“Half of the Sites” Binding of D-Glyceraldehyde-3-phosphate Dehydrogenase Folding Intermediate with GroEL*

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Two D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) folding intermediate subunits bind with chaperonin 60 (GroEL) to form a stable complex, which can no longer bind with additional GAPDH intermediate subunits, but does bind with one more lysozyme folding intermediate or one chaperonin 10 (GroES) molecule, suggesting that the two GAPDH subunits bind at one end of the GroEL molecule displaying a “half of the sites” binding profile. For lysozyme, GroEL binds with either one or two folding intermediates to form a stable 1:1 or 1:2 complex with one substrate on each end of the GroEL double ring for the latter. The 1:1 complex of GroEL-GroES binds with one lysozyme or one dimeric GAPDH folding intermediate to form a stable ternary complex. Both complexes of GroEL-lysozyme, and GroEL-GAPDH1z bind with one GroES molecule only at the other end of the GroEL molecule forming a trans ternary complex. According to the stoichiometry of GroEL binding with the GAPDH folding intermediate and the formation of ternary complexes containing GroEL-GAPDH1z, it is suggested that the folding intermediate of GAPDH binds, very likely in the dimeric form, with GroEL at one end only.

D-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH,1 EC 1.2.1.12) is a homotetrameric enzyme playing a key role in glycolysis. It has been used as a model for studies on unfolding, refolding, dissociation and association of oligomeric proteins (1–4), but little is known about details of its folding and association. Kinetic analysis of activity recovery of denatured GAPDH indicated that dimerization of the dimer is the rate-limiting step in the process of reactivation of the denatured enzyme (5). At low temperatures, tetrameric GAPDH can be induced by ATP to dissociate into inactive but structurally compact monomers, useful for the study of its reassociation and reactivation (6). A cold intermediate of a hyperthermophilic GAPDH was characterized by Jaenicke’s group to represent a native-like “assembled molten globule” displaying a reversible and highly cooperative conformational transition to the unfolded state (7, 8). GAPDH has also been used as a target protein to examine the chaperone activity of eukaryotic protein disulfide isomerase (9, 10) and bacterial DsbA (11) and DsbC,2 independent from their disulfide isomerase activity. Recently, a burst-phase intermediate of denatured rabbit muscle GAPDH during refolding has been characterized in this laboratory to be similar to the relatively stable unfolding intermediate of the enzyme denatured in 0.5–1.0 M guanidine hydrochloride (GdnHCl). This intermediate binds to GroEL with suppression of both its reactivation and aggregation. The stable complex with GroEL can be dissociated in the presence of ATP resulting in the reactivation of GAPDH to a level considerably higher than that obtained by spontaneous reactivation of the denatured GAPDH upon dilution and to a still higher level if GroES is also present (13).

Molecular chaperones such as GroEL play an essential role in assisting the folding of nascent peptides to form biologically functional proteins by binding with folding intermediate and thereby preventing reactions that lead to aggregation (14, 15). Stoichiometric analysis of the suppression of GAPDH reactivation by binding with GroEL suggested that the tetradecameric GroEL binds with one GAPDH dimer or two monomers (13). In this paper we have examined the binding profiles of GroEL itself and various GroEL complexes, such as GroEL-GroES, GroEL-GAPDH, and GroEL-lysozyme with different ligands, and the results are consistent with the suggestion that GroEL binds two lysozyme intermediates symmetrically but with a dimeric species of the GAPDH folding intermediate only at one end of the double ring.

EXPERIMENTAL PROCEDURES

Materials—D-Glyceraldehyde-3-phosphate was prepared from its diethyl acetal monobarium salt (Sigma) by the method provided. Hen egg white lysozyme, ADP, and oxidized glutathione were purchased from Serva. Bovine serum albumin (98–99% albumin, Fraction V), Micrococuss lysodeikticus dried cells, and GdnHCl were from Sigma. Dithiothreitol was from Promega. Reduced glutathione was from Boehringer Mannheim. Tris was from Amresco. All other chemicals were local products of analytical grade. In all experiments, unless otherwise specified, 100 mM Tris-HCl buffer (pH 7.5) containing 200 mM KCl, 5 mM MgCl2, and 2 mM EDTA was employed and referred to hereafter simply as the Tris buffer.

Preparation and Determination of Proteins—The multicopy plasmid pGroES containing the coding sequences of GroEL and GroES, a gift from Dr. S. L. Yang of the Shanghai Research Center of Biotechnology, Academia Sinica, was overexpressed in Escherichia coli HB101. The expression product was purified basically according to Landry and Gierash (16). Further purification of the GroES fraction was carried out by hydroxyapatite chromatography using 5–200 mM phosphate gradient to remove the co-expressed chloramphenicol acylase, and the GroEL fraction was treated by ATP according to Schmidt et al. (17) to remove the endogenously bound polypeptides. GroEL and GroES thus purified both showed one band on SDS-PAGE with the expected molecular mass (MM). The purified GroEL preparation had very low intrinsic fluorescence, especially with an excitation wavelength of 295 nm. Prep-

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1 The abbreviations used are: GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase; GroEL, chaperonin 60; GroES, chaperonin 10; DHFR, dihydrofolate reductase; GdnHCl, guanidine hydrochloride; MM, molecular mass; PAGE, polyacrylamide gel electrophoresis.

2 J. Chen, J.-L. Song, S. Zhang, Y. Wang, D.-F. Cui, and C.-C. Wang, unpublished data.
aration and activity assay of rabbit muscle GAPDH were as described by Liang et al. (2).

Protein concentrations were determined by measuring the absorbance at 280 nm with the following absorption coefficients (A°280): 1.00 for GAPDH (18), 0.66 for bovine serum albumin, 2.63 for native lysozyme, and 2.37 for denatured lysozyme (19). The concentrations of GroEL and GroES were determined by the method of Bradford (20) with bovine serum albumin as a standard. Unless otherwise specified, GroEL, GroES, and GAPDH were considered as tetradesamer, heptamer, and monomer, respectively, in the calculations of concentrations and molar ratios as denatured GAPDH is presumably fully dissociated.

Denaturation of Lysozyme and GAPDH—Lysozyme at 20 mg/ml was completely denatured and reduced in 100 mM sodium phosphate (pH 8.0) containing 8 M GdnHCl and 150 mM dithiothreitol at room temperature for 4 h. The reaction mixture was brought to pH 2.0 with 6 M HCl, dialyzed first against 10 mM HCl, and then against 100 mM acetic acid at 4 °C thoroughly. The denatured and reduced lysozyme was aliquoted at 200 μM and stored at −20 °C. Denaturation of GAPDH was carried out by incubation of 140 or 200 μM enzyme in the Tris buffer containing 3.0 M GdnHCl and 1 mM dithiothreitol for at least 4 h at 4 °C.

Preparation of Complexes of GroEL with Different Ligands—The complex of GroEL-GroES was prepared according to Azem et al. (21) by incubation of 2 μM GroEL, 2 μM GroES, and 1 mM ATP in the Tris buffer at 37 °C for 30 min. The complexes of GroEL-GAPDH, GroEL-lysozyme, and GroEL-lysozyme were obtained by 50-fold dilution of 200 μM denatured GAPDH and 100- or 50-fold dilution of 200 μM denatured and reduced lysozyme, respectively, into the Tris buffer containing 2 μM GroEL and incubation at 25 °C for 30 min. The complexes of GroEL-GroES-GAPDH, and GroEL-GroES-lysozyme, were made by rapid dilution of denatured GAPDH to 4 μM and denatured and reduced lysozyme to 2 μM, respectively, in the Tris buffer containing 2 μM GroEL-GroES and 50 μM ATP and incubation at 25 °C for 30 min. The complexes of GroEL-GAPDH-lysozyme, and GroEL-lysozyme-GAPDH were prepared, respectively, by dilution of denatured and reduced lysozyme to 2 μM in the Tris buffer containing 2 μM GroEL-GAPDH and denatured GAPDH to 4 μM in the Tris buffer containing 2 μM GroEL-lysozyme and incubation at 25 °C for 30 min. The complexes of GroEL-GAPDH-GroES and GroEL-lysozyme-GroES were obtained by incubating 2 μM GroES and 1 mM ATP with 2 μM GroEL-GAPDH and GroEL-lysozyme, respectively, at 37 °C for 30 min. To examine the construction of the complexes of GroEL-GroES-GAPDH and GroEL-G-APDH-GroES, denatured and reduced lysozyme was diluted to 2 μM in the Tris buffer containing either complex at 2 and 50 μM ATP and incubated for 30 min at 25 °C. Similarly, denatured GAPDH was diluted to 4 μM in the Tris buffer containing 2 μM GroEL-GroES-lysozyme, or GroEL-lysozyme-GroES and 50 μM ATP, or 2 μM GroEL-lysozyme, respectively, to examine the construction of three complexes.

In all the reactions involving GroES-containing complexes, 50 μM ATP was added to stabilize the complexes (22). All the reactions involving GroES binding were carried out at 37 °C and contained 1 mM ATP.

Identification of the Complexes—Each complex was loaded on a Sephacryl S-200 (Amersham Pharmacia Biotech) gel filtration column (1.2 × 50 cm) and eluted at 4 °C using the Tris buffer at a flow rate of 0.5 ml/min. The elution buffer contained also 50 μM ATP if the complex reaction contained GroES. The protein peak in void volume was collected, concentrated by Centrincon-10 (Amicon), and analyzed by SDS-PAGE of 15% polyacrylamide gel.

Refolding of GAPDH and Lysozyme—Refolding of denatured GAPDH was initiated by 200-fold dilution to 0.69 μM at 4 °C in the Tris buffer containing 5 mM dithiothreitol, with or without different concentrations of GroEL, GroEL-GroES, GroEL-lysozyme1, GroEL-lysozyme2, GroEL-lysozyme1-GroES, or GroEL-lysozyme2-GroES as specified. The activity recovery was determined according to Liang et al. (2) after incubation first at 4 °C for 30 min and then at 25 °C for 3 h after dilution (23), and the reactivation yield was defined as percentage of the activity of native GAPDH. Oxidative refolding of reduced and denatured lysozyme was carried out by 100-fold dilution to 2 μM in the Tris buffer containing 1 mM oxidized glutathione and 2 mM reduced glutathione with or without different concentrations of the complexes of GroEL, GroEL-GroES, GroEL-GAPDH, GroEL-lysozyme1-GroES, or GroEL-GAPDH-GroES at 25 °C for 2 h. If the complex contained GroES, 50 μM ADP was also present in the buffer. Lysozyme activity was determined at 30 °C by following absorbance decrease at 450 nm of a 0.25 mg/ml M. lysodeikticus suspension in 67 mM sodium phosphate buffer (pH 6.2) containing 100 mM NaCl (19, 24). Reactivation yield was defined as percentage of the activity of the native lysozyme. Aggregation of GAPDH during refolding was monitored continuously at 25 °C by 90° light scattering at 488 nm in a Hitachi F4010 spectrofluorometer.

RESULTS

Identification of Complexes of GroEL with Different Ligands—By Sephacryl S-200 chromatography with a MM fractionation range of 250–5 kDa, GAPDH (14 × 60 kDa) was eluted in the void volume, and GAPDH (4 × 36 kDa), GroES (7 × 10 kDa), and lysozyme (1 × 14.3 kDa) were eluted successively in well separated peaks. Fig. 1 shows the SDS-PAGE patterns of the proteins collected in void volume peaks of a Sephacryl S-200 column loaded with reaction products of GroEL with GAPDH, folding intermediates of GAPDH, or lysozyme at various proportions. The results identified the formation of all the stable complexes of GroEL with the bound components, and the bands in each lane appear at MM positions of the expected components. The band of GroES subunit appeared above the lysozyme band at the position corresponding to an apparent MM of 15 kDa but not 10 kDa as also noted by Tilly et al. (25). The order of addition of the ligands had no effect on the formation of all the ternary complexes.

Effects of GroEL and GroEL-GroES on the Reactivation and Aggregation during Refolding of Denatured GAPDH—As shown in Fig. 2A, the spontaneous reactivation of denatured GAPDH at 0.69 μM decreased with increasing concentration of GroEL in the dilution buffer and was completely suppressed at a ratio of GroEL/GAPDH of 0.5. When the GroEL-GroES complex was used instead of GroEL, the reactivation of GAPDH was suppressed at the same ratio of 0.5. Denatured GAPDH aggregated rapidly upon dilution as monitored by light scattering (Fig. 2B). Both the rate and the extent of aggregation decreased with increasing concentration of GroEL or GroEL-GroES complex, with full suppression of the aggregation at the same molar ratio of 0.5 in both cases.

The suppression of both the spontaneous reactivation and the aggregation of GAPDH during refolding in the presence of GroEL indicated the formation of a stable complex between GroEL and a folding intermediate of GAPDH (13). The above
results indicate that one tetradecameric GroEL binds stoichiometrically with two monomeric or one dimeric species of the GAPDH folding intermediate. The stable complexes of GroEL-GroES, GroEL-GAPDH, and GroEL-GroES-GAPDH thus formed were eluted in the void volume peak of Sephacryl S-200 chromatography and had the expected components as shown in Fig. 1 (lanes 1, 2, and 3).

Effects of GroEL and GroEL-GroES on the Reactivation of Denatured and Reduced Lysozyme—In contrast to GAPDH, the lysozyme folding intermediate binds to GroEL and the GroEL-GroES complex with different stoichiometry. As shown in Fig. 3, the spontaneous reactivation of 18% for lysozyme at 2 μM decreased with increasing concentration of GroEL in the refolding solution and was fully suppressed at a ratio of GroEL/lysozyme of 0.5. The reactivation of lysozyme also declined in the presence of the GroEL-GroES complex but became fully suppressed only when the molar ratio of GroEL/GroES/lysozyme reached 1.0. The above indicates the formation of stable complexes between one GroEL molecule and two lysozyme folding intermediates, GroEL-lysozyme, and between one GroEL-GroES and one lysozyme folding intermediate, GroEL-GroES-lysozyme. The components of the above complexes have also been demonstrated directly (Fig. 1, lanes 4 and 6).

The formation of stable ternary complexes of GroEL-GroES with folding intermediate of GAPDH or lysozyme indicates that the substrate binding does not induce the release of GroES from the GroEL-GroES complex. Moreover, the stability of the ternary complexes were not effected by ADP from 50 μM to 1 mM present in the Tris buffer (data not shown).

Effects of GroEL-Lysozyme, GroEL-Lysozyme2, GroEL-GroES-Lysozyme, and GroEL-Lysozyme-GroES on GAPDH Reactivation—As shown in Fig. 4, the presence of GroEL-lysozyme1 (Fig. 1, lane 5) in the refolding buffer suppressed the refolding of denatured GAPDH at a molar ratio of 0.5, indicating the formation of the complex of one GroEL-lysozyme1 with one dimeric or two monomeric GAPDH subunits, consistent with the result shown in Fig. 1, lane 7. However, the presence of GroEL-lysozyme2, GroEL-GroES-lysozyme1, or GroEL-lysozyme2-GroES in the refolding buffer had no effect at all on the reactivation of GAPDH up to a molar ratio of 0.5, indicating full occupancy on both ends of these GroEL complexes to preclude binding with GAPDH. For the GroEL-lysozyme1 complex, hence, there must be one lysozyme intermediate on each end of the GroEL molecule, and for the ternary complexes of GroEL-GroES-lysozyme1 and GroEL-lysozyme2-GroES, a lysozyme at one end and a GroES at the other were also consistent with the SDS-PAGE patterns of the respective complexes (Fig. 1, lanes 4, 6, and 9). GAPDH band was not detected from the reaction products of GroEL-lysozyme2, GroEL-GroES-lysozyme1, or GroEL-lysozyme1-GroES with GAPDH folding intermediates (Fig. 1, lanes 13, 14, and 15).

Effects of GroEL-GAPDH, GroEL-GroES-GAPDH, and GroEL-GAPDH-GroES on Lysozyme Reactivation—In contrast to GroEL-lysozyme, the presence of GroEL-GAPDH complex fully suppressed the refolding of denatured and reduced lysozyme at the molar ratio of 1.0 (Fig. 5), indicating that the complex of GroEL-GAPDH had one substrate binding site free for binding with one lysozyme, i.e., both subunits of the GAPDH folding intermediates are bound at only one end of the GroEL molecule. The components of the complex of GroEL-GAPDH-lysozyme were confirmed by SDS-PAGE in Fig. 1, lane 8. The complexes of GroEL-GroES-GAPDH and GroEL-GAPDH-GroES (Fig. 1, lanes 3 and 10) even at a molar ratio of 1.0 had no effect at all on the refolding of lysozyme, and no lysozyme band could be detected in the respective reaction products as shown in lanes 11 and 12 in Fig. 1. Moreover, in the presence of different concentrations of ADP from 50 μM to 1 mM, the complexes of GroEL-GroES-lysozyme and GroEL-GroES-GAPDH showed no effect on the reactivation of GAPDH and lysozyme, respectively (data not shown). GroES bound to complex of GroEL-GAPDH or GroEL-lysozyme only at the trans end of the complex but not at the cis end to cover the bound substrate, therefore no free

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**Fig. 2.** Effects of GroEL and GroEL-GroES on the reactivation and aggregation of denatured GAPDH. A, reactivation of 0.69 μM denatured GAPDH in the presence of different concentrations of GroEL (●) or GroEL-GroES complex (○). B, aggregation during the refolding of 0.69 μM denatured GAPDH monitored by light scattering at 488 nm. 1, no addition; 2 and 3, in the presence of GroEL at the ratio of 0.25 and 0.5, respectively; 4, 5, and 6, with GroEL-GroES complex at the ratio of 0.125, 0.25, and 0.5, respectively.

**Fig. 3.** Effects of GroEL and GroEL-GroES on the reactivation of denatured and reduced lysozyme. Reactivation of 200 μM denatured and reduced lysozyme was triggered by dilution to 2 μM in the presence of different concentrations of GroEL (●) or GroEL-GroES complex (○).
ends were available in GroEL-GAPDH$_2$-GroES and GroEL-lysozyme$_1$-GroES complexes.

**DISCUSSION**

The three-dimensional structure of GroEL (26) reveals that the GroEL molecule has two substrate-binding sites on each end of the double ring. In the presence of ADP, one GroEL molecule binds with only one GroES molecule to form the bullet-like complex of GroEL-GroES; no football-like GroEL-GroES$_2$ is detected even in the presence of 3-fold excess of GroES (27). The complex of GroEL-GroES has a free binding site and can bind with either lysozyme or GAPDH folding intermediate to form a stable 1:1 complex of GroEL-GroES-lysozyme$_1$ or 1:2 complex of GroEL-GroES-GAPDH$_2$. The binding of GAPDH or lysozyme intermediate in the presence of 50 $\mu$m ADP does not trigger the release of GroES from GroEL. It is consistent with the report by Sparrer and Buchner (28) for the complex of GroEL-GroES-multrose binding protein (Y283D) but is different from the report by Hartl and co-workers (29) that GroEL-bound substrate polypeptide can induce GroES cycling on and off GroEL in the presence of 0.2 mM ADP. In fact, ADP at concentrations from 50 $\mu$m to 1 mM has been found to show no effect on the stability of the ternary complexes of GroEL-GroES-lysozyme$_1$ and GroEL-GroES-GAPDH$_2$.

It has been reported that GroES binds with similar efficiency to either ring of the GroEL-rhodanese complex in the presence of physiological concentration of ADP, 0.2 mM, resulting in a mixture of trans- and cis-complexes (29), however, the fact that the stable 1:1 complex of GroEL-lysozyme$_1$-GroES or the 1:2 complex of GroEL-GAPDH$_2$-GroES can no longer bind additional substrate strongly suggests that GroES binds with the complex of GroEL-lysozyme$_1$, or GroEL-GAPDH$_2$ in the presence of 1 mM ADP on the free end only. Similarly, the complex of GroEL-malate dehydrogenase-GroES also appears to be in a trans form (27).

One GroEL molecule binds with one lysozyme folding intermediate to form a stable 1:1 complex, GroEL-lysozyme$_1$, which can further bind an additional lysozyme folding intermediate at the other side to form the 1:2 complex. As this complex GroEL-lysozyme$_2$ can no longer bind with any additional substrate, the two lysozyme molecules bind, most likely, one on each end of the double ring. The GroEL-lysozyme$_1$ complex can also bind GroES or GAPDH folding intermediate at the free side.

In contrast to the GroEL-lysozyme complex, although the GroEL-GAPDH$_2$ complex has the stoichiometry of 1:2 and no longer binds with additional GAPDH folding intermediate, it can still bind with either one lysozyme or one GroES molecule, indicating the GroEL-GAPDH$_2$ complex has indeed a free binding site left on the other end, and the two subunits of the GAPDH folding intermediate are bound at the same end of the double ring. It seems that as in the case of allosteric proteins, the binding with GAPDH folding intermediate at one end of GroEL molecule prevents the binding of another GAPDH folding intermediate at the other end, or in other words, the binding of GAPDH folding intermediate is a “half of the sites” reaction. The volume of the cavity of the GroEL molecule is estimated to be 85,000 Å$^3$ and might accommodate a native protein of 70 kDa or a much smaller nonnative polypeptide (22). However, as Chen et al. (27) and Thiyagarajan et al. (30) pointed out, portions of the bound polypeptide can protrude from the cavity so as to allow the binding of a larger nonnative molecule, such as two subunits of GAPDH folding intermediate of 72 kDa or in a similar way alcohol oxidase of 75 kDa (31). It is known that some other proteins, such as subtilisin with MM of 27.7 kDa (32), rhodanese of 33 kDa (31, 33), malate dehydrogenase of 35 kDa (34), rat liver ornithine transcarbamylase of 36 kDa (35), and mitochondrial aspartate aminotransferase of 46 kDa (36) bind with GroEL in a 1:1 stoichiometry, but it is not known whether the free end is still available for binding of another substrate. On the other hand, the relatively small molecules of lysozyme (14.3 kDa) and dihydrofolate reductase (DHFR, 20 kDa) show multiple binding. However molecular mass does not seem to be the only factor as the binding of maltose-binding protein (40 kDa) to GroEL shows a 1:2 stoichiometry. Presumably, molecular shape rather than molecular mass and the mode of interactions between GroEL and ligands are the crucial factors. “Half of the sites” binding probably results from the conformational change at the other end of the GroEL molecule induced by the bound ligand at one end.

It is known that the closely packed crystalline homotetrameric GAPDH exists as a dimer of dimers (37). During the unfolding at low denaturant concentrations, the dimer of GAPDH subunit exists as a partially unfolded and aggregation-prone form (2), which is most likely the species formed in the burst phase during its refolding and prone to GroEL binding (13). It is therefore likely that, in the GroEL-GAPDH$_2$ complex, the two GAPDH subunits exist as a dimer but not two monomers.

It is interesting to note that one GroEL molecule can rapidly bind with four barnase molecules composed of 110 amino acid

![](image.png)

**FIG. 4. Effects of GroEL-lysozyme$_1$, GroEL-lysozyme$_2$, GroEL-GroES-lysozyme$_1$, and GroEL-GroES-lysozyme$_2$ on GAPDH reactivation.** Reactivation of 0.69 $\mu$m denatured and reduced lysozyme in the presence of different concentrations of GroEL-lysozyme$_1$ (●), GroEL-lysozyme$_2$ (○), GroEL-GroES-lysozyme$_1$ (■), and GroEL-GroES-lysozyme$_2$/GroES (□).

![](image.png)

**FIG. 5. Effects of GroEL-GAPDH$_2$, GroEL-GroES-GAPDH$_2$, and GroEL-GAPDH$_2$/GroES on lysozyme reactivation.** Reactivation of 2 $\mu$m denatured and reduced lysozyme in the presence of different concentrations of GroEL-GAPDH$_2$ (●), GroEL-GroES-GAPDH$_2$ (■), and GroEL-GAPDH$_2$/GroES (□).
residues with barnase in great excess to GroEL, but the binding stoichiometry becomes 1:1 with GroEL in excess to barnase (38). Two or four molecules of mutated E. coli DHFR can be bound to one GroEL depending on different mutants (39). Also the binding stoichiometry of chymotrypsin inhibitor 2, a very small protein of only 64 residues (12), with GroEL varies from 1:1 for wild type to 1:8 for some mutants with the substrate in excess. However, in all the above cases, it has not been stated whether the multiple molecules of substrate were bound at the same end or both ends of the GroEL double ring. It has been pointed out that the 1:4 complex of GroEL with mutant DHFR is not significantly populated, only <10%, even with the ligand in excess, and the predominating complex formed has a ratio of 1:2 (39). The lysozyme molecule is only slightly bigger than barnase and smaller than DHFR, but only one lysozyme molecule can bind at each end of a GroEL molecule.

Scheme 1 is a model consistent with all the observations for the unfolding and refolding of GAPDH and the binding of the folding intermediate with GroEL, where M is the denatured monomer; D, D', and D'' are dimeric folding intermediates with different conformations; T is tetrameric native GAPDH; and A is aggregated enzyme.

A tetrameric GAPDH is fully denatured to be four unfolded monomeric subunits by GdnHCl under the conditions employed. Upon dilution, a dimeric folding intermediate formed very fast in a burst phase, which is partially folded with secondary structure between that of the native and the denatured species and aggregation-prone (13). GroEL recognizes and binds with this dimeric folding intermediate through hydrophobic interactions at one end of the double ring to form a stable asymmetric complex and thus prevent the aggregation between the folding intermediates. The intermediate is released only in the presence of ATP or ATP/GroES for either further folding and association to become an active tetrameric molecule or aggregation or rebinding with GroEL.

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