Prevention of in Vitro Protein Thermal Aggregation by the Sulfolobus solfataricus Chaperonin

EVIDENCE FOR NONEQUIVALENT BINDING SURFACES ON THE CHAPERONIN MOLECULE*

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We have studied the effects of the Sulfolobus solfataricus chaperonin on the aggregation and inactivation upon heating of four model enzymes: chicken egg white lysozyme (one 14.4-kDa chain), yeast α-glucosidase (one 68.5-kDa chain), chicken liver malic enzyme (four 65-kDa subunits), and yeast alcohol dehydrogenase (four 37.5-kDa subunits). When the proteins were heated in the presence of an equimolar amount of chaperonin, 1) the aggregation was prevented in all solutions; 2) the inactivation profiles of the single-chain enzymes were comparable with those detected in the absence of the chaperonin, and enzyme activities were regained in the solutions heated in the presence of the chaperonin upon ATP hydrolysis (78 and 55% activity regains for lysozyme and α-glucosidase, respectively); 3) the inactivation of the tetrameric enzymes was completely prevented, whereas the activities decreased in the absence of the chaperonin. We demonstrate by gel filtration chromatography that the chaperonin interacted with the structures occurring during thermal denaturation of the model proteins and that the interaction with the single-chain proteins (but not that with the tetrameric proteins) was reversed upon ATP hydrolysis. The chaperonin had nonequivalent surfaces for the binding of the model proteins upon heating: the thermal denaturation intermediates of the single-chain proteins share Surfaces I, while the thermal denaturation intermediates of the tetrameric proteins share Surfaces II. ATP binding to the chaperonin induced a conformation that lacked Surfaces I and carried Surfaces II. These data support the concept that chaperonins protect native proteins against thermal aggregation by two mechanistically distinct strategies (an ATP-dependent strategy and an ATP-independent strategy), and provide the first evidence that a chaperonin molecule bears functionally specialized surfaces for the binding of the protein substrates.

The term “chaperonins” denotes a family of ubiquitous, essential proteins endowed with ATPase activity that are involved in the folding, transport, and assembly of newly synthesized proteins (for reviews, see Ellis and van der Vies (1991), Ellis et al. (1992), and Horwich and Willison (1993)).

Eubacterial chaperonins are structurally and functionally closely related to eukaryotic intraorganellar chaperonins. They have similar amino acid sequences and consist of two stacked homo-oligomeric rings of seven subunits of ~60 kDa each that delimitate a central cavity; sequence-related co-chaperonins, consisting of a single homo-oligomeric ring of seven subunits of ~10 kDa each, have been isolated together with these chaperonins. The crystal structure determination of GroEL (the Escherichia coli chaperonin) and a mutational analysis revealed three domains in each subunit. The apical domain faces the central cavity, and its flexible regions are involved in the binding of the polypeptide substrate and the co-chaperonin GroES. The equatorial domain provides most of the intratoroidal side-to-side contacts and all of the ring-to-ring contacts and contains the ATP-binding site; the domain intermediate between the two other probably allows allosteric domain movement (Braig et al., 1994; Fenton et al., 1994; Hartl et al., 1994). GroEL promotes correct folding by binding to non-native structures that are folding intermediates, thus preventing the aggregation of hydrophobic surfaces, and coupling their release in a correctly folded form to the binding and/or hydrolysis of ATP; in some, but not all, cases the formation of native proteins requires the presence of GroES.

Trent et al. (1991) reported that the chaperonin of the thermophilic archaeon Sulfolobus shibatae shows no significant relationship to GroEL-like chaperonins but has a 40% sequence identity with the chaperonins of the eukaryotic cytosol (named CCTs), reflecting the evolutive lineage of their sources. Archaeal chaperonins and CCTs do not have co-chaperonin equivalents; they show the typical double-ring structure with an 8- or 9-fold symmetry and a homo- or hetero-oligomeric structure. CCTs have a domain arrangement similar to that of GroEL; analysis of sequence relatedness to GroEL revealed a high level of identity in the regions corresponding to the ATP binding site, but no significant identity in the region corresponding to the polypeptide binding site (Kim et al., 1994; Kubota et al., 1994). In a functional regard, CCTs promote correct protein folding by an ATP-dependent mechanism similar to that exerted by GroEL but without the requirement for a co-chaperonin component (Frydman et al., 1992; Gao et al., 1992; Melki and Cowan, 1994).

The chaperonin of the thermophilic archaeon S. solfataricus (Ssco),1 has a 9-fold symmetry (Knap et al., 1994; Marco et al., 1994) and two subunit species that differ in size and N-terminal sequences (Knap et al., 1994), or one subunit of ~60 kDa (Guagliardi et al., 1994; Marco et al., 1994). Ssco displays a K+-dependent ATPase activity and promotes correct refolding of several thermophilic and mesophilic proteins in an

1 The abbreviations used are: Ssco, S. solfataricus chaperonin; ME, malic enzyme; ADH, alcohol dehydrogenase; PAGE, polyacrylamide gel electrophoresis.

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ATP hydrolysis-dependent manner (Guagliardi et al., 1994). In an effort to enlarge our knowledge about archaeal chaperonins, we investigated the effects of Ssocpn on the aggregation and inactivation upon heating of four model enzymes: chicken egg white lysozyme (a monomer of 14.4 kDa), yeast α-glucosidase (a monomer of 68.5 kDa), chicken liver malic enzyme (a homotetramer of 37.5 kDa subunits), and yeast alcohol dehydrogenase (a homodimer of 65 kDa subunits). This study provides, for the first time, biochemical evidence that a chaperonin molecule possesses nonequivalent surfaces for the binding of the protein substrates.

**EXPERIMENTAL PROCEDURES**

**Materials—**ATP, NAD, NADP, ME from chicken liver (29 units/mg), ADH from yeast (200 units/mg), and lysozyme from chicken egg white (183 units/mg) were purchased from Sigma; α-glucosidase from yeast (133 units/mg) and p-nitrophenyl-α-β-maltoside were from Boehringer Mannheim. The other chemicals were of the highest grade available.

**Miscellaneous—**Protein concentration was determined by the assay of Bradford (1976) using bovine serum albumin as the standard. SDS-PAGE analysis was carried out according to Laemmli (1970) after the run, the proteins were revealed by Coomassie staining. Protein aggregation was monitored as turbidity at 450 nm; the maximal turbidity was taken as 100% aggregation. Molar concentrations were calculated on the basis of the following molecular mass values: 920 kDa for Ssocpn; 68.5 kDa for lysozyme; 65 kDa for a-glucosidase; 260 kDa for ME; 150 kDa for ADH.

**Enzymatic Assays—**The enzymes were assayed at 25 °C (lysozyme, ME, and ADH) or at 30 °C (α-glucosidase) by a Cary 1E Varian recording spectrophotometer equipped with a thermostated cell compartment. Absorbance variations were always linear within 2 min; each activity assay was performed in duplicate. The assay mixture for chicken egg white lysozyme consisted of 1 ml of a fresh suspension 0.1 mg/ml of lyophilized E. coli cells in 50 mM Tris-HCl buffer, pH 7.4. After the addition of lysozyme, the time required for an absorbance decrease of A_{\text{350 nm}} = 0.1 was measured; the specific activity of the enzyme was calculated by the formula: specific activity = (absorbance decrease/1.44) (1 mg/ml protein) × (mg/ml protein) × (1 mg/ml protein)/1.44 (mg/ml protein). The enzyme activity was calculated from the continuous increase of absorbance at 400 nm was monitored, and the extinction coefficient for p-nitrophenol was 9.6 mM. Chicken liver ME was assayed in 20 mM Tris-HCl buffer, pH 7.5, 0.05 mM NADP, 1 mM MgCl_2, 1 mM L-malate (1 ml final volume). Yeast ADH was assayed in 50 mM sodium phosphate buffer, pH 8.0, 2 mM NAD, 1 mM ethanol (1 ml final volume).

**Purification of the Chaperonin—**The chaperonin was purified from crude extracts of S. solfataricus strain MT-4 as described previously (Guagliardi et al., 1994). Briefly, the crude extract (20 mg) was dialyzed against 10 mM Tris-HCl buffer, pH 8.4 (Buffer A) and loaded onto a Superose 6 column (Pharmacia Biotech Inc., 2.6 x 30 cm) eluted with 1 ml of the same buffer supplemented with 0.1 mM NaCl at a flow rate of 0.5 ml/min; the fractions containing the chaperonin were pooled, concentrated by a vacuum centrifuge, and dialyzed against Buffer A. The sample (1.4 mg) was loaded onto a Matrex Gel Red A affinity chromatography column (Amicon, 1 x 5 cm) equilibrated in Buffer A and eluted with a linear 0–0.4 M NaCl gradient in Buffer A at a flow rate of 0.5 ml/min; the fractions containing the chaperonin were pooled, dialyzed against Buffer A, and stored at 4 °C (1 mg). SDS-PAGE analysis revealed one band at 57 kDa, which showed the preparation was homogeneous.

**RESULTS**

Effects of Ssocpn on the Thermal Aggregations and Inactivations of Lysosome and α-Glucosidase—Temperatures and protein concentrations were chosen as to obtain the aggregation of chicken egg white lysozyme and yeast α-glucosidase. Light scattering measurements showed that aggregation occurred in solutions of 80 μg/ml lysozyme and 200 μg/ml α-glucosidase upon heating at 70 and 40 °C, respectively (Fig. 1). The addi-
tion of excess Ssocpn to the solutions after they had precipitated did not exert any effect. When identical solutions of lysozyme and α-glucosidase were heated in the presence of Ssocpn at an equimolar ratio between the chaperonin oligomer and the polypeptide chain, light scattering did not increase. The effect of Ssocpn in preventing thermal aggregation of lysozyme and α-glucosidase was specific; the presence of an excess of bovine serum albumin in the place of Ssocpn in the solutions did not prevent the occurrence of precipitates. Following the addition of ATP/Mg/K (0.5 mM ATP, 0.5 mM MgCl₂, 10 mM KCl), light scattering increased in the solutions of lysozyme and α-glucosidase heated in the presence of Ssocpn.

In a reasonable interpretation of the results, Ssocpn did not rescue the protein aggregates, but rather it interacted with lysozyme or α-glucosidase before their precipitation and so maintained them in solution. The hydrophobic surfaces of Ssocpn contributed to the binding, as demonstrated by the finding that aggregation occurred as in the absence of Ssocpn if the solutions of lysozyme and α-glucosidase contained the chaperonin plus 0.1% Triton X-100. The addition of ATP/Mg/K discharged the proteins that proceeded to aggregate at the temperatures of the incubation. No aggregation was detected if ATP/Mg (0.5 mM ATP, 0.5 mM MgCl₂) was added instead of ATP/Mg/K; that is, because Ssocpn displays a K⁺-dependent ATPase activity (Guagliardi et al., 1994), ATP hydrolysis was required to disrupt the binding between Ssocpn and the protein chain. Lysozyme and α-glucosidase discharged from Ssocpn after ATP hydrolysis reaggregated, even though the test tube contained Ssocpn; in other words, under the experimental conditions described, Ssocpn was not viable for rebinding the denaturation intermediates. We are currently investigating the recycling of Ssocpn by studying the effects of ATP hydrolysis on its conformation, and the chaperonin affinity toward the target protein, potassium ions, and ATP during in vitro functioning.

We determined the time courses of inactivations of lysozyme and α-glucosidase at the designated temperatures in the absence and in the presence of Ssocpn oligomer at an equimolar ratio with the protein chain (Fig. 2). Ssocpn did not modify the kinetics of inactivation; that is, Ssocpn did not prevent the loss of activity of the two enzymes. Following the addition of ATP/Mg/K (but not of ATP/Mg), final activity regains of 78 and 55% were obtained in the solutions of lysozyme and α-glucosidase heated in the presence of Ssocpn. Lysozyme and α-glucosidase activities decreased when the incubations were continued after the addition of ATP/Mg (not reported in the figure), which is in accordance with the intrinsic heat lability of the native enzymes. These results show that the molecules remained in a folding-competent conformation while bound to the chaperonin and that correctly folded molecules were released in solution upon ATP hydrolysis.

The final percents of active lysozyme or α-glucosidase did not vary when the chaperonin was present in the solutions at a molar excess over the protein chain or when a molar ratio of 1:2 between Ssocpn and the protein chain was used. At a molar ratio of Ssocpn to protein chain of 1:10, lysozyme activity was restored by 37% and α-glucosidase activity by 20%.

Effects of Ssocpn on the Thermal Aggregations and Inactivations of Malic Enzyme and Alcohol Dehydrogenase—We chose conditions of temperature and concentrations of chicken liver ME or yeast ADH that ensured their precipitation. Solutions of 6 μg/ml ME and 6 μg/ml ADH promptly aggregated upon heating at 50 °C (Fig. 3). Ssocpn was without effect if added

![Graph showing thermal inactivations of chicken egg white lysozyme (upper panels) and yeast α-glucosidase (lower panels) in the absence (open symbols) and in the presence (closed symbols) of Ssocpn.](http://www.jbc.org/)

Lysozyme and α-glucosidase were heated exactly as described in the legend to Fig. 1. At the times defined, the enzyme activity was assayed as described under "Experimental Procedures" on aliquots drawn from each mixture; the activity regains were calculated as percentages with respect to the specific activities of the native enzymes. The scale of the x axis changed at the time indicated by the arrow.
when the proteins had aggregated. Aggregation was completely suppressed when Ssocpn was present in the solutions from the beginning of the heating in a molar ratio of one chaperonin oligomer to one ME or ADH molecule. The presence of excess bovine serum albumin in place of Ssocpn did not prevent the increases in light scattering. The addition of ATP/Mg/K or ATP/Mg in the solutions of ME and ADH heated in the presence of Ssocpn did not exert any effect.

These results suggest that Ssocpn interacted with ME and ADH and prevented their precipitation upon heating; the hydrophobic nature of the interaction was demonstrated by the fact that 0.1% Triton X-100 in the solutions of ME and ADH containing Ssocpn abolished the protection against aggregation. The bound molecules were not released upon ATP binding or hydrolysis.

During heating, ME and ADH very rapidly lost their activity in the absence of Ssocpn but retained total activity in the presence of the chaperonin; the addition of ATP/Mg/K did not exert any effect (Fig. 4). We verified that the addition of Ssocpn to native solutions of ME and ADH did not affect their specific activities. In conclusion, the binding of ME or ADH to Ssocpn during heating did not cause any perturbation of the enzyme active sites, and the active conformations of the two enzymes were preserved.

No inactivation at all was detected when Ssocpn was incubated with ME or ADH at a molar ratio of 1:10; when Ssocpn and ME or ADH were heated at a molar ratio of 1:20, residual ME and ADH activities of about 30% were calculated after 30 min.

Interaction of Ssocpn with the Model Proteins—Fig. 5A shows that the Ssocpn-mediated suppression of protein thermal aggregation involved the interaction of the chaperonin with the protein molecule (see legend for experimental details). The solutions were heated and then injected onto a Superose 6 column; only one peak eluted from the column with the void volume. SDS-PAGE analysis revealed the presence of two molecular species in the peak: Ssocpn and the protein used as substrate. A peak of activity was superimposable to that of absorption at 280 nm in the run that showed the interaction of Ssocpn with ME or ADH; no activity was found in the peak of the run that showed the interaction of Ssocpn with lysozyme or a-glucosidase.

Exploiting the same experimental tool, we demonstrate that 1) the interaction of Ssocpn with the single-chain proteins was reversed upon ATP hydrolysis, free lysozyme, or a-glucosidase being catalytically active and 2) the interaction of Ssocpn with the tetrameric proteins was unaffected by ATP hydrolysis (Fig. 5B).

We demonstrate that Ssocpn does not interact with the native conformations of the model proteins (Fig. 5C). The chromatography on Superose 6 of nonheated solutions separated the protein used (eluted in an active form in a position corresponding to its native molecular weight) from Ssocpn (eluted in the void volume); SDS-PAGE analysis of the first eluting peak showed only the band corresponding to the Ssocpn subunit (not shown). Therefore, Ssocpn prevents the thermal aggregation of the model proteins by coating with its hydrophobic surfaces the interactive hydrophobic patches exposed on the intermediates of the thermal denaturation process.

Binding Surfaces on Ssocpn for the Model Proteins—The results described above led to the discovery of two different strategies by which Ssocpn prevents the thermal aggregation of
the model proteins: an ATP hydrolysis-dependent strategy common to the single-chain enzymes and an ATP-independent strategy common to the tetrameric enzymes. This prompted the question: do the molecules that share the same strategy, share the same binding surfaces on the chaperonin?

To ascertain whether lysozyme and \( \alpha \)-glucosidase have the same binding surfaces, we saturated Ssocpn with the denaturation intermediate of \( \alpha \)-glucosidase and tested if this chaperonin could prevent the thermal aggregation of lysozyme. Ssocpn and \( \alpha \)-glucosidase in a molar ratio of 1:50 were heated at 40°C for 2 h; an ultracentrifugation at 50,000 rpm for 1 h pelleted visible aggregates. Gel filtration of the supernatant on Superose 6 column (1 × 48 cm; eluent 10 mM Tris-HCl, pH 8.4, 0.2 M NaCl; flow rate of 12 ml/h at 4°C) yielded the Ssocpn/\( \alpha \)-glucosidase complex (as judged by SDS-PAGE analysis) in the void volume. We verified that all Ssocpn molecules in the peak were saturated with \( \alpha \)-glucosidase. In fact, the inclusion of a protein excess from the peak in a solution of \( \alpha \)-glucosidase heated at 40°C did not prevent the occurrence of aggregates (no aggregation was detected when only the aliquot from the peak was heated).

Ssocpn saturated with \( \alpha \)-glucosidase (briefly Ssocpn/\( \alpha \)-glucosidase) was included in a solution of lysozyme in a protein excess over the lysozyme molecule, and the light scattering was continuously monitored during heating at 70°C, a light scattering increase similar to that obtained when lysozyme was incubated in the absence of the chaperonin was recorded (Ssocpn/\( \alpha \)-glucosidase alone did not aggregate over the time of the heating under the same conditions). This result means that the thermal denaturation intermediate of lysozyme did not find surfaces available for the binding to Ssocpn/\( \alpha \)-glucosidase. In a control experiment, we included in the solution of lysozyme to be heated an Ssocpn/\( \alpha \)-glucosidase complex, which was prepared at a molar ratio of 1:0.5 between the chaperonin and the \( \alpha \)-glucosidase chain and was subjected to the same experimental protocol: in this case, lysozyme aggregation was completely suppressed.

By the same strategy, we demonstrated that the denaturation intermediates of ME and ADH have the same binding surfaces on Ssocpn. We prepared a chaperonin saturated with the thermal denaturation intermediate of ME by heating at 50°C for 1 h a solution in which the molar ratio between Ssocpn and the substrate molecule was 1:80. The aggregates were removed by ultracentrifugation, and the saturated form of Ssocpn (Ssocpn/ME unable to prevent the aggregation of free ME) was recovered in the peak eluting with the void volume from the Superose column loaded with the supernatant (see above for details). Ssocpn/ME was unable to prevent the aggregation at 50°C of a solution of ADH; vice versa, an Ssocpn-ME complex, which was prepared at a molar ratio of 1:1 and was subjected to the same experimental protocol as that used to prepare Ssocpn/ME, suppressed ADH aggregation.

Surprisingly, when Ssocpn/ME was included in solutions of lysozyme and \( \alpha \)-glucosidase and the solutions were heated at 50°C, no aggregations were detected; active molecules were regained upon the addition of ATP/Mg/K. When Ssocpn/\( \alpha \)-glucosidase was included in solutions of ME and ADH and the solutions were heated at 50°C, aggregations and inactivations were prevented. Since no artifact seemed responsible for these results, Ssocpn has surfaces specific for the binding of the denaturation intermediates of the single-chain proteins (Surfaces I), which are not saturable by the
thermaldenaturationintermediatesofthetetramericproteins; other surfaces specific for the binding of the denaturation in-
termediates of the tetrameric proteins (Surfaces II) are not
saturable by the thermal denaturation intermediates of the
single-chain proteins. Consequently, it appears that Ssocpn
has nonequivalent surfaces for the binding of the model pro-
tein during their heating.

FIG. 5. A, solutions (0.3 ml) of lysozyme (10 μg), α-glucosidase (20 μg), ME (30 μg) and ADH (30 μg) containing Ssocpn at an equimolar ratio with each protein molecule were heated at the designated temperatures; after a 2-h incubation, each solution was chromatographed on a Superose 6 column (1 × 48 cm; flow rate of 12 ml/h at 4 °C; eluent 10 mM Tris-HCl, pH 8.4, 0.2 M NaCl). Left, the chromatographic profiles relative to the runs:

- Enzymatic activity associated with the peak
- No activity associated with the peak

thermal denaturation intermediates of the tetrameric proteins;
other surfaces specific for the binding of the denaturation in-
termediates of the tetrameric proteins (Surfaces II) are not
saturable by the thermal denaturation intermediates of the
single-chain proteins. Consequently, it appears that Ssocpn
has nonequivalent surfaces for the binding of the model pro-
teins during their heating.

DISCUSSION

Some native proteins form visible aggregates when subjected
to heating, as a consequence of hydrophobic intermolecular
interactions; protein thermal aggregation is responsible for an
irreversible loss of biological activity. We studied the effects of
the chaperonin from the archaean S. solfataricus, Ssocpn, on
the in vitro thermal aggregation of two single-chain enzymes
of 14.4 and 68.5 kDa and two tetrameric enzymes of 260 and
150 kDa.

Like other chaperons, Ssocpn does not rescue proteins once
they aggregate and does not bind the native molecules. Ssocpn
prevents protein aggregation by interacting with the hydropho-
bic surfaces of the thermal denaturation intermediates of the
model proteins, thus suppressing the intermolecular interac-
tions that lead to aggregation. Because the tetrameric proteins
retain their enzymatic activity upon binding to Ssocpn, the
structure of the intermediate bound by Ssocpn is probably very
similar to that of the native molecule. Ssocpn does not prevent
the thermal denaturation of the single-chain enzymes that
leads to their loss of activity, rather these enzymes are kept in
a form that is refoldable upon ATP hydrolysis. As demon-
strated for GroEL (Jackson et al., 1993; Hartl, 1994; Todd et al.,
1994; Weissman et al., 1994), the binding and hydrolysis of
ATP could trigger cycles of release from and rebinding to
Ssocpn of the molecules until they have reached a correctly
folded form.

The binding of the model proteins to Ssocpn during heating
is not casual in the sense that the chaperonin surfaces are not
indifferently available for the binding of any molecule; thermal
denaturation intermediates of the single-chain proteins share
Surfaces I, while thermal denaturation intermediates of the
tetrameric proteins share Surfaces II.

There is evidence that the binding of ATP to Ssocpn drives a
conformational change in the overall structure of the chapero-
nin (Guagliardi et al., 1994; Knapp et al., 1994). To gain insight
into the functional consequence of this change, we heated ly-
sozyme, α-glucosidase, ME, and ADH in the presence of the
ATP-induced form of Ssocpn and found that only the aggrega-
tions of the tetrameric enzymes were suppressed, not the ag-
gregations of the single-chain enzymes, which proceeded as in
the absence of the chaperonin (not shown). Therefore, the ATP-
induced form of Ssocpn does not bind the thermal denaturation intermediates of the single-chain enzymes, but it is still able to bind the thermal denaturation intermediates of the tetrameric enzymes. This finding implies that ATP binding to Ssocpn leads to a disappearance or modification of Surfaces I such that the interaction with the proper molecules is prevented; Surfaces II are probably less (or not at all) affected by the structural rearrangement. It is noteworthy that the ATP-induced form of Ssocpn is unable to bind the refolding intermediates of several chemically denatured proteins (Guagliardi et al., 1994); we are currently investigating whether the flexible Surfaces I are implicated also in the binding of the interactive structures occurring during folding.

The domain organization of archaeal chaperonins is unknown. We suspect that Surfaces I could correspond to the flexible regions of the apical domain in GroEL involved in the binding of polypeptide substrate: these regions undergo a strong motion upon ATP-binding (Chen et al., 1994), and there is compelling evidence in the literature that the ATP-bound form of GroEL has a low affinity for unfolded protein substrates. It is unlikely that Surfaces II are located in the same region. The absence of any functional interference between the two Surfaces could imply that they are not in close vicinity on the chaperonin cylinder, and surfaces suited to bind molecules that exert catalytic activity could be surfaces exposed to the milieu; electron microscopy showed that the external envelope of GroEL binds the protein molecules (Azem et al., 1994). Our data show that Surfaces I and Surfaces II can bind more than one polypeptide chain during heating, which is consistent with the idea that chaperonins have multiple substrate binding sites. How many binding sites there are on Surfaces I and Surfaces II remains an open question, although it seems that the latter surfaces bind a higher number of substrate molecules than the former ones.

The activity of GroEL in preventing the thermal aggregation of native proteins has been poorly investigated in comparison with the activities in preventing the aggregation during refolding of unfolded proteins. Upon heating in the presence of GroEL, native α-glucosidase (Holl-Neugebauer et al., 1991), rhodanese (Mendoza et al., 1992), dihydrofolate reductase (Martin et al., 1992), and malate dehydrogenase (Hartman et al., 1993) are protected against aggregation but not against inactivation, and active molecules are released in solution upon ATP hydrolysis. In an ATP-independent manner, GroEL keeps RNA polymerase active (Ziemienowicz et al., 1993), interacts with a conformation of 6-hydroxy-2-oxo-3-pyridinone oxidase close to the native state (Brandsch et al., 1992) and with a form of rhodanese that is active (Mendoza et al., 1992).

Taken together, the aforementioned results about GroEL and the results of this study strongly suggest that chaperonins protect the native proteins against aggregative damage by two mechanistically distinct strategies. The cellular concentration of free ATP, which is high under physiological conditions and dramatically decreases upon different kinds of stress, may reflect the biological significance of the in vitro ATP-dependent strategy by which chaperonins prevent aggregation; thermal aggregation of proteins increases in ATP-depleted cells (Nguyen and Bensaude, 1994). The proteins that interact with the chaperonins in an ATP-independent fashion and in a conformation close to the native state probably undergo their cellular turnovers while bound to the chaperonin; active Ssocpn-bound ME and ADH lose their activity following trypsin digestion, and the inactivation profiles are identical to those of the free native molecules at the same protein concentrations (data not shown). In this scenario, the chaperonin-bound protein has the life-span of the free protein, which devised a strategy to avoid a sticky situation. The role of “hydrophobic support” played by the chaperonins in the cell is well suited to proteins that are very abundant also under physiological conditions, and, in terms of cell utility, one molecule that plays different roles via nonequivalent surfaces seems very advantageous.

Finally, we would like to underline an aspect of the research on chaperonins from thermophilic Archaea. The protein named Hsp70 in Eukarya and DnaK in Eubacteria plays a central role in the heat shock response of these cells as a molecular chaperone involved in protein folding and transport; in E. coli, DnaK and related proteins cooperate with GroEL in a common pathway to facilitate protein folding (Gragerov et al., 1992; Hartl et al., 1994). Thermophilic Archaea do not possess an equivalent to DnaK, and chaperonins are the main proteins expressed under conditions of heat shock (Conway de Macario and Macario, 1994). Investigating the activity of archaeal chaperonins might strengthen the hypothesis that in Archaea chaperonins fulfill the functions of 70 kDa-chaperones in Eubacteria and Eukarya.

REFERENCES

Azem, A., Kessel, M., and Goloubinoff, P. (1994) Science 265, 653–656
Braford, M. M. (1976) Anal. Biochem. 72, 249–254
Braig, K., Otwinowski, Z., Hegde, R., Bolswert, D. C., Jachimick, A., Horwich, A. L., and Sigler, P. B. (1994) Nature 371, 578–586
Brandsch, R., Bichler, V., Schmidt, M., and Buchner, J. (1992) J. Biol. Chem. 267, 20844–20849
Chen, S., Roseman, A. M., Hunter, A. S., Woo, S. P., Burston, S. G., Ranson, N. C., Clarke, A. R., and Saibl, H. R. (1994) Nature 371, 261–264
Conway de Macario, E., and Macario, A. J. L. (1994) Trends Biotech. 12, 512–518
Ellis, R. J., and van der Vies, S. M. (1991) Annu. Rev. Biochem. 60, 321–347
Fenton, W. A., Kashi, Y., Furtak, K., and Horwich, A. L. (1994) Nature 371, 590–599
Frydman, J., Nimmegsner, E., Erdjument-Bromage, H., Wall, J. S., Tempst, P., and Hartl, F. U. (1992) EMBO J. 11, 4767–4778
Gao, Y., Thomas, J. O., Chow, R. L., Lee, G., and Cowan, N. J. (1992) Protein Sci. 1, 1043–1050
Gragerov, A., Nucler, E., Komissarova, N., Gatanian, A. G., Gottesman, M. E., and Nikiforov, V. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10341–10344
Guagliardi, A., Cortelezzi, E., Bartolucci, S., and Resi, M. (1994) Protein Sci. 3, 1436–1443
Halverson, H. (1996) Methods Enzymol. 268, 359–562
Hartl, F. U. (1994) Nature 371, 557–559
Hartl, F. U., Martin, J. N., and Neupert, W. (1992) Annu. Rev. Biophys. Biomol. Struct. 21, 293–322
Hartl, F. U., Hixidan, R., and Langer, T. (1994) Trends Biochem. Sci. 19, 20–25
Hartman, D. J., Surin, B. P., Dixon, N. E., Hoogenaad, N. J., and Hoj, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2276–2280
Holl-Neugebauer, B., Rudolph, R., Schmidt, M., and Buchner, J. (1991) Biochem. J. 270, 1109–1114
Horwich, A. L., and Willison, K. R. (1993) Phil. Trans. R. Soc. Lond. 339, 313–326
Jackson, G. S., Staniforth, R. A., Halsall, D. J., Atkinson, T., Holbrock, J. J., Clarke, A. R., and Burdon, S. G. (1993) Biochimica et Biophysica Acta 1160, 685–687
Kim, S., Willison, K. R., and Horwich, A. L. (1994) Trends Biochem. Sci. 19, 543–548
Knap, S., Schmidt-Krey, I., Hebert, H., Berman, T., Jornvall, H., and Ladenstein, R. (1994) J. Biol. Chem. 269, 392–407
Kubota, H., Hynes, G., Carne, A., Ashworth, A., and Willison, K. (1994) Current Biol. 4, 89–99
Laemmli, U. K. (1970) Nature 227, 680–685
Marcot, S., Urena, D., Carassoca, J. L., Waldmann, T., Peters, J., Hegeli, R., Pfeifer, G., Sack-Konehl, H., and Baumeister, W. (1994) FEBS Lett. 341, 152–155
Martin, J. H., Horwich, A. L., and Hartl, F. U. (1992) Science 256, 995–998
Mekel, R., and Cowan, N. J. (1994) Mol. Cell. Biol. 14, 2895–2904
Mendoza, J. A., Lorimer, G. H., and Horowitz, P. M. (1992) J. Biol. Chem. 267, 17631–17634
Nguyen, V. T., and Bensaude, O. (1994) Eur. J. Biochem. 220, 239–246
Todd, M. J., Viitanen, P. V., and Lorimer, G. H. (1994) Science 265, 659–666
Trent, J. D., Nimmesgern, E., Wall, J. S., Hartl, F. U., and Horwich, A. L. (1991) Nature 354, 490–493
Tsugita, A., Inouye, M., Terazaki, E., and Streisinger, G. (1986). Nature 323, 391–397
Weissman, J. S., Kashi, Y., Fenton, W. A., and Horwich, A. L. (1994) Cell 78, 693–702
Ziemienowicz, A., Skowrya, D., Zielstra-Ruyljas, J., Faylor, O., Georgopoulos, C., and Zylicz, M. (1993) J. Biol. Chem. 268, 25425–25431
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