Activation of the ATPase activity of heat-shock proteins Hsc70/Hsp70 by cysteine-string protein

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DnaJ proteins are characterized by a 'J' domain which is homologous to a region of the *Escherichia coli* protein DnaJ. DnaJ has been shown to interact with the chaperone protein DnaK, and a number of eukaryotic DnaJ-like proteins have been found to interact with the 70 kDa heat-shock protein/70 kDa heat-shock cognate protein (Hsp70/Hsc70), the eukaryotic homologues of DnaK. Cysteine-string proteins (Csps) are believed to function in calcium-stimulated exocytosis and in this paper we describe a specific ATP-dependent interaction between a Csp (Csp1) and Hsc70/Hsp70. We also show that Csp1 can stimulate the ATPase activity of both Hsc70 and Hsp70 several-fold. Furthermore, we demonstrate that Csp2, a Csp variant

found in adrenal chromaffin cells, can enhance the ATPase activity of Hsc70 to a similar extent as Csp1, whereas Csp(137–198), a truncated protein lacking the 'J' domain of Csp1 is unable to stimulate the ATPase activity of Hsc70. This suggests that the functions of Csp1 and Csp2 must differ in some aspect other than interaction with Hsc70. This study is also important from a general view of DnaJ/Hsc70 interactions, as Csps lack a G/F-rich region which has been suggested to be essential for activation of the ATPase activity of DnaK by DnaJ. Thus, this work would imply that a G/F-rich region is not an essential feature of DnaJ proteins for stimulation of the ATPase activity of Hsp70 proteins.

INTRODUCTION

Cysteine string proteins (Csps) are unique cysteine-rich proteins which were originally identified in *Drosophila* [1] and are localized to synaptic vesicle membranes [2,3]. These proteins are so called because they contain a cysteine-rich region, which in *Drosophila* consists of a contiguous string of 11 cysteine residues flanked on either side by another pair of cysteines. Csp null mutants of *Drosophila* have a partially lethal phenotype with only a small number of flies escaping but with impaired neurotransmission [4,5]. It has been suggested that the function of Csps may be to modulate the activity of presynaptic calcium channels, regulating neurotransmitter release at the synapse [6], but they may have a more direct role in synaptic vesicle exocytosis. It has recently been found that adrenal chromaffin cells express two variant Csps which are identical, except that Csp2 is truncated lacking the C-terminal 31 amino acid residues of Csp1 [7]. Csps are present on the large dense-core granules of chromaffin cells [8] and as such are implicated in the calcium-stimulated release of catecholamines from this cell type, in addition to neurotransmitter release from nerve terminals. The Csps possess a 'J' domain [1,6,7] characteristic of DnaJ proteins and involved in the interaction of these proteins with the 70 kDa heat-shock protein (Hsp70) chaperone family.

Proteins of the Hsp70 chaperone family (DnaK in *Escherichia coli*) are involved in a wide range of cellular processes, such as protein folding, translocation across membranes and the assembly or disassembly of protein complexes [9]. Hsp70 proteins are believed to function by binding to exposed regions of unfolded polypeptide chains, and in many cases the activity of Hsp70 is directed by partner proteins known as DnaJ proteins [10]. For example, the DnaJ-like protein auxillin recruits the constitutive

Hsp70 protein, Hsc70 (a 70 kDa heat-shock cognate protein), to endocytic vesicles where the latter protein functions in ATP-dependent clathrin uncoating [11]. In *E. coli*, DnaK interaction with both DnaJ and GrpE is required for ATP-dependent initiation of bacteriophage λ DNA replication, in addition to central roles in protein folding and transport [9].

DnaJ-like proteins share a number of features in common with the well-characterized bacterial DnaJ [10,12]. All DnaJ-like proteins have a signature 'J' domain, normally found in the N-terminal region of the protein and believed to incorporate the Hsp70/DnaK binding site. Other motifs found in many, but not all, DnaJ-like proteins are a glycine- and phenylalanine-rich region (G/F-rich region) and a cysteine repeat motif. Evidence suggests that the first 108 residues of DnaJ (which include the 'J' and G/F-rich domains) are able to activate the ATPase activity of DnaK [13,14], and indeed one study found that a DnaJ mutant lacking amino acids 77–107 and the G/F-rich domain, could stimulate the ATPase activity of DnaK to a similar extent as wild-type DnaJ but was unable to activate the substrate-binding activity of DnaK [15]. However, more recent studies employing a number of DnaJ mutant proteins have shown that both the 'J' domain and G/F-rich region of DnaJ are required for activation of the ATPase activity of DnaK [16,17]. There are thus conflicting data concerning the role of the G/F-rich segment in DnaJ function and in particular in the stimulation of the ATPase activity of DnaK. The cysteine-rich motif of DnaJ, which takes the form of a zinc finger-like domain, appears to be involved in binding to denatured protein substrates, as demonstrated by its ability to prevent aggregation of denatured rhodanese [17]. Finally, whilst the first 108 amino acids of DnaJ have been found to be sufficient for replication of the bacteriophage λ *in vitro* [15] other work suggests that the re-folding

Abbreviations used: Csp, cysteine-string protein; DTT, dithiothreitol; Hsc70, 70 kDa heat-shock cognate protein; Hsp70, 70 kDa heat-shock protein; NTA, nitrilotriacetic acid.

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of denatured luciferase requires an intact DnaJ protein [17]. Thus, DnaJ may have different functional roles in these two reactions, with the zinc finger-like domain and the extreme C-terminal domain being required for certain substrate interactions.

The presence of a 'J' domain in Csps suggests that they may interact with Hsc70, however, as yet, no such interaction has been described. In addition, the absence of a G/F-rich domain would suggest that Csps may not stimulate the ATPase activity of Hsc70. In this work we demonstrate that immobilized recombinant Csp1 [7] interacts specifically with Hsc70 from a rat brain protein extract in an ATP-dependent manner. Under normal cellular conditions Hsc70 is present in two forms in brain, the majority being the constitutively expressed Hsc70, while 10% represents the heat shock-inducible form of the protein, Hsp70; we show that Csp1 can bind to both Hsc70 and Hsp70 and can also stimulate the ATPase activity of these proteins several-fold. In addition, we demonstrate that Csp2 can also stimulate the ATPase activity of Hsc70. Therefore, the physiological significance of chromaffin cells expressing two variant Csps does not reside in the ability of these proteins to interact with Hsc70. As well as demonstrating a *bona fide*, ATP-dependent interaction between Csp and Hsc70, this work also suggests that the G/F-rich region present in some DnaJ proteins is not a prerequisite for activation of the ATPase activity of Hsp70 proteins by all DnaJ proteins.

MATERIALS AND METHODS

Materials

Ni²⁺-nitrilotriacetic acid (Ni²⁺-NTA)-agarose and *E. coli* M15 cells were obtained from Qiagen (Surrey, UK). Recombinant human Hsp70 and purified bovine brain Hsc70 were obtained from StressGen (Victoria, Canada). Additional Hsc70 purified from bovine brain by the protocol of Schlossman et al. [18] was a gift from Dr. M. Clague (Physiological Laboratory, University of Liverpool, Liverpool, U.K.). Hsc70 antiserum and all other chemicals were of an analytical grade from Sigma (Poole, UK).

Preparation of proteins from rat brain

A rat brain was homogenized in 5 ml of buffer A [20 mM Tris/HCl/1 M KCl/250 mM sucrose/2 mM MgCl₂/1 mM dithiothreitol (DTT)/1 mM PMSF, pH 8.0] and centrifuged at 100 000 *g* for 60 min at 4 °C. The supernatant which contained cytosolic proteins was removed and the pellet was resuspended in 10 ml of buffer A by homogenization and spun at 100 000 *g* for 30 min at 4 °C. The pellet from this centrifugation step was resuspended in 10 ml of buffer B (10 mM Hepes/100 mM KCl/2 mM MgCl₂/1 mM DTT, pH 7.8) and spun at 100 000 *g* for 30 min at 4 °C. The pellet was then resuspended in 10 ml of buffer B and Triton-X 100 was added to a final concentration of 4% (v/v) and incubated at 4 °C for 45 min with frequent mixing. After centrifugation at 100 000 *g* for 30 min at 4 °C the supernatant, which contained solubilized membrane proteins, was removed. The cytosolic and solubilized membrane protein fractions were then dialysed against buffer C [25 mM Tris/HCl, 50 mM KCl, 1 mM DTT and 1% (v/v) Triton-X 100 (membrane fraction only), pH 7.8] overnight and clarified by centrifugation at 100 000 *g* for 60 min at 4 °C.

Generation of plasmid constructs

The coding sequences of Csp1 and Csp2 were amplified by PCR from bovine adrenal chromaffin cell cDNA and cloned into the

pQE30 expression vector (Qiagen) as previously described [7]. Csp (137–198) was generated by PCR using the Csp1, pQE30 construct as the template. The primers had *Bam*H1 and *Hind*III restriction sites to facilitate sub-cloning into the pQE30 vector. The sense and antisense primers used were (5'-dGGCGGATCCAAGCCCAAGGCACCTGAG) and (5'-dC-TCAAGCTTTTAGTTGAACCCGTCGGTG) respectively, and the *Bam*H1 and *Hind*III restriction sites are underlined. The identities of the plasmid inserts were confirmed by automated sequencing.

Expression and purification of His₆-tagged recombinant proteins

Isopropyl-1-thio- β -D-galactopyranoside (2 mM) was added to a 1-litre culture of *E. coli* M15 cells containing the cloned *csp* sequences, and incubated at 37 °C for 5 h with shaking, inducing expression of the recombinant proteins. The induced cells were washed and resuspended in 20 ml of breaking buffer (5 mM ATP/100 mM Hepes/5 mM MgCl₂/2 mM 2-mercaptoethanol/500 mM KCl/1 mM PMSF/10 μ g/ml leupeptin/1 μ M pepstatin A, pH 7). The cells were stored at -80 °C overnight and thawed the following morning. Lysozyme (1 mg/ml) was added and the cells were left on ice for 30 min. Following ultrasonication, 2 μ g/ml DNase was added and the cells were left on ice for 15 min. Centrifugation at 100 000 *g* removed cell debris and the supernatant was loaded onto an Ni²⁺-NTA-agarose column and washed with 50 mM imidazole buffer [50 mM imidazole/20 mM Hepes/200 mM KCl/2 mM 2-mercaptoethanol/0.6 mM ATP/2 mM MgCl₂/10% (v/v) glycerol, pH 7] to remove unbound protein. MgATP was added to this buffer to remove any bacterial DnaK bound to the recombinant Csps. His₆-tagged Csps were eluted by application of a gradient of 50–500 mM imidazole, and peak fractions containing the recombinant protein were identified by SDS/PAGE. Chromatography was performed at 4 °C using a Pharmacia FPLC system.

Protein binding studies

The prepared cytosolic and solubilized membrane protein fractions were re-combined and Triton-X 100 added to give a final concentration of 1% (v/v). The combined protein fraction was then incubated with Ni²⁺-NTA-agarose (200 μ l/ml of protein) at 4 °C for 60 min with regular mixing to remove proteins bound non-specifically to the agarose. Proteins not bound to the agarose were recovered by centrifugation at 13 000 *g* for 10 min, passed through a 0.2 μ m filter and incubated (mixing regularly) for 90 min with 1 mg of His₆-Csp1 immobilized on an Ni²⁺-NTA-agarose column or with a control column (minus Csp1). Following this, the columns were washed with dialysis buffer (25 mM Tris/HCl/50 mM KCl/1 mM DTT/1% Triton-X 100, pH 7.8) to remove any unbound proteins. Imidazole (50 mM in dialysis buffer) was then added to elute proteins bound non-specifically to the agarose. After washing with dialysis buffer, 2 mM MgATP (in dialysis buffer) was added and the eluted proteins were collected. The column was then washed with dialysis buffer and finally 500 mM imidazole was added to remove bound Csp1 from the agarose. The eluted protein fractions were then precipitated with an equal volume of methanol at -20 °C, resuspended in SDS dissociation buffer, separated by SDS/PAGE and visualized by Coomassie Blue or silver staining. The same protocol was followed when using recombinant Hsp70 or purified Hsc70 rather than total protein, however there was no need to pre-clear Hsc70 and Hsp70 by prior incubation with Ni²⁺-NTA-agarose.

ATPase assays

ATPase assays were performed in a buffer containing 25 mM Tris, 100 mM KCl, 0.5 mM DTT, 2 mM $MgCl_2$, 0.6 mM ATP and 10% (v/v) glycerol, pH 9. Assays were performed at 37 °C for 1 h and activity was determined by assaying the release of inorganic phosphate using the spectrophotometric method of Lanzetta et al. [19] with modifications [20]. Corrections were made for non-enzymic breakdown of ATP by running duplicate assays on ice and subtracting these from the values obtained at 37 °C. Samples were read at A_{660} and their values were compared with a calibration curve using KH_2PO_4 as a standard.

RESULTS

Comparison of Csp1, Csp2 and the truncated mutant Csp(137–198)

Csp 1 and Csp2 are shown schematically in Figure 1. We also generated a Csp truncation mutant which consists of amino acids 137–198 of Csp1 and lacks the N-terminal 'J' domain and the cysteine-string region (Figure 1). These three proteins were purified as His₆-tagged recombinant proteins and their migration on an SDS polyacrylamide gel is shown in Figure 2. The apparent

Figure 1 Comparison of the domains of *E. coli* DnaJ, Csp1, Csp2 and Csp(137–198)

G/F represents the glycine- and phenylalanine-rich region of DnaJ.

Figure 2 SDS/PAGE analysis of Csp1, Csp2 and Csp(137–198)

Csp1 (a), Csp2 (b) and Csp(137–198) (c) were run on a 15% (w/v) SDS polyacrylamide gel [Tris-glycine with 1% (v/v) mercaptoethanol in sample buffer] and visualized by Coomassie Blue staining. The arrows point to monomeric forms of the proteins. The arrowheads indicate dimer forms of Csp1 and dimer and higher oligomers of Csp2. The positions of molecular-mass standards (in kDa) are shown on the left.

Figure 3 Analysis of MgATP-dependent binding of cellular proteins to immobilized Csp1

A preparation of cytosolic and solubilized membrane proteins from rat brain was added to His₆-tagged Csp1 immobilized on Ni²⁺-NTA-agarose or a control column. The column was initially washed with 50 mM imidazole in dialysis buffer and then with 2 mM MgATP in dialysis buffer and the eluted proteins were collected from this step. The eluant was separated by SDS/PAGE and the proteins were visualized by Coomassie Blue staining (CBB) (A). The arrows highlight proteins eluted from the Csp1 column but not the control column. The MgATP-eluted proteins were transferred to nitrocellulose and probed with Hsc70 antiserum (B).

molecular masses of Csp1 and Csp2 are approx. 7 kDa and 3 kDa larger than the expected masses of 22.2 and 18.9 kDa respectively. It has previously been established that Csps migrate anomalously on SDS polyacrylamide gels [7,21,22], however, Csp(137–198), which has an expected molecular mass of 6790 Da, runs considerably larger than forecast, at around 24 kDa, and this suggests that the C-terminal region of Csps may be responsible for the abnormal migration of these proteins on SDS polyacrylamide gels. The preparations of recombinant Csp1 and Csp2 contain higher-molecular-mass polypeptides which we [7], and others [21,22], have shown by immunoblotting to be dimers or higher oligomers of Csps (Figure 2). However, Csp(137–198) does not appear to form these higher-molecular-mass complexes, suggesting that the dimerization/oligomerization domain is absent from this region of Csp.

Binding of Hsc70 to Csp1

The initial step in this study was to determine whether Csp can specifically interact with Hsc70 from a complex pool of different proteins. To examine this, 2 ml of a rat brain protein-extract was incubated with 1 mg of His₆-tagged recombinant Csp1 immobilized on an Ni²⁺-NTA-agarose chromatography column and also with a control column (minus Csp1). The columns were then washed with dialysis buffer containing 50 mM imidazole to elute proteins bound non-specifically to the agarose. Evidence suggests that eukaryotic Hsp70–DnaJ complexes dissociate in the presence of MgATP [23], therefore dialysis buffer containing MgATP was subsequently added to the columns and this eluted a small number of proteins from the Csp1 and from the control columns. In addition, an increased amount of a protein of approx. 70 kDa was eluted by ATP from the column containing immobilized Csp1 (Figure 3A). The size of this protein suggested that it might be Hsc70, and this was confirmed by immuno-

Figure 4 MgATP-dependent binding of Hsc70 and Hsp70 to immobilized Csp1

Purified Hsc70 and recombinant Hsp70 were added to columns containing Ni^{2+} -NTA-agarose-bound Csp1. The columns were initially washed with 50 mM imidazole in dialysis buffer to remove proteins bound non-specifically to the agarose. Dialysis buffer containing 2 mM MgATP was then added to the columns, and subsequently 500 mM imidazole in dialysis buffer, and the proteins eluted from these two steps were collected. Samples of the loaded proteins, the MgATP eluants and the proteins remaining on the column were separated by SDS/PAGE and visualized by silver staining.

blotting with an Hsc70 antiserum (Figure 3B), demonstrating that Hsc70 can bind to Csp1 in an ATP-dependent and specific manner. Furthermore, an additional protein of around 27 kDa was eluted by ATP from the Csp1 column (Figure 3A), and we found by immunoblotting (results not shown) that this was in fact recombinant Csp1, suggesting that ATP-dependent release of Hsc70 from immobilized Csp1 also causes release of some His₆-Csp1 from the Ni^{2+} -NTA-agarose.

Hsc70 is present in brain in two forms, the constitutive form known as Hsc70 and the heat-shock inducible form, Hsp70, which is present at 10-fold lower levels under normal cellular conditions. We tested the ability of recombinant Hsp70 and purified Hsc70 to bind immobilized Csp1. The load samples in Figure 4 show the homogeneity of the Hsc70 and Hsp70 preparations and that Hsc70 migrates as a slightly larger polypeptide. Both Hsc70 and Hsp70 were bound to immobilized Csp1 and were released in the presence of MgATP. This finding agrees with a recent study showing that an antibody raised against the DnaJ protein, Hsp40, can precipitate both Hsc70 and Hsp70 from a human oral squamous cell carcinoma cell line (HOC815) lysate [23]. From Figure 4 it can be seen that ATP-dependent release of Hsc70 and Hsp70 from immobilized Csp1 did not result in Csp1 release, as was found when a brain extract was added (Figure 3A).

It has been shown previously that the ATP-induced dissociation of Hsc70 from substrate proteins is dependent on K^+ but not Na^+ [24]. When the 50 mM KCl in the column buffer was replaced with the same concentration of NaCl, immunoblotting revealed that MgATP did not dissociate either bound Hsc70 or Hsp70 from immobilized Csp1. Indeed, only when 500 mM imidazole, which releases bound His₆-tagged proteins from Ni^{2+} -NTA-agarose, was added could Hsc70 and Hsp70 be detected in the eluant (results not shown); this result confirms the specificity of the Csp1 interaction with the chaperone proteins.

Effect of Csp1 on the ATPase activity of Hsc70

Having demonstrated an ATP-dependent interaction, we proceeded to test the ability of Csp1 to stimulate the ATPase activity of Hsc70 and Hsp70. The assay used was based on the spectro-

Table 1 Stimulation of the ATPase activity of Hsc70 and Hsp70 by Csp1

Hsc70 (10 mg; experiment 1) or Hsp70 (10 mg; experiment 2) was incubated either alone or in the presence of 20 μg Csp1 in ATPase assay buffer (see the Materials and methods section). The release of free phosphate was measured using a spectrophotometric method (A_{660}) and converted into nmoles of free phosphate liberated by comparison with a KH_2PO_4 standard. Data are expressed as means $\pm$ S.E.M. ($n = 6$).

Protein	ATPase activity (nmol phosphate released/min per mg of protein)
Experiment 1	
Hsc70	1.08 ± 0.05
Csp1	0.33 ± 0.04
Hsc70 + Csp1	13.98 ± 0.45
Experiment 2	
Hsp70	0.50 ± 0.30
Csp1	0.21 ± 0.04
Hsp70 + Csp1	4.66 ± 0.20

photometric method of Lanzetta et al. [19] with modifications [20], and has previously been used to show stimulation of the ATPase activity of the *N*-ethylmaleimide-sensitive fusion protein (NSF) by soluble NSF attachment proteins (SNAPs) [25,26]. Hsc70/Hsp70 (10 μg) was incubated either alone or in the presence of 20 μg of Csp1 and the release of free phosphate was measured. It was found that Csp1 enhanced the ATPase activity of Hsc70 from 1.08 to 14 nmol of free phosphate liberated/min per mg of protein, a 13-fold increase (Table 1). Csp1 also stimulated the ATPase activity of Hsp70, increasing the amount of free phosphate liberated from 0.5 to 4.6 nmol/min per mg of protein, a 9-fold increase (Table 1).

Comparison of the effects of Csp1, Csp2 and Csp(137–198) on ATPase activation of Hsc70

We were interested in comparing the effects of Csp1, Csp2 and Csp(137–198) on the ATPase activity of Hsc70, the constitutive protein likely to interact with Csps. To test this we incubated 10 μg of Hsc70 alone or in the presence of 20 μg of each of the three proteins and measured the release of free phosphate. Csp1 and Csp2 had nearly identical effects on Hsc70, both stimulating its ATPase activity by around 14-fold (Table 2). In contrast Csp(137–198) had little effect on the ATPase activity of Hsc70, stimulating slightly in the experiments shown in Table 2 and having no effect in other experiments (results not shown). Thus, the increased ATPase activity of Hsc70 is not a property of the His₆-tag of Csp1 and 2, but a *bona fide* interaction probably occurring within the 'J' domain which is absent from Csp(137–198).

DISCUSSION

In this study we demonstrate a specific, ATP-dependent interaction between the chaperone protein Hsc70, in a brain extract, and Csp1. In addition, we have shown that Csp1 can also bind to the heat-shock inducible form of Hsc70, Hsp70. Csp1 was able to stimulate the ATPase activity of both Hsc70 and Hsp70 by 13- and 9-fold respectively. It is possible that the different levels of activation of these proteins by Csp1 and the different residual activities of Hsc70 and Hsp70 (1.08 compared with 0.5 nmol phosphate liberated/min per mg of protein) is due

Table 2 Comparison of the effects of Csp1, Csp2 and Csp(137–198) on the ATPase activity of Hsc70

Hsc70 (10 µg) was incubated alone or in the presence of 20 mg of Csp1, Csp2 or Csp(137–198) in ATPase assay buffer (see the Materials and methods section) and release of free phosphate was measured by a spectrophotometric method (A_{660}). The levels of phosphate released for each condition were converted into fold stimulation above the level for Hsc70 alone, after subtraction of any values for Csp alone. Results are expressed as means $\pm$ S.E.M. ($n = 6$).

Protein	Fold stimulation of ATPase activity of Hsc70
Csp1	14.45 $\pm$ 0.94
Csp2	14.70 $\pm$ 0.33
Csp(137–198)	1.64 $\pm$ 0.27

to different levels of protein activity achieved during purification and may not be physiologically meaningful.

We found that Csp2 was able to stimulate ATP hydrolysis by Hsc70 to a similar extent as Csp1, suggesting that if the function of these two proteins differs then this is not as a consequence of the ability of the proteins to interact with Hsc70. In contrast, the peptide Csp(137–198), which lacks the 'J' domain and cysteine-string region of Csp1, was unable to enhance the ATPase activity of Hsc70 to any significant extent. This strengthens our findings as it confirms that ATPase activation is not a general property of His₆-tagged proteins but represents a specific interaction between the DnaJ proteins and Hsc70, probably involving the 'J' domain of Csp1 and Csp2.

It has been suggested that Csps function in calcium-dependent exocytosis [2,4,5]. The present work suggests that Csp involvement in these processes may be manifested through its interaction with Hsc70. Indeed Hsc70 has previously been shown to function in ATP-dependent clathrin uncoating of endocytic vesicles, and it may be that Csp also functions in this process [27] or that Hsc70 is required for some other aspect of vesicular traffic. An important goal in dissecting the function of the Csp–Hsc70 interaction is to identify the substrate protein with which these proteins interact.

Another interesting finding of this work was the unexpected migration of Csp(137–198) on SDS polyacrylamide gels. Previous studies have found that Csps run anomalously when separated by SDS/PAGE, however, the apparent molecular mass of the peptide fragment was around 24 kDa, substantially larger than the expected mass of 6.8 kDa. We, therefore, suggest that the abnormal migration of Csps on SDS polyacrylamide gels is largely a consequence of the C-terminal region of these proteins. Supporting this idea, Csp2 only has the first 30 amino acids of the 61 residue peptide Csp(137–198) and migrates to a position approx. 3 kDa larger than its expected molecular mass, whereas Csp1 (which contains the whole peptide) has an apparent molecular mass around 7 kDa larger than expected. A possible explanation for the abnormal migration of Csp(137–198) could be its large number of proline residues (10 % by frequency). It also appears that the dimerization/oligomerization domain is absent from residues 137–198.

Recent work has suggested that the G/F-rich region of DnaJ is crucially required for activation of the ATPase activity of DnaK [16,17]. The conjecture from this might be that this region is required for Hsc70 activation by all DnaJ proteins. However, not all DnaJ proteins contain a G/F-rich region and it could be implied that such proteins would be unable to stimulate the

ATPase activity of Hsc70. Csps do not have a G/F-rich region and are thus an ideal model on which to test this hypothesis. We have demonstrated that Csps are able to enhance substantially the ATPase activity of Hsc70, and this suggests that some other feature of Csp (and probably other DnaJ proteins) is able to substitute for the G/F-rich region of DnaJ. Indeed, the primary sequence of this domain may be unimportant, as it has been suggested that the G/F-rich region may play an auxiliary role in stimulating the ATPase activity of DnaK by stabilizing the active conformation of the 'J' domain [17], and this region has been shown by NMR spectroscopy to be 'flexibly disordered' in solution [28].

Substrate interactions by DnaJ require the zinc finger-like domain as well as the extreme C-terminal domain of DnaJ [17]. Although Csps have a cysteine-rich motif, unlike DnaJ this does not take the form of a zinc finger-like domain, which has been found to be essential for interaction of DnaJ with denatured protein substrates [17]. The unique cysteine-string and C-terminal domains of Csps may, therefore, result in their interaction along with Hsc70 with specific substrate proteins. The identification of these substrates will be required to increase our understanding of the role of Csps in vesicular traffic.

Note added in proof (received 23 January 1997)

While this paper was under review, a paper by Braun et al. [29] also reported activation of Hsc70 ATPase activity by Csp.

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REFERENCES

- Zinsmaier, K. E., Hofbauer, A., Heimbeck, G., Pflugfelder, G. O., Buchner, S. and Buchner, E. (1990) *J. Neurogenet.* **7**, 15–29
- Mastrogriacomo, A., Parsons, S. M., Zampighi, G. A., Jenden, D. J., Umbach, J. A. and Gundersen, C. B. (1994) *Science* **263**, 981–982
- Van de Goor, J., Ramaswami, M. and Kelly, R. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5739–5743
- Zinsmaier, K. E., Eberle, K. K., Buchner, E., Walter, N. and Benzer, S. (1994) *Science* **263**, 977–980
- Umbach, J. A., Zinsmaier, K. E., Eberle, K. K., Buchner, E., Benzer, S. and Gundersen, C. B. (1994) *Neuron* **13**, 899–907
- Gundersen, C. B. and Umbach, J. A. (1992) *Neuron* **9**, 527–537
- Chamberlain, L. H. and Burgoyne, R. D. (1996) *J. Biol. Chem.* **271**, 7320–7323
- Chamberlain, L. H., Henry, J. and Burgoyne, R. D. (1996) *J. Biol. Chem.* **271**, 19514–19517
- Gething, M.-J. and Sambrook, J. (1992) *Nature (London)* **355**, 33–45
- Cyr, D. M., Langer, T. and Douglas, M. G. (1994) *Trends Biochem. Sci.* **19**, 176–181
- Ungewickell, E., Ungewickell, H., Holstein, S. E. H., Linder, R., Prasad, K., Barouch, W., Martin, B., Greene, L. E. and Eisenberg, E. (1995) *Nature (London)* **378**, 632–635
- Rassow, J., Voos, W. and Pfanner, N. (1995) *Trends Cell Biol.* **5**, 207–212
- Wall, D., Zyllicz, M. and Georgopoulos, C. (1994) *J. Biol. Chem.* **269**, 5446–5451
- Cheetham, M. E., Jackson, A. P. and Anderton, B. H. (1994) *Eur. J. Biochem.* **226**, 99–107
- Wall, D., Zyllicz, M. and Georgopoulos, C. (1995) *J. Biol. Chem.* **270**, 2139–2144
- Karza, A. W. and McMacken, R. (1996) *J. Biol. Chem.* **271**, 11236–11246
- Szabo, A., Korszun, R., Hartl, F. U. and Flanagan, J. (1996) *EMBO J.* **15**, 408–417
- Schlossman, D. M., Schmid, S. L., Braell, W. A. and Rothman, J. E. (1984) *J. Cell Biol.* **99**, 723–733
- Lanzetta, P. A., Alvarez, L. J., Reinach, P. S. and Candia, O. A. (1979) *Anal. Biochem.* **100**, 95–97
- Lill, R., Dowhan, W. and Wickner, W. (1990) *Cell* **60**, 271–280
- Mastrogriacomo, A. and Gundersen, C. B. (1995) *Mol. Brain Res.* **28**, 12–18
- Braun, J. E. A. and Scheller, R. H. (1995) *Neuropharmacology* **34**, 1362–1369

- 23 Sugito, K., Yamane, M., Hattori, H., Hayashi, Y., Tohnai, I., Ueda, M., Tsuchida, N. and Ohtsuka, K. (1995) *FEBS Lett.* **358**, 161–164
- 24 Palleros, D. R., Reid, K. L., Shi, L., Welch, W. J. and Fink, A. L. (1993) *Nature (London)* **365**, 664–666
- 25 Morgan, A., Dimaline, R. and Burgoyne, R. D. (1994) *J. Biol. Chem.* **269**, 29347–29350
- 26 Barnard, R. J. O., Morgan, A. and Burgoyne, R. D. (1996) *Mol. Biol. Cell* **7**, 693–701
- 27 Sudhof, T. C. (1995) *Nature (London)* **375**, 645–653
- 28 Szyperski, T., Pellicchia, M., Wall, D., Georgopoulos, C. and Wuthrich, K. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11343–11347
- 29 Braun, J. E. A., Wilbanks, S. M. and Scheller, R. H. (1995) *J. Biol. Chem.* **271**, 25989–25993

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