The Disordered Mobile Loop of GroES Folds into a Defined β-Hairpin upon Binding GroEL*

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The GroES mobile loop is a stretch of ~16 amino acids that exhibits a high degree of flexible disorder in the free protein. This loop is responsible for the interaction between GroES and GroEL, and it undergoes a folding transition upon binding to GroEL. Results derived from a combination of transferred nuclear Overhauser effect NMR experiments and molecular dynamics simulations indicate that the mobile loop adopts a β-hairpin structure with a Type I, G1 Bulge turn. This structure is distinct from the conformation of the loop in the co-crystal of GroES with GroEL-ADP but identical to the conformation of the bacteriophage-panned “strongly binding peptide” in the co-crystal with GroEL. Analysis of sequence conservation suggests that sequences of the mobile loop and strongly binding peptide were selected for the ability to adopt this hairpin conformation.

Chaperonins and co-chaperonins are ring-shaped molecular chaperones. Escherichia coli GroEL and GroES are, respectively, the prototypical chaperonin and co-chaperonin pair. Together, they comprise the GroES intracellular machine that binds, unfolds, and refolds nascent and misfolded proteins (for review, see Refs. 1–6). GroEL and GroES undergo cyclic interactions of binding and releasing, which are under the control of an “ATP clock,” in which ATP hydrolysis is coupled to cycling (7). Each functional cycle starts with binding of substrate to hydrophobic sites on GroEL (8). Subsequent binding of ATP and GroES to GroEL causes a major conformational change in GroEL involving a rotation and twist of the GroES-binding apical domains of GroEL. This conformational change and GroES binding sequester the hydrophobic sites on GroEL (8). Subsequent binding of ATP and GroES to GroEL causes a major conformational change in GroEL involving a rotation and twist of the GroES-binding apical domains of GroEL. This conformational change and GroES binding sequester the hydrophobic sites on GroEL (8). Subsequent binding of ATP and GroES to GroEL causes a major conformational change in GroEL involving a rotation and twist of the GroES-binding apical domains of GroEL. This conformational change and GroES binding sequester the hydrophobic sites on GroEL (8).

The mobile loop of GroES adopts a “loop” conformation, which does not conform to a particular secondary structure (12, 13, 20). The increase in affinity was explained as being a result of decreasing the disorder of the mobile loop or, conversely, preordering the binding structure of the mobile loop. Likewise, changes in GroEL-GroES binding affinity of the bacteriophage T4 co-chaperonin were shown to be controlled by amino acid preferences for β-sheet, which modulate the formation of a GroEL binding β-hairpin conformation (16). Therefore, an understanding of the structure and folding of the mobile loop is essential to understanding the functional interaction between co-chaperonins and chaperonins.

Two very different conformations have been described for the GroEL-bound GroES mobile loop. Landry et al. (21) determined the conformation of a GroEL-bound mobile loop peptide using transferred nuclear Overhauser effect (trNOE) NMR spectroscopy. Eighty-four trNOE distances constrained the central nine residues of the peptide into a β-hairpin. Subsequent crystallographic studies on the GroES-GroEL-ADP complex indicated that the mobile loop of GroES adopts a “loop” conformation, which does not conform to a particular secondary structure (12, 22). Here, we refine the GroEL-bound GroES mobile loop peptide conformation determined by trNOE NMR using unrestrained molecular dynamics simulation in solvent water. We find that the peptide retains a β-hairpin conformation but adopts a standard Type I, G1 Bulge-turn geometry, a structure that explains the sequence conservation in co-chaperonin mobile loops. NMR studies show that substitution of a conserved glycine weakens binding of a synthetic mobile loop peptide and reduces turn formation by the mobile loop in GroES. The refined β-hairpin conformation is virtually identical to the conformation described by x-ray crystallography for the GroEL-bound strongly binding peptide (SBP) selected by bacteriophage-panning (22). We propose that SBP more precisely mimics a co-chaperonin mobile loop rather than a typical GroEL substrate.

EXPERIMENTAL PROCEDURES

Molecular Dynamics Simulations—The molecular dynamics simulations of GroES mobile loop residues 19–27 were performed using the AMBER force field (23) and the particle mesh Ewald (24) method for calculation of long range electrostatic interactions. Explicit aqueous

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† The abbreviations used are: trNOE, transferred nuclear Overhauser effect; SBP, strongly binding peptide; TOCSY, two-dimensional total correlation spectroscopy; NOESY, nuclear Overhauser effect spectroscopy.
The GroES Mobile Loop Adopts a β-Hairpin

FIG. 1. Selected attributes of the mobile loop peptide as it progressed through molecular dynamics simulation. For clarity, only the first 2400 ps of Run2 are displayed. A, root mean square deviation (Rmsd) of backbone atoms for each molecule compared with the starting structure. The hairpin determined by NMR stays much closer to the starting conformation than does the loop conformation determined by x-ray crystallography. B, distance between the H atoms of Lys-20 and Val-26. C, dihedral angles that change during the crankshaft conformational change in Run1. D, dihedral angles that change during the crankshaft conformational change in Run2. E–G, the starting structures (gray) and the resulting structures (black) for Run1 (E), Run2 (F), and the simulation beginning with the co-crystal loop structure (G).

Protein Purification—GroEL and GroES were expressed in E. coli with the same buffer. The proteins were eluted with a 0–1 M NaCl gradient. Fractions containing GroES were dialyzed into Buffer B (50 mM Tris-Cl, pH 7.5, 125 mM NaCl). The GroEL solution was then made 2.5 mM in MgCl₂ and 1.0 mM in ATP before passing over a Cibracon Blue 3GA column (Sigma). GroEL eluted in the flow-through and appeared to be greater than 90% pure by visual inspection after SDS-polyacrylamide gel electrophoresis. The fractions containing GroEL from the Q-Sepharose column were collected, pooled, and exchanged into Buffer B (50 mM Tris-Cl, pH 7.5, 125 mM NaCl). The GroEL solution was then made 2.5 mM in MgCl₂ and 1.0 mM in ATP before passing over a Cibracon Blue 3GA column (Sigma). GroEL eluted in the flow-through and appeared to be greater than 90% pure by visual inspection after SDS-polyacrylamide gel electrophoresis.

The GroES mobile loop peptides (Table I) were synthesized as carboxamides using Fmoc (9-fluorenylmethoxycarbonyl) chemistry, acetylated off-line, and purified by reverse-phase high performance liquid chromatography. Identities of the peptides were checked by matrix-assisted laser desorption ionization mass spectrometry.

NMR Spectroscopy—All NMR data were recorded on a GE Omega PSG 500 MHz spectrometer and processed using Felix 98.0 (Molecular Simulations, Inc.). Chemical shifts were referenced to internal 3-(trimethylsilyl)propionate (0 ppm).

For analysis of trNOEs in GroES mobile-loop peptides (Table I), two-dimensional NOESY spectra (30, 31) were acquired at 30 °C with mixing times of 75, 150, and 300 ms. The NMR samples contained 40 mM potassium phosphate buffer in 8% (w/v) D₂O, pH 6.0, 2 mM GroES.
RESULTS

Molecular Dynamics of the GroES Mobile Loop Peptide—None of the 20 structures previously generated by Landry et al. (21) exhibited standard geometry in the turn formed by GroES residues 21–24. This might be due to a paucity of trNOE restraints in the turn or to NMR restraints that derive from an ensemble of conformations. Thus, we chose a single member of the family of 20 structures (frame 17 of Protein Data Bank: code 1EGS) for further refinement by molecular dynamics simulations in solvent water without NMR restraints, reasoning that in the context of the otherwise well defined β-hairpin, the turn may adopt a single preferred conformation. The β-hairpin structure consisted of GroES residues 19–27, with an acetyl group capping the amino terminus and an amino group capping the carboxyl terminus. These nine residues contain the hydrophobic tripeptide that makes direct contact with GroEL. Beginning with this β-hairpin, two simulations (Run1 and Run2) were conducted at 25 °C for 1.3 and 4.2 ns, respectively, using AMBER 5.0.

Throughout both simulations, the peptides remained as β-hairpins with the original antiparallel hydrogen-bonding register (Fig. 1, A, E, and F). Previous trNOE NMR data indicated that the H atoms of residues corresponding to GroES Lys-20 and Val-26 are near each other when the mobile loop peptide is in the GroEL-bound conformation (11). For both Run1 and Run2, the H atoms of Lys-20 and Val-26 remain in close proximity (Fig. 1B). Deviation from the NMR-determined conformation was assessed in terms of the violations of long range trNOE restraints (spanning at least three residues in the sequence). The starting structure violated none of the 12 long range restraints by more than 0.5 Å (21). After 1.25 and 2.25 ns, respectively, the structures in Run1 and Run2 violated none of the 12 long range restraints by more than 2.0 Å. This tolerance level is sufficient to accommodate side-chain dynamics but distinguish alternate backbone conformers. For comparison, the GroES-GroEL-ADP loop conformation violated 10 long range restraints by more than 2.0 Å.

During both simulations, identical crankshaft conformational changes occurred that involved the rotations of the ϕ dihedral angle of Ala-22 and the ϕ dihedral angle of Gly-23 (Fig. 1, C and D). The conformational changes finished −1 ns into Run1 and −2 ns into Run2, and the geometry of each turn had changed to that of a Type I turn with a G1 Bulge. Once adopted, this structure remained throughout the duration of each simulation.

To compare the intrinsic stability of alternative conformations in the simulation environment, another molecular dynamics simulation was performed using the loop crystal structure determined by Xu et al. (12) as the starting conformation. This simulation ran for 1.4 ns, and the initial loop structure was lost early in the simulation (Fig. 1, A, B, and G).

Mobile Loop Sequence Conservation—The GroEL binding loop sequences of 77 GroES homologues were examined to determine if sequence conservation is consistent with the formation of a β-hairpin with a Type I, G1 Bulge turn. A schematic of the consensus sequence corresponding to GroES residues 18–28 is shown in Fig. 2.

The peptide segment representing the consensus sequence is clearly amphiphatic, with polar and charged residues before the turn and hydrophobic residues after the turn. The first and last residues within the turn, positions i and i+4, are predominately occupied by threonine and isoleucine, respectively. A β-hairpin structure with a Type I, G1 Bulge turn requires that the amino acids at these positions adopt dihedral angles within β-sheet φ/ψ space (35). Studies on the β-sheet propensity of the 20 amino acids show that threonine has the greatest propensity at a site on the solvent-exposed edge of a β-sheet and that isoleucine has the greatest propensity at a site in the hydrophobic interior (36, 37). Indeed, position i is not immobilized as much as position i+4 in GroEL-bound mobile-loop peptides (11, 21). In the crystal structure, position i is solvent-exposed in the GroEL-bound state, whereas position i+4 is packed against the apical domain (12). Therefore, conservation at these sites in the mobile loop is compatible with a GroEL-bound hairpin conformation.

The glycine observed at position i+3 of the proposed Type I turn is the most highly conserved residue and occurs at this position in nearly 95% of the 77 co-chaperonin sequences. This is consistent with a statistical study that found glycine to be significantly favored at the i+3 position of all Type I turns (38). The predominance of glycine is explained by its ability to more easily adopt a positive ϕ angle, which is necessary at this site within Type I turns. The positive ϕ angle probably facilitates the reversal of the polypeptide backbone to run in the antiparallel direction (38). The alanine at i+1 and the glycine at i+2 are less well conserved; likewise, there is a high degree of amino acid variation at these sites in all Type I turns (38).

Transferred Nuclear Overhauser Effect NMR—TrNOE experiments using synthetic peptides and GroEL were conducted to test the hypothesis that peptide sequences that most favor the formation of a Type I turn with a G1 Bulge bind most effectively to GroEL. Four synthetic peptides corresponding to GroES mobile loop residues 13–32 were designed (Table I).
Positions 23 and 24 are, respectively, the $i/H_{11001}^2$ and $i/H_{11001}^3$ sites of the Type I turn in the proposed GroEL-bound hairpin conformation. Alanine at position 24 should be detrimental to the turn conformation, whereas alanine at position 23 or $d$-alanine ($dA$) at position 24 should be compatible with the turn conformation. The compatibility or incompatibility of the substitutions should be manifest as changes in the affinity of the peptides for GroEL.

All the trNOE volumes in Fig. 3 were found to be increasing at 75 ms of NOESY mixing time; thus, the relative affinities of the different peptides for GroEL can be determined by the degree of cross-relaxation observed for each peptide (39–41). Qualitative inspection of the spectra suggests that the G24A peptide does not bind GroEL as well as the wild type peptide, whereas the G24dA peptide and possibly even the G23A peptide bind more effectively.

The $^1$H NMR spectrum of each peptide was assigned by standard methods (42) at 10 °C. The trNOE experiments were performed at 30 °C, and the volumes of the nonoverlapping $H^{i}/H^{i+1}$ trNOE cross-peaks were calculated. Spectra of the peptides in the absence of GroEL yielded no trNOE cross-peaks. The relative volumes of selected trNOE cross-peaks corresponding to interactions within the proposed $\beta$-hairpin structure are shown in Fig. 4. Relative to the wild type peptide, a lesser degree of cross-relaxation is observed for the G24A peptide, and a greater degree of cross-relaxation is observed for the G24dA peptide. The values determined for the G23A peptide are almost identical to those of the wild type peptide.

The $^1$H NMR spectroscopy of GroES, GroES(G23A), and GroES(G24A)—Due to the flexible disorder of the loop, the spectrum of GroES contains sharp $^1$H NMR signals. Two-dimensional NMR spectroscopy was used to compare structural

| GroES residue No.: | 13 | 14 | 15 | 16 | 17 | 18$^*$ | 19 | 20 | 21 | 22 | 23 | 24 | 25$^*$ | 26$^*$ | 27$^*$ | 28 | 29 | 30 | 31 | 32 | 33 |
|-------------------|----|----|----|----|----|-------|----|----|----|----|----|----|-------|-------|-------|----|----|----|----|----|----|
| GroES:            | Ac-| K  | R  | K  | E  | V   | E   | T   | K   | S   | A   | A   | G     | I     | V     | L   | T   | G   | S   | A   | A   | -NH$_2$ |
| GroES (G23A):     | Ac-| K  | R  | K  | E  | V   | E   | T   | K   | S   | A   | A   | G     | I     | V     | L   | T   | G   | S   | A   | A   | -NH$_2$ |
| GroES (G24A):     | Ac-| K  | R  | K  | E  | V   | E   | T   | K   | S   | A   | G   | $dA$  | I     | V     | L   | T   | G   | S   | A   | A   | -NH$_2$ |
| GroES (G24dA):    | Ac-| K  | R  | K  | E  | V   | E   | T   | K   | S   | A   | G   | $dA$  | I     | V     | L   | T   | G   | S   | A   | A   | -NH$_2$ |

Turn position: $i +1 +2 +3 +4$

| FIG. 3. Selected regions of the NOEST spectra (75-ms mixing time) for the indicated peptides in the presence of GroEL. TrNOEs quantified in Fig. 4 are indicated by arrows. |
|FIG. 4. The relative volumes of selected $H^i/H^{i+1}$ trNOE cross-peaks (75-ms mixing time), indicating peptide affinity for GroEL. WT, wild type. |
The characteristics of the mobile loops of GroES and two GroES mobile loop mutants, GroES(G23A) and GroES(G24A).

$^1$H resonance assignments were obtained for residues 18–32 of GroES(G23A) and GroES(G24A). As expected for disordered loops, no long range NOE cross-peaks in the NOESY spectra were found for either protein. Furthermore, as previously observed for the GroES mobile loop (11), all the $^1$H chemical shifts are very close to random coil values (43). However, for equivalent residues surrounding the nascent $\beta$-hairpin, the $^1$H chemical shifts of GroES(G24A) tend to be up-field of the GroES $^1$H chemical shifts (Fig. 5), whereas the $^1$H chemical shifts of GroES(G23A) are almost identical to those of GroES (Fig. 5). Upfield deviations in $^1$H chemical shift are indicative of less $\beta$-sheet structure (43, 44).

Since the degree of cross-relaxation in the NOESY experiment is related to interproton distance, we measured the volumes for nonoverlapping cross-peaks in the $^1$H/$^1$H$_{i+1}$ region of the spectrum. These protons are in close proximity when a polypeptide is in a $\beta$-sheet conformation (42). Three amino acid pairs produced $^1$H/$^1$H$_{i+1}$ cross-peaks that were well isolated and measurable for all three proteins: Ile-25/Val-26, Val-26/Leu-27, and Leu-27/Thr-28. These volumes were normalized against the $^1$H/$^1$H cross-peaks of Thr-19 and Thr-28 within the same spectrum. The normalized volumes indicate that a higher degree of $\beta$-sheet structure is present at these positions of GroES and GroES(G23A) than in GroES(G24A) (Fig. 6). Furthermore, as was seen with the chemical shift deviations, GroES(G23A) appears to be much more similar to GroES than does GroES(G24A).

**DISCUSSION**

**A Type I, G1 Bulge Turn in the GroEL-bound $\beta$-Hairpin**—A combination of experiment and simulation has produced a detailed model for the structure of the GroEL-bound GroES mobile loop. Beginning with a mobile loop conformation determined by trNOE NMR, two unrestrained molecular dynamics simulations produced identical conformational changes. In both cases the $\beta$-hairpin adopted a Type I, G1 Bulge-turn conformation. All of the long range NMR restraints remain satisfied at a tolerance of 2 Å, indicating that the relaxed conformation retains the features imposed by GroEL binding. Analysis of 77 co-chaperonin homologues revealed that the amino acid sequence conservation is consistent with this GroEL binding mode.

The trNOE experiments revealed that substitutions within the GroES mobile loop peptides favored or disfavored binding to GroEL as predicted by their effect on the GroEL-bound hairpin conformation. When an alanine was substituted for glycine at the $i+3$ position (G24A), there was a significant decrease in binding. However, if $d$-alanine was substituted for glycine at the $i+3$ position (G24dA), there was an increase in binding of the peptide to GroEL. The $i+3$ site of a Type I turn possesses atypical dihedral angles, $\phi \sim 90^\circ$ and $\psi \sim 0^\circ$. This region of Ramachandran space is occupied most often by glycine because it possesses no side chain (35). However, $d$-alanine is also compatible with these dihedral angles, and therefore, it favors GroEL binding when substituted for glycine at position 24. The structural role of glycine at this position explains why it is almost perfectly conserved among GroES homologs.
In contrast, substitution with alanine at the i+2 site has little consequence because there are no stringent structural requirements at this position. The dihedral angles of the i+2 site of a Type I turn characteristically occupy an intermediate region between the α-helix and β-sheet spaces of the Ramachandran plot. Any amino acid other than proline should be acceptable at this site (38, 45). The slightly enhanced binding of the G23A peptide may be the result of decreased conformational dynamics imposed by the addition of a side chain or possibly a slight increase in the turn propensity. It is probably not due to new intermolecular interactions since a substitution with a polar side chain, serine, had the same effect (data not shown).

Since the molecular simulations and trNOE studies dealt with mobile loop peptides, it is important to consider whether the substitution at position 23 and 24 were examined in the context of the entire GroES protein. A comparison of H2O chemical shifts shows that the substitution at position 24 affects the structure of the mobile loop, whereas the substitution at position 23 has almost no effect. In GroES(G24A), for residues that are biased toward β-sheet in wild-type GroES, H2O chemical shifts move up-field, and H2O/2H2O+1 NOE volumes decrease, indicating that the G24A substitution reduces the bias toward the GroEL-binding β-hairpin conformation by disfavoring the Type I, G1 Bulge turn. However, the extent that binding of GroES(G24A) to GroEL is affected remains to be determined. In preliminary studies, GroES(G24A) has reduced function in vivo and in vitro, whereas GroES(G23A) is nearly indistinguishable from wild type GroES (data not shown).

The effects resulting from the substitutions of alanine for glycine at position 23 or 24 are similar to in vivo observations of two groES mutant E. coli strains that each possess a single aspartate substitution for glycine at position 23 or 24. When the alleles are present in single copy, the groES(G24D) strain does not grow at 43 °C, whereas the groES(G23D) strain grows nearly as well as wild type at 43 °C (11).

**Superimposition with GroEL-bound Strongly Binding Peptide—**The Type I, G1 Bulge-turn conformation is shared by

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**TABLE II**

Sequence alignments of the 12-residue SBP and segments of several co-chaperonin binding loops

|                     | Identity to GroES | Identity to SBP |
|---------------------|-------------------|-----------------|
| SBP:                | S W M T T P H G F L H P | 1/12            |
| GroES:              | V E T K S A G I V L T | 12/12           |
| T4 Hsp10:           | D E E V T E S G L I G | 2/12            |
| RB43 Hsp10:         | S E I V S E S G F L I G | 3/12            |
| M. lep. Hsp10:      | A E T M T P S G L V I P | 4/12            |
another molecule that binds to GroEL. Chen and Sigler (22) selected the SBP by biopanning with the apical domain of GroEL. The crystal structure of the GroEL-bound SBP is a β-hairpin and was discussed in terms of what it reveals about binding of substrates to GroEL. Interestingly, the backbone conformation of SBP and the structures resulting from the simulations Run1 and Run2 are nearly indistinguishable (Fig. 7). The SBP structure has a Type I turn with a G1 Bulge and has the same antiparallel hydrogen-bonding register as the NMR-derived GroES mobile loop hairpin conformation. Backbone atoms of the central seven residues from Run1 (1.25 ns) and Run2 (2.25 ns) superimpose on SBP with root mean square deviations of 0.6 and 0.4 Å, respectively. When superimposed on SBP, the highly conserved isoleucine of the GroES hydrophobic tripeptide projects directly into the substrate/GroES-binding site of the apical domain of GroEL. This contrasts with the poor similarity to the loop conformation in the GroES-GroEL-ADP structure, on which they superimpose with root mean square deviations of 2.3 and 2.5 Å, respectively.

Perhaps NMR and crystallography have converged on a mode of co-chaperonin binding. Table II displays an alignment of sequences for SBP and the homologous regions of GroES and three other co-chaperonins. These diverse co-chaperonins have as much identity to SBP as they do to GroES. Since SBP, GroES, bacteriophage T4 Hsp10, and bacteriophage RB43 Hsp10 all interact with GroEL, it is possible that SBP is a co-chaperonin mimic in that it possesses sequence elements that are employed by these co-chaperonins for binding GroEL. These features include the highly conserved glycine at position i+3 in the Type I turn, threonine at position i, and two of three hydrophobic residues in the GroEL binding tripeptide. Furthermore, the proline at the last position in SBP is in the same position as a proline in yeast Hsp10. The affinity of Hsp10 for Hsp60 decreased when the proline was changed to histidine or serine (14, 20), and conversely, the affinity of GroES for GroEL and Hsp60 increased when the corresponding threonine was changed to proline (19).

These observations suggest that the GroEL-bound conformation of SBP represents the GroEL-bound conformation of co-chaperonin mobile loops. The manner of SBP binding to GroEL was originally proposed to be indicative of a theoretical substrate. However, the backbone conformation of SBP is identical to that of our independently determined GroES mobile loop structure, and SBP is similar in sequence to co-chaperonin mobile loops. Therefore, the manner of SBP binding to GroEL might be better described as that of a theoretical co-chaperonin, thus corroborating the conclusion that the mobile loop of GroES employs a β-hairpin structure with a Type I, G1 Bulge turn when interacting with GroEL. However, this conclusion does not repudiate the reverse argument that SBP and co-chaperonin mobile loops bind to GroEL in the same manner as some substrates.

Plasticity in the GroES-GroEL Interaction—The GroES mobile loop β-hairpin described here differs from the mobile loop observed in the GroES-GroEL-ADP co-crystal structure, which was described as having a loop conformation (22). Perhaps, conformational plasticity in GroEL binding loops of GroES and other co-chaperonins is functionally significant. Alternate modes of binding were observed for CheA and CheY, which are involved in the chemotaxis signal transduction pathway of E. coli. A crystal structure of a heterodimer, consisting of CheY and the CheY binding domain of CheA, revealed two different binding modes within the same asymmetric unit (48). It was proposed that the plasticity at the interface was essential to function. Plasticity in the mobile loop might facilitate GroES binding to GroEL in the course of nucleotide- and substrate-dependent conformational transitions by the GroEL apical domains. Given the large displacement of the GroES-binding sites of GroEL during a functional cycle (49, 50), plasticity of the mobile loop would permit a dynamic interaction between GroES and GroEL. Furthermore, the binding mode of the mobile loop could be different for alternate GroEL conformations. The GroEL conformational transitions could affect the GroES-GroEL interaction in a way that would not affect the peptide-GroEL complexes. Although the β-hairpin conformation is accessible to the mobile loop in the GroEL-GroEL-ADP crystal structure, the observed loop conformation may be stabilized by constraints imposed by the ADP state of GroEL and/or the crystal lattice. We note that the ATP state of GroEL is thought to have the highest affinity for GroES (7).

We have previously proposed that disorder in the loops modulates binding affinity while preserving specificity (21); however, the existence of alternate GroEL-bound conformations suggests an additional role. It is possible that, after the mobile loops make the initial hydrophobic interaction with the apical domains of GroEL, subsequent folding of the loops into the preferred β-hairpin promotes the coordinated translational and rotational transitions of the GroEL apical domains.

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