Intrinsic Fluorescence Studies of the Chaperonin GroEL Containing Single Tyr → Trp Replacements Reveal Ligand-induced Conformational Changes*

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Two mutants of GroEL containing the single tyrosine to tryptophan replacement of either residue 203 or 360 in the apical domain have been purified, characterized, and used for fluorescence studies. Both mutants can facilitate the in vitro refolding of rhodanese in an ATP- and GroES-dependent manner, producing yields of recoverable activity comparable to the wild-type chaperonin. Y203W shows some increased hydrophobic exposure and easier urea-induced disassembly compared with wild-type or Y360W, although the unfolding of all the species was similar at high concentrations of urea. Intrinsic fluorescence studies of the two mutants reveal that nucleotide binding (ADP or AMP-PNP (adenosine 5′-(β,γ-imino)triphosphate)) induces conformational changes in the tetradecamer that are independent of the presence of the co-chaperonin, GroES. The $K_{1/2}$ for this transition is approximately 5 μM for both mutants. Energy transfer experiments show that the tryptophan fluorescence of the Y360W mutant is partially quenched (∼50%) upon binding of the fluorescent, hydrophobic probe 4,4′-bis(1-anilino-8-naphthalenesulfonic acid), while the fluorescence of the Y203W mutant is significantly quenched (∼75%). These results are discussed in relation to the molecular mechanism for GroEL function.

The molecular chaperones are a class of proteins that have been shown to facilitate the folding and transport of nascent polypeptides in vivo (1, 2). GroEL from Escherichia coli is a widely studied member of the chaperonins, one class of molecular chaperones that have been found in prokaryotes, mitochondria, and chloroplasts (3). GroEL has been demonstrated to promote the in vitro refolding and assembly of many chemically denatured proteins, including rhodanese (4, 5), ribulose-bisphosphate carboxylase (6), glutamine synthetase (7), and dihydrofolate reductase (8). The ability of GroEL to interact with a wide variety of proteins is attributed to nonspecific hydrophobic interactions with protein folding intermediates (4, 9).

GroEL is a homotetradecamer (14-mer) of 57.2-kDa monomers. The 14 subunits are arranged as two, stacked heptameric rings (10, 11). The 2.8-Å crystal structure reveals a complicated architecture, with each monomer organized into three domains: equatorial (residues 6–133 together with 409–523), intermediate (residues 134–190 and 377–408), and apical (residues 191–376). The inter-heptamer contacts are entirely defined by the equatorial domains, while interactions between monomers within each ring are mediated by the equatorial domains and by conserved regions at the intermediate-apical domain interface (11). The apical domains line the opening to a central cavity at both ends of the tetradecamer and contain a region (residues 200–263) that has been implicated as the site of polypeptide and GroES binding by site-directed mutagenesis and the use of the hydrophobic probe bis-ANS, which becomes photoincorporated between residues 203 and 249 (12, 13). A more recent crystal structure shows ATPγS bound to a site in the equatorial domain, at the base of the intermediate domain, with Mg$^{2+}$ and another ion (possibly K$^+$) coordinating the ligation (14).

It has been suggested that exposure of the hydrophobic surfaces on the apical domains of GroEL, which account for interaction with substrate and GroES, may need to be triggered by substrate or by other ligand-induced conformational changes (15). In fact, several studies using cryo-electron microscopy have demonstrated quaternary structural changes in the tetradecamer where the monomers of one ring pivot with respect to the other ring and the apical domains swing outward upon ATP and GroES binding (16, 17). Similarly, recent work showed that a unique proteolytic site for trypsin at Arg-268 becomes exposed in the apical domains of one heptamer upon nucleotide binding (15). These results all confirm the original finding that nucleotides induce asymmetry between the two heptameric rings, and partially explain the finding in terms of structural changes in the oligomer (18).

In this report, use was made of two mutants of GroEL containing single tyrosine to tryptophan replacements (Y203W and Y360W). Changes in the intrinsic tryptophan fluorescence for each mutant reveal a conformational change upon nucleotide binding (ADP or AMP-PNP) that is unaffected by the presence of GroES. The results of fluorescence experiments suggest that nucleotide binding to the GroEL tetradecamer results in conformational changes in the apical domain, which are reported by changes in the environment of the tryptophan at 203 or 360. Since these conformational changes precede
binding of the co-chaperonin GroES, it suggests that nucleotides alone can play a critical role in the regulation of the quaternary structure of GroEL.

MATERIALS AND METHODS

Reagents and Proteins—All reagents used were of analytical grade. 4,4′-Bis(1-anilino-8-naphthalenesulfonic acid) was obtained from Molecular Probes (Eugene, OR).

Tryptophan mutations were constructed in the E. coli groE gene using the method of Stansens et al. (19). The groE operon was cloned as an EcoRI-HindIII fragment from the plasmid pOF39 (20) into the plasmids pMe5-8 (19), to give the plasmid pMeESL. Gapped duplexes were prepared by annealing purified ThrIII-HpaI-cut (mutant 203) or ClaI-NotI-cut (mutant 203) pMeESL plasmid DNA with single-stranded DNA of pMa5-8 (19). The gapped duplexes were hybridized with phosphorylated oligonucleotide 203 (5′-GCTTTGTTGAACAGGACGAGACCGTTA-3′) or oligonucleotide 360 (5′-GCCTTTTACCGTGCCCA-GTACAGATTG-3′), and the gaps filled in and ligated as described (19). The ligation mix was transformed into WK6mutS (19), and chloramphenicol-resistant transformants were selected and used to transform WK6. Mutants were identified by sequencing DNA from several transformants from this mix; a mutation frequency of approximately 50% was obtained. No other mutations occurred within the gapped region.

To test for in vivo function, the plasmids carrying the mutations were transformed into a strain carrying a temperature-sensitive mutant of groE. Growth of the strains at 43 °C was identical to the same strain containing pMeESL, whereas no growth took place with pMe5-8 in the same strain.

Wild-type GroEL was purified from lysates of E. coli cells bearing the multicopy plasmid pND5 (21). The purification was by a disassembly/reassembly process as described previously (Method I, in Ref. 22). The Y203W and Y360W tryptophan mutants of GroEL were purified from lysates of E. coli bearing the appropriate vectors (see above). The purification utilized the same two first steps as for the wild-type (a DEAE-Sephadex column and gel filtration on a Sephacryl S-400 column). Subsequent purification was essentially as described previously by Mizobata and Kawata (23). Briefly, GroEL and GroES were mixed in equimolar amounts (based on oligomer concentrations) in a solution containing 50 mM Tris-HCl, pH 7.8, 0.1 mM NaCl, 10 mM KCl, 10 mM Mg(CH3COO)2, 0.5 mM DTT, and 4 mM ATP. After a 1 h incubation at room temperature, the mixture was subjected to filtration chromatography on a Sephacryl S-200 column equilibrated with a buffer of 50 mM Tris-HCl, pH 7.8, 0.1 mM NaCl, 10 mM KCl, 10 mM Mg(CH3COO)2, 0.5 mM DTT, and 4 mM ATP. Fractions containing the GroEL-GroES complex were pooled, concentrated (Centricon-30) with a buffer change, and applied to the same column (Sephacryl S-200) equilibrated with a buffer containing 50 mM Tris-HCl, pH 7.8, 50 mM KCl, 0.5 mM DTT, and 0.1 mM EDTA. The fractions containing GroEL were collected, concentrated using a Centricon-30, and then dialyzed against 50 mM Tris-HCl, pH 7.8, 0.1 mM DTT. GroEL was then made 10% with respect to glycerol and frozen at −70 °C. This purification method produces wild-type GroEL with very low tryptophan content and produced mutants with little or no bound tryptophan contaminants (Ref. 23 and Fig. 1).

The co-chaperonin, GroES, was purified from lysates of E. coli cells bearing the multicopy plasmid pND5, using a previously published protocol (24). After purification, GroES was stored at 4 °C in 70% ammonium sulfate. Prior to use, GroES was centrifuged in a microcentrifuge, the pellet was resuspended in 50 mM triethanolamine hydrochloride, pH 8.0, and dialyzed overnight against the same buffer. Theprotomer concentration of GroEL was measured under denaturing conditions, and GroES was measured under non-denaturing conditions using the bicinchoninic acid assay (Pierce) according to the procedure recommended by the manufacturer.

Chaperonin-assisted refolding of the rhodanese was measured as described previously by monitoring the regain of enzyme activity (25). In all cases, 109 n mole-adenurated rhodanese was refolded using 2.5 μM GroEL (protomer) in addition to the other components of the chaperonin assay mixture.

Fluorescence Measurements—All the fluorescence measurements were made using an SLM 500C fluorometer (SLM Instruments, Urbana, IL). For the intrinsic tryptophan fluorescence, excitation was at either 280 or 290 nm, as indicated in the figure legends. Experiments containing nucleotides were excited at 290 nm, where controls showed that the presence of nucleotides interfered the least with excitation of the tryptophanes. Controls with N-acetyltryptophanamide (NATA) showed that nucleotide concentrations below ~5 mM exhibited negligible inner filter effect. The spectra were therefore not corrected for nucleotide concentration. Fluorescence intensities were measured at the maximum of the spectra (~348 nm for Y203W and ~352 nm for Y360W), which were scanned from 300 to 400 nm. Excitation for the bis-ANS experiments was at 394 nm, with the emission monitored at 500 nm.

Curve Fitting—Data were fit using a nonlinear least squares fitting procedure implemented in the software program, SigmaPlot (Jandel Scientific, San Rafael, CA). Where data were fit to a simple binding curve, the following equation was used: $F_{\text{obs}} = (mS/Kd + 1)$, where $F_{\text{obs}}$ represents the measured intrinsic tryptophan fluorescence and $S$ represents the concentration of ligand. The form of the Hill equation employed for fitting the cooperative transitions was: $F_{\text{obs}} = F_{\text{m}} \cdot [(F_{\text{m}} - F_{\text{m}}^0)S/(Kd + S^0)]$, where $F_{\text{obs}}$ represents the measured intrinsic tryptophan fluorescence, $F_{\text{m}}$ and $F_{\text{m}}^0$ represent the maximum and minimum fluorescence values taken as the base-line values, and $S$ represents the ligand concentration.

RESULTS

The Y203W and Y360W Mutants Have the Expected Intrinsic Tryptophan—Fig. 1 shows a comparison of the intrinsic fluorescence of equimolar amounts of the two mutants and NATA in Tris-HCl, pH 7.6. The wavelength maxima are 342 nm for Y203W and 345 for Y360W compared with 352 nm for NATA, a model compound for tryptophan, indicating that the mutants contain partially buried tryptophan residues. The tryptophan content of each mutant was analyzed by comparing spectra in 8 M urea of 2 μM NATA with spectra of 2 μM solutions of the mutants in the same buffer. As previously reported, the wild-type contains very little intrinsic fluorescence, mostly attributable to tyrosines rather than tryptophans (22). Both denatured mutants have spectra comparable to the NATA standard (wavelength maxima and relative intensities: NATA, 352 nm, I = 1; Y203W, 351 nm, I = 1.09; and Y360W, 352 nm, I = 0.84). These results are consistent with the presence of only the single tryptophan residues which were substituted.

The Y203W and Y360W Mutants of GroEL Exhibit Functional and Structural Characteristics Similar to the Wild-type Protein—Both mutants were capable of complementing a groE+ mutant strain, indicating that neither of the single tryptophan substitutions disrupted the ability of the chaperonin to function in vivo (data not shown). This suggests that the mutants were capable of interacting with substrates and the co-chaperonin, as well as nucleotides, in vivo. The ability of the mutants to facilitate protein refolding in vitro was also assessed by their ability to arrest the spontaneous folding of rhodanese and by their ability to participate in the complete chaperonin-assisted folding of rhodanese that requires the co-chaperonin GroES as described previously (25). Both mutants as well as the wild-type protein could arrest the spontaneous...
folding of rhodanese > 96%. The mutants were able to facilitate the refolding of rhodanese in an ATP- and GroES-dependent manner, producing yields of recoverable activity similar to that previously reported with wild-type GroEL (25). Thus, the wild-type GroEL led to 41% refolding in the assay described under “Materials and Methods” (compared with 36% in Ref. 25), while the mutants gave 30% (Y203W) and 38% (Y360W).

The structural stabilities of the mutants were determined by subjecting them to urea disassembly and denaturation, as described previously (26). Fig. 2A shows the results for both mutants and wild-type GroEL. The data for the wild-type GroEL (shown as closed squares) are consistent with that previously reported and are provided for comparison. The rise in bisANS fluorescence with increasing [urea] to ~3 m has been ascribed to the disassembly of the GroEL tetradecamers, while the decrease in intensity at higher [urea] correlates with previous observations that GroEL monomers unfold under these conditions. Y203W appears to have more hydrophobic exposure than the other forms, and the subsequent increase occurs at lower [urea]. However, the decrease at high [urea] is very similar for all the species. The effects of nucleotide binding on the urea-induced dissociation of the oligomer are shown in Fig. 2B. Nucleotide binding destabilizes the oligomer, such that it disassembles and the monomers unfold at lower urea concentrations. These results are compatible with those reported for wild-type GroEL liganded by ADP or ATP (26, 27). To further characterize the effects of nucleotide binding to the mutants, use was made of the recent report that nucleotide binding to wild-type GroEL results in selective trypsin proteolysis at a site in the apical domains (15). Consistent with this previous report for wild type, the mutants were protected from trypsin digestion when liganded by Mg2+ and became susceptible to proteolysis upon addition of nucleotide (data not shown). These results suggest that these mutants of GroEL have the appropriate intrinsic fluorescence properties that would be expected from their tryptophan content, and, although they may show some differences, they retain the essential structural and functional properties of wild-type chaperonin.

The Fluorescent, Hydrophobic Probe Bis-ANS Can Bind to Both Tryptophan Mutants, Resulting in Energy Transfer—Previous experiments have shown the binding of hydrophobic probes to GroEL in the presence of various perturbants (26), or the photoincorporation of bis-ANS into a region of the protein between residues 203 and 249 (13). To extend these results, energy transfer experiments were conducted using the tryptophan mutants of GroEL and bis-ANS. The tryptophan mutants and bis-ANS are an appropriate donor-acceptor pair for energy transfer because the tryptophan fluorescence has an emission spectrum that overlaps with the excitation spectrum of bis-ANS. Therefore, bis-ANS binding to or appropriately near the region containing the tryptophan should result in quenching of the intrinsic tryptophan fluorescence.

Fig. 3A shows the results for the Y360W mutant. In the absence of bis-ANS, the tryptophan fluorescence produces a peak with a maximum at 352 nm. Upon addition of bis-ANS to a final concentration of 10 μM, the tryptophan fluorescence is quenched ~50%. By contrast, Fig. 3B shows the results for the Y203W mutant. Absent bis-ANS, a strong tryptophan fluorescence signal is seen with an emission maximum at 348 nm. But upon addition of bis-ANS, the tryptophan fluorescence is significantly quenched (~75%). Given the report of photoincorporation of bis-ANS into the region of the apical domain between residues 203 and 249, where mutations of hydrophobic residues affected substrate and/or GroES binding, the results presented here are consistent with bis-ANS binding to the region of the apical domain that lines the opening to the central cavity, allowing for efficient transfer from Trp-203 and moderate transfer from Trp-360 (12, 13).

The energy transfer for the Y203W was used to construct a binding isotherm based on the amount of tryptophan quenching at increasing bis-ANS concentrations and yielded an apparent K_D of ~3.5 μM (data not shown). This value is consistent with that previously reported for the binding of bis-ANS to

**Fig. 2** Urea-induced disassembly and unfolding of wild-type GroEL and tryptophan mutants. A, fluorescence intensity of bis-ANS bound to unliganded protein as a function of urea concentration. Individual samples of wild-type or mutant GroEL at each of the indicated urea concentrations were prepared, containing 1 μM chaperonin, 50 mM Tris-HCl, pH 7.8, and 10 μM bis-ANS. Fluorescence spectra were recorded, and the intensities at the maxima are plotted versus urea concentration. Filled circles, Y203W mutant; filled triangles, Y360W mutant. Wild-type GroEL (closed squares) are provided for comparison. The lines are drawn to aid the eye and connect the data for the mutant proteins. Fluorescence was excited at 394 nm and emission monitored at 500 nm. B, fluorescence intensity of bis-ANS bound to ATP-Mg-ligated protein as a function of urea concentration. Individual samples of wild-type or mutant GroEL at each of the indicated urea concentrations were prepared, containing 1 μM chaperonin, 50 mM Tris-HCl, pH 7.8, 50 mM MgCl_2, 2 mM ATP, and 10 μM bis-ANS. Fluorescence measurements and curve denotations are as in A.
Conformational Changes in Tryptophan Mutants of GroEL

The Tryptophan Mutants Report Conformational Changes in the Tetradecamer upon Nucleotide Binding—Since both tryptophan substitutions are in the apical domain of GroEL and since it has been reported that nucleotide binding results in reorientation of the apical domains of at least one heptamer (15, 16), the intrinsic fluorescence of each mutant was monitored as a function of nucleotide concentration. Fig. 4 (A and B) shows the results for the Y203W mutant in the presence of ADP and AMP-PNP, respectively. In panel A, the intrinsic tryptophan fluorescence decreases in a concentration-dependent fashion indicative of ligand binding (filled squares). The transition was fit with the Hill equation (see “Materials and Methods”) for a cooperative transition, yielding a \( K_{1/2} = 3 \, \mu M \) and a Hill coefficient \( (n_H) \) of 3.54 ± 1.36. The presence of the co-chaperonin, GroES, did not significantly change the observed transition (open circles); \( K_{1/2} = 5 \, \mu M \) and \( n_H = 2.03 \pm 0.41 \). The lack of an effect by the co-chaperonin was consistent with other results showing that increasing amounts of GroES, at a fixed nucleotide concentration, did not change the intrinsic tryptophan fluorescence of GroEL (data not shown). In panel B (AMP-PNP), both transitions were again indistinguishable (± GroES) within experimental error, with values of \( K_{1/2} = 5 \, \mu M \) and \( n_H = 1.48 \pm 0.25 \) (− GroES) or \( n_H = 1.66 \pm 0.28 \) (+ GroES).

Fig. 5 shows the results of the analogous experiments for the Y360W mutant in the presence of AMP-PNP. The experiments were also performed with ADP, producing results indistinguishable from AMP-PNP (not shown). In this case the transitions are best described by a simple binding isotherm (see “Materials and Methods” for equation), with an apparent \( K_D = 5.1 \, \mu M \) (open squares). This value is consistent with that for a similar transition reported by Burston et al., using a pyrene maleimide-labeled GroEL (28). Again, the presence of GroES did not alter the observed transitions (filled triangles). The differences between the transitions reported by the two mutants suggest that one tryptophan monitors nucleotide binding (Y360W), while the other monitors a subsequent, cooperative structural change upon nucleotide binding (Y203W). Since the presence of the co-chaperonin did not affect the observed transitions, this conformational change apparently precedes (and may allow for) GroES binding.

Although the tryptophan mutants of GroEL could facilitate rhodanese refolding in a GroES-dependent fashion, there was a concern that the mutants might not form a stable complex with GroES, which could skew the interpretation of the fluorescence experiments. To be certain that a stable GroES-GroEL complex could be formed with both mutants, GroES was reductively methylated using \( ^{14}C \)-formaldehyde, by a previously described procedure (29). The labeled GroES was used for complex formation in the presence of ADP and the subsequent complexes were detected on native (nondenaturing) polyacrylamide gels. Both GroEL mutants formed stable binary complexes with \( ^{14}C \)-labeled GroES, to the same degree as wild-type GroEL.2

Trp-203 and Trp-360 Are Exposed to Solvent before and after the Nucleotide-induced Conformational Change—To better understand the conformational changes in GroEL that result in decreased intrinsic tryptophan fluorescence upon binding of ADP or AMP-PNP, the quenching of the tryptophan fluorescence by acrylamide was measured. Acrylamide quenching gives an estimation of the exposure of tryptophans in a protein to the bulk solution. Fig. 6 shows the acrylamide quenching of the tryptophan fluorescence for the Y203W and Y360W mutants. The lines in Fig. 6 were obtained by fitting the data to the Stern-Volmer equation, \( F_o/F = 1 + K_q [Q] \), where \( F_o \) and \( F \) are the fluorescence intensities in the absence and presence of the quenching agent, \( K_q \) is the apparent quenching constant and \( [Q] \) is the concentration of the quencher. NATA was used as a model compound for tryptophan and served as a positive control in these experiments (closed squares). The curvature of the data, which is apparent for the mutants in the absence of ligands, suggests that there is a static component to the quenching. These data were analyzed in terms of dynamic (\( K_D \)) and static (\( K_S \)) contributions using the \( K_q \) values at each acrylamide concentration by the method described by Lakowicz (42). Y360W gave \( K_D = 7.46 \, m^{-1} \) and \( K_S = 0.79 \, m^{-1} \); while Y203W gave \( K_D = 4.99 \, m^{-1} \) and \( K_S = 1.51 \, m^{-1} \). By comparison, NATA gave \( K_D = 14.78 \, m^{-1} \) and \( K_S = 3.58 \, m^{-1} \). In the presence of various ligands (\( Mg^{2+} \), \( Mg^{2+} \), and \( K^+ \); or \( K^+ \) and nucleotide-Mg), both mutants gave similar results. Only the data for

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2 J. Ybarra, personal communication.
Conformational Changes in Tryptophan Mutants of GroEL

GroEL in the presence of Mg$^{2+}$ and K$^+$ are shown (filled circles and open diamonds), but, in each of the three ligand conditions tested, the mutants displayed accessibility of the tryptophans to solvent that was somewhat reduced compared to the unliganded chaperonin which was reasonably well described with only dynamic quenching ($K_q = 5.2 \text{ M}^{-1}$ for Y360W and $K_q = 4.6 \text{ M}^{-1}$ for Y203W). This degree of solvent accessibility for tryptophans 203 and 360 in GroEL is significant compared to other native proteins, e.g. the $K_q$ for native rhodanese has been reported as 1.0 $\text{ M}^{-1}$ (30, 31). Based on these results, the unliganded GroEL oligomer has greater solvent accessibility of both Trp-203 and Trp-360 compared to any of the Mg$^{2+}$-liganded states of the protein, which is most apparent in the static quenching components. This is consistent with the reports of divalent cations stabilizing the tetradecamer (26, 32, 33). Despite the apparent conformational changes that nucleotide binding induces, subsequent binding of nucleotide (ADP or AMP-PNP) to a Mg$^{2+}$-liganded GroEL oligomer does not further alter the solvent accessibility of either tryptophan.

By contrast, when the same experiment was performed using KI as the quencher (data not shown), neither of the tryptophan mutants was readily quenched by the I$^-$ ($K_q = 1–2 \text{ M}^{-1}$). The disparity between the results is most likely due to the ionic nature of the iodide, since the experiments with acrylamide and K$^+$ had an approximately equivalent ionic strength of 100 mM KI. Both tryptophans are located in hydrophobic patches of the apical domain which may prevent good quenching by the ionic quencher as compared to the acrylamide.

**DISCUSSION**

The results presented here support earlier studies that implicate ligand-induced conformational changes in GroEL (15, 16, 18, 28, 34) by showing that nucleotide binding alone (ADP or AMP-PNP) causes a single transition in the intrinsic tryptophan fluorescence of both mutants. For the Y203W mutant, the cooperative transition is most consistent with models in which nucleotides bind to one of the heptameric rings of a GroEL oligomer, producing a concerted conformational change in the apical domains of that heptamer while preventing nucleotide from binding to the opposite ring. The transition in the
are in different regions of the protein structure; residue 360 is located close to the hinge region between the intermediate and apical domains of a monomer, and residue 203 is located under the tip of the apical domain facing into the central cavity. Thus, the tryptophan fluorescence transitions might give an indication of how a message can be communicated from the nucleotide binding pocket in the equatorial domains, via the intermediate domains, to the interactive surfaces of the apical domains. Further, it is significant that GroES is not necessary and does not facilitate this apparent conformational change. This result extends the initial observations that nucleotide alone produces an asymmetric GroEL tetradecamer, and it can help explain (along with the ability of nucleotides to differentially modulate the affinity of GroEL for substrate) why some proteins require nucleotide, but not GroES, for their facilitated refolding by GroEL.

That GroES does not alter the observed intrinsic fluorescence transitions might at first appear to be contradictory with the view that GroES increases the cooperativity of nucleotide hydrolysis by GroEL (35–37). However, this may be an insight into the real effect of GroES in the system, to coordinate ATP hydrolysis and thereby facilitate the inherent ability of nucleotide to modulate the quaternary structure of GroEL. The co-chaperonin is not necessary for the cooperative conformational change induced by nucleotide binding as shown here, and, in fact, GroES appears to bind after the nucleotide binding-induced change, but prior to ATP hydrolysis (28). Some evidence also suggests that a transient conformational state exists upon nucleotide hydrolysis, which GroES binding would induce due to its ability to coordinate hydrolysis (28). The design of the experiments herein do not address this possibility. Given recent evidence that substrates affect the cooperativity of ATP hydrolysis by GroEL and the fact that non-native substrates stabilize the tetradecamer, we also cannot rule out the possibility that GroES affects the conformational changes in GroEL differently when the chaperonin is part of a ternary complex with non-native substrate (38, 39).

The conformational changes reported by the two single tyrosine to tryptophan mutants appear to be directly relevant to the functioning of the chaperonin based on the ability of the fluorescent, hydrophobic probe, bis-ANS, to quench the tryptophan fluorescence. This probe has been used in a number of studies to report the exposure of hydrophobic surfaces on the chaperonin or on non-native substrates (4). It was also shown that this probe could be photoincorporated into the region of GroEL between residues 203 and 249 (13). This is the region of GroEL rich in hydrophobic residues shown to be important for interaction with substrates and GroES (11, 12). The ability of this probe to bind and effectively quench the tryptophan fluorescence of the Y203W mutant and to partially quench the fluorescence of the Y360W mutant is consistent with bis-ANS binding within the hydrophobic region revealed by the crystal structure and mutation analysis. In fact, the larger distance of Tyr-360 from the region of photoincorporation explains why the quenching of Y360W is less than that observed for Y203W. The quenching experiments with acrylamide and KI also suggest that these putative sites of interaction do not significantly change their solvent exposure (being only slightly regulated by Mg\(^{2+}\) binding) and that their hydrophobic nature may be such that highly ionic molecules such as I\(^-\) are prevented from approaching.

The results presented here are consistent with the mechanisms of GroEL activity proposed by a number of groups, in which the two heptameric rings of GroEL alternate between high and low affinity states (28, 38, 40, 41). In these models, the transition from low to high affinity states is determined by
nucleotide binding and subsequent binding of non-native protein or GroES. Thus, nucleotides can induce cooperative changes in the GroEL structure that expose sites in the apical domains, allowing for protein-protein interactions.

REFERENCES
1. Gething, M.-J., and Sambrook, J. (1992) Nature 355, 33–45
2. Ellis, R. J., van der Vies, S. M., and Hemmingsen, S. M. (1990) Biochem. Soc. Symp. 53, 145–153
3. Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. (1988) Nature 333, 330–334
4. Mendoza, J. A., Rogers, E., Lorimer, G. H., and Horowitz, P. M. (1991) J. Biol. Chem. 266, 13044–13049
5. Martin, J., Langer, T., Boteva, R., Horwich, A. L., and Hartl, R.-U. (1991) Nature 352, 36–42
6. Goloubinoff, P., Christeller, J. T., Gatenby, A. A., and Lorimer, G. H. (1989) Nature 342, 884–889
7. Fisher, M. T. (1992) Biochemistry 31, 3955–2963
8. Viitanen, P. V., Donaldson, G. K., Lubben, T. H., and Gatenby, A. A. (1991) Biochemistry 30, 9716–9723
9. Lin, Z., Schwarz, F. P., and Eisenstein, E. (1995) J. Biol. Chem. 270, 1011–1014
10. Hendrix, R. W. (1979) J. Mol. Biol. 129, 375–392
11. Braig, K., Otwinowski, Z., Hedge, W., Boisvert, D. C., Joachimiak, A., Horwich, A. L., and Sigler, P. B. (1994) Nature 371, 578–586
12. Fenton, Q. A., Kashi, Y., Furtak, K., and Horwich, A. L. (1994) J. Mol. Biol. 259, 1197–1207
13. Seale, J. W., Martinez, J. L., and Horowitz, P. M. (1995) J. Biol. Chem. 270, 28551–28556
14. Boisvert, D. C., Wang, J., Otwinowski, Z., Horwich, A. L., and Sigler, P. B. (1994) Nature 371, 578–586
15. Goloubinoff, P., Christeller, J. T., Gatenby, A. A., and Lorimer, G. H. (1989) Nature 342, 884–889
16. Saibil, H. R., Zheng, D., Roseman, A. M., Hunter, A. S., Watson, G. M. F., Barnett, L. K., and Ellis, R. J. (1993) Curr. Biol. 3, 285–273