

Dissociation of the GroEL–GroES Asymmetric Complex Is Accelerated by Increased Cooperativity in ATP Binding to the GroEL Ring Distal to GroES[†]

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ABSTRACT: A kinetic analysis of the ATP-dependent dissociation of wild-type GroEL and mutants from immobilized GroES was carried out using surface plasmon resonance. Excellent fits of the data were obtained using a double-exponential equation with a linear drift. Both the fast and slow observed dissociation rate constants are found to have a sigmoidal dependence on the concentration of ATP. The values of the Hill coefficients corresponding to the fast and slow observed rate constants of dissociation of wild-type GroEL and the Arg197→Ala mutant are in good agreement with the respective values of the Hill coefficients previously determined for these proteins from plots of initial rates of ATP hydrolysis as a function of ATP concentration, in the presence of GroES. Our results are consistent with a kinetic mechanism for dissociation of the GroEL–GroES complex according to which GroES release takes place after an ATP-induced conformational change in the trans ring that is preceded by ATP hydrolysis and a subsequent conformational change in the cis ring. It is shown that the rate of complex dissociation increases with increasing positive cooperativity in ATP binding by the GroEL ring distal to GroES in the GroEL–GroES complex.

The *Escherichia coli* GroE system facilitates protein folding both in vivo and in vitro (for recent reviews, see, for example, refs 1–3). It comprises GroEL, an oligomer of 14 identical subunits of 57.3 kDa that form 2, stacked back-to-back, heptameric rings with 7-fold symmetry (4), and its helper-protein GroES which is a 7-membered ring of identical subunits of 10 kDa (5). Each subunit of GroEL consists of three domains: (i) an equatorial domain that forms all of the inter-ring contacts and many of the intra-ring contacts between subunits; (ii) an apical domain that forms the opening of the central cavity; and (iii) an intermediate domain that connects the two domains. GroEL undergoes allosteric transitions induced by binding of ATP and GroES (for a review, see ref 6) which are important for its cycling between protein-acceptor and release states and for its folding function (7; for a review, see ref 3). Each ring of GroEL is in equilibrium between **T** and **R** states which interconvert in an ATP-dependent and concerted manner (8, 9), in accordance with the Monod–Wyman–Changeux model (10). In the absence of ligands, GroEL is predominantly in the **TT** state. In the presence of ATP, the equilibrium is shifted toward the **TR** ($L_1 = [\text{TR}]/[\text{TT}]$) and **RR** states ($L_2 = [\text{RR}]/[\text{TR}]$) (11).

Binding of GroES to the apical domains of GroEL in the **R** state induces a further large conformational change which involves rigid-body domain movements that pivot around the two hinges at the junctions between the apical, intermedi-

ate, and equatorial domains (12, 13). GroES binding to a ring (the cis ring) displaces polypeptide substrates bound to that ring into the central cavity and isolates them from bulk solution (1–3). The GroES-induced allosteric transition leads to doubling of the volume of the central cavity of the cis ring and to a switch in the nature of its surface from being predominantly hydrophobic to hydrophilic (13), thus favoring folding. Binding of GroES also modulates the ATPase activity of the distal (trans) ring of GroEL (14, 15) and causes ATP bound to the cis ring to become committed to hydrolysis (16). In addition, it has been shown that GroES facilitates the **T** to **R** transition of the distal ring (15), thereby promoting release of polypeptides bound to that ring (17). Formation and dissociation of the GroEL–GroES complex is regulated by ATP binding and hydrolysis. GroES binds only to ATP-bound (or ADP-bound) rings of GroEL (18). Dissociation of GroES requires ATP hydrolysis in the cis ring followed by ATP binding to the trans ring (19). More recently, fluorescence energy transfer experiments have indicated that a conformational change with a rate constant of 0.04 s^{-1} takes place in the ADP-bound cis ring before ATP binds to the trans ring (20). A kinetic analysis of allosteric effects of ATP binding to the trans ring on the rate of GroES dissociation has, however, not been reported. Here, we present such an analysis using surface plasmon resonance and show that the rate and, in particular, the cooperativity of the allosteric transition of the trans ring control the rate of dissociation of GroES from the cis ring. A model according to which GroES dissociation takes place after an ATP-induced conformational change in the trans ring that is preceded by ATP hydrolysis and a subsequent conformational change in the cis ring is found to be consistent with our kinetic data.

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EXPERIMENTAL PROCEDURES

Molecular Biology and Biochemical Procedures. Construction of the GroEL single mutants Arg197→Ala (21), Phe44→Trp (22), and Arg501→Ala (23) as well as the double mutants Arg13→Gly, Ala126→Val (24) and Phe44→Trp, Thr522→Ala (25) has been described. The Phe44→Trp, Glu67→Ala and Phe44→Trp, Arg18→Ala double mutants were generated as before (26) using single-stranded DNA containing the gene for the Phe44→Trp GroEL mutant and the oligonucleotides: Glu67→Ala, 5'-GCGCACCCATATTTGCGAACTTGCTTCC-3'; Asp18→Ala, 5'-GCGCACCCATATTTGCGAACTTGCTTCC-3'. Expression and purification of GroEL and GroES were achieved as before (15). ATPase assays were carried out at 25 °C as previously described (26).

Surface Plasmon Resonance Measurements. ATP-dependent formation and dissociation of the GroEL–GroES complex was monitored by surface plasmon resonance with a BIAcore 2000 apparatus (BIAcore AB, Sweden). GroES was immobilized on a CM5 chip (BIAcore AB, Sweden) by amine coupling chemistry using standard protocols with HBS (10 mM HEPES with 0.15 M NaCl, 3.4 mM EDTA, and 0.005% surfactant P20 at pH 7.4) as running buffer. After activation with a freshly prepared mixture of *N*-hydroxysuccinimide (50 mM in water) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (195 mM in water) for 4 min (flow rate 10 μ L/min), GroES (5 μ g/mL in 150 mM sodium acetate buffer, pH 3.8) was injected for 5–10 min (flow rate 10 μ L/min). Remaining activated carboxylic groups were deactivated by injection of 1 M ethanolamine hydrochloride, pH 8.6, for 5 min (flow rate 10 μ L/min). A total of 1000 RU of GroES were immobilized by this method. After coupling, the buffer was changed to 50 mM Tris-HCl (pH 7.5) containing 1 mM DTT, 10 mM MgCl₂, 10 mM KCl (buffer A) and ATP at different fixed concentrations. This buffer was used in all the binding and dissociation experiments. The association of ATP-bound GroEL with GroES was monitored by adding different final concentrations of ATP to 10 nM GroEL in buffer A and immediately injecting this solution for 5 min at a flow rate of 20 μ L/min. The dissociation of GroEL from GroES was investigated by injecting different concentrations of ATP in buffer A for 15–30 min at a flow rate of 20 μ L/min. Controls are described under Results.

Data Analysis. In the case of transient kinetic analysis, it is not feasible to take into account all the possible ligation states of a GroEL ring with respect to ATP. We, therefore, consider a scheme (Figure 1) where it is assumed (as in the original formulation of the Hill equation) that *n* molecules of ATP bind to the trans ring of GroEL in an all-or-none fashion. ATP binding occurs only after the ADP-bound cis ring in the GroEL–GroES–(ADP)₇ ‘bullet’ complex (designated by **TD**ES) has undergone a conformational change (20) designated by a prime. Binding of ATP shifts the equilibrium of the trans ring from a **T**-like state with low affinity for ATP to an **R**-like state with high affinity for ATP and causes a conformational change in the cis ring designated by a double prime. Dissociation of GroES takes place from the **R_nD'**ES state which has an ADP-bound cis ring and an ATP-bound trans ring. We assume that ATP binding and GroES release occur much more rapidly than the conforma-

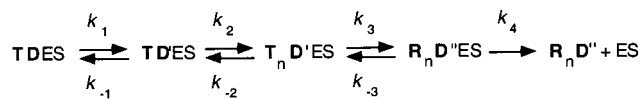


FIGURE 1: Scheme for different states of the GroEL–GroES complex considered in the kinetic analysis of its dissociation. The low- and high-affinity states for ATP and the ADP-bound conformation of GroEL rings are designated by **T**, **R**, and **D**, respectively. GroES is designated by ES. ATP binding occurs after the ADP-bound cis ring in the GroEL–GroES–(ADP)₇ ‘bullet’ complex (designated by **TD**ES) has undergone a conformational change designated by a prime. The trans ring of the **TD'**ES complex then binds *n* molecules of ATP in an all-or-none fashion. Binding of ATP shifts the equilibrium of the trans ring from a **T**-like state with low affinity for ATP to an **R**-like state with high affinity for ATP and causes a conformational change in the cis ring designated by a double prime. GroES then dissociates from the **R_nD'**ES complex. The **TD'**ES and **R_nD'**ES species are assumed to be in steady-state.

tional switches between the **T** and **R** states and the **D** and **D'** states and, thus, that the concentrations of the **TD'**ES and **R_nD'**ES species are in quasi-steady-state. Their concentrations are, therefore, given by the following expressions:

$$[\text{TD}'ES] = (k_1[\text{TD}ES] + k_{-2}[\text{T}_n\text{D}'ES]) / (k_{-1} + k_2[S]^n) \quad (1a)$$

$$[\text{R}_n\text{D}'ES] = k_3[\text{T}_n\text{D}'ES] / (k_{-3} + k_4) \quad (1b)$$

The concentrations of the other species in the scheme in Figure 1 are given by the following set of linear differential equations:

$$d[\text{TD}ES]/dt = -k_1k_2[S]^n[\text{TD}ES]/(k_{-1} + k_2[S]^n) + k_{-1}k_{-2}[\text{T}_n\text{D}'ES]/(k_{-1} + k_2[S]^n) \quad (2)$$

$$d[\text{T}_n\text{D}'ES]/dt = k_1k_2[S]^n[\text{TD}ES]/(k_{-1} + k_2[S]^n) - \{k_{-1}k_{-2}/(k_{-1} + k_2[S]^n) + k_3k_4/(k_{-3} + k_4)\}[\text{T}_n\text{D}'ES] \quad (3)$$

$$d[\text{ES}]/dt = k_3k_4[\text{T}_n\text{D}'ES]/(k_{-3} + k_4) \quad (4)$$

where *t* stands for time, [S] for the concentration of ATP, and [ES] for the concentration of free GroES. Solving eqs 2–4 yields the following expressions for the sum and product of the apparent rate constants, *k*_{obs(1)} and *k*_{obs(2)}, as follows:

$$k_{\text{obs}(1)} + k_{\text{obs}(2)} = (k_1k_2[S]^n + k_{-1}k_{-2}) / (k_{-1} + k_2[S]^n) + k_3k_4 / (k_{-3} + k_4) \quad (5)$$

$$k_{\text{obs}(1)}k_{\text{obs}(2)} = k_1k_2k_3k_4[S]^n / \{(k_{-1} + k_2[S]^n)(k_{-3} + k_4)\} \quad (6)$$

Assuming that the first step is fast relative to the second step yields the following expressions for *k*_{obs(1)} and *k*_{obs(2)}:

$$k_{\text{obs}(1)} = (k_1k_2[S]^n + k_{-1}k_{-2}) / (k_{-1} + k_2[S]^n) \quad (7a)$$

$$k_{\text{obs}(2)} = k_1k_2k_3k_4[S]^n / \{(k_1k_2[S]^n + k_{-1}k_{-2})(k_{-3} + k_4)\} \quad (7b)$$

Inspection of eqs 7a and 7b shows that the values of the two apparent rate constants depend on the concentration of ATP in a sigmoidal manner for *n* > 1. Data of resonance response units (RU) as a function of time (sensorgrams) for the dissociation phase were fitted to a double-exponential

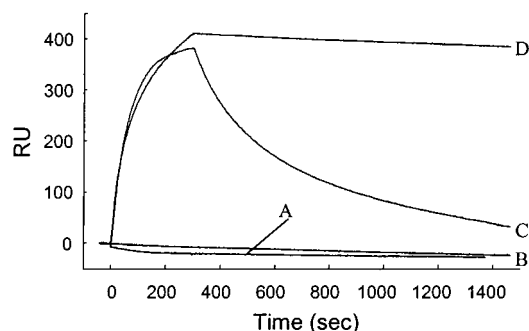


FIGURE 2: Resonance response units (RU) as a function of time for the ATP-dependent interaction of wild-type GroEL and the Asp398→Ala mutant with immobilized GroES. The experiments were carried out in buffer A containing 10 nM GroEL with or without 100 μ M ATP as described under Experimental Procedures. No binding is observed if ATP is injected alone (A) or if GroEL is injected without ATP (B). Wild-type GroEL binds to the immobilized GroES and is released in the presence of ATP (C). The Asp398→Ala GroEL mutant which is defective in ATP hydrolysis can bind to the immobilized GroES but is not released (D).

equation plus linear drift using the BIAcore software package. The linear baseline drift most likely reflects slow dissociation or inactivation of the immobilized GroES. Reported values of apparent rate constants represent the average of the values determined by analysis of 3–6 sensorgrams for each concentration of ATP. The concentration dependence of the apparent rate constants was analyzed by directly fitting the data in Figures 4 and 5 for the fast and slow phases to eqs 7a and 7b, respectively. For the purpose of data fitting, eqs 7a and 7b were rewritten in the respective compact forms $(a[S]^n + c)/([S]^n + b)$ and $a'[S]^n/([S]^n + b')$.

Initial velocities of ATP hydrolysis as a function of ATP concentration were fitted to the previously derived equation (11):

$$V_0 = \frac{0.5V_{\max(1)}L_1([S]/K_R)(1 + [S]/K_R)^{N-1} + V_{\max(2)}L_1L_2([S]/K_R)(1 + [S]/K_R)^{2N-1}}{1 + L_1(1 + [S]/K_R)^N + L_1L_2(1 + [S]/K_R)^{2N}} \quad (8)$$

where L_1 and L_2 are the respective apparent allosteric constants for the transitions **TT**→**TR** and **TR**→**RR**, V_0 is the initial rate of ATP hydrolysis, $V_{\max(1)}$ and $V_{\max(2)}$ are the respective maximal initial rates of ATP hydrolysis of the **TR** and **RR** states, and K_R is the dissociation constant of ATP for rings in the **R** state. All data fitting was carried out using Kaleidagraph [version 2.1 Synergy Software (PCS Inc.)]. Estimates of parameters (\pm standard errors) are reported.

RESULTS

Binding of GroEL to GroES is adenosine nucleotide-dependent (for a review, see ref 6). Thus, as expected, no binding to immobilized GroES was observed when wild-type GroEL was injected without ATP (Figure 2). No signal was observed also when only buffer containing ATP was injected. In a further control experiment (Figure 2), it was observed that the Asp398→Ala GroEL mutant (which is defective in ATP hydrolysis) can bind to GroES in a manner similar to wild-type GroEL but cannot dissociate (19). In

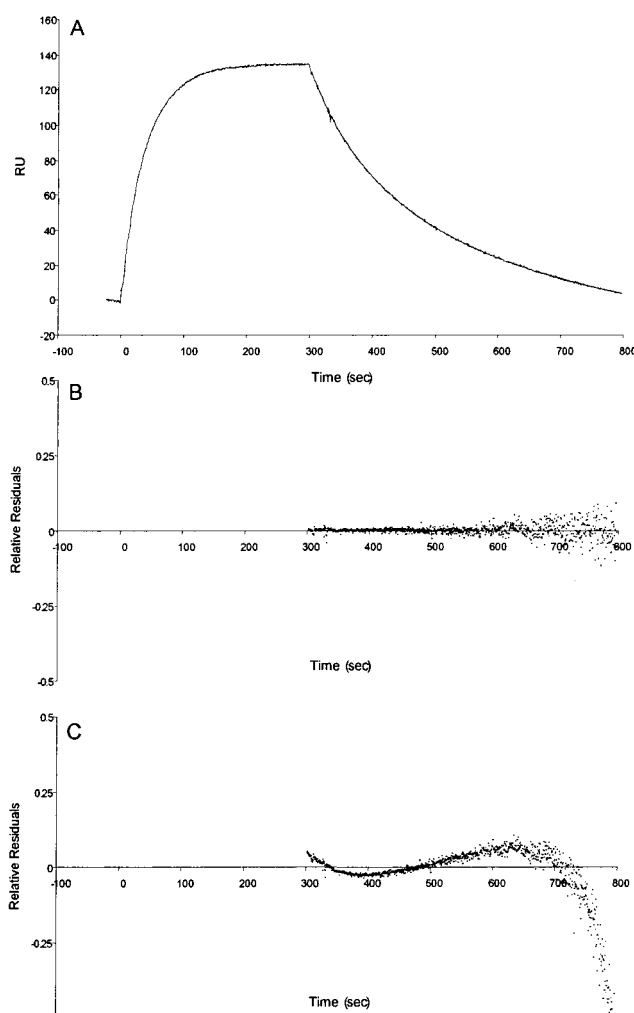


FIGURE 3: Fits of the dissociation phase of the ATP-dependent interaction of wild-type GroEL with immobilized GroES. The ATP-dependent interaction of 10 nM wild-type GroEL with immobilized GroES was measured in buffer A containing 500 μ M ATP as described under Experimental Procedures (A). The fit to a double-exponential equation with linear drift of the data for the dissociation phase is completely hidden by the experimental curve. Plots of residuals with small and random deviations about zero were obtained for this fit (B). A single-exponential equation with linear drift failed to fit the data for the dissociation phase as indicated by the nonrandom deviations of the residuals from zero (C).

previous studies on the interaction of GroEL with GroES using the BIAcore instrument, the sensorgram data were fitted to a simple 1:1 binding model (27) and to such a model with mass transport limitation or to a heterogeneous surface model (two types of complexes are formed) with mass transport limitation (28). Fitting our data for wild-type GroEL to a 1:1 binding model with one on-rate and two off-rates yielded estimates of $0.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and $11 \times 10^{-3} \text{ s}^{-1}$ for the values of the on-rate and fast off-rate, respectively, that are very similar to the values of $1.1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and $19 \times 10^{-3} \text{ s}^{-1}$ reported by Hayer-Hartl et al. (27). The resulting fits to all of these models were found, however, to be unsatisfactory, in agreement with the findings of Nieba et al. (28). Failure of a parallel reaction model (one analyte and two independent ligands) indicates that the data do not reflect heterogeneous immobilization. Here, we analyze only the dissociation phase, and the data are fitted to a double-exponential equation with linear drift (Figure 3). The quality

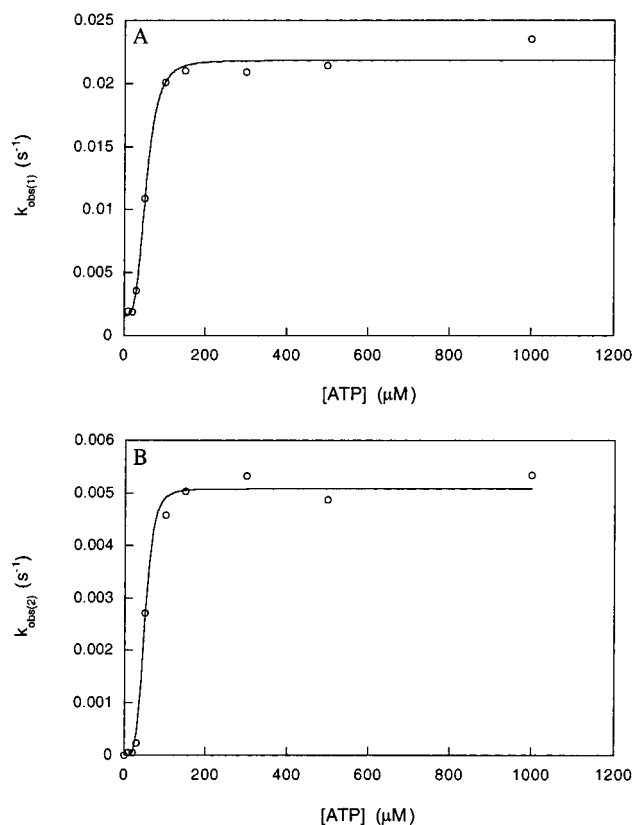


FIGURE 4: Observed rate constants of dissociation of wild-type GroEL from immobilized GroES at different concentrations of ATP. Data of resonance response units (RU) as a function of time were fitted to a double-exponential equation with linear drift using the BIAcore software package. The observed rate constants of the fast (A) and slow (B) phases are plotted as a function of the concentration of ATP, and the data were fitted to eqs 7a and 7b, respectively. The errors in $k_{\text{obs}(1)}$ and $k_{\text{obs}(2)}$ are up to 3% except in the case of $k_{\text{obs}(2)}$ at 10, 20, and 30 μM ATP where they are 20, 60, and 7%, respectively, but the absolute errors are very small and, thus, have almost no effect on the estimates of parameter values. The experiments were carried out at 25 °C as described under Experimental Procedures.

of the fits is excellent as reflected by residuals with random deviations about zero (shown in Figure 3B for a typical fit) and the low chi-square values (≤ 0.2). The data could not be fitted to a single-exponential equation with linear drift as reflected by residuals with nonrandom deviations about zero (Figure 3C). Previously, data for dissociation of the GroEL–GroES complex in solution also fitted well to a double-exponential equation (20).

ATP-Dependent Dissociation of Wild-Type GroEL from Immobilized GroES. The value of the observed rate constant corresponding to the fast phase of dissociation of wild-type GroEL from immobilized GroES is found to have a sigmoidal dependence on ATP concentration, but it is not zero in the absence of ATP (Figure 4A). Hence, these data were fitted to eq 7a. Assignment of this phase to the first two steps in the reaction scheme in Figure 1 is also based on the lack of correlation between the value of this observed rate constant and the extent of inter-ring cooperativity with respect to ATP (see Discussion). The value of the Hill coefficient corresponding to this phase is found to be 3.7 (± 0.8). It may be seen by inspection of eq 7a that the value of k_{-2} is equal to that of the observed rate constant of the fast phase at [ATP] = 0. It is found to be about 0.002 s⁻¹.

The value of k_1 is found to be 0.02 s⁻¹, which is in very good agreement with the value of 0.04 s⁻¹ previously determined for this step in solution studies (20, 29). The values of the rate constants k_{-1} and k_2 cannot be determined separately in the analysis here owing to the large errors involved.

The value of the observed rate constant corresponding to the slow phase of dissociation of wild-type GroEL from immobilized GroES is also found to have a sigmoidal dependence on ATP concentration, but it approaches zero at low ATP concentrations (Figure 4B). Hence, these data were fitted to eq 7b. Assignment of this phase to the last two steps in the reaction scheme in Figure 1 is also based on the correlation we find between the value of the logarithm of this observed rate constant and $\ln L_2$ and the extent of inter-ring allostery (see Discussion). The value of the Hill coefficient corresponding to this phase is found to be 4.8 (± 1.0). It may be seen by inspection of eq 7b that values of the individual rate constants cannot be determined from the fit to this equation.

ATP-Dependent Dissociation of the Arg197→Ala GroEL Mutant from Immobilized GroES. The values of the observed rate constants corresponding to the fast and slow phases of dissociation of the Arg197→Ala GroEL mutant from immobilized GroES are also found to have a sigmoidal dependence on ATP concentration (Figure 5). The phases were assigned as in the case of wild-type GroEL. The data for the fast phase were, therefore, fitted to eq 7a. The value of the Hill coefficient corresponding to this phase is found to be 1.6 (± 0.4). The value of k_1 is found to be 0.025 s⁻¹, which is somewhat higher than the value for wild-type GroEL. The value of k_{-2} is 0.002 s⁻¹, which is the same as that for wild-type GroEL. The data for the slow phase were fitted to eq 7b. The value of the Hill coefficient corresponding to this phase is found to be 2.95 (± 0.35).

Allosteric Properties of the Phe44→Trp, Arg18→Ala and Phe44→Trp, Glu67→Ala GroEL Double Mutants. Initial velocities of ATP hydrolysis were measured at different ATP concentrations (data not shown). The data were fitted to eq 8 (11). In the case of the Phe44→Trp, Arg18→Ala mutant, the values of the intrinsic allosteric constants, L_1 and L_2 , and K_R are 0.013 (± 0.006), $1.8 (\pm 1.6) \times 10^{-8}$, and 12 (± 1) μM, respectively. In the case of the Phe44→Trp, Glu67→Ala mutant, the values of the intrinsic allosteric constants, L_1 and L_2 , and K_R are found to be $1.3 (\pm 0.6) \times 10^{-3}$, $1.2 (\pm 0.9) \times 10^{-10}$, and 6.3 (± 0.6) μM, respectively.

Effects of Mutations That Alter the Allosteric Properties of GroEL on the Observed Rate Constants of Dissociation of GroEL from Immobilized GroES. Values of the observed rate constants corresponding to the dissociation of different GroEL mutants from immobilized GroES were determined in the presence of 100 μM ATP. This concentration of ATP is sufficient to promote a T to R transition in the trans ring of GroEL in complex with GroES (ref 21 and data not shown). A linear relationship ($r = 0.85$) with a negative slope is observed between the logarithms of the observed rate constants corresponding to the slow phase of dissociation of the different GroEL mutants and the logarithms of their respective allosteric constant, L_2 (Figure 6A). A linear relationship ($r = 0.81$) is also observed between the logarithms of the observed rate constants corresponding to the slow phase of dissociation of the different GroEL mutants

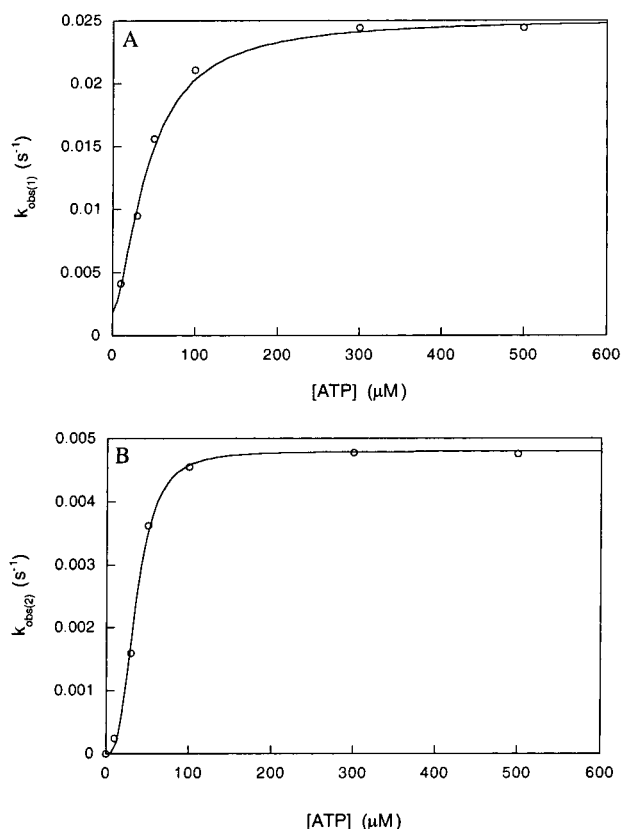


FIGURE 5: Observed rate constants of dissociation of the Arg197→Ala GroEL mutant from immobilized GroES at different concentrations of ATP. Data of resonance response units (RU) as a function of time were fitted to a double-exponential equation with linear drift using the BIAcore software package. The observed rate constants of the fast (A) and slow (B) phases are plotted as a function of the concentration of ATP, and the data were fitted to eqs 7a and 7b, respectively. The errors in $k_{\text{obs}(1)}$ and $k_{\text{obs}(2)}$ are up to 5% except in the case of $k_{\text{obs}(2)}$ at 10 μM ATP where it is about 30%, but the absolute error is very small and, thus, has almost no effect on the estimates of parameter values. The experiments were carried out at 25 °C.

and the extent of inter-ring negative cooperativity, $\ln(L_1/L_2)$, of the respective GroEL mutants (Figure 6B). In both cases, the data for the Arg13→Gly, Ala126→Val are outliers that are not included in the linear fits. No relationship was observed between values of the observed rate constants corresponding to the fast phase of dissociation of these mutants and values of their allosteric constants (not shown).

DISCUSSION

The values of both the fast and the slow observed rate constants of dissociation of wild-type GroEL and the Arg197→Ala mutant from GroES are found to display a sigmoidal dependence on the concentration of ATP (Figures 4 and 5). These findings lend support to the assumptions in our model (Figure 1) that the concentrations of the **TD**'ES and **R_nD**'ES species are in steady-state and that the first two steps in the model are fast relative to the last two steps. Otherwise, only one or neither of the observed rate constants would show a sigmoidal dependence on the concentration of ATP. The values of the Hill coefficients corresponding to the fast and the slow observed rate constants of dissociation of wild-type GroEL are found to be higher than those

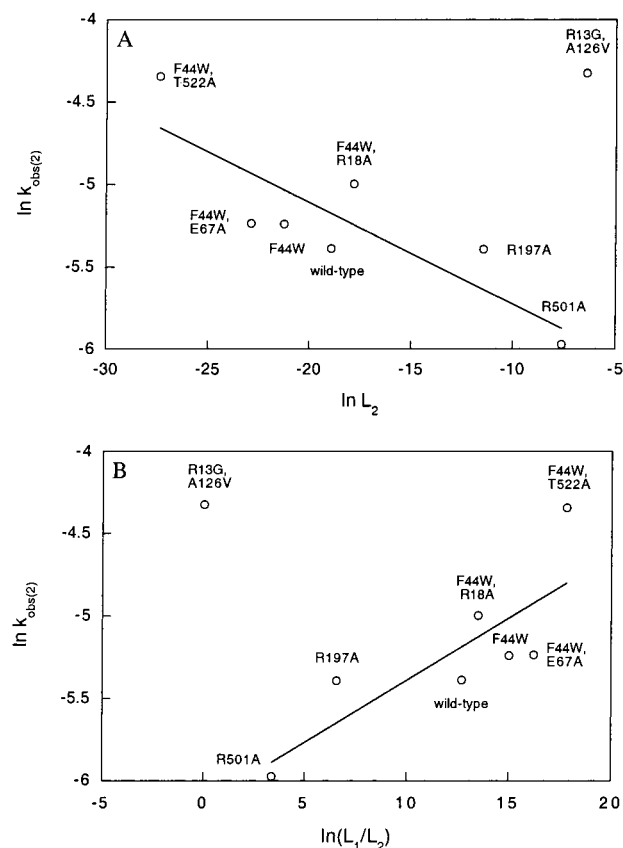


FIGURE 6: Allosteric effects of ATP on the observed rate constant corresponding to the slow phase of dissociation of GroEL from immobilized GroES. Values of the observed rate constants corresponding to the slow phase of dissociation of different GroEL mutants from immobilized GroES were determined in the presence of 100 μM ATP at 25 °C as described under Experimental Procedures. Their logarithms are plotted against the logarithms of the allosteric constant, L_2 (A), and the extent of inter-ring negative cooperativity, $\ln(L_1/L_2)$ (B), of the respective GroEL mutants. The values of L_1 and L_2 for wild-type GroEL (11) and the mutants Arg197→Ala (21), Phe44→Trp (22), Arg501→Ala (23), Arg13→Gly, Ala126→Val (24), and Phe44→Trp, Thr522→Ala (25) have been reported previously. The errors in $k_{\text{obs}(2)}$ are up to 7%. The single-letter notation for amino acids is used.

of the Arg197→Ala mutant, in accord with previous observations that this mutant has reduced intra-ring cooperativity (21). The values of the Hill coefficients corresponding to the respective fast and the slow observed rate constants of dissociation of wild-type GroEL [$3.7 (\pm 0.8)$ and $4.8 (\pm 1.0)$] and the Arg197→Ala mutant [$1.6 (\pm 0.4)$ and $2.95 (\pm 0.35)$] are in good agreement with the respective values of $3.83 (\pm 0.37)$ and $2.35 (\pm 0.12)$ of the Hill coefficients determined for these proteins from plots of initial rates of ATP hydrolysis as a function of ATP concentration, in the presence of GroES (21). This agreement provides further support for the model in Figure 1 and for the assertion (15) that plots of initial rates of ATPase activity as a function of ATP concentration, in the presence of GroES, provide information about cooperativity in the trans ring (and not the cis ring). Our model is also supported by the fact that the values of the Hill coefficients corresponding to the fast and the slow observed rate constants of dissociation are similar to each other in the case of both wild-type GroEL and the Arg197→Ala mutant. Such a similarity which is predicted by the model would not necessarily be observed in the case of a parallel kinetic

model for complex dissociation in which the two species in steady-state are on different pathways.

Strikingly, a linear relationship with a negative slope is observed between the logarithms of the observed rate constants corresponding to the slow phase of dissociation of the different GroEL mutants and the logarithms of their respective allosteric constant, L_2 , for the transition **TR**→**RR** (Figure 6A). In other words, the rate of complex dissociation increases with increasing positive cooperativity in ATP binding by the GroEL ring distal to GroES in the GroEL–GroES complex. This finding mirrors an earlier observation of ours that GroES binding promotes the allosteric transition of the trans GroEL ring (15). It may be seen from inspection of eq 7b for the observed slow rate constant that, in the case of increasing positive cooperativity, k_3 may decrease but the Hill coefficient, n , will increase, which may, therefore, give rise to an overall increase in the value of $k_{\text{obs}(2)}$. A double-logarithmic transformation of eq 7b shows that a correlation between $\ln k_{\text{obs}(2)}$ and n is to be expected, but there is no reason to assume that the values of the different intrinsic rate constants in this equation will be the same for the different mutants. Hence, the correlation between $\ln k_{\text{obs}(2)}$ and the extent of positive cooperativity in the second ring as reflected by L_2 is not expected to be perfect.

No correlations are observed between the values of the observed rate constants corresponding to the fast and slow phases of dissociation of the different GroEL mutants and those of the allosteric constant L_1 for the transition **TT**→**TR**. This is to be expected since ATP binding to the GroEL–GroES complex induces a **T** to **R**-like allosteric transition in the second ring and not in the first ring which is GroES-bound. The correlation between $k_{\text{obs}(2)}$ and L_2 and the absence of a correlation between $k_{\text{obs}(2)}$ and L_1 are also reflected in the somewhat weaker correlation between $k_{\text{obs}(2)}$ and the extent of inter-ring cooperativity (Figure 6B). No correlation is also observed between the values of the observed rate constants corresponding to the fast phase of dissociation of the different GroEL mutants and those of the allosteric constant L_2 . The lack of correlation between $k_{\text{obs}(1)}$ and L_2 is expected, in part, because of the constant term in the numerator of eq 7a. It is also expected given that $k_{\text{obs}(2)}$ (and not $k_{\text{obs}(1)}$) corresponds to the reaction steps which involve the allosteric transition (Figure 1). The linear fits in Figure 6 do not include the outlier data for the Arg13→Gly, Ala126→Val mutant. Outliers in linear free energy relationships often indicate that the perturbation (mutation) has caused a change in mechanism. Cells containing the Arg13→Gly, Ala126→Val double mutant which has defective inter-ring cooperativity (24) remain viable (30, 31) although some of GroEL's function is compromised in the double mutant (31), suggesting that the mechanism of GroES release from this mutant is different.

In conclusion, our results are consistent with a kinetic mechanism for dissociation of the GroEL–GroES complex according to which GroES release takes place after an ATP-induced conformational change in the trans ring that is preceded by ATP hydrolysis and a subsequent conformational change in the cis ring (19, 20). The importance of allostery in GroEL for its function still remains not fully understood. Here, we have found that the dissociation rate of the GroEL–GroES complex increases with increasing positive intra-ring cooperativity in ATP binding by the trans

ring. This observation explains why binding of polypeptide substrates to the trans ring, which increases positive intra-ring cooperativity in ATP binding by stabilizing it in a **T**-like state, accelerates GroES release from the cis ring (20). Previously, it was shown that the rate of GroEL-assisted folding of mouse dihydrofolate reductase, in the absence of GroES, decreases with increasing positive intra-ring cooperativity in the substrate-bound ring (7). The opposite effect in the case of GroES release may reflect, in part, the more ordered and multivalent nature of its association with GroEL as compared with most polypeptide substrates.

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