Residual Structure in Urea-Denatured Chaperonin GroEL†

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Received June 19, 1995; Revised Manuscript Received August 28, 1995⊗

ABSTRACT: The urea denaturation of the chaperonin GroEL has been studied by circular dichroism, intrinsic tyrosine fluorescence and fluorescence of the hydrophobic probe, 1,1′-bis(4-anilino)naphthalene-5,5′-disulfonic acid (bisANS). It is shown that GroEL denaturation, monitored by CD and intrinsic fluorescence measurements, can be well described by a two-state transition that is complete by 3−3.1 M urea. The beginning of this transition overlaps the urea concentrations where the oligomeric protein starts to dissociate into individual monomers. Subsequent addition of the denaturant leads to complete unfolding of the monomers. Monomers unfolded at urea concentrations higher than 3.1 M are not competent to form their native conformations under the conditions employed here, and they are not able to reassemble to oligomers upon dilution of urea. In contrast to the CD and intrinsic fluorescence measurements, bisANS bound to GroEL exhibits considerable fluorescence intensity under conditions where the CD and intrinsic fluorescence signals have already reached their minimum values (>3.1 M urea). This binding of bisANS, under conditions where the majority of the secondary structure of GroEL has already unfolded, indicates the existence of hydrophobic residual structure. This structure cannot be detected by CD measurements, but it can be unfolded by raising further the urea concentration. The existence of this structure does not depend on the source or method of the protein preparation. Intrinsic fluorescence and trypsin digestion demonstrate no difference between the bisANS-bound form of GroEL and the free form of the protein, showing that the GroEL structure is not greatly affected by the interaction with bisANS. Analysis of the chymotryptic fragments of GroEL, photolabeled with bisANS, suggests that photoincorporation of the probe at 3.1 M urea occurs within amino acid residues 203−249 in the apical domain, suggesting this portion of GroEL is the region that contains the residual structure. This residual structure may be important as a nucleation site for folding and/or an interactive region that can lead to misfolding under some conditions. The existence and location of this residual structure may facilitate the registration of the regions of the primary sequence that interact to achieve the interesting fold of GroEL, and they may help understanding of reports that preformed chaperonins can assist refolding/reassembly of the fully unfolded chaperonin.

Molecular chaperonins are proteins that can, as one of their functions, mediate protein folding in an ATP dependent manner (Hartl et al., 1994). The best studied chaperonin is the *Escherichia coli* protein GroEL[1] (cpn60), which is homologous to Hsp60 found in the mitochondrial matrix. GroEL is an oligomeric protein containing 14 identical 60 kDa subunits arranged in two stacked seven-membered rings to form a cylinder.

Each subunit is folded into three domains (Braig et al., 1994): (1) The equatorial domain, consisting of residues 6−133 together with 409−523, is highly α-helical and well ordered (Braig et al., 1994). It provides most of the contacts between subunits in one ring and all contacts between the rings. (2) The small intermediate domain contains residues 134−190 together with 377−408. (3) The apical domain, containing residues 191−376, has been suggested to be responsible for the interaction with partially folded polypeptides (Fenton et al., 1994). It contains many hydrophobic residues, and the apical domain was the least well resolved portion of the crystal structure because of its possible flexibility (Braig et al., 1994). It has been shown that in solution GroEL exhibits significant flexibility, which is modulated by binding of polypeptides (Gorovits & Horowitz, 1995).

Partial unfolding of GroEL leads first to the dissociation of the oligomeric protein to monomers at about 2.5 M urea (Mendoza et al., 1994) or 1.5 M guanidinium chloride (Price et al., 1993; Mizobata & Kawata, 1994). These monomers are assembly competent, and, compared with their properties in the tetradecamer, they have increased hydrophobic exposure and flexibility (Horowitz et al., 1995). It was suggested that hydrophobic exposure can play a significant role in the binding of the unfolded substrate (Horowitz et al., 1995), and this exposure can be modulated by factors such as ionic strength and pH (Gibbons & Horowitz, 1995).

The GroEL monomer in the tetradecamer, as noted above, presents an interesting folding problem, since the first biosynthesized sequence must extensively interact with the last biosynthesized segment to form the largest and most structured equatorial domain. *In vitro* studies performed to date have failed to refold and reassemble GroEL after denaturation (Price et al., 1993; Mizobata & Kawata, 1994). It has been suggested that pre-existing chaperonins and MgATP are needed to assist the correct assembly of the

† This research was supported by research grants GM 25177 and ES 05729 from the NIH and research grant AQ 723 from the Robert A. Welch Foundation to P.M.H.

⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1995.

[1] Abbreviations: GroEL, chaperonin 60 or groEL; CD, circular dichroism; bisANS, 1,1′-bis(4-anilino)naphthalene-5,5′-disulfonic acid; EDTA, ethylenediaminetetraacetic acid.

0006-2960/95/0434-13928$09.00/0

GroEL tetradecamer after unfolding in 4 M urea (Lissin et al., 1990, Lissin & Hemmingsen, 1993).

It has been presumed in many studies that denatured proteins are completely unfolded after the observed major structural transitions induced by urea or guanidinium hydrochloride. However, there are several systems in which intermediates have been demonstrated. For example, some mutants of staphylococcal nuclease have an intermediate, compact denatured state that breaks down in a cooperative, first-order transition (Gittis et al., 1993). Residual structure was detected near histidine-92 of the α subunit of tryptophan synthase (Saab-Rincón et al., 1993). A hydrophobic cluster has been observed at 7 M urea in the 63-residue domain of the 434-repressor (Neri et al., 1992). Similarly, a large fragment of barnase (23−110), showing a very weak signal in the near-UV CD measurements, contains a small amount of pre-existing structure, which can be enhanced upon binding with a peptide inhibitor of the intact protein or with substrate (Kippen et al., 1994).

In the present study, we show, through the comparison of the unfolding transitions of GroEL monitored by CD and tyrosine intrinsic fluorescence transitions with the transition obtained by using bisANS fluorescence, that there exists some residual hydrophobic structure in urea-denatured GroEL at moderate concentrations of urea. This structure is maximized at ~3.1 M urea where there is little detectable secondary structure as judged by CD or intrinsic fluorescence. This residual structure can be denatured by increasing further the concentration of urea. Through the use of covalent photoincorporation of bisANS, we have identified a portion of the apical domain of GroEL (203−249) as the region containing the residual structure.

MATERIALS AND METHODS

Reagents. Electrophoresis purity urea was purchased from Bio-Rad. Urea concentrations were determined by measuring the refractive index of a stock solution and comparing it with standard data (CRC Handbook of Chemistry and Molecular Biology, 1974).

Protein Purification. The chaperonin GroEL was purified from lysates of *E. coli* cells bearing the multicopy plasmid pGroESL (Goloubinoff et al., 1989) as previously described (Mendoza et al., 1994). After purification, GroEL was dialyzed against 50 mM Tris−HCl, pH 7.6, containing 1 mM dithiothreitol, and was then brought to 10% (v/v) in glycerol, rapidly frozen, and stored at −70 °C. The protomer concentration of GroEL was measured by the bicinchoninic acid protein assay (Pierce) using the procedure recommended by the manufacturer and assuming a molecular mass of 60 kDa.

Standard Buffer. The following solution was used as a standard buffer: 50 mM Tris−HCl, pH 7.8, 50 mM KCl, 10 mM β-mercaptoethanol, and 2 mM EDTA.

Circular Dichroism Spectroscopy. Circular dichroism measurements were made using a Jasco J-500C spectropolarimeter at 25 °C. Time-dependent measurements of the elipticity at 222 nm indicated that samples were at equilibrium. All data shown (recorded at 0.2 nm intervals; 4 s time constant) are the averages of three scans. Signals of the buffer blanks were subtracted from the corresponding samples. Samples containing 1 μM GroEL in 50 mM Tris−HCl, pH 7.8, and different concentrations of urea (0−6 M) were incubated for 90−120 min prior to measurements. Data describing the structural transitions of GroEL by CD and by scattering (see below) were analyzed using a nonlinear least-squares fit to the equation described by Pace (1990).

Fluorescence Titration for bisANS Binding to GroEL. Fluorescence measurements were made on a SLM 48000 spectrofluorometer (SLM Instruments, Urbana, IL). Separate samples for each point were made with 1 μM GroEL and 10 μM bisANS in the standard buffer. Final concentration of urea was varied from 0 to 8 M. Samples were incubated for 90−120 min prior to measurements. For the concentration dependence of the transition, the bisANS concentration was fixed at the following concentrations: 1.4, 5, 10, 15, 20, 30, and 40 μM. Fluorescence emission was measured at 500 nm with excitation at 399 nm.

Intrinsic Tyrosine Fluorescence Measurements. Fluorescence measurements were made at 25 °C on a SLM 48000 spectrofluorometer (SLM Instruments, Urbana, IL). Separate samples for each point were made with 1−1.7 μM GroEL in the standard buffer. Urea concentration was varied from 0 to 6 M. Samples were incubated for 90−120 min prior to measurements. In some cases, solutions contained 10 μM bisANS, as noted in the text. Samples were excited at 280 nm and fluorescence was detected at 310 nm.

Light-Scattering Measurements. Light-scattering measurements were made at 25 °C on a SLM 48000 spectrofluorometer (SLM Instruments, Urbana, IL). Samples were excited at 323 nm, and scattered light was detected at 90° to the incident beam. Signals were corrected for the blank values. Urea concentration was varied from 0 to 6 M. Samples were incubated for 90−120 min prior to measurements.

Sedimentation Analysis. Samples of GroEL (5.1 μM) in 50 mM Tris−HCl, pH 7.8, were subjected to sedimentation velocity analyses using a Beckman XL-A analytical ultracentrifuge. The runs were performed utilizing 12 mm double-sector cells in a four-hole Ti-60 rotor. The temperature was kept at 25 °C. Solutions had an $A_{230} = 0.8-1.0$, and the rotor speed in different runs was between 27 000 and 40 000 rpm. The scans were analyzed by the method of van Holde and Weischet (1978) using the UltraScan ultracentrifuge data collection and analysis program (Mendoza et al., 1994). All data were corrected to standard conditions.

Analysis of the Ability of GroEL to Reassemble. Samples of GroEL (63 μM) were denatured in the standard buffer at various concentrations of urea (0.8, 1.52, 2.4, 2.64, 2.8, 2.96, 3.04, 3.12, 3.2, 3.32, 3.48, 3.68, 3.92, 4.24, and 4.8 M) for 90 min at 25 °C. Samples were then diluted with the standard buffer, maintaining final protein concentration 8.3 μM (7.65 times dilution). After incubation for 90 min, samples were analyzed by electrophoresis on 6% native Laemmeli gels followed by Coomassie staining.

Photoincorporation of GroEL with bisANS. GroEL was photolabeled with bisANS as described previously (Seale et al., 1995). Briefly, GroEL (18 μM) in 50 mM Tris, pH 7.8, 50 μM bisANS, and either 2.5 or 3.1 M urea was irradiated with UV light (254 nm) for 60 min using a model UVS-11, Mineralight lamp (Ultra-Violet Products, Inc., San Gabriel, CA). Next, the 3.1 M treated sample was diluted to 2.5 M urea by the addition of buffer for chymotrypsin digestion. Both samples were then treated with chymotrypsin (0.1% w/w, final concentration). Aliquots were removed at 0, 15,

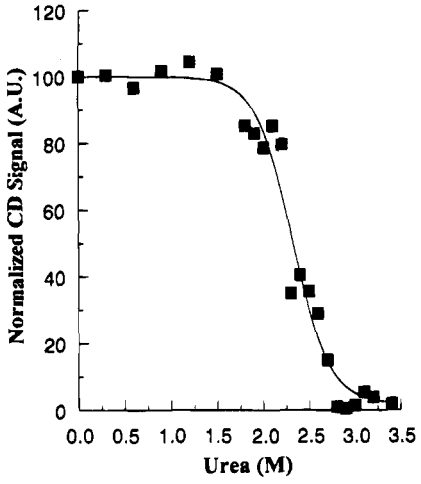


FIGURE 1: Dependence of GroEL ellipticity measured at 218 nm as a function of urea concentration. Circular dichroism measurements were made using a Jasco J-500C spectropolarimeter at 25 °C. All data shown (recorded at 0.2 nm intervals; 4 s time constant) are the averages of three measurements. Signals of the buffer blanks were subtracted from the corresponding samples, and the ellipticities were normalized. Samples contained 1 μM GroEL in 50 mM Tris–HCl, pH 7.8, and different urea concentrations and prepared as in Materials and Methods.

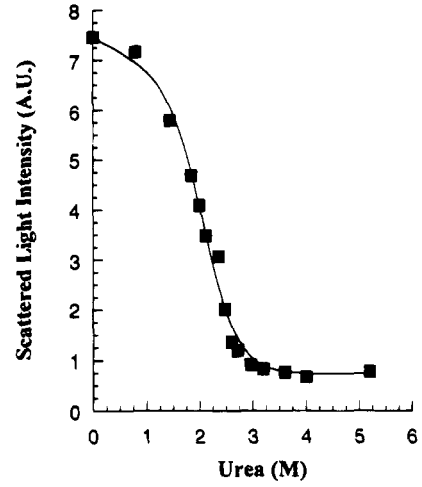


FIGURE 2: Intensity of scattered light for the GroEL solution as a function of urea concentration. The protein concentration was 360 μg/mL in the standard buffer, containing different amount of urea. Samples were prepared as in Materials and Methods. Intensity of the scattered light was detected at 323 nm and corrected for the intensity of the corresponding blank solution. The solid line here represents a fit of the experimental data to a two-state model (Pace, 1990).

30, and 45 min. PMSF was added to a 10 mM final concentration, and the sample was incubated on ice for 10 min. Samples were then heated at 100 °C for 4 min, cooled to room temperature, and analyzed by electrophoresis on 12% SDS Laemelli gels. Fluorescence of the labeled protein bands was excited with a model TM40UV transilluminator (Ultra-Violet Products, Inc., San Gabriel, CA). Fluorescence was recorded on Polaroid 55 Sheet Film (Polaroid) at f 8 for 30–60 s though a Nikon Y3 yellow filter.

RESULTS

Urea-Induced Changes in Secondary Structure of GroEL. The circular dichroism spectrum of native GroEL in the far UV was the same as previously described (Price et al., 1993; Mendoza et al., 1994), and the CD signal at 218 nm was used to follow the GroEL unfolding process (Figure 1). The normalized ellipticities describe a transition between 1.75 and 3 M urea that leads to the complete loss of detectable regular secondary structure. It was previously shown by centrifugation (Mendoza et al., 1994) and by light scattering here (Figure 2) that urea induces dissociation of the oligomer and GroEL is present mainly as monomers at greater than 2.6 M urea. Thus, the decrease in the CD signal between 2.5 and 3 M urea represents unfolding of individual monomers, since by 2.9–3 M urea the protein has lost most of its detectable secondary structure. The urea-induced structural transition followed by monitoring the intrinsic fluorescence from the tyrosine residues of GroEL occurs at somewhat higher urea concentrations, with the urea concentration at the midpoint of the transition at ~3 M urea rather than at ~2.2 M urea (data not shown and Gorovitz and Horowitz, submitted for publication). This would be consistent with there being some structure that can influence the tyrosine environments while there is little secondary structure.

Fluorescence of the BisANS–GroEL Complex is Maximum in a State with Little Detectable Secondary Structure. BisANS has been used as a reporter of hydrophobic surfaces

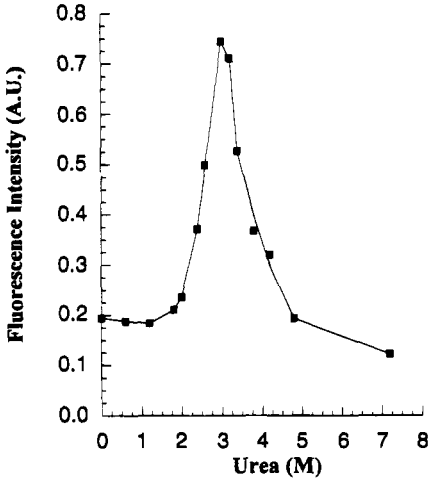


FIGURE 3: Fluorescence intensity of bisANS bound to GroEL as a function of the urea concentration. Individual samples at each of the indicated urea concentrations were prepared as in the Materials and Methods with a protein concentration of 1 μM and a bisANS concentration of 10 μM. Fluorescence was excited at 399 and detected at 500 nm.

on GroEL (Horowitz et al., 1995). The two-step process of dissociation and unfolding of GroEL with increasing urea concentrations has been suggested to be reflected in the biphasic response of the fluorescence of bisANS as shown in Figure 3 (Horowitz et al., 1995; Gorovits et al., 1995). The profile shows a sharp increase in the bisANS fluorescence between 2 and 3 M urea, demonstrating that a large amount of hydrophobic surface is hidden in the native (unperturbed) conformation of GroEL.

The dissociation detected by scattering (Figure 2) is reflected in the increasing phase of the urea-induced bisANS fluorescence response (Figure 3, 1.5–3 M urea). Under conditions used in the present study the protein is completely dissociated at 2.5 M urea, as was demonstrated by analytical ultracentrifugation which is consistent with previous reports (Mendoza et al., 1994). At 2.86, 3, 3.33, 3.46, or 5.06 M urea, the $s_{20,w}$ was 2–2.2S, values that are close to 1.95S

calculated for a 60 kDa polypeptide in a random coil conformation. Hence, at concentrations of urea, higher than 3−3.2 M, GroEL is monomeric and lacks those structural elements that are detectable by CD or by tyrosine intrinsic fluorescence.

Comparison of the CD and bisANS Fluorescence Transitions. Comparison of the CD denaturation profile (Figure 1) and bisANS fluorescence transition (Figure 3) shows that, while the CD signal has already reached its minimum at 2.9−3 M urea, the bisANS fluorescence is at its maximum. The ability of the probe to bind to GroEL in a state with very little secondary structure indicates that there must be some hydrophobic structure that is stable at moderate (>3 M) urea concentrations. This structure can be unfolded by further addition of denaturant as indicated by the decrease of fluorescence in Figure 3. BisANS binding to partially unfolded GroEL could be observed up to 4.5−5 M urea, showing that the hydrophobic residual structure can exist up to those concentrations of urea. Previous studies of the effects of urea on the fluorescence of bisANS bound to gamma cyclodextrin or the enzyme rhodanese indicate that the decrease in fluorescence observed here is related to structural properties of GroEL, and it is not due to a general loosening of bisANS binding at the higher urea concentrations (Horowitz & Butler, 1993).

BisANS Transitions Are Not Due to Interactions of the Probe with GroEL. Several approaches were used to evaluate the possibility that the interaction of bisANS with GroEL could shift the equilibrium between different conformational states of the protein thereby contributing to the observations (Li Shi et al., 1994). These tests indicate that there was no significant stabilizing influence due to the binding of bisANS.

The intrinsic fluorescence of GroEL was measured as a function of the urea concentration in the presence of 10 μM bisANS. The transition followed very closely the transition shown above measured in the absence of the probe.

Possible stabilization of the GroEL molecule due to the binding to the bisANS was examined using proteolysis. It has been shown previously that native GroEL is resistant to the proteolysis by trypsin or chymotrypsin (Horowitz et al., 1995). Partial urea denaturation allows for the digestion of GroEL, and several distinct polypeptides are produced (Horowitz et al., 1995). In the present study, proteolysis was carried out at 2.5, 3.2, and 4.2 M urea at 3% trypsin (w/w) for various times. There were no significant differences detected between samples that did or did not contain bisANS, showing the absence of a stabilizing effect of the probe on the structure of GroEL.

The slow kinetics of bisANS binding was used to demonstrate that significant changes in the conformation of DnaK occur upon interaction with the probe (Li Shi et al., 1994). In the present study, the response in the fluorescence signal observed upon addition of bisANS to the protein solution was complete within the time of manual mixing (10−20 s), and no additional increase was seen over 20−30 min.

The dependence of the fluorescence of the bisANS−GroEL complex on the urea concentration was studied as a function of the probe concentration. The concentration of the probe was varied between 1.4 and 40 μM. Although the fluorescence intensities changed, there were no significant differences in the shapes of the urea-induced transitions.

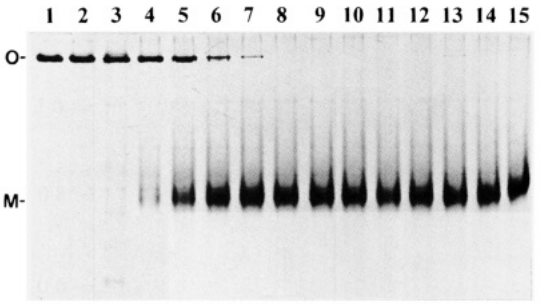


FIGURE 4: Analys of GroEL reassembly. Protein was dissolved in the standard buffer, containing different urea concentrations, and incubated for 90 min. Urea concentrations are, from left to right, 0.8, 1.52, 2.4, 2.64, 2.8, 2.96, 3.04, 3.12, 3.2, 3.32, 3.48, 3.68, 3.92, 4.24, and 4.8 M urea. Protein was then diluted by the standard buffer, maintaining constant protein concentration. After 90 min of incubation, samples were analyzed by native polyacrylamide gel (6%) electrophoresis followed by staining with Coomassie R-250. Oligomeric GroEL is denoted by "O", and monomeric GroEL is denoted by "M".

Thus, the biphasic response and the position of the maximum remained unchanged.

GroEL Loses the Ability to Reassemble at >3 M Urea. The ability of urea-pretreated GroEL to reassemble into tetradecamers was assessed using native gel electrophoresis (Figure 4). Samples of GroEL were first unfolded at various urea concentrations for 90 min, prepared as described in Materials and Methods and electrophoresed under nondenaturing conditions that separate monomeric and oligomeric forms of GroEL. Since there is no urea in the gel, the procedure measures the ability of GroEL to reassemble; it does not measure the state of the chaperonin in the given urea concentration (Mendoza et al., 1995). Figure 4 shows that, under the conditions used in this study, GroEL fails to reassemble if incubated at concentrations of urea higher than 3.04 M (lane 7). This urea concentration coincides with the point where GroEL loses the majority of its detectable regular secondary structure (see above). Thus, monomers formed at 2.86 M urea significantly reassemble, while there is no reassembly from monomers formed at 3.25 M urea. These samples both have minimal structure by CD, light scattering and intrinsic fluorescence, but they are on opposite limbs of the biphasic bisANS detected transitions.

Apical Domain is the Likely Site of the Residual Structure. In order to gain information about the region(s) within GroEL that might be associated with the residual structure, the protein was photolabeled with bisANS. It has been shown that bisANS, although a noncovalent probe, can be covalently photoincorporated into a protein following UV irradiation (Seale et al., 1995). Because photoincorporation preferentially occurs near the site of probe binding, it is possible to gain information about the region where the probe binds.

Figure 5 shows the digestion pattern of GroEL, photolabeled by bisANS, where lanes 1, 3, 5, and 7 represent samples pretreated at 2.5 M urea and lanes 2, 4, 6, and 8 represent samples pretreated at 3.1 M urea. In order to compare proteolytic fragments from the photolabeled GroEL, all samples were brought to 2.5 M urea for the digestion and subsequent analysis (see Materials and Methods). There were no significant differences in the number and positions of covalently labeled polypeptides between these two samples. It has been shown by sequencing of photolabeled fragments of GroEL (Seale et al., 1995) that at 2.5 M urea

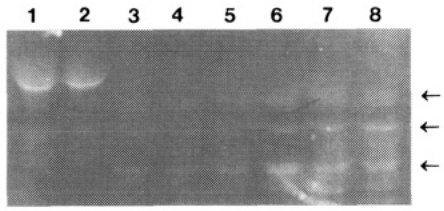


FIGURE 5: GroEL photolabeling. GroEL was photolabeled with bisANS in the presence of 2.5 M (lanes 1, 3, 5, and 7) and 3.1 M (lanes 2, 4, 6, and 8) urea as discribed in the Materials and Methods. Labeled GroEL was then digested by 0.1% (w/w) of chymotrypsin. Aliquots were removed at 0 (lanes 1 and 2), 15 (lanes 3 and 4), 30 (lanes 5 and 6), and 60 min (lanes 7 and 8), and separated on an SDS polyacrylamide gel (12%). Fluorescence of the gel was recorded on the Polaroid Sheet Film as discribed in the Materials and Methods. The bright bands in lanes 1 and 2 correspond to undigested GroEL. The arrows denote the positions of the most prominent fluorescent peptides.

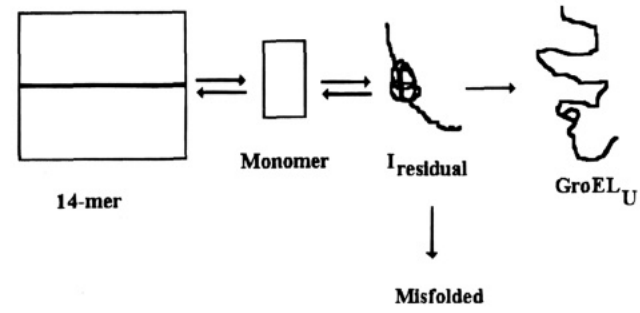
photoincorporation of the bisANS occurs in the apical domain of GroEL within the sequence 203−249. The similarity of the two digestion patterns suggests that photoincorporation of bisANS at 3.1 M urea also occurs within the apical domain.

DISCUSSION

Comparison of structural transitions of GroEL monitored by a number of parameters including CD and intrinsic fluorescence with transitions monitored by using the hydrophobic probe, bisANS, demonstrates a considerable shift in the denaturant concentrations that appear to be necessary to completely unfold the structure. Organized hydrophobic surfaces appear to persist, or can form, under conditions where the other parameters indicate that virtually all structure is lost. These hydrophobic surfaces do not lead to significant protein association under the conditions where they were detected. The results of a number of tests suggest that appearance of hydrophobic residual structure was a property of GroEL, and it was not due to the interactions with the probe, e.g., the transitions monitored by intrinsic fluorescence were the same in the presence or absence of bisANS, and the biphasic character of the bisANS detected transitions were independent of the bisANS concentrations. This residual structure could be completely unfolded when the urea concentration was raised to 4.5−5 M. Analysis of this unfolding process, performed as described by Ikegami (1981), shows that this is a cooperative, two-state transition (not shown).

The photoincorporation studies here, compared with the results of previous work, suggest that the apical domain of GroEL is a major contributor to the hydrophobic residual structure. Hydropathy plots emphasize that the amino acid sequence centered around residue 250 in the apical domain contains long stretches of hydrophobic residues (Horowitz et al., 1995); this same region contains the hydrophobic residues that were identified by site-directed mutagenesis as likely sites for the functionally relevant binding of folding intermediates and the cochaperonin GroES (Fenton et al., 1994). The same region of the apical domain, especially between residues 220 and 360, is the least well resolved portion of the structure in X-ray studies, indicating some degree of flexibility (Braig et al., 1994). It is possible, therefore, that the residual structure represents a region of the apical domain that is stabilized mainly by hydrophobic

Scheme 1



interactions. This structure may be a remnant of the structure within the folded protein, or it may form when the constraints of the native structure are relieved at moderate concentrations of urea.

Residual structure has been detected in a number of other systems, and it has been suggested that these regions in otherwise unfolded states can be important for initiating protein folding (Kippen et al., 1994). Fersht and colleagues showed that small areas of stable residual structure are present in the pH-denatured form of barnase (Arcus et al., 1994). These regions had been predicted to be formed early in barnase folding (Fersht, 1993). A similar conclusion was made in the case of the residual structure in the urea unfolded 63 amino acid containing domain of the 434-repressor, studied by nuclear Overhauser effect (Neri et al., 1992). The nonrandom hydrophobic cluster, formed by the side chains of Val^{54}, Val^{56}, Trp^{58}, and Leu^{59}, was proposed to be a "nucleation cluster" for the folding of the protein. Garcia et al. (1995) suggested that residual structure could form around tryptophans 308 and 333 in phosphoglycerate kinase, and they speculated that these clusters might serve as nucleation sites for the folding of this enzyme. It is thus possible that the residual structure detected here in GroEL could, under the proper conditions, constitute a nucleation site for the folding of GroEL monomers.

To date, it has not been reported possible to refold and reassemble GroEL after denaturation at high concentrations of urea (8 M) or guanidinium hydrochloride (6 M). It has been suggested that the facile formation of native-like 14mers requires chaperonins and nucleotide (Lissin et al., 1990, 1993). In the present study, there was no significant refolding and reassembly of samples treated at urea concentrations higher than 3.1−3.2 M. This coincides with the concentration of urea, where protein loses detectable secondary structure. Thus, the hydrophobic residual structure may be involved in interactions that could lead to misfolded states of GroEL that are kinetic traps in its refolding/reassembly pathway. This would be consistent with suggestions that folding of GroEL, just as with many other proteins, could benefit from interactions with preformed chaperonins, as has been observed (Lissen et al., 1990). The residual structure detected here is the type that has been suggested to be capable of binding to native GroEL, thereby preventing kinetic trapping (Horowitz et al., 1995).

The results of this work can be summarized in Scheme 1. This scheme suggests that moderate concentrations of urea can induce the formation of monomers that can reassemble to functional 14mers. Increasing the urea concentration leads partial unfolding to produce an ensemble of states with little regular secondary structure, but which contain hydrophobic clusters within the portions of the sequence that form the

apical domain. These structures can lead to misfolded forms that kinetically trap GroEL and prevent the facile acquisition of native structure. Further additions of urea would unfold the residual structure and produce states that are more completely unfolded. This scheme is consistent with the observations that completely unfolded GroEL has not been observed to reform 14mers, although the oligomers can be formed from GroEL that has been denatured under less harsh conditions. Thus, this residual structure might have important consequences, including the following: (1) it could nucleate tertiary structure during folding; (2) its position in the center of the sequence could contribute to the registration of the N- and C- terminal sequences of GroEL that are required for the acquisition of its interesting native fold; and/or (3) it could represent an organized hydrophobic region that could interact with partially folded proteins as part of the functional cycle of this chaperonin.

NOTE ADDED IN PROOF

It has recently been demonstrated that GroEL can be refolded from the completely denatured state. [Ybarra, J., & Horowitz, P. M. (1995) *J. Biol. Chem.* (in press).]

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BI9513867