Binding of an N-terminal Rhodanese Peptide to DnaJ and to Ribosomes*

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A peptide corresponding to the N-terminal 17 amino acids of bovine rhodanese was fluorescently labeled with a coumarin derivative at its primary amino group(s) and then purified by high performance liquid chromatography. This peptide interacted with the molecular chaperone DnaJ in the absence of other chaperones and ATP. In the presence of ATP, the molecular chaperone DnaJ bound to the DnaJ-peptide complex, but not to the peptide alone. The chaperone GrpE appeared to cause the release of the peptide bound to the ternary complex in the presence of ATP but not in the presence of ADP. This nucleotide apparently stabilized the complex. The peptide also bound to salt-washed Escherichia coli 70 S ribosomes, specifically to 50 S ribosomal subunits, not to 30 S subunits. DnaJ plus DnaK interacted with the peptide on the ribosome. GrpE caused dissociation of the peptide from the ribosome; ATP was required for this reaction. It was inhibited by ADP. A comparable series of chaperone-mediated reactions is assumed to occur with the N-terminal segment of the nascent polypeptide to facilitate its folding on ribosomes.

Molecular chaperones have been shown in many studies to facilitate refolding of proteins from their denatured state. However, the mechanism by which nascent proteins are initially folded into their native conformation is not known. Several reports indicate that proteins fold on ribosomes during translation (Kudlicki et al., 1995a, 1995b, 1995c; Fedorov and Baldwin, 1995; Kolb et al., 1994). However, the mechanism by which this folding takes place and the role of molecular chaperones in this process has not been established. Our previous results demonstrated that Escherichia coli ribosomes isolated from a cell-free transcription/translation reaction mixture alter synthesis of bovine rhodanese contain appreciable amounts of full-length, inactive rhodanese bound to the ribosomes as peptidyl-tRNA. These ribosome-bound polypeptides could be released in enzymatically active form by incubation with all five of the bacterial chaperones (DnaJ, DnaK, GrpE, GroEL, and GroES). DnaJ appeared to interact with the N-terminal segment of the nascent protein on the ribosome as the first step in a chaperone-mediated reaction sequence (Kudlicki et al., 1994b). However, Hartl and his co-workers (Langer et al., 1992) concluded that DnaJ binds first to the nascent protein followed by DnaK and GrpE as the growing peptide emerges from the ribosome during translation. Later, Hartl and co-workers reported that DnaJ may be the first of the molecular chaperones to interact with the nascent peptide on ribosomes as they observed cross-linking between DnaJ and luciferase peptides emerging from wheat germ ribosomes (Hendrick et al., 1993).

Binding of peptides to DnaK has been studied extensively. Stimulation of DnaK's intrinsic low ATPase activity was usually taken as a measure of interaction. By this measure it was determined that the minimal length of the peptide for activity was 8–9 amino acids (Jordan and McMacken, 1995). These authors also found that DnaJ in a 1000-fold lower concentration than the peptide used would stimulate the ATPase activity of DnaK. Peptides with hydrophobic residues had relatively high activity (Gragerov et al., 1994).

The role of ATP in the functional cycle of the DnaK system was analyzed kinetically by McCarty et al. (1995). Their main results relevant to the data presented here was that DnaJ stabilized the DnaK-peptide interaction in the presence of ATP. Several other reports indicated high affinity of DnaK for the peptide only in the absence of ATP or in the presence of ADP (Landry et al., 1992; Puillerm et al., 1994; Farr et al., 1995; Buchberger et al., 1995). Another point relevant to data presented below is the observed conformational change of DnaK induced by ATP affecting the ATPase activity and the substrate binding domains (Buchberger et al., 1995).

Direct binding of a small peptide to the molecular chaperone DnaJ has not been demonstrated. Hartl and co-workers described interaction with DnaJ of partially folded rhodanese and of casein but not of reduced carboxymethylated α-lactalbumin (Langer et al., 1992). More recently, denatured luciferase was shown to bind first to DnaJ; then DnaK formed a ternary complex with the former two components (Szabo et al., 1994). To investigate further the interaction of the molecular chaperones with the N terminus of nascent rhodanese, we used a peptide corresponding in sequence to the N-terminal 17 amino acids of native bovine rhodanese: VHQLYRALVSTKWLAE. Coumarin was then covalently attached to this peptide at its free amino group(s). The fluorescently labeled peptide was purified by HPLC. Interaction of this peptide with molecular chaperones and with ribosomes in the absence and presence of the chaperones was monitored by fluorescence. The results presented here demonstrate that the N-terminal peptide of rhodanese has high affinity for DnaJ. In the absence of DnaK and GrpE, nucleotides have no apparent effect on this interaction. Similarly, the peptide by itself appears to have a binding site on ribosomes that is on the 50 S ribosomal subunit. An ATP-dependent reaction cycle involving DnaJ, DnaK, and GrpE is demonstrated in the presence and absence of 70 S ribosomes.

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1 The abbreviations used are: HPLC, high performance liquid chromatography; DCC, 7-diethylaminocoumarin-3-carboxylic acid; AMP-PCP, adenosine 5′-β,γ-methylene-triphosphate; Rel Q, relative fluorescence quantum yield.

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EXPERIMENTAL PROCEDURES

Materials—Nucleoside triphosphates and E. coli tRNA were purchased from Boehringer Mannheim; the succinimidyl ester of 7-diethylamino-4-carboxyfluorescein (DCC) was from Molecular Probes, Inc. (Eugene, OR). tRNA$^{met}$, rifampicin, sparsomycin, and all other biochemicals were from Sigma. $[1^{14}C]$Leucine was purchased from Du- pont NEN. The chaperones DnaK, DnaJ, GrpE, GroEL, and GroES were bought from Epicentre Technologies (Madison, WI). DnaJ was also obtained from StressGen (Victoria, Canada). The plasmid used was pSP65 containing the rhodanese coding sequence under the SP6 promoter (Kudlicki et al., 1994a).

Methods—Preparation of 70 S salt-washed ribosomes as well as the 50 and 30 S ribosomal subunits was carried out as described previously (Odom et al., 1980). A peptide corresponding to the N-terminal 17 amino acids of bovine rhodanese (Miller et al., 1991) was synthesized and purified as described in Merrill et al. (1992). The purified peptide was evaporated to dryness under vacuum and redissolved in H$_2$O. It has an absorbance maximum at 276 nm where the molar extinction coefficient is estimated to be about 6,000 on the basis of 1 tyrosine and 1 trypto- phan residue.

The purified peptide was labeled at its N-terminal α-amino group and/or at the ε-amino group of its lysine residue as follows. The fluorescent probe used was the succinimidyl ester of DCC. The reaction was conducted in 100 mM Hepes-KOH (pH 7.0), 40% dimethylformamide, 0.35 mM peptide, and 4 mM DCC ester for 30 min at 37 °C. The peptide was then precipitated by addition of 9 volumes of acetone. Unreacted DCC ester and free DCC remain in solution and thus are separated from the precipitated peptide at this step. The precipitate was collected, washed once with acetone, and taken up in 70% aqueous dimethylformamide. The sample was applied to a C$_3$ reversed-phase column (Beckman Ultraprep, 0.46 × 7.5 cm). Then the column was washed with 5% aqueous acetonitrile. At this point a complex gradient of solution B (85% aqueous acetone + 0.1% trifluoroacetic acid) at a flow rate of 1 ml/min with monitoring of the eluate absorbance at 220 nm, until it fell below 0.2. At this point a gradient of HPLC solution B (85% aqueous acetone + 0.1% trifluoroacetic acid) was begun: from 0 to 20% over a period of 5 min, constant at 20% for 5 min, then from 20 to 40% over 10 min, and finally constant at 40% until the first peak of labeled peptide emerged (about 10 min after 40% solution B was reached). Two additional peaks of labeled peptide were obtained by increasing solution B to 50% over a period of 10 min, but the first peak is the one used for the studies reported here. Peptide from this peak was evaporated to dryness under vacuum and redissolved in H$_2$O. It has an absorbance maximum at 276 nm where the molar extinction coefficient is estimated to be about 6,000 on the basis of 1 tyrosine and 1 tryptophan residue.

Fluorometry techniques were used to monitor changes in the local environment and mobility of the DCC-labeled peptide after its interaction with molecular chaperones and ribosomes. Fluorescence measurements were carried out on a model 8000C photon-counting spectrophotometer from SLM-Aminco Instruments, Inc. (Urbana, IL) as detailed previously (Kudlicki et al., 1994b). The excitation wavelength was 390 nm. The coumarin-labeled peptide was used at 0.13 μM. Its fluorescence quantum yield determined from the integrated emission spectrum was arbitrarily set at 1.00. The average variability of fluorescence anisotropy for nine independent determinations with the coumarin-labeled N-terminal peptide (see Table I) was 0.141 ± 0.011. Unless stated otherwise, additions were made into the cuvette containing 420 μl of solution C (55 mM Tris-OAc (pH 7.8), 2 mM dithiothreitol, 1.9% polyethylene glycol (M, 6,000), 27 mM phospho(enol)pyruvate, 30 mM NH$_4$OAc, 72 mM KOAc, 10 mM Mg(OAc)$_2$.0.5$ mM EDTA). Chaperones, nucleotides, and ribosomes were added in amounts and in the order as indicated in the legends to the tables and figures. After incubation at 25 °C for the indicated time (5 min unless otherwise specified), spectra were taken. When ribosomes with bound N-terminal peptide and chaperones were used, the samples were centrifuged in an Airfuge (Beckman) for 45 min at 150,000 × g. After centrifugation, the supernatants were carefully removed, the ribosomal pellets rinsed, then resuspended in solution C in a volume equal to that of the supernatant. Both fractions were analyzed. The distribution of the N-terminal peptide between both fractions was analyzed by fluorescence. Standard corrections for the fluorescence measurements were performed. Background values were subtracted for the buffer solution, for unlabeled peptide, for the ribosomes, and for the chaperones by themselves. In the titration experiments, volume changes were corrected by additions of solution to the blank cuvette.

RESULTS

Chaperone Interaction with the N-terminal Peptide in the Absence of Ribosomes—Experiments were carried out in the absence of ribosomes to determine whether a direct interaction of the N-terminal rhodanese peptide with any of the E. coli molecular chaperones could be detected by changes in fluorescence from the coumarin-labeled peptide. The fluorescence emission spectra given in Fig. 1A indicate that a large increase in fluorescence intensity occurred when the coumarin-labeled peptide was incubated with DnaJ. The relative quantum yield, fluorescence anisotropy, and emission maxima for this set of measurements are presented in the inset to Fig. 1A. A relative quantum yield of 1.00 was arbitrarily assigned to the fluorescence emission obtained with 0.13 μM of the coumarin-labeled peptide under standard conditions in the absence of chaperones or other components from E. coli. The changes in both fluorescence quantum yield and anisotropy indicate that DnaJ bound directly to the N-terminal peptide.

The stoichiometry of binding between DnaJ and the peptide was determined by monitoring the increase in fluorescence intensity caused by successive addition of DnaJ to known amounts of the coumarin-labeled peptide (Fig. 2). The rate of
intensity change was too rapid to be measured with the system used. The results from repeated experiments with different preparations of DnaJ indicated binding in a ratio of 0.6–1.1 peptide:DnaJ. The calculated ratio of DnaJ:peptide for the experiment represented by Fig. 2 for which the amount of both DnaJ and peptide were carefully determined was 0.98. Considering the inherent accuracy of the methods used and lack of information about the proportion of the DnaJ protein that is inactive, these results strongly indicate that 1 molecule of peptide is tightly bound per molecule of DnaJ. The linearity of the binding curve is striking and indicates that the $K_d$ for the peptide-DnaJ complex is considerably below the concentration of the peptide (0.13 μM) and the concentration range of DnaJ that was used in these experiments. It was not possible to reliably determine $K_d$ for the complex because of the limits in sensitivity of the system used. However, the results indicate that the $K_d$ for the peptide-DnaJ complex is at least an order of magnitude below the concentrations used for the experiments of Fig. 2, i.e. below 13 nM.

Subsequent addition of DnaK to the reaction mixture containing peptide and DnaJ (Fig. 1A) caused a decrease in the quantum yield but an increase in the anisotropy indicating the formation of a peptide-DnaJ-DnaK complex. Addition of GrpE to this reaction mixture caused an additional large decrease in both these parameters (Fig. 1A). The anisotropy decreased to near the value observed for peptide in the absence of chaperones, but the quantum yield remained significantly above that for free peptides. These data indicate that GrpE caused a major change in the peptide-DnaJ-DnaK complex, probably dissociation of peptide and/or DnaJ-peptide. If DnaK was added first (Fig. 1B), no effect on fluorescence from the peptide was detected during a 30-min period. The effects of DnaJ added subsequently after 5 min of incubation were substantially reduced (Rel Q of 2.62 versus 3.18 when DnaJ was added first). These results indicate that DnaK did not detectably bind directly to the peptide under these conditions used, but rather interacted with DnaJ or the DnaJ-peptide complex. Farr et al. (1995) found a large activation energy barrier for interaction of DnaK with a peptide corresponding to the N terminus of the cro repressor. It is possible that the interaction of DnaK directly with the rhodanese peptide might occur at a rate that would be detected under other conditions. However, it is clear that the interaction is greatly accelerated by DnaJ. It should be noted that there are differences in the quantum yield and anisotropy depending on whether DnaJ or K were added first to the peptide. These differences remained unchanged even if the incubation time was extended to 60 min. The subsequent addition of GrpE after first DnaK then DnaJ caused a smaller decrease in fluorescence intensity than when DnaJ was added first (Rel Q of 2.37 versus 2.63). GrpE alone or added in the presence of either DnaK or DnaJ had little or no detectable effect on fluorescence from the peptide, indicating that it affects the peptide-DnaJ-DnaK complex.

The reactions represented by Fig. 1, A and B, were carried out in the presence of ATP and an ATP-regenerating system. When otherwise identical experiments were repeated in the absence of ATP, phospho(enol)pyruvate and pyruvate kinase, the binding of the peptide to DnaJ in the absence of the other chaperones was very similar to that seen in the presence of ATP (Fig. 3A versus Fig. 1A). However, in the absence of ATP, the subsequent addition of DnaJ caused a small increase in fluorescence intensity rather than a decrease as was observed in the presence of ATP. When DnaK was added first (no DnaJ), the observed small increases in fluorescence intensity and anisotropy indicate some direct interaction of the peptide and DnaK (Fig. 3B). This result is in agreement with reports in the literature (Landry et al., 1992; Palleros et al., 1994; McCarty et al., 1995).
The effects with time of ATP, ADP, and AMP-PCP on the fluorescence from peptide-chaperone complex. Fluorescence intensity and anisotropy were measured at the indicated times for three identical reaction mixtures containing peptide, DnaK, DnaJ, and GrpE as described for Fig. 3B. To one of them, ATP was added to give 1 mM concentration; the second and third received ADP and AMP-PCP, respectively, in the same amount.

The results indicate that GrpE does not promote the release of the peptide from the complex when ATP is absent.

To investigate this point further, a set of experiments was carried out in which ADP or the nonhydrolyzable analog, AMP-PCP, was tested in comparison to ATP; pyruvate kinase was omitted from the reaction mixture. Three samples were prepared that contained the coumarin-labeled peptide and the three chaperones in the absence of any nucleotide. Fluorescence measurements were taken and anisotropy determined. The fluorescence parameters were very similar to those given for spectrum 4 of Fig. 3B. Then either ATP, ADP, or AMP-PCP was added to the samples. The results given in Fig. 4A show a large increase in quantum yield with ADP compared with ATP or AMP-PCP. Rel Q is at a maximum at 1 min (the shortest time at which the measurement could be carried out under the conditions used) then declines with ATP, whereas it remains nearly constant with time for ADP and AMP-PCP. Rel Q at 1 min is much higher with ADP than with ATP or AMP-PCP (6.4 versus 4.4–4.6), indicating that the ADP form of the chaperone complex has a larger effect on the N-terminal coumarin probe. The changes in anisotropy, A, with time (Fig. 4B) for ADP show a sharp decline at 1 min (0.22–0.14) then increase to levels that are higher than the corresponding values with AMP-PCP or ATP at 1 min. Anisotropy is nearly constant over time with AMP-PCP but with ATP declines in parallel with the decline in Rel Q. Considered together, the results indicate that ATP and ADP cause pronounced differences in the interaction of DnaK with the peptide-DnaJ complex that is likely to reflect hydrolysis of ATP during the function of DnaK. Nucleotide-induced changes in the conformation of DnaK have been reported (Buchberger et al., 1995), and it has been shown that binding of ATP to DnaK induces dissociation of the chaperone from its substrate (McCarty et al., 1995). The results reported here are consistent with these observations.

Coumarin is relatively hydrophobic and might cause nonspecific binding of the peptide to the chaperones. Another peptide was labeled with coumarin and used as a control. This peptide (amino acids 142–156 of bovine rhodanese) represents the tether region in the rhodanese tertiary structure connecting the two globular regions of the enzyme (Ploegman et al., 1978). Fluorescence from the coumarin-labeled tether peptide indicated that it did not bind to DnaJ and was unaffected by DnaK or GrpE (data not shown).

Binding of the N-terminal Peptide to Salt-washed Ribosomes and Ribosomal Subunits—The results presented above appear to reflect a sequential order of chaperone interaction with the N-terminal rhodanese peptide and the dependence of these reactions on adenosine phosphates. It should be emphasized that they were carried out in the absence of ribosomes. However, previous work has demonstrated that all five of the bacterial chaperones (DnaJ, DnaK, GroE, GroEL, and GroES) are required with nascent full-length rhodanese bound to ribosomes as peptidyl-tRNA to efficiently promote the codon-directed release of active enzyme from the ribosomes. DnaJ, DnaK and GrpE were shown to affect fluorescence from coumarin linked to the N terminus of nascent polypeptides on ribosomes. Our hypothesis is that the DnaJ, DnaK, and GrpE-mediated reactions described above with the synthetic peptide in the absence of ribosomes are directly related to the function of these chaperones in folding the ribosome-bound nascent enzyme. Accordingly, experiments were carried out to investigate the relation of these reactions to binding of the coumarin-labeled N-terminal rhodanese peptide to ribosomes.

Changes in fluorescence intensity, anisotropy and emission maxima indicate that the coumarin-labeled N-terminal peptide binds directly to 70 S ribosomes or 50 S ribosomal subunits but not to 30 S ribosomal subunits in the absence of chaperones (Table I). The relative fluorescence quantum yield and anisotropy increase from 1.0 and 0.14 to 4.2 and 0.29, respectively, with an amount of ribosomes that gave a near maximum effect.

**Table I**

| Additions                                      | Fluorescence characteristics<sup>a</sup> |
|-----------------------------------------------|-----------------------------------------|
|                                               | A | λ<sub>max</sub> | Rel Q |
| A. Coumarin-labeled N-terminal peptide        |   |                |       |
| Peptide only                                  | 0.14 | 481 | 1.0 |
| 70 S ribosomes                                | 0.29 | 473 | 4.2 |
| 50 S ribosomes                                | 0.27 | 476 | 3.7 |
| 30 S ribosomes                                | 0.16 | 480 | 1.2 |
| 70 S ribosomes plus DnaJ<sup>b</sup>          | 0.32 | 473 | 7.7 |
| B. Coumarin-labeled tether peptide            |   |                |       |
| Peptide only                                  | 0.13 | 476 | 1.0 |
| 70 S ribosomes                                | 0.14 | 476 | 1.6 |
| 50 S ribosomes                                | 0.13 | 476 | 1.5 |
| 30 S ribosomes                                | 0.13 | 476 | 1.2 |
| 70 S ribosomes plus DnaJ<sup>b</sup>          | 0.14 | 476 | 1.7 |

<sup>a</sup> A, fluorescence anisotropy; λ<sub>max</sub>, wavelength of emission maximum; Rel Q, relative fluorescence quantum yield.

<sup>b</sup> Ribosomes were preincubated with DnaJ (0.13 μM) for 5 min at 37 °C.
The results with different preparations of ribosomes were somewhat variable but generally gave a maximum effect with a peptide-to-ribosome molar ratio in the range of 0.5. This low ratio may reflect damaged or otherwise inactive ribosomes.

When ribosomal subunits were analyzed individually in similar experiments, 50 S ribosomal subunits gave values for quantum yield and anisotropy that approached those observed with 70 S ribosomes. In contrast, much lower values for quantum yield and anisotropy were obtained with similar molar amounts of 30 S ribosomal subunits. When incubated with the ribosomes or ribosomal subunits, fluorescence intensity from the tether peptide was 1.6 and there was virtually no change in anisotropy or shift in the wavelength of maximal fluorescence (Table I). We interpret the results to indicate that the N-terminal peptide is bound to the 50 S ribosomal subunit.

The Effect of DnaJ, DnaK, and GrpE on the Interaction of the N-terminal Peptide with Ribosomes—The results described above indicate that in the absence of ribosomes, DnaJ, and the labeled N-terminal peptide form a tight complex that is not detectably affected by adenosine phosphates. Accordingly experiments were carried out to determine the effects of DnaJ on binding of the peptide to ribosomes. The experiments were comparable to those for binding of the peptide to 70 S ribosomes (Table I) except for the addition of DnaJ to the reaction mixture. The results are included in Table I. In the presence of DnaJ and ribosomes, both the anisotropy and the quantum yield increased markedly to levels that were higher than with either the ribosomes or with DnaJ alone. The results appear to indicate the formation of a ternary complex between DnaJ, the N-terminal peptide, and the ribosome.

In the next set of experiments, the peptide was incubated with ribosomes, then subsequently with DnaJ and with DnaK plus GrpE in the presence of either ATP or ADP. Binding of the peptide to the ribosomes was determined after sedimenting the ribosomes by centrifugation through a cushion of sucrose. The percentage of the total fluorescence that was recovered in both the supernatant and ribosome fractions are given in Table II. The fluorescence anisotropy for each fraction is given in parentheses. It should be noted that since the quantum yield for each sample is unknown, the absolute amount of peptide recovered in the fraction cannot be calculated. However, the results show that about 95% of the fluorescence and presumably a high portion of the peptide remains associated with the ribosomes in the presence of DnaJ and with either ATP or ADP, or with DnaJ, DnaK, and GrpE in the presence of ADP. However, with ATP in the presence of DnaK and GrpE, most of the fluorescence is released from the ribosomes into the supernatant fraction.

DISCUSSION

The results presented above demonstrate a series of reactions that take place between the molecular chaperones DnaJ, DnaK, and GrpE and N-terminal (coumarin)-17 amino acid peptide corresponding to the N terminus of rhodanese. The reactions take place in the absence of ribosomes but are assumed to correspond to the function of these chaperones in folding nascent rhodanese bound to ribosomes as peptidyl-tRNA. The results indicate that DnaJ is the first of the molecular chaperones to bind to this peptide in a reaction that is not affected by ATP (Fig. 2, A and B). Similarly, binding of the peptide to the ribosome is enhanced by DnaJ (Table I). DnaJ was shown to be the first of the molecular chaperones to interact with nascent polypeptides on prokaryotic or eukaryotic ribosomes as demonstrated by fluorescence (Kudlicki et al., 1994b) and by cross-linking experiments (Hendrick et al., 1993). DnaK forms a stable complex when added to the peptide-DnaJ complex. However, differences were observed in both anisotropy and quantum yield depending on whether the complex was formed in the presence or absence of ATP. This may reflect ATP-induced conformational changes of DnaK (Buchberger et al., 1995). McCarty et al. (1995) reported stable complex formation in the presence of ATP between DnaK-peptide and DnaJ.

A striking difference, depending on whether ATP or ADP is present, is observed in the subsequent reactions of GrpE with the DnaJ-DnaK-peptide complex (see Fig. 4). This may be due to the effects of GrpE on ATP and its hydrolysis by DnaK, or nucleotide exchange which may affect the stability of this complex and the interaction of GrpE with it. A recent study of the ATPase cycle with DnaK mutants suggests that both DnaJ and GrpE may synergistically activate DnaK’s ATPase activity and induce a conformational change in DnaK (Kamath-Loeb et al., 1995). These results with those reported here and from previ-
ous published work indicate that DnaJ, DnaK, and GrpE function together in a series of reactions which can take place with nascent chains on ribosomes. However, they do not provide a definitive answer as to whether chaperones are required for folding of all nascent proteins on ribosomes or even every molecule of nascent rhodanese.

Recently, we demonstrated that ribosomes themselves, specifically the RNA of the large ribosomal subunit, can act as a “chaperone” in refolding denatured rhodanese (Kudlicki et al., 1996). Based on these studies, we suggest that the molecular chaperones do not have an obligatory role in folding of nascent rhodanese on ribosomes. They may function to renature and thereby recover nascent rhodanese that is not folded correctly during translation. This improperly folded full-length rhodanese appears to accumulate on ribosomes as peptidyl-tRNA that does not efficiently undergo codon-directed termination and release. Why folding of rhodanese and some other proteins fail during translation and how these reactions are coupled to codon and release factor-dependent termination and release of the nascent protein is not known. However, our speculation is that DnaJ interacts as the first of the molecular chaperones to bind to an improperly folded nascent peptide as it emerges from the ribosomal tunnel (Eisenstein et al., 1994) followed by the cycle of reactions indicated above with the N-terminal rhodanese peptide. These results are summarized in Fig. 5.

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