Residue Lysine-34 in GroES Modulates Allosteric Transitions in GroEL†

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ABSTRACT: The conserved residue Lys-34 in GroES was replaced by alanine and glutamic acid using site-directed mutagenesis. This residue is near the carboxy terminus of the mobile loop in GroES (residues 17−32) which becomes immobilized upon formation of the GroEL/GroES complex [Landry et al. (1993) *Nature 364*, 255−258]. Both charge neutralization (Lys-34→Ala) and charge reversal (Lys-34→Glu) at this position have little effect on the binding constant of GroES to GroEL, but they increase the enhancement by GroES of cooperativity in ATP hydrolysis by GroEL. This is reflected by a change in the Hill coefficient (at 10 mM K^+) from 4.10 (±0.22) in the presence of wild-type GroES to 5.17 (±0.24) and 4.46 (±0.14) in the presence of the GroES mutants Lys-34→Ala and Lys-34→Glu, respectively. The results are interpreted using the Monod−Wyman−Changeux (MWC) model for cooperativity [Monod et al. (1965) *J. Mol. Biol. 12*, 88−118]. They suggest that Lys-34 in GroES modulates the allosteric transition in GroEL by stabilizing a relaxed (**R**)-like state.

Chaperonins are ubiquitous proteins that facilitate protein folding both *in vivo* and *in vitro* [for reviews, see, for example, Georgopoulos and Welch (1993), Hendrick and Hartl (1993), and Ellis (1994)]. The *Escherichia coli* GroE chaperonin system comprises GroEL, an oligomer of 14 identical subunits of 57.3 kDa (Hemmingsen et al., 1988) which form 2 stacked heptameric rings (Hendrix, 1979; Hohn et al., 1979; Saibil et al., 1991), and its helper-protein GroES, which is a 7-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 GroEL substrates [see, for example, Goloubinoff et al. (1989) and Martin et al. (1991)] in a manner that depends on the precise nature of the refolding conditions (Schmidt et al., 1994a). GroEL has an ATPase activity which is K^+-dependent (Viitanen et al., 1990) and cooperative with respect to ATP (Gray & Fersht, 1991) and K^+ ions (Todd et al., 1993). In the presence of GroES, the ATPase activity of GroEL is inhibited (Chandrasekhar et al., 1986; Viitanen et al., 1990; Gray & Fersht, 1991; Langer et al., 1992; Jackson et al., 1993), and positive cooperativity in ATP hydrolysis by GroEL with respect to ATP is increased (Gray & Fersht, 1991).

GroES forms a 1:1 bullet-shaped (Langer et al., 1992; Saibil et al., 1993) or a 2:1 football-shaped (Llorca et al., 1994; Azem et al., 1994; Schmidt et al., 1994b) complex with GroEL in the presence of the adenine nucleotides ATP (Chandrasekhar et al., 1986) and ADP (Bochkareva et al., 1992). Recently (Landry et al., 1993), it was shown that a highly flexible loop in GroES comprising residues 17−32 in the GroES sequence becomes immobilized upon formation of the GroEL/GroES complex. Limited proteolysis of GroES with trypsin showed that this segment is accessible in GroES but not in the GroEL/GroES complex (Landry et al., 1993). In addition, mutations in the *groES* structural gene that were originally isolated (Georgopoulos et al., 1973) on the basis of their ability to block bacteriophage λ growth were found to be located in the carboxy-terminal region (residues 23−31) of this segment (Zeilstra-Ryalls et al., 1993). It was concluded from these results, and from comparison of the sequences of the mobile loop region in GroES homologues [see, for example, Hartman et al. (1992)], that there is a direct interaction between GroEL and the mobile loop region of GroES (Landry et al., 1993). The recent observation (Höhfeld & Hartl, 1994) that the mutation Pro-36→Ser in yeast hsp10 (this position in yeast hsp10 corresponds to Val-28 in the mobile loop of GroES) reduces its binding affinity for hsp60 further supports this conclusion.

Cooperativity in GroEL function reflects adenine nucleotide- and GroES-induced conformational changes (Langer et al., 1992; Saibil et al., 1993) in its structure which are important for the release of protein ligands in a form committed to reach the native state (Badcoe et al., 1991; Creighton, 1991; Baneyx & Gatenby, 1992; Langer et al., 1992). We were interested in identifying residues in GroES that are involved in the modulation of the allosteric transitions in GroEL. Here, we report the effects of the mutations Lys-34→Ala and Lys-34→Glu in GroES on the allosteric properties of GroEL. Residue Lys-34 was targeted and mutated since it is next to the mobile loop in GroES (Landry et al., 1993) and, also, because conserved charged residues often play a key role in allosteric transitions (Perutz, 1989; Yifrach & Horovitz, 1994). The effects of the mutations in GroES on its interaction with GroEL were determined by measuring the ATPase activity of GroEL in the presence of the different GroES mutants. The mutations Lys-34→Ala and Lys-34→Glu in GroES were found to increase the enhancement by GroES of cooperativity in ATP hydrolysis by GroEL but to have little effect on the binding affinity of GroES to GroEL. The results are interpreted using the conceptual framework of the MWC model (Monod et al., 1965) for cooperativity.

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EXPERIMENTAL PROCEDURES

Materials. Molecular biology reagents were purchased from New England Biolabs and radiochemicals from Amersham International or New England Nuclear (Du Pont). Citrate synthase (pig heart) used in the reactivation experiments was from Boehringer Mannheim, and all other reagents were from Sigma or Aldrich.

Mutagenesis. Single-stranded DNA of the plasmid pOA (Horovitz et al., 1993a) containing the genes coding for GroEL and GroES was obtained by infecting *E. coli* TG2 cells harboring this plasmid with M13KO7 helper-phage (Pharmacia, Sweden). Site-directed mutagenesis was carried out as before (Horovitz et al., 1993a) using the following mutagenic oligonucleotide: Lys-34→Ala or Glu: 5′-GCCGCGGGTGGAC*(GT)*C*AGCCGCTGCAGA-3′; an asterisk follows the mismatched bases. Parentheses indicate equimolar mixed base additions. Mutants were identified by direct sequencing of single-stranded DNA of the full GroES gene. The groEL gene in pOA plasmids containing wild-type or mutant groES genes was inactivated by introducing a frameshift mutation at the *Bst*BI restriction site of this gene by digestion with *Bst*BI followed by a fill-in and ligation reaction.

Protein Expression and Purification. Starter cultures (5 mL) of *E. coli* TG2 cells harboring either wild-type or mutant pOA plasmid were grown overnight at 37 °C in 2× TY medium containing 50 μg/mL ampicillin. These cultures were used to inoculate 0.5 L of 2× TY medium containing ampicillin. GroEL and GroES were purified from cells grown overnight at 37 °C without IPTG induction. GroEL was purified as described by Todd et al. (1993) but with some modifications. Cell pellets were resuspended in buffer containing 50 mM Tris-HCl, pH 7.5, 60 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, and 0.5 mM PMSF (buffer A) and lysed by sonication. The lysate was clarified by centrifugation at 30000*g* for 30 min at 4 °C. The supernatant was subjected to a 30−55% ammonium sulfate cutoff step. Protein pellet was resuspended in buffer containing 50 mM Tris-HCl, pH 7.2, 1 mM DTT, and 0.1 mM EDTA (buffer B) and desalted using a PD-10 Sephadex column equilibrated with buffer B. Samples were then applied to a DEAE-Sepharose CL-6B column equilibrated with buffer B. GroEL-enriched fractions were pooled, concentrated to less than 2 mL using a Centriprep-30 (Amicon), incubated with buffer B containing 0.1 mM ATP (pH 7.2) for 20 min at 25 °C, and applied to a Sephacryl S-300 gel-filtration column (Pharmacia) equilibrated with buffer B (without ATP). Combined GroEL fractions were next applied to a Mono-Q HR 5/5 column equilibrated with 50 mM MES, pH 6.0, 1 mM DTT, and 1 mM EDTA. Pure GroEL fractions were concentrated by Centriprep-30, frozen in liquid nitrogen, and stored at −80 °C in buffer containing 20 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 2 mM DTT, and 0.1 mM EDTA. GroES-enriched fractions from the DEAE-Sepharose column were pooled, subjected to a 60% ammonium sulfate cutoff step, and then applied to a Sephacryl S-300 column equilibrated with buffer B. GroES-containing fractions were then applied to a Mono-Q HR 5/5 column equilibrated in the same buffer. GroES was finally purified on a Mono-S column equilibrated in 50 mM sodium succinate (pH 4.6) and 0.1 mM EDTA. Pure GroES fractions were stored in buffer A (without PMSF) at −80 °C. The homogeneity of GroEL and GroES preparations was judged from 7.5% native and SDS−PAGE as described earlier (Horovitz et al., 1993b). GroEL and GroES concentrations were determined using the absorbance values A_{280nm}(0.1%, 1cm) of 0.25 and 0.17, respectively (Bochkareva et al., 1992).

ATPase Assays. The ATPase activity of GroEL was measured according to the procedure described by Viitanen et al. (1990) with some modifications (Horovitz et al., 1993a). The reactions were carried out at 25 °C in a buffer of 50 mM Tris-HCl (pH 7.5), 10 mM KCl, 10 mM $MgCl_2$, and 1 mM DTT.

Data Analysis. Inhibition of the ATPase activity of GroEL by GroES may be analyzed using the equation (Todd et al., 1993):

$$\nu/V_{max} = \frac{[ATP]^n(1-\alpha)}{K' + [ATP]^n} + \frac{[ATP]^n\alpha}{2(K'' + [ATP]^n)} \quad (1)$$

$$\alpha = \{K_d + [GroEL]_T + [GroES]_T \pm [(K_d + [GroEL]_T + [GroES]_T)^2 - 4[GroEL]_T[GroES]_T]^{1/2}\}/2[GroEL]_T$$

where $\alpha = [GroEL/GroES]/[GroEL]_T$, [GroEL/GroES] is the concentration of the asymmetric GroEL/GroES complex, $[GroEL]_T$ and $[GroES]_T$ are the total concentrations of GroEL and GroES, respectively, K' and K'' are the binding constants of ATP to GroEL and the asymmetric GroEL/GroES complex, respectively, and K_d is the dissociation constant between GroEL and GroES. In the presence of excess ATP ($[ATP]^n \gg K'$ and $[ATP]^n \gg K''$), eq 1 reduces to

$$\nu/V_{max} = 1 - \alpha/2 \quad (2)$$

Analysis of the inhibition of the ATPase activity of GroEL by wild-type GroES and the Lys-34→Ala and Lys-34→Glu mutants was carried out by directly fitting the data in Figure 1 to eq 2 using Kaleidagraph [version 2.1 Synergy Software (PCS Inc.)].

Analysis of cooperativity in ATP hydrolysis by GroEL 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:

$$V_0 = V_{max}K[S]^n/(1 + K[S]^n) \quad (3)$$

where V_0 and V_{max} are the initial and maximal initial ATPase reaction velocities, respectively, [S] is the concentration of substrate (ATP), K is the apparent ATP binding constant, and n is the Hill coefficient. Linear plots were obtained by replotting the data according to the logarithmic form of the Hill equation:

$$\log[V_0/(V_{max} - V_0)] = \log K + n \log [S] \quad (4)$$

Initial rates of ATP hydrolysis by GroEL were also directly fitting to the MWC equation (Monod et al., 1965) for exclusive binding to the **R** state assuming 7 sites ($N = 7$):

$$V_0 = \{V_{max}[S]/K_R(1 + [S]/K_R)^{N-1}\}/[L'_2 + (1 + [S]/K_R)^N] \quad (5)$$

where K_R is the intrinsic dissociation constant between ATP and the **R** form (which in our model is the **RR**ES species as shown in Figure 3) and L'_2 (=[**TR**ES]/[**RR**ES]) is the allosteric constant.

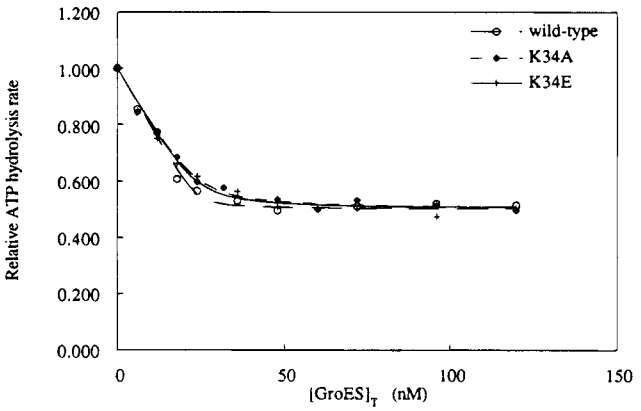

FIGURE 1: Inhibition of the ATPase activity of GroEL by wild-type and mutant GroES's. Initial maximal rates of ATP hydrolysis by GroEL were measured at various concentrations of wild-type GroES and the Lys-34→Ala and Lys-34→Glu mutants. The rates are all normalized relative to the rate of ATP hydrolysis by GroEL in the absence of GroES. The concentrations of ATP and GroEL are 100 μM and 24 nM (oligomer), respectively. The reactions were carried out at 25 °C as described under Experimental Procedures. The data were fitted to eq 2.

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.

Citrate Synthase Reactivation Assays. Reactivation of denatured citrate synthase by GroE was carried out according to Buchner et al. (1991) with some modifications. Citrate synthase was denatured at a concentration of 7.5 μM (monomer) in a buffer of 0.1 M Tris-HCl (pH 8.0), 6 M urea, and 20 mM DTT for 2 h at 25 °C. Refolding was initiated by rapid 100-fold dilution into buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 2 mM ATP, 0.26 μM GroEL (oligomer), and 1.30 μM GroES (oligomer) at 25 °C. At different time points, aliquots were removed and assayed for citrate synthase activity in a buffer of 0.1 M Tris-HCl (pH 8.0), 2 mM EDTA, 0.5 mM 5,5'-dithiobis(2-nitrobenzoic acid), 0.5 mM acetyl-CoA, and 0.5 mM oxaloacetate following the procedure described by Srere et al. (1963). The yields and half-times of reactivation in the presence of wild-type GroES and the Lys-34→Ala and Lys-34→Glu GroES mutants were found to be similar (data not shown).

RESULTS

Inhibition of the ATPase Activity of GroEL by Wild-Type GroES and the Lys-34→Ala and Lys-34→Glu GroES Mutants. Initial rates of ATP hydrolysis by GroEL were measured in the presence of different concentrations of wild-type or mutant GroES (Figure 1). The mutations Lys-34→Ala and Lys-34→Glu in GroES are found to have little effect on its inhibition of the ATPase activity of GroEL (Figure 1). The data in Figure 1 were directly fitted to eq 2. The dissociation constant between wild-type GroES and GroEL at 10 mM KCl is found to be 0.3 (±0.2) nM. This value is in agreement with the value of 0.5−3 nM reported by Jackson et al. (1993) and is somewhat lower than the value of 1.9 (±0.8) nM at 100 mM KCl reported by Todd et al. (1993). The dissociation constants between the Lys-34→Ala and Lys-34→Glu GroES mutants and GroEL, also at 10 mM KCl, are 1.1 (±0.3) nM and 1.4 (±0.3) nM, respectively. The maximal inhibition reached at high ratios of GroES to GroEL is potassium-dependent (Todd et al., 1993). In the case of both wild-type GroES and the Lys-34→Ala and Lys-34→Glu mutants, we find that in the presence of 10 mM KCl, a maximum of 50% inhibition of the ATPase activity of GroEL is reached, as previously observed by others (Chandrasekhar et al., 1986; Gray & Fersht, 1991; Jackson et al., 1993).

Cooperativity in ATP Hydrolysis by GroEL in the Presence of GroES Mutants. Initial rates of ATP hydrolysis by GroEL as a function of ATP concentration were measured in the absence of GroES and in the presence of wild-type or mutant GroES (Figure 2). The data were directly fitted to the Hill equation (eq 3). The values of k_{cat} of ATP hydrolysis in the absence and in the presence of wild-type GroES at 10 mM K^+ are 0.0280 (±0.0002) s^{-1} and 0.0144 (±0.0002) s^{-1} (per monomer), respectively, in good agreement with earlier studies (Horovitz et al., 1993a; Jackson et al., 1993; Yifrach & Horovitz, 1994). The value of k_{cat} of ATP hydrolysis by GroEL in the presence of the Lys-34→Ala and Lys-34→Glu GroES mutants is in both cases 0.013 (±0.001) s^{-1} (per monomer). In the presence of wild-type GroES, cooperativity in ATP hydrolysis by GroEL is increased (Gray & Fersht, 1991). The Hill coefficients in the absence and in the presence of wild-type GroES (at 10 mM K^+) were here found to be 2.76 (±0.15) and 4.10 (±0.22), respectively (Figure 2A). In the presence of the GroES mutants Lys-34→Glu (Figure 2B) and Lys-34→Ala (Figure 2C), cooperativity in ATP hydrolysis by GroEL is increased more than in the presence of wild-type GroES, as reflected by Hill coefficients of 4.46 (±0.14) and 5.17 (±0.24), respectively.

Analysis of Cooperativity in ATP Hydrolysis by GroEL According to the MWC Model. The data in Figure 2 were directly fitted to the MWC equation (eq 5) for exclusive binding to the **R** state assuming seven ATP binding sites ($N = 7$). The values of V_{max}, the intrinsic ATP binding constants (K_R), and the allosteric constants (L'_2) obtained from the fit are given in Table 1. The mutations have a small effect on k_{cat} and little if any effect on the intrinsic ATP binding constant (K_R) but large effects on the allosteric constant, L'_2, which is defined (Figure 3) as follows: L'_2 = [**TRES**]/[**RRES**]. The change upon mutation in the free energy corresponding to the transition from the **TRES** state to the **RRES** state is given by: $\Delta\Delta G_{coop} = -RT \ln [L'_2(wt)/L'_2(mut)]$. In the case of the Lys-34→Ala mutant, the value of $\Delta\Delta G_{coop}$ is 2.4 kcal mol^{-1} whereas in the case of the Lys-34→Glu mutant the value of $\Delta\Delta G_{coop}$ is 1.5 kcal mol^{-1} (both relative to wild-type GroES).

DISCUSSION

A scheme for the different possible states of GroEL in the presence of ATP (Yifrach & Horovitz, 1994) is here extended to include GroES (see Figure 3). According to this scheme, which is based on the MWC model for cooperativity (Monod et al., 1965), each ring of GroEL is in equilibrium between two states: a tense (**T**) state with a relatively low affinity for ATP (the substrate) and a relaxed (**R**) state with relatively high affinity for ATP.[1] In the absence of adenine nucleotides, GroEL is mainly in the symmetrical **TT** state. In the presence of ATP, the equi-

[1] 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 not only depends on its ligation state with respect to different ligands but also on the ligation state of the adjacent ring.

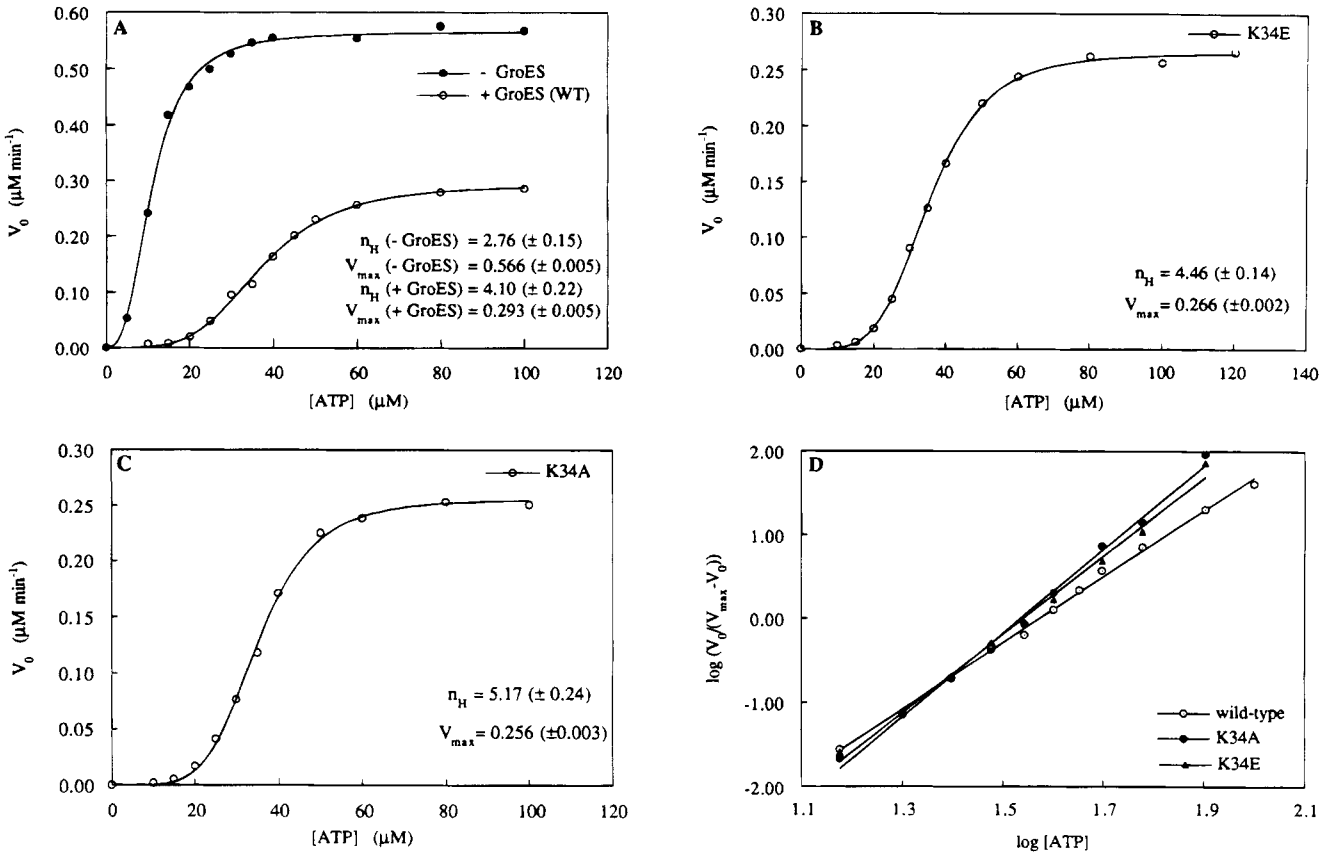

FIGURE 2: Initial velocity of ATP hydrolysis by GroEL at different ATP concentrations in the absence or presence of wild-type GroES (A) and in the presence of the Lys-34→Glu (B) and Lys-34→Ala (C) mutants of GroES. The data were fitted to eq 3. In panel D, the data for wild-type GroES and the Lys-34→Glu and Lys-34→Ala mutants are plotted according to the logarithmic form of the Hill equation (eq 4). The oligomer concentrations of GroEL and GroES are 24.1 and 144.6 nM, respectively. The reactions were carried out at 25 °C as described under Experimental Procedures.

Table 1: Analysis of the Effects of Mutations in GroES on Cooperativity of ATP Hydrolysis by GroEL According to the MWC Model[a]

GroES	V_{max} (μM min^{-1})	K_R (μM)	L'_2
−GroES	0.616 (±0.011)	5.55 (±0.66)	
+GroES (wild-type)	0.344 (±0.012)	17.8 (±2.7)	1605
+GroES (Lys-33→Ala)	0.278 (±0.008)	8.0 (±1.8)	105237
+GroES (Lys-33→Glu)	0.296 (±0.006)	12.1 (±1.6)	9080

[a] Estimates for V_{max}, the dissociation constant of ATP (K_R), and the allosteric constant (L'_2), as defined in Figure 1 and in the main text, that were obtained after fitting the data shown in Figure 2 to the MWC equation (eq 5) for 7 sites ($N = 7$) assuming exclusive binding to the **R** state. In the absence of GroES, the allosteric constant, L_1, is 810.

librium is shifted toward the **TR** state (L_1 = [**TT**]/[**TR**]). Positive cooperativity in ATP hydrolysis by wild-type GroEL is due to this shift in equilibrium. A simplifying assumption here and in previous work (Yifrach & Horovitz, 1994) is that binding of ATP occurs exclusively to the **R** state. A further shift in the equilibrium toward the symmetrical **RR** state (L_2 = [**TR**]/[**RR**]) is usually not observed owing to negative cooperativity between the two rings ($L_1 \ll L_2$) which requires description by a KNF-type model for cooperativity (Koshland et al., 1966). Thus, only seven sites of GroEL hydrolyze ATP with positive cooperativity (Bochkareva et al., 1992).

Binding of GroES to GroEL requires the presence of adenine nucleotides (Chandrasekhar et al., 1986; Bochkareva et al., 1992). We, therefore, assume that GroES binds to the adenine nucleotide bound **TR** and **RR** states of GroEL with binding constants of K_{ES} and K'_{ES}, respectively, and

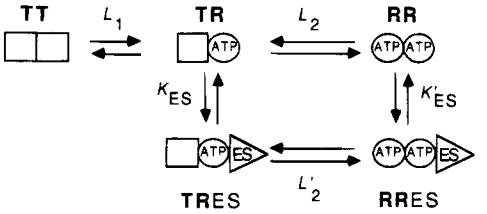

FIGURE 3: Scheme for the different states of GroEL in the presence of ATP and GroES. In the absence of ligands, GroEL is predominantly in the symmetrical **TT** state. In the presence of ATP, the equilibrium is shifted toward the **TR** state (L_1 = [**TT**]/[**TR**]). A further shift in the equilibrium toward the symmetrical **RR** state is usually not observed at relatively low ATP concentrations (<100 μM) since $L_1 \ll L_2$ (L_2 = [**TR**]/[**RR**]). GroES binds to the adenine nucleotide bound **TR** state of GroEL with a binding constant of K_{ES} and to the **RR** state of GroEL with a binding constant of K'_{ES}. The symmetric football-shaped species is not formed at relatively low K^+ and ATP concentrations (Azem et al., 1994; Schmidt et al., 1994b) and is, therefore, not included in this scheme. The notation of **T** and **R** is used to designate, respectively, all the possible low- and high-affinity states for ATP of one ring of GroEL.

that it does not bind to the **TT** state (see Figure 3). Binding of GroES to the **TR** state of GroEL inhibits the ATPase activity of GroEL (Chandrasekhar et al., 1986; Viitanen et al., 1990; Gray & Fersht, 1991; Langer et al., 1992; Jackson et al., 1993) and, also, increases positive cooperativity in ATP hydrolysis by GroEL (Gray & Fersht, 1991). According to our model, positive cooperativity in ATP hydrolysis by GroEL in the presence of GroES arises from a shift in the equilibrium from the inactive **TRES** state to the active **RRES** state (L'_2 = [**TRES**]/[**RRES**]). We assume that GroES binds to the ring in the **R** conformation in the **TR**

state of GroEL since positive cooperativity in ATP hydrolysis by GroEL in the presence of GroES would not be observed if GroES was bound to the ring in the **T** conformation.

In this study, residue Lys-34 in GroES was replaced by both alanine and glutamic acid. Both charge neutralization (Lys-34→Ala) and charge reversal (Lys-34→Glu) at this position have little effect on the binding constant of GroES to GroEL, but they increase the enhancement by GroES of cooperativity in ATP hydrolysis by GroEL. The most straightforward explanation of these results is that Lys-34 is involved in interactions that stabilize the **RR**ES state and that it is not involved in interactions which stabilize the **TR**ES state. The binding of GroES to GroEL is thus not affected by the mutation of Lys-34 [K_{ES}(wt) ≈ K_{ES}(mutants)], and cooperativity in ATP hydrolysis by GroEL is increased owing to destabilization of the **RR**ES state relative to the **TR**ES state [L'_2(mutants) ≫ L'_2(wt)]. Landry et al. (1993) showed that a mobile segment in GroES, spanning residues 17−32 in the GroES sequence, becomes immobilized upon formation of the GroEL/GroES complex and concluded, on the basis of this and other evidence, that binding of GroES to GroEL is mediated, at least in part, by this segment. Lys-34 is near, but not in, this mobile segment, and, therefore, our results indicating that this residue is not involved in the binding to GroES are consistent with the conclusions of Landry et al. (1993).

How does Lys-34 in GroES stabilize the **RR**ES state? One possibility is that Lys-34 in GroES forms a stabilizing intermolecular interaction with GroEL in the **RR**ES state but not in the **TR**ES state. Another possibility is that Lys-34 forms an intramolecular interaction in GroES in the **RR**ES state. The value of $\Delta\Delta G_{coop}$ for the Lys-34→Ala mutant is expected to reflect seven such stabilizing interactions either between GroES and GroEL monomers or within the GroES monomers and is, therefore, more consistent with this interaction being a solvent-exposed electrostatic interaction (Horovitz et al., 1990; Dao-pin et al., 1991) than a buried one (Anderson et al., 1990). To our knowledge, this paper describes the first example of a mutation in one protein that affects the allosteric properties of a second protein.

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