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Hsp105 but not Hsp70 family proteins suppress the aggregation of heat-denatured protein in the presence of ADP

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Abstract **Hsp105α and Hsp105β are mammalian members of the Hsp105/110 family, a diverged subgroup of the Hsp70 family. Here, we show that Hsp105α and Hsp105β bind non-native protein through the β-sheet domain and suppress the aggregation of heat-denatured protein in the presence of ADP rather than ATP. In contrast, Hsc70/Hsp40 suppressed the aggregation of heat-denatured protein in the presence of ATP rather than ADP. Furthermore, the overexpression of Hsp105α but not Hsp70 in COS-7 cells rescued the inactivation of luciferase caused by ATP depletion. Thus, Hsp105/110 family proteins are suggested to function as a substitute for Hsp70 family proteins to suppress the aggregation of denatured proteins in cells under severe stress, in which the cellular ATP level decreases markedly.**

Key words: Hsp105α; Hsp105β; Hsc70; Thermal aggregation; ATP; ADP

1. Introduction

The 70-kDa heat shock protein (Hsp70) family is a major and well-characterized group of heat shock proteins, and most eukaryotes have at least several different species of Hsp70 in a variety of cellular compartments. These proteins play a key role in the folding, translocation and degradation of cellular proteins as molecular chaperones [1,2]. The chaperone activity of Hsp70 and Hsc70 (a constitutive form of Hsp70) relies on cycles of substrate binding and release, which are driven by conformational changes of Hsp70. The ATP-bound form of Hsp70 binds and releases peptide rapidly, resulting in a low affinity for substrate, whereas the ADP-bound Hsp70 can bind substrate slowly but more stably, resulting in a high affinity for substrate [3–6]. The conformational changes of Hsp70 are induced by its intrinsic ATPase activity, which is facilitated by co-chaperones of the Hsp40 family [7].

Hsp105α and Hsp105β are mammalian members of the Hsp105/110 family, a diverged subgroup of the Hsp70 family. Hsp105α is expressed constitutively and induced by various forms of stress, while Hsp105β is an alternatively spliced form of Hsp105α that is specifically produced following heat shock at 42°C [8]. Hsp105α and Hsp105β exist as complexes associated with Hsp70 and Hsc70 in mammalian cells [9,10]. Recently, we reported that Hsp105α and Hsp105β not only suppress the aggregation of denatured proteins, but also regulate the Hsc70 chaperone system [11]. Furthermore, Hsp105α and Hsp105β are phosphorylated at Ser^{509} by protein kinase CK2 (CK2) in vitro and in vivo [12,13], and the CK2-mediated phosphorylation modulates the inhibitory effect of Hsp105α on Hsp70/Hsc70 chaperone activity [13].

In this study, we examined the effects of adenine nucleotides on the suppression by Hsp105α and Hsp105β of the thermal aggregation of luciferase, and showed that Hsp105α and Hsp105β strongly suppressed the aggregation in the presence of ADP rather than ATP. In contrast, Hsc70 with Hsp40 suppressed the thermal aggregation of luciferase in the presence of ATP rather than ADP. Furthermore, the overexpression of Hsp105α but not Hsp70 in COS-7 cells rescued the inactivation of luciferase caused by ATP depletion. These findings suggested that Hsp105 but not Hsp70 family proteins play important roles in protein disaggregation in cells in which the cellular ATP level decreases markedly under conditions of stress.

2. Materials and methods

2.1. Plasmids

Expression plasmids for His-tagged wild-type mouse Hsp105α and Hsp105β (pTrcHis105-1 and pTrcHis105-2) in *Escherichia coli* have been described previously [11]. For the construction of deletion mutants of Hsp105, a polymerase chain reaction (PCR) was performed with pTrcHis105-1 as the template and 5′ end-phosphorylated specific primers: His-Hsp105Δβ (deleted region: 393–511 aa), 5′-CTCGAGGCAGACATGGAATGTCCA-3′ and 5′-AGAAAGAATTGCACACTGCAGTGCAC-3′; His-Hsp105ΔL (deleted region: 512–608 aa), 5′-GGGAGAGACCTTCTTAACATGTATATTG-3′ and 5′-AGAGGAGCCATCCTCTTCCTCGGT-3′; His-Hsp105ΔC5 (deleted region: 854–858 aa), 5′-CTAGACAGAGCCCTTCTCATTAGGGTG-3′ and 5′-GGTACCAAGCTTGGCTGTTTTGGCGGATGA-3′. The PCR products were digested with *Dpn*I and self-circularized. Expression plasmids for His-Hsp105N2 (residues 1–511 aa), His-Hsp105C3 (residues 386–858 aa) and His-Hsp105αC (residues 444–858 aa) have been described previously [13]. All constructs were transformed into *E. coli* BL21.

Expression plasmids in mammalian cells for human Hsp70 (pCMV70) [14], mouse Hsp105α (pcDNA105α) [15] and firefly luciferase (pGL3-control, Promega) were used.

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Abbreviations: Hsp, heat shock protein; CK2, protein kinase CK2; PCR, polymerase chain reaction; DTT, dithiothreitol; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; CBB, Coomassie brilliant blue; BSA, bovine serum albumin; RCMLA, reduced carboxymethylated α-lactalbumin; LA, α-lactalbumin

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2.2. Protein purification

Recombinant His-tagged proteins were purified by nickel-chelating agarose column chromatography (Invitrogen) followed by Mono Q anion-exchange column chromatography (Amersham Bioscience), as described previously [13]. Hsc70 was purified from bovine brain by DEAE-CL6B Sepharose column chromatography (Amersham Bioscience) and then ATP-agarose column chromatography (Sigma), as described previously [11]. These purified proteins were dialyzed against buffer A (25 mM Tris–HCl, pH7.5, 20 mM NaCl, 0.1 mM EDTA and 0.1 mM dithiothreitol (DTT)), concentrated by ultrafiltration using an Ultrafree centrifugal filter device (Millipore), and stored at −80°C. Purified proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and Coomassie brilliant blue (CBB)-stained bands were quantified by densitometry. The concentration of purified proteins was estimated with bovine serum albumin (BSA) as a standard. All of the purified proteins were detected as a single band of predicted molecular mass and were more than 95% pure.

2.3. Suppression of thermal aggregation of luciferase by chaperones

Firefly luciferase (0.5 μM) was mixed with chaperone(s) (3.5 μM each) in buffer B (25 mM HEPES–KOH, p H7.5, 50 mM KCl, 5 mM $MgCl_2$ and 5 mM DTT) containing 5 mM ATP or ADP, and then incubated at 25 or 45°C for 30 min. The reaction mixtures were centrifuged at 20 000×*g* for 20 min, and the resultant supernatant and pellet fractions were subjected to SDS–PAGE. Proteins in gels were electrotransferred onto nitrocellulose membranes, and membranes were blocked with 5% skim milk in Tris-buffered saline (20 mM Tris–HCl, pH 7.6, and 137 mM NaCl) containing 0.1% Tween 20, and incubated with anti-luciferase antibody (Sigma). The membranes were then incubated with horseradish peroxidase-conjugated anti-mouse IgG antibody (Santa Cruz), and the antibody–antigen complexes were detected using the ECL Western blot detection system (Amersham Bioscience).

2.4. Inactivation of cytoplasmic luciferase in ATP-depleted cells

African green monkey kidney COS-7 cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM, Nissui Pharmaceutical) supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 in air at 37°C. For transfection experiments, cells were grown in a 24-well plate to 70–80% confluence and washed twice with Opti-MEM (Invitrogen). Then, 0.05 μg of pGL3-control was transfected into the cells with 0.5 μg of pcDNA3 vector, pCMV70 or pcDNA105α, with DMRIE-C reagent (Invitrogen) according to the manufacturer's instructions. The following day, cells were trypsinized and divided equally into four wells of a 24-well plate. At 48 h after transfection, cells were incubated in glucose-free DMEM supplemented with 10% fetal bovine serum containing 2 μM rotenone (Sigma) and 5 mM 2-deoxyglucose for up to 180 min at 37°C. As a control, cells were incubated in DMEM supplemented with 10% fetal bovine serum. Cells were then washed with phosphate-buffered saline (PBS), lysed in 20 μl of Cell Culture Lysis Reagent (Promega) and centrifuged at 20 000×*g* for 5 min at 4°C. Aliquots (2 μl) of supernatants were mixed with 50 μl of Luciferase Assay Reagent (Promega) and the luciferase activity was measured in a luminometer (TD-20/20; Turner Designs). Relative levels of cellular ATP were measured with CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's instructions.

2.5. Binding of Hsp105α with a substrate protein, RCMLA

The binding of Hsp105α with a substrate protein was analyzed using native gel electrophoresis or gel filtration chromatography. Reduced carboxymethylated α-lactalbumin (RCMLA, Sigma) and α-lactalbumin (LA, Sigma) were used as a non-native and a native substrate protein, respectively. Radiolabeling of RCMLA and LA was performed with carrier-free Na[^{125}I] (Amersham Bioscience) and IodoBeads according to the manufacturer's instructions (Pierce), and the reaction mixture was subjected to gel filtration chromatography on a Sephadex G-25 column using PBS containing 150 mM NaCl to remove free Na[^{125}I].

CK2-phosphorylated Hsp105α was prepared as described previously [13]. Briefly, His-Hsp105α (100 μg) was incubated with 10 U of CK2 (Sigma) in 500 μl of buffer C (20 mM HEPES-KOH, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 200 mM NaCl, 20 mM β-glycerophosphate, 20 mM NaF and 200 μM ATP) for 3 h at 37°C. After the incubation, the reaction mixture was subjected to gel filtration chromatography on a Sephadex G-50 column using buffer A to remove free excess ATP.

For analysis using gel filtration chromatography, Hsp105α or CK2-phosphorylated Hsp105α (7 μM each) was incubated with RCMLA (140 or 3.5 μM) or LA (140 μM) in buffer D (20 mM sodium phosphate, pH 7.0, and 150 mM KCl) containing 5 mM ATP or ADP at 25°C for specific periods. After centrifugation at 20 000×*g* for 15 min, the supernatant was subjected to gel filtration chromatography on a G3000SW column (Tosoh) in buffer D at a flow rate of 0.5 ml/min. The elution profile was monitored by measuring absorbance at 215 nm. Pellet fractions were subjected to SDS–PAGE and proteins were detected by CBB staining.

For the analysis using native gel electrophoresis, Hsp105α, its mutants or Hsc70 (5 μM) was mixed and incubated with ^{125}I-labeled RCMLA or LA (1.7 μM each) in buffer B containing 5 mM ATP or ADP at 25°C for 3 h, and electrophoresed on 5% polyacrylamide gel containing 1×TBE (89 mM Tris, 89 mM boric acid and 2 mM EDTA) at 4°C. Since the ^{125}I-labeled RCMLA or LA solution contained 150 mM NaCl, the reaction mixtures for the binding assay of Hsp105 or Hsc70 contained a final concentration of approximately 30 mM NaCl. The specificity of the Hsp105α–RCMLA complex was determined by adding a 100-fold excess of non-labeled RCMLA to the ^{125}I-labeled RCMLA. The gel was then dried and ^{125}I-labeled RCMLA was detected by autoradiography.

3. Results

3.1. Hsp105 but not Hsc70/Hsp40 prevents the thermal aggregation of luciferase in the presence of ADP

We have shown that the thermal aggregation of luciferase is substantially suppressed by Hsp105α or Hsp105β [11]. Here, we examined the effects of adenine nucleotides on this suppression (Fig. 1A). All luciferase existed in the supernatant fraction at 25°C, whereas approximately 80% was aggregated and recovered in the pellet fraction after incubation at 45°C for 30 min in the presence of either ATP or ADP (lanes 1 and 2). However, the thermal aggregation of luciferase was more markedly suppressed by Hsp105α and Hsp105β in the presence of ADP rather than in the presence of ATP (lanes 6 and 7). On the other hand, although Hsp40 or Hsc70 alone failed to suppress the thermal aggregation of luciferase in the presence of ATP or ADP (lanes 3 and 4), together they suppressed the aggregation in the presence of ATP but not ADP (lane 5). Furthermore, when luciferase was incubated at 45°C with Hsc70, Hsp40 and Hsp105α/Hsp105β, the aggregation of luciferase was suppressed in the presence of ATP or ADP (lanes 8 and 9).

Furthermore, when the effect of ATP/ADP ratio on the suppression of the aggregation of heat-denatured protein by Hsp105α, Hsp105β and Hsc70/Hsp40 was examined, Hsp105α and Hsp105β suppressed the thermal aggregation of luciferase markedly dependent on the decreasing ratio of ATP/ADP (Fig. 1B). On the other hand, Hsc70/Hsp40 suppressed the thermal aggregation of luciferase markedly at the ATP/ADP ratio of 1:4, but not at the ratio of 1:9, at which Hsp105α and Hsp105β could suppress the thermal aggregation markedly. Thus, Hsp105 and Hsc70/Hsp40 seemed to suppress the thermal aggregation of protein differently.

3.2. Overexpression of Hsp105α but not Hsp70 rescued the inactivation of luciferase caused by ATP depletion in COS-7 cells

As Hsp105 but not Hsp70 prevented the aggregation of heat-denatured protein in the presence of ADP in vitro, we next examined the effect of Hsp105α and Hsp70 on the in-

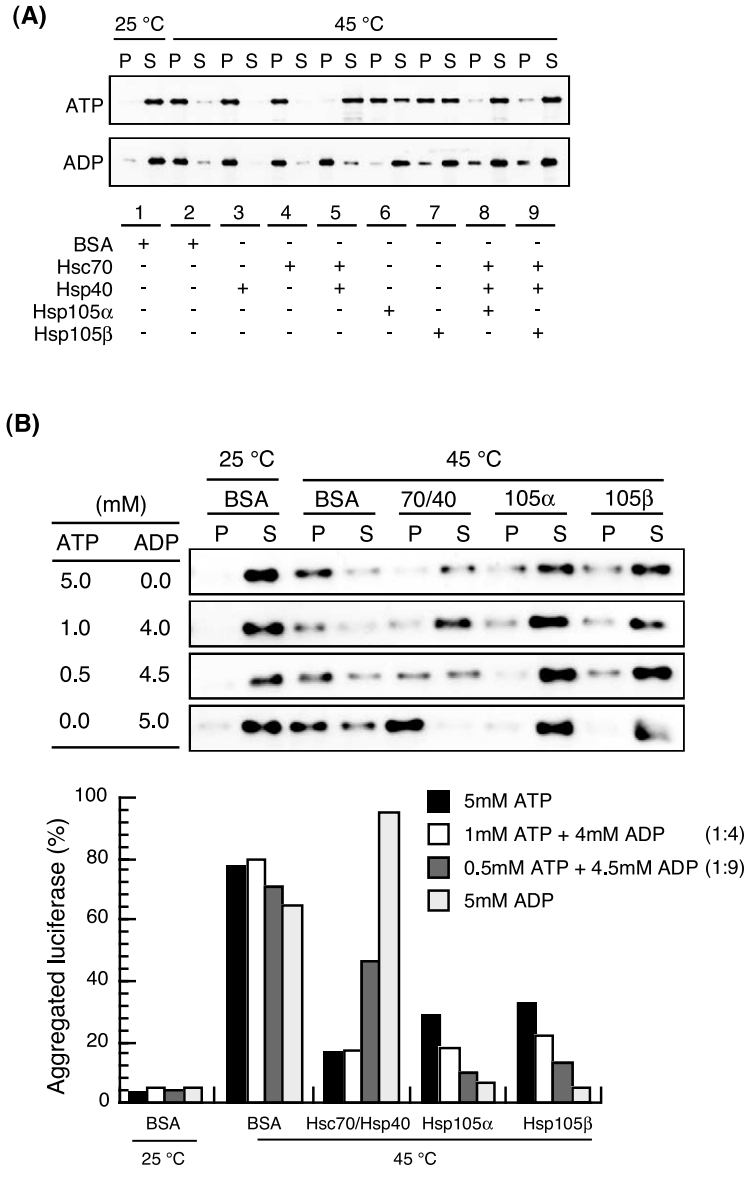

Fig. 1. Effects of adenine nucleotides on the suppression of aggregation of heat-denatured protein by Hsp105α and Hsp105β. A: Luciferase (0.5 μM) was incubated with chaperone(s) or BSA (3.5 μM each) at 25 or 45°C for 30 min in the presence of 5 mM ATP or ADP, then reaction mixtures were centrifuged. The resultant supernatant (S) and pellet (P) fractions were subjected to 10% SDS–PAGE, and luciferase protein was detected by immunostaining using anti-luciferase antibody. B: Luciferase (0.5 μM) was incubated with chaperone(s) or BSA (3.5 μM each) at 25 or 45°C for 30 min in the presence of ADP and/or ATP at different ratios, then the aggregation of luciferase was analyzed as described in A. The density of bands was quantified by densitometry, and the aggregated luciferase is shown as ratios to total amount of luciferase.

activation of proteins in ATP-depleted cells in vivo. When COS-7 cells were incubated in glucose-free medium containing rotenone and 2-deoxyglucose, cellular ATP levels were reduced to less than 20% of control cells within 30 min and further diminished gradually to approximately 3% of control cells after 180 min (Fig. 2A). Overexpression of Hsp105 or Hsp70 did not affect the reduction of cellular ATP levels. Under these conditions, the activity of luciferase expression in the cells was reduced to approximately 55, 50 and 35% of control cells after 1, 2 and 3 h of ATP depletion, respectively. However, the inactivation of luciferase caused by ATP depletion in the cells was significantly suppressed by the overexpression of Hsp105α but not Hsp70 (Fig. 2B). Thus, Hsp105 and Hsp70 also seemed to suppress the denaturation of protein differently in vivo.

3.3. *Effects of adenine nucleotides on the substrate binding of Hsp105α*

Since Hsp105α and Hsp105β suppressed the thermal aggregation of luciferase, we next examined whether Hsp105α binds and forms a complex with substrate protein using gel-filtration chromatography (Fig. 3A–C). RCMLA was used as a soluble non-native protein, and LA as a native protein. When Hsp105α was incubated with RCMLA at a ratio of 2:1 in the presence of ADP, the Hsp105α–RCMLA complex was clearly observed (Fig. 3A). In the presence of ATP, however, even if Hsp105α was incubated with a 20-fold molar excess of RCMLA, the Hsp105α–RCMLA complex was not detected (Fig. 3B). In contrast, the Hsp105α–LA complex was not detected in the presence of either ADP (Fig. 3C) or ATP (data not shown). Thus, it was suggested that Hsp105α bound

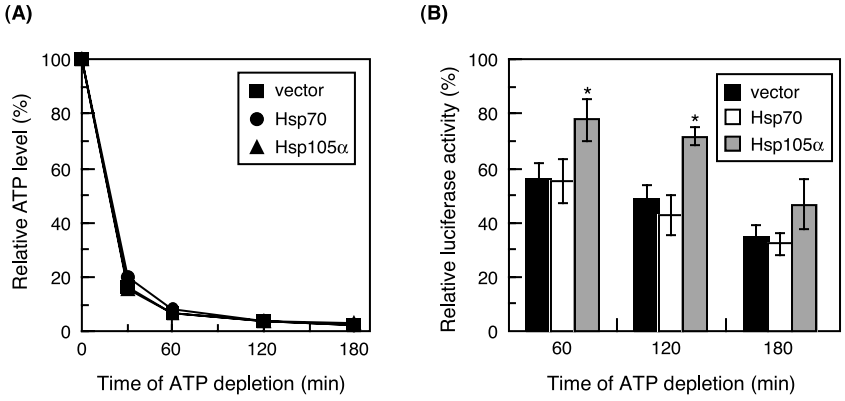

Fig. 2. Effects of Hsp105α on the inactivation of luciferase in ATP-depleted cells. A: Relative ATP levels in COS-7 cells subjected to ATP depletion. Cells transfected with pcDNA3, pCMV70 or pcDNA105α were incubated in glucose-free medium containing 2 μM rotenone and 5 mM 2-deoxyglucose for up to 180 min at 37°C. Relative cellular levels of ATP are shown as ratios to those of respective control cells without ATP depletion. B: The pGL3-control plasmid expressing luciferase was transfected into COS-7 cells with pcDNA3, pCMV70 or pcDNA105α. At 48 h after transfection, cells were incubated in the glucose-free medium containing rotenone and 2-deoxyglucose for up to 180 min at 37°C. Cells were then lysed, and the activity of luciferase was measured. Relative luciferase activities are shown as ratios to those of respective control cells without ATP depletion, and each value represents the mean of three independent experiments with standard errors. Statistical significance was determined by unpaired Student's *t*-test: $*P < 0.05$.

to and formed a complex with non-native protein in the presence of ADP but not in the presence of ATP.

Furthermore, the binding of Hsp105α with RCMLA was also confirmed using native gel electrophoresis (Fig. 3D,E). When Hsp105α was incubated with RCMLA or LA at a ratio of 3:1, the complex of Hsp105α and RCMLA was detected in the presence of ADP rather than ATP. The complex of Hsp105α and LA was not detected in the presence of either ADP or ATP. In contrast, Hsc70 formed a complex with RCMLA in the presence of ATP rather than ADP, and did not form a complex with LA in the presence of ADP or ATP.

Since Hsp105α bound the non-native protein in the pres-

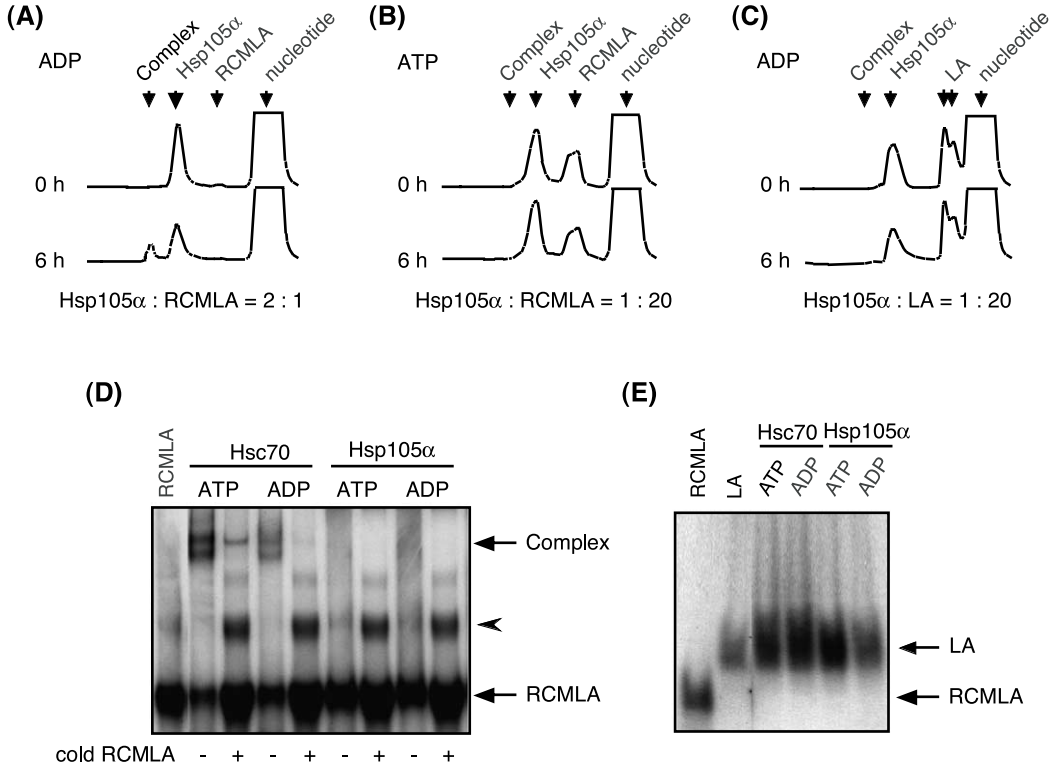

Fig. 3. Effects of adenine nucleotides on the binding of Hsp105α to non-native protein substrate. A: Hsp105α (7 μM) was incubated with RCMLA (3.5 μM) in the presence of 5 mM ADP at 25°C for 0 or 6 h. B: Hsp105α (7 μM) was incubated with RCMLA (140 μM) in the presence of 5 mM ATP at 25°C for 0 or 6 h. C: Hsp105α (7 μM) was incubated with LA (140 μM) in the presence of 5 mM ADP at 25°C for 0 or 6 h. After centrifugation, the supernatants were analyzed by gel filtration chromatography using a G3000SW column. D,E: Hsp105α or Hsc70 (5 μM each) was mixed with ^{125}I-labeled RCMLA (D) or LA (E) (1.66 μM each) and incubated at 25°C for 3 h in the presence of ATP or ADP. Reaction mixtures were then subjected to native gel electrophoresis, and [^{125}I]RCMLA was detected by autoradiography. The specificity of the Hsp105α–RCMLA complex was determined by adding a 100-fold excess of non-labeled RCMLA. An arrowhead indicates the non-specific bands.

ence of ADP, we next analyzed the substrate-binding region of Hsp105α. Hsp105α is composed of an amino-terminal ATP-binding domain, followed by β-sheet, loop and carboxy-terminal α-helix domains similar to HSP70 family proteins [16,17]. A series of deletion mutants of Hsp105α were constructed, and the binding of these mutants with RCMLA was analyzed using native gel electrophoresis (Fig. 4). Among these deletion mutants, Hsp105αC and Hsp105Δβ lacking the β-sheet domain failed to bind to RCMLA. Thus, Hsp105α seemed to bind non-native protein via the β-sheet domain of Hsp105α.

3.4. *Solubility of Hsp105α–substrate complex was regulated by phosphorylation of Hsp105α*

When an excess of Hsp105α was incubated with RCMLA (at a ratio of 2:1) in the presence of ADP, the complex of Hsp105α and RCMLA was soluble and recovered in the supernatant fractions (Fig. 3A). However, when Hsp105α was incubated with an excess of RCMLA (at a ratio of 1:20) in the presence of ADP, gel-filtration chromatography revealed that the peaks of both Hsp105α and RCMLA decreased, whereas the peak of the Hsp105α-RCMLA complex did not increase during the incubation (Fig. 5A). The amounts of Hsp105α and RCMLA in the pellet fractions increased during the incubation as determined by SDS–PAGE analysis. Thus, the Hsp105α–RCMLA complex formed under conditions of an excess of substrate seemed to become insoluble.

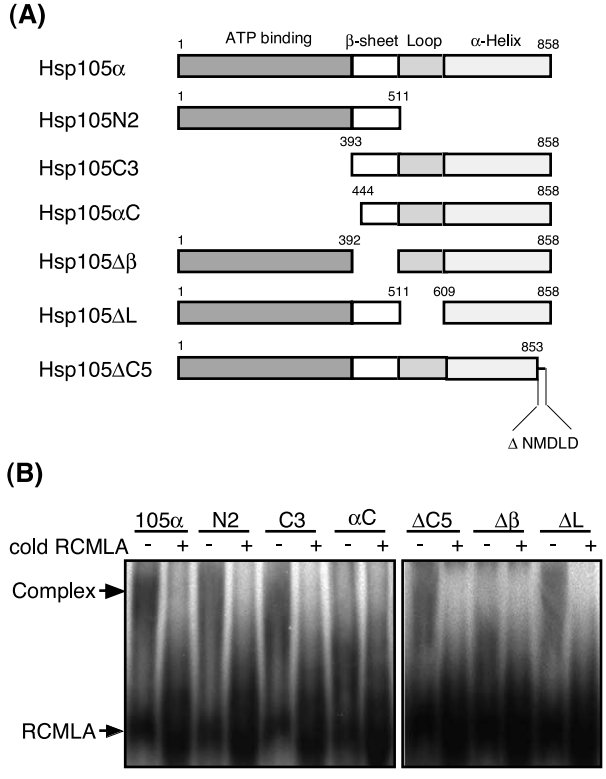

Fig. 4. Determination of substrate-binding region of Hsp105α. A: A schematic diagram of the domain structures of Hsp105α and its deletion mutants. B: Hsp105α or its mutants (5 μM each) was mixed with ^{125}I-labeled RCMLA (1.66 μM) and incubated at 25°C for 3 h in the presence of ADP. Reaction mixtures were analyzed by native gel electrophoresis, followed by autoradiography. The specificity of the Hsp105α–RCMLA complex was determined by adding a 100-fold excess of non-labeled RCMLA.

Since Hsp105α and Hsp105β are phosphorylated by CK2 in vitro and in vivo [12,13], we next examined the effects of the phosphorylation of Hsp105α on the formation of Hsp105α–RCMLA complex. As shown in Fig. 5B, when the CK2-phosphorylated Hsp105α was incubated with RCMLA at a ratio of 1:20, the peak of the Hsp105α–RCMLA complex increased with a concomitant decrease of the peaks of both Hsp105α and RCMLA, and the amounts of Hsp105α and RCMLA in the pellet fractions did not increase during the incubation. These results suggested that the solubility of the Hsp105α–substrate complex might be modulated by the phosphorylation of Hsp105α.

4. Discussion

Here, we showed that Hsp105α and Hsp105β bound to a non-native protein substrate and prevented the aggregation of heat-denatured protein in the presence of ADP, and the β-sheet domain of Hsp105α and Hsp105β was necessary for the binding. Consistent with these findings, the β-sheet domain of Hsp110 (the hamster homologue of Hsp105α) is required for the suppression of the thermal aggregation of luciferase [18]. In addition, the overexpression of Hsp105α but not Hsp70 in COS-7 cells rescued the inactivation of luciferase caused by depletion of ATP, suggesting that Hsp105 suppresses the denaturation of protein under ATP-depleted conditions in vivo and in vitro.

Hsp70 family proteins bind to non-native proteins via the central β-sheet domain [19–21]. The ATP-bound form of Hsp70 binds and releases peptide rapidly resulting in a low affinity for substrate, whereas the ADP-bound form of Hsp70 can bind substrate slowly but more stably resulting in a high affinity for substrate [3–6]. In the present study, however, Hsc70 bound to a non-native protein, RCMLA, in the presence of either ATP or ADP, and the amount of Hsc70–RCMLA complex detected in the presence of ATP was more than that in the presence of ADP (Fig. 3D). More Hsc70–substrate formed in the presence of ATP than ADP possibly due to the suppression of the dissociation of the Hsp70–RCMLA complex in the reaction mixture containing 30 mM NaCl used in this experiment, as the dissociation of Hsp70–substrate is prevented in the presence of Na^+ [4].

However, Hsc70 or Hsp40 alone failed to suppress the thermal aggregation of luciferase in the presence of either ATP or ADP, while together Hsc70 and Hsp40 had a suppressive effect in the presence of ATP but not ADP, as reported by Minami et al. [7]. Since Hsp40 promotes the conformational changes and chaperone activity of Hsp70 by enhancing its ATPase activity, the chaperone activity of Hsp70 seemed to be essential for the suppression of the thermal aggregation of luciferase by Hsp70/Hsp40. In contrast, Hsp105α and Hsp105β alone suppressed the aggregation in the presence of ADP rather than ATP. In addition, although Hsp105α and Hsp105β have an ATP-binding consensus sequence similar to those of Hsp70 family proteins, these proteins did not show any ATPase activity [11]. Since ATP hydrolysis of Hsp105α and Hsp105β is not essential for the suppression by these proteins of the aggregation of heat-denatured protein, the Hsp105 and Hsc70/Hsp40 chaperone systems seem to suppress the aggregation of heat-denatured protein differently.

Phosphorylation of heat shock proteins results in the mod-

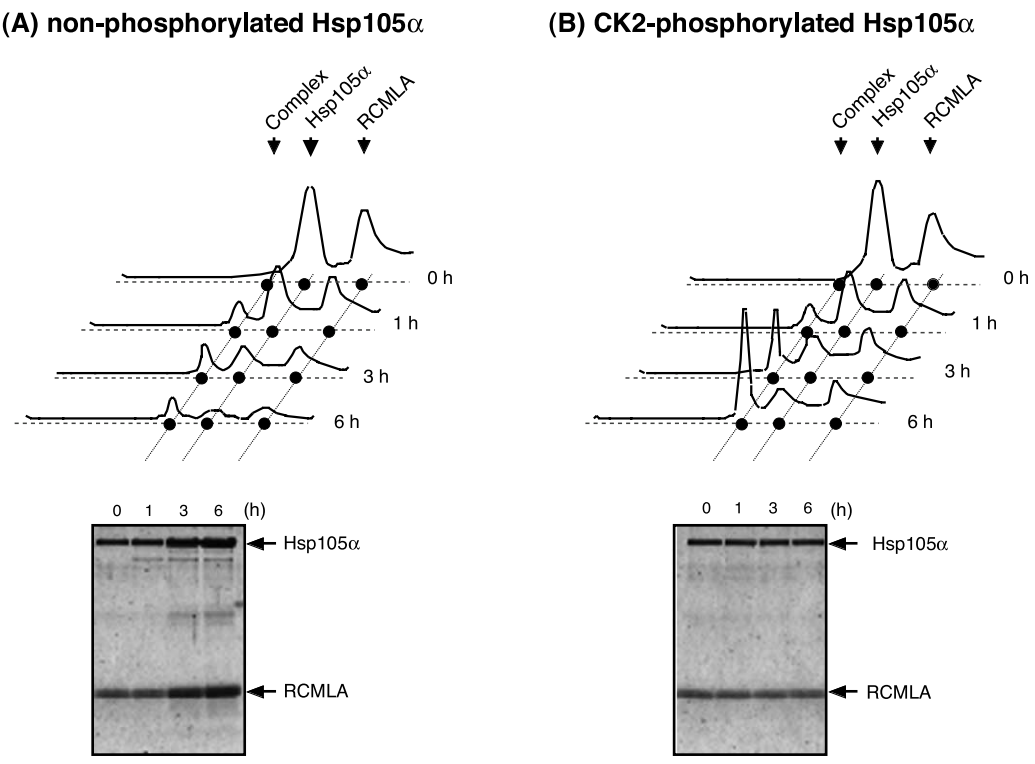

Fig. 5. Effect of phosphorylation of Hsp105α on the solubility of Hsp105α–substrate complex under substrate excess conditions. Non-phosphorylated (A) or CK2-phosphorylated (B) Hsp105α (7 μM each) was incubated with RCMLA (140 μM) in the presence of ADP at 25°C for up to 6 h, and the reaction mixtures were then centrifuged. The supernatants were analyzed by gel filtration chromatography using a G3000SW column, and the pellet fractions were subjected to SDS–PAGE and proteins were detected by CBB staining.

ification of their functions. Small heat shock proteins are phosphorylated by mitogen-activated protein kinase-activated protein kinase-2, and the phosphorylation of Hsp27 results in a dissociation of multimeric sHsp complexes and a subsequent down-regulation of chaperone activity [22,23]. Protein kinase A-catalyzed phosphorylation of Hsp60 regulates its attachment to histone 2B in T lymphocyte plasma membrane [24]. Recently, we have demonstrated that Hsp105α is phosphorylated at Ser^{509} by CK2 in vitro and in vivo, and that the phosphorylation suppresses the inhibitory effect of Hsp105α on Hsc70/Hsp70 chaperone activity [13]. In addition, we showed here that the solubility of the complex of Hsp105α and non-native protein substrate was controlled by the phosphorylation of Hsp105α. Thus, the phosphorylation of Hsp105 also plays an important role in the physiological functions of Hsp105, although further studies will be required for a complete understanding.

ATP is a main energy source used by cells to assume fundamental functions (e.g. respiration, proliferation, differentiation, and apoptosis). However, cellular ATP levels decrease rapidly under conditions of stress such as ischemia, infection and inflammation as well as heat shock [25,26]. Furthermore, ATP depletion leads to the aggregation of intracellular proteins (in particular, actin cytoskeletal constituents), destabilization of the plasma membrane (blebbing), and necrotic cell death [27]. Since Hsp105 but not Hsp70/Hsc70 prevented the aggregation and/or denaturation of protein in the ATP-depleted conditions in vitro and in vivo, Hsp105 family proteins may play important roles in protein disaggregation as a substitute for Hsp70 family proteins in cells under severe stress, in which the cellular ATP level decreases, cellular ADP level drastically increases and Hsp70 chaperone system cannot function.

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References

[1] Hendrick, J.P. and Hartl, F.-U. (1993) Annu. Rev. Biochem. 62, 349–384.
[2] Bukau, B. and Horwich, A.L. (1998) Cell 92, 351–366.
[3] Palleros, D.R., Welch, W.J. and Fink, A.L. (1991) Proc. Natl. Acad. Sci. USA 88, 5719–5723.
[4] Palleros, D.R., Reid, K.L., Shi, L., Welch, W.J. and Fink, A.L. (1993) Nature 365, 664–666.
[5] Greene, L.E., Zinner, R., Naficy, S. and Eisenberg, E. (1995) J. Biol. Chem. 270, 2967–2973.
[6] McCarty, J.S., Buchberger, A., Reinstein, J. and Bukau, B. (1995) J. Mol. Biol. 249, 126–137.
[7] Minami, Y., Hohfeld, J., Ohtsuka, K. and Hartl, F.-U. (1996) J. Biol. Chem. 271, 19617–19624.
[8] Hatayama, T., Honda, K. and Yukioka, M. (1986) Biochem. Biophys. Res. Commun. 137, 957–963.
[9] Hatayama, T., Yasuda, K. and Yasuda, K. (1998) Biochem. Biophys. Res. Commun. 248, 395–401.
[10] Wakatsuki, T. and Hatayama, T. (1998) Biol. Pharm. Bull. 21, 905–910.
[11] Yamagishi, N., Nishihori, H., Ishihara, K., Ohtsuka, K. and Hatayama, T. (2000) Biochem. Biophys. Res. Commun. 272, 850–855.
[12] Ishihara, K., Yasuda, K. and Hatayama, T. (2000) Biochem. Biophys. Res. Commun. 270, 927–931.
[13] Ishihara, K., Yamagishi, N. and Hatayama, T. (2003) Biochem. J. 371, 917–925.
[14] Michels, A.A., Karon, B., Konings, A.W.T., Ohutsuka, K., Ben-

saude, O. and Kampinga, H.H. (1997) J. Biol. Chem. 272, 33283–33289.
[15] Ishihara, K., Yamagishi, N., Saito, Y., Adachi, H., Kobayashi, Y., Sobue, G., Ohtsuka, K. and Hatayama, T. (2003) J. Biol. Chem. 278, 25143–25150.
[16] Yasuda, K., Nakai, A., Hatayama, T. and Nagata, K. (1995) J. Biol. Chem. 270, 29718–29723.
[17] Ishihara, K., Yasuda, K. and Hatayama, T. (1999) Biochim. Biophys. Acta 1444, 138–142.
[18] Oh, H.J., Easton, D., Murawski, M., Kaneko, Y. and Subjeck, J.R. (1999) J. Biol. Chem. 274, 15712–15718.
[19] Flaherty, K.M., DeLuca-Flaherty, C. and McKay, D.B. (1990) Nature 346, 623–628.
[20] Wang, T.F., Chang, J.H. and Wang, C. (1993) J. Biol. Chem. 268, 26049–26051.
[21] Morshauser, R.C., Wang, H., Flynn, G.C. and Zuiderweg, E.R. (1995) Biochemistry 34, 6261–6266.
[22] Rogalla, T., Ehrnsperger, M., Preville, X., Kotlyarov, A., Lutsch, G., Ducasse, C., Paul, C., Wieske, M. and Arrigo, A.P. (1999) J. Biol. Chem. 274, 18947–18956.
[23] Fernando, P., Megeney, L.A. and Heikkila, J.J. (2003) Exp. Cell Res. 286, 175–185.
[24] Khan, I.U., Wallin, R., Gupta, R.S. and Kammer, G.M. (1998) Proc. Natl. Acad. Sci. USA 95, 10425–10430.
[25] Findly, R.C., Gillies, R.J. and Shulman, R.G. (1983) Science 219, 1223–1225.
[26] Mallouk, Y., Vayssier-Taussat, M., Bonventre, J.V. and Polla, B.S. (1999) Int. J. Mol. Med. 4, 463–474.
[27] Gabai, V.L., Kabakov, A.E. and Mosin, A.F. (1992) Tissue Cell 24, 171–177.