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Biochemistry

β-Lactamase binds to GroEL in a conformation highly protected against hydrogen/deuterium exchange

(molecular chaperones/protein folding/mass spectrometry)

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ABSTRACT ***Escherichia coli* RTEM β-lactamase reversibly forms a stable complex with GroEL, devoid of any enzymatic activity, at 48°C. When β-lactamase is diluted from this complex into denaturant solution, its unfolding rate is identical to that from the native state, while the unfolding rate from the molten globule state is too fast to be measured. Electrospray mass spectrometry shows that the rate of proton exchange in β-lactamase in the complex at 48°C is slower than in the absence of GroEL at the same temperature, and resembles the exchange of the native state at 25°C. Similarly, the final number of protected deuterons is higher in the presence of GroEL than in its absence. We conclude that, for β-lactamase, a state with significant native structure is bound to GroEL. Thus, different proteins are recognized by GroEL in very different states, ranging from totally unfolded to native-like, and this recognition may depend on which state can provide sufficient accessible hydrophobic amino acids in a suitably clustered arrangement. Reversible binding of native-like states with hydrophobic patches may be an important property of GroEL to protect the cell from aggregating protein after heat-shock.**

GroEL is probably the best characterized molecular chaperone and it interacts with many natural and recombinant proteins in *Escherichia coli* both during folding and under heat-shock conditions (1, 2). The crystal structure of GroEL (3) shows it to exist in a 14-mer double-donut form, with hydrophobic residues lining the inside of the toroid. Mutations of these residues abolish polypeptide binding (4). It is now generally accepted (5) that GroEL binds to non-native states of its substrate proteins, and this is plausibly explained by the fact that the hydrophobic amino acids of soluble, globular proteins become accessible for binding to the chaperone only in non-native states.

Experiments with different substrate proteins have led to very different proposals about the nature of the bound state, however. Barnase (6), lactate dehydrogenase (7), and α-lactalbumin (8) have been proposed to be bound in denaturated states, with the latter protein also having been suggested to be bound exclusively in a molten globule state (9, 10). In the cases of other proteins, various folding intermediates have been proposed to lead to binding (11, 12), such as a very late folding intermediate of an antibody Fab fragment, with native-like domains, which was shown to bind to GroEL (13).

Amide proton exchange (for a review, see ref. 14 and references therein) has been a particularly useful technique in determining the folding state of the substrate in the GroEL complex. Such H/D exchange experiments have been carried out using NMR and the stable cyclophilin-GroEL complex and have shown that all substrate protons quantitatively exchange in the complex under conditions where there is no exchange in the absence of the chaperone (15). This necessitates that a globally destabilized state is bound and is thus inconsistent with the occurrence of a native-like structure in the complex. In the case of α-lactalbumin, in which one of its disulfide bonds was reduced and the other three were reshuffled, an exchange rate slower than that of a random coil was observed (9), leading to the proposal that this protein is bound in a molten globule state. For barnase, in contrast, it was shown (6) that transient binding to GroEL accelerates the exchange of those protons that do not exchange by local breathing but only by global unfolding (16, 17), leading to the conclusion that barnase is transiently bound in a fully unfolded state. Thus, the proton exchange experiments reported to date all demand bound states with no protection against exchange, or at most, protection as high as in the molten globule state.

We have now investigated this problem, using H/D exchange detected by electrospray ionization mass spectrometry (ESI MS) (for review, see ref. 18 and references therein) and by monitoring the kinetics of unfolding of the *E. coli* RTEM β-lactamase. This 263-amino acid protein has been shown to exist in three stable states in urea or guanidinium chloride (Gdn·Cl) equilibrium folding experiments: at 3.5 M urea or at 1.5 M Gdn·Cl, an enzymatically inactive β-lactamase with a native-like fluorescence maximum was identified (19–21), similar to the enzyme from *Staphylococcus aureus* (22–24). A related, but probably somewhat different compact state, the A state, is observed at low pH and high salt concentration (25–27). This latter state shows the characteristics that are commonly used to define the molten globule state.

While the precursor of β-lactamase, which contains the hydrophobic signal sequence, forms stable and reversible complexes with GroEL starting from the native or denatured state under most conditions, reversible binding of mature β-lactamase to GroEL has only been detected at 48°C. At this temperature, the enzyme is still fully active in the absence of GroEL (β-lactamase unfolds with a transition midpoint of 51°C; ref. 20), but loses its activity upon binding to GroEL and regains it upon lowering the temperature or the addition of ATP. At room temperature or low temperature, no evidence for binding to GroEL, either transient (by retardation of its folding) or stable (by direct measurements), could be detected for mature β-lactamase (20, 28).

In this study, we examined the unfolding rates and the H/D exchange characteristics of the disulfide-containing mature β-lactamase in the presence and absence of GroEL and conclude that a native-like conformation of β-lactamase binds

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Abbreviations: Gdn·Cl, guanidinium hydrochloride; H/D, hydrogen/deuterium; ESI, electrospray ionization; ANS, 8-anilino-1-naphthalene-sulfonate.

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to the chaperonin at 48°C. Thus, GroEL can recognize native-like states even of relatively small proteins.

MATERIALS AND METHODS

Proteins. β-Lactamase was purified (29) from *E. coli* strain BL21(DE3) harboring the plasmid pET-E11. The main steps in this procedure were Q-Sepharose FF chromatography (Pharmacia) followed by affinity chromatography on phenylboronic acid agarose (MoBiTec). The chaperonin GroEL was purified from the *E. coli* strain W3110 (pOF39) as described previously (20). The concentrations of β-lactamase and GroEL were measured using the bicinchoninic acid assay (Pierce). β-Lactamase was deuterated at all exchangeable sites by exposure to 4 M Gdn·Cl in ^2H_2O for 2 hr at pH 7.0 (values given in the text for pH and pD were taken directly from the pH-meter). The protein was then freeze-dried, redissolved in ^2H_2O (pH 7.2), and desalted on a PD-10 column (Pharmacia) equilibrated with 100 mM potassium phosphate (pH 7.2) in ^2H_2O. This procedure was repeated three times to ensure complete deuteration af all labile protons. β-Lactamase was then refolded in 100 mM potassium phosphate (pH 7.2) in ^2H_2O. The yield of correctly refolded β-lactamase was checked by enzymatic activity using the chromogenic substrate nitrocefin as described previously (28).

Fluorescence Kinetics. Denaturation kinetics of GroEL-bound β-lactamase were determined by protein fluorescence, and measured with a Shimadzu model RF-5000 fluorescence spectrometer. The excitation wavelength was 290 nm and the emission was monitored at 340 nm, using a spectral bandwidth of 5 nm for both excitation and emission. All solutions were stirred during measurement and the temperature was kept constant with a thermostatically controlled water bath. To form the complex, β-lactamase was incubated for 30 min at 48°C in 100 mM potassium phosphate (pH 7.2) in the presence of a 3-fold molar excess of GroEL, and complex formation was tested by the disappearance of β-lactamase activity (28). The reference samples were treated identically in the absence of GroEL. To populate the molten globule form of β-lactamase, the protein was incubated in 10 mM HCl and 50 mM KCl (pH 2.0) for 30 min at 20°C. Formation of the β-lactamase molten globule was followed by 8-anilino-1-naphthalene-sulfonate fluorescence as previously described (26). Denaturation was initiated by 10-fold dilution of the protein solutions into 1.5 M Gdn·Cl (pH 7.2) at 48°C. The final concentration of β-lactamase in the denaturing buffer was 0.4 μM for all experiments.

H/D Exchange. To initiate the H/D exchange reaction, 20 μM β-lactamase, deuterated at all exchangeable sites, was diluted 20-fold into H_2O (pH 6.0) in the presence or absence of 1 molar equivalent of GroEL at 48°C and at 25°C. Aliquots were taken at different time points within a 2-hr period and were rapidly mixed with an equal volume of 50% methanol containing 1% acetic acid precooled to 4°C. The quenched samples were injected into the mass spectrometer within 15 sec, and the degree of deuterium incorporation into β-lactamase was deduced from the protein's mass shift during the course of an experiment. The conditions of the ESI mass spectrometer were carefully controlled to study hydrogen exchange in labile states. This includes recording the mass spectra without source heating, continually flushing the enclosed ionization chamber with dry nitrogen and precooling the delivery solvent to 4°C. In all experiments, Milli-Q purified water was used and the pH was adjusted to 6.0 using ammonium acetate (J.T. Baker, HPLC grade) and acetic acid (HPLC grade).

ESI mass spectra were collected using a TSQ 700 mass spectrometer (Finnigan-MAT, San Jose, CA). The spectra represent the average of 3-sec scans, from 800 to 1800 m/z, acquired during 1 min. The protein solutions were infused by a syringe-pump (Harvard Apparatus) at a flow rate of 5 μl/min and the instrument was calibrated using myoglobin (10 μM; molecular weight 16,951.5 Da).

For measuring H/D exchange at equilibrium, 20 μM protonated β-lactamase in the presence of 1 molar equivalent of GroEL in 100 mM potassium phosphate buffer, H_2O (pH 7.2) was diluted 20-fold into 100 mM potassium phosphate, ^2H_2O (pH 7.2) at 4°C. Controls from which GroEL was omitted were measured under identical conditions. The samples were subjected to three cycles of heating to 48°C for 1 hr and cooling to 4°C for 1 hr. After standing at 4°C for 1 hr following the last temperature shift, the protein solutions were diluted 20-fold into H_2O (pH 5.0), and washed five times at 4°C on a Centricon 10 concentrator (Amicon) with H_2O (pH 5.0) to quantitatively exchange all the fast-exchanging protons. The protein solutions were then concentrated to 10 μM, diluted 1:1 with precooled 50% acetonitrile containing 1% acetic acid, and immediately measured for deuteron incorporation. The number of deuterons incorporated into β-lactamase was identified using a Sciex (Thornhill, ON, Canada) API III$^+$ mass spectrometer. The ion spray voltage was 5000 V, the dwell time was 0.7 msec, and the scan step size was 0.15 atomic mass unit. The samples were injected into the ion source at a flow rate of 7 μl/min. Spectra represent averages of 5-sec scans averaged over 1 min.

RESULTS

Kinetics of β-Lactamase Unfolding. To establish whether β-lactamase is bound to GroEL in a form that still contains a significant degree of structure, we determined its unfolding kinetics starting from the GroEL-bound state and compared these with the unfolding kinetics of the native protein under the same conditions. At 48°C, β-lactamase is enzymatically fully active in the absence of a denaturant, but forms a complex with GroEL, devoid of any activity, which can be dissociated with ATP or by lowering the temperature (20). Thus, β-lactamase can be bound reversibly by GroEL. The activity of β-lactamase was measured before dilution of the GroEL:β-lactamase complex into denaturing buffer; absence of its activity confirmed complete complex formation (data not shown) as expected from the excess of GroEL over β-lactamase. A comparison of the denaturation kinetics of native β-lactamase in 1.5 M Gdn·Cl at 48°C with the denaturation kinetics of the GroEL:β-lactamase complex under the same conditions, followed by Trp-fluorescence, showed very similar curves (Fig. 1). GroEL, which lacks Trp, makes no contribution (data not shown). The unfolding kinetics of β-lactamase could be fitted to a single exponential (21). The denaturation of β-lactamase bound to GroEL gives an unfolding rate constant of $3.32 \times 10^{-2}\ s^{-1}$ with an amplitude of 52 (Fig. 1*A*). This rate constant is comparable to that measured for the denaturation of β-lactamase at 48°C under the same conditions but in the absence of GroEL, giving rate constant of $3.58 \times 10^{-2}\ s^{-1}$ with an amplitude of 75 (Fig. 1*B*). The similar rate constants suggest that the same transition state has to be overcome when starting from the GroEL-bound or the native state and clearly show that β-lactamase must have significant residual structure when bound to GroEL. The smaller amplitude for the unfolding of the GroEL:β-lactamase complex (69% of the amplitude of the free β-lactamase) can be explained as a quenching effect by GroEL on the bound β-lactamase as reported (20). In contrast, when the reaction was started from HCl/KCl, pH 2.0, where β-lactamase is largely in a molten globule state (27), and diluted into the 1.5 M Gdn·Cl solution at pH 7.2, 48°C, the kinetics were not resolvable, indicating that the process is too fast to be detectable by manual mixing. The quantitative formation of the molten globule state before denaturation was followed and confirmed by 8-anilino-1-naphthalene-sulfonate fluorescence. As expected, a 20-fold increase in the fluorescence at 470 nm at pH 2.0 compared with pH 7.0 was observed

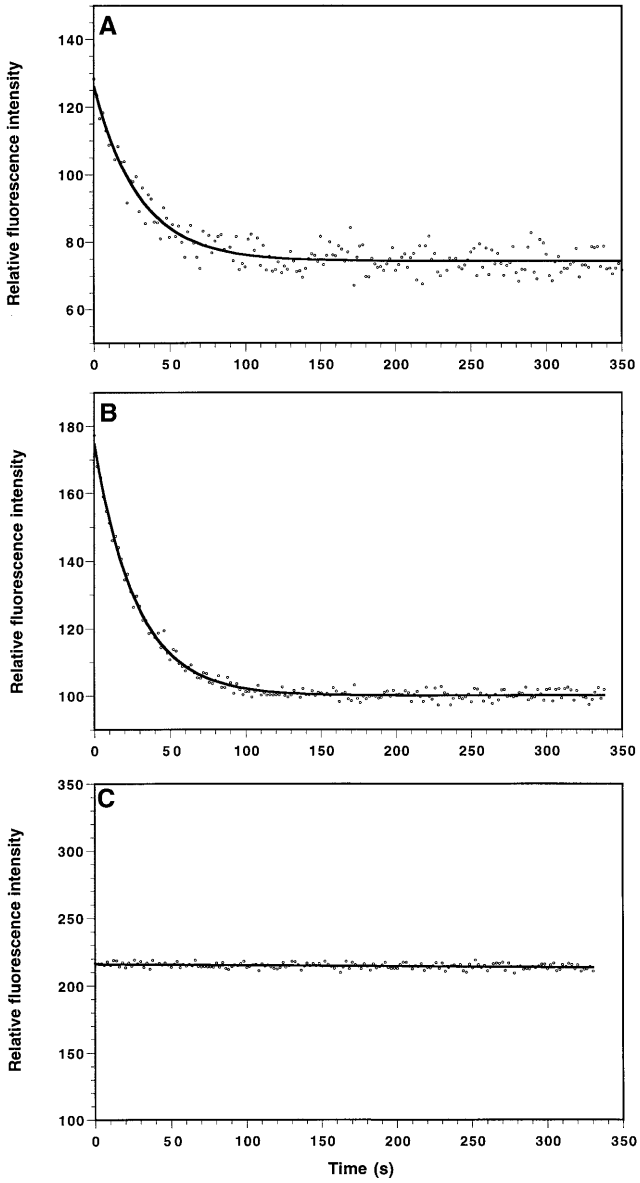

FIG. 1. Denaturation kinetics of 0.4 μM β-lactamase in 1.5 M Gdn·Cl at 48°C in the presence (*A*) and absence (*B*) of 1.2 μM GroEL (14-mer), as described under *Materials and Methods.* The denaturation kinetics were followed by Trp-fluorescence. For comparison, the denaturation curve of β-lactamase starting from the molten globule state is shown in *C*.

(data not shown). Because of the slower denaturation rate both native and GroEL-bound β-lactamase are therefore more structured than the molten globule form.

H/D Exchange Kinetics. Before H/D exchange, the stoichiometry of the complex GroEL:β-lactamase was determined by isothermal titration calorimetry. With this GroEL preparation (20), the stoichiometry of 1.1 ± 0.02 β-lactamase molecules bound to 1 GroEL 14-mer at 48°C was confirmed by three independent measurements (P.G., I. Jelezarov, and A.P., unpublished data). Hydrogen exchange was measured for the complex GroEL:β-lactamase at 48°C and for β-lactamase in the absence of the chaperonin at 25°C and 48°C as described in *Materials and Methods*. β-Lactamase, deuterated at all exchangeable sites, was diluted 20-fold into buffered H_2O and the number of deuterons was determined at different time points by ESI MS. Fig. 2 shows the kinetics of H/D exchange, when the exchange was performed at 25°C with native β-lactamase. After 20 min incubation in H_2O, there was no further decrease in the number of protected deuterons. Under these conditions (dilution into water, pH 6.0, 25°C, 5% residual ^2H_2O) 60 deuterons remain protected after 2 hr (Fig. 2, ○). However, when the exchange was performed at 48°C (Fig. 2, ◇) only 26 deuterons were still protected after 2 hr. In contrast, when the kinetic traces of H/D exchange of the GroEL-bound β-lactamase were measured at 48°C, this bound β-lactamase was found to be more protected (Fig. 2, ●) from exchange than the unbound species under the same conditions. After 2 hr incubation in H_2O in the presence of GroEL, an average of 49 deuterons remained protected compared with only 26 in the free protein at the same temperature. This difference of 23 deuterons suggests that binding to GroEL partially protects β-lactamase from H/D exchange. Furthermore, these data suggest that β-lactamase is not fully denaturated upon binding to GroEL. If the protection observed was exclusively due to GroEL, which would make denaturated β-lactamase inaccessible to solvent, slow H/D exchange would also have been seen with other GroEL-bound substrates. However, this is not the case (6, 9, 15). β-Lactamase may therefore be in a conformation that itself is protected against exchange. In comparison, the curves in Fig. 2 show the predicted exchange kinetics in a completely unstructured β-lactamase at 25°C (solid line) and at 48°C (dashed line). These data were calculated from the amino acid sequence of the protein (30), which contains a total of 250 amide protons. No effort was made to include in the simulated curves the contribution from the exchangeable amino acid side chain deuterons, which is important only at early time points. The exchange is calculated for a deuterated protein diluted into H_2O, at the temperature of the experiment (31, 32).

The ESI mass spectrum of denaturated β-lactamase in 50% acetonitrile, 1% acetic acid is shown in Fig. 3*A*. Under these conditions, β-lactamase produces a charge state distribution ranging from +17 to +35 in the m/z range 800-1800, with a peak maximum at +29. The deconvoluted spectrum gives a molecular weight of 28,907 ± 2.6, which is in excellent agreement with the molecular weight of β-lactamase calculated from the amino acid sequence (28,906.8 Da; ref. 30). As described by Robinson *et al.* (9), a prerequisite for detecting the extent of labeling of the GroEL-bound substrate protein by ESI MS is the dissociation of the complex in the gas phase as the naked pseudomolecular ions are formed. Fig. 3*B* shows the MS spectra of the GroEL:β-lactamase complex after 8 min exchange against H_2O as described in *Materials and Methods*.

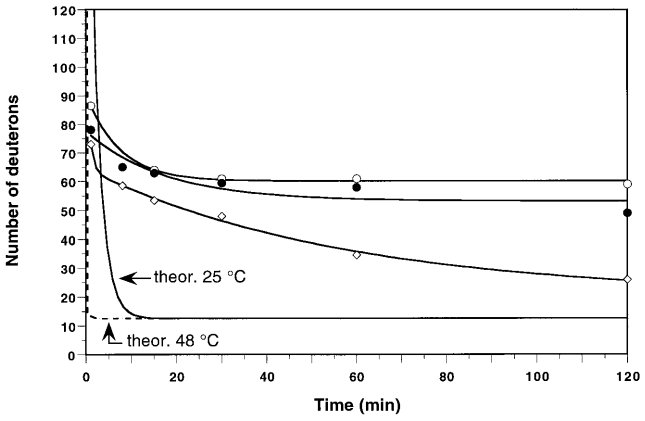

FIG. 2. Hydrogen exchange kinetics of β-lactamase at pH 6.0. (○) 25°C, no GroEL; (◇) 48°C, no GroEL; (●) 48°C β-lactamase:GroEL complex; solid line, curve simulated for β-lactamase in a completely unstructured conformation at 25°C; dashed line, the same at 48°C. For comparison, all simulations are normalized to 5% residual ^2H_2O. The mass of β-lactamase was determined within an average error of ± 3 mass units, except for the 1 min time point where the error was ± 7 mass units.

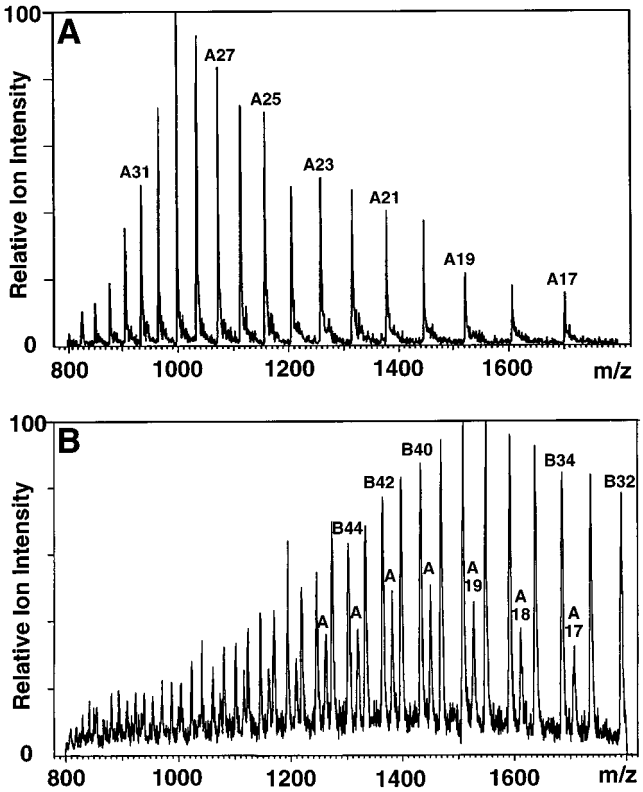

FIG. 3. ESI mass spectra of β-lactamase in 50% acetonitrile, 1% acetic acid (*A*) and of the complex GroEL:β-lactamase, 8 min after initiating H/D exchange in water at 48°C (*B*). The charge state series of β-lactamase are labeled A, those of GroEL are labeled B.

The charge state series of β-lactamase (labeled with A) ranges from +17 to +26 with a maximum at +20, and is thus clearly different from the spectra of denaturated β-lactamase (Fig. 3*A*), suggesting that the conformation of β-lactamase is at least partially preserved until injected into the mass spectrometer. Furthermore, the charge state series of GroEL (labeled B) ranges from +31 to +54, with a peak maximum at +37, which provides evidence that the chaperone is not completely denatured (9). We were thus able to preserve the conformation of β-lactamase until analysis by ESI MS. As described elsewhere (9, 33), this strategy permits the detection and quantification even of the protons that are only partially protected.

To determine whether repeated cycles of binding to and release from GroEL can decrease the number of protected protons in β-lactamase, three experiments were performed. First, protonated β-lactamase was subjected to three cycles of 1 hr heating to 48°C in the presence of 1 molar equivalent of GroEL (20) (binding phase to GroEL) and 1 hr of cooling to 4°C (dissociation phase) in ^2H_2O. This strategy ensures that each β-lactamase molecule has bound at least once to the chaperone (15), and even though the above experiments (Fig. 2) do indicate quantitative binding of β-lactamase to GroEL, we wanted to exclude any molecule escaping GroEL contact. Second, protonated β-lactamase underwent the same cycles but in the absence of GroEL. Third, protonated β-lactamase was subjected to three cycles of heating to only 25°C and cooling to 4°C in ^2H_2O. Incubation of all protein solutions at 4°C in ^2H_2O after the last temperature shift ensured complete dissociation of the GroEL:β-lactamase complex and refolding to the native conformation, as was confirmed by the complete recovery of β-lactamase activity (data not shown). Washing of the protein solutions in water before mass spectrometry caused a back-exchange of both the labile side chain protons and the nonprotected amide protons. This experiment allowed quantitation of those protons that are protected in native β-lactamase but are exchanged only after binding to GroEL or by local unfolding at 48°C.

β-Lactamase subjected to three 1-hr cycles of heating to 25°C in ^2H_2O has a molecular weight of 28,918.8 ± 1.8, only 12 Da higher than that of the protonated β-lactamase (molecular weight 28,906.8). This suggests that, at 25°C and pH 7.2, the structure of β-lactamase is stable and that only 12 protons, which remain protected after washing the protein in H_2O, pH 5.0, at 4°C, can be exchanged by thermal breathing into β-lactamase. This small difference in the molecular weight proves that the deuterons of the amino acid side chains and the non-protected amide protons are back-exchanged rapidly upon exposure to H_2O, and that the structurally informative protected amide deuterons can be measured. β-Lactamase subjected to three 1-hr cycles of heating to 48°C and cooling to 4°C in ^2H_2O has a molecular weight of 28,972.4 ± 1.4 (Fig. 4*A*). Therefore, 66 protons exchange when the temperature is increased from 25°C to 48°C, 54 more than at 25°C, suggesting an increased destabilization of the structure of β-lactamase at this temperature. Fig. 4*B* shows the mass spectrum of the GroEL:β-lactamase complex subjected to the same cycles at 48°C. The molecular weight of 28,952.9 ± 3.1 for β-lactamase is 19.5 Da lower than that of β-lactamase which was subjected to temperature cycling between 48°C and 4°C in the absence of GroEL (Fig. 4*A*). These results indicate once more that the GroEL-bound β-lactamase shows a higher degree of protection toward H/D exchange than does β-lactamase free in solution under the same conditions.

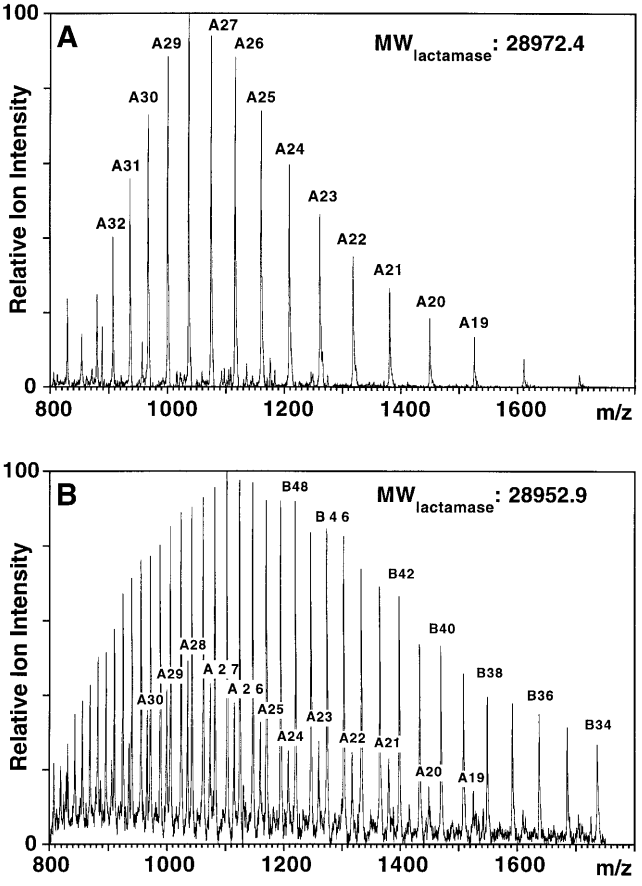

FIG. 4. ESI mass spectra of the equilibrium H/D exchange experiments. (*A*) β-Lactamase subjected to three 1-hr cycles of heating to 48°C and cooling to 4°C in ^2H_2O; (*B*) GroEL:β-lactamase complex treated as in *A*. The charge state series of β-lactamase are labeled A, those of GroEL B. The molecular weight of β-lactamase calculated from the charge series between +19 and +32 is shown in the figures.

DISCUSSION

Our experiments show that β-lactamase has to overcome an activation barrier when unfolding from the GroEL-bound state, and that this barrier is similar to that encountered when unfolding is initiated from the native state. In contrast, when unfolding into the same Gdn·Cl-containing buffer is started from a HCl/KCl mixture, in which β-lactamase has been shown to fulfill the criteria of a molten globule (27), the activation barrier is so low that the reaction proceeds too quickly to be measured by manual kinetics. The GroEL-bound β-lactamase thus must contain a significant amount of native structure, and the state bound at 48°C is not consistent with a molten globule.

To obtain more detailed information about the bound state of β-lactamase than is possible from the unfolding kinetics, we used mass spectrometry to investigate the H/D exchange of the bound protein. Neither the final state nor the kinetics were found to be consistent with a molten globule or a totally unfolded state, since both the number of protons which remained protected and the slow exchange kinetics were surprisingly similar to the characteristics of the native protein at 25°C. In addition, the binding of GroEL actually slowed the exchange and increased the number of exchange-resistant protons. It follows, therefore, that β-lactamase must retain a considerable amount of native-like structure when bound to GroEL.

β-Lactamase binds to GroEL only at 48°C; there is no detectable binding at 25°C or at lower temperatures (20, 28). Stable complexes of β-lactamase with GroEL are not observed starting from either native or urea-denatured β-lactamase, nor can a retardation of folding be measured at low temperature. This has been interpreted as implicating hydrophobic interactions as the crucial determinant for binding. Calorimetric studies with other substrate proteins have confirmed that their association with GroEL is primarily mediated by hydrophobic interactions (34). The stoichiometry of the GroEL–β-lactamase complex was determined by isothermal titration calorimetry, with one β-lactamase molecule being found to be bound to one 14-mer GroEL (P.G., I. Jelezarov, and A.P., unpublished data). This indicates the absence of thermally induced aggregates of the chaperone-bound substrate.

β-Lactamase is thus different from previously investigated small protein substrates, and these differences demonstrate that a range of folding states of GroEL-bound proteins exists. Barnase, which, like β-lactamase, does not form stable complexes with GroEL, has been suggested to transiently bind in a completely unfolded state, since the exchange of those amide protons which can only exchange from the globally unfolded state is accelerated (6). It is possible that barnase can provide hydrophobic interactions sufficient for GroEL binding only when it is globally unfolded, but even in this case the interaction is weak and only transient. Similarly, cyclophilin loses all protons upon complex formation with GroEL (15), which is again very different from the observations made with β-lactamase under similar conditions. It is possible that the β-barrel structure of cyclophilin has to unfold completely to allow access of GroEL to its hydrophobic amino acids, and that in the resulting complex no native hydrogen bonds are preserved. In the case of barnase, the H-bonds of the β-sheet also become accessible only upon global unfolding, but not by local breathing (16, 17). α-Lactalbumin, which in its 3-disulfide reshuffled state also forms a stable complex with GroEL, shows much faster H/D exchange kinetics upon binding than are observed for β-lactamase. From this exchange behavior it has been suggested that the conformation of the GroEL bound α-lactalbumin must be very similar to its bona fide molten globule state (9). Again, α-lactalbumin may provide sufficient interactions only when it is in a molten globule state. It must be noted, however, that the proton exchange of β-lactamase is slowed down when it is bound to GroEL, suggesting either a stabilization of the native state of β-lactamase structure at a temperature just below its thermal denaturation or some slowing down of exchange due to limited solvent accessibility in the complex. However, since the effect of GroEL is most pronounced on the slowly exchanging protons (Fig. 2), a stabilization of a native-like structure is the simplest explanation.

In conclusion, different proteins appear to form transient or stable complexes with GroEL in rather different states. The preservation of native structure in the complex, with concomitant slowly exchanging protons, may be possible if hydrophobic patches occur in native-like states, most likely at the interface between domains. An antibody Fab fragment, a four-domain protein linked by a disulfide bond between its two two-domain chains, was also found to show a significant kinetic barrier upon unfolding from the GroEL complex, suggesting that it too contains a significant amount of native-like structure (13). Alternatively, one subdomain of a protein may unfold and lead to binding to the chaperone while the other is responsible for the slowly exchanging protons. The crystal structure of the *E. coli* TEM1 β-lactamase at 1.8 Å resolution (35) reveals quite a hydrophobic cavity, consisting of Phe-72, Leu-76, Ala-135, Leu-139, Pro-145, Leu-148, and Leu-162 (numbering according to Ambler *et al.*, ref. 36) that could become exposed, without global unfolding of the protein, by thermal fluctuations at higher temperatures, and thus be responsible for the interaction with GroEL. Alternatively, an interaction of the residues at the interface between both domains with GroEL could account for the data. We are currently unable to distinguish between these alternatives for β-lactamase binding to GroEL, but because of the presence of preserved slowly exchanging protons, the spatial resolving power of NMR will help to delineate this question and will allow further insights into the conformation of β-lactamase that is bound to GroEL. Since very different results have been found to date upon probing the GroEL-bound states of different proteins using proton exchange, the great diagnostic power of this method is further demonstrated. We believe that the ability of GroEL to reversibly bind to native-like states, exposing hydrophobic surfaces by thermal breathing just below the thermal transition midpoint, is an important property in protecting the cell against heat-shock induced aggregation.

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1. Lorimer, G. H. (1996) *FASEB J.* **10,** 5–9.
2. Ellis, R. J. & Hartl, F.-U. (1996) *FASEB J.* **10,** 20–26.
3. Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D. C., Joachimiak, A., Horwich, A. L. & Sigler, P. B. (1994) *Nature (London)* **371,** 578–586.
4. Fenton, W. A., Kashi, Y., Furtak, K. & Horwich, A. L. (1994) *Nature (London)* **371,** 614–619.
5. Jaenicke, R. (1993) *Curr. Opin. Struct. Biol.* **3,** 104–112.
6. Zahn, R., Perrett, S., Stenberg, G. & Fersht, A. R. (1996) *Science* **271,** 642–645.
7. Badcoe, I. G., Smith, C. J., Wood, S., Halsall, D. J., Halbrook, J. J., Lund, P. & Clarke, A. R. (1991) *Biochemistry* **30,** 9195–9200.
8. Okazaki, A., Ikura, T., Nikaido, K. & Kuwaijama, K. (1994) *Nat. Struct. Biol.* **1,** 439–447.
9. Robinson, C. V., Gross, M., Eyles, S. J., Ewbank, J. J., Mayhew, M., Hartl, F.-U., Dobson, C. M. & Radford, S. E. (1994) *Nature (London)* **372,** 646–651.
10. Hayer-Hartl, M. K., Ewbank, J. J., Creighton, T. E. & Hartl, F.-U. (1994) *EMBO J.* **13,** 3192–3202.
11. Martin, J., Langer, T., Boteva, R., Schramel, A., Horwich, A. L. & Hartl, F.-U. (1991) *Nature (London)* **52,** 36–42.
12. Peralta, D., Harman, D. J., Hoogenraad, N. J. & Høj, P. B. (1994) *FEBS Lett.* **339,** 45–49.

13. Lilie, H. & Buchner, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8100–8104.
14. Englander, S. W., Sosnick, T. R., Englander, J. J. & Mayne, L. (1996) *Curr. Opin. Struct. Biol.* **6,** 18–23.
15. Zahn, R., Spitzfaden, C., Ottiger, M., Wüthrich, K. & Plückthun, A. (1994) *Nature (London)* **368,** 261–265.
16. Clarke, J., Hounslow, A. M., Bycroft, M. & Fersht, A. T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9837–9841.
17. Perrett, S., Clarke, J., Hounslow, A. M. & Fersht. A. R. (1995) *Biochemistry* **34,** 9288–9298.
18. Miranker, A., Robinson, C. V., Radford, S. E. & Dobson, C. M. (1996) *FASEB J.* **10,** 93–101.
19. Zahn, R., Axmann, S. E., Rücknagel, K.-P., Jaeger, E., Laminet, A. A. & Plückthun, A. (1994) *J. Mol. Biol.* **242,** 150–164.
20. Zahn, R. & Plückthun, A. (1994) *J. Mol. Biol.* **242,** 165–174.
21. Vanhove, M., Raquet, X. & Frère, J.-M. (1995) *Proteins Struct. Funct. Genet.* **22,** 110–118.
22. Mitchinson, C. & Pain, R. H. (1985) *J. Mol. Biol.* **184,** 331–342.
23. Uversky, V. N. (1993) *Biochemistry* **32,** 13288–13298.
24. Uversky, V. N. & Ptitsyn, O. B. (1994) *Biochemistry* **33,** 2782–2791.
25. Goto, Y., Calciano, L. J. & Fink, A. L. (1990) *Proc. Natl. Acad. Sci. USA* **87** 573–577.
26. Goto, Y., Takahashi, N. & Fink, A. L. (1990) *Biochemistry* **29,** 3480–3488.
27. Goto, Y. & Fink, A. L. (1989) *Biochemistry* **28,** 945–952.
28. Laminet, A. A. & Plückthun, A. (1989) *EMBO J.* **8,** 1469–1477.
29. Cartwright, S. J. & Waley, S. G. (1984) *Biochem. J.* **221,** 505–512.
30. Ambler, R. P. & Scott, G. K. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3732–3736.
31. Bai, Y., Milne, J. S., Mayne, L. & Englander, S. W. (1993) *Proteins Struct. Funct. Genet.* **17,** 75–86.
32. Connelly, C. P., Bai, Y., Jeng, M.-F & Englander, S. W. (1993) *Proteins Struct. Funct. Genet.* **17,** 87–92.
33. Miranker, A., Robinson, C. V., Radford, S. E., Aplin, R. T. & Dobson, C. M. (1993) *Science* **262,** 896–900.
34. Lin, Z., Schwarz, F. P. & Eisenstein, E. (1995) *J. Biol. Chem.* **270,** 1011–1014.
35. Jelsch, C., Mourey, L., Masson, J.-M. & Samama, J.-P. (1993) *Proteins Struct. Funct. Genet.* **16,** 364–383.
36. Ambler, R. P., Coulson, A. F. W., Frère, J.-M., Ghuysen, J. M., Joris, B., Forsman, M., Levesque, R. C., Tiraby, G. & Waley, S. G. (1991) *Biochem. J.* **276,** 269–272.