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Role of Heat Shock Cognate 70 Protein in Import of Ornithine Transcarbamylase Precursor into Mammalian Mitochondria

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The roles of the 70-kDa cytosolic heat shock protein (hsp70) in import of precursor proteins into the mitochondria were postulated to be related to (i) unfolding of precursor proteins in the cytosol, (ii) maintenance of the import-competent state, and (iii) unfolding and transport of precursor proteins through contact sites, in cooperation with matrix hsp70. We examined roles of cytosolic hsp70 family members in import of ornithine transcarbamylase precursor (pOTC) into rat liver mitochondria, using an in vitro import system and antibodies against hsp70. Immunoblot analysis using an hsc70 (70-kDa heat shock cognate protein)-specific monoclonal antibody and a polyclonal antibody that reacts with both hsc70 and hsp70 showed that hsc70 is the only or major form of hsp70 family members in the rabbit reticulocyte lysate. The hsc70 antibody did not inhibit pOTC import when added prior to import assay. However, when pOTC was synthesized in the presence of the antibody and then subjected to import assay, pOTC import was markedly decreased. pOTC import was also decreased when the precursor was synthesized in the lysate depleted for hsc70 by treatment with hsc70 antibody-conjugated Sepharose. This reduction was almost completely restored by readdition of purified mouse hsc70 during pOTC synthesis. The readdition of hsc70 after pOTC synthesis and only during the import assay was not effective. Thus, once import competence of pOTC was lost, hsc70 was ineffective for restoration. Newly synthesized pOTC lost import competence in the absence of hsc70 somewhat more rapidly than in its presence. These results indicate that hsc70 is required during pOTC synthesis and not during import into the mitochondria. hsc70 presumably binds to pOTC polypeptide and maintains it in an import-competent form.

Most mitochondrial proteins are encoded in the nuclear genome and are synthesized on cytosolic free ribosomes as larger precursors with presequences on their amino termini. Newly synthesized precursor proteins are released into the cytosolic pool, and the half-life in the cytosol is less than a few minutes in vivo (19). Precursor proteins are loosely folded in the cytosol, bind to a mitochondrial surface receptor(s), and are then transported across the outer and inner membranes at the contact site. The presequence portions of the transported precursor proteins are proteolytically cleaved in the mitochondrial matrix, and the mature portions are folded into their native conformations (reviewed in reference 35).

Studies in our laboratory (18, 20) and elsewhere (22, 26, 27) have shown that a cytosolic factor(s) present in rabbit reticulocyte lysate or yeast cytosol is important for the import of precursor proteins into the mitochondria. Recently, mitochondrial presequence-specific cytosolic factors have been identified in rabbit reticulocyte lysate or rat liver cytosol (8, 21, 23, 28). Other studies showed that the 70-kDa heat shock protein (hsp70) is involved in the transport of mitochondrial precursor proteins in yeasts (5, 22) and in mammals (31).

hsp70 is not specific to mitochondrial import, as it is also involved in translocation of secretory precursor proteins (2), intracellular proteins for lysosomal degradation (1), nuclear proteins (12, 32), and peroxisomal proteins (33). The precise role of hsp70 in protein targeting to these organelles is not well understood. Recently, Frydman et al. (6) reported that protein folding in the cytosol is mediated by sequential action of hsp70-hsp40 and chaperonin complex TriC. Cytosolic hsp70 also mediates various cellular events, in addition to protein translocation into various organelles (34). Thus, the manner in which hsp70 specifically facilitates mitochondrial import of proteins that are destined for the organelle remains to be determined.

The roles of cytosolic hsp70 in intracellular protein sorting were originally studied with *Saccharomyces cerevisiae* hsp70 (5, 22). The yeast cytosol contains two classes of hsp70, the *SSA* subfamily (Ssa1 to Ssa4) and the *SSB* subfamily (Ssb1 and Ssb2) (3). Ssa facilitates translocation of precursor proteins into both mitochondria and microsomes, and Ssb seems to play a role in translation. In mammals, two hsp70 family members, hsp70 and the 70-kDa heat shock cognate protein (hsc70), have been speculated to be involved in both translocation and translation processes.

Here we tested these postulated roles for mammalian hsp70 family members in protein import into mammalian mitochondria, using antibodies against hsp70s. We developed an in vitro import system that was depleted for hsc70 and which depended on the readdition of hsc70. We report here that hsc70 is required during synthesis of the precursor protein but apparently not during its import into mitochondria.

MATERIALS AND METHODS

Materials. The nuclease-treated rabbit reticulocyte lysate and in vitro transcription kit were purchased from Promega Corp. Cyanogen bromide-activated Sepharose 4FF resin and the Q-Sepharose HP column were obtained from Pharmacia Biotech. [^{35}S]methionine (>48 TBq/mmol) and the enhanced chemiluminescence Western blotting (immunoblotting) detection reagent were purchased from Amersham. Rabbit polyclonal antibody against hsp70 used in this study was that described elsewhere (11). Rat 1B5 monoclonal antibody against Chinese hamster hsc70 (11) was purified from rat ascitic fluid by Q-Sepharose

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HP column chromatography, essentially as described previously (9). Mouse hsc70 was purified from Ehrlich ascites tumor cells as described previously (12).

Depletion of hsc70 from rabbit reticulocyte lysate and in vitro translation. mRNA for rat ornithine transcarbamylase precursor (pOTC) was synthesized by in vitro transcription of plasmid pSPT18/pOTC as described previously (20). Depletion of hsc70 from rabbit reticulocyte lysate was done by treatment with 1B5 antibody-resin. Ten milligrams of the purified antibody was coupled to 1 ml of cyanogen bromide-activated Sepharose 4FF resin, according to the protocol provided by the manufacturer. The 1B5 antibody-resin was equilibrated with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH and 120 mM potassium acetate (pH 7.6), and an equal volume of reticulocyte lysate was added to the pelleted resin. The mixture was incubated at 4°C for 90 min with occasional agitation, and the resin was removed by centrifugation. The supernatant was used as hsc70-depleted lysate for in vitro translation in the presence of [^{35}S]methionine, as described previously (20).

Import of in vitro-synthesized precursor protein into isolated mitochondria. The import mixture (50 μl) containing 3.0 μl of the lysate and ^{35}S-labelled pOTC (0.8×10^4 to 3.0×10^4 dpm) was incubated with isolated rat liver mitochondria (100 μg of protein) at 25°C, as described previously (23). The reaction was stopped by diluting the import mixture into 1.0 ml of the ice-cold mitochondrion isolation buffer [210 mM mannitol, 70 mM sucrose, 3 mM HEPES-KOH (pH 7.4), 0.2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.5 mg of bovine serum albumin per ml] containing 0.1 mM dinitrophenol. The mitochondria were reisolated by centrifugation at 9,000 × *g* for 10 min in a refrigerated microcentrifuge. The pelleted mitochondria were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by fluorography and imaging plate analysis using a FUJIX BAS2000 analyzer.

Other methods. Rat liver mitochondria were prepared from male Wistar rats (150 to 200 g) as described previously (30). Two-dimensional gel electrophoresis was performed according to the method of O'Farrell et al. (25) by combining isoelectric focusing (4:1 mixture of pH 5 to 7 and pH 3.5 to 10 Ampholine; Pharmacia) in the first dimension with SDS–8% polyacrylamide gel electrophoresis in the second dimension. For immunoblotting, the proteins separated by gel electrophoresis were transferred to a nitrocellulose membrane (Schleicher & Schuell, Inc.) with a semidry blotter (Sartorius), according to the protocol supplied by the manufacturer. Immunodetection was performed with purified 1B5 monoclonal antibody (0.5 μg of immunoglobulin G per ml) or anti-hsp70 polyclonal antiserum (1/400 dilution) as the first antibody (11). A biotin-avidin system (ABC kit; Vector Laboratories) was used in the second antibody reaction. Peroxidase activity was detected with the enhanced chemiluminescence kit (Amersham).

RESULTS

Antibody against hsc70 inhibits mitochondrial import of pOTC when added during translation. We first identified hsp70 family members present in rabbit reticulocyte lysate. The rodent hsp70 family in the cytosol is composed of hsp70 and hsc70 (29). Since these hsp70 family members differ in their pIs, two-dimensional gel electrophoresis was done to identify hsp70 family members in the lysate. For this we used two different antibodies (11); one was an anti-mouse hsp70 polyclonal antibody that recognizes both hsp70 and hsc70 from many sources, including humans and drosophilas, and the other was rat monoclonal antibody 1B5 against Chinese hamster hsc70 that is specific for hsc70 in humans, rodents, and chicks. Immunoblot analysis with the hsp70 polyclonal antibody gave only one spot of polypeptide with an estimated molecular mass of 73 kDa and pI of 5.5 (Fig. 1A). The same polypeptide was detected with the 1B5 monoclonal antibody (Fig. 1B). This means that hsc70 is the only or major form of hsp70 family members present in the rabbit reticulocyte lysate.

We next examined effect of antibodies on import of pOTC into rat liver mitochondria. When the 1B5 monoclonal antibody was added prior to import, pOTC import was not inhibited (Fig. 2). Several other anti-hsc70 antibodies, including N27F3-4 monoclonal antibody (Stress Gen Biotechnologies Corp.), had little effect on pOTC import (data not shown). On the other hand, when the monoclonal antibody was added prior to translation, the import was markedly decreased. The import was less than one-fourth of that of pOTC synthesized in the untreated lysate. Neither nonimmune rabbit immunoglobulin G nor an unrelated monoclonal antibody inhibited pOTC import when added prior to translation. Addition of the 1B5 antibody had little effect on translation.

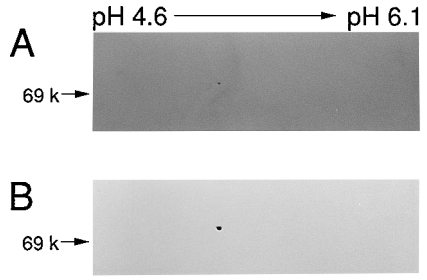

FIG. 1. Immunoblot analysis of hsp70 family members in rabbit reticulocyte lysate. Rabbit reticulocyte lysate (800 μg of protein) was subjected to isoelectric focusing–SDS-polyacrylamide two-dimensional gel electrophoresis, and proteins were transferred to nitrocellulose membranes as described in Materials and Methods. The membranes were probed with the polyclonal antibody against hsp70 (0.2 mg of immunoglobulin G per ml) (A) or with the purified 1B5 monoclonal antibody against hsc70 (0.5 μg/ml) (B). The membranes were then treated with biotinylated second antibody and streptavidin-horseradish peroxidase, and peroxidase activity was detected with an enhanced chemiluminescence kit (Amersham), following to the protocol provided by Amersham. 69 k, bovine serum albumin.

Depletion of hsc70 from rabbit reticulocyte lysate inhibits pOTC import. To investigate in detail the role of hsc70 in the import of pOTC into mammalian mitochondria, rabbit reticulocyte lysate was depleted of hsc70 by using antibody-coupled

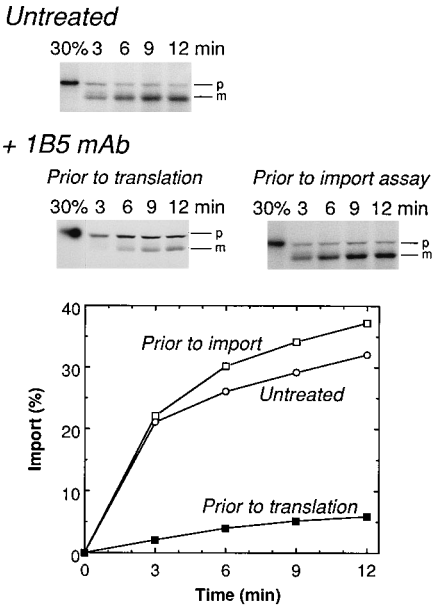

FIG. 2. Effect of addition of the 1B5 monoclonal antibody on import of in vitro-synthesized pOTC into rat liver mitochondria. Rat pOTC was synthesized in rabbit reticulocyte lysate without (Untreated and Prior to import assay) or with (Prior to translation) addition of 3.6 μg of the 1B5 monoclonal antibody purified from culture supernatant of the hybridoma cells. In the latter case, the antibody was added to the lysate, the mixture was incubated for 30 min at 4°C, and pOTC translation was conducted. pOTC synthesis was little affected by the addition of the antibody (2.7×10^3 to 3.0×10^3 dpm/μl). (Prior to import assay) After translation, the antibody was added to the translation mixture containing the in vitro-synthesized pOTC and incubated for 30 min at 4°C. The import mixtures (50 μl) containing 3 μl of the lysate and about 2×10^4 dpm of pOTC were incubated with isolated rat liver mitochondria (100 μg of protein) at 25°C for the indicated times. The import was halted by diluting the import mixture into 1.0 ml of ice-cold mitochondrion isolation buffer containing 0.1 mM dinitrophenol. The mitochondria were reisolated and subjected to SDS-polyacrylamide gel electrophoresis followed by fluorography. Portions of the fluorograms are shown in the upper panels. p, pOTC; m, mature form of OTC; 30%, 30% of the input pOTC. The results were quantitated by imaging plate analysis using a FUJIX BAS2000 analyzer and are shown in the bottom panel.

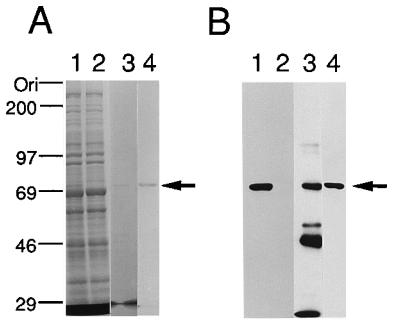

FIG. 3. Depletion for hsc70 of rabbit reticulocyte lysate with 1B5 monoclonal antibody-coupled Sepharose. hsc70 depletion was performed as described in Materials and Methods. Proteins were separated by SDS–8% polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue (A) or subjected to immunoblot analysis (B). Immunodetection was performed with 1B5 monoclonal antibody as the first antibody as described for Fig. 1. Lanes 1, untreated lysate (250 μg of protein); lanes 2, depleted lysate (250 μg of protein); lanes 3, the proteins absorbed to and extracted from the 1B5-coupled Sepharose; lanes 4, 0.2 μg of purified mouse hsc70. Protein molecular weight markers; myosin (200K), phosphorylase *b* (97K), bovine serum albumin (69K), ovalbumin (46K), and carbonic anhydrase (29K). Arrows indicate hsc70.

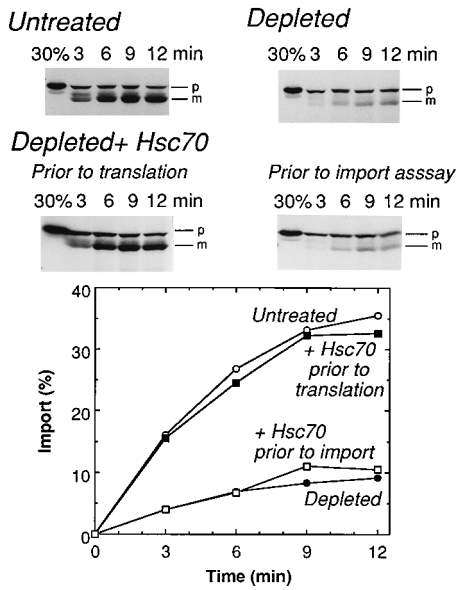

FIG. 4. Effect of hsc70 depletion and readdition on import of pOTC into mitochondria. Rat pOTC was synthesized in the untreated rabbit reticulocyte lysate (1.6×10^4 dpm) or the hsc70-depleted lysate without (0.8×10^4 dpm) or with readdition of 100 μg/ml of the purified mouse hsc70. hsc70 was readded prior to translation (0.9×10^4 dpm) or prior to import reaction (0.9×10^4 dpm). The import reaction and subsequent analysis were done as described for Fig. 2. Portions of the fluorograms are shown in the upper panels. p, pOTC; m, mature form of OTC; 30%, 30% of the input pOTC. The results were quantitated by imaging plate analysis using a FUJIX BAS2000 analyzer and are shown in the bottom panel.

resin. The purified 1B5 monoclonal antibody was covalently coupled to cyanogen bromide-activated Sepharose resin, and hsc70 was depleted from the lysate with the 1B5 antibody-coupled resin. In Coomassie brilliant blue staining, a polypeptide that comigrated with the purified mouse hsc70 disappeared specifically from the lysate by treatment with the antibody-resin, and this polypeptide was recovered from the resin (Fig. 3A). Immunoblot analysis showed that hsc70 was completely removed from the lysate by the antibody-resin (Fig. 3B). When the absorbed proteins were extracted from the antibody-resin, the antibody (rat 1B5) was partially removed from the resin and gave polypeptide bands of the heavy chain (50 kDa) and the light chain (29 kDa) (Fig. 3B, lane 3). These immunoglobulin G chains were not present in the depleted lysate.

Rabbit hsc70 comigrated with the purified mouse hsc70 (lane 4). Mammalian hsc70 shows more than 99% amino acid identity among the human, rat, and mouse proteins. Assuming that the rabbit and mouse hsc70s gave immunoreactive bands of the same intensity in the immunoblot analysis (lanes 1 and 4), the concentration of hsc70 in the reticulocyte lysate was estimated to be about 200 μg/ml (0.1% of total protein). This value agrees well with documented data (36).

Cotranslational readdition of purified hsc70 to the depleted lysate restores pOTC import. Rat pOTC synthesized in the untreated or the hsc70-depleted reticulocyte lysate was subjected to import assay. When hsc70 was depleted with the 1B5 antibody-Sepharose, the efficiency of pOTC synthesis was decreased to one-third to one-half of that in the untreated lysate. This reduction can be attributed to the irreversible binding of ribosomes to the dextran gel (13). Import of pOTC synthesized in the hsc70-depleted lysate was about one-fourth of that of the precursor synthesized in the untreated lysate (Fig. 4). This result is consistent with that obtained with the addition of the antibody prior to translation (Fig. 2). It is notable that a low activity of pOTC import remained in the depleted lysate though hsc70 was completely depleted from the lysate. Therefore, hsc70 is probably not an indispensable cytosolic factor for the import of pOTC into the mitochondria, but it does increase the import efficiency.

When purified hsc70 (100 μg/ml) was readded to the hsc70-depleted lysate after translation and prior to import assay, the decreased pOTC import was not restored. On the other hand, the pOTC import was almost completely recovered when hsc70 was readded prior to translation. The restoration of pOTC import depended on the amount of added hsc70, and nearly complete restoration was achieved with 100 μg of hsc70 per ml (Fig. 5). This concentration was similar to that in rabbit reticulocyte lysate (see above). Heat-treated hsc70 was not effective. These results indicate that hsc70 interacts with the nascent precursor protein emerging from the ribosomes and maintains the precursor protein in an import-competent state. Since hsc70 was unable to restore the import competence of pOTC once it was lost, hsc70 may exert its action by preventing aggregation or misfolding (even correct folding) of the precursor protein at the translational stage.

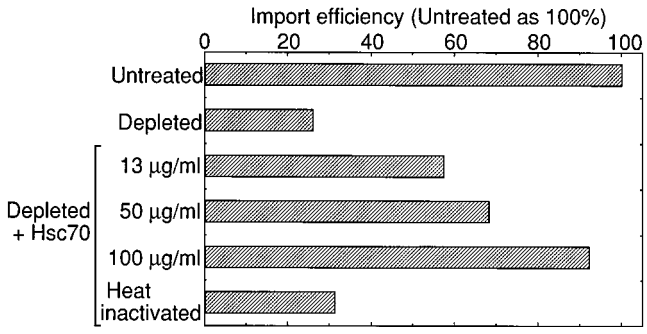

FIG. 5. Effects of various amounts of readded hsc70 on pOTC import. Rat pOTC was synthesized in the untreated rabbit reticulocyte lysate (1.6×10^4 dpm) or the hsc70-depleted lysate without (0.8×10^4 dpm) or with (0.9×10^4 dpm) readdition of indicated amounts of the purified hsc70 or 100 μg of heat-inactivated (95°C, 3 min) hsc70 per ml. hsc70 was readded prior to translation. Import was carried out for 12 min and was analyzed as described for Fig. 2.

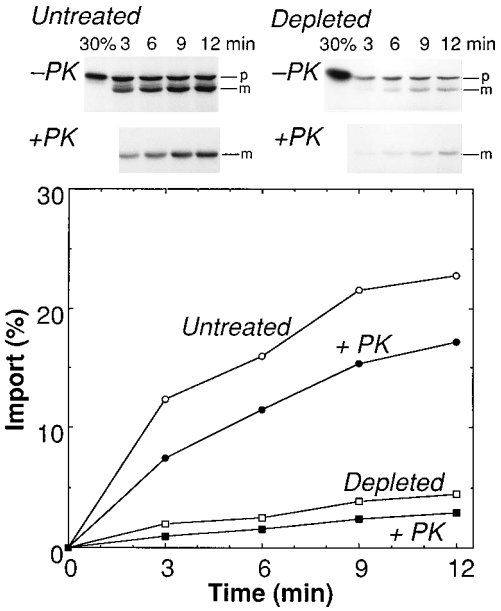

FIG. 6. Proteinase K treatment of mitochondria after import of pOTC synthesized in the hsc70-depleted lysate. pOTC synthesized in the untreated lysate (2.7×10^4 dpm) or the hsc70-depleted lysate (1.0×10^4 dpm) was subjected to import assay as described for Fig. 4. The import reaction was stopped by diluting the import mixture into 1.0 ml of ice-cold mitochondrion isolation buffer containing 0.1 mM dinitrophenol. Half of the sample was treated with 40 μg of proteinase K (PK) per ml for 30 min on ice, and digestion was terminated with 1 mM phenylmethylsulfonyl fluoride. The following analysis was as described for Fig. 2.

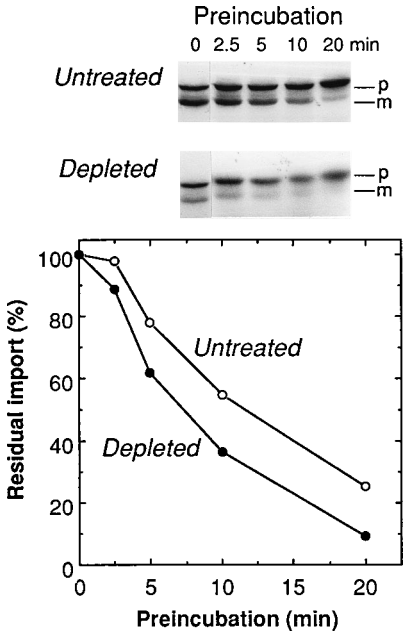

FIG. 7. Effect of preincubation on import competence of pOTC in the presence and absence of hsc70. pOTC was synthesized in the untreated lysate or the hsc70-depleted lysate as described for Fig. 6. The import assay mixtures without mitochondria were preincubated at 25°C for the indicated times. Import was started by the addition of mitochondria, performed for 12 min at 25°C, and analyzed as described for Fig. 2. Percent import was plotted versus time of preincubation.

Evaluation of the role of cytosolic hsc70 in pOTC import into mitochondria. In the import pathway of the precursor proteins from the cytosol into the mitochondrial matrix, cytosolic hsp70 and mitochondrial hsp70 have been postulated to interact cooperatively with the precursors passing through the import channel at the contact site (24). According to this model, stacking of the precursor proteins passing through the channel or delay of the import can happen without cytosolic hsc70. To test these possibilities, pOTC synthesized in the hsc70-depleted lysate was subjected to import assay, and the reisolated mitochondria were treated with proteinase K to digest any incompletely translocated protein (Fig. 6). The proportion of the proteinase K-resistant form in the processed OTC (about two-thirds) was similar to that obtained with the untreated lysate. Therefore, a substantial amount of pOTC could be imported completely into the mitochondria even in the absence of hsc70, without severe stacking. Lithgow et al. (15) reported that hsc70 is also located in the outer membrane of rat liver mitochondria. Since the 1B5 antibody did not inhibit pOTC import when added during the import, no evidence was obtained for involvement of this membrane-associated hsc70 in pOTC import.

Half-lives of import-competent pOTC in the presence and absence of cytosolic hsc70. Cytosolic hsp70s have been postulated to maintain import competence of mitochondrial precursor proteins in the cytosol. To test this hypothesis, pOTC synthesized in the untreated lysate or in the hsc70-depleted lysate was incubated for various periods in the import mixture without mitochondria at 25°C. Loss of the import competence of the precursor was monitored by import into the mitochondria. As shown in Fig. 7, import competence was lost a little more rapidly in the absence of hsc70 than in its presence. The half-life of the import-competent pOTC was about 8 min in the absence of hsc70 and 12 min in its presence. Therefore, hsc70 maintains import competence of pOTC, but the action appears to be transient or of short range. The transient action of hsc70 may be effective, considering the short half-life of pOTC of less than a few minutes in vitro (Fig. 2) and in vivo (19).

Sucrose gradient centrifugation analysis of pOTC synthesized in the presence of the absence of hsc70. When pOTC synthesized in the untreated reticulocyte lysate was subjected to sucrose gradient centrifugation, it sedimented broadly with a peak of 11S (Fig. 8). A similar complex was observed in our previous experiments (17). On the other hand, when pOTC synthesized in the hsc70-depleted lysate was centrifuged, the complex of 11S disappeared almost completely and pOTC distributed along the entire gradient (Fig. 8). pOTC did not always form a large aggregate in the absence of hsc70, because the proportions of pOTC sedimented to the bottom of the tube were similar (about 5%) whether pOTC was synthesized in the presence or absence of hsc70. Whether the 11S complex represents the import-competent form remains to be elucidated.

DISCUSSION

We developed an in vitro translation-mitochondrial import system in which cytosolic hsc70 was completely depleted. We found that cytosolic hsc70 maintains import competence of pOTC when present during synthesis and that hsc70 is not required during pOTC import. To our knowledge, this is the first in vitro study in which molecular mechanisms of hsc70 during synthesis and mitochondrial import of a precursor protein were analyzed.

hsp70 was postulated to maintain import competence of a urea-denatured precursor protein into mitochondria together with other cytosolic factors (31). Urea-denatured proteins and polypeptides elongating from ribosomes are similar in that

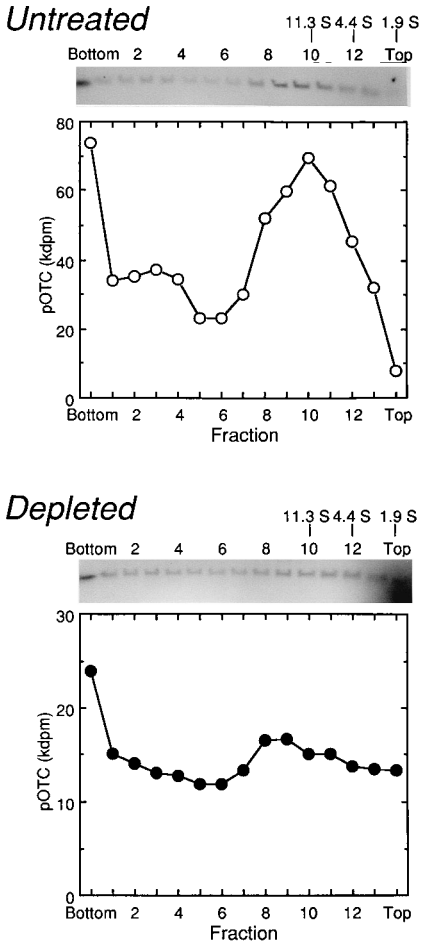

FIG. 8. Sucrose gradient centrifugation of pOTC synthesized in the untreated or hsc70-depleted lysate. Samples (0.10 ml) of rat pOTC synthesized in the untreated lysate (1.4×10^6 dpm) or the hsc70-depleted lysate (5.1×10^5 dpm) were layered on linear 5 to 20% sucrose gradients (4.9 ml) containing 50 mM HEPES-KOH (pH 7.5), 2 mM ATP, 5 mM magnesium acetate, 50 μM antipain, and 50 μM leupeptin. Centrifugation was performed for 2.5 h at 20°C at 50,000 rpm in a Hitachi 55P ultracentrifuge with an RPS55T rotor. Fractions were collected through the bottom of the tubes and subjected to SDS-polyacrylamide gel electrophoresis followed by fluorography. Portions of the fluorograms are shown over the graphs, and the results were quantitated by imaging plate analysis. The recoveries of ^{35}S-labeled pOTC were about 60% in both cases. Vertical lines show the position of bovine catalase (11.3S), rabbit hemoglobin (4.4S), and hen egg white lysozyme (1.9S).

they have loose conformations. The loose conformations formed from the urea-denatured state may be recognized by hsc70, as are polypeptides elongating from ribosomes. hsc70 may assist the polypeptides in forming secondary structures. Secondary structures without tightly folded tertiary structures are candidate products of the action of hsp70.

Generally, precursor proteins synthesized in wheat germ extract cannot be translocated into mitochondria. Recently, Lain et al. (14) reported that aspartate aminotransferase precursor synthesized in wheat germ extract forms a stable complex with wheat hsp70 present in the extract. Although this complex did not show import competence, import competence of the precursor protein was restored by the addition of rabbit reticulocyte lysate. Thus, it is possible that mammalian hsp70 and other cytosolic factors in rabbit reticulocyte lysate compete for the precursor protein with wheat hsp70. Competition for the precursor protein is a likely event in yeast systems reported by Murakami et al. (22).

hsc70 was not an indispensable cytosolic factor for the import of pOTC into mitochondria. Rather it seems that the role of hsc70 is to enrich import-competent precursor proteins. Although it has been conceptually postulated that cytosolic hsp70 unfolds translocating precursor protein (29), a direct demonstration that a catalytic amount of hsp70 protein can unfold or disaggregate proteins is lacking. Gething and Sambrook (7) presented a working model that stabilization of unfolded proteins is a common action mechanism for hsp70. The action of hsp70 in the mitochondrial protein import can be understood by this model.

hsc70 has been shown to function in assembling cytosolic proteins along their folding pathway (10). It is to be noted that in vitro-synthesized aspartate aminotransferase precursor, a mitochondrion-targeted protein, can fold properly by extended incubation in reticulocyte lysate (16). This finding strongly suggests that hsc70 functions in a similar manner for both cytosolic and mitochondrion-targeted proteins during synthesis.

Recently, Frydman et al. (6) showed that hsc70 and hsp40, one of the mammalian DnaJ homologs, mediate the initial folding process in the cytosol, and they proposed that hsc70-hsp40 transfers the growing polypeptide chain to the chaperonin complex TriC. Since cytosolic proteins are folded properly by the set of these chaperones, mitochondrion-targeted proteins may bypass the subsequent folding processes. We speculate that another member(s) of the DnaJ family (4) and putative organelle-targeting factors such as presequence binding factor (21, 23) in the lysate prevent further folding of the mitochondrial precursor proteins in the cytosol, to maintain import competence.

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