Hsp31, the *Escherichia coli* yedU Gene Product, Is a Molecular Chaperone Whose Activity Is Inhibited by ATP at High Temperatures*

M. S. R. Sastry, Konstantin Korotkov, Yan Brodsky, and François Baneyx

From the Department of Chemical Engineering, University of Washington, Seattle, Washington 98195-1750

Received for publication, June 12, 2002, and in revised form, September 3, 2002

Published, JBC Papers in Press, September 15, 2002, DOI 10.1074/jbc.M205800200

The *Escherichia coli* chromosome contains several uncharacterized heat-inducible loci that may encode novel molecular chaperones or proteases. Here we show that the 31-kDa product of the *yedU* gene is an efficient homodimeric molecular chaperone that is conserved in a number of pathogenic eubacteria and fungi. Heat shock protein (Hsp) 31 relies on temperature-driven conformational changes to expose structured hydrophobic domains that are likely responsible for substrate binding. Complementing the function of refolding, remodeling, and holding chaperones, Hsp 31 preferentially interacts with early unfolding intermediates and rapidly releases them in an active form after transfer to low temperatures. Although Hsp 31 does not appear to exhibit intrinsic ATPase activity, binding of ATP at high temperatures restricts the size or availability of the substrate binding site, thereby modulating chaperone activity. The possible role of ATP in coordinating the function of the cellular complement of molecular chaperones is discussed.

In the cellular environment, the *de novo* folding of short, single-domain proteins is thought to proceed rapidly and efficiently whereas that of large, multidomain proteins and slow folding polypeptides often requires the assistance of molecular chaperones. In *Escherichia coli*, nascent and newly synthesized chains that rely on chaperones to reach a proper conformation are engaged by either trigger factor or the DnaK-DnaJ-GrpE system (and in some cases are transferred to the GroEL-GroES team) before being released in a native form. Chaperones interact with their client proteins by binding to solvent-exposed hydrophobic stretches that would normally be buried within the substrate core. By shielding interactive surfaces that give rise to misfolded and aggregated species, chaperones promote on-pathway folding without accelerating folding rates or becoming part of the final structure (for recent reviews, see Refs. 1–4).

Another role of molecular chaperones is to repair host proteins that have misfolded as a result of temperature increase or other forms of stress. The main players in this process are the

* This work was supported by Research Project Grant MBC-99-335-01 from the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Chemical Engineering, Box 351750, University of Washington, Seattle, WA 98195-1750. Tel.: 206-685-7659; Fax: 206-685-3451; E-mail: baneyx@u.washington.edu.

‡ Present address: Immunex, 51 University St., Seattle, WA 98101.

The *Escherichia coli* chromosome contains several uncharacterized heat-inducible loci that may encode novel molecular chaperones or proteases. Here we show that the 31-kDa product of the *yedU* gene is an efficient homodimeric molecular chaperone that is conserved in a number of pathogenic eubacteria and fungi. Heat shock protein (Hsp) 31 relies on temperature-driven conformational changes to expose structured hydrophobic domains that are likely responsible for substrate binding. Complementing the function of refolding, remodeling, and holding chaperones, Hsp 31 preferentially interacts with early unfolding intermediates and rapidly releases them in an active form after transfer to low temperatures. Although Hsp 31 does not appear to exhibit intrinsic ATPase activity, binding of ATP at high temperatures restricts the size or availability of the substrate binding site, thereby modulating chaperone activity. The possible role of ATP in coordinating the function of the cellular complement of molecular chaperones is discussed.

In the cellular environment, the *de novo* folding of short, single-domain proteins is thought to proceed rapidly and efficiently whereas that of large, multidomain proteins and slow folding polypeptides often requires the assistance of molecular chaperones. In *Escherichia coli*, nascent and newly synthesized chains that rely on chaperones to reach a proper conformation are engaged by either trigger factor or the DnaK-DnaJ-GrpE system (and in some cases are transferred to the GroEL-GroES team) before being released in a native form. Chaperones interact with their client proteins by binding to solvent-exposed hydrophobic stretches that would normally be buried within the substrate core. By shielding interactive surfaces that give rise to misfolded and aggregated species, chaperones promote on-pathway folding without accelerating folding rates or becoming part of the final structure (for recent reviews, see Refs. 1–4).

Another role of molecular chaperones is to repair host proteins that have misfolded as a result of temperature increase or other forms of stress. The main players in this process are the

* This work was supported by Research Project Grant MBC-99-335-01 from the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Chemical Engineering, Box 351750, University of Washington, Seattle, WA 98195-1750. Tel.: 206-685-7659; Fax: 206-685-3451; E-mail: baneyx@u.washington.edu.

‡ Present address: Immunex, 51 University St., Seattle, WA 98101.
Hsp31, an ATP-modulated Chaperone

46027

Hsp31 Is a Molecular Chaperone—Most cytoplasmic Hsps characterized to date function either as molecular chaperones or heat shock proteases. Because one of the hallmarks of molecular chaperone function is an ability to suppress the aggregation of unfolding intermediates, we tested whether Hsp31 would prevent the heat-induced misfolding of two model substances, CS (Fig. 2A) and ADH (Fig. 2B). In both cases, a significant concentration-dependent decrease in light scattering was ob-

Other Analytical Techniques—CD spectra of Hsp31 (0.3 mg/ml in 10 mM phosphate buffer, pH 7.5) were recorded on a thermostated Aviv 62A DS spectropolarimeter by using a 1-mm pathlength cuvette. For sizing experiments 1.2 μg of Hsp31 was injected on a thermostated Biosep S2000 column developed in 150 mM Tris-HCl, pH 7.4. The column was calibrated by using the low weight calibration kit from Amersham Biosciences. Dynamic light-scattering measurements were performed at 20 °C with 1 mg/ml Hsp31 in 10 mM NaPO4, pH 7.5 by using a DynaPro99 instrument (Protein Solutions, Lakewood, NJ) illu-

influenced at 832.8 nm with a 25-milliwatt solid state laser. Data analysis was performed by using the instrument software. Inverse Laplace transform analysis was used to find the mean and standard deviation (polydispersity) of the protein hydrodynamic radius distribution. For intrinsic tryptophan fluorescence experiments, 0.8 μM Hsp31 in 1 ml of 100 mM phosphate buffer, pH 7.5, and 5 mM MgCl2 was incubated at the indicated temperatures for 30 min. ATP, AMP-PNP, or ADP was added on 2 ml final concentration. Intrinsic fluorescence was measured in a thermostated Hitachi F4500 spectrophotometer 15 min after nucleotide addition by using an excitation wavelength of 295 nm and slit widths at 2.5 nm. Spectra were fitted with a fifth-degree polynomial function to obtain maximum fluorescence intensities and emission wavelengths. ATPase assays were performed essentially as described (13). Briefly, 2 μl of Hsp31 (1 mg/ml) was mixed at 25 or 45 °C with 148 μl of assay buffer (20 mM HEPES pH 7.0, 5 mM MgCl2, and 50 mM KCl) and 10 μl of ATP mixture consisting of 4.8 μl of 2.5 mM ATP, 8 μl of γ[32P]ATP (3,000 Ci mmol; Amersham Biosciences), and 67.2 μl of assay buffer. Samples (25 μl) were collected immediately after mixing and every 10 min thereafter and subjected to trichloroacetic acid precipitation and molybdate extraction (15). In some experiments, MDH was added at a 3:1 molar excess over Hsp31. Purified GroEL was used as a positive control.

RESULTS

Purification and Secondary and Quaternary Structure of Hsp31—In an effort to assign a function to the putative product of the heat-inducible (14) and H-NS-regulated (15) yedU gene, we amplified the yedU open reading frame and placed it under transcriptional control of the bacteriophage T7 promoter. Induced BL21(DE3) transformants accumulated large amounts of a soluble 51-kDa protein that matched the expected molec-

ular weight of 51,194. The protein was designated Hsp31 and purified to near homogeneity by three chromatography steps (Fig. 1A). Hsp31 was found to assemble as a homotrimer by size exclusion chromatography (Fig. 1B). Quaternary structure assignment was confirmed by dynamic light-scattering measurements conducted in triplete. At 1 mg/ml, the hydrodynamic radius of Hsp31 was 3.28 ± 0.10 nm and the polydispersity was 10%. This corresponds to a molecular mass of 62 ± 5.6 kDa that is fully consistent with a dimeric structure. No appreciable variation was found in elution position when sizing experiments were repeated between 25 and 50 °C or in the presence of 100 mM dithiothreitol (data not shown), suggesting that changes in temperature do not affect Hsp31 quaternary structure and that Cys185 and Cys207 are unlikely to form an intermo
eron disulfide bridge.

Far-UV CD analysis showed that Hsp31 contains a significant amount of α-helical structure (54%) and is about 25% β-pleated. The protein exhibited little change in secondary structure between 25 and 55 °C (Fig. 1C) and appeared remarkably thermostable because the molar ellipticity at 220 nm remained constant at temperatures as high as 90 °C (data not shown).

Hsp31 Is a Molecular Chaperone—Most cytoplasmic Hsps characterized to date function either as molecular chaperones or heat shock proteases. Because one of the hallmarks of molecular chaperone function is an ability to suppress the aggregation of unfolding intermediates, we tested whether Hsp31 would prevent the heat-induced misfolding of two model sub-

strates, CS (Fig. 2A) and ADH (Fig. 2B). In both cases, a concentration-dependent decrease in light scattering was ob-

been incubated at 23 or 45 °C for 30 min. ATP, AMP-PNP, or ADP was added on 2 ml final concentration. Intrinsic fluorescence was measured in a thermostated Hitachi F4500 spectrophotometer 15 min after nucleotide addition by using an excitation wavelength of 295 nm and slit widths at 2.5 nm. Spectra were fitted with a fifth-degree polynomial function to obtain maximum fluorescence intensities and emission wavelengths. ATPase assays were performed essentially as described (13). Briefly, 2 μl of Hsp31 (1 mg/ml) was mixed at 25 or 45 °C with 148 μl of assay buffer (20 mM HEPES pH 7.0, 5 mM MgCl2, and 50 mM KCl) and 10 μl of ATP mixture consisting of 4.8 μl of 2.5 mM ATP, 8 μl of γ[32P]ATP (3,000 Ci mmol; Amersham Biosciences), and 67.2 μl of assay buffer. Samples (25 μl) were collected immediately after mixing and every 10 min thereafter and subjected to trichloroacetic acid precipitation and molybdate extraction (15). In some experiments, MDH was added at a 3:1 molar excess over Hsp31. Purified GroEL was used as a positive control.

RESULTS

Purification and Secondary and Quaternary Structure of Hsp31—In an effort to assign a function to the putative product of the heat-inducible (14) and H-NS-regulated (15) yedU gene, we amplified the yedU open reading frame and placed it under transcriptional control of the bacteriophage T7 promoter. Induced BL21(DE3) transformants accumulated large amounts of a soluble 51-kDa protein that matched the expected molecular weight of 51,194. The protein was designated Hsp31 and purified to near homogeneity by three chromatography steps (Fig. 1A). Hsp31 was found to assemble as a homotrimer by size exclusion chromatography (Fig. 1B). Quaternary structure assignment was confirmed by dynamic light-scattering measurements conducted in triplete. At 1 mg/ml, the hydrodynamic radius of Hsp31 was 3.28 ± 0.10 nm and the polydispersity was 10%. This corresponds to a molecular mass of 62 ± 5.6 kDa that is fully consistent with a dimeric structure. No appreciable variation was found in elution position when sizing experiments were repeated between 25 and 50 °C or in the presence of 100 mM dithiothreitol (data not shown), suggesting that changes in temperature do not affect Hsp31 quaternary structure and that Cys185 and Cys207 are unlikely to form an intermonomer disulfide bridge.

Far-UV CD analysis showed that Hsp31 contains a significant amount of α-helical structure (54%) and is about 25% β-pleated. The protein exhibited little change in secondary structure between 25 and 55 °C (Fig. 1C) and appeared remarkably thermostable because the molar ellipticity at 220 nm remained constant at temperatures as high as 90 °C (data not shown).

Hsp31 Is a Molecular Chaperone—Most cytoplasmic Hsps characterized to date function either as molecular chaperones or heat shock proteases. Because one of the hallmarks of molecular chaperone function is an ability to suppress the aggregation of unfolding intermediates, we tested whether Hsp31 would prevent the heat-induced misfolding of two model substrates, CS (Fig. 2A) and ADH (Fig. 2B). In both cases, a concentration-dependent decrease in light scattering was ob-

Hsp31 Is a Molecular Chaperone—Most cytoplasmic Hsps characterized to date function either as molecular chaperones or heat shock proteases. Because one of the hallmarks of molecular chaperone function is an ability to suppress the aggregation of unfolding intermediates, we tested whether Hsp31 would prevent the heat-induced misfolding of two model substrates, CS (Fig. 2A) and ADH (Fig. 2B). In both cases, a concentration-dependent decrease in light scattering was ob-
served, and Hsp31 was quite efficient at suppressing CS and ADH aggregation when provided at a 6-fold molar excess (based on monomers) over substrate proteins.

We next investigated whether Hsp31 would promote the reactivation (or prevent the inactivation) of thermally unfolded proteins following stress abatement. For these experiments, ADH, CS, and MDH were incubated at 45 °C for 30 min in the presence or absence of Hsp31, and enzymatic activities were assayed after 30 min at 23 °C. Controls were held for 60 min at 23 °C without additive. A.U., arbitrary units.

High Temperatures Promote the Exposure of Structured Hydrophobic Domains in Hsp31 That Are Likely Substrate Binding Sites—Because molecular chaperones typically associate with partially folded proteins through hydrophobic-hydrophobic interactions, we investigated the possibility that temperature modulates the degree of Hsp31 surface hydrophobicity. For these experiments, Hsp31 was incubated at four different

---

**Fig. 1.** Purification and structure of Hsp31. A, silver-stained SDS-polyacrylamide gel of Hsp31 fractions after cell lysis (lane 1) and following ion exchange (lane 2) hydroxyapatite (lane 3) and size exclusion chromatography (lane 4). Lane M contains molecular mass markers. B, Hsp31 elutes as a dimer on a BioSep S2000 column. The elution position of protein standards, albumin (67 kDa), chymotrypsinogen A (25 kDa), and ribonuclease A (13.7 kDa), is shown by arrows. A.U., arbitrary units. C, far-UV CD spectra of Hsp31 were recorded at 25 °C (trace 1), 35 °C (trace 2), 45 °C (trace 3) and 55 °C (trace 4).

**Fig. 2.** Hsp31 exhibits molecular chaperone activity. The aggregation of CS at 45 °C (A) or ADH at 41.5 °C (B) was monitored without additive (traces 1) or in the presence of BSA at a 6:1 molar excess (based on protomers; traces 2), or Hsp31 at a 1:1 (traces 3), 3:1 (traces 4), or 6:1 molar excess over CS/MDH (traces 5). C, ADH, CS, and MDH were incubated for 30 min at 45 °C in the presence or absence of the indicated molar excess of Hsp31, and enzymatic activities were assayed after 30 min at 23 °C. Controls were held for 60 min at 23 °C without additive. A.U., arbitrary units.
temperatures and photolabeled with bis-ANS, a molecule that exhibits little intrinsic fluorescence in its free form but becomes highly fluorescent when bound to solvent-exposed structured hydrophobic patches. Although high temperatures do not influence the efficiency of bis-ANS photoincorporation (17), the quantum yield of the dye exhibits significant temperature dependence. The change in Hsp31 hydrophobicity was therefore quantified by injecting the labeled products on a gel filtration column developed at 23 °C and by simultaneously monitoring the fluorescence emission at 477 nm and its absorbance at 280 nm. A.U., arbitrary units.

Fig. 3. High temperatures lead to the progressive exposure of structured hydrophobic domains in Hsp31. Bis-ANS was photoincorporated into Hsp31 at 23 °C (trace 3), 30 °C (trace 4), 37 °C (trace 5), or 45 °C (trace 6). Trace 1 corresponds to Hsp31 incubated with bis-ANS at 45 °C without irradiation, and trace 2 corresponds to Hsp31 alone irradiated at 45 °C. Samples were fractionated at 23 °C on a BioSep S2000 gel filtration column, and fluorescence emission at 477 nm was monitored online. Inset, sample absorbance at 280 nm. A.U., arbitrary units.

Fig. 4. Bis-ANS photoincorporation inhibits Hsp31 chaperone function. Hsp31 was pretreated (+) or not (−) at 45 °C for 15 min in the presence (+) or absence (−) of bis-ANS and subjected (+) or not (−) to UV irradiation for 30 min at the same temperature. All samples were subjected to dialysis and transferred to ice. MDH was incubated for 30 min at 45 °C in the presence or absence of the various Hsp31 preparations, and enzymatic activities were assayed after 30 min at 23 °C. The control was held for 60 min at 23 °C without additive.
was photoincorporated into Hsp31 by UV irradiation at 45 °C for 30 min, and the free probe was removed by dialysis. The MDH activity experiment of Fig. 2 was then repeated with the chemically modified chaperone. Fig. 4 shows that pretreatment of Hsp31 at 45 °C for 30 min in the presence or absence of UV irradiation had no effect on chaperone function because the recovery levels of active MDH were comparable to the control. However, activity yields were reduced by \( \frac{1}{10} \) fold when the assay was conducted with Hsp31 covalently coupled to bis-ANS. When UV irradiation was omitted, the yields of MDH activity were slightly reduced, presumably because a small amount of bis-ANS is not removed on dialysis and remains noncovalently associated with Hsp31. We conclude that the structured hydrophobic domains exposed by Hsp31 at high temperatures and bound by bis-ANS are in close proximity and most likely correspond to the substrate binding site(s) of the chaperone. Taken together, the above results suggest that Hsp31 makes use of temperature-driven exposure of structured hydrophobic domains to capture, and possibly release, non-native protein substrates.

ATP Inhibits Hsp31 Activity at High Temperatures—Molecular chaperones that actively refold or remodel proteins (e.g. GroEL, DnaK, and the Clp ATPases) use conformational changes driven by ATP hydrolysis to perform their function (1, 2, 4, 5). In the case of Hsp31, we did not detect ATPase activity at 23 or 45 °C using \( [\gamma^{32P}]ATP \) with or without MDH (at a 1:3 ratio to Hsp31) in the assay mix (data not shown). Thus, Hsp31 has very weak or no ATPase activity. However, when the experiments of Fig. 2 were repeated in the presence of 2 mM ATP, the recovery of active CS or MDH was reduced by approximately half (data not shown).

To gain further information on the role of adenosine nucleotides and the nature of the (un)folding intermediates bound by Hsp31, we monitored the time course of MDH activity recovery after chemical or thermal denaturation. When the refolding of GdnHCl-unfolded MDH was initiated by rapid dilution at 23 °C, approximately 10% of the original activity was spontaneously recovered after 180 min of incubation (Fig. 5A, G). Addition of Hsp31 to the refolding buffer increased reactivation yields to \( \frac{1}{2} \) 20% without significantly influencing refolding rates (○) but ATP had no impact on either process (Fig. 5A, ●). The yield improvement in the presence of Hsp31 likely reflects the transient stabilization of MDH folding intermediates via interactions with the substrate binding site of Hsp31 (the so-called buffering effect).

When MDH was incubated for 30 min at 45 °C, only traces of enzymatic activity were detected on transfer to 23 °C (Fig. 5B, ●). However, if thermal inactivation was carried out in the
presence of Hsp31, 20% of the original MDH activity was recovered immediately after temperature downshift and only a small activity gain was observed thereafter (Fig. 5B, E). Identical results were obtained with CS (data not shown). Addition of ATP to the MDH inactivation mixture reduced the beneficial effect of Hsp31 by 50% (Fig. 5B, H18554), whereas ADP had essentially no influence on recovery yields ('). High temperature conditioning of Hsp31 by ATP was essential to achieve inhibition of chaperone activity because addition of either ATP or ADP 90 min after transfer to 23°C did not affect the yields of enzymatic activity (Fig. 5B, f and Œ).

To confirm the above results, aggregation suppression experiments were repeated in the presence of 2 mM ATP or ADP. In agreement with the activity data, addition of ATP to the reaction buffer inhibited the ability of Hsp31 to suppress the heat-induced aggregation of CS by about 50% (Fig. 6A, trace 3) and that of ADH by ~35% (Fig. 6B, trace 3). ADP had a smaller but discernible effect (Fig. 6, trace 4). Overall, these results suggest 1) that Hsp31 binds to and stabilizes early unfolding intermediates of MDH and CS at high temperatures and rapidly releases them in an active form upon stress abatement, and 2) that ATP inhibits the chaperone activity of Hsp31 under heat shock conditions by interfering with its ability to interact with partially folded substrate proteins.

ATP Binding Induces Conformational Changes in Hsp31

Fig. 6. Influence of adenine nucleotides on the ability of Hsp31 to suppress the heat-induced aggregation of CS and ADH. The aggregation of CS at 45°C (A) or ADH at 41.5°C (B) was monitored with no additive (traces 1), a 3:1 molar excess of Hsp31 (traces 2), a 3:1 molar excess of Hsp31 and 2 mM ATP (traces 3), or a 3:1 molar excess of Hsp31 and 2 mM ADP (traces 4). A.U., arbitrary units.

The simplest explanation for the results of Figs. 5B and 6 is that the binding of ATP to Hsp31 at high temperatures reduces the availability of binding sites for non-native protein substrates. To test this hypothesis, Hsp31 was incubated with bis-ANS at 45°C in the absence or presence of various nucleotides, and fluorescence emission spectra were recorded at
Fig. 7. Binding of ATP or AMP-PNP to Hsp31 restricts the exposure of structured hydrophobic domains at high temperature. A, Hsp31 was diluted in bis-ANS-containing buffer held at 45 °C and supplemented with no additive (trace 1), 2 mM ADP (trace 2), 2 mM AMP-PNP (trace 3), or 2 mM ATP (trace 4). Bis-ANS fluorescence emission spectra were recorded immediately. B, fluorescence spectra of samples containing no additive (○), 2 mM ADP (△), 2 mM AMP-PNP (■), or 2 mM ATP (□) were recorded after 15 and 30 min incubation at 45 °C. Maximum fluorescence intensities were normalized to those of the Hsp31-only samples at the same time point. C, BSA was diluted in bis-ANS-containing buffer, held at 45 °C, and supplemented with no additive (trace 1), 2 mM ADP (trace 2), or 2 mM ATP (trace 3). Bis-ANS fluorescence emission spectra were recorded immediately. Similar results were obtained after 15 and 30 min of incubation at 45 °C (data not shown). All spectra are corrected for background fluorescence of buffer, bis-ANS, and nucleotides. A.U., arbitrary units.

Various time points after excitation at 340 nm. Fig. 7A shows that whereas little difference existed between the bis-ANS emission spectra recorded in the absence or presence of ADP (Fig. 7, traces 1 and 2), ATP reduced the maximum bis-ANS fluorescence intensity by about 30% (Fig. 7, trace 4) with no appreciable time dependence (Fig. 7B). This effect was only apparent at high temperatures, and no statistically significant effect of ATP addition on bis-ANS fluorescence between 23 and 37 °C was seen (data not shown). The nonhydrolyzable ATP analog AMP-PNP had an intermediate effect with an about 20% reduction in maximum fluorescence intensity (Fig. 7, trace 3). In contrast, ATP and ADP had no effect on the bis-ANS emission spectrum of the control protein BSA (Fig. 7C).

To determine whether the ATP-mediated quenching of bis-ANS fluorescence at 45 °C was the result of a structural rearrangement in Hsp31, we took advantage of the presence of three tryptophan residues at positions 107, 173, and 229 to monitor Hsp31 conformation at various temperatures in the presence or absence of adenosine nucleotides. Fig. 8 shows that the λmax of Hsp31 tryptophans was about 9 nm lower than that of free tryptophan (reflective of the fact that they experience a hydrophobic environment) and remained essentially unchanged at up to 37 °C under all experimental conditions. A small blue shift (~4 nm) that was unaffected by the presence of nucleotides was observed at 45 °C and most likely corresponds to a temperature-induced, fine conformational rearrangement in Hsp31. On the other hand, both nucleotides reduced tryptophan maximum fluorescence intensity, with ATP being approximately three times more effective than ADP at all temperatures (Fig. 8, □ and △). Although these results should be considered with care because Hsp31 dimers contain six tryptophans, they are consistent with the idea that, at high temperatures, the binding of adenosine nucleotides (and particularly that of ATP) induces a conformational change in Hsp31. Because the relative magnitude of intrinsic fluorescence intensity quenching by ATP is comparable at 23 and 45 °C but Hsp31 activity is not affected by ATP at the former temperature (Fig. 5A), ATP is likely to function by interfering with the temperature-driven opening of the substrate binding site. In summary, the binding of ATP nucleotides to Hsp31 appears to exert an antagonistic effect on the heat-induced exposure of structured hydrophobic domains by the chaperone and thereby modulates Hsp31 activity.

Hsp31 Is a Representative Member of a New Family of Molecular Chaperones—Similarity searches with the basic local alignment sequence tool (18) were conducted to determine whether Hsp31 orthologs were present in the data bases of nonredundant and unfinished microbial genomes. Fig. 9 shows that Hsp31 is highly conserved (greater than 55% identity at the amino acid levels) in several human pathogens including Vibrio cholerae, the enterohemorrhagic E. coli O157:H7, and the opportunistic bacteria Staphylococcus aureus and Pseudomonas aeruginosa. More divergent orthologs (40–45% homology) were identified in a number of other eubacteria and fungi including Sinorhizobium meliloti, Agrobacterium tumefaciens, Enterococcus faecalis, Xylella fastidiosa, Coccidiodes immitis, Schizosaccharomyces pombe, and Brucella melitensis (data not shown). Of interest was the fact that two of these weak Hsp31 homologs, E. faecalis CAC41347 and B. melitensis NP_541994, have been annotated as proteases on the basis of their homol-
ogy to *Pyrococcus furiosis* protease I (PfpI), a member of the disparate ThiJ (DJ-1)/PfpI family. Although we cannot rule out the possibility that Hsp31 is a protease (particularly if additional cofactors are required for its function), several lines of evidence argue against it. First, the homology between Hsp31 and PfpI is quite low (15% identity) and could not be found by using direct algorithms (analysis with basic local alignment sequence tool 2 failed to produce an alignment). Second, PfpI-type proteases are dimers of trimers and only form a catalytic triad in their hexameric state (19, 20). In contrast, Hsp31 is a dimer. Third, we were unable to detect any substrate degradation when Hsp31 was incubated with MDH at either high or low temperatures (data not shown). On the other hand, the body of our data is consistent with a molecular chaperone function for Hsp31.

**DISCUSSION**

It is now well established that molecular chaperones play an important role in cellular protein folding by promoting the proper isomerization of newly synthesized polypeptides and the remodeling and refolding of misfolded proteins (1–5, 7). Because these tasks become more critical when cells are exposed to heat or other forms of stress, most molecular chaperones residing in the *E. coli* cytoplasm are Hsps whose genes are transiently transcribed at an elevated level by the *E. coli* H9268 holoenzyme after temperature upshift (21). Genome-wide expression profiling has revealed that 77 genes are induced when *E. coli* is heat shocked at 50 °C (14). Although most of these genes correspond to known members of the cytoplasmic and extracytoplasmic heat shock stimulons, 23 open reading frames of unknown function were also identified, raising the possibility that some of these may encode molecular chaperones of novel function. Bardwell and co-workers (16) capitalized on these findings by demonstrating that Hsp33, the *yrfI* (*hslO*) gene product, is a redox-activated chaperone that plays an important role in oxidative stress and by assigning a function to Hsp15 (the *yrfH* gene product) in the recycling of 50 S ribosomal subunits that still carry a nascent chain (22).

Here we have shown that the *yedU* open reading frame, now
Hsp31, an ATP-modulated Chaperone

referred to as hchA for heat-inducible chaperone, encodes an α-helix-rich homodimeric protein that exhibits chaperone activity in vitro. Homolog searches suggest that Hsp31 belongs to a new family of cytoplasmic molecular chaperones that are conserved in eubacteria and fungi. Although the significance of the observation remains unclear, it is interesting to note that many Hsp31-containing organisms are human and plant pathogens.

Like E. coli HspB (23) and other small Hsps (7), Hsp31 appears to rely on the temperature-driven exposure of structured hydrophobic domains to capture non-native protein substrates (Figs. 3 and 4). However, although small Hsps have been proposed to maintain partially folded proteins on their surface to await active refolding by the Hsp70 system (17, 24, 25), Hsp31 seems to preferentially bind and stabilize early unfolding intermediates and to rapidly release them in an active form once stress has abated (Fig. 5B). This activity would complement the function of folding chaperones (e.g. DnaK-DnaJ-GrpE and GroEL-GroES) that refold relatively unstructured intermediates, remodeling chaperones (e.g. ClpB) that disentangle early (6) and late (26) protein aggregates before transferring them to the DnaK-DnaJ-GrpE team (27, 28), and holding chaperones (e.g. IbpB) that may serve as a reservoir of partially folded polypeptides awaiting refolding.

One of the most intriguing aspects of Hsp31 function is that it does not appear to exhibit intrinsic ATPase activity. Binding of ATP at 45 °C induces a conformational change that reduces Hsp31 surface hydrophobicity (Fig. 7) and interferes with its ability to capture substrate proteins (Figs. 5B and 6), possibly by precluding opening of the binding site. The fact that ATP does not affect Hsp31 hydrophobicity at temperatures ≥37 °C or the ability of the chaperone to promote the reactivation of chemically denatured MDH at 23 °C (Fig. 5A) suggests that this regulatory mechanism preferentially operates under heat shock conditions. Some precedent exists for ATP tuning of molecular chaperone activity in non-ATPase Hsps. For instance, ATP binding induces conformational changes in human αB-crystallin that enhances its chaperone function (29, 30).

Paradoxically, another small Hsp, tobacco Hsp18, experiences a decrease in chaperone activity in the presence of ATP (31). Hsp31 appears to behave like the latter protein. Why would such negative modulation of activity be necessary and only involve ATP? A possible explanation is that the cell cytoplasm exhibits heterogeneous unfolding/folding microenvironments after heat shock. In locations where host protein misfolding is acute, ClpB together with the DnaK-DnaJ-GrpE and GroEL-GroES systems may be recruited at high concentrations to repair stress-damaged polypeptides. Because heat shock leads to a rapid decrease in intracellular ATP concentration in some organisms (32), and because all of these Hsps are ATPases, a local depletion of the ATP pool may follow that reduces the ability of folding and remodeling chaperones to turn over their substrates but concomitantly maximizes the capture of early unfolding intermediates by Hsp31. This activity would prevent additional loading of the refolding systems because Hsp31 substrates would have to be handled by DnaK-DnaJ-GrpE or GroEL-GroES if allowed to undergo significant unfolding. On the other hand, in a microenvironment where protein misfolding is less severe, or if heat shock is mild, ATP may not become limiting. Under these conditions, the rapid repair of early unfolding intermediates by Hsp70/Hsp60, rather than their transient stabilization in an inactive form by Hsp31, would be physiologically more advantageous to the cell.

REFERENCES
1. Bukau, B., and Horwich, A. L. (1998) Cell 92, 351–366
2. Hartl, F. U., and Hayer-Hartl, M. (2002) Science 295, 1852–1858
3. Lund, P. A. (2001) Adv. Microb. Physiol. 44, 93–140
4. Therumulai, D., and Lorimer, G. H. (2001) Annu. Rev. Biophys. Biomol. Struct. 30, 245–269
5. Ben-Zvi, A. P., and Goloubinoff, P. (2001) J. Struct. Biol. 135, 84–93
6. Thomas, J. G., and Baneyx, F. (2000) Mol. Microbiol. 36, 1360–1370
7. Naberhaus, F. (2002) Microbiol. Mol. Biol. Rev. 66, 64–93
8. Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Ciesielski, S., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Ma, Y., and Shao, Y. (1997) Science 277, 1453–1474
9. Cannon, D. J., and Mikey, R. H. (1968) Biochem. Biophys. Res. Commun. 35, 463–469
10. Singh, M., Brooks, G. C., and Srere, P. A. (1970) J. Biol. Chem. 245, 4636–4640
11. Srere, P. A. (1966) J. Biol. Chem. 241, 2157–2165
12. Valle, B., and Hoch, F. (1955) Proc. Natl. Acad. Sci. U. S. A. 41, 327–330
13. Chevalier, M., King, L., and Blond, S. (1998) Methods Enzymol. 290, 384–409
14. Richmond, C. S., Glasser, J. D., Bau, R., Jia, H., and Blattner, F. R. (1999) Nucleic Acids Res. 27, 3821–3835
15. Yoshida, T., Ueguchi, C., Yamada, H., and Mizuno, T. (1993) Mol. Gen. Genet. 237, 113–122
16. Jakob, U., Muse, W., Eser, M., and Bardwell, J. C. A. (1999) Cell 96, 341–352
17. Lee, G. J., Rosenman, A. M., Sabil, H. R., and Vierling, E. (1997) EMBO J. 16, 653–671
18. Alschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
19. Du, X., Chiu, T.-C., Kim, R., Wang, W., Jancarik, J., Yokota, H., and Kim, S.-H. (2001) Proc. Natl. Acad. Sci. U. S. A. 97, 14079–14084
20. Halio, S. B., Blumentals, I. I., Short, S. A., Merrill, B. M., and Kelly, R. M. (1996) J. Bacteriol. 178, 2605–2612
21. Grese, C. A. (1996) in Escherichia coli and Salmonella Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) pp. 1382–1399, ASM Press, Washington, D. C.
22. Karcher, P., Stahl, J. M., Nierhaus, K. H., and Bardwell, J. C. (2000) EMBO J. 19, 741–748
23. Shearstone, J. R., and Baneyx, F. (1999) J. Biol. Chem. 274, 9937–9945
24. Ehrenberger, M., Graber, S., Gaestel, M., and Buchner, J. (1997) EMBO J. 16, 221–229
25. Veinger, L., Diamant, S., Buchner, J., and Goloubinoff, P. (1998) J. Biol. Chem. 273, 11032–11037
26. Migaki, A., Tomoyasu, T., Goloubinoff, P., Rudiger, S., Roder, D., Langen, A., and Bukau, B. (1999) EMBO J. 18, 6934–6949
27. Goloubinoff, P., Migaki, A., Ben-Zvi, A. P., Tomoyasu, T., and Bukau, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13732–13737
28. Zolkiewski, M. (1999) J. Biol. Chem. 274, 28083–28086
29. Muchowski, P. J., and Clark, J. I. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1094–1099
30. Muchowski, P. J., Hays, L. G., Yates, J. R., III, and Clark, J. I. (1999) J. Biol. Chem. 274, 30190–30195
31. Symchuk, P., Masin, J., Konopasek, L., and Zársky, V. (2000) Plant J. 23, 703–713
32. Findlay, R. C., Gillies, R. J., and Shulman, R. G. (1983) Science 219, 1222–1225
33. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876–4882
Hsp31, the *Escherichia coli* yedU Gene Product, Is a Molecular Chaperone Whose Activity Is Inhibited by ATP at High Temperatures
M. S. R. Sastry, Konstantin Korotkov, Yan Brodsky and François Baneyx

*J. Biol. Chem.* 2002, 277:46026-46034.
doi: 10.1074/jbc.M205800200 originally published online September 15, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205800200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 32 references, 17 of which can be accessed free at [http://www.jbc.org/content/277/48/46026.full.html#ref-list-1](http://www.jbc.org/content/277/48/46026.full.html#ref-list-1)