation of myosin, but also suppresses to some extent the heat-induced inactivation of myosin ATPase. These

*Corresponding author. Address: A. N. Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky prosp. 33, Moscow 119071, Russia. Fax: +7 495 954 2732.
E-mail address: levitsky@inbi.ras.ru (D.I. Levitsky).

Abbreviations: DLS, dynamic light scattering; DSC, differential scanning calorimetry; Hsp27, recombinant human heat shock protein with apparent molecular mass 27 kDa; Hsp27 wt, wild type Hsp27; Hsp27-3D, pseudophosphorylated Hsp27 with mutations S15D, S78D and S82D; sHSP, small heat shock proteins; S1, myosin subfragment 1

0014-5793/$34.00
doi:10.1016/j.febslet.2008.03.035

results, together with electron microscopy observations on rotary shadowed myosin molecules, allowed Melkani and coauthors to suppose that αB-crystallin and probably other mammalian sHSPs may interact with the heads of myosin molecules under heat-shock conditions and suppress their thermal unfolding leading to aggregation and ATPase inactivation [21].

To check this assumption and to elucidate how sHSPs interact with myosin, we studied the effects of Hsp27 and its pseudophosphorylated mutant Hsp27-3D on the thermal unfolding of isolated myosin head termed subfragment 1 (S1), as well as on its aggregation and inactivation of its ATPase under heat-shock conditions. We found that, upon heating at 43 °C, the wild type Hsp27 and especially Hsp27-3D effectively suppress S1 aggregation. However, in contradiction with the effect of αB-crystallin described by Melkani et al. [21], we did not observe any effects of Hsp27 on the S1 thermal unfolding as measured by differential scanning calorimetry (DSC) as well as on the heat-induced inactivation of the S1 ATPase.

2. Materials and methods

2.1. Proteins

S1 from rabbit skeletal myosin was prepared by digestion of myosin filaments with α-chymotrypsin [22]. The concentration of S1 was estimated spectrophotometrically using extinction coefficient $E^{1\%}$ at 280 nm of 7.5 cm^{-1}. Recombinant human Hsp27 and its pseudophosphorylated mutant Hsp27-3D were cloned, expressed and purified as described earlier [16,23]. All proteins were homogeneous according to SDS–PAGE [24].

2.2. ATPase inactivation

The thermally induced inactivation of S1 ATPase was measured after heating the protein (0.5 mg/ml) at 43 °C in the medium containing 30 mM Hepes (pH 7.3), 1 mM $MgCl_2$, and 100 mM KCl, in the presence or in the absence of Hsp27. S1 aliquots were heated for appropriate periods of time, then cooled and subjected to ATPase measurements. The experiments on myosin ATPase were performed under similar conditions except that lower protein concentration (0.04 mg/ml) and Tris–HCl (pH 7.5) buffer containing 5 mM $CaCl_2$ was used instead of Hepes. The ATPase activity of S1 (or myosin) was determined by P_i release [25] at 25 °C in the medium containing S1 or myosin (0.04 mg/ml), 1 mM ATP, and either 0.5 M KCl, 5 mM EDTA, and 50 mM Tris–HCl (pH 7.5) for K^+-EDTA-ATPase, or 5 mM $CaCl_2$ and 25 mM Tris–HCl (pH 7.5) for Ca^{2+}-ATPase. Reaction was initiated by addition of ATP and stopped after 10 min of incubation by addition of $HClO_4$ to final concentration of 2.5%.

2.3. DSC studies

DSC experiments were performed on a DASM-4M differential scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia) as described earlier [18,26,27]. All measurements were performed in 30 mM Hepes-KOH (pH 7.3) containing 1 mM $MgCl_2$ and 100 mM KCl. Protein solution was heated with constant rate of 1 K/min from 5 to 90 °C. The reversibility of the thermal transitions was tested by checking the reproducibility of the calorimetric trace in the second heating of the sample immediately after cooling. The thermal denaturation of S1 was fully irreversible while the denaturation of Hsp27 was completely reversible. Calorimetric traces were corrected for instrumental background by using special DSC approach described earlier [28]. Transition temperatures (T_m) were determined from the maximum of the thermal transition.

2.4. S1 aggregation

Thermally induced aggregation of S1 was determined by changes in either turbidity or light scattering upon incubation of S1 at 43 °C in the absence or in the presence of Hsp27. Turbidity was measured as apparent optical density at 360 nm on a 3100 Pro spectrophotometer (Amercham Pharmacia). Light scattering measurements were performed at 90° on a Cary Eclipse fluorescence spectrophotometer (Varian) equipped with a temperature controller and thermoprobes. The light scattering at 350 nm was measured with both excitation and emission slits equal to 1.5 nm. All experiments were performed in 30 mM Hepes (pH 7.3) containing 1 mM $MgCl_2$ and 100 mM KCl.

2.5. Dynamic light scattering (DLS)

DLS measurements were performed on a Photocor Complex apparatus (Photocor Instruments Inc., USA) equipped with a temperature controller. The sample was illuminated by a 633 nm laser light, and the scattering signal was observed at an angle of 90°. DLS data were accumulated and analyzed with multifunctional real-time correlator Photocor-FC. DynaLS software (Alango, Israel) was used for polydisperse analysis of DLS data. The kinetics of S1 aggregation was studied by measuring an increase in the mean hydrodynamic radius of the particles (R_h) upon incubation of S1 at 43 °C in the absence or in the presence of Hsp27 in 30 mM Hepes (pH 7.3) containing 1 mM $MgCl_2$ and 100 mM KCl.

2.6. Cosedimentation experiments

S1 (0.5 mg/ml) in the absence or in the presence of different quantities of Hsp27-3D was heated at 43 °C in the medium containing 30 mM Hepes (pH 7.3), 1 mM $MgCl_2$, and 100 mM NaCl. During heating, aliquots of the sample were withdrawn, cooled down, and subjected to ultracentrifugation at 140,000 g for 20 min on Beckman airfuge (Beckman Instruments Inc., USA). The protein composition of the supernatants was determined by SDS–PAGE [24].

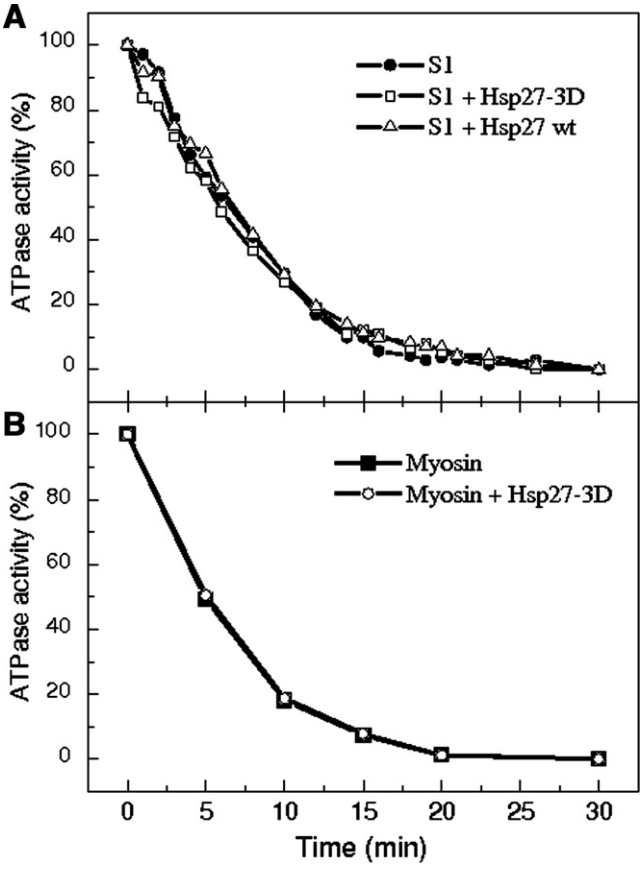

Fig. 1. Kinetics of heat-induced inactivation of S1 K^+-EDTA-ATPase (A) and myosin Ca^{2+}-ATPase (B) in the course of incubation of S1 (0.5 mg/ml) or myosin (0.04 mg/ml) at 43 °C in the absence or in the presence of Hsp27 (or Hsp27-3D). The weight ratios of sHSPs to S1 or myosin in the incubation mixture varied from 1:8 to 1:1. In all cases, the inactivation curves obtained in the absence or in the presence of sHSPs were nearly the same, independently of the concentration of sHSP added. 100% ATPase activity was 0.49 and 0.44 μmol P_i/min per mg of protein for S1 and myosin, respectively.