GroES Promotes the T to R Transition of the GroEL Ring Distal to GroES in the GroEL−GroES Complex†

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ABSTRACT: Curves of initial rates of ATP hydrolysis by GroEL as a function of ATP concentration, in the presence of fixed concentrations of GroES, were found to deviate from sigmoidal kinetics. Instead of the lag phase typical of sigmoidal curves, a linear phase is observed at low ATP concentrations. Consequently, a good fit of the data to the Hill equation could not be achieved. Such curves could be simulated using a linear combination of Hill equations, thus indicating that more than one allosteric transition is taking place in the ATP concentration range studied. The data were fitted to a fractional saturation equation for ATP binding to GroEL based on a partition function that includes both GroES and ATP-liganded states of GroEL. Using this equation, it was possible to estimate in a reliable manner the value of the allosteric constant, L_2', for the transition of the ring distal to GroES in the GroEL−GroES complex from the low (T)- to the high (R)-affinity state for ATP. The value of L_2' is found to be 4×10^{-5} whereas the value of the allosteric constant, L_2, for the transition of the second ring of GroEL from the T to R state is 2×10^{-9} [Yifrach, O., & Horovitz, A. (1995) *Biochemistry 34*, 5303−5308]. Comparison of these values shows that GroES promotes the T to R transition of the ring distal to GroES in the GroEL−GroES complex. Owing to the relatively low affinity of the R conformation for nonfolded proteins, this transition will lead to release of protein substrates from *trans* ternary complexes of GroEL, GroES, and protein substrate. The role of this release mechanism may be to assist the folding of relatively large proteins that cannot form *cis* ternary complexes and/or to facilitate degradation of damaged proteins which cannot fold.

The *Escherichia coli* GroE system facilitates protein folding both *in vivo* and *in vitro* [for recent reviews see, for example, Clarke (1996), Martin and Hartl (1997), and Fenton and Horwich (1997)]. It comprises GroEL, an oligomer of 14 identical subunits that form two stacked heptameric rings with 7-fold symmetry (Braig et al., 1994, 1995) and its helper protein GroES which is a seven-membered ring of identical subunits (Hunt et al., 1996; Mande et al., 1996). GroES modulates the ATPase activity of GroEL (Gray & Fersht, 1991) and is required for the reactivation of certain protein substrates in a manner that depends on the precise folding conditions (Schmidt et al., 1994). The relationship between the modulation of the ATPase activity of GroEL by GroES and its role in protein release is, however, poorly understood. The current view of the steps involved in GroE-assisted folding can be summarized, as follows (Weissman et al., 1995; Mayhew et al., 1996): (i) polypeptide substrate becomes bound to the ring distal to GroES in a 1:1 GroEL−GroES complex thus forming a ternary *trans* complex; (ii) release of GroES from the *trans* ring and its rebinding to the *cis* ring (or binding of an additional GroES molecule to GroEL) leads to formation of a productive *cis* ternary complex in which the polypeptide substrate and GroES are bound to the same ring; (iii) the bound polypeptide substrate is released into the space sequestered underneath GroES where it can partially or fully fold before being discharged. According to this model (Weissman et al., 1995; Mayhew et al., 1996), only *cis* ternary complexes lead to productive folding. The role of *trans* complexes in folding remains in question although it is clear that some relatively large proteins such as the phage P22 tailspike protein are able to form only *trans* complexes (Gordon et al., 1994).

The role of allostery in GroE-assisted protein folding also remains unclear. GroEL has 14 ATP binding sites and a K^+-dependent ATPase activity (Viitanen et al., 1990) which displays positive cooperativity within rings and negative cooperativity between rings (Yifrach & Horovitz, 1995). A nested model describing the mixed cooperativity in ATP hydrolysis by GroEL, with respect to ATP, was recently put forward (Yifrach & Horovitz, 1995). According to this model which is now supported also by electron microscopy studies (Roseman et al., 1996), each ring of GroEL is in equilibrium between a T state, with low affinity for ATP and high affinity for nonfolded proteins, and an R state with high affinity for ATP and low affinity for nonfolded protein substrates (Yifrach & Horovitz, 1995; 1996). The concerted (Monod et al., 1965) switch of individual GroEL rings from the T to R state which is manifested in the positive cooperativity in ATP binding may, therefore, facilitate concerted release of bound nonfolded protein substrates. A second level of allostery is between the rings of GroEL, which undergoes sequential transitions (Koshland et al., 1966), in the presence of increasing concentrations of ATP, from the TT state *via* the TR state to the RR state. Negative cooperativity between rings is responsible for the stability of the TR state relative to the RR state and, therefore, also

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for the formation of ternary *trans* complexes. In this paper, we analyze the effect of GroES on cooperativity in ATP hydrolysis by GroEL. We show that GroES facilitates the T to R transition of the GroEL ring distal to GroES in 1:1 GroEL−GroES complexes, thereby promoting protein release from ternary *trans* complexes. The implications of this mechanism for GroE-assisted folding are discussed.

THEORY

According to the MWC model (Monod et al., 1965), cooperativity in ligand binding is due to an equilibrium between two unligated conformations of the protein: a tense (T) conformation with relatively low affinity for the ligand, which is the predominant form in the absence of ligand, and a relaxed (R) conformation with relatively high affinity for the ligand. In the case of nonexclusive binding of the ligand to both the T and R states, the extent of cooperativity is determined by the allosteric constant L (= [R]/[T]) and by the ratio of affinities of the ligand to the R and T states.

The sum of the concentrations of all the different species considered by the MWC model, normalized relative to the concentrations of the unligated forms, is given by the binding polynomial, P, as follows (Wyman, 1964):

$$P = \frac{1}{[\mathrm{R}] + [\mathrm{T}]} \sum_{i=0}^{N} ([\mathrm{T}]_i + [\mathrm{R}]_i) \qquad (1)$$

where $[\mathrm{R}]_i$ and $[\mathrm{T}]_i$ are the concentrations of species in the R or T conformations to which i substrate molecules are bound, [R] (≡$[\mathrm{R}]_0$) and [T] (≡$[\mathrm{T}]_0$) are the concentrations of the unligated species, and N is the total number of sites. The fractional saturation binding equation, $\bar{Y}$, may be derived from the binding polynomial using the relationship

$$\bar{Y} = \frac{1}{N} \frac{\partial \ln P}{\partial \ln[\mathrm{S}]} = \frac{1}{N} \frac{[\mathrm{S}]}{P} \frac{\partial P}{\partial [\mathrm{S}]} \qquad (2)$$

where [S] is the substrate (ATP) concentration and N is the total number of sites.

A nested model for cooperativity in ATP hydrolysis by GroEL was recently developed (Yifrach & Horovitz, 1995). In this model, there are two levels of allostery: one within each ring of GroEL and the second between the two rings. In the first level, each heptameric ring is in equilibrium between the T and R states, in accordance with the MWC model of cooperativity. A second level of allostery is between the two rings of the GroEL particle which undergoes sequential KNF-type transitions from the TT state *via* the nonsymmetrical TR state to the RR state. The binding polynomial for this model is given by (Yifrach & Horovitz, 1995)

$$P = \frac{1}{1 + 2L_1 + L_1L_2}\{(1 + [\mathrm{S}]/K_T)^{2N} + 2L_1(1 + [\mathrm{S}]/K_T)^N(1 + [\mathrm{S}]/K_R)^N + L_1L_2(1 + [\mathrm{S}]/K_R)^{2N}\} \qquad (3)$$

where L_1 (= [TR]/[TT]) and L_2 (= [RR]/[TR]) are the intrinsic allosteric constants and K_R and K_T are the dissociation constants of ATP from the rings in the R and T states, respectively.

In the presence of GroES (denoted by ES), additional states (TRES and R′RES) need to be included in the partition function, as follows:

$$P = \frac{1}{1 + L_1 + L_1[\mathrm{ES}]K_{\mathrm{ES}} + L_1L_2'[\mathrm{ES}]K_{ES}}\{(1 + [\mathrm{S}]/K_T)^{2N} + L_1(1 + [\mathrm{S}]/K_T)^N(1 + [\mathrm{S}]/K_R)^N + L_1[\mathrm{ES}]K_{ES}(1 + [\mathrm{S}]/K_T)^N(1 + [\mathrm{S}]/K_R)^N + L_1L_2'[\mathrm{ES}]K_{ES}(1 + [\mathrm{S}]/K_R)^N(1 + [\mathrm{S}]/K_R')^N\} \qquad (4)$$

where K_{ES} (=[TRES]/[TR][ES]) is the binding constant of GroES to GroEL in the TR state, L_2' is equal to [R′RES]/[TRES] and L_1 is the apparent allosteric constant. The prime is used to indicate that the conformations of the GroEL rings *cis* and *trans* to GroES are not identical. By combining eqs 2 and 4 for the case of exclusive binding ($K_\mathrm{R}/K_\mathrm{T} = 0$) of ATP to rings in the R or R′ states, one obtains

$$\bar{Y} = [L_1\alpha(1 + \alpha)^{N-1} + L_1\alpha\beta(1 + \alpha)^{N-1} + L_1L_2'\alpha\beta(1 + \alpha)^{N-1}(1 + \gamma)^N + L_1L_2'\beta\gamma(1 + \alpha)^N(1 + \gamma)^{N-1}]/[2\{1 + L_1(1 + \alpha)^N + L_1\beta(1 + \alpha)^N + L_1L_2'\beta(1 + \alpha)^N(1 + \gamma)^N\}] \qquad (5)$$

where $\alpha = [\mathrm{S}]/K_\mathrm{R}$, $\beta = [\mathrm{ES}]K_{\mathrm{ES}}$, $\gamma = [\mathrm{S}]/K'_\mathrm{R}$ and $N = 7$. The assumption of exclusive binding to the R state in the derivation of eq 5 is supported by the 3 orders of magnitude difference in affinities of ATP for the T and R states (Jackson et al., 1993). By assuming that only the ring distal to GroES in the R′RES species has steady-state ATPase activity and that the TRES species has no steady-state ATPase activity, one obtains

$$V_0 = 0.5[V_{\mathrm{max}(1)}([\mathrm{S}]/K_\mathrm{R})(1 + [\mathrm{S}]/K_\mathrm{R})^{N-1} + V_{\mathrm{max}(2)}L_2'[\mathrm{ES}]K_{ES}([\mathrm{S}]/K_R')(1 + [\mathrm{S}]/K_\mathrm{R})^N(1 + [\mathrm{S}]/K_R')^{N-1}]/\left[\frac{1}{L_1} + (1 + [\mathrm{S}]/K_\mathrm{R})^N + [\mathrm{ES}]K_{ES}(1 + [\mathrm{S}]/K_\mathrm{R})^N + L_2'[\mathrm{ES}]K_{ES}(1 + [\mathrm{S}]/K_\mathrm{R})^N(1 + [\mathrm{S}]/K_R')^N\right] \qquad (6)$$

where V_o is the observed initial rate of ATP hydrolysis, $V_{\mathrm{max}(1)}$ and $V_{\mathrm{max}(2)}$ are the respective maximal initial rates of ATP hydrolysis of the TR and R′RES species, K_R is the dissociation constant of ATP from the ring in the R conformation of the TR state, K'_R is the dissociation constant of ATP from the ring in the R′ conformation of the R′RES species, and L_1 and L_2' are defined as before. In this expression $\bar{Y} = \bar{Y}_1 + \bar{Y}_2$, where the subscripts 1 and 2 refer to the TR and R′RES states, respectively.

EXPERIMENTAL PROCEDURES

Materials. Radiochemicals were purchased from Amersham International or New England Nuclear (Du Pont). All other reagents were from Sigma or Aldrich.

Methods. Expression of GroEL was carried out as before (Horovitz et al., 1993) and purification was achieved as described earlier (Yifrach and Horovitz, 1994) but with some modification (Matthew Todd & George Lorimer, personal communication). GroEL-enriched fractions after gel filtation

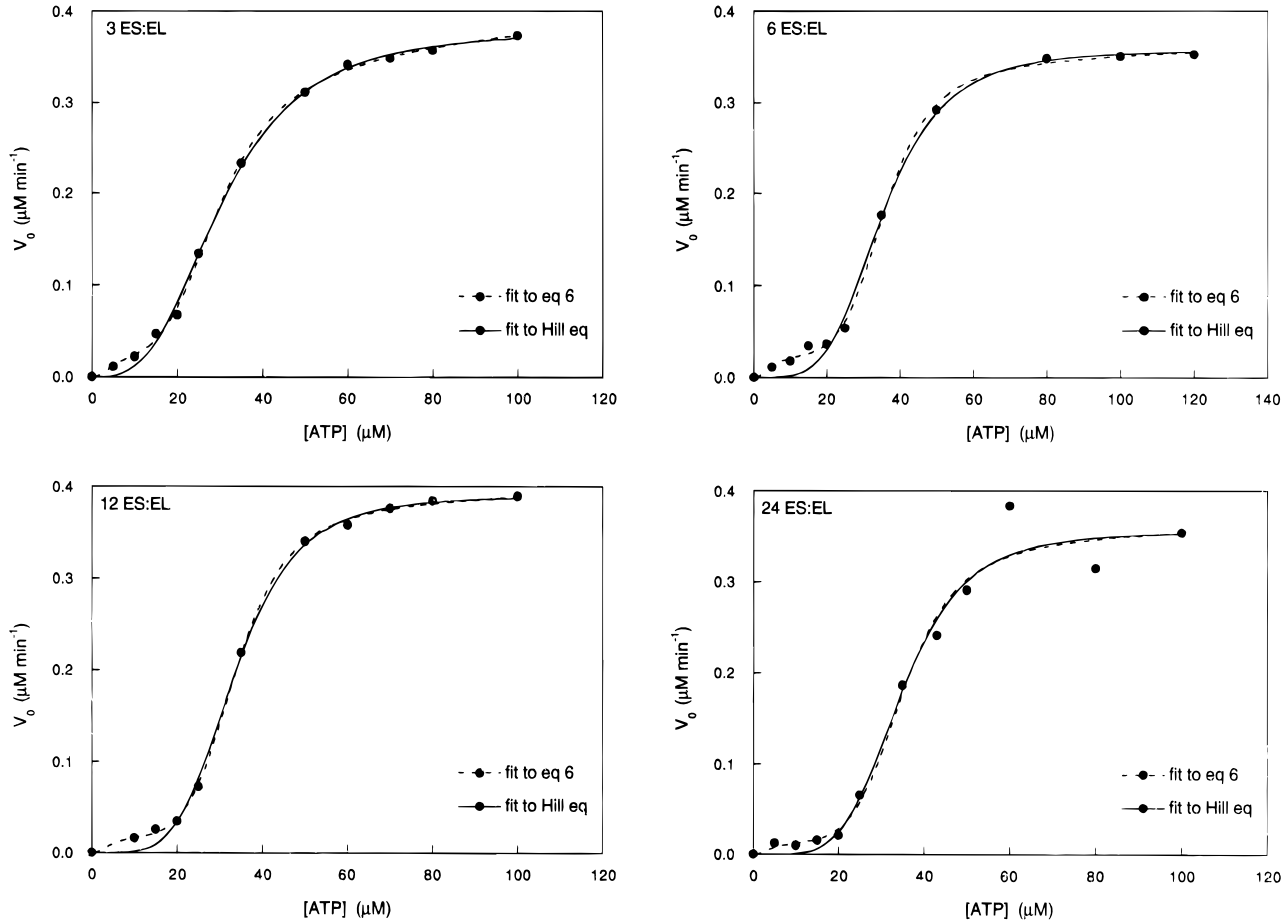

FIGURE 1: Initial velocity of ATP hydrolysis by GroEL as a function ATP concentration in the presence of different fixed concentrations of GroES. The data were fitted to both the Hill equation and to eq 6. Data fitting was carried out using fixed average values for the parameters $V_{max(1)}$, L_1 (=[TR]/[TT]) and K_R that were determined from six independent experiments carried out in the absence of GroES. The values used in the data fitting were 1.42 μM min^{-1} for $V_{max(1)}$, 1.7 $\times$ 10^{-3} for L_1, and 5.3 μM for K_R in good agreement with values reported earlier (Yifrach & Horovitz, 1995). Experiments were carried out at 25 °C in the presence of 25 nM GroEL as described under Experimental Procedures.

were combined and applied to a Mono-Q HR 5/5 column. A linear gradient from 0.15 to 0.35 M NaCl (35 min, 1 mL/min) in 50 mM MES buffer (pH 6.0) containing 1 mM EDTA, 1 mM DTT, and 25% methanol was used to elute GroEL. GroEL purified in this manner contained less than 0.8 mol of tryptophan/mol of GroEL oligomer. The kinetic parameters of wild-type GroEL purified in this way were identical to those reported earlier (Yifrach & Horovitz, 1995). The ATPase activity of GroEL was measured as described by Viitanen et al. (1990) with some modifications (Horovitz et al., 1993). Initial rates of ATP hydrolysis were determined by measuring the amount of inorganic phosphate present after four 1 min intervals following the start of the reaction.

Data Analysis. Computer simulations and data fitting were carried out using Kaleidagraph [version 2.1 Synergy Software (PCS Inc.)]. Computer-simulated data for two simultaneous allosteric transitions were generated using the Hill equation:

$$V_0 = V_{max}K[S]^n/(1+ K[S]^n) \quad (7)$$

where V_0 and V_{max} are the initial and maximal initial ATPase reaction velocities, K is the apparent association constant, and n is the Hill coefficient. Analysis of cooperativity in ATP hydrolysis by GroEL in the presence of GroES was performed by fitting the data to eq 6. Estimates of parameters (±standard errors) are reported.

RESULTS AND DISCUSSION

ATP Hydrolysis by GroEL in the Presence of GroES. Initial rates of ATP hydrolysis by GroEL were measured as a function of ATP concentration in the presence of different fixed concentrations of GroES (Figure 1). In contrast with earlier studies by us and others (Gray & Fersht, 1991; Kovalenko et al., 1994), a linear phase was observed at low ATP concentrations instead of the lag phase that is characteristic of sigmoidal curves. Consequently, a good fit of the data to the Hill equation (eq 7) could not be achieved as shown in Figure 1. A plot of the residuals as a function of the ATP concentration shows that in the case of the fit to the Hill equation there is a clear nonrandom distribution of the residuals about zero in particular for the data at the low ATP concentrations (Figure 2A). The linear phase was absent in experiments carried out without GroES. In addition, GroES by itself had no measurable ATPase activity (data not shown). We, therefore, reasoned that the observed deviation from sigmoidal kinetics reflects the fact that more than one ATP-induced allosteric transition is simultaneously taking place. This conclusion was further strengthened by noting that a linear combination (with different weights) of two Hill equations with different values for the parameters in the equation could generate a kinetic profile similar to the one observed here (Figure 3). It should be mentioned that in the earlier studies, the linear phase was probably not

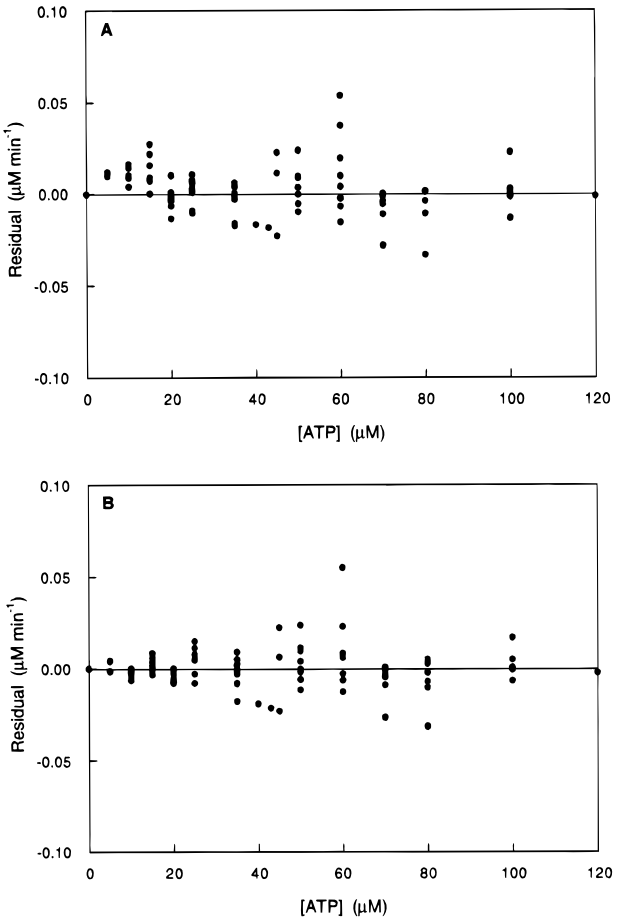

FIGURE 2: Plot of the residuals as a function of the ATP concentration. The residuals are the differences between the experimental data and the fits to the Hill equation (panel A) and eq 6 (panel B) calculated for each concentration of ATP that was employed. The plot is based on the data shown in Figure 1 and on other similar data not shown. In the case of the fit to eq 6, there is a random distribution of the residuals about zero indicating a good fit. In the case of the fit to the Hill equation, there is a clear nonrandom distribution of the residuals about zero in particular for the data at the low ATP concentrations.

observed owing to insufficient data and the difficulty in obtaining highly reliable data at low ATP concentrations.

In the absence of GroES, two ATP-induced allosteric transitions take place: TT → TR and TR → RR (Yifrach & Horovitz, 1995). In the presence of GroES, the additional allosteric transition TRES → R′RES takes place (Kovalenko et al., 1994). As the above three allosteric transitions are coupled, the data could not be fitted to a linear combination of Hill equations (Figure 3) for each of the respective transitions. We, therefore, extended the partition function previously derived for ATP alone (Yifrach & Horovitz, 1995) for the case where both GroES and ATP are present (eq 5). The data were fitted to eq 6 which was derived from the partition function. For simplicity, it is assumed in eqs 4−6 that in the ATP concentration range 0−120 μM the RR and ESR′R′ES species may be neglected. Data fitting was carried out using fixed average values for the parameters $V_{max(1)}$, L_1, and K_R that were determined from six independent experiments carried out in the absence of GroES. The values used were 1.42 μM min^{-1} for $V_{max(1)}$, 1.7×10^{-3} for L_1, and 5.3 μM for K_R in good agreement with values reported earlier (Yifrach & Horovitz, 1995). Estimates of $k_{cat(2)}$ (the rate constant of ATP hydrolysis by the R′RES species), K'_R (the dissociation constant of ATP from the R′ ring of the R′RES species), K_{ES} (the binding constant of GroES to the TR state), and L'_2 (= [R′RES]/[TRES]) obtained from the fits to eq 6 were found to be essentially independent of the concentration of GroES (Table 1), thereby supporting the validity of the analysis presented in this paper. Also in support of the analysis presented here are the plots of the residuals as a function of ATP concentration which show that in the case of the fit to eq 6 (but not the Hill equation) there is a random distribution of the residuals about zero for the entire range of ATP concentrations employed (Figure 2B). The value of $k_{cat(2)}$ was found to be about 32 min^{-1} (per GroEL−GroES oligomer complex) and the value of K_{ES} was found to be about 0.16 nM^{-1} (Table 1). These estimates differ somewhat from the values reported by Kovalenko et al. (1994) probably owing to the different equations used to fit the data. Interestingly, the value of K'_R, which is found to be about 9 μM, is similar to the value of K_R indicating that GroES does not affect the intrinsic binding of ATP to the ring distal to GroES in the GroEL−GroES complex.

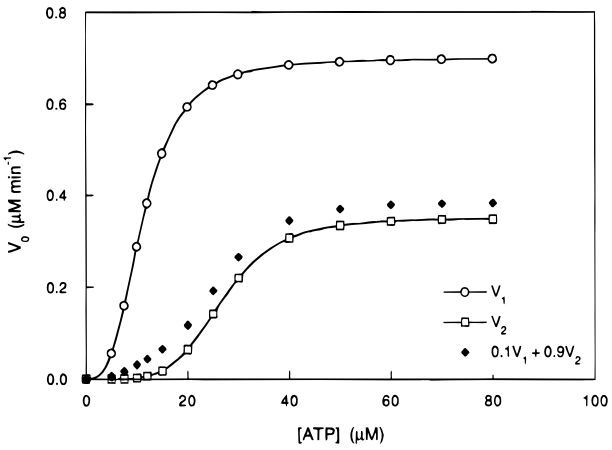

FIGURE 3: Simulated curves of initial rates of ATP hydrolysis as a function of ATP concentration for two allosteric transitions that are taking place separately and also together. The data corresponding to the transitions that are taking place separately were generated using the Hill equation (eq 7). The values of the parameters in these simulations were (*i*) $V_{max} = 0.70$, $K = 7 \times 10^{-4}$, and $n = 3$ and (*ii*) $V_{max} = 0.35$, $K = 7 \times 10^{-8}$, and $n = 5$. The data for these transitions when they are taking place together were generated using a linear combination of Hill equations with weights of 0.1 and 0.9 for the two respective transitions.

Table 1: Analysis of the Effect of GroES on Cooperativity in ATP Hydrolysis by GroEL with Respect to ATP[a]

[GroES]/[GroEL]	$k_{cat(2)}$ (min^{-1})	K_{ES} (nM^{-1})	K'_R (μM)	$\Delta G'_2$ (kcal mol^{-1})
3	34 (±1)	0.6 (±0.4)	14 (±2)	4.4 (±0.3)
6	30 (±1)	0.3 (±0.2)	10 (±3)	6.1 (±0.4)
12	33.6 (±0.6)	0.08 (±0.02)	6 (±1)	7.9 (±0.3)
24	33 (±2)	0.06 (±0.06)	8 (±5)	7 (±1)
average[b]	32.4 (±0.8)	0.16 (±0.06)	9 (±2)	6.3 (±0.3)

[a] Estimates for the rate constant of ATP hydrolysis by the R′RES species [$k_{cat(2)}$], the binding constant of GroES to GroEL in the TR state (K_{ES}), the dissociation constant of ATP from the ring in the R′ conformation of the R′RES species and the allosteric constant L'_2 (as defined in the main text and in Figure 4) were determined by fitting the data in Figure 1 (and other data not shown) to eq 6. The free energies of the allosteric transition TRES → R′RES were calculated from $\Delta G'_2 = -RT\ln L'_2$. Each value represents an average determined from two independent experiments. Experiments were carried out at 25 °C in the presence of 25 nM GroEL oligomer as described under Experimental Procedures. [b] The average rate and binding constants were calculated from the corresponding average free energies.

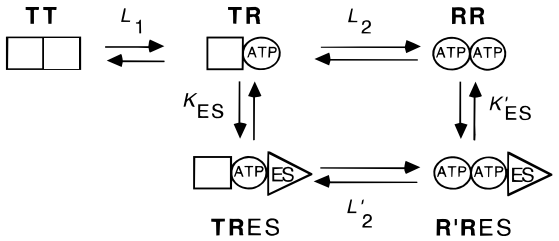

FIGURE 4: Scheme for different states of GroEL in the presence of ATP and GroES. In the absence of ATP, GroEL is predominantly in the TT state. In the presence of ATP, the equilibrium is shifted toward the TR and RR states (L_1 = [TR]/[TT] and L_2 = [RR]/[TR]). GroES binds to the ring in the R conformation of the TR and RR states with affinities K_{ES} (= [TRES]/[TR][ES]) and K'_{ES} (=[R′RES]/[RR][ES]), respectively. In the presence of GroES and ATP, the equilibrium is shifted toward the R′RES state (L'_2 = [R′RES]/[TRES]). For simplicity, the notation of T and R is used to designate all the low affinity and high affinity states for ATP of one ring of GroEL. Prime is used to designate the distal ring of GroEL with respect to GroES in the GroEL−GroES complex. The symmetric football-shaped GroEL−$GroES_2$ species is not formed at the relatively low ATP concentrations employed in the experiments reported here and is, therefore, not included in this scheme. A measure of the effect of GroES on the allosteric transition of the distal ring of GroEL is given by $\Delta\Delta G = -RT \ln(L'_2/L_2)$.

Effect of GroES on Cooperativity in ATP Hydrolysis by GroEL. A common misconception in the chaperonin field is that GroES increases cooperativity in ATP hydrolysis by GroEL with respect to ATP. This misconception is due to the fact that the value of the Hill coefficient for the plot of initial rates of ATP hydrolysis by GroEL, as a function of ATP concentration, is increased in the presence of GroES (Gray & Fersht, 1991). This comparison of Hill coefficients is misleading since the Hill coefficient in the absence of GroES is for the transition of the first ring of GroEL from T to R (i.e., for the process TT → TR) whereas the Hill coefficient in the presence of GroES is mainly for the transition of the second ring of GroEL from T to R (i.e., for the process TRES → R′RES). Instead of comparing the values of the Hill coefficients in the absence and in the presence of GroES, one should compare the values of L_2 and L'_2 which correspond to the transition from T to R of the second ring of GroEL, either in the absence or in the presence of GroES, respectively (Figure 4). Previously, it was not possible to estimate the value of the allosteric constant L'_2 in a reliable manner since the transition TRES → R′RES cannot be studied in isolation. This problem was overcome here by deriving a fractional saturation equation for ATP binding to GroEL (eq 6) that is based on a partition function that includes both the GroES and ATP-liganded states of GroEL. Using this equation, the value of the allosteric constant L'_2 is found to be about 4×10^{-5}. A similar estimate of the value of L'_2 was previously obtained by assuming that the transition TRES → R′RES is the only one taking place (Kovalenko et al., 1994). Comparison with the value of the allosteric constant L_2, previously estimated to be about 2×10^{-9} (Yifrach & Horovitz, 1995), shows that GroES promotes the T to R transition of the distal ring of GroEL. In other words, GroES reduces cooperativity (with respect to ATP) in ATP hydrolysis by the distal ring of GroEL. In terms of free energy, GroES stabilizes the R state of the distal ring of GroEL relative to its T state by about 6 kcal mol^{-1} at 25 °C [$\Delta\Delta G = -RT\ln(L'_2/L_2)$].

Relative Affinities for GroES of the TR and RR States of GroEL. Owing to free energy conservation in the cycle in Figure 4, one has: $L_2/L'_2 = K_{ES}/K'_{ES}$. From this relation and the finding that $L_2 << L'_2$ it follows that: $K_{ES} << K'_{ES}$, i.e. the affinity of GroES to the ring in the R conformation of the TR state is significantly lower than it is to the rings in the R conformation of the RR state. Two biochemical criteria, therefore, indicate that the R conformation is different in the TR and RR states: (i) the different affinities they have for GroES and (ii) the different rate constants for ATP hydrolysis of the TR and RR states (Yifrach & Horovitz, 1995).

Implications for GroE-Assisted Folding. According to the current view of the mechanism of GroE-assisted folding (Weissman et al., 1995; Mayhew et al., 1996) only *cis* ternary complexes lead to productive folding. The role of GroES in this mechanism is to cap the cavity of GroEL thereby creating a protected environment in which substrates can fold without the risk of aggregation. The role of *trans* ternary complexes in GroE-assisted folding has remained in question, although it is clear that some relatively large proteins such as the phage P22 tailspike protein are able to form only *trans* complexes with GroEL−GroES (Gordon et al., 1994). The analysis presented in this paper suggests that GroES has a role in release of protein substrates from *trans* complexes. We have shown here that GroES promotes the T to R transition of the GroEL ring distal to GroES in 1:1 GroEL−GroES complexes. Owing to the relatively low affinity of the R conformation for nonfolded proteins (Staniforth et al., 1994; Yifrach & Horovitz, 1996), this transition will lead to release of protein substrates from *trans* complexes. The biological importance of protein substrate release from *trans* ternary complexes may be to assist the folding of relatively large proteins and/or to facilitate degradation of damaged proteins that cannot reach the native state. In addition, promotion by GroES of the T to R transition of the GroEL ring distal to GroES may facilitate binding of another GroES molecule to that protein-bound ring. Thus, promotion of the T to R transition of the GroEL ring distal to GroES in 1:1 GroEL−GroES complexes may facilitate folding from both *cis* and *trans* complexes. Finally, it should be mentioned that small proteins are able to form *cis* complexes with GroES not only because they do not interfere sterically with GroES binding but maybe also because they stabilize the T conformation to a lesser extent owing to fewer contacts with GroEL.

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