Biochemistry

 Volume 34, Number 16 April 25, 1995

Accelerated Publications

Nested Cooperativity in the ATPase Activity of the Oligomeric Chaperonin GroEL†

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Received February 1, 1995; Revised Manuscript Received March 8, 1995®

ABSTRACT: Initial rates of ATP hydrolysis by wild-type GroEL were measured as a function of ATP concentration from 0 to 0.8 mM. Two allosteric transitions are observed: one at relatively low ATP concentrations (≤ 100 μM) and the second at higher concentrations of ATP with respective midpoints of about 16 and 160 μM. Two allosteric transitions were previously observed also in the case of the Arg-196 → Ala GroEL mutant [Yifrach, O., & Horovitz, A. (1994) *J. Mol. Biol. 243*, 397−401]. On the basis of these observations a mathematical model for nested cooperativity in ATP hydrolysis by GroEL is developed in which there are two levels of allostery: one within each ring and the second between rings. In the first level, each heptameric ring is in equilibrium between the **T** and **R** states, in accordance with the Monod−Wyman−Changeux (MWC) model of cooperativity [Monod et al. (1965) *J. Mol. Biol. 12*, 88−118]. A second level of allostery is between the rings of the GroEL particle which undergoes sequential Koshland−Némethy−Filmer (KNF)-type transitions from the **TT** state *via* the **TR** state to the **RR** state [Koshland et al. (1966) *Biochemistry 5*, 365−385]. Using our model, we estimate the values of the Hill coefficient for the negative cooperativity between rings in wild-type GroEL and the Arg-196 → Ala mutant to be 0.003 (±0.001) and 0.07 (±0.02), respectively. The inter-ring coupling free energies in wild-type GroEL and the Arg-196 → Ala mutant are −7.5 (±0.4) and −3.9 (±0.3) kcal mol^{-1}, respectively.

The *Escherichia coli* GroE chaperonin system facilitates protein folding *in vivo* and *in vitro* [for reviews see, for example, Georgopoulos and Welch (1993), Hendrick and Hartl (1993), and Ellis (1994)]. It comprises GroEL, an oligomer of 14 identical subunits of 57.3 kDa (Hemmingsen et al., 1988), and its helper protein GroES, which is a seven-membered ring of identical subunits (Chandrasekhar et al., 1986) of 10 kDa (Hemmingsen et al., 1988). GroES is required for the successful reactivation of certain polypeptide substrates in a manner that depends on the refolding conditions (Schmidt et al., 1994). Electron microscopy studies have shown that the GroEL particle consists of two stacked heptameric rings with a central hole (Hendrix, 1979; Hohn et al., 1979; Saibil et al., 1991; Langer et al., 1992). The recently solved crystal structure of a double mutant of GroEL (Braig et al., 1994) shows that each subunit consists of three domains: (i) a large α-helical equatorial domain (residues 6−133 and 409−523) that forms all of the inter-ring contacts and most of the intra-ring side-to-side contacts between subunits; (ii) a large, loosely held, apical domain (residues 191−376) which forms the opening of the central channel; and (iii) an intermediate domain (residues 134−190 and 377−408) which connects the apical and equatorial domains.

GroEL has 14 ATP binding sites and a weak K^+-dependent (Viitanen et al., 1990) ATPase activity which is cooperative

† This work was supported by a research grant from the Angel Faivovich Foundation for ecological research. A.H. is an incumbent of the Robert Edward and Roselyn Rich Manson Career Development Chair.

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® Abstract published in *Advance ACS Abstracts,* April 1, 1995.

0006-2960/95/0434-5303$09.00/0

with respect to ATP (Gray & Fersht, 1991; Bochkareva et al., 1992) and K^+ ions (Todd et al., 1993). In the presence of GroES, the ATPase activity of GroEL is inhibited (Chandrasekhar et al., 1986), and cooperativity in the ATPase activity of GroEL with respect to ATP is increased (Gray & Fersht, 1991; Kovalenko et al., 1994). It has been shown that adenine nucleotides induce conformational changes in GroEL (Langer et al., 1992; Saibil et al., 1993) that destabilize its oligomeric structure (Horovitz et al., 1993). These conformational changes are required for the release of polypeptide substrates in a form committed to reach the native state (Badcoe et al., 1991; Langer et al., 1992; Baneyx & Gatenby, 1992; Jackson et al., 1993). Recently (Yifrach & Horovitz, 1994), we reported the effects of the mutation Arg-196 → Ala[1] in GroEL on its allosteric properties with respect to ATP. This residue is in the apical domain and is in contact with Glu-385 in the neighboring intermediate domain (Braig et al., 1994). Our results indicated that cooperativity in the ATPase activity of GroEL, with respect to ATP, is positive within each ring and negative between rings (Yifrach & Horovitz, 1994), and they suggested, therefore, the existence of nested cooperativity.

Several models, in particular the Monod–Wyman–Changeux (MWC) model (Monod et al., 1965) and the Koshland–Némethy–Filmer (KNF) model (Koshland et al., 1966), have been developed to describe cooperativity in ligand binding by oligomeric proteins. In both models, such cooperativity is due to ligand-induced conformational changes which may be either concerted (MWC), sequential (KNF), or a combination of both (Eigen, 1967). Nested allosteric models were first put forward in order to explain certain linkage phenomena in hemoglobin (Wyman, 1964, 1968). The concept of nested allostery is, however, particularly useful in the case of large oligomeric proteins with hierarchical structure. The hierarchical structure in these proteins suggests that a corresponding hierarchy in allosteric interactions may exist. For example, the individual hexameric units of arthropod hemocyanins bind oxygen with less positive cooperativity than the higher oligomers, which consist of 2, 4, or 8 hexameric units, for which the value of the Hill coefficient may be greater than 7 [see, for example, van Holde and Miller (1982)]. Nested allosteric models that were previously developed include some that are based only on the MWC formalism (Robert et al., 1987; Decker et al., 1988) and others in which KNF-type allosteric transitions are nested inside MWC transitions (Smith & Ackers, 1985; Brunori et al., 1986). Here, we present a model for nested cooperativity in ATP hydrolysis by GroEL, with respect to ATP, in which MWC-type transitions are nested inside a KNF model. We show that this allosteric model applies to wild-type GroEL and that effects of ADP and ATPγS on the allosteric properties of wild-type GroEL that we measure experimentally are consistent with this model. This model enables us to estimate for the first time the extent of negative cooperativity between rings in ATP hydrolysis by GroEL with respect to ATP.

THEORY

According to the MWC model, cooperativity in ligand binding is due to an equilibrium between two unligated states: a tense (**T**) state with relatively low affinity for the substrate (ATP), which is the predominant form in the absence of substrate, and a relaxed (**R**) state with relatively high affinity for the substrate. In the case of exclusive binding to the **R** state, the extent of cooperativity is determined only by the equilibrium constant L (=[**T**]/[**R**]). In the case of nonexclusive binding, the extent of cooperativity also depends on the relative affinities of the substrate for the **T** and **R** states. The sum of all the different macromolecular species considered by the MWC model can be represented using a grand partition function or binding polynomial, P, that is normalized relative to the concentrations of the unligated forms (Wyman, 1964):

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

where N is the total number of sites, $[\mathbf{R}]_i$ and $[\mathbf{T}]_i$ are the concentrations of the species in the **R** or **T** conformations to which i substrate molecules are bound, and [**R**] ($\equiv[\mathbf{R}]_0$) and [**T**] ($\equiv[\mathbf{T}]_0$) are the concentrations of the unligated species. By expressing the concentrations of the various $\mathbf{T}_i$ and $\mathbf{R}_i$ species in terms of the substrate concentration ([S]) and the statistically corrected microscopic binding constants, one obtains the following binding polynomial for the MWC model:

$$P = \frac{1}{[\mathbf{R}] + [\mathbf{T}]}\{[\mathbf{R}](1 + [\mathrm{S}]/K_\mathrm{R})^N + [\mathbf{T}](1 + [\mathrm{S}]/K_\mathrm{T})^N\} \tag{2}$$

where the terms $(1 + [\mathrm{S}]/K_\mathrm{R})^N$ and $(1 + [\mathrm{S}]/K_\mathrm{T})^N$ correspond respectively to N identical and independent sites with intrinsic affinities of K_R and K_T. The macroscopic equilibrium constants for each binding step are related to the intrinsic dissociation constants K_R and K_T *via* the statistical factors given by the binomial coefficients upon expansion of $(1 + [\mathrm{S}]/K_\mathrm{R})^N$ and $(1 + [\mathrm{S}]/K_\mathrm{T})^N$. By replacing [**T**] in eq 2 with L[**R**], one obtains

$$P = \frac{1}{1 + L}\{(1 + [\mathrm{S}]/K_\mathrm{R})^N + L(1 + [\mathrm{S}]/K_\mathrm{T})^N\} \tag{3}$$

The saturation binding curve, Y, may be easily derived from the binding polynomial using the relationship:

$$Y = \frac{\partial \ln P}{\partial \ln [\mathrm{S}]} = \left(\frac{[\mathrm{S}]}{P}\right)\frac{\partial P}{\partial [\mathrm{S}]} \tag{4}$$

The fractional saturation curve for the MWC model in the case of nonexclusive binding, obtained by combining eqs 3 and 4, is

$$\bar{Y} = \frac{\alpha(1 + \alpha)^{N-1} + Lc\alpha(1 + c\alpha)^{N-1}}{(1 + \alpha)^N + L(1 + c\alpha)^N} \tag{5}$$

where $\bar{Y} = Y/N$, $\alpha = [\mathrm{S}]/K_\mathrm{R}$, and $c = K_\mathrm{R}/K_\mathrm{T}$. The expression for exclusive binding is obtained by substituting $c = 0$ in eq 5.

In our model of nested cooperativity in GroEL, with respect to ATP, there are two levels of allostery. The first level is within each ring, and the second level is between the two rings. The two levels in allosteric interactions correspond to the hierarchy in the structure of GroEL which

[1] Sequence numbering does not include the N-terminal methionine residue which is cleaved.

is a dimer of heptamers. 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.[2] These two allosteric levels may be described by the following binding polynomial:

$$P = \frac{1}{[TT] + [TR] + [RR]}\{[TT](1 + [S]/K_T)^{2N} + [TR](1 + [S]/K_T)^{N}(1 + [S]/K_R)^{N} + [RR](1 + [S]/K_R)^{2N}\} \quad (6)$$

Upon expressing the concentrations of the unligated forms in terms of the intrinsic[3] allosteric constants L_1 and L_2 (L_1 = [TR]/[TT] and L_2 = [RR]/[TR]), one obtains

$$P = \frac{1}{1 + 2L_1 + L_1L_2}\{(1 + [S]/K_T)^{2N} + 2L_1(1 + [S]/K_T)^{N}(1 + [S]/K_R)^{N} + L_1L_2(1 + [S]/K_R)^{2N}\} \quad (7)$$

The fractional saturation binding curve obtained by combining eqs 4 and 7 is given by

$$\bar{Y} = \{c\alpha(1 + c\alpha)^{2N-1} + L_1c\alpha(1 + c\alpha)^{N-1}(1 + \alpha)^{N} + L_1\alpha(1 + c\alpha)^{N}(1 + \alpha)^{N-1} + L_1L_2\alpha(1 + \alpha)^{2N-1}\}/\{(1 + c\alpha)^{2N} + 2L_1(1 + c\alpha)^{N}(1 + \alpha)^{N} + L_1L_2(1 + \alpha)^{2N}\} \quad (8)$$

where $\alpha = [S]/K_R$, $c = K_R/K_T$, and $2N = 14$. In the case of exclusive binding ($c = 0$) one has

$$\bar{Y} = \frac{L_1\alpha(1 + \alpha)^{N-1} + L_1L_2\alpha(1 + \alpha)^{2N-1}}{1 + 2L_1(1 + \alpha)^{N} + L_1L_2(1 + \alpha)^{2N}} \quad (9)$$

Using the relationship $\bar{Y} = V_0/V_{max}$, we may rewrite eq 9, as

$$V_0 = \{0.5V_{max(1)}L_1([S]/K_R)(1 + [S]/K_R)^{N-1} + V_{max(2)}L_1L_2([S]/K_R)(1 + [S]/K_R)^{2N-1}\}/\{1 + L_1(1 + [S]/K_R)^{N} + L_1L_2(1 + [S]/K_R)^{2N}\} \quad (10)$$

where L_1 and L_2 are apparent allosteric constants and K_R is the dissociation constant of ATP. In this expression, $\bar{Y} = \bar{Y}_1 + \bar{Y}_2$, where the subscripts 1 and 2 refer to the **TR** and **RR** states, respectively. In the case of the Arg-196 → Ala mutant, eq 10 can be further simplified by assuming that the unligated **TT** state can be ignored, in which case one has

$$V_0 = \frac{0.5V_{max(1)}[S] + V_{max(2)}L_2[S](1 + [S]/K_R)^{N}}{K_R + [S] + K_RL_2(1 + [S]/K_R)^{N+1}} \quad (11)$$

Equation 11 was previously applied to kinetic data for the Arg-196 → Ala GroEL mutant using $2N = 14$ (Yifrach & Horovitz, 1994).

Analysis of the second level of allostery between the two GroEL rings may be performed using previously derived expressions for symmetric dimers. It may be shown [see, for example, Levitzki (1978)] (that, in the case of a symmetric dimer, the Hill coefficient at 50% saturation, n_{50}, is given by

$$n_{50} = \frac{2}{1 + (K_1/K_2)^{1/2}} \quad (12)$$

where K_1 and K_2 are the binding constants of the ligand to the first and second sites in the protein. According to the KNF model applied to the GroEL double ring, the binding constants K_1 and K_2 are given by

$$K_1 = K_sK_tK_{TR}/K_{TT} \quad (13)$$

$$K_2 = K_sK_tK_{RR}/K_{TR} \quad (14)$$

where K_{TT}, K_{TR} (=K_{RT}), and K_{RR} are the equilibrium constants for the association of the respective monomers (single rings) into dimers (double rings), K_t is the equilibrium constant for the conformational change **T** → **R** in the monomer (single ring) state (see Figure 1), and K_s is the binding constant of the substrate, S (ATP), to the single ring in the **R** state. Combining eqs 12−14 yields

$$n_{50} = \frac{2}{1 + ((K_{TR})^2/K_{RR}K_{TT})^{1/2}} \quad (15)$$

The values of the equilibrium constants K_{TT}, K_{TR} (=K_{RT}), and K_{RR} are difficult to determine, but it is possible to estimate the values of the intrinsic allosteric constants L_1 and L_2. It may be seen upon inspection of Figure 1 that $L_1 = K_tK_{RT}/K_{TT}$ and $L_2 = K_tK_{RR}/K_{TR}$. Introducing these expressions into eq 15 yields

$$n_{50} = \frac{2}{1 + (L_1/L_2)^{1/2}} \quad (16)$$

In this equation (eq 16), the Hill coefficient for the interaction between the two rings is a function only of the intrinsic allosteric constants L_1 and L_2.

EXPERIMENTAL PROCEDURES

Materials. Molecular biology reagents were purchased from New England Biolabs and radiochemicals from Amersham International or New England Nuclear (Du Pont). All other reagents were from Sigma or Aldrich.

Methods. The construction of the Arg-196 → Ala mutant has been reported previously (Yifrach & Horovitz, 1994). Expression and purification of GroEL were achieved as before (Yifrach & Horovitz, 1994). The ATPase activity of GroEL was measured according to the procedure described by Viitanen et al. (1990) with some modifications (Horovitz et al., 1993).

[2] For simplicity, we use the notation of **T** and **R** to designate respectively all the various conformations of the low- and high-affinity states for ATP of an individual ring. The conformation of a ring in the **T** or **R** state may depend not only on its ligation state with respect to different ligands but also on the ligation state of the adjacent ring.

[3] The transition **T** → **R** of either ring in the **TT** state will bring about the formation of the **TR** state since the **TR** and **RT** states are indistinguishable. Likewise, the transition **R** → **T** of either ring in the **RR** state will bring about the formation of the **TR** state. The intrinsic allosteric constants L_1 and L_2 are therefore related to the apparent allosteric constants, L_1(app) and L_2(app), as follows: L_1(app) = $2L_1$; L_2(app) = $L_2/2$.

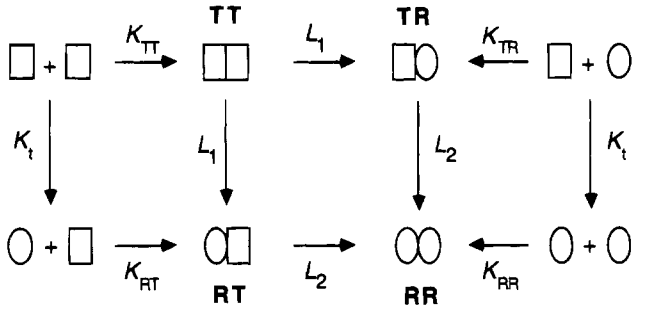

FIGURE 1: Scheme for the nested cooperativity in GroEL with respect to ATP. The scheme is based on both the MWC (Monod et al., 1965) and the KNF (Koshland et al., 1966) models of cooperativity. In the absence of ligands, GroEL is predominantly in the symmetrical **TT** state. In the presence of ATP, the equilibrium is shifted toward the **TR** (≡**RT**) state (L_1 = [**TR**]/[**TT**]). A further shift in the equilibrium toward the symmetrical **RR** state occurs at higher ATP concentrations (L_2 = [**RR**]/[**TR**]). Analysis of the allosteric changes within each ring is based on the MWC model. Analysis of the interaction between rings is based on the KNF model since symmetry is broken; *i.e.*, the changes which occur at the level of the double ring are sequential and not concerted. According to the KNF model, cooperativity is a function of K_{TT}, K_{TR} (=K_{RT}), and K_{RR}, which are the equilibrium constants for the association of the respective monomers (single rings) into dimers (double rings). The equilibrium constant, K_t, for the conformational change **T** → **R** in the monomer (single ring) state does not affect the level of allostery between rings.

Data Analysis. Analysis of cooperativity in ATP hydrolysis by GroEL at low ATP concentrations (≤100 μM) was performed by directly fitting initial ATPase velocities at different ATP concentrations, using Kaleidagraph [version 2.1 Synergy Software (PCS Inc.)], to the Hill equation:

$$V_0 = V_{max}K[S]^n/(1 + K[S]^n) \quad (17)$$

where V_0 and V_{max} are the initial and maximal initial ATPase reaction velocities, [S] is the concentration of substrate (ATP), K is the apparent ATP binding constant, and n is the Hill coefficient. Analysis of cooperativity in ATP hydrolysis by wild-type GroEL and the Arg-196 → Ala mutant over the entire range of ATP concentrations (0−0.8 mM in the case of wild-type GroEL) was carried out using eq 10. Identical estimates for the values of the different parameters were obtained when data fitting to eq 10 was carried out either with or without fixing the values for $V_{max(1)}$ and $V_{max(2)}$ (*i.e.*, three or five free parameters, respectively).

Throughout this paper, we report estimates of parameters ± standard errors as given by Kaleidagraph which are 68% confidence intervals for the true values of the parameters. R^2 values for all the fits in this study were greater than 0.99.

RESULTS

Positive Cooperativity in ATP Hydrolysis by Wild-Type GroEL. Initial rates of ATP hydrolysis by GroEL were measured as a function of ATP concentration from 0 to 0.8 mM. Two allosteric transitions are observed: one at relatively low ATP concentrations (≤100 μM) and the second at higher concentrations of ATP[4] (Figure 2). The midpoints of the two transitions are at ATP concentrations

[4] Both wild-type and the Arg-196 → Ala mutant GroEL particles are stable and do not dissociate into monomers at ATP concentrations higher than the midpoint of the second transition (Yifrach & Horovitz, 1994).

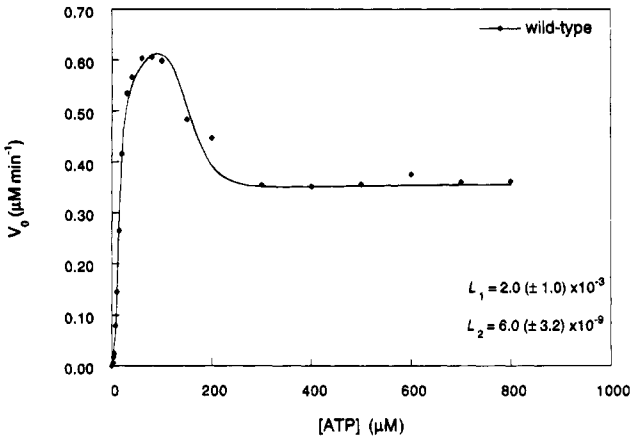

FIGURE 2: Initial velocity of ATP hydrolysis by wild-type GroEL at different ATP concentrations. The data were fitted to eq 10. The oligomer concentration of GroEL is 25 nM. The reactions were carried out at 25 °C as described under Experimental Procedures.

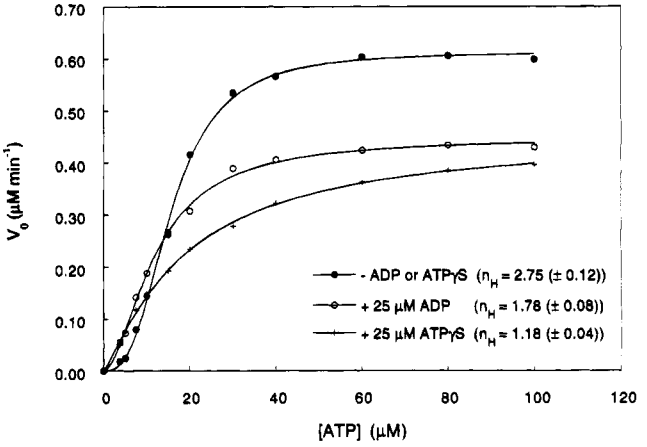

FIGURE 3: Initial velocity of ATP hydrolysis by wild-type GroEL at different ATP concentrations in the absence or presence of 25 μM ATPγS or ADP. The data were fitted to the Hill equation (eq 17). The oligomer concentration of GroEL is 25 nM. The reactions were carried out at 25 °C as described under Experimental Procedures.

of about 16 and 160 μM, respectively. The data for the low ATP concentrations (Figure 3) were directly fitted to the Hill equation (eq 17). The value of the Hill coefficient for the allosteric transition at the low ATP concentrations (≤100 μM), in the presence of 10 mM K^+ ions, is found to be 2.75 (±0.12), which is in good agreement with the values previously reported (Todd et al., 1993; Yifrach & Horovitz, 1994). In our model, positive cooperativity in ATP hydrolysis by GroEL with respect to ATP at this concentration range (≤100 μM) reflects the transition from the **TT** state to the **TR** state. The value of k_{cat} for ATP hydrolysis, calculated for seven sites in the **TR** state assuming exclusive binding to the **R** state, is 0.058 (±0.001) s^{-1}. The data for the entire range of ATP concentrations (0−0.8 mM) were fitted to eq 10 ($2N$ = 14). The value of the intrinsic allosteric constant, L_1, for the first transition (**TT** → **TR**) is 0.002 (±0.001) whereas the value of the intrinsic allosteric constant, L_2, for the second transition (**TR** → **RR**) is 6.0 (±3.2) × 10^{-9}. The values of k_{cat} for ATP hydrolysis of the **TR** and **RR** states obtained from this fit and calculated for 7 and 14 sites, respectively, are 0.132 s^{-1} and 0.016 s^{-1}, and the value of K_R is 10 μM. The higher value of k_{cat} for ATP hydrolysis

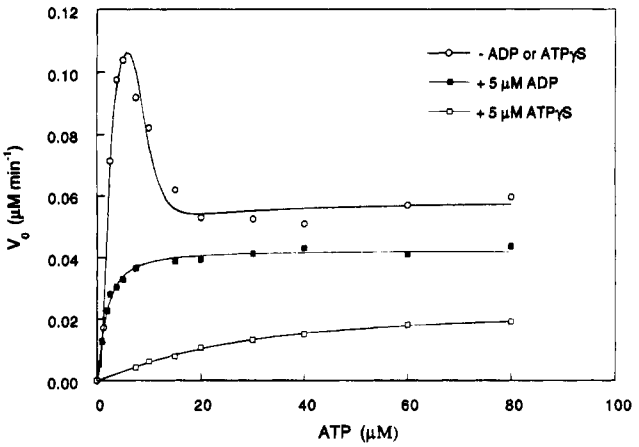

FIGURE 4: Initial velocity of ATP hydrolysis by the Arg-196 → Ala GroEL mutant at different ATP concentrations in the absence or in the presence of ADP or ATPγS. The data in the absence of ADP or ATPγS were fitted to eq 10. The data in the presence of ADP or ATPγS were fitted to the Hill equation (eq 17). The values of the intrinsic allosteric constants L_1 and L_2, in the absence of ADP or ATPγS, are $L_1 = 7.6\ (\pm 3.4) \times 10^{-3}$ and $L_2 = 1.1\ (\pm 0.4) \times 10^{-5}$. The values of the Hill coefficients in the presence of 5 μM ATPγS or ADP are 1.30 (±0.09) and 1.35 (±0.09), respectively. The oligomer concentration of GroEL is 10.6 nM. The reactions were carried out at 25 °C as described under Experimental Procedures.

of the **TR** state obtained from the fit to eq 10 may reflect the true k_{cat} for ATP hydrolysis of the GroEL single ring.

Positive Cooperativity in ATP Hydrolysis by the Arg-196 → Ala GroEL Mutant. Initial rates of ATP hydrolysis by GroEL were measured as a function of ATP concentration. Two allosteric transitions are observed also in the case of the Arg-196 → Ala GroEL mutant. The midpoints of the two transitions are at ATP concentrations of about 2.5 and 12 μM (Figure 4). The data for the Arg-196 → Ala mutant were previously fitted to eq 11 (Yifrach & Horovitz, 1994). Here, the data is fitted to eq 10 ($2N = 14$) which was derived without assuming that the concentration of the **TT** state may be ignored. The value of the intrinsic allosteric constant, L_1, for the first transition is $7.6\ (\pm 3.4) \times 10^{-3}$ whereas the value of the intrinsic allosteric constant, L_2, for the second transition is $1.1\ (\pm 0.4) \times 10^{-5}$. The values of k_{cat} for ATP hydrolysis of the **TR** and **RR** states obtained from this fit and calculated for 7 and 14 sites, respectively, are 0.067 s^{-1} and 0.008 s^{-1}, and the value of K_R is 2 μM.

Effects of ATPγS and ADP on the Extent of Cooperativity in ATP Hydrolysis by Wild-Type GroEL and the Arg-196 → Ala Mutant. Initial rates of ATP hydrolysis by wild-type GroEL were measured as a function of ATP concentration in the presence of 25 μM ATPγS or ADP (Figure 3). The data were directly fitted to the Hill equation (eq 17). In the presence of 25 μM ATPγS, the value of the Hill coefficient is 1.18 (±0.04); *i.e.*, under these conditions the kinetics of ATP hydrolysis by GroEL are essentially Michaelis–Menten. In the presence of 25 μM ADP, the value of the Hill coefficient is 1.78 (±0.08).

Initial rates of ATP hydrolysis by the Arg-196 → Ala mutant were measured as a function of ATP concentration in the presence of 5 μM ATPγS or ADP (Figure 4). The data were directly fitted to the Hill equation (eq 17). The values of the Hill coefficients in the presence of 5 μM ATPγS or ADP are 1.30 (±0.09) and 1.35 (±0.09), respectively.

Negative Cooperativity between the Two Rings of Wild-Type GroEL and the Arg-196 → Ala Mutant. The extent of negative cooperativity between rings in ATP hydrolysis by GroEL with respect to ATP was estimated using eq 16. The values of the Hill coefficient for this level of allostery are 0.003 (±0.001) and 0.07 (±0.02) for wild-type GroEL and the Arg-196 → Ala mutant, respectively. The coupling free energy (Horovitz, 1995) between the two rings is given by (see Figure 1) $\Delta\Delta G_{int} = -RT \ln(L_1/L_2)$. In the case of wild-type GroEL, the value of $\Delta\Delta G_{int}$ is −7.5 (±0.4) kcal mol^{-1} whereas in the case of the Arg-196 → Ala mutant the value of $\Delta\Delta G_{int}$ is −3.9 (±0.3) kcal mol^{-1}, both at 25 °C.

DISCUSSION

Our results suggest a nested allosteric model for cooperativity in ATP hydrolysis by GroEL. According to this model, there are two levels of allostery in GroEL: one within each ring and the second between the two rings. These two levels of allostery correspond to the hierarchical structure of GroEL which, in the absence of ligands, is a symmetric dimer of heptamers. In the first level of allostery, each heptameric ring is in equilibrium between the **T** and **R** states, in accordance with the MWC representation (Monod et al., 1965). The transition of the first ring from the **T** state to the **R** state, *i.e.*, the transition of the GroEL particle from the **TT** state to the **TR** state (L_1 = [**TR**]/[**TT**]), is reflected by the positive cooperativity in ATP binding and hydrolysis by GroEL at relatively low ATP concentrations (≤100 μM) in the absence of GroES (Gray & Fersht, 1991; Bochkareva et al., 1992). In the presence of 25 μM ATPγS (or ADP), a concentration higher than the midpoint of the first transition (16 μM) but far smaller than the midpoint of the second transition (160 μM), the equilibrium is shifted toward the **TR** state, thus leading to a decrease in the extent of cooperativity (Figure 3).

The transition of the second ring from the **T** state to the **R** state, *i.e.*, the transition of the GroEL particle from the **TR** state to the **RR** state (L_2 = [**RR**]/[**TR**]) which has more high-affinity sites for ATP but a lower k_{cat}, was previously observed in the case of the Arg-196 → Ala mutant (Yifrach & Horovitz, 1994). In the case of wild-type GroEL, this second transition has been observed only indirectly in the presence of GroES (Gray & Fersht, 1991; Kovalenko et al., 1994) or ADP (Bochkareva & Girshovich, 1994). Here, by extending (to 0.8 mM) the range of ATP concentrations at which we measured initial ATPase velocities, we were able to determine the value of the allosteric constant, L_2, for the transition of the second ring of wild-type GroEL in the absence of GroES or other ligands. The values of both L_1 and L_2 for wild-type GroEL are significantly smaller than the corresponding values for the Arg-196 → Ala mutant. In other words, positive cooperativity in ATP hydrolysis with respect to ATP within each of the rings of the Arg-196 → Ala mutant is decreased relative to wild-type GroEL. Positive cooperativity with respect to ATP in ATP hydrolysis by the Arg-196 → Ala mutant is decreased (Figure 4) in the presence of 5 μM ATPγS or ADP, a concentration between the midpoints of the two allosteric transitions of this mutant, owing to a shift in the equilibrium toward the **TR** and **RR** states. Arg-196 makes an interaction with Glu-385 in the intermediate domain of a neighboring subunit in the same ring (Braig et al., 1994). The mutation Arg-196 → Ala is,

therefore, expected to affect the allosteric transition of each of the rings as observed in this study.

The 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 . By determining the values of L_1 and L_2 for wild-type GroEL and the Arg-196 → Ala mutant, and using eq 16 which was previously derived for symmetric dimers, it is here possible for the first time to estimate the values of the Hill coefficients for the negative inter-ring cooperativity in ATP hydrolysis with respect to ATP. The values of these Hill coefficients for wild-type GroEL and the Arg-196 → Ala mutant are 0.003 (±0.001) and 0.07 (±0.02), respectively. Although both values are ≪1 (thus reflecting strong negative cooperativity), the value for wild type is significantly smaller. The mutation Arg-196 → Ala, therefore, reduces not only the positive cooperativity within each ring but also the negative cooperativity between rings. The coupling free energy, $\Delta\Delta G_{int}$, is a measure of the extent to which the free energy associated with the transition **T** → **R** depends on the conformational state of the adjacent ring (Horovitz, 1995). The change upon the mutation Arg-196 → Ala in $\Delta\Delta G_{int}$ is −3.6 (±0.5) kcal mol^{-1}, and it reflects the weaker coupling between rings in the mutant. The structural basis for the reduction of the inter-ring negative cooperativity owing to this mutation is, however, not yet clear.

In conclusion, a model for nested cooperativity in ATP hydrolysis by GroEL has been developed in which MWC transitions (Monod et al., 1965) within each ring are nested inside a KNF model (Koshland et al., 1966) for the interaction between the two rings. The two levels in allosteric interactions correspond to the hierarchy in the unique double-toroid structure of GroEL. A key question which remains unanswered is whether ATP-induced conformational changes in GroEL actually occur in the cell where the concentration of ATP is high (in the millimolar range) and relatively constant? One intriguing possibility is that the concentration of ATP in the cavity of GroEL is not in equilibrium with the rest of the cell since GroEL's ATP binding sites are buried (Braig et al., 1994) and thus may not be accessible when GroES and nonfolded protein are bound. Cooperativity in ATP hydrolysis by GroEL may have evolved so that a small change in the local concentration of ATP, during its reaction cycle, may trigger switching between different functional states, thereby promoting binding or release of protein substrates.

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BI950232O