Conformational Cycle of the Archaeosome, a TCP1-like Chaperonin from Sulfolobus shibatae*

Elise Quaite-Randall, Jonathan D. Trent, Robert Josephs‡, and Andrzej Joachimiak§

From the Argonne National Laboratory, Argonne, Illinois 60439 and the University of Chicago, Chicago, Illinois 60637

The major heat shock proteins in the archaeon Sulfolobus shibatae are similar to the cytosolic eukaryotic chaperonin and form an 18-subunit bitoroidal complex. Two sequence-related subunits constitute a functional complex, named the archaeosome. The archaeosome exists in two distinct conformational states that are part of chaperonin functional cycle. The closed archaeosome complex binds ATP and forms an open complex. Upon ATP hydrolysis, the open complex dissociates into subunits. Free subunits reassemble into a two-ring structure. The equilibrium between the complexes and free subunits is affected by ATP and temperature. Denatured proteins associate with both conformational states as well as with free subunits that form an intermediate complex. These unexpected observations suggest a new mechanism of archaeosome-mediated thermostolerance and protein folding.

Molecular chaperonins assist protein folding in the cell, during which the chaperonin recognizes and binds the unfolded protein substrate (1–3). The bound protein assumes a molten globule-like state and changes conformation in a poorly understood process, resulting in the formation of its native state (4–9). Recently published x-ray structure and mutational analysis of the bacterial tetradecameric GroEL, protein from Escherichia coli suggest that chaperonin's principal role is to provide an interactive surface for unfolded proteins and their cofactors (10, 11). The interaction with this surface presumably limits the possible polypeptide conformations to those that are committed to fold into the native structure. The mechanism by which this is achieved and the exact role of chaperonin is the subject of extensive study (12). However, it is generally accepted that in the process of folding, both protein and chaperonin undergo major conformational changes (3, 4, 7–9, 13–15).

Much less is known about protein folding mediated by the TCP1/TRiC family of chaperonins, which includes the cytosolic chaperonins of eukaryota and archaea. As many as nine different subunits of eukaryotic TCP1 protein can be associated in a double-ring structure (12, 16). Assisting protein factors, analogous to GroES, have been identified, but their role in protein folding remains unclear (17). Archaeal chaperonins share a high sequence similarity with eukaryotic TCP1 chaperonins, but they are composed of two subunits (18–20). These chaperonins also form double-ring structures with 8/2 or 9/2 symmetry (18–21). Archaeal chaperonins have ATPase activity, recognize and bind unfolded proteins (22, 23), and can contribute to the thermostability and folding of enzymes (22). Furthermore, chaperonins isolated from hyperthermophilic organisms show remarkable chemical and thermal stability (18, 22, 23).

We report here that the major heat shock protein in S. shibatae (called also TFSS (23) and rosettasome (1a)), unlike the bacterial GroEL, exists in two distinct, stable conformational states (closed and open) that appear to be part of the protein folding pathway. This protein will be referred to here as the "archaeosome." The closed archaeosome complex binds ATP and forms an open complex, which upon ATP hydrolysis specifically dissociates into subunits. Free subunits reassemble to an 18-subunit bitoroidal complex and complete the cycle. The equilibrium between complex and subunits is affected by ATP and temperature. Heat-denatured proteins associate with both conformational states. The subunits also bind denatured proteins and form an intermediate complex.

MATERIALS AND METHODS

Reagents and Proteins—Dephosphorylated bovine α-S1-casein, alcohol oxidase from Hansenula sp., ATP, ADP, trifluoroethanol (TFE), and spectrally pure sodium phosphate were purchased from Sigma, human casein kinase II was from Boehringer Mannheim, and [γ-32P]ATP (3000 Ci/mmol) was from DuPont. All other reagents were of analytical grade.

Dephosphorylated bovine α-S1-casein and pure α and β subunits of the archaeosome were labeled with [32P] using [γ-32P]ATP and human casein kinase II. Excess of proteins over [γ-32P]ATP were used to ensure a single labeling event per polypeptide chain. Human casein kinase II was activated by heat and [32P]-labeled proteins were purified from unreacted [γ-32P]ATP on cellulose GF5 gel filtration columns from Pierce. The specific radioactivity of radiolabeled proteins was determined by liquid scintillation counting and UV spectroscopy. Protein concentration was determined by spectrophotometry using calculated extinction coefficients. Molar extinction coefficients of chaperonin and its subunits were calculated from the protein sequence using the method of Gill and von Hippel (24) and were 9.08 × 10^4 M^−1 cm^−1 for 278 nm for the α subunit, 3.26 × 10^4 M^−1 cm^−1 for the β subunit at 280 nm, and 3.75 × 10^4 M^−1 cm^−1 for the αβ archaeosome at 280 nm.

Purification of archaeosome and glutamate dehydrogenase from Sulfolobus shibatae was done using the following procedure. S. shibatae DSM strain 5389 was grown at 80 °C in liquid medium (containing yeast extract and Brox's salts) in a 160-liter fermenter as described previously (23). Cells were lysed by raising the pH to 7.5 with 0.1 M NaOH in the presence of 0.25% Triton X-100 (v/v) in 50 mM Tris/HC1, pH 7.5, 10 mM 2-mercaptoethanol, and 1 mM EDTA. Soluble crude extract was chromatographed on a FastQ FPLC column, and proteins were eluted with 20 mM Tris/HCl, pH 7.5, 1 mM DTT, and 0–500 mM NaCl gradient. Fractions containing chaperonin were concentrated on YM100 membrane from Amicon, and chaperonin was further purified by gel permeation chromatography on a Sephacryl 300 column in 20 mM Tris/HCl pH 7.5 buffer containing 1 mM DTT and 250 mM NaCl. A protein peak that eluted close to void volume comprised the archaeosome. Finally, chaperonin was separated on a high resolution MonoQ column.

* This project was supported by the U. S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-Eng-38. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Argonne National Laboratory, 9700 S. Cass Ave., Argonne, IL 60439.

§ University of Chicago, Chicago, Illinois 60637

1 The abbreviations used are TFE, trifluoroethanol; DTT, dithiothreitol; FPLC, fast protein liquid chromatography; PAGE, polyacrylamide gel electrophoresis; CD, circular dichroism; ATP-γ-S, adenosine 5′-O-(3-thiotriphosphate).

*28818
(16/10) column using FPLC and eluted with 20 mM Tris/HCl pH 7.5 buffer, 1 mM DTT, and 0–500 mM NaCl gradient. Fractions containing α and β subunits were concentrated on YM30 membrane from Amicon and were further purified by gel permeation chromatography on a Sephacryl 300 column using FPLC and eluted with 20 mM Tris/HCl pH 7.5 buffer, 1 mM DTT, and 0–150 mM NaCl gradient. Under these conditions α and β subunits were separated on a high resolution MonoQ (16/10) column using FPLC and eluted with 20 mM Tris/HCl, pH 7.5, 1 mM DTT, and 0–150 mM NaCl gradient. A protein peak that eluted close to two void volumes contained archaeosome subunits. Finally, α and β subunits were separated on a high resolution MonoQ (16/10) column using FPLC and eluted with 20 mM Tris/HCl pH 7.5 buffer, 1 mM DTT, and 0–500 mM NaCl gradient. Fractions containing α and β subunits were concentrated on YM30 membrane from Amicon and were further purified by gel permeation chromatography on a Sephacryl 300 column in 20 mM Tris/HCl pH 7.5 buffer, 1 mM DTT, and 250 mM NaCl. A protein peak that eluted close to two void volumes contained archaeosome subunits. Finally, α and β subunits were separated on a high resolution MonoQ (16/10) column using FPLC and eluted with 20 mM Tris/HCl, pH 7.5, 1 mM DTT, and 0–150 mM NaCl gradient. Under these conditions α and β subunits can be separated from each other. Alternatively, pure archaeosome was dissociated to subunits by a 1-h incubation at 75°C with 2 mM ATP, and α and β subunits were separated on a high resolution MonoQ (16/10) column using FPLC, as described above. The fractions were judged to be pure by gel electrophoresis and stained with silver. Thermus aquaticus chaperonin was purified using a procedure similar to that described for the archaeosomes.

Glutamate dehydrogenase from S. shibatae (DSM strain 5389) was purified using the modified procedure of Robb et al. (25), in which Sepharose CL-6B column was replaced with Sephacyr 300 column and Phenyl-Sepharose CL-6B column was replaced with MonoQ (16/10). The purity and the identity of the protein were confirmed by sequencing of the N terminus (W. M. Keck Foundation, Biotechnology Resource Laboratory, Yale University) and by activity assay (22).

Native Gel Electrophoresis—Chaperonin complexes were separated on 4, 6, 10, and 6–10% gradient polyacrylamide (75/15) gels that were prerun at 25 V/cm at constant power for 2 h in 11 mM Tris/HCl pH 7.5 buffer, pH 7.5, at 4°C. Gels were run at 25 V/cm with buffer recirculation at constant power for 2 h at 4°C in a gel system from Hoefer. Gels were stained for 5 min with Coomassie Brilliant Blue to visualize proteins, vacuum dried, and autoradiographed. Archaeosome closed and open complexes were purified in milligram scale by native gel electrophoresis under native conditions. Two-dimensional Gel Electrophoresis—Two-dimensional gel electrophoresis was done as described previously (26). Protein samples were mixed with an equal volume of 9 M urea, 4% (v/v) Nonidet P-40, 2% 2-mercaptoethanol, and 2% ampholytes (pH 9–10; from LKB). First-dimension isoelectric focusing was done using 40-cm rod gels containing 50% pH 3–10 and 50% pH 5–7 ampholytes from Bio-Rad. After isoelectric focusing, the tube gels were equilibrated in a buffer containing SDS. Second-dimension SDS-polyacrylamide gel electrophoresis was run in slab gels containing 10–17% linear gradient acrylamide. Gels were fixed in 50% (v/v) ethanol with 0.1% formaldehyde and 1% acetic acid and stained with silver.

**RESULTS**

Composition and Properties of Archaeosome Complexes—Trent et al. (23) reported that the native TF55 protein migrates as a double band in native polyacrylamide gel electrophoresis (PAGE). We have found that pure S. shibatae archaeosome separates into two major components on native PAGE (Fig. 1, lane 1). The protein complexes representing the two major components, the slower migrating top band (TB) and the faster migrating bottom band (BB), were purified to homogeneity using preparative native PAGE and were characterized further. Two-dimensional gel electrophoresis of the native archaeosome shows that it is composed of two polypeptides that separate into several charge variants (Fig. 1, 2-DE panel). Similar heterogeneity has been observed for other thermostable proteins (26), and is most likely a result of deamidation at Asn and Gln side chains at high temperature (27). It is also possible that some of the heterogeneity can be attributed to phosphorylation, which has been reported for the Sulfolobus solfataricus chaperonin (18).

The two polypeptides are sequence-related and have molecular masses of 59.8 and 59.9 kDa, respectively, as derived from amino acid sequences of their genes (19, 23). The 59.8 kDa protein (β) is identical to the TF55 protein (23), and the 59.9...
kDa protein (α) will be characterized in detail elsewhere (19). Pure top and bottom complexes were examined by two-dimen-sional gel electrophoresis and showed identical protein composi-tion (Fig. 1, 2-DE panel). In both complexes, α and β subunits were present in roughly stoichiometric amounts (19 and below). Both complexes are free of nucleotides, as indicated by their UV spectra (not shown).

Using electron microscopy and circular dichroism (CD), we examined the possibility that conformational differences in the chaperonin complex could account for the observed difference in gel mobility. Electron micrographs showed that the pure top band complex forms a nine-fold, double-ring structure that has an electron dense core, i.e. it stains preferentially in the center with uranyl acetate (Fig. 1, micrographs panel). This complex appears identical to the TF55 structures published earlier (23).

We describe top band as an “open” complex because it appears to contain an open cavity when viewed down the nine-fold axis of the particle (Fig. 1, symmetry panel). The side view shows the characteristic four-band striation pattern reported for other hsp60 chaperonins having 7/2, 8/2, and 9/2 symmetries (3, 20, 21, 23). In contrast, the faster moving BB complex showed no obvious symmetry on electron micrographs and forms a “closed” complex with a poorly defined central cavity (Fig. 1, micrographs panel), although it is similar to the open complex in size and appearance both in top and side views. CD spectra of the purified closed and open complexes show quite remark-able differences (Fig. 2). The archaeosome closed complex shows ellipticity typical of proteins with high α-helix content. The CD spectrum of the open complex has minimum at 224 nm shifts to 220 nm perhaps suggesting lower α-helical content.

Relative Amount of Closed and Open Complexes Varies During Heat Shock—In S. shibatae the relative amounts of closed and open complexes are affected by growth temperature (Fig. 3A). Under normal growth conditions (75 °C) the closed complex is more abundant. Under stress conditions (heat shock temperature >85 °C), more open complex is detected. Under lethal conditions (>90 °C), the open complex disappears, and only the more temperature-stable closed complex persists. Under both normal and heat shock conditions S. shibatae cells also contain free subunits (data not shown).

Trent et al. (23) reported previously that TF55 protein binds guanidine/HCl-denatured dihydrofolate reductase and unspecified E. coli proteins. We found that both the open and closed complexes bind heat-denatured 32P-α-51-casein (Fig. 3B). The binding of casein is temperature-dependent and occurs only above 50 °C. The association of casein with the archaeosome complexes is weak, as compared with the binding of 32P-α-51-casein to thermophilic GroEL-like chaperonin from T. aquat i cus (Fig. 3C and Ref. 28), and the ternary complex appears to be formed transiently. Similar results have been reported recently for binding of β-actin to c-cpn60 cytosolic chaperonin of eu-karyota (29). C-cpn60 chaperonin binds β-actin ten times more weakly than E. coli GroEL, but in contrast to GroEL c-cpn60 supports effective folding of β-actin.

Specific Dissociation of Archaeosome Complexes—Like other chaperonins, the archaeosome shows ATPase activity (18, 22, 23). We investigated the effect of ATP and its analogs on the equilibrium between closed and open complexes. Purified closed complex of archaeosome was incubated with ATP, ADP, or ATP-γS (a slowly hydrolyzable analog of ATP) (Fig. 4A). In some experiments, small amounts (5%) of TFE were added; TFE and other alcohols have been reported to affect protein conformation by weakening hydrophobic interactions and disrupting oligomeric structures (30).

In the presence of ATP, ADP or ATP-γS, the closed complex can be partly converted to the open complex (Fig. 4A, lanes 4, 5, 8, and 9). The open complex appears to be stabilized by the addition of ADP or ATP-γS (Fig. 4A, lanes 4, 5, 8, and 9). The binding of ATP and subsequent hydrolysis of the phosphoester bond lead to complex dissociation (Fig. 4A, lanes 6 and 7). As expected, the addition of 5% TFE further destabilizes the com-plexes, causing dissociation of the complex to subunits (Fig. 4A, lanes 3 and 7). More complex dissociates in the presence of ATP.

The observed effect of ATP is very specific, because the closed complex resists treatment with 7 M urea, and both complexes are stable for weeks at ambient temperatures with or without bound nucleotide (data not shown and Ref. 18).

Reconstitution of Archaeosome from Free Subunits—We pu-rified α and β subunits of the archaeosome to homogeneity directly from cell extracts of S. shibatae as described under “Materials and Methods.” These subunits can be reconstituted into the full-size complex by incubating mixtures of subunits at concentrations greater than 10 μM at 20 °C in phosphate buffer and at neutral pH (Fig. 4B, left panel). Small amounts of intermediate-size complexes that behave like a single ring were also observed on overloaded gels. Electron micrographs of the reconstituted archaeosome display normal bitoroidal structures (Fig. 4B, right panel) that bind 32P-α-51-casein (data not shown). Neither ATP nor peptides are required for the recon-stitution, but both affect it (see below).

To study the composition of the archaeosome, purified α and β subunits were 32P-labeled, and the relative composition of the reconstituted archaeosome was determined. When a stoichio-metric mixture of α and β subunits was reconstituted with a small amount of 32P-labeled α subunit, 18.4 ± 3.2% of 32P-label was found in the reconstituted chaperonin. Under identical conditions 15.0 ± 3.3% 32P-labeled β subunit reconstituted into the chaperonin complex. This result suggests an apparent 1:1 stoichiometry for α and β subunits in archaeosome. Each sub-unit alone, however, can also reconstitute a high molecular weight complex; these complexes have gel mobilities slightly different from that of the heterologeric archaeosome (19). β subunits form a complex both in the presence and absence of ATP, whereas α subunits only assemble in the absence of ATP. In the presence of both α and β subunits, only the stable αβ heterologeric complex is formed with and without ATP (19). Hence, the bitoroidal archaeosome seems to be asymmetric in respect to ATP-dependent ring stability.

When α and β subunits reconstitute in the presence of heat-denatured 32P-α-casein, the 32P-casein is found mainly bound to subunits and to an intermediate complex, whereas only small amounts are associated with the open and closed forms of full-size archaeosome (Fig. 4B, right panel). Similar weaker binding of denatured proteins to TCP-1-like chaperonin was reported by Tian et al. (29). We evaluated the relative affinity...
of α and β subunits for 32P-α-casein in titration experiments (Fig. 4C). Both subunits bind 32P-casein, but the β subunit appears to bind casein 5-10 times more strongly than the α subunit. The subunits-32P-casein complex migrated in gels slower than free subunits but faster than the archaeosome complex, suggesting that this complex may represent an intermediate. As judged by its gel mobility, it could represent a single ring composed of identical subunits with bound α-casein. Single rings of thermophilic chaperonin have been reported recently (31), and the equilibria between chaperonin, single rings, and subunits have been observed for three different chaperonins including GroEL (32).

**DISCUSSION**

Extensive efforts are underway in many laboratories to fully characterize the eukaryotic chaperonin TCP1/TRiC. In contrast to bacterial GroEL, where the underlying mechanisms of protein binding and folding have been studied in great depth, less is known about the mechanism by which the eukaryotic TCP1 chaperonin folds proteins. The most obvious difference is the presumed multishubunit complexity of the TCP1 chaperonin. Archaeobacterial chaperonins show extensive sequence similarity to eukaryotic cytosolic chaperonins (TCP1) and represent a simpler model with which to study folding in eukaryotic cells because the archaeal chaperonin complex is composed of just two subunits (18, 19).

We have shown that the S. shibatae chaperonin exists in vitro and presumably also in vivo as two distinct complexes that can be separated by native PAGE. We believe that these two complexes can be detected because the complex is frozen in these two states by lowering the temperature from 75°C to room temperature. Two distinct conformations of TCP1 chaperonin from mouse testis have been observed by Hynes et al. (16) using specific antibodies. These authors suggested that binding or hydrolysis of ATP acts as a switch between two conformational forms of chaperonin. Knap et al. (18) observed similar complexes of S. solfataricus chaperonin on electron micrographs. Guagliardi et al. (22) reported that the chaperonin from S. solfataricus (termed SsoCpn) in the presence of Mg-ATP undergoes a large conformational rearrangement (as observed by change in tryptophan fluorescence). Conformational changes in E. coli GroEL (33) and complexes of GroEL with bound ATP, GroES, and protein-substrate (3) have been reported. The magnitude of the conformational changes in the archaeosome structure is pertinent to the structure of chaperonin from thermophilic archaeabacteria obtained with electron microscopy by Phipps et al. (21). This structure shows a large mass of protein blocking the entrance to the chaperonin central cavity. These EM reconstructions could represent the structure of closed complex described here or complex with bound protein substitute.

The CD spectra of purified complexes showed remarkable differences, implying that the open complex is structurally altered (Fig. 2). Both the electron microscopy and the CD results suggest that there is a major conformational difference between the two complexes. The structural changes in the open complex that allow uranyl acetate to bind within the central cavity also increase the effective cross-section of the complex, which reduces its mobility in native polyacrylamide gels. The change in the CD spectrum reflects more extensive structural rearrangement than just domain movement. If the domain organization of archaeosome is similar to that of E. coli GroEL (as suggested by limited but significant sequence similarity), then our data would imply conformational changes in the apical region. This domain was proposed by Horwich and coworkers (11) to be involved in protein binding and folding.

We have shown here that the conformational changes are related to conversion of closed complex to open complex and dissociation to subunits. The equilibrium between three states, open and closed complexes and free subunits, is affected in vitro by temperature and by Mg-ATP and its derivatives, ADP and ATP γS. It appears that in vivo under mild heat shock, the amount of open complex increases, suggesting a response to a stress. Under lethal heat shock conditions only the closed complex remains. Our data suggest that this dissociation is controlled by ATP hydrolysis. A large concentration of free subunits may provide an advantage to the cell by capturing unfolded polypeptides under heat shock conditions and arresting protein aggregation. The full role of free subunits in the protein folding cycle has yet to be established, and an hsp70-like function of the α and β subunits cannot be excluded. A similar function in heat shock response has been attributed to yeast hsp104 protein, which is believed to assist protein solu-
Parsell et al. (34) recently reported that yeast hsp104 can rescue proteins from aggregates once they have formed. Strikingly, it has been noted earlier that the stability of hsp104 hexamer in vitro, similar to the archaeosome, is ATP-dependent (35). Thus the dissociation of the archaeosome to subunits could be an important part of a functional cycle that links protein-mediated thermotolerance with protein folding.

Several protein folding cycles have been proposed for bacterial chaperonin (7–9, 13, 14). These cycles postulate the formation of specific bi-, ter-, and quaternary complexes that facilitate protein folding and chaperonin regeneration. The cycle that we propose in Fig. 5 includes a change in conformation and in oligomerization state. The cell maintains all the chaperonin components (closed complex, open complex, and subunits) in equilibrium. Both complexes and subunits appear to bind denatured proteins. Our data imply that in the archaea, unfolded protein enters the cycle by binding to subunits, proceeds through an intermediate that is composed of individual subunits, and continues to the double-ring complex. We believe
that the folded protein is released when the open complex dissociates into subunits (Fig. 5).

There is a direct analogy between the archaeosome open complex and the GroEL-GroES complex as high energy states and the free subunits and GroEL as the low energy states (7). The dissociation of the archaeosome to subunits is the ultimate relaxation of the high energy state. The closed complex appears to represent an intermediate energy state. We suggest that as previously reported for GroEL (7), the thermodynamic barriers separating protein-bound and free archaeosome states are overcome by ATP hydrolysis. The dissociation of bound protein is most likely accomplished by a change in the binding affinities of the chaperonin for a non-native protein. The extreme way to achieve this is to break up the structure of the complex into its subunits. Clearly the chaperonin complex and free subunits must present different interactive surfaces for unfolded proteins.

We propose that, as an unfolded protein assumes its native structure, the archaeosome undergoes conformational changes. The high entropy of the unfolded protein is assimilated by the chaperonin as the protein folds, the archaeosome acting as an "entropic sink." After ATP hydrolysis, the ternary complex dissociates, releasing folded protein and subunits (Fig. 5). Free subunits reassemble into complexes, completing the cycle. It is likely that the archaeosome folds proteins in a quite different way than the GroEL-like chaperonins. In fact, Cowan and co-workers (29) showed recently that a distinct set of folding intermediates is released from different chaperonins. The hyperthermophilic archaeabacteria must protect and fold proteins that are already quite thermostable, and therefore archaeo-

![Conformational cycle of archaeosome](image)

**Fig. 5. Conformational cycle of archaeosome.** Proposed model for conformational cycle and protein binding of the *S. shibatae* chaperonin during thermotolerance and protein folding.

some may require higher energy to overcome thermodynamic barrier in folding of these proteins. It is likely that proteins and chaperonins coevolved to optimize folding requirements of the cell. This is reflected in the properties of chaperonin and the dynamics and degree of structural changes during chaperonin-mediated protein folding.

Acknowledgments—We thank Carol Gionetti and Sandra Tollekens for running two-dimensional gel electrophoresis, Michael Garavito for providing preparative gel electrophoresis unit, Randy Knowlton for helping with purification of archaeosome complexes and the binding assay, and John Sutherland for use of U98 at National Synchrotron Light Source, Brookhaven National Laboratory and for collecting CD spectra. We also thank Fred Stevens and Mark Donnelly for critical reading of this manuscript.

REFERENCES

1. Braig, K., Hainfield, J., Simon, M., Furuya, F., and Horwich, A. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3978–3982
2. Langer, T., Pfeffer, G., Martin, J., Baumeister, W., and Hartl, F.-U. (1992) EMBO J. 11, 4757–4761
3. Chen, S., Roseman, A. M., Hunter, A. S., Wood, S. P., Burston, G. S., Ranson, N. A., Clarke, A. R., and Saibil, R. H. (1994) Nature 368, 261–264
4. Henderick, J. P., and Hartl, F. U. (1993) Annu. Rev. Biochem. 62, 349–384
5. Robinson, C. V., Gross, M., Eyles, S. J., Ewbank, J. J., Mayhew, M., Hartl, F. U., Dobson, C. M., and Radford, S. E. (1994) Nature 372, 646–651
6. Zahn, R., Spatzheden, C., Ottiger, M., Wuthrich, K., and Pluckthun, A. (1994) Nature 368, 261–265
7. Todd, M. J., Viitanen, P. V., and Lorimer, G. H. (1994) Science 265, 659–666
8. Martin, J., Mayhew, M., Langer, T., and Hartl, F. U. (1993) Nature 366, 228–233
9. Weissman, J. S., Kashi, Y., Fenton, W. A., and Horwich, A. L. (1994) Cell 78, 693–702
10. Braig, K., Otwinowski, Z., Hegde, R., Bolowert, D. C., Jachimiahi, A., Horwich, A. L., and Sigler, P. B. (1994) Nature 371, 578–586
11. Fenton, W. A., Kashi, Y., Furtak, K., and Horich, A. L. (1994) Nature 371, 614–619
12. Craig, E. A., Weissman, J. S., and Horwich, A. L. (1994) Cell 78, 365–372
13. Mendoza, J. A., Demeler, B., and Horowit, P. M. (1994) J. Biol. Chem. 269, 2447–2451
14. Schmidt, M., Rutkak, K., Rachel, R., Pfeffer, G., J aenickie, R., Viitanen, P. V., Lorimer, G., and Buchner, J. (1994) Science 265, 656–659
15. Azem, A., Kessel, M., and Goloubinoff, P. (1994) Nature 365, 653–656
16. Hynes, G., Kuboka, H., and Willison, K. R. (1995) FEBS Lett. 358, 129–132
17. Gao, Y., Melki, R., Walden, P. D., Lewis, S. A., Ampa, C., Rommel, E., Vandekerckhove, J., and Cowan, N. J. (1994) J. Cell Biol. 125, 989–996
18. Knapp, S., Schmidt-Krey, I., Herbert, H., Bergman, T., Jornvall, H., and Lindoestin, R. (1994) J. Biol. Chem. 269, 10, 1711–1722
19. Phipps, B. M., Hoffmann, A., Stetter, K. O., and Baumeister, W. (1991) EMBO J. 10, 341–349
20. Phipps, B. M., Tyype, D., Hegert, V., Volker, S., Hoffmann, A., Stetter, K. O., and Baumeister, W. (1993) Nature 361, 474–477
21. Guagliard, A., Cerchia, L., Bartolucci, S., and Roes, M. (1994) Protein Sci. 3, 1436–1443
22. Cot, J. D., Nimmegsen, E., Wall, J. S., Hartl, F. U., and Horwich, A. L. (1991) Nature 349, 490–493
23. Gill, S. C., and von Hippel, P. H. (1989) Anal. Biochem. 182, 319–326
24. Robb, F. T., Park, J. B., and Adams, W. W. (1992) Biochim. Biophys. Acta 1120, 267–272
25. Giometti, C. S., Tollekens, S. L., Mukund, S., Zhou, H. Z., Ma, K., Huhong, M., and Adams, W. W. (1994) J. Chromatogr. 698, 341–349
26. Brennan, T. V., and Clarke, S. (1993) Protein Sci. 2, 331–338
27. Jachimiac, A., Knowlton, J., and Quaiti-Randall, E. (1995) FASEB J. 9, 54
28. Tian, G., Vainberg, I. E., Tap, W. D., Lewis, S. A., and Horwich, A. L. (1994) Nature 372, 250–253
29. Thomas, P. D., and Dill, K. (1993) Protein Sci. 2, 2050–2065
30. Ishii, N., Taguchi, H., Sasabe, H., and Yosida, M. (1995) FEBS Lett. 362, 121–125
31. Lissin, N. M. (1995) FEBS Lett. 361, 55–60
32. Zahn, R., Harris, J. R., Pfeffer, G., Pluckthun, A., and Baumeister, W. (1993) J. Biol. Chem. 268, 579–584
33. Parsell, D. A., Kowal, A. S., Singer, M. A., and Lindquist, S. (1994) Nature 372, 475–478
34. Parsell, D. A., Kowal, A. S., and Lindquist, S. (1994) J. Biol. Chem. 269, 4480–4487