Reversible Oligomerization and Denaturation of the Chaperonin GroES†

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ABSTRACT: The chaperonin GroEL can assist protein folding and normally acts with the co-chaperonin GroES. These *Escherichia coli* proteins are encoded on the same operon, with GroES positioned first. In this report, we have investigated the reversible folding of GroES. Using fluorescence anisotropy of dansyl-labeled GroES, intrinsic fluorescence, bis-ANS binding, sedimentation velocity, and limited proteolysis, we show that GroES unfolds in a single, two-state transition. Importantly, intrinsic fluorescence and sedimentation velocity analyses show that GroES is capable of refolding and reassembling from a urea denatured state. The refolded GroES is fully active as shown by its ability to assist GroEL in the refolding of rhodanese. These results indicate that chaperonins may not require other chaperonins for successful folding/assembly. We also show that GroES is capable of assisting in the refolding/reassembly of fully denatured GroEL. The reversible folding of GroES coupled with the ability of GroES to assist the refolding/reassembly of GroEL suggest that the *groE* operon may be organized in a manner that provides a structural role in GroES/GroEL assembly as well as a functional role.

The chaperonins are a diverse class of proteins whose functions include roles in protein trafficking, protein assembly, and protein folding (Gething & Sambrook, 1992). The most widely studied chaperonins are the GroE proteins of *Escherichia coli*, GroEL and GroES (Georgopoulos & Ang, 1990). Both chaperonins are encoded by separate genes contained within the *groE* operon (Zeilstra-Ryalls et al., 1991). Of the two proteins, GroEL has been the most extensively studied. GroEL is a homotetradecamer of 57 kDa subunits arranged in two heptameric rings that are stacked together. A 2.8 Å X-ray crystal structure was recently determined for GroEL (Braig et al., 1994). GroEL possesses a weak K^+ dependent ATPase activity that is partially inhibited by the presence of GroES (Hendrix, 1979; Chandrasekhar et al., 1986; Todd et al., 1994). The current models for chaperonin action suggest that the partially folded polypeptides bind to GroEL and that their release is modulated by GroES (Azem et al., 1994; Weissman et al., 1994; Schmidt et al., 1994).

Functionally, GroES has been shown to modulate the ATPase activity of GroEL (Hendrix, 1979; Chandrasekhar et al., 1986; Todd et al., 1994). Structurally, GroES is a homoheptamer composed of 10 kDa subunits. Unlike the double-toroidal arrangement of GroEL, GroES is organized as a single-ring structure. An X-ray crystal structure of GroES has recently been determined (Hunt et al., 1996). The core of the subunit structure forms a topologically irregular β-barrel.

The question arises that if chaperonins assist the folding of newly synthesized polypeptides, what chaperones the chaperonins? Until recently, attempts at the refolding/reassembly of GroEL oligomers have been unsuccessful (Price et al., 1993). Lissin and co-workers showed that GroEL can facilitate its own self-assembly in an ATP dependent fashion (Lissin et al., 1990; Lissin, 1995). Recent results in this lab have shown that structured, monomeric GroEL can be reassembled into a functional tetradecamer without the requirement of preassembled GroEL from a folded, monomeric state (Mendoza et al., 1994) or from a urea-denatured state in the presence of stabilizing agents (Ybarra & Horowitz, 1995).

The successful chemical synthesis of functional GroES suggests that the sequence of GroES contains all the information required for successful folding (Mascagni et al., 1991). In those experiments, a heterogeneous population of GroES oligomers was generated. This heterogeneity may be a consequence of the chemistry of peptide synthesis, or the multiple species may result from kinetic "traps" in the folding/assembly pathway of GroES. In light of these considerations, we have further investigated the urea induced dissociation and unfolding of GroES.

In the present work, we show that urea can dissociate and unfold GroES in a single, two-state transition as monitored by several different techniques. Analytical ultracentrifugation and intrinsic fluorescence show that the dissociation and unfolding are reversed upon removal or dilution of the urea. GroES that has been refolded and reassembled is fully capable of assisting GroEL in the refolding of denatured rhodanese. In addition, GroES is capable of assisting the refolding of urea denatured GroEL. The spontaneous refolding of GroES coupled with its ability to assist the refolding of GroEL hints at a functional role for the organization of the GroESL operon.

EXPERIMENTAL PROCEDURES

Reagents. Dansyl chloride[1] and bis-ANS were purchased from Molecular Probes Inc. (Eugene, OR). All reagents were of analytical grade.

Protein Purification. GroES was purified essentially as described previously (Staniforth et al., 1994). GroES that

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[1] Abbreviations: dansyl chloride, 2-(dimethylamino)naphthalene-5-sulfonyl chloride; dns-ES, dansyl-labeled GroES; bis-ANS, 4,4′-bis-(1-anilino-8-naphthalenesulfonic acid); TEA, triethanolamine acetate.

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had been purified according to this method was further purified by gel filtration on a Sephacryl S-200 column. Fractions containing purified GroES were pooled, concentrated, and stored in 70% ammonium sulfate at 4 °C. GroEL was purified as described previously (Seale et al., 1995). Bovine liver rhodanese was purified as previously described (Miller et al., 1992). Protein concentrations were determined by the method of Bradford (1976).

Dansyl Chloride Labeling of GroES. GroES (7.5 mg/mL) was dialyzed against 0.1 M carbonate buffer, pH 9.5, overnight to remove any traces of Tris-HCl and ammonium sulfate. A slight molar excess of dansyl chloride was added to the protein solution, and the mixture was incubated at room temperature for 2 h. The solution was then dialyzed against 50 mM Tris HCl, pH 7.8, at 4 °C overnight to remove any unincorporated dansyl chloride. For anisotropy measurements, the samples were excited at 374 nm and the emission was measured at 480 nm. Each measurement was the average of 25 readings.

Equilibrium Unfolding/Refolding of GroES. GroES (0.5 mg/mL) in 10 mM Tris-HCl, pH 7.8, 100 mM NaCl, was incubated in various concentrations of urea for 1 h at room temperature. For bis-ANS binding studies, GroES was incubated for 1 h at room temperature in buffer containing urea. Bis-ANS was then added to a final concentration of 10 μM, and the fluorescence of the bound bis-ANS at 500 nm was recorded. To allow for refolding, unfolded GroES was dialyzed against buffer without urea for 4 h before sedimentation velocity experiments. For measurement of refolding activity and intrinsic fluorescence, GroES was unfolded in 8 M urea and diluted to the appropriate final urea concentrations. These samples were incubated at room temperature for 2 h prior to recording activity or intrinsic fluorescence at 310 nm.

Sedimentation Velocity Analysis. Samples of GroES were subjected to sedimentation velocity analysis using a Beckman XL-A analytical ultracentrifuge. For each run, GroES concentration was 0.5 mg/mL. The temperature was kept constant at 25 °C. The rotor speed was 40 000 rpm for all samples. The scans were analyzed by the method of van Holde and Weishet (1978) using the UltraScan data collection and analysis program (B. Demeler, Department of Biochemistry, University of Texas Health Sciences Center, San Antonio, TX). All data were corrected for buffer density and viscosity.

Limited Proteolysis of GroES. GroES (1 mg/mL) was incubated in 50 mM NH_4HCO_3, pH 7.8, containing various concentrations of urea and 5% (w/w) V8 protease. Aliquots were removed at various time points and boiled for 4 min in SDS loading buffer, and the peptides were separated on Tricine−SDS gels as described by Schägger and von Jagow (1987).

Refolding of GroEL in the Presence of GroES. GroEL (10 mg/mL) was denatured in 50 mM triethanolamine acetate, pH 7.5, 1 mM DTT, 0.1 mM EDTA, and 8 M urea at room temperature for 4 h. An aliquot of denatured GroEL was then diluted into 50 mM TEA containing GroES and MgATP to give the following final concentrations: 50 mM TEA, pH 7.5, 0.5 M urea, 10 mM $MgCl_2$, 5 mM ATP, 0.1 mg of GroES/mL, and 0.625 mg of GroEL/mL. These samples were then incubated at room temperature for various times. Aliquots from each sample were then analyzed by non-denaturing polyacrylamide electrophoresis (Neahoff et al., 1986).

Activity Measurement of Refolded GroES. GroES was incubated at various urea concentrations for 1 h at room temperature. These samples were then diluted into 50 mM Tris-HCl (pH 7.5) supplemented with urea to make all samples 0.45 M urea. The diluted protein was then incubated at room temperature for at 2 h. The sample designated "fresh GroES" was kept concentrated and cold prior to the rhodanese assay. Rhodanese activity was measured as described (Miller et al., 1992). The results are presented as the percent recovery of native rhodanese activity. All results are the average of three trials with standard deviations of less than 5%.

RESULTS

Unfolding of GroES Can Be Followed by Several Physical Techniques. To follow the urea denaturation of GroES, we chose physical techniques that would monitor changes in quaternary structure as well as the overall integrity of the protein structure. Figure 1A shows the urea denaturation of GroES as followed by various fluorescence techniques. The denaturation of GroES as monitored by tyrosine intrinsic fluorescence (Figure 1A, open squares), fluorescence anisotropy of dansyl-labeled GroES (Figure 1A, open circles), and bis-ANS fluorescence (Figure 1A, open triangles) can be described as a two-state process. These data were fitted according to the method described by Pace (1990). Thermodynamic parameters were derived from these fits and are summarized in Table 1. These parameters are all very similar, suggesting that the same transition is being monitored by each technique and that there is no evidence for intermediates. The midpoint of these transitions lies near 3 M urea. In addition, the midpoints of these transitions are shifted to higher [urea] as the GroES concentration is increased. Increasing the GroES concentration 5-fold results in a shift in the midpoint of the transitions approximately 0.5 M higher in [urea] (data not shown). This result suggests that an oligomerization process is being monitored.

Sedimentation velocity measurements were used to monitor the oligomerization state of GroES. Figure 1B shows the urea dependence of the sedimentation velocity of GroES. At urea concentrations corresponding to the initial plateau in Figure 1A, GroES is homogeneous with an $S_{20,w}$ value near 4.1 S (Figure 1B, open data points). Likewise, at urea concentrations corresponding to the post-transition baseline in Figure 1A, GroES is homogeneous with an $S_{20,w}$ value near 1.0 S (Figure 1B, closed diamonds). Within the transition region followed by the fluorescence techniques, sedimentation velocity analysis reveals that the protein is composed of populations of both large and smaller components (Figure 1B, closed circles, closed squares, closed triangles). Finite element analysis (Borries Demeler, in preparation) confirmed that GroES before the transition has a molecular mass of approximately 70 kDa (data not shown). These data also appear to be following the same process monitored by the fluorescence techniques. In total, the fluorescence and sedimentation velocity results suggest that GroES unfolds with no apparent equilibrium intermediates.

Proteolytic susceptibility indicates that the overall integrity of the protein structure is lost during these transitions (Figure 2). GroES was incubated at various [urea] in the presence of 5% (w/w) V8 protease. Proteolysis was carried out for 5 min. There is only one proteolysis site exposed at [urea] of 1M or less (Figure 2, lanes B and C). Before the transition

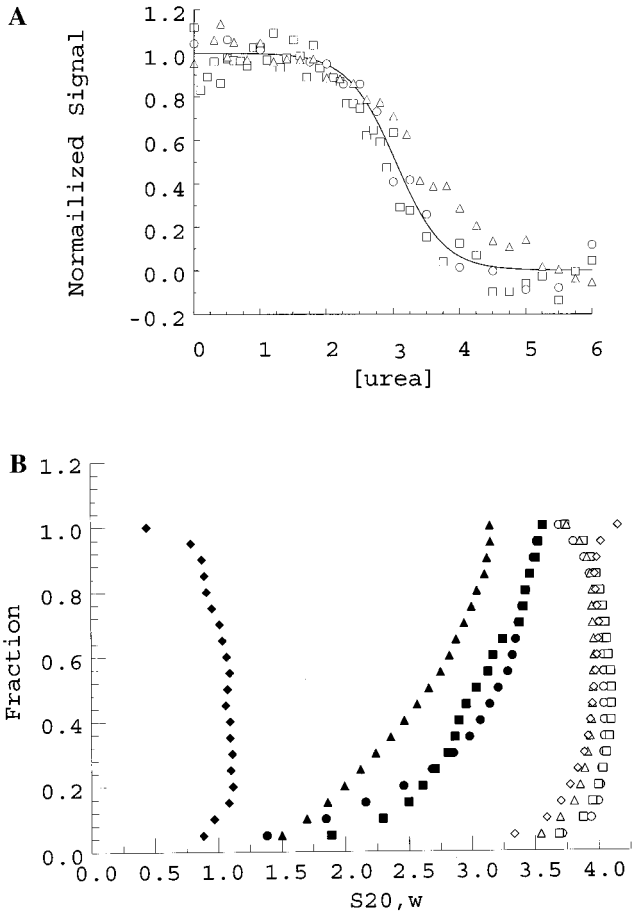

FIGURE 1: Reversible folding of GroES. (A) GroES (0.5 mg/mL in 10 mM Tri- HCl, pH 7.8, 100 mM NaCl) was incubated in various concentrations of urea for a minimum of 1 h at room temperature. Fluorescence measurements were recorded at room temperature. Tyrosine intrinsic fluorescence is illustrated by the open squares. Fluorescence anisotropy of dansyl-labeled GroES is shown by the open circles. The binding of the non-covalent hydrophobic probe bis-ANS (final concentration of 10 μM) is depicted with open triangles. The solid line corresponds to the fit of the anisotropy data. (B) For sedimentation velocity measurements, samples were dialyzed against the appropriate buffer overnight prior to centrifugation. During centrifugation, the absorbance of the cells was monitored at 230 nm. Data were collected and analyzed as described in Experimental Procedures. Symbols correspond to GroES dialyzed against the following urea concentrations (M): 0.0, open circles; 1.0, open squares; 1.5, open triangles; 2.0, open diamonds; 2.5, closed squares; 2.75, closed circles; 3.0, closed triangles; 4.0, closed diamonds.

Table 1: Derived Thermodynamic Parameters from GroES Unfolding Transitions

method[a]	ΔG_{H_2O}[b]	m[c]	$[urea]_{1/2}$[d]
intrinsic fluorescence	4.43 ± 0.4	1.53 ± 0.2	2.90
intrinsic (reversibility)	5.77 ± 1.3	1.83 ± 0.4	3.15
bis-ANS binding	3.53 ± 0.4	1.03 ± 0.1	3.43
DNS anisotropy	4.94 ± 0.6	1.63 ± 0.2	3.03

[a] All methods refer to the curves shown in Figure 1A except for intrinsic (reversibility) which corresponds to Figure 3A (open squares). [b] In kcal mol^{-1}. [c] In kcal mol^{-1} M^{-1}. [d] $[urea]_{1/2} = \Delta G_{H_2O}/m$ (molar).

(2 M urea), GroES becomes much more susceptible to V8 protease (Figure 2, lane D), as shown by an increase in the number of peptide fragments generated. This [urea] corresponds to the point before dissociation as monitored by sedimentation velocity (Figure 1B). At 4 M urea (lane E), there is an increase in the amount of proteolysis, but no detectable, new fragments. By 6 M urea (lane F), the V8

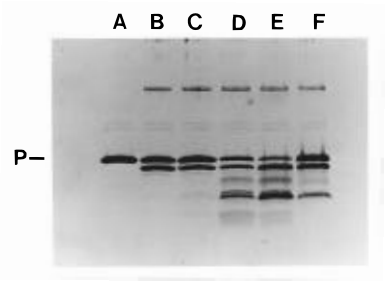

FIGURE 2: Proteolytic susceptibility of GroES. Proteolysis of GroES in the presence of urea was performed as described in Experimental Procedures. Proteolysis was carried out for 5 min at room temperature. Lane A, undigested GroES; lane B, 0 M urea; lane C, 1 M urea; lane D, 2 M urea; lane E 4 M urea; lane F, 6 M urea. The parent GroES (MW 10 000) is denoted by P. The band near the top of the gel in lanes B−F corresponds to the V8 protease.

protease begins to denature as evidenced by decreased proteolysis. Similar proteolytic susceptibility of GroES as a function of [urea] was observed with proteinase K and chymotrypsin (data not shown).

GroES Denaturation Is Reversible. Figure 3 illustrates that the denaturation of GroES can be reversed by removal or dilution of the urea. Figure 3A shows the unfolding and refolding of GroES followed by intrinsic fluorescence. GroES was unfolded in urea, and the intrinsic fluorescence was measured after 2 h to generate the unfolding curve (Figure 3A, open circles). For refolding, urea denatured GroES was diluted into buffer to give various final urea concentrations. After 2 h of refolding, the intrinsic fluorescence was recorded (Figure 3a, open squares). The derived thermodynamic parameters (Table 1) show that the transitions are similar. There is an increase in the intrinsic fluorescence at urea concentrations greater than 6 M. To insure that this was not another unfolding transition, the fluorescence of *N*-acetyltyrosine ethyl ester was followed as a function of [urea]. This model tyrosine compound also showed a similar increase in fluorescence intensity at urea concentrations above 6 M (data not shown), indicating that this is not another structural transition. This result is similar to that reported for tyrosine by Schmid (1989). Sedimentation velocity measurements were also used to monitor the oligomeric structure of GroES along the refolding transition. Native GroES has a sedimentation coefficient of approximately 4.1 S (Figure 3B, squares). GroES incubated in 8M urea has a sedimentation coefficient near 1.0 S (Figure 3b, triangles). Samples of GroES that have been unfolded in 8 M urea regain a sedimentation coefficient similar to native GroES when dialyzed against buffer without urea (Figure 3B, circles). Unlike the work of Mascagni et al. (1993) where multiple oligomeric species were formed during chemical synthesis of GroES, the refolding from a urea denatured state appears to give a single oligomeric species as indicated by the uniformity of the $S_{20,w}$ profile. The shape of the $S_{20,w}$ plot of the native GroES indicates that there is some heterogeneity in the original sample (van Holde & Weishet, 1978). It is interesting that this heterogeneity is reduced after denaturation and refolding of the GroES.

Refolded GroES Is Active. Another indication of the reversibility of GroES denaturation is given by the regain of GroES "activity". Table 2 shows the ability of refolded GroES to assist GroEL in refolding denatured rhodanese. GroES samples that had been incubated at [urea] below the denaturation transition (i.e., 0, 1, and 2 M) display activity equal to the controls. Interestingly, the control activity in

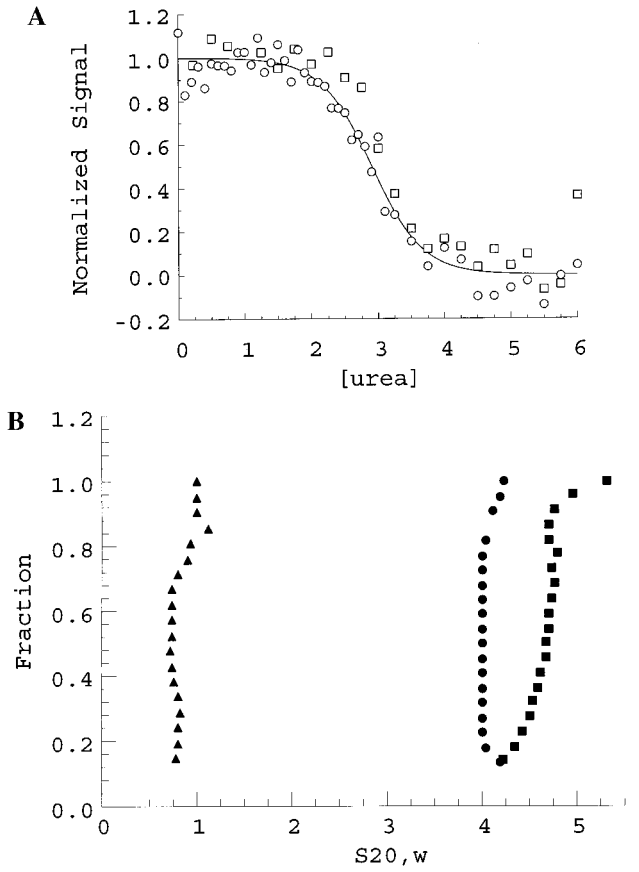

FIGURE 3: GroES denaturation is reversible. (A) GroES was denatured and renatured in urea as described in Experimental Procedures. The intrinsic fluorescence of the single tyrosine was followed by excitation at 274 nm and measuring the emission at 310 nm. Emission intensities were recorded for 30 s (60 data points) and the points presented as an average of that measurement. Denaturation is indicated by the open circles, refolding is shown with the open squares. The solid line corresponds to the fit of the unfolding data. (B) GroES was incubated in various [urea] for at least 4 h and subsequently dialyzed overnight against buffer without urea. Data were collected and analyzed as described in Experimental Procedures. Analysis is presented as the integral distribution of S values for GroES. The y-axis indicates the fraction of material with $S_{20,w}$ values less than or equal to the values given on the *x*-axis. A single, pure component displaying ideal behavior would be represented by a vertical line that intercepts the *x*-axis at the appropriate $S_{20,w}$ value. Samples correspond to native GroES (squares), GroES in 8 M urea (triangles), and GroES refolded from 8 M urea (circles).

Table 2: Ability of Refolded GroES to Assist GroEL in Rhodanese Refolding

GroES sample	% native rhodanese activity regained
"fresh GroES"	38
0 M urea	25
1 M urea	31
2 M urea	26
4.5 M urea	41
6.4 M urea	58

these experiments is less than the activity of GroES that is kept cold and concentrated prior to activity measurements (Table 2, "fresh GroES"). This suggests that GroES undergoes some changes in dilute solution at room temperature that may result in the loss of activity. GroES that is incubated at [urea] higher than the denaturation transition (i.e., 4.5 and 6.4 M) and which is then refolded displays activity higher than either the control or GroES that is freshly diluted from the stock. These results indicate that refolding

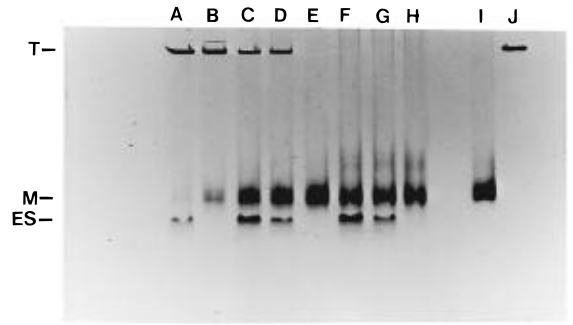

FIGURE 4: GroES can assist the refolding of GroEL. GroEL was denatured and refolded as described in Experimental Procedures. Lane A, GroEL diluted into buffer + MgATP, 0.1 M ammonium sulfate and a stoichiometric amount of monomeric GroES; lane B, GroEL diluted into buffer + MgATP and 0.1 M ammonium sulfate; lane C, GroEL diluted into buffer + MgATP and a 2-fold excess of monomeric GroES; lane D, GroEL diluted into buffer + MgATP and a stoichiometric amount of monomeric GroES; lane E, GroEL diluted into buffer + MgATP; lane F; GroEL diluted into buffer + 2 fold excess of monomeric GroES; lane G, GroEL diluted into buffer + stoichiometric amount of monomeric GroES; lane H, GroEL diluted into buffer only; lane I, unfolded GroEL; lane J, native GroEL. Native GroEL is denoted by T, monomeric GroEL is denoted by M, and GroES is denoted by ES.

after urea denaturation can reverse the effects of incubating GroES at room temperature for an extended period of time.

GroES Is Able To Assist the Refolding/Reassembly of GroEL. Previous studies have suggested that Mg−nucleotide is required for the self assembly of urea-denatured GroEL and that this process is stimulated by GroES (Lissin et al., 1990; Lissin, 1995). In those studies complete regain of native GroEL was unsuccessful. With those studies in mind, we investigated the ability of GroES to assist the refolding/assembly of GroEL. Figure 4 shows that GroES can assist the refolding/assembly of urea denatured GroEL. GroEL was unfolded in 8 M urea, and then diluted into buffer so that the final [urea] was 0.5 M. Conditions were selected to determine the effects of MgATP as well as GroES. GroEL diluted from 8 M urea into buffer containing no MgATP or GroES gives no GroEL tetradecamer (Figure 4, lane H). Likewise, the addition of a stoichiometric amount of monomeric GroES (Figure 4, lane G) or a 2-fold excess of monomeric GroES (Figure 4, lane F) results in no recovery of native GroEL. However, when GroES and MgATP are added to the refolding buffer, tetradecameric GroEL is produced (Figure 4, lanes C and D). Analysis by gel filtration HPLC indicates that approximately 75% of the monomeric GroEL is reassembled into tetradecameric GroEL when GroES is added to the buffer containing MgATP (data not shown). Dilution of GroEL into buffer containing MgATP does not result in recovery of detectable amounts of native GroEL (Figure 4, lane E). It does not appear that increasing the amount of GroES increases the recovery of GroEL further (Figure 4, lane D vs lane C).

A recent study has shown that GroEL can be refolded from 8 M urea by dilution into buffer containing small amounts of the stabilizing agent $(NH_4)_2SO_4$ (Ybarra & Horowitz, 1995). Figure 4, lane B, shows the refolding of GroEL under such conditions. Using gel filtration HPLC, it was determined that 75% of the native GroEL was recovered upon dilution into buffer containing $(NH_4)_2SO_4$ and MgATP (data not shown). However, a "new" higher molecular weight species appears in this sample (Figure 4, lane B). The addition of a stoichiometric amount of monomeric GroES to the $(NH_4)_2SO_4$ buffer results in almost complete recovery

(98%) in the amount of native GroEL (Figure 4, lane A), as determined by HPLC (data not shown). Interestingly, the higher molecular weight species does not appear when GroES is added to the $(NH_4)_2SO_4$ refolding buffer (lane A vs lane B). It is tempting to speculate that the inclusion of GroES helps to reduce nonproductive folding/assembly of GroEL. These results suggest that GroES can play an active role in assisting the proper refolding/reassembly of GroEL.

DISCUSSION

Because the chaperonins possess the ability to aid in the folding of newly synthesized proteins, the question has been asked, "what helps the chaperonins to fold?" The successful chemical synthesis of GroES suggested that GroES could spontaneously fold (Mascagni et al., 1993). However, multiple species were formed by this method. Whether these other species were products of the peptide chemistry or represented kinetic traps in the folding was unclear. The present study demonstrates that GroES can be completely refolded from a urea-denatured state without the generation of misfolded species. In addition, the refolded GroES is fully capable of assisting GroEL in the refolding of rhodanese. In summary, GroES is able to refold without the assistance of any other chaperonins and it appears that there are no "traps" that result in misfolded GroES.

The successful refolding of GroES also has implications for the refolding of GroEL. Until very recently, it has been difficult to refold urea denatured GroEL (Price et al., 1993; Mendoza et al., 1994; Lissin et al., 1990; Ybarra & Horowitz, 1995). Previous studies have demonstrated that GroEL that had been disassembled in 4 M urea could reassemble in the presence of MgATP (Lissin et al., 1990). It is unclear whether GroEL is completely unfolded at this urea concentration. In fact, a recent study indicates that GroEL contains some hydrophobic residual structure at urea concentrations as high as 4 M (Gorovits et al., 1995). In the present work, GroEL was unfolded completely in 8 M urea under conditions where it was not able to reassemble in the presence of MgATP. However, when GroES was included in the refolding buffer, native GroEL was recovered (Figure 4, lanes C and D). The requirement of MgATP for this GroES stimulated recovery hints at a role for the "self-chaperoning" mechanism proposed by Lissin et al (1990).

Another recent report has demonstrated that GroEL can be refolded from 8 M urea upon dilution into buffer containing the MgATP and the stabilizing agent $(NH_4)_2SO_4$ (Ybarra & Horowitz, 1995). However, under these circumstances, higher molecular weight species are also generated (Figure 4, lane B). Interestingly, when a stoichiometric amount of GroES is added, the higher molecular weight species are no longer formed. The ammonium sulfate does not appear to be required to form native GroEL. Figure 4, lanes C and D, shows that GroES and MgATP are able to produce tetradecameric GroEL. Interestingly, the amount of native GroEL produced (75%) is the same for refolding in ammonium sulfate or GroES and MgATP. This result supports the suggestion that GroES can assist the correct refolding and assembly of GroEL.

The reversible folding and assembly of GroES has interesting implications about the function of GroES. The dual abilities of GroES to spontaneously refold/reassemble and to assist the refolding/reassembly of GroEL suggests a structural as well as a functional role for the organization of the *groE* operon. Not only does the organization of the groE genes into a single operon have importance in genetic regulation, it may also provide an important structural function at the protein level.

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REFERENCES

Azem, A., Kessel, M., & Goloubinoff, P. (1994) *Science 265*, 653−656.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
Braig, K., Otwinowski, Z., Hedge, R., Boisvert, D. C., Joachimiak, A., Horwich, A. L., & Sigler, P. B. (1994) *Nature 371*, 578−586.
Chandrasekhar, G. N., Tilly, K., Woolford, C., Hendrix, R. W., & Georgopolous, C. (1986) *J. Biol. Chem. 261*, 12414−12419.
Gething, M. J., & Sambrook, J. (1992) *Nature 355*, 33−45.
Georgopoulos, C., & Ang, D. (1990) *Semin. Cell Biol. 1*, 19−25.
Gorovits, B. M., Seale, J. W., & Horowitz, P. M. (1995) *Biochemistry 34*, 13928−13933.
Hendrix, R. W. (1979) *J. Mol. Biol. 129*, 375−392.
Hunt, J. F., Weaver, A. J., Landry, S. J., Gierasch, L., & Deisenhofer, J. (1996) *Nature 379*, 37−45.
Lissin, N. M., (1995) *FEBS Lett. 361*, 55−60.
Lissin, N. M., Venyaminov, S. Y., & Girshovich, A. S. (1990) *Nature 348*, 339−342.
Mascagni, P., Tonolo, M., Ball, H., Lim, M., Ellis, R. J., & Coates, A. (1991) *FEBS Lett. 286*, 201−203.
Mendoza, J. A., Demeler, B., & Horowitz, P. M. (1994) *J. Biol. Chem. 269*, 2447−2451.
Miller, D. M., Kurzban, G. P., Mendoza, J. A., Chirgwin, J. M., Hardies, S. C., & Horowitz, P. M. (1992) *Biochim. Biophys. Acta 1121*, 286−292.
Neahoff, V., Cheong-Kim, K., & Altland, K. (1986) *Electrophoresis 7*, 56−57.
Pace, C. N. (1990) *Trends Biotechnol. 8*, 93−98.
Price, N. C., Kelley, S. M., Thomson, G. J., Coggins, J. R., Wood, S., & auf der Mauer, A. (1993) *Biochim. Biophys. Acta 1161*, 52−58.
Schägger, H., & von Jagow, G. (1987) *Anal. Biochem. 166*, 368−379.
Schmid, F. X. (1989) in *Protein Structure: A Practical Approach* (Creighton, T. E., Ed.) pp 251−285, IRL Press, Oxford, England.
Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P., Lorimer, G. H., & Buchner, J. (1994) *Science 265*, 656−659.
Seale, J. W., Martinez, J. L., & Horowitz, P. M. (1995) *Biochemistry 34*, 7443−7449.
Staniforth, R. A., Cortes, A., Burston, S. G., Atkinson, T., Holbrook, J. J., & Clarke, A. R. (1994) *FEBS Lett. 344*, 129−135.
Todd, M. J., Viitanen, P. V., & Lorimer, G. H. (1993) *Biochemistry 32*, 8560−8567.
van Holde, K. E., & Weischet, W. O. (1978) *Biopolymers 17*, 1387−1403.
Weissman, J. S., Kashi, Y., Fenton, W. A., & Horwich, A. L. (1994) *Cell 78*, 693−702.
Ybarra, J., & Horowitz, P. M. (1995) *J. Biol. Chem. 270*, 22113−22115.
Zeilstra-Ryalls, J., Fayet, O., & Georgopolous, C. (1991) *Annu. Rev. Microbiol. 45*, 301−325.

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