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Nucleotide binding-promoted conformational changes release a nonnative polypeptide from the *Escherichia coli* chaperonin GroEL

(chaperones/surface plasmon resonance)

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ABSTRACT **The *Escherichia coli* chaperonins GroEL and GroES facilitate the refolding of polypeptide chains in an ATP hydrolysis-dependent reaction. The elementary steps in the binding and release of polypeptide substrates to GroEL were investigated in surface plasmon resonance studies to measure the rates of binding and dissociation of a nonnative variant of subtilisin. The rate constants determined for GroEL association with and dissociation from this variant yielded a micromolar dissociation constant, in agreement with independent calorimetric estimates. The rate of GroEL dissociation from the nonnative chain was increased significantly in the presence of 5′-adenylylimidodiphosphate (AMP-PNP), ADP, and ATP, yielding maximal values between 0.04 and 0.22 s^{-1}. The sigmoidal dependence of the dissociation rate on the concentration of AMP-PNP and ADP indicated that polypeptide dissociation is limited by a concerted conformational change that occurs after nucleotide binding. The dependence of the rate of release on ATP exhibited two sigmoidal transitions attributable to nucleotide binding to the distal and proximal toroid of a GroEL–polypeptide chain complex. The addition of GroES resulted in a marked increase in the rate of nonnative polypeptide release from GroEL, indicating that the cochaperonin binds more rapidly than the dissociation of polypeptides. These data demonstrate the importance of nucleotide binding-promoted concerted conformational changes for the release of chains from GroEL, which correlate with the sigmoidal hydrolysis of ATP by the chaperonin. The implications of these findings are discussed in terms of a working hypothesis for a single cycle of chaperonin action.**

An increasing body of evidence has implicated several heat shock proteins as molecular chaperones that control intracellular protein folding (1). Among these proteins, the GroES and GroEL chaperonins have been intensively studied at the genetic, biochemical, enzymological, and structural levels (2, 3). Much insight into the manner whereby GroEL and GroES participate in the acquisition of native polypeptide structure comes from extensive *in vitro* protein-folding studies, which have given rise to a number of suggestions for the ways that chaperones could facilitate protein folding in cells (4–8). These studies have described in general terms the multiple rounds of polypeptide binding to and release from GroEL that is controlled by ATP hydrolysis. However, numerous questions remain to be addressed for a molecular description of the complex process of chaperonin-promoted protein folding.

While protein folding studies offer the advantage of directly observing the functional properties of chaperonins, the use of substrates that are progressively adopting native structure during the course of a reaction may obscure important information about the elementary steps of chaperonin function. We have therefore taken an alternative approach by investigating the interaction of GroEL with a nonnative variant of subtilisin BPN′ that is unable to refold but which is fully soluble in neutral buffers without denaturants (9). In this way, we sought to distinguish between the elementary rates of binding and release of nonnative substrates from the rate of polypeptide refolding. The surface plasmon resonance studies described here have been used to demonstrate that a rate-limiting, concerted conformational change in GroEL occurs after nucleotide binding to promote the release of nonnative chains. A consideration of the principles of coupled vectorial processes (10) has been used to explain the unusual effect of ATP on the rate of substrate dissociation and to suggest that the energy available from nucleotide hydrolysis is utilized by GroEL to promote an unfolding of nonnative folding intermediates.

EXPERIMENTAL PROCEDURES

The interaction of GroEL with subtilisin BPN′ PJ9 was analyzed with a BIAcore biosensor (Pharmacia). The nonnative subtilisin variant was immobilized on the dextran surface to increase the relative ease of surface regeneration, to minimize nonspecific aggregation of the nonnative chain on the matrix, and to increase the signal amplitude upon binding GroEL to the lower molecular weight substrate. Importantly, the level of subtilisin immobilization was kept at a low level, typically between 100 and 150 resonance units (RU) to minimize the effects of GroEL rebinding to the surface after dissociation. Subtilisin BPN′ PJ9 was immobilized on a CM5 sensor chip by using the amine coupling kit (11). HBS buffer [10 mM Hepes, pH 7.4/150 mM NaCl/3.4 mM EDTA/0.005% nonionic surfactant Polysorbate 20 (P-20)] was used for coupling at a flow rate of 1 μl/min. The matrix was activated with 1 μl of a mixture of 0.05 M *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride (EDC)/0.2 M *N*-hydroxysuccinimide (NHS), and subtilisin was injected at a concentration of 0.12 mg/ml in 10 mM sodium acetate, pH 5.0, containing 5% dimethyl sulfoxide, 100 mM NaCl, and 0.005% P-20. Unreacted esters on the dextran surface were subsequently deactivated three times with 14 μl of 1 M ethanolamine, pH 8.5, washed one time with 0.5% SDS, and treated once more with 14 μl of ethanolamine. A final step that includes two, 2-μl washes with 6 M guanidinium chloride (GdmCl) was incorporated to dissociate any subtilisin that was noncovalently associated with the matrix. Although this method doubtless resulted in a heterogeneous mixture of immobilized substrate,

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Abbreviations: RU, resonance units; P-20, non-ionic surfactant Polysorbate 20; AMP-PNP, 5′-adenylylimidodiphosphate; ATP-γ-S, adenosine 5′-O-(3-thiotriphosphate); EDC, *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide.

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GroEL binding and dissociation could be adequately described by single-phase kinetics.

All binding and dissociation measurements were performed in 50 mM Tris·HCl, pH 7.8/150 mM KCl/0.5 mM EDTA/5 mM $MgCl_2$/0.1 mM dithiothreitol/0.005% P-20, at 25°C, where GroEL binding to immobilized subtilisin was specific, showing negligible affinity for the dextran matrix (~8 RU). Association and dissociation phases were analyzed with BIAevaluation software 2.0 (Pharmacia) to yield observed rate constants with less than 5% error. Dissociation rates were measured by first allowing GroEL to bind to subtilisin at a flow rate of 5 μl/min, after which the flow rate was increased to 40 μl/min to reduce the uncertainty attendant to analysis of the initial phase of dissociation. Typically, only the initial 10 s of the dissociation curves (comprising about 50 data points) were used to minimize complications due to rebinding.

The dependence of the observed rate constants for chaperonin dissociation from immobilized subtilisin in the presence of nucleotides were analyzed empirically in terms of one or two sigmoidal transitions. The following equation was used in analyses of the effect of ADP and 5′-adenylylimidodiphosphate (AMP-PNP) on the observed dissociation rates:

$$k_{obs} = k_0 + k_2[\text{nucleotide}]^{n_H}/(K_{0.5}{}^{n_H} + [\text{nucleotide}]^{n_H}), \quad [1]$$

where k_0 is the dissociation rate constant observed in the absence of nucleotides, k_2 is the maximal value for the observed rate constant, corresponding to the maximal rate for the conformational change that promotes release, $K_{0.5}$ is the midpoint of the curve, reflecting the nucleotide concentration that promotes half-maximal polypeptide dissociation from the chaperonin, and n_H, the Hill coefficient, empirically reflects the degree of sigmoidality of the curves. This analysis omits a term for k_{-2}, the rate constant for a reversible conformational change, since it was estimated to be negligibly low relative to k_0. The effect of ATP on the dissociation of GroEL from subtilisin was analyzed in terms of two sigmoidal transitions as indicated in the following equation:

$$k_{obs} = k_0 + k_2'[\text{nucleotide}]^{n_H'}/(K_{0.5}^{\prime n_H'} + [\text{nucleotide}]^{n_H'}) + k_2''[\text{nucleotide}]^{n_H''}/(K_{0.5}^{\prime\prime n_H''} + [\text{nucleotide}]^{n_H''}), \quad [2]$$

where the parameters are defined as above and the single- and double-primed terms correspond the first and second sigmoidal transitions. The effect of GroES on the GroEL–nucleotide–polypeptide complex was analyzed in terms of a two-step binding model, where the overall association constant, K_a, is equivalent to K_1 (K_2 + 1), where K_1 is the association constant for the encounter complex determined by the half-maximal GroES concentration and K_2 is the isomerization equilibrium constant given by k_2/k_0 since k_{-2} was negligibly small.

Subtilisin BPN′ PJ9 and GroEL were prepared as described (9); wild-type GroES was prepared as recently described (12).

RESULTS

Binding and Dissociation of GroEL to Immobilized, Nonnative Subtilisin. The addition of GroEL to a sensor chip with the nonnative subtilisin variant PJ9 covalently immobilized on the dextran layer yielded a time-dependent exponential increase in the effective refractive index, as seen in Fig. 1. The apparent rate of chaperonin association with subtilisin PJ9 was a linear function of GroEL concentration to about 2 μM oligomer, yielding an association rate constant of 5.4×10^3 $M^{-1}\cdot s^{-1}$. Higher concentrations of GroEL resulted in a low but reproducible level of nonspecific binding and were avoided. The dissociation of GroEL from PJ9 was measured by washing the surface with buffer and following the time-dependent exponential decrease in refractive index, yielding a dissociation rate constant of 2×10^{-3} s^{-1}. These estimates yield a value for the equilibrium dissociation constant for GroEL binding to nonnative PJ9 of about 0.4 μM, in reasonable agreement with that measured calorimetrically (9), and suggest a simple mode of chaperonin binding. Apparently, just a fraction of the immobilized subtilisin variant was available for GroEL binding since only about 20% of the expected RU signal was observed under maximal binding conditions, possibly due to chaperonin association solely with PJ9 immobilized at the surface of the dextran layer.

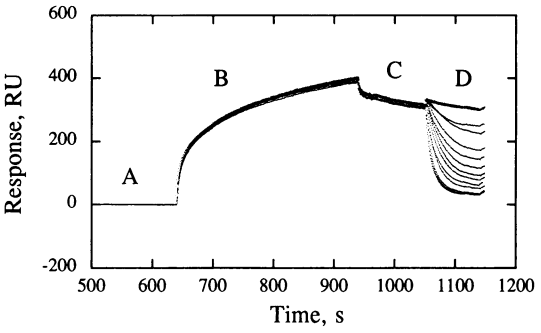

FIG. 1. Four phases in typical sensograms for the nucleotide-dependent interaction of GroEL and immobilized subtilisin BPN′ PJ9. The change in RU is proportional to the GroEL–subtilisin complex. (*A*) Baseline of buffer flow over immobilized subtilisin BPN′ PJ9. (*B*) Binding phase of 0.5 μM GroEL (oligomer) to subtilisin measured by continuous flow at a rate of 5 μl/min for 5 min. (*C*) A 2-min buffer wash of the GroEL–subtilisin complex that was formed on the sensor chip surface. (*D*) Dissociation of the bound GroEL from subtilisin BPN′ PJ9 upon the addition of nucleotides in the absence or presence of GroES at a flow rate of 40 μl/min. The actual traces represent a series of experiments in which the nucleotide concentration was varied in the presence of 0.63 μM GroES oligomers. Responses are given as increases over baseline values.

Effect of Nonhydrolyzable Nucleotides and GroES on the Dissociation of GroEL from Nonnative Subtilisin. The mechanism whereby nucleotides promote the release of nonnative chains from GroEL was investigated by measuring the concentration dependence of the observed monophasic rate for chaperonin dissociation from the immobilized, nonnative substrate. As can be seen in Fig. 2, the increase in the rate of GroEL dissociation follows a sigmoidal dependence on AMP-PNP concentration, reaching a maximum value of 0.035 s^{-1}. These results indicate that substrate dissociation from GroEL is limited by a concerted conformational change that follows nucleotide binding. The following scheme was therefore used

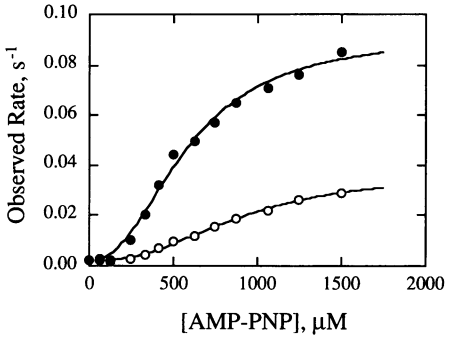

FIG. 2. Effect of AMP-PNP on the observed rate of GroEL dissociation from the nonnative subtilisin variant PJ9. The nucleotide dependence of the dissociation rates was analyzed by using Eq. **1**, with $k_0 = 2 \times 10^{-3}$ s^{-1} to yield values of $k_2 = 0.035$ s^{-1}, $K_{0.5} = 907$ μM, and $n_H = 2.4$ in the absence of GroES (○) and $k_2 = 0.090$ s^{-1}, $K_{0.5} = 584$ μM, and $n_H = 2.3$ in the presence of 0.63 μM GroES (●).

to analyze the nucleotide concentration dependence of the observed rate constants:

$$\text{EL-PJ9} + \text{AMP-PNP} \underset{k_{-1n}}{\overset{k_{1n}}{\rightleftharpoons}} \text{(AMP-PNP)-EL-PJ9} \underset{k_{-2n}}{\overset{\text{slow } k_{2n}}{\rightleftharpoons}}$$

$$\text{(AMP-PNP)-EL*-PJ9} \xrightarrow{\text{fast}} \text{(AMP-PNP)-EL*} + \text{PJ9},$$

where (AMP-PNP)-EL*-PJ9 is a conformational isomer of GroEL promoted by analog binding, k_{1n} and k_{-1n} represent nucleotide binding and dissociation steps, respectively, and k_{2n} and k_{-2n} correspond to a slow conformational transition which is followed by rapid nonnative substrate release.

The effect of GroES on this cooperative conformational change was to increase its rate, such that the limiting value of the release rate increases from $\approx 0.035\ s^{-1}$ to $0.090\ s^{-1}$ (Fig. 2). This effect was analyzed in terms of the following scheme:

$$\text{ES} + \text{(AMP-PNP)-EL-PJ9} \underset{k_{-1s}}{\overset{k_{1s}}{\rightleftharpoons}} \text{ES-(AMP-PNP)-EL-PJ9} \underset{k_{-2s}}{\overset{k}{\rightleftharpoons}}$$

$$\text{ES(AMP-PNP)-EL*-PJ9} \xrightarrow{\text{fast}} \text{ES-(AMP-PNP)-EL*} + \text{PJ9},$$

where k_{1s} and k_{-1s} are association and dissociation rate constants, respectively, for the formation of an encounter complex between GroES and the GroEL–nucleotide–polypeptide chain complex, and k_{2s} and k_{-2s} are the rate constants for the isomerization of the chaperonin complex. Because k_{-2s} was estimated to be negligibly small relative to the release that occurs in the absence of nucleotides ($\approx 0.002\ s^{-1}$), the effect of GroES is to increase k_{2s}, the rate of the isomerization, in a hyperbolic manner (Fig. 3). This result indicates that GroES binds more rapidly than the conformational change that promotes substrate release and yields an average dissociation constant for the initial binding of GroES to the GroEL–nucleotide complex of approximately 20 nM and an overall binding constant of 4.2×10^{-10} M. No increase in the rate of GroEL dissociation from subtilisin was detected upon the addition of GroES in the absence of nucleotides. Analysis of the data in Fig. 2 in terms of Eq. **1** indicated that GroES had little effect on the sigmoidality of the observed rate on AMP-PNP concentration, although the cochaperonin promoted a decrease in the half-maximal concentration of nucleotide required to populate the species that leads to rapid substrate dissociation.

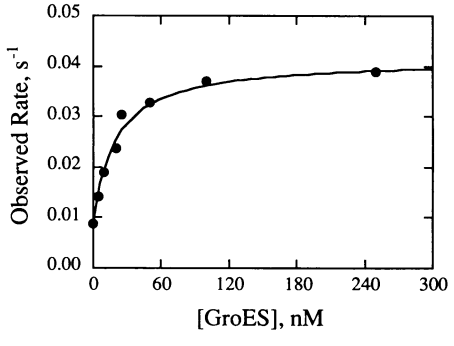

FIG. 3. Effect of GroES on the nucleotide-promoted dissociation of GroEL from nonnative subtilisin. The dependence of the observed dissociation rate on GroES concentration was measured at 500 μM AMP-PNP and was analyzed in terms of two-step binding to yield a dissociation constant for an encounter complex of 19.3 nM and an overall binding constant of 4.2×10^{-10} M.

Increasing ADP concentration similarly led to a sigmoidal increase in the rate of GroEL dissociation from the nonnative polypeptide. As can be seen in Fig. 4, the maximal rate of GroEL dissociation from PJ9 was estimated to be $0.083\ s^{-1}$, which was increased to $\approx 0.135\ s^{-1}$ in the presence of GroES. These must be considered lower limits for the dissociation rate of GroEL from nonnative subtilisin since the values approach the instrumental upper limit of BIAcore. In this case, there was little effect of GroES on the concentration of nucleotide required to reach half-maximal rates of chain release. The hyperbolic dependence of the polypeptide dissociation rate was half maximal at an oligomer concentration of 187 nM GroES, yielding an overall dissociation constant of GroES binding to the GroEL–ADP–polypeptide complex of 2.7 nM, consistent with previous estimates (7, 13).

Effect of ATP in the Absence and Presence of GroES on the Dissociation of GroEL from Nonnative Subtilisin. The effect of ATP on the rate of polypeptide release from GroEL was more unusual. As can be seen in Fig. 5, a "bisigmoidal" dependence of k_{obs} on ATP concentration was detected. These data were therefore analyzed empirically in terms of a sum of two sigmoidal transitions as presented in Eq. **2**, which describes sequential nucleotide binding-promoted conformational changes in each of the two GroEL toroids. This analysis yielded values of k'_2 of $0.021\ s^{-1}$ and k''_2 of $0.133\ s^{-1}$. The two sigmoidal transitions can be more readily seen in the presence of GroES, yielding values for k'_2 of $0.068\ s^{-1}$ and k''_2 of $0.218\ s^{-1}$. As in the case with ADP, the values for k''_2 must be considered lower limits. However, given the sigmoidal dependence of k_{obs} on AMP-PNP concentration and in light of the maximal increase in cooperativity of ATP hydrolysis by GroEL in the presence of GroES (4, 13), a Hill coefficient of 7 was assumed for the second sigmoidal transition, which enabled an analysis in terms of Eq. **2.** It is of interest, however, that there was little effect of GroES on the values estimated for the concentration of ATP required to reach half-maximal rates in each of the two transitions.

A similar hyperbolic dependence of the rate of release on the GroES concentration was observed for ATP as for analogs, which yielded average binding constants at both reduced (5 μM) and elevated (17.5 μM) ATP concentrations of about $1.5–4.5 \times 10^{-10}$ M (data not shown).

DISCUSSION

The surface plasmon resonance studies described here provide several clues regarding the elementary steps of chaperonin-facilitated protein folding. The dependence of the observed

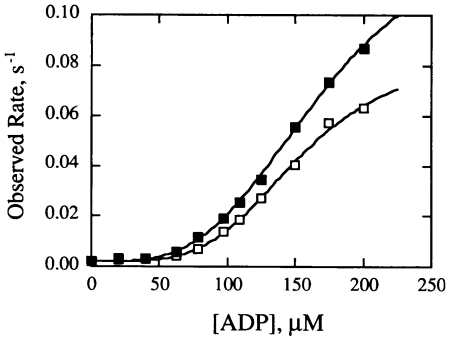

FIG. 4. Effect of ADP on the observed dissociation of GroEL from nonnative subtilisin. The theoretical curves were drawn as described in the legend to Fig. 2 by using values of $k_2 = 0.083\ s^{-1}$, $K_{0.5} = 153$ μM, and $n_H = 4.1$ in the absence of GroES (□) and $k_2 = 0.135\ s^{-1}$, $K_{0.5} = 170$ μM, and $n_H = 3.4$ in the presence of 0.63 μM GroES (■).

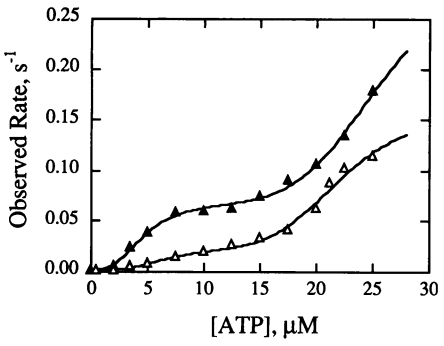

FIG. 5. Effect of ATP on the observed dissociation of GroEL from nonnative subtilisin. The ATP dependence of the dissociation rates in the absence of GroES (△) was analyzed by using Eq. **2**, with $k_0 = 2 \times 10^{-3}\ s^{-1}$ and $n_{H''} = 7$ to yield values of $k'_2 = 0.021\ s^{-1}$, $K'_{0.5} = 6.6\ \mu M$, and $n_{H'} = 3.6$ for the first transition and $k''_2 = 0.133\ s^{-1}$ and $K''_{0.5} = 21.9\ \mu M$ for the second transition. In the presence of 0.63 μM GroES (▲), analysis in terms of Eq. **2** yielded $k'_2 = 0.068\ s^{-1}$, $K'_{0.5} = 4.73\ \mu M$, and $n_{H'} = 2.8$ for the first transition and $k''_2 = 0.218\ s^{-1}$ and $K''_{0.5} = 25.1\ \mu M$ for the second transition.

rate of dissociation of the GroEL–subtilisin complex on the nonhydrolyzable nucleotides AMP-PNP and ADP indicates that a rate-limiting conformational change promotes nonnative protein release (Figs. 2 and 4). Since the dependence of the observed rate of release on nucleotide concentration must be proportional to the fraction of GroEL that has undergone a conformational change, its striking sigmoidal profile suggests that the conformational change that leads to substrate dissociation is highly concerted. Alternatively, the fraction of GroEL which undergoes this conformational change may reflect the chaperonin population in which one of the two toroids of GroEL is fully occupied with nucleotides. The concentration of this species would be a sigmoidal function of nucleotide concentration, even if they were bound in a simple, hyperbolic fashion, as seen for ADP binding to GroEL in microcalorimetric titrations (Z. W. White, F. P. Schwarz, and E.E., unpublished observations). However, any increase in the cooperativity of nucleotide binding would increase this species fraction as a function of ligand concentration, linking the cooperative binding of nucleotides to the conformational change that promotes substrate protein release. Although the rate of a "global" conformational change upon nucleotide binding to pyrene-labeled GroEL has been reported to be 180 s^{-1} (7) and may be required for GroES binding, a slower, subsequent conformational change apparently leads to the dissociation of nonnative chains.

A functional asymmetry in GroEL, in which the hydrolysis of ATP sequentially alternates between each of the two toroids of the chaperonin (4, 14–16), provides an essential framework to explain the two sigmoidal transitions of chain dissociation seen in the presence of ATP (Fig. 5). These two transitions, which are only barely detectable in the absence of GroES, can be rationalized in terms of synchronized conformational changes in each of the two toroids of GroEL which, to varying effect, increase the rate of dissociation of GroEL–polypeptide chain complexes. Interestingly, these transitions may be related to the two lines of cooperativity seen in nucleotide hydrolysis by GroEL (16), which have been interpreted in terms of concerted, intratoroidal nucleotide hydrolysis, with sequential, intertoroidal effects on activity. We attribute the first sigmoidal transition to a nucleotide binding-promoted conformational change in the ring opposite that containing bound polypeptide and, therefore, assign the second sigmoidal transition in the nucleotide dependent dissociation rate to an ATP-promoted conformational change in the ring that is associated with the bound substrate. This assignment resides in part on the observation of an asymmetric chaperonin complex with malate dehydrogenase associated with the end of GroEL opposite that to which GroES is bound (17), as well as from the fact that the second transition gives rise to an increased rate of release, suggesting a more direct effect of the conformational change in decreasing GroEL affinity for the substrate protein. Thus, the binding of ATP to the first ring promotes a conformational change in GroEL that leads to moderate rates of polypeptide chain release ($\approx 0.02\ s^{-1}$), corresponding to the single sigmoidal transitions seen for the nonhydrolyzable nucleotides AMP-PNP (Fig. 2) and ADP (Fig. 4). Subsequent binding of ATP on the second (polypeptide-bound) ring of GroEL then more rapidly facilitates the conformational change that results in chain release ($\approx 0.13\ s^{-1}$), thereby accounting for the two transitions seen in plots of the rate of release versus ATP concentration.

The effect of GroES on the rate limiting conformational change in GroEL promoted by nucleotide binding is to increase its rate and, therefore, the rate of chain dissociation. It is of interest that our preliminary estimate ($\approx 0.22\ s^{-1}$) for the rate of nonnative substrate dissociation in the presence of GroES is faster than the rate of ATP hydrolysis in the absence of polypeptides (4, 13), which have been seen to stimulate the ATPase activity of GroEL several fold (7, 8, 18). The increase in separation of the two transitions of substrate protein release in the presence of GroES is probably due to the effect of the cochaperonin on increasing the cooperativity of the GroEL ATPase to the limit to which it becomes "quantized" (4, 13). Thus, the high degree of sigmoidality seen in the second substrate dissociation transition doubtless reflects a very highly concerted conformational change that leads to substrate dissociation.

These findings, as well as a wide body of evidence on the GroEL chaperonin system (2, 4–7), are compatible with a simple working hypothesis for the elementary steps of a single cycle of chaperonin action, presented diagrammatically in Fig. 6. In the first step, there is formation of an asymmetric GroES–GroEL-substrate complex (17) by ATP and GroES binding to the first toroid, which, under limiting nucleotide concentration, may lead to (relatively slow) polypeptide dissociation. At physiological concentrations, however, ATP may bind on the second ring that contains nonnative chains, possibly after stimulated ATP hydrolysis on the first ring (7, 8, 18). Presumably, a second mole of GroES then binds to form a transient, symmetrical chaperonin complex (4, 19, 20), suggesting a pivotal role of GroES in polypeptide dissociation. Nucleotide binding to the second toroid promotes the relevant conformational change that leads to the more rapid release of chains. Subsequent hydrolysis of ATP in the second ring results in the dissociation of GroES and ADP from the first ring (4, 15), leading to an asymmetric complex. This asymmetric complex, which may possess altered affinity for nonnative polypeptides (21), is then able to bind chains to facilitate the dissociation of the second mole of GroES and remaining ADP (6). In this view, the association of second mole of GroES prevents chain rebinding to the same site on the chaperonin, enabling it either to refold in solution or to rebind at the opposite end of an asymmetric complex.

The observation that the energies available from ATP, ADP, and AMP-PNP binding were all effective in promoting an increase in the rate of nonnative chain dissociation from GroEL suggests that the requirement for ATP and GroES under conditions that are unfavorable for spontaneous *in vitro* folding may be that chaperonins facilitate protein folding by unfolding nonnative intermediates. The idea that GroEL can unfold nonproductive folding intermediates that are prone to aggregation has been suggested previously (5, 22–24). Two recent experimental results that support this role for energy transduction concern the ATP-dependent refolding of an intermediate of malate dehydrogenase which is susceptible to aggregation (25, 26), and the release of assembly-defective

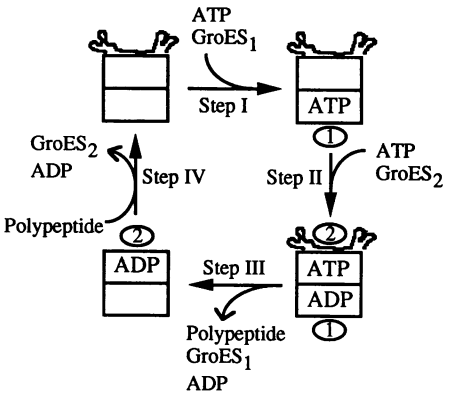

FIG. 6. Hypothesis for ATP-promoted release of nonnative polypeptides from chaperonin complexes. A cartoon of possible elementary steps in a single cycle of nonnative protein binding to GroEL and its release in the presence of ATP and GroES is shown. A polypeptide chain is schematically represented on the edge of the apical domain of GroEL, represented as a side view of the two stacked rings. Step I: ATP and the first mole of GroES bind to a high affinity ring of GroEL which is opposite to that containing bound polypeptide. Step II: Upon hydrolysis of the 7 ATPs in the high affinity ring, 7 additional ATPs and a second mole of GroES bind to the polypeptide-containing ring to form a symmetrical chaperonin complex with bound polypeptide. Step III: Hydrolysis of the newly bound ATP results in $GroES_1$ and ADP dissociation from the first ring and polypeptide dissociation from the second ring of GroEL. Step IV: A polypeptide chain rebinds to the asymmetric chaperonin complex, promoting the dissociation of $GroES_2$ and ADP to generate the initial GroEL–polypeptide chain complex, but with the chain bound to the second toroid of GroEL. A different conformation of GroEL or the polypeptide chain may exist at each step in this cycle.

monomers of ornithine transcarbamoylase in the presence of the nonhydrolyzable nucleotide ATP-γ-S (27). Apparently, the energy available from binding nonhydrolyzable nucleotides can readily lead to nonnative substrate dissociation from GroEL, at rates comparable to those seen in the presence of ATP, but is insufficient to allow chaperones to unwrap nonnative folding intermediates into a conformational isomer that has a lower kinetic barrier for folding. Thus, the GroEL/GroES chaperonin system may be quite unlike other folding catalysts, such as the propeptide domain of the subtilisin, which are thought to bind a unique intermediate to accelerate folding (28), and may explain the failure of GroEL to facilitate subtilisin refolding in the absence of its propeptide (data not shown).

Chaperonin complexes may transduce the chemical energy available from ATP in an analogous manner to other systems that perform biochemical work, referred to by Jencks (10) as coupled vectorial processes. The minimal, ordered cycle presented in Fig. 6, which requires the transient formation of a symmetrical chaperonin complex, is coupled in that ATP hydrolysis must occur for the work of intermediate unfolding to be performed. Until it is established how the energy of ATP hydrolysis is released in a cycle of chaperonin action, it is impossible to say whether the unfolding of compact intermediates occurs in a single step or in several steps in a cycle of substrate protein binding and dissociation. However, if work is performed to bind a compact intermediate and release it in a less compact conformation, the intermediates depicted in Fig. 6 suggest two steps that may represent the equivalent of a "power stroke" in the chaperonin cycle. Either the asymmetric chaperonin complex formed after step III (transiently) possesses increased affinity for compact, nonnative folding intermediates by exposing latent hydrophobic surfaces in GroEL (17, 18, 20) or the transient association of GroES with a GroEL ring containing bound polypeptide after step II leads to a conformational change in the polypeptide to a less compact conformation that allows the chain to reenter productive folding pathways.

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