Real Time Kinetics of the DnaK/DnaJ/GrpE Molecular Chaperone Machine Action*

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Applying stopped-flow fluorescence spectroscopy for measuring conformational changes of the DnaK molecular chaperone (bacterial Hsp70 homologue) and its binding to target peptide, we found that after ATP hydrolysis, DnaK is converted to the DnaK*(ADP) conformation, which possesses limited affinity for peptide substrates and the GrpE cochaperone but efficiently binds the DnaJ chaperone. In the presence of DnaJ (bacterial Hsp40 homologue), the DnaK*(ADP) form is converted back to the DnaK conformation, and the resulting DnaJ-DnaK(ADP) complex binds to peptide substrates more tightly. Formation of the DnaJ (substrate-DnaK(ADP)) complex is a rate-limiting reaction. The presence of GrpE and ATP hydrolysis promotes the fast release of the peptide substrate from the chaperone complex and converts DnaK to the DnaK*(ADP) conformation. We conclude that in the presence of DnaJ and GrpE, the binding-release cycle of DnaK is stoichiometrically coupled to the adenosine triphosphatase activity of DnaK.

The Hsp70 molecular chaperone (Ellis, 1993), by transient binding to different denatured (Gragerov et al., 1994; Fourie et al., 1994) or native polypeptides (Wawrzyniak and Zylicz, 1995), makes these substrates suitable for transport (Hendrick et al., 1993) and proteolysis (Sherman and Goldberg, 1992), sequesters unfolded peptides thus promoting their correct folding (Szabo et al., 1994), and protects or reactivates heat labile enzymes (Skowyra et al., 1990; Schroder et al., 1993; Ziemienowicz et al., 1993, 1995). Hsp70 also disassembles the clathrin-coated vesicles during receptor-mediated endocytosis (Schlossman et al., 1984, 1987) and activates the formation of protein complexes involved in the initiation of transcription (Blaszczak et al., 1995), DNA replication (Alfano and McMacken, 1989; Zylicz et al., 1989; Wickner et al., 1992), or the assembly of the glucocorticoid receptor complex (Hutchison et al., 1992).

The Hsp70 cooperates in these reactions with two other cochaperones, Hsp40 (the bacterial DnaJ homologue; Zylicz et al., 1985; Silver and Way, 1993) and Hsp24 (the bacterial GrpE homologue; Zylicz et al., 1987, 1994; Bolliger et al., 1994; Lalaraya et al., 1994). The Escherichia coli DnaK/DnaJ/GrpE chaperone system can therefore serve as a paradigm for the analysis of the Hsp70 chaperone machine.

The bacterial DnaK protein, like its eukaryotic Hsp70 homologue, possesses a weak K\(^{+}\)-dependent ATPase activity (Zylicz et al., 1983; O’Brien and McKay, 1995), which is stimulated 2–20-fold in the presence of peptide substrates (Zylicz et al., 1983; Jordan and McMacken, 1995). In the absence of peptide substrates, under steady-state conditions, the ATPase of DnaK is highly stimulated in the joint presence of the GrpE and DnaJ chaperones (Liberek et al., 1993a; Jordan and McMacken, 1995; McCarty et al., 1995). The situation is different in the presence of a protein substrate. In this case, DnaJ does not affect the peptide-dependent ATPase activity of DnaK, but the presence of GrpE vigorously stimulates this activity (jordan and McMacken, 1995).

In this paper using methodology that allowed us to follow the real-time kinetics of DnaK/DnaJ/GrpE molecular chaperone action, we were able to show that the presence of DnaJ induces such DnaK conformations that the stable complex between peptide substrates and DnaK is formed. GrpE and ATP hydrolysis are required for the recycling of DnaK from this complex, thus allowing multiple substrate binding-release cycles of DnaK chaperone action.

EXPERIMENTAL PROCEDURES

Reagents, Materials, and Experimental Conditions—All experiments described in this paper were performed in buffer A containing 25 mM Hepes/KOH, pH 7.4, 150 mM KCl, 25 mM NaCl, and 5 mM MgCl\(_2\). All experiments were performed at 27 °C. The ap1 peptide substrate (Cys-Ala-Leu-Leu-Gln-Ser-Arg) was synthesized and labeled with acrylodan by Dr. Robert Schackman (University of Utah Core Facility).

E. coli Chaperone Proteins—In all experiments presented in this paper, highly purified (more than 95% pure) proteins were used. DnaK was purified as described by Zylicz et al. (1987). An additional size exclusion fast protein liquid chromatography step on Superose 6 (Pharmacia Biotech Inc.) column, equilibrated with buffer A, was included to separate monomers from the oligomeric form of DnaK. The DnaJ protein was purified as described by Zylicz et al. (1985), with an additional size exclusion fast protein liquid chromatography step as described for the DnaK protein, with the exception that buffer A was supplemented with KCl to a final concentration of 250 mM. GrpE was purified as described in Zylicz et al. (1987). Protein concentration was determined spectrophotometrically as described by Lang et al. (1992).

Fluorescence Spectra—Fluorescence measurements were performed on a Perkin-Elmer Instruments LS-50 spectrometer in a 1-mL fluorescence cuvette. The excitation wavelength was set at 300 nm to eliminate nucleotide absorbance, and emission was recorded in the range of 310–450 nm.

Stopped-flow Measurements—Real time kinetics of DnaK conformational changes were performed using a dual monochromator, a stopped-flow spectrophluorimeter (Applied Photophysics DX.17 MV; sequential mixing option) with excitation wavelength at 285 nm (10 nm bandwidth). Fluorescence emission was monitored using a 320-nm cut-off filter. In the case of monitoring the binding of DnaK to the ap1 peptide, the excitation wavelength was at 370 nm and emission wavelength at 520 nm.

RESULTS

ATP Hydrolysis Leads to the Induction of DnaK’s Conformational Changes—When the DnaK chaperone is preincubated with ATP, the fluorescence of its single tryptophan located near its ATP binding site decreases, and simultaneously, the maximum of fluorescence is shifted to a shorter wavelength (Fig. 1).

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DnaK (Liberek et al., 1992; Banek et al., 1992). This result combined with the effect of ATP on the trypsin digestion pattern of DnaK (Liberek et al., 1991b) and its FTIR spectra (Banecki et al., 1992) strongly supports the idea that in the presence of ATP, the DnaK chaperone undergoes dramatic conformational changes.

In this paper the conformation occurring after incubation of DnaK with ATP is referred as DnaK*. To monitor such conformational changes of DnaK we used the stopped-flow technique and measured the kinetics of the decrease of DnaK's tryptophan fluorescence after rapidly mixing DnaK with ATP (Fig. 2A). The rate of this reaction ($k_{tr}$, time of 50% amplitude change $t_{50} = 30.3 \pm 0.1$ s; assuming pseudo first-order kinetics $k_{cat} = 0.023 \pm 0.0016$ s$^{-1}$) does not change with an increase in ATP concentration (Fig. 2A and result not shown). The substitution of ATP by either ADP, ADP + Pi, or AMP-PCP$^1$ did not result in any changes of the tryptophan fluorescence of DnaK (Figs. 1 and 2A and results not shown), suggesting that the ATP hydrolysis, rather than nucleotide binding, is a major factor influencing change of the tryptophan fluorescence of DnaK. According to the previously published results, one molecule of ATP is hydrolyzed by DnaK every 10–15 min (Zyltcz et al., 1983; Liberek et al., 1991a; Palleros et al., 1993; Schmid et al., 1994; Jordan and McMacken, 1995; McCarty et al., 1995), whereas the observed DnaK conformational changes occur much more rapidly ($t_{50} = 30.3$ s). To solve this discrepancy, we showed that the purified monomeric form of DnaK (nucleotide-free), used in these experiments, hydrolyzed ATP molecule at a much faster rate ($k_{cat} = 0.025 \pm 0.005$ s$^{-1}$; see Fig. 2B). However, after a single round of ATP hydrolysis, the rate of the reaction decreased and reached the previously estimated steady-state value (Fig. 2B). This burst of ATP hydrolysis could not be detected previously, because in those cases at least 10-fold lower concentrations of DnaK (which was a mixture of monomeric and multimeric forms) were used.

Results presented in Figs. 1 and 2 reveal that the hydrolysis of ATP occurs with a pre-steady state burst and that the dissociation of Pi and ADP is rate-determining. Supporting this, the burst of product formation is detected in the absence of exogenous Pi, but inhibited in the presence of Pi$^2$ (Fig. 2B). The diagram of the DnaK ATPase reaction is proposed in Fig. 3A. The binding of ATP to the DnaK is a fast reaction ($t_{50} < 0.5$ s; $K_d = 35$ mM) (Montgomery et al., 1993). This induces conformational changes of DnaK (Palleros et al., 1992; Banek et al., 1992) that cannot be monitored by intrinsic fluorescence of the tryptophan (Figs. 1 and 2 and result not shown). The binding of ATP to DnaK ($k_1$; Fig. 3A) is not inhibited by the presence of exogenous Pi (result not shown). After the ATP hydrolysis ($k_2$; Fig. 3A) both products (ADP and P), which are present in the active site of DnaK ATPase dissociate from DnaK complex at different rates. After the conformational change of DnaK (conversion to DnaK* form), the P product is released ($k_3$; Fig. 3A).

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$^1$The abbreviations used are: AMP-PCP, adenylyl 5′-yl imidodiphosphate; ATP→S, adenosine 5′-O-(3-thiotriphosphate); eATP, 1′N′-ethanoadenosine 5′-triphosphate; FTIR, Fourier transform infrared.

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![Intrinsic fluorescence of the DnaK's tryptophan.](image1)

**Fig. 1.** Intrinsic fluorescence of the DnaK's tryptophan. The fluorescence of DnaK (2 μM) was measured as described under “Experimental Procedures” in the absence or presence of 1 mM ATP or 1 mM ADP supplemented with 1 mM K/HPO$_4$. Similar results were obtained when ADP and K/HPO$_4$ were added after the first turnover of DnaK ATPase (result not shown).

![Kinetics of conformational changes of DnaK during the ATP hydrolysis.](image2)

**Fig. 2.** Kinetics of conformational changes of DnaK during the ATP hydrolysis. A, real time kinetics of conformational changes of DnaK were measured directly by following the relative fluorescence of the single tryptophan residue located at the N-terminal domain of the DnaK chaperone. The ATP (or ADP) was injected (final concentration, 100 μM) into a highly purified, monomeric DnaK (5 μM), and the change of tryptophan's fluorescence was monitored using stopped-flow spectrophotometer as described under “Experimental Procedures.” The insert represents the same data plotted in the scale from 0 to 5 s to show that fast reaction of ADP binding does not change the DnaK's tryptophan fluorescence. B, ATP hydrolysis catalyzed by DnaK. DnaK protein (5 μM) and ATP (100 μM) was incubated with K/HPO$_4$ or without 500 μM K/HPO$_4$. At the desired time, the reaction was stopped using two volumes of 8 M urea, and the amount of ADP formed during the hydrolysis reaction was estimated as described by Gao et al. (1993). The insert represents the data obtained following longer times of incubation. The DnaK monomer and buffer conditions were as described under “Experimental Procedures.”
exogenous Pi (Fig. 4A). Interestingly, the preincubation of DnaK with both ADP and Pi does not change the tryptophan fluorescence of DnaK (Fig. 1), suggesting that the DnaK*(ADP) conformation could only be reached after ATP hydrolysis.

DnaJ Converts a DnaK*(ADP) Form Back to the Basic Conformation, Characteristic for DnaK before the ATP Hydrolysis—When DnaJ was added to DnaK in the presence of ATP (Fig. 3B, reaction 1) we observed an increase in the tryptophan fluorescence of DnaK. This increase of fluorescence depends on the preincubation time of DnaK with ATP (Fig. 4B). When no preincubation was performed, no fluorescence change was observed (Fig. 4B, delay 0 s). However, after a 150-s preincubation of DnaK with ATP, when the majority of DnaK molecules should have been converted to the DnaK*(ADP) form (see Fig. 4A, delay 150 s), the addition of DnaJ resulted in a rapid increase in tryptophan’s fluorescence ($t_{\frac{1}{2}} = 1.4 \pm 0.2$ s; Fig. 4B, delay 150 s). The amplitude of these changes is similar to those observed during incubation of the DnaK with ATP (Fig. 2A), suggesting that DnaJ is able to convert stoichiometric amount of DnaK*(ADP) form back to the basic DnaK conformation (Fig. 3B, reaction 1). In control experiments, we showed that the highly purified DnaJ protein used in these experiments is devoid of any tryptophan fluorescence, in agreement with the fact that the dnaJ gene sequence does not predict the presence of any tryptophan residue (Bardwell et al., 1986). The effect of DnaJ on conformational changes of DnaK is proportional to the concentration of the DnaJ. A 4-fold decrease in DnaJ concentration decreased in half the DnaJ-dependent effect on DnaK’s fluorescence (result not shown), suggesting that binding of DnaJ to DnaK is the rate-limiting step. To support these conclusions, we showed that ATP hydrolysis is important for the DnaJ-DnaK(ADP) complex formation. Moreover, this reaction can also occur in the absence of protein substrate (Wawrzynow and Zyllicz, 1995; Wawrzynow et al., 1995). To confirm the idea that DnaJ could bind to DnaK*(ADP) conformation and that this reaction occurs after the ATP hydrolysis step, we showed that 500 $\mu$M K/HPO$_4$ should inhibit the ATP hydrolysis (Fig. 2A) and the transition of DnaK to DnaK*(ADP) conformation (Fig. 4A), does not alter kinetics of DnaJ-dependent conformational changes, under the condition where K/HPO$_4$ was added after the DnaK*(ADP) conformation was reached (Fig. 4C). These results suggest that the presence of DnaJ induces the DnaK conformational changes such that Pi (and to some extend also ADP) can dissociate from the complex. This idea can be supported by the fact that the presence of DnaJ partially releases the $\epsilon$ADP fluorescence analogue from the DnaK-$\epsilon$ADP complex formed after incubation of $\epsilon$ATP with DnaK. However, when DnaJ in this reaction is substituted by the GrpE-nucleotide exchange factor, the release of $\epsilon$ADP from the complex is
much more efficient.\textsuperscript{2} GrpE and ATP Change the DnaJ-DnaK(ADP) Form Back to the DnaK*(ADP) Conformation—It has been shown previously that the GrpE protein releases either ADP or ATP from DnaK (Liberek et al., 1991a) and forms a tight GrpE-DnaK complex (Zylicz et al., 1987, Buchberger et al., 1994). At the same time, the presence of ATP disrupts the GrpE-DnaK complex (Zylicz et al., 1987).

To monitor the conformational changes of DnaK induced by the presence of GrpE, we mixed DnaK, ATP with GrpE. In the case where DnaK was not converted to DnaK*(ADP) form (Fig. 5A, delay 0 s), a fast decrease of DnaK’s tryptophan fluorescence was observed ($t_{1/2} = 0.24 \pm 0.05$ s; Fig. 5B). In contrast, in the absence of GrpE, the reaction was much slower (Fig. 5A, $t_{1/2} = 30.3$ s). The presence of GrpE appears to accelerate conformational changes of DnaK induced after the ATP hydrolysis. In the same time, GrpE (in the absence of protein substrates) does not accelerate the rate of ATP hydrolysis (Zylicz and McMacken, 1995), suggesting that GrpE works mostly as a nucleotide exchange factor. An 8-fold decrease in GrpE concentration did not significantly affect this reaction (result not shown), suggesting that GrpE can be recycled. Probably, when all three components are mixed together (DnaK, GrpE, and ATP), the reaction proceeds with additional intermediary steps. First GrpE binds to DnaK, and then, upon ATP hydrolysis, DnaK is converted to the DnaK*(ADP) form resulting in the release of GrpE. However, when the DnaK protein was first preincubated with ATP, DnaK’s tryptophan fluorescence was less sensitive to the presence of GrpE (Fig. 5B, delay 150 s). These results suggest that DnaK*(ADP) conformation possesses limited affinity to GrpE cochaperone.

A different situation is observed when DnaJ is added to this reaction (Fig. 5C). The preincubation of DnaK, ATP, and DnaJ resulted in the formation of the DnaJ-DnaK(ADP) complex (Fig. 3, reactions 1). In the continuous presence of ATP, the addition of GrpE to this complex triggered the very fast conformational changes of DnaK ($t_{1/2} = 0.18 \pm 0.05$ s) to the DnaK*(ADP) form (Fig. 5C). We suggest that GrpE releases ADP from the DnaJ-DnaK(ADP) complex first (Fig. 3B, reaction 2; see also Liberek et al. (1991a)) and then an intermediate [DnaJ-DnaK-GrpE] complex is formed. The formation of this transient intermediate is suggested also by the fact that the simultaneous presence of GrpE and DnaJ stimulates DnaK’s ATPase activity from 50- up to 180-fold (Liberek et al., 1991a; McCarty et al., 1995). Subsequently after the ATP hydrolysis (Fig. 3B, reaction 3), DnaK is converted to the DnaK*(ADP) form and GrpE is released from the chaperone complex. In the control experiment, we showed that ATPγS could trigger the release of DnaJ from DnaK complex (Wawrzynów and Zylicz, 1995). This facts suggest that DnaJ could leave chaperone complex before GrpE dissociation. The DnaK*(ADP) Form Possesses Different Affinity toward the Peptide Substrate Than DnaJ-DnaK(ADP)—Previously it was established that DnaK binds tightly to the synthetic seven-residue polypeptide (ap1) possessing the amino acid sequence derived from the presequence of the mitochondrial aspartate aminotransferase protein (Schmid et al., 1994). Christen and co-workers (Schmid et al., 1994) used this acrylodan-labeled peptide as a fluorescence substrate to monitor its binding to the DnaK protein. Due to the enhancement of fluorescence of the acrylodan residue upon binding of the ap1 peptide to the DnaK, it was possible to establish the kinetic parameters of this reaction. However, the effect of GrpE or/and DnaJ was not previously tested (Schmid et al., 1994). In this paper we used the same experimental system to elucidate which conformational form of DnaK possesses the highest affinity for the peptide substrate.

\textsuperscript{2} B. Banecki and M. Zylicz, unpublished results.
As reported previously by Schmid et al. (1994), in the absence of ATP, DnaK binds efficiently to ap1 peptide (Fig. 6A). To monitor the ATP-dependent release of DnaK from its substrate complex, we preincubated DnaK with ap1 peptide and then added ATP. When all three factors were rapidly mixed, the increase of DnaK’s binding to ap1 was observed (Fig. 6B, 0 s delay). When DnaK and ap1 were incubated for 100 s before the addition of ATP, we observed the release of DnaK from its complex with ap1 peptide (t_{1/2} = 51760.1 s; Fig. 6B). The increase in the rate of this reaction (k_{obs} = 0.042 ± 0.002 s^{-1}) comparable with the rate of conformational changes of DnaK induced after ATP hydrolysis, observed in the absence of the peptide substrate (Fig. 2A; k_{obs} = 0.027 s^{-1}) could be due to the peptide-dependent-stimulation of DnaK’s ATPase (Jordan and McMacken, 1995).

In the situation when DnaK is converted to the DnaK*(ADP) conformation, the binding to the peptide substrate was faster, but the amplitude of the fluorescence changes was much smaller (Fig. 6C, delay 100 s) than in the absence of ATP (Fig. 6A). These observations suggest that the presence of hydrolyzable ATP simple shifts the equilibrium of DnaK/substrate reaction in favor of dissociation.

The presence of DnaJ changes the kinetics of this reaction. In the presence of ATP, DnaJ substantially stabilizes the ap1-DnaK complex (Fig. 6C). In this case DnaJ probably inhibits the dissociation of ap1 from ap1-DnaK complex. An increase in substrate-DnaK complex stability in the presence of DnaJ was previously noted using different experimental approaches (for review, see Wawrzynow et al. (1995)).

GrpE and ATP Promote the Fast Release of the Substrate Peptide from the DnaJ^*DnaK(ADP) Complex—it was postulated previously that GrpE is involved in the recycling of the DnaK protein, thus reducing the amount of DnaK needed in these in vitro reactions (Alfano and McMacken, 1998; Zylicz et al., 1989). In addition, during the folding of various polypeptides, the presence of GrpE protein and ATP hydrolysis is required for their efficient transfer from DnaK/DnaJ to the GroEL/ES chaperone systems (Langer et al., 1992).

To test the kinetics of GrpE action, we preincubated DnaK, DnaJ, and ATP with ap1 peptide and then injected GrpE (Fig. 6D). After a short preincubation of 0–10 s, almost no effect was observed (Fig. 6D, delay 0 and 10 s). As shown in Fig. 6C, at least 100–200 s is required for DnaJ [ap1-DnaK(ADP)] complex formation. When the preincubation was prolonged to 100 or 200 s, the addition of GrpE (in the presence of ATP) triggered an efficient and fast release of the substrate (Fig. 6D, delay 200 s). As shown in Fig. 5, in this case DnaK is converted back to the DnaK*(ADP) conformation. The GrpE-dependent disassocia-
tion of DnaK from ap1 complex is fast ($t_{1/2} = 0.3 \pm 0.05$ s), suggesting that the action of both cochaperones, DnaJ and GrpE, is coupled to ATP hydrolysis and probably accomplished through a DnaJ-(ap1-DnaK-GrpE) intermediate.

DISCUSSION

According to the data presented in this paper, the reaction of the DnaK-dependent ATP hydrolysis occurs with a pre-steady state burst, when initiated in the presence of nucleotide-free monomeric DnaK protein. The products of this reaction (ADP + P$_i$) suppress the turnover of the subsequent hydrolysis reaction, and the productsofthisreaction(ADP + P$_i$)suppresstheturnoverofthesubsequenthydrolysisreaction. After the first round of ATP hydrolysis, DnaK is converted to the DnaK*(ADP) conformation. Three facts suggest that this DnaK conformational change occurs not during the binding of ATP to DnaK but after the ATP hydrolysis.

1) The rate of DnaK’s conformational change is not influenced by the increase in ATP concentration (this paper).
2) The exogenous P$_i$ inhibits both ATP hydrolysis and DnaK conformational change but does not influence the binding of ATP to DnaK (this paper).
3) The absence of K+ inhibits DnaK’s ATPase activity and conformational change but not the binding of ATP to DnaK (Palleros et al., 1993).

Assuming a high concentration of ATP and the presence of both cochaperones (DnaJ and GrpE), most of DnaK in vivo should then be in the DnaK*(ADP) form. This suggests that the entry (Fig. 3A) to the DnaK/DnaJ/GrpE cycle is significant only in an in vitro situation where DnaK-nucleotide-free form could be reached.

The scheme in Fig. 7 summarizes the results presented in this paper. In the presence of DnaJ, the DnaK*(ADP) form is converted to the DnaK conformation (Fig. 7, reaction 1), and the DnaJ-DnaK(ADP) complex binds to its peptide substrate tightly (Fig. 7, reaction 2). In the control experiments, we have shown that the DnaK*(ADP) form binds protein substrates in a very fast reaction but that in the absence of DnaJ, DnaK rapidly dissociates from its substrate-DnaK complex. The DnaJ-dependent transition of DnaK*(ADP) to DnaK conformation is not inhibited by the exogenous P$_i$, suggesting that the ATP hydrolysis is not required for this transition reaction. The formation of the active DnaJ-DnaK(ADP) complex is limited by the DnaJ concentration. In vivo, coli bacterial cells there is at least 10-fold less DnaJ than DnaK (Bardwell et al., 1986). In the cell, probably most of DnaK protein is in the DnaK*(ADP) form, which binds and releases protein substrates very fast.

Only this DnaK, which is activated by DnaJ (or these substrates that are "tagged" by DnaJ) will form a stable substrate-DnaK complex.

The ap1-DnaK(ADP) complex, which is probably transiently bound to DnaJ, is being formed after the DnaJ-dependent activation of DnaK*(ADP) (Fig. 6, reaction 2). This is a rate-limiting step in DnaK/DnaJ/GrpE cycle. The rate of this reaction is dependent on the peptide concentration and its amino acid sequence or/and peptide conformation. In this case, the affinity of the peptide to DnaJ is important (Wawrzynow and Zylicz, 1995; Wawrzynow et al., 1995). The presence of ADP stabilizes the DnaJ-{-apl-DnaK(ADP)} complex (Wawrzynow et al., 1995). The mechanism of ADP-dependent stabilization of DnaK-substrate complex should be significant in vivo. During stress conditions, the ATP concentration dramatically drops down and ADP concentration increases (Findly et al., 1983). Such situations can "freeze" Hsp70-substrate complexes helping the Hsp70 to protect other protein from inactivation.

The GrpE, after the ATP hydrolysis, triggers the fast release of DnaK from its protein substrate and converts DnaK back to the DnaK*(ADP) form (Fig. 7, reaction 3). The GrpE is known as a nucleotide exchange factor, which after release of ADP from DnaK complex could lead to the formation of [substrate-DnaKGrpE] intermediate (Osiipiuk et al., 1993). The GrpE also, in the presence of protein substrate stimulates DnaK’s ATPase activity (Jordan and McMacken, 1995). Our data do not exclude the existence of any additional intermediate complexes during the binding of ATP and ATP hydrolysis. DnaJ probably dissociates first from the substrate-chaperone complex.

The experiments described in this paper were performed using the ap1 peptide, which does not directly interact with the DnaJ protein (result not shown). In the case where the protein substrate binds to DnaJ (AP, denatured luciferase, RepA, s$_{12}$), the DnaJ chaperone in addition to the DnaK-substrate stabilization effect, can also contribute in delivery of the protein substrate to DnaK (Liberek et al., 1990; Wickner et al., 1992; Garner et al., 1992; Langer et al., 1992; Szabo et al., 1994; Wawrzynow et al., 1995). In these cases the substrate-DnaJ complex will directly bind to DnaK*(ADP) (Fig. 7, reaction 2). DnaJ remaining tightly bound to the substrate can promote several ATP-dependent cycles of DnaK conformational changes (Szabo et al., 1994). Such multiple binding and release steps of DnaK from the DnaJ-substrate complex can induce conformational changes to the protein substrates, thus leading to the activation of some proteins or reactivation of heat-inactivated enzymes. Using FTIR spectroscopy we found that 13C-labeled DnaK protein indeed induces (or freezes) conformational changes to the 12C-labeled RepA protein substrate.2

Recently, we were able to show, using two independent experimental approaches (size high performance liquid chromatography and the enzyme-linked immunosorbent assay), that the presence of ATP and DnaJ stabilizes the DnaK bound not only to the denatured but also to the native polypeptide substrates (Wawrzynow et al., 1995). Therefore, the results presented in this paper represent general phenomena, which describe the role of DnaJ and the GrpE cochaperones in DnaK-substrate complex formation and dissociation.

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