Significance of chaperonin 10-mediated inhibition of ATP hydrolysis by chaperonin 60

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ABSTRACT Chaperonins are essential for the folding of proteins in bacteria, mitochondria, and chloroplasts. We have functionally characterized the yeast mitochondrial chaperonins hsp60 and hsp10. In the presence of ADP, one molecule of hsp10 binds to hsp60 with an apparent $K_d$ of 0.9 nM and a second molecule of hsp10 binds with a $K_d$ of 24 nM. In the presence of ATP, the purified yeast chaperonins mediate the refolding of mitochondrial malate dehydrogenase. Hsp10 inhibits the ATPase activity of hsp60 by about 40% (Hsp10(P36H) is a point mutant of hsp10 that confers temperature-sensitive growth to yeast. Consistent with the in vivo phenotype, refolding of mitochondrial malate dehydrogenase in the presence of purified hsp10(P36H) and hsp60 is reduced at 25°C and abolished at 30°C. The affinity of hsp10(P36H) to hsp60 as well as to Escherichia coli GroEL is reduced. However, this decrease in affinity does not correlate with the functional defect, because hsp10(P36H) fully assists the GroEL-mediated refolding of malate dehydrogenase at 30°C. Refolding activity, rather, correlates with the ability of hsp10(P36H) to inhibit the ATPase of GroEL but not that of hsp60. Based on our findings, we propose that the inhibition of ATP hydrolysis is mechanically coupled to chaperonin-mediated protein folding.

Group I chaperonins (cpns) are a subclass of molecular chaperones that are found in eubacteria, mitochondria, and chloroplasts. CPns consist of two interacting partner proteins, cpn60 and cpn10. Cpn60 and cpn10 from Escherichia coli; also termed GroEL and GroES, have been characterized in great detail and have served as a paradigm for chaperonin action (1). GroEL is composed of 14 subunits of 60 kDa that form two stacked heptameric rings. The GroEL oligomer encloses a central cavity in which substrate protein is supposed to bind. GroES is a single heptameric ring of 10 kDa subunits. In the presence of adenine nucleotides, GroEL and GroES form an asymmetric 1:1 complex. A symmetric structure consisting of two GroES molecules bound to both ends of the GroEL cylinder has been observed in the presence of ATP (6–8). The functional importance of this symmetric complex is currently under debate. Complex formation between the two partner chaperonins is mediated, at least in part, by the mobile loop in each of the seven GroES subunits (12, 16). GroEL itself is an ATPase with a low turnover number of $\sim$5 min$^{-1}$ per monomer (17). GroES inhibits ATP hydrolysis by GroEL by about 50%, suggesting that binding of GroES induces half-of-the-site-reactivity in GroEL (17–19). More recently it has been shown that even in the absence of GroES only one ring of GroEL at a time hydrolyzes ATP (20). The phenomenon of cpn10-mediated inhibition of ATP hydrolysis by cpn60 has also been observed with the homologs of GroEL/ES from Thermus aquaticus brockii; however, its significance is not known (21). The idea that the energy of ATP hydrolysis is required for chaperonin-dependent protein folding has recently been challenged. Protein folding, although with delayed kinetics, was shown to proceed in the presence of ADP, suggesting that the role of ATP hydrolysis is to optimize the rate of GroES cycling (22).

Here we address the question of whether the inhibition of the ATP hydrolysis rate influences the refolding efficiency of a substrate protein in vitro. To that end we have employed the yeast mitochondrial chaperonins hsp60 and hsp10, the homologs of GroEL and GroES, and hsp10(P36H), a temperature-sensitive mutant of hsp10. At the nonpermissive temperature for growth, the refolding of denatured mitochondrial malate dehydrogenase (mMDH) was mediated by hsp60 in combination with wild-type but not with mutant hsp10. However, under the same conditions, GroEL was functional in refolding mMDH irrespective of whether combined with hsp10 or hsp10(P36H). The ability of cpn10 to support mMDH refolding correlated with the ability to inhibit the ATPase activity of the partner chaperonin: Hsp10(P36H) failed to inhibit the ATPase of its in vivo partner hsp60 but did inhibit that of E. coli GroEL. The results suggest that an important function of cpn10 in the folding cycle is to regulate the rate of ATP hydrolysis by cpn60.

EXPERIMENTAL PROCEDURES

Materials. All chemicals, enzymes, and protease inhibitors were from Fluka or Sigma, unless stated otherwise. mMDH, GroEL, and GroES were from Boehringer Mannheim, and [y-32P]-ATP was from Amersham. Purification of hsp60, hsp10, and hsp10(P36H) will be described elsewhere.

Protein-Refolding Assays. Denaturation and refolding of mitochondrial malate dehydrogenase was as described (23). Kinetic experiments were fitted to a uni-bimolecular folding mechanism (24).

Determination of Binding Constants by Centrifugation Through Sucrose Cushions. Centrifugation experiments were performed in buffer A containing 50 mM Mes-NaOH (pH 6.5), 20 mM KCl, and 20 mM MgCl$_2$ supplemented with 2 mM of the nucleotide as indicated in the Figure 2 legend. Sucrose cushions were prepared in siliconized centrifuge tubes (11 × 43 mm; Beckman) by underlaying 150 μl buffer A with 800 μl 20% (wt/wt) sucrose in buffer A. The concentration of chaperonins is given per 1 ml, the final volume of the sucrose cushion. Chaperonins were mixed as indicated in the Figure 2 legend in a volume of 50 μl buffer A and incubated at 25°C for 5 min.

Abbreviations: mMDH, mitochondrial malate dehydrogenase; PMSF, phenylmethylsulfonyl fluoride.

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†To prevent confusion when analyzing chaperonins from different sources, we use the following nomenclature: GroEL and GroES are the chaperonins from Escherichia coli; hsp60 and hsp10 are the corresponding homologs from Saccharomyces cerevisiae; and cpn60 and cpn10 designate any member of the chaperonin 60 or chaperonin 10 family, respectively.

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5 min. Centrifugation was performed at 356,000 × g for 1 h at 25°C or at 30°C. After centrifugation, samples were split into a top (850-μl) and bottom (150-μl) fraction. Samples were analyzed by SDS/PAGE (10% Tris-tricine gels) and immunoblotting followed by autoradiography and quantification with a densitometer. The fraction of cpn10 in the bottom (bound) was expressed as a percentage of total cpn10 concentration (bottom + top). Recovery was between 75 and 100%.

ATPase Assay. ATP hydrolysis was assayed in the linear range with [γ-32P]-ATP (1–3 Ci mmol⁻¹; 1 Ci = 37 GBq) as substrate, using the orthophosphate–molybdate extraction method (25). Unless stated otherwise, experiments were carried out at the indicated temperatures with 1 μM cpn60 protomer (71.4 nM oligomer) in standard buffer containing 2 mM ATP, 50 mM HEPES-NaOH (pH 7.4), 20 mM KCl, and 20 mM MgCl₂. The activity is expressed as a percentage of the maximal turnover rate of uninhibited cpn60 in the absence of co-chaperonin.

Miscellaneous. SDS/PAGE was performed using a tricine-based buffer system (26). Immunoblotting was performed using polyclonal antisera against the corresponding proteins based on the method (25). Unless stated otherwise, experiments were carried out at the indicated temperatures with 1 μM cpn60 protomer (71.4 nM oligomer) in standard buffer containing 2 mM ATP, 50 mM HEPES-NaOH (pH 7.4), 20 mM KCl, and 20 mM MgCl₂. The activity is expressed as a percentage of the maximal turnover rate of uninhibited cpn60 in the absence of co-chaperonin.

RESULTS

Yeast hsp10(P36H) Does Not Assist hsp60 in the Refolding of Mitochondrial Malate Dehydrogenase at 30°C but Does Assist Bacterial GroEL. A major role of yeast hsp10 is to assist the hsp60-mediated folding of newly imported precursor proteins inside the mitochondrial matrix (28). We have generated a point mutant of hsp10, hsp10(P36H), that confers temperature-sensitive growth to yeast. Analysis of purified hsp60, hsp10, and hsp10(P36H) revealed that hsp60 was assembled into a 14-mer whereas hsp10 and hsp10(P36H) were assembled into a 7-mer. The thermal stability of hsp10(P36H) was identical to the wild-type protein (unpublished work). To get a better understanding of the defect induced by the point mutation in hsp10 we tested the ability of hsp10(P36H) to assist hsp60-mediated folding of mMDH that strictly requires the co-chaperonin (23, 29–34).

If denatured mMDH was allowed to refold in the presence of hsp60, hsp10, Mg-ATP, and potassium ions, active enzyme was recovered at 25 or 30°C, respectively (Fig. 1A and B, + hsp10). After 150 min, the yield of refolded mMDH was maximal (≈40%) at a 2- to 5-fold molar ratio of hsp10 heptamer to hsp60 14-mer. With hsp10(P36H), however, the maximal yield of active mMDH was reduced to ≈30% at 25°C [Fig. 1A, + hsp10(P36H)] and to less than 10% at 30°C [Fig. 1B, + hsp10(P36H)]. In the absence of chaperonins, less than 10% of mMDH regained activity at 25°C and less than 5% regained activity at 30°C (Fig. 1A–C, spont.). Surprisingly, GroEL functioned equally well with hsp10 or with hsp10(P36H) at 30°C (Fig. 1C) and even at 37°C (data not shown). The maximal yield of active mMDH at 30°C with either hsp10 or hsp10(P36H) as co-chaperonin was ≈60%. Refolding in the presence of GroEL was maximal at a 2- to 5-fold molar excess of either wild-type or mutant hsp10 (Fig. 1C). However, at substoichiometric levels of co-chaperonin, the yield of active mMDH obtained in the presence of hsp10(P36H) was significantly lower [Fig. 1C; compare + hsp10 and + hsp10(P36H)]. This observation might reflect the slightly decreased affinity of hsp10(P36H) for cpn60 proteins (see Table 1 and below).

The spontaneous refolding of mMDH has been studied in detail and can be separated into two partial reactions with two

![Fig. 1. Chaperonin-mediated refolding of mitochondrial malate dehydrogenase (mMDH). At the indicated temperatures denatured mMDH was diluted to a final concentration of 71.5 nM into refolding buffer (50 mM HEPES-NaOH, pH 7.4/20 mM KCl/20 mM MgCl₂/2 mM DTT) containing 0.1 μM cpn60 (oligomer) and increasing amounts of cpn10 (0.5- to 10-fold molar excess with respect to hsp60 oligomer). Refolding was initiated by the addition of 2 mM ATP, and activity of mMDH was determined after 150 min. The yield of each refolding reaction is expressed as the percentage of activity displayed by an aliquot of mMDH that was treated identically but had not been denatured before dilution into the refolding buffer. The first bar of each graph represents the fraction of mMDH that regains activity in the absence of chaperonins (spont.). (Upper) Refolding reactions in the presence of wild-type hsp10. (Lower) Refolding reactions in the presence of hsp10(P36H). Individual refolding experiments were performed in triplicate. Results are given as mean ± SD (error bars). (A) Refolding in the presence of hsp60 at 25°C. (B) Refolding in the presence of hsp60 at 30°C. (C) Refolding in the presence of GroEL at 30°C. (D) Kinetic analysis of the refolding of mMDH in the presence of hsp60 plus hsp10 or hsp10(P36H), respectively. Denatured mMDH was diluted into refolding buffer containing hsp60 (.), hsp60 plus hsp10 (○), or hsp60 plus hsp10(P36H) (△). Refolding was initiated by the addition of 4 mM ATP. Concentrations of hsp60 and mMDH were as described in A–C, and a 5-fold molar excess of cpn10 over hsp60 was used. The value corresponding to 100% mMDH activity was determined individually for each time point in a mock refolding reaction containing hsp60, hsp10, and nondenatured mMDH. Evaluation of mMDH refolding kinetics was performed using the program FACSIMILE (23).](image-url)
Table 1. Apparent dissociation constants ($K_d$) determined for the various chaperonin complexes in the presence of ADP and ATP

<table>
<thead>
<tr>
<th>Chaperonin combination</th>
<th>Nucleotide,</th>
<th>$K_d$, nM</th>
<th>$n$</th>
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<tr>
<td>hsp60 + hsp10</td>
<td>ADP</td>
<td>0.88 ± 0.05</td>
<td>0.6</td>
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<td></td>
<td></td>
<td>24.2 ± 4.4</td>
<td>1.4</td>
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<tr>
<td></td>
<td>ATP</td>
<td>4.4 ± 0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>hsp60 + hsp10(P36H)</td>
<td>ADP</td>
<td>19.2 ± 1.6</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>21.8 ± 2.2</td>
<td>0.7</td>
</tr>
<tr>
<td>GroEL + hsp10(P36H)</td>
<td>ADP</td>
<td>7.4 ± 0.8</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>9.0 ± 2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>GroEL + GroES</td>
<td>ADP</td>
<td>0.19 ± 0.04</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.1 ± 6.3</td>
<td>1.8</td>
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$K_d$ values and the number of independent binding sites for cpn10 proteins ($n$) were derived from linear regression analysis of Scatchard plots as exemplified in Fig. 2. Correlation coefficient $R$ for all linear regressions was >0.95.

Independent rate constants (23, 24). In the presence of GroEL/ES and ATP the bimolecular rate constant ($k_2$) was not altered, whereas the unimolecular rate constant ($k_1$) was significantly increased (31, 34). We compared $k_1$ of mMDH refolding at 25°C in the absence of chaperonins with $k_1$ in the presence of hsp60 and hsp10 or hsp10(P36H), respectively. In our assay, $k_1$ for the spontaneous refolding of mMDH was $4.2 \times 10^{-4}$ s$^{-1}$. The rate constant remained unaltered when hsp60 and ATP were present ($k_1 = 3.4 \times 10^{-4}$ s$^{-1}$, Fig. 1D). However, when hsp10 was present as well, $k_1$ increased from $3.4 \times 10^{-4}$ s$^{-1}$ to $7 \times 10^{-4}$ s$^{-1}$ (Fig. 1D). This result resembles the reported increase of $k_1$ in the presence of GroEL and GroES (34). In contrast, there was no significant rate enhancement in the presence of hsp10(P36H) ($k_1 = 3.9 \times 10^{-4}$ s$^{-1}$).

Thus, at 25°C, hsp10(P36H) has lost the ability to accelerate refolding of mMDH, but it is still capable of increasing its yield (Fig. 1A and D).

**Hsp10 and GroEL Have a Reduced Affinity for hsp10(P36H).** Cpn60 and cpn10 can be completely separated by their sedimentation rate. After centrifugation through sucrose cushions in the absence of nucleotide, hsp60 was recovered in the bottom fraction and hsp10 was recovered in the top fraction (Fig. 2A, - nucl.). Hsp10 is recovered in the bottom fraction only in the presence of nucleotide when bound to hsp60 (Fig. 2A). Scatchard analysis as exemplified in Fig. 2 B and C yielded apparent $K_d$ values for the various chaperonin combinations at 25°C (Table 1). The corresponding values at 30°C were essentially identical to those measured at 25°C (data not shown). Binding of hsp10(P36H) was reduced ~20-fold with hsp60 (compared with hsp10) and ~40-fold with GroEL (compared with GroES) in the presence of ADP (Table 1). In the presence of ATP, the apparent $K_d$ values were changed to a lesser extent (Table 1) and were similar to the previously determined $K_d$ of 17 nM for GroEL/ES in the presence of ATP (12).

Unexpectedly, the Scatchard plots of the homologous chaperonin combinations in the presence of ADP indicate the existence of a second binding site of lower affinity (Fig. 2B and Table 1) (35). No second binding site could be detected for hsp10(P36H) on either hsp60 or GroEL.

**Hsp10(P36H) Inhibits the ATPase Activity of GroEL but Not That of hsp60.** The ATPase activity of GroEL is dependent on potassium ions (17, 25). The potassium concentration at which the rate of ATP hydrolysis is half-maximal ($K_{act}$) was determined for yeast hsp60 by measuring the initial velocity of nucleotide hydrolysis at different potassium concentrations (Fig. 3A). In the presence of 0.5 mM ATP $K_{act}$ was 1.3 mM (±...
0.5 mM). The maximal ATPase rate was $2.5 \pm 0.1 \text{ min}^{-1}$ (based on protomer). At 25°C, binding of either hsp10 or hsp10(P36H) inhibited the ATPase of GroEL by $\approx 40\%$, whereas GroES inhibited the ATPase by 50%, as previously reported (Fig. 3B, + GroEL; e.g., ref. 17). The inhibitory effect was unaltered at 30°C, indicating that temperature did not affect the function of hsp10(P36H) in a way that can be sensed by GroEL (Fig. 3C, + GroEL). Hsp10 inhibited the ATPase of hsp60 by $\approx 40\%$, at 25°C and at 30°C (Fig. 3B and C, + hsp60). In contrast, hsp10(P36H) did not inhibit the ATPase activity of hsp60 at all 30°C and inhibited it by about 10% at 25°C (Fig. 3B and C, + hsp60). The apparent dissociation constant of the hsp60/hsp10(P36H) complex was 22 nM in the presence of ATP (Table 1). Based on this dissociation constant and the concentrations used in our experiments, it can be calculated that at a 5-fold molar excess of hsp10(P36H) more than 97% of hsp60 has formed a 1:1 complex with the co-chaperonin. Even when we increased the concentration of hsp10(P36H) up to a 50-fold molar excess over hsp60, no inhibition of the ATPase was detected at 30°C (data not shown). Thus, binding of hsp10(P36H) to hsp60 under these conditions fails to inhibit the ATPase activity of hsp60.

**DISCUSSION**

Formulation of the Holochaperonin Complex in the Presence of Nucleotide. Separation of free and bound ligand by sucrose density centrifugation has been previously applied to determine dissociation constants ($K_d$) of protein–protein interactions (36, 37). This method does not yield $K_d$ values under strict equilibrium conditions. However, it can be applied to determine the affinity between cpn60 and cpn10 in the presence of ADP, where the chaperonin complex has a half-life of $\approx 5$ h (12, 38). We find that the $K_d$ for the *E. coli* GroEL/GroES complex determined by sucrose density centrifugation agrees well with published data (12, 39, 40). The yeast hsp60/hsp10 complex is of similar stability. We also applied the sucrose density gradients to determine the stability of the different chaperonin complexes in the presence of ATP. Under these conditions chaperonin complexes are of dynamic nature. However, the apparent $K_d$ values allow a direct comparison between the stability of the different complexes. Our results indicate that the binding affinity of hsp10(P36H) is less affected in the presence of ATP (that is, under active folding conditions) than in the presence of ADP. The $K_d$ of the nonfunctional combination of hsp60/hsp10(P36H) is almost identical to the $K_d$ of GroEL/ES (Hay-Hartel al. (12); compare also Results and Table 1).

It has been suggested that football-shaped chaperonin complexes, consisting of two GroES heptamers attached to one GroEL 14-mer, are involved in the chaperonin reaction cycle. The existence of such structures in the presence of ATP is now generally accepted, and only their functional significance is being debated (6, 8–12, 38, 41–44). Football-shaped holochaperonins so far have not been detected in the presence of ADP by electron microscopy, crosslinking, or surface plasmon resonance techniques. Our finding that a second molecule of GroES or hsp10, respectively, can bind to its homologous partner protein in the presence of ADP suggests the existence of football-shaped structures, but we cannot tell whether these structures are involved in the folding cycle. Because chaperonins can also function in the presence of ADP, the existence of ADP-induced footballs might indeed be functionally relevant (22). We did not detect a second binding site in the presence of ATP. Our failure to reproduce the well established binding of a second cpn10 under these conditions is most likely due to the dynamic nature of the complex in the presence of ATP. It will be interesting to determine the stoichiometry and affinity of the different chaperonin combinations in the presence of ATP when the complex should be stable.

**Effect of the P36H Mutation in the Mobile Loop of hsp10.**

The point mutation in hsp10(P36H) is located in the region that is believed to bind to cpn60. It is thus attractive to hypothesize that the *in vivo* phenotype of hsp10(P36H) reflects a reduced stability of the chaperonin complex under nonpermissive conditions for growth. In fact, it was shown that hsp10(P36S), a mutant of yeast hsp10 isolated in an independent screen, showed decreased affinity for *E. coli* GroEL (28).

![Fig. 3. Effect of cpn10 on the ATPase activity of hsp60 or GroEL, respectively. The rate of ATP hydrolysis was determined at the indicated temperatures as described in Experimental Procedures. (A) Initial rates of ATP hydrolysis by hsp60 at different potassium concentrations. The concentration of ATP was 500 μM and the temperature was 25°C. Data were fitted to the Michaelis–Menten equation $V = V_{max} \times [KCl]/([KCl] + K_{cat})$. (B) Inhibition of the ATPase activity of hsp60 (Upper) or GroEL (Lower) at 25°C. Cpm60 oligomer (11.4 nM) was incubated in the absence or presence of a 5-fold molar excess of hsp10, hsp10(P36H), or GroES, respectively. (C) ATPase activity of hsp60 (Upper) or GroEL (Lower) at 30°C, as described in B.](image-url)

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**Table 1.**

<table>
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<th>Condition</th>
<th>ATPase Activity (%)</th>
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<td>hsp60</td>
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<td>hsp10</td>
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<tr>
<td>hsp10(P36H)</td>
<td>20</td>
</tr>
<tr>
<td>GroEL</td>
<td>50</td>
</tr>
<tr>
<td>GroES</td>
<td>30</td>
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</table>

**Fig. 3.** Effect of cpn10 on the ATPase activity of hsp60 or GroEL, respectively. The rate of ATP hydrolysis was determined at the indicated temperatures as described in Experimental Procedures. (A) Initial rates of ATP hydrolysis by hsp60 at different potassium concentrations. The concentration of ATP was 500 μM and the temperature was 25°C. Data were fitted to the Michaelis–Menten equation $V = V_{max} \times [KCl]/([KCl] + K_{cat})$. (B) Inhibition of the ATPase activity of hsp60 (Upper) or GroEL (Lower) at 25°C. Cpm60 oligomer (11.4 nM) was incubated in the absence or presence of a 5-fold molar excess of hsp10, hsp10(P36H), or GroES, respectively. (C) ATPase activity of hsp60 (Upper) or GroEL (Lower) at 30°C, as described in B.
We conclude that the defect of hsp10(P36H) is not related to the lower affinity for hsp60 but reflects a more subtle defect of allosteric communication with hsp60. A similar, but less dramatic effect has previously been reported for a K34A mutant of GroES (45). Lysine-34 is located in close proximity to the mobile loop of GroES. The GroES(K34A) mutant was not affected in its ability to inhibit the ATPase of GroEL and fully supported GroEL in refolding citrate synthase. However, the mutation increased the GroES-mediated enhancement of cooperativity in ATP hydrolysis by GroEL.

**Implications for the Role of cpn10 in Chaperonin-Mediated Protein Folding.** Recent studies on the chaperonin cycle indicate that substrate proteins are productively released from a cis ternary complex in which cpn10 and substrate are bound to the same ring of cpn60 (46, 47). Weissman et al. (47) have suggested that ATP hydrolysis by cpn60 acts as a timer for the release of substrate protein and cpn10 from such a complex. Based on our results, we propose that cpn10 modulates the rate of ATP hydrolysis by cpn60 thereby timing substrate release. According to this model, the inability of hsp10(P36H) to assist folding correlates with its inability to inhibit the ATPase of hsp60 (Fig. 4). Although binding of hsp10(P36H) to hsp60 closes the cavity, the co-chaperonin fails to act as a negative effector on the opposite ring (38). ATP hydrolysis in this ring continues at its uninhibited rate, and only a small fraction of mMDH reaches the native state during one cycle. As a result, a larger fraction of unfolded substrate protein will be able to escape into the bulk solution (48). Because folding of mMDH depends on rebinding to hsp60, an increased number of release events will increase the chance for aggregation. In agreement with this hypothesis we find that the P36H mutation decreases not only the rate but also the yield of mMDH refolding. The model implies further that an average time of productive folding is optimal for most, if not all, authentic substrate proteins, a hypothesis previously suggested by others (22, 47). It is also consistent with the finding that GroEL/ES-mediated folding can occur with a slow rate in the presence of ADP. Under these conditions GroES dissociates from GroEL with a half-time of 8–10 min (22). It might also account for our observation that the defect of hsp10(P36H) is more pronounced at higher temperatures: The rate of ATP hydrolysis by hsp60 increases with temperature (data not shown), and the time for productive folding inside the hsp60 cavity will thus be shorter. In addition to its inability to time ATP hydrolysis, hsp10(P36H) might also fail to induce other allosteric transitions in hsp60. This defect could reduce the enlargement of the closed cavity and possibly the release of substrate protein bound to the walls of cpn60 into the lumen of the cavity. Determination of the hsp60/hsp10(P36H) structure (e.g., by cryo-electron microscopy) should shed light on this aspect and its relation to the function of chaperonins in the folding of proteins.

The program FACSIMILE was kindly provided by Drs. S. G. Burston and A. R. Clarke. We are indebted to Prof. G. Schatz for critical comments and his generous help and support throughout the project. We further thank Dr. R. Sterner and Dr. A. Matouschek for help with the fitting procedures and all the members of the Schatz laboratory for critical reading of the manuscript. This study was supported by Grant 31–40510.94 from the Swiss National Science Foundation.
