Structure-Function Analysis of the Zinc Finger Region of the DnaJ Molecular Chaperone*

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DnaJ is a molecular chaperone, which not only binds to its various protein substrates, but also can activate the DnaK cochaperone to bind to its various protein substrates as well. DnaJ is a modular protein, which contains a putative zinc finger motif of unknown function. Quantitation of the released Zn(II) ions, upon challenge with p-hydroxymercaprylphenylsulfonic acid, and by atomic absorption showed that two Zn(II) ions interact with each monomer of DnaJ. Following the release of Zn(II) ions, the free cysteine residues probably form disulfide bridge(s), which contribute to overcoming the destabilizing effect of losing Zn(II). Supporting this view, infrared and circular dichroism studies show that the DnaJ secondary structure is largely unaffected by the release of Zn(II). Moreover, infrared spectra recorded at different temperatures, as well as scanning calorimetry, show that the Zn(II) ions help to stabilize DnaJ's tertiary structure. An internal 57-amino acid deletion of the cysteine-rich region did not noticeably affect the affinity of this mutant protein, DnaJ Δ144–200, to bind DnaK nor its ability to stimulate DnaK's ATPase activity. However, the DnaJ Δ144–200 was unable to induce DnaK to a conformation required for the stabilization of the DnaK-substrate complex. Additionally, the DnaJ Δ144–200 mutant protein alone was unimpaired in its ability to interact with its 32 transcription factor substrate, but exhibited reduced affinity toward its P1 RepA and AP substrates. Finally, these in vitro results correlate well with the in vivo observed partial inhibition of bacteriophage λ growth in a DnaJ Δ144–200 mutant background.

The Hsp70 family of proteins, the DnaK for Escherichia coli being the prototype, participate in a variety of cellular functions, such as protein folding, proteolysis, protein transport, the activation of various transcriptional and replication factors to bind to specific DNA sequences, as well as the protection and renaturation of some heat-labile proteins. These observations have led to their classification as molecular chaperones (for review, see Georgopoulos et al. (1994) and Hendrick and Hartl (1993)). However, recent findings have made it clear that DnaK does not function alone. Two other heat shock proteins, DnaJ (the prototype of the eukaryotic Hsp40 proteins) and GrpE (the prototype of the eukaryotic Hsp24 proteins), are also known to participate in these reactions (Georgopoulos et al., 1994). The initial studies of bacteriophage λ (Alfano and McMacken, 1989; Zyllicz et al., 1989; Zyllicz, 1993) and P1 DNA replication (Wickner et al., 1992), followed by detailed in vitro protein folding experiments (Langer et al., 1992; Hendrick et al., 1993), have revealed the intricate interactions of the DnaK/DnaJ/GrpE machinery, whose activity is stoichiometrically coupled to ATP hydrolysis (Banecki and Zyllicz, 1996). Following ATP hydrolysis, DnaJ changes its conformation to the DnaK*-ADP form (the * indicates that this conformation cannot be reached by simply preincubating DnaJ with ADP), which destabilizes its complex with protein substrates. In this form DnaJ binds and releases protein substrates very fast. In order to stabilize the DnaK-substrate complex, the presence of DnaJ is required. In this case, DnaJ changes DnaK* conformation in such a way that the affinity of DnaJ for both native and denatured protein substrates is now increased (Banecki and Zyllicz, 1996). In addition, DnaJ, which alone can bind to several protein substrates (Wawrzynow and Zyllicz, 1995) and perform molecular chaperone functions (Schröder et al., 1993), can "target" DnaJ to those substrates that directly interact with DnaJ (Szabo et al., 1994; Wawrzynow et al., 1995). After the DnaJ (substrate-DnaJ-ADP) complex formation, the GrpE protein and ATP hydrolysis are required to release and recycle DnaJ from this complex (Banecki and Zyllicz, 1996). Thus, the DnaJ chaperone, in a GrpE/ATP-dependent reaction, dissociates from the substrate complex and is converted back to the DnaJ*-ADP conformation, which is ready to rebind (in a DnaJ-dependent reaction) to its protein substrates (Banecki and Zyllicz, 1996).

Both genetic and biochemical studies of various eukaryotic DnaJ-like proteins indicate that most, if not all, of the activities of E. coli DnaJ have been functionally conserved throughout evolution (Caplan et al., 1993; Silver and Way, 1993; Cyr et al., 1994). Members of the Hsp40 family are structurally diverse, containing different combinations of four domains. All Hsp40 members contain a "J-domain" (approximately 70 amino acids long), which is the most highly conserved and is responsible for stimulating the ATPase activity of the DnaK chaperone (Wall et al., 1994). Recently, using NMR methodology, the tertiary structure of the "J-domain" has been determined (Szymerski et al., 1994). Following the J-domain is a 35-amino acid-long region that is rich in both the Gly and Phe amino acids (the so-called G/F module). The deletion of the G/F module drastically interferes with the DnaJ-dependent stabilization of the DnaK-substrate complexes (Wall et al., 1995). Downstream of the G/F-rich module of some DnaJ-like proteins (for a review, see Caplan et al. (1993)) is a Cys-rich region that contains four

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repeats of the sequence CXXCGXXG (Bardwell et al., 1986; Ohki et al., 1986), which strongly resembles one of the zinc finger motifs (Table I). The COOH-terminal region of all DnaJ-like proteins is much less conserved and could be responsible for substrate binding.

**Materials and Methods**

**Protein Purification—**DnaJ, DnaK, rpoC, and J proteins were purified according to Zylicz et al. (1985), Zylicz et al. (1987, 1988), and Zylicz et al. (1989), respectively. The P1 replication protein, RepA, was the kind gift of Dr. Dhruba K. Chattoraj (National Institutes of Health). The purity of all enzymes was greater than 95%. The DnaJ concentration was estimated by performing the amino acid analysis.

The DnaJ Δ144–200 cysteine-rich internal deletion mutant was constructed by polymerase chain reaction using the plj 10(5′-CCCGATTCCATATGGTCCATGGTCGTG-3′) and plj 11(5′-CCCGATCCCATATGCGATTCATGGTCGTG-3′) primers. Flanking primers to plj 10 and 3′ to plj 11 were used to prepare the DNA. Both plj 10 and plj 11 were engineered with NdeI restriction sites (underlined). The polymerase chain reaction products were then digested with NdeI and ligated together. The gel-purified products were then digested with Sall and DnaI and cloned into the corresponding sites of the pBluescript plasmid (Wall et al., 1994), generating DnaJ Δ144–200. The resulting DnaJ Δ144–200 protein has a 57-amino acid deletion (residues 144–200) and an insertion of 2 amino acids (His-Met). The accuracy of the plj Δ144–200 construct was verified by DNA sequence analysis. DnaJ Δ144–200 was purified essentially as wild type DnaJ, except that the urea extraction step was replaced by increasing the ionic strength of DnaJ (20 mM Tris/HCl, pH 7.4, 120 mM NaCl) prepared in D2O, pD 7.2 (pD 3) primers. Flanking primers to pJ10 and GCATGGTCATGGTCGTG-3′. The plasmid was transformed into E. coli JM109, and the transformants were selected by screening for ampicillin resistance. Plasmids were purified from Escherichia coli F− strains harboring the plasmids by the method reported by Bluem et al. (1988). The number and position of amide I components were taken from second derivative and deconvoluted absorption spectra (Banecki et al., 1992).

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1 The abbreviations used are: PMPS, p-hydroxymercuriphenylsulfonic acid; PAR, 4-(2-pyridylazo)resorcinol; DTT, dithiothreitol; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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**Determination of the Zinc Content of DnaJ—**Nucleotide sequence analysis of the dnaJ gene predicts the presence of two putative metal binding sites, which resemble one of the zinc finger motifs (Table I). To determine how many Zn(II) molecules may bind to DnaJ, we incubated a highly purified DnaJ protein sample (Zylicz et al., 1985), which is fully active in λ DNA replication system (Zylicz et al., 1989) with PMPS, which is known to release Zn(II) from zinc-binding proteins (Hunt et al., 1984). The formation of a mercaptide bond between the free Cys residues and PMPS can be monitored by absorbance at 250 nm (Fig. 1A). The addition of a high affinity metallochromic indicator (PAR), which changes color (absorbance at 500 nm) after the formation of the PAR/Zn(II) complex, allowed the monitoring of the amount of Zn(II) released from DnaJ after PMPS treatment (Fig. 1B). As described by Hunt et al. (1984) 4 molecules of PMPS release one Zn(II) ion. As shown in Fig. 1, the absorption at 250 nm (monitoring mercaptide bond formation) and at 500 nm (monitoring PAR/Zn(II) complex formation) reached a plateau at a ratio of 8 PMPS for each DnaJ monomer, suggesting that 8 Cys residues are involved in binding two Zn(II) ions. Thus, the use of PMPS allowed the purification of the Zn(II)-free DnaJ apoprotein, which we designate as DnaJ(–).

By following the absorbance of PAR at 500 nm as a function of increasing amounts of free Zn(II) (results not shown), we calculated that the absorption A<sub>500nm</sub> = 0.56, which is obtained for a PMPS/DnaJ ratio higher than 8 (see Fig. 1B), corresponds to 1.95 ± 0.2 ions of Zn(II) per monomer of DnaJ. Similar results, 2.01 ± 0.1 Zn(II) ions/monomer of DnaJ, were obtained using atomic absorption spectroscopy (results not shown). We conclude that a monomer of DnaJ binds 2 molecules of Zn(II) ions.

The DnaJ protein possesses 10 Cys residues. To estimate if all of these Cys are involved in the binding of Zn(II) ions, we calculated the content of free sulfydryl groups in denatured and native DnaJ protein in the presence or absence of Zn(II). After extensive dialysis of the DnaJ protein against a buffer that did not contain DTT, we added an excess of DTNB and measured the absorbance at 412 nm as described previously by Qui et al. (1994). Under non-denaturing conditions, native DnaJ protein contains 2 Cys residues, which could interact with DTNB. Denaturation of DnaJ by 8 M Urea exposes all 10 Cys residues to DTNB. Surprisingly, DnaJ(–) apoprotein possesses only 4 Cys that could interact with DTNB (results not shown). These
results suggest that, following the release of Zn(II) ions, some of the free Cys residues can form Cys-Cys bridges.

The near-ultraviolet circular dichroism spectra of native DnaJ and the DnaJZn(II) apoprotein support this hypothesis (Fig. 2). In the range of 200–250 nm, we could not detect any substantial changes in the DnaJ or DnaJZn(II) CD spectra (Fig. 2A), suggesting that the release of Zn(II) did not substantially affect DnaJ’s secondary structure. However, some differences could be seen in the 250–290 nm range. Following the release of Zn(II) ions, the ellipticity at 270 nm increases (Fig. 2B). This reaction is reversible because subsequent addition of ZnCl₂ (10 mM) partially reverses this effect (Fig. 2B). Interestingly, only when ZnCl₂ is used in the presence of 2 mM DTT does the reaction fully reverse, supporting our previous hypothesis that after release of Zn(II) some of the Cys residues of DnaJ could form Cys-Cys bridges, which are disrupted only in the presence of DTT. A similar effect has been described previously for the T4 gp32 metalloprotein (Qiu et al., 1994).

Infrared Spectroscopy Studies of DnaJ and DnaJ Zn(–) apoprotein—To further characterize DnaJ, we measured its infrared absorbance spectra in H₂O and D₂O (Fig. 3A). The spectrum (amide I band) obtained in H₂O buffer exhibits a maximum at 1650 cm⁻¹. The amide II band, due to NH bending and CH stretching vibrations, is present at 1550 cm⁻¹. In a D₂O-containing buffer, the amide I band is shifted to 1641 cm⁻¹ and the amide II band is shifted to about 1450 cm⁻¹. This latter effect is probably due to a H/D exchange reaction. The deconvoluted spectra of the DnaJ protein, shown in Fig. 3B, suggest that the amide I band is composed of at least seven components (Fig. 3B). The assignment of these bands to a particular protein secondary structure was done according to well established criteria (Byler and Susi, 1986), leading to the description of the secondary structure for the DnaJ and DnaJ Zn(–) apoproteins (Table II). After the release of Zn, the amount of random coil and α-helical structures slightly increase, but, at the same time, the amount of β-structure proportionally decreases. As was suggested by the CD spectra results, the differences in secondary structure between DnaJ and DnaJ Zn(–) apoprotein are minor and probably local (Table II).

Infrared spectroscopy may also provide indirect information on the tertiary structure of a protein. This information could be obtained from H/D exchange studies and/or infrared spectra recorded at different temperatures in D₂O (Banecki et al., 1992). The infrared spectra of DnaJ and DnaJ Zn(–) apoprotein taken at different temperatures, shown in Fig. 4, provide information about their thermal denaturation. The wild type
DnaJ protein starts to denature at the temperature range between 50 and 55 °C. At this temperature range, the intensity of 1655 cm\(^{-1}\) peak decreases while the intensity of 1635 cm\(^{-1}\) peak, which reflects the \(\beta\)-structure content, is not changed. In the temperature range of 65–70 °C, the protein is extensively unwound and is found predominantly in a random coil structure (peak 1644 cm\(^{-1}\)). When the temperature is further increased, two new peaks are found (at 1618 and 1684 cm\(^{-1}\)) (Fig. 4), which probably correspond to the aggregated state of the protein (Casal et al., 1988). In the case of DnaJ Zn\((-)\), the corresponded peaks appear at a lower temperature (Fig. 4), suggesting that the release of Zn(II) could partially destabilize the tertiary structure of DnaJ.

Differential Scanning Calorimetry of DnaJ and DnaJ Zn\((-)\)—The conclusions reached with infrared spectroscopy on the stability of DnaJ and DnaJ Zn\((-)\) apoprotein were supported by the use of the differential scanning calorimetry tech-

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**FIG. 3.** Infrared absorbance spectrum of DnaJ in the 1800–1500 cm\(^{-1}\) region. Panel A, the infrared absorbance spectrum of DnaJ (5 \(\mu\)M) in buffer containing H\(_2\)O (solid line) or D\(_2\)O (dashed line) was measured as described under “Materials and Methods.” Panel B, the same, as described in panel A, absorption spectrum of DnaJ amide I band (in the presence of D\(_2\)O) after band narrowing by Fourier deconvolution using a 18 cm\(^{-1}\) half-bandwidth and a resolution enhancement factor of 2.7. The shape of the deconvoluted amide I bands were simulated by gaussian/lorentzian functions. The best fit was obtained with a 30% gaussian proportion for each amide I component band. The symbols \(\alpha\), \(\beta\), t, and r refer to \(\alpha\)-helices, \(\beta\)-structure, turns, and random (non-ordered) structure, respectively.

**FIG. 4.** Deconvoluted spectra (amide I) of DnaJ (panel A) and DnaJ Zn\((-)\) apoprotein (panel B) in D\(_2\)O at different temperatures. The infrared spectra were recorded at 20, 40, 45, 50, 55, 60, 65, and 70 °C. The protein concentration (5 \(\mu\)M) and buffer conditions were as described under “Materials and Methods.” The deconvolution parameters for amide I band were as described in Fig. 3B.

**TABLE II**

Calculated positions and fractional areas of the amide I component bands for the DnaJ and DnaJ Zn\((-)\) chaperone proteins

| Structure | DnaJ | Fractional area | DnaJ Zn\(-) | Fractional area |
|-----------|------|----------------|-------------|----------------|
|           | Position | cm\(^{-1}\) | % | Position | cm\(^{-1}\) | % |
| \(\beta\) | 1676 | 6 | \(\beta\) | 1676 | 5.6 |
| t         | 1666 | 9.6 | t         | 1666 | 8.0 |
| \(\alpha\) | 1655 | 28.6 | \(\alpha\) | 1655 | 30.1 |
| r         | 1642 | 16.1 | r         | 1642 | 19.5 |
| \(\beta\) | 1635 | 38.2 | \(\beta\) | 1635 | 35.6 |
| \(\beta\) | 1618 | 1.5 | \(\beta\) | 1618 | 1.2 |
nique (Fig. 5). The calorimetric profile of DnaJ shows three peaks centered at 53, 57.3, and 67.3 °C (Fig. 5 and Table III), arguing that the denaturation process of DnaJ does not conform to a two-state mechanism, the transition most likely involving partially folded intermediates. This result suggests that DnaJ consists of two or more domains. As was already noted in the case of the Fourier transformed infrared differentiation spectra, the release of Zn(II) from the DnaJ protein decreases its stability since the transition points are centered at 49.6, 56.2, and 68.0 °C (Fig. 6, Table III). Deletion of the 57-amino acid Cys-rich region, consisting of residues 144–200 (DnaJΔ144–200), leads to a further decrease in DnaJ’s transition points (results not shown), suggesting that the binding of Zn(II) ions, or the disulfide bridges formed following the release of Zn(II) from the DnaJ protein are involved in the stabilization of DnaJ’s tertiary structure. In control experiments, using size high pressure liquid chromatography under the conditions described by Wawrzynow and Zylicz (1995), we showed that all three forms, DnaJ, DnaJ Zn(−), and DnaJ Δ144–200, are dimeric (results not shown), which suggests that Zn(II) is not involved in the formation of DnaJ dimers.

Interaction of DnaJ, DnaJ Zn(−), and DnaJ Δ144–200 with DnaK—Recently, we showed that in the presence of ATP, DnaJ can interact with the DnaK molecular chaperone and stabilize DnaK complexed with its various denatured or native protein substrates (Wawrzynow and Zylicz, 1995; Wawrzynow et al., 1995). The conclusion from these and other studies is that DnaJ protein can induce conformational changes in the DnaK protein, which lead to the stabilization of the substrate-DnaK complex. Such conformational changes of DnaK can be monitored by the changes in the fluorescence of the single tryptophan 102 residue, located in the amino-terminal domain of DnaK, near the ATP binding site (Banecki and Zylicz, 1996). In contrast, DnaJ does not possess any tryptophan residues. As shown in Fig. 6, both DnaJ and DnaJ Zn(−) apoprotein exhibit similar properties in modulating DnaK’s conformation.

The DnaJ Δ144–200 exhibits a significant reduction in its ability to change DnaK’s conformation (Fig. 6). This observation can be explained by assuming that the internal 57-amino acid deletion causes a conformational change in DnaJ, which results in its inability to interact efficiently with the DnaK chaperone. To test this possibility, we used the very sensitive ELISA assay to probe for weak interactions between the DnaJ and DnaK proteins (Wawrzynow and Zylicz, 1995). In this assay, DnaJ or DnaJ Δ144–200 were first bound to the ELISA plates, and following extensive washing and blocking with excess of BSA, increasing concentrations of the DnaK protein (in the presence of ATP and BSA) were added. Interestingly, using this assay, both DnaJ and DnaJ Δ144–200 interacted with DnaK with identical affinities (Fig. 7). As a negative control for the specificity of the ELISA assay we used DnaKc94, a truncated protein lacking the 94 carboxy-terminal amino acids. As published previously (Wawrzynow and Zylicz, 1995), the substitution of DnaK by DnaKc94 abolished the DnaK-DnaJ interaction (Fig. 7).

In a second approach, we monitored DnaJ-DnaK interactions using the microcalorimetry method. The raw data for the calorimetric titration of DnaK and a mixture of DnaJ and DnaK with ATP-Mg²⁺ are shown in Fig. 8. The area of the downward peak is proportional to the heat released during three distinct processes, namely the binding of ATP to DnaK, the hydrolysis of ATP, and the conformational changes of the DnaK protein. The presence of the DnaJ protein substantially increases the heat released during DnaK’s interaction with ATP (Fig. 8B), supporting the previous findings that DnaJ, in the absence of protein substrate, accelerates ATP hydrolysis (Liberek et al., 1993; Jordan and McMacken, 1995). In control experiments we could not detect any heat release when ATP was injected to DnaJ protein (results not shown), suggesting that DnaJ does not interact with ATP. No enthalpy changes were detected when ATP was omitted, and DnaJ was injected to DnaK (re-

**Fig. 5. Heat release during the denaturation of the DnaJ (panel A) or DnaJ Zn(−) apoprotein (panel B).** Experimental data are represented by a continuous line. The theoretical curve calculated with the parameters in Table III is represented by filled squares. Three states (dotted line) are the minimum number of states that accurately describe the denaturation process.

**Table III.** Thermodynamic parameters associated with the thermal unfolding of DnaJ and DnaJ Zn(−) apoprotein.

| Transition points °C | ΔH kcal mol⁻¹ | Hcal/vHoff | Transition points °C | ΔH kcal mol⁻¹ | Hcal/vHoff |
|----------------------|---------------|------------|----------------------|---------------|------------|
| 53.0                 | 71.6          | 0.96       | 49.6                 | 71.3          | 0.94       |
| 57.3                 | 102           | 0.94       | 56.2                 | 111.0         | 1.03       |
| 67.3                 | 62.5          | 0.59       | 68.8                 | 32.1          | 0.55       |
sults not shown), suggesting that the presence of ATP is required for the DnaJ-DnaK interaction. In support of these conclusions, we showed that ATP hydrolysis is important for the DnaJ-DnaK-ADP complex formation, and such a complex can only be detected when ATP was present during both the preincubation and mobile phase of size high pressure liquid chromatography (Wawrzynów and Zylicz, 1995). Additionally, we found that deletion of the Cys-rich region does not significantly change the DnaJ- and GrpE-dependent stimulation of DnaK’s ATPase activity (results not shown). Thus, in agreement with our previous findings, the Cys-rich region of DnaJ does not play a significant role in the binding of DnaK to DnaJ (Wall et al., 1995). We conclude that the deletion of Cys-rich region does not change the ability of DnaJ to interact with DnaK, but blocks the DnaJ-dependent induction of a change in DnaK’s conformation.
DnaJ and DnaJ Δ144–200 Binding to Their Protein Substrates—DnaJ is a molecular chaperone and can interact with several seemingly native proteins, e.g. σxy, RepA, λP, and λO (Wawrzynow and Zylicz, 1995). Using the ELISA assay we found that DnaJ Δ144–200 can bind to the σxy substrate, but its ability to interact with other physiologically relevant substrates, such as λP or RepA, was significantly reduced (Fig. 9, A and B). These results suggest that DnaJ Δ144–200 is not only blocked in the induction of DnaK’s active conformational state, but itself may bind less efficiently to some of its protein substrates.

DnaJ Δ144–200 Is Affected in Its Ability to Stimulate DnaK-Substrate Complex Formation—As shown previously, the wild type DnaJ protein and the presence of ATP is required for substrate-DnaK complex formation (Langer et al., 1992; Liberek and Georgopoulos, 1993; Wawrzynow et al., 1995). We found that DnaJ Δ144–200 does not efficiently stimulate the λP-DnaK and σxy-DnaK complex formation (Fig. 10). The failure of DnaJ Δ144–200 to efficiently stabilize the λP-DnaK complex is probably due to two effects, namely a block in the DnaJ-dependent induction of DnaK to an active conformation and a decrease in the affinity of DnaJ Δ144–200 for its λP substrate. In contrast, σxy interacts with DnaJ Δ144–200 with the same efficiency as does wild type DnaJ, so the inhibition of the σxy-DnaK complex formation in the presence of DnaJ Δ144–200 is probably due mostly to a block in the transition of DnaK to the DnaK conformation.

In support of all these findings and conclusions, we find that DnaJ Δ144–200 is only partially active in an in vitro λ DNA replication system (results not shown). A similar effect is also found in vivo. As shown in Fig. 11, at 42°C dnanj Δ144–200 mutant bacteria do not support bacteriophage λ growth to the same extent as do the wild type isogenic bacteria.

**DISCUSSION**

Previous studies have established that zinc fingers and other metal-binding protein domains are involved in protein/DNA interactions, protein interactions with damaged DNA, and DNA-protein interactions (for a review, see Berg (1990)). In this paper we show that the native DnaJ molecular chaperone is indeed a metalloprotein, which binds two Zn(II) metal ions/DnaJ monomer. The zinc binding motif described in this paper closely resembles the C4 zinc binding domain of certain DNA-binding proteins (for review, see Berg (1990)).

The characterization of Zn(II) ions in DnaJ’s structure-function is complicated, because the release of Zn(II) from DnaJ is probably followed by the in vitro formation of S-S bridges between some of the free Cys residues, which result in a stabilization of the DnaJ Zn(−) apoprotein structure. Nevertheless, the release of Zn(II) ions from the DnaJ protein results in minor and probably local changes in secondary structure.

Both infrared spectroscopy and scanning calorimetry show that the release of Zn(II) ions from DnaJ results in the destabilization of DnaJ’s tertiary structure, as evidenced by the decrease in the transition points during the melting of DnaJ,
while simultaneously increasing its aggregation state. The role of Zn(II) ions in stabilizing the tertiary structure of a protein was originally suggested by Giedroc et al. (1987). The finding that DnaJ has three melting transition points (53.0, 57.3, and 67.3 °C) suggests that DnaJ has a complicated domain structure. The release of Zn(II) ions or deletion of the Cys-rich region results in the decrease of the temperature transition points, suggesting that the structure of DnaJ is rather compact and that the changes in one domain may influence the structure of other domains.

The DnaJ molecular chaperone is known to interact with several native proteins. For example, it interacts with high affinity with RepA and α² and with low affinity with the λO and λP proteins (for review, see Wawrzynow and Zyllicz (1995)). In this paper we have shown that the mutant DnaJ 144–200 (deletion of the entire Cys-rich region) protein interacts with wild type efficiency with α², but interacts with reduced efficiency with its RepA and λP substrates. These results suggest that the binding of DnaJ to its substrates is complex and may involve various modes or conformations. In control experiments we found that the DnaJ 144–200 mutant protein exists as a dimer in solution and binds to DnaK with normal efficiency. This last result supports our previous findings, namely that the amino-terminal "J domain," which lacks the Cys-rich region, is responsible for binding to the DnaK protein and for stimulating of DnaK's ATPase activity (Wall et al., 1994).

Recently, it has become clear that, in addition to the role that DnaJ plays in targeting the DnaK chaperone to the DnaJ-substrate complexes, DnaJ is also required for the stabilization of the DnaK-substrate complex. Hartl and colleagues have previously suggested that the DnaK-ATP form could bind to the DnaJ-modified substrates, and following ATP hydrolysis form a stable DnaJ (substrate-DnaK-ADP) complex (Szabo et al., 1994) (see also McCarty et al. (1995)). However, the results presented in this paper show that the situation is far more complex. For example, the DnaJ 144–200 mutant protein, which stimulates DnaK's ATPase activity like wild type DnaJ+, cannot efficiently change DnaK's conformation, resulting in a decrease in the stability of the DnaK-λP and DnaK-α² complexes. This, in turn, suggests that perhaps the major role of DnaJ in the formation of a stable substrate-DnaK complex is not the stimulation of ATP hydrolysis but rather a change in DnaK's conformation, which allows the stable substrate-DnaK complex formation. This interpretation is in agreement with our recent stopped flow data, according to which DnaK (in the absence of DnaJ) during ATP hydrolysis changes its own conformation to the so-called DnaK*-ADP form, which possesses limited affinity for its protein substrates, but binds efficiently to DnaJ. In the presence of DnaJ, the DnaK*-ADP form is converted to a different conformation, capable of forming a stable complex with DnaK's substrates (Banecki and Zyllicz, 1996). Moreover, such DnaJ-dependent activation of DnaK's structure can occur in the absence of protein substrate (Wawrzynow et al., 1995).

**REFERENCES**

Alfano, C., and McMadden, R. (1989) J. Biol. Chem. 264, 10709–10718

Banecki, B., and Zyllicz, M. (1996) J. Biol. Chem. 271, 6337–6414

Banecki, B., Zyllicz, M., Bertoli, E., and Tanfani, F. (1992) J. Biol. Chem. 267, 25051–25058

Bardwell, J. C. A., Tilly, K., Craig, E., King, J., Zyllicz, M., and Georgopoulos, C. (1986) J. Biol. Chem. 261, 1762–1785

Berg, J. M. (1990) J. Biol. Chem. 265, 6513–6516

Blume, E. E., Hubner, W., and Messner, G. (1988) Biochemistry 27, 8239–8249

Byler, D. M., and Susi, H. (1996) Biopolymers 25, 469–487

Capián, A. J., Cyr, D., and Douglas, M. G. (1993) Mol. Biol. Cell 4, 555–563

Casal, H. L., Kohler, U., and Mantsch, H. H. (1988) Biochem. Biophys. Acta 957, 123–130

Cyr, D. M., Langer, T., and Douglas, M. (1994) Trends Biochem. Sci. 19, 176–181

Georgopoulos, C., Liberek, K., Zyllicz, M., and Ang, D. (1994) In The Biology of Heat Shock Proteins and Molloyd Chaperones (Morimoto, R. I., Tissieres, A., and Georgopoulos, C., eds) pp. 209–249, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Gruber, D. P., Keating, K. M., Williams, K. R., and Coleman, J. E. (1987) Biochemistry 26, 5251–5259

Hendrick, J. P., and Hartl, F.-U. (1993) Annu. Rev. Biochem. 62, 349–384

Hendrick, J. P., Langer, T., Davis, T. A., Hartl, F.-U., and Wiedmann, M. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10216–10220

Hunt, J. B., Neece, S. H., Schachman, H. K., and Ginsburg, A. (1984) J. Biol. Chem. 259, 14793–14803

Jordan, R., and McMacken, R. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 1778–1781

Kodadek, T., and Giedroc, D. P. (1994) J. Biol. Chem. 269, 2773–2781

McCarty, J. S., Buchberger, A., Reinstein, J., and Bukau, B. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 8351–8355

McCarthy, J. S., Buchberger, A., Reinstein, J., and Bukau, B. (1995) J. Biol. Chem. 270, 11019–11023

Ohki, M., Tamura, F., Nishimura, S., and Uchida, H. (1986) J. Biol. Chem. 261, 126–137

Schroder, H., Langer, T., Hartl, F.-U., and Bukau, B. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10216–10220

Silver, P. A., and Way, J. C. (1993) Cell 74, 5–6

Sehl, L. C., and Castellino, F. J. (1990) J. Biol. Chem. 265, 5482–5486

Szabo, A., Korszun, R., Hartl, F.-U., and Flanagan, J. (1996) EMBO J. 15, 408–417

**Addendum**—While this article was in preparation, a study by Szabo et al. (1996) appeared that also demonstrates the presence of two zinc atoms in DnaJ monomer, using atomic absorption and extended x-ray absorption fine structure spectroscopy. In addition, using various DnaJ fragments, these authors proposed that cysteine-rich portion of the DnaJ protein is involved in binding to denatured protein substrates. This conclusion was based on the fact that cysteine-rich domain was the only shared by two DnaJ fragments, each capable of preventing the aggregation of Rhodanase. Our data substantially expand this work since they demonstrate that the role of the cysteine-rich domain is not as simple as the one proposed by Szabo et al. (1996). Our results show that DnaJ 144–200, which lacks this cysteine-rich region, binds perfectly well to its λP but less well to its RepA protein substrate. Additionally, the DnaJ 144–200 is restricted in its capacity to activate DnaK to bind to either α² or λP.

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Structure-Function Analysis of the Zinc Finger Region of the DnaJ Molecular Chaperone

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