Facilitated Protein Aggregation

EFFECTS OF CALCIUM ON THE CHAPERONE AND ANTI-CHAPERONE ACTIVITY OF PROTEIN DISULFIDE-ISOMERASE*

(Received for publication, August 29, 1996, and in revised form, October 7, 1996)

Todd P. Primm, Kenneth W. Walker, and Hiram F. Gilbert‡

From the Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030

Protein disulfide-isomerase (PDI) catalyzes the formation and isomerization of disulfides during oxidative protein folding in the eukaryotic endoplasmic reticulum. At high concentrations, it also serves as a chaperone and inhibits aggregation. However, at lower concentrations, PDI can display the unusual ability to facilitate aggregation, termed anti-chaperone activity (Puig, A., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 7764–7771). Under reducing conditions (10 mM dithiothreitol) and at a low concentration (0.1–0.3 μM) relative to the unfolded protein substrate, PDI facilitates aggregation of alcohol dehydrogenase (11 μM) that has been denatured thermally or chemically. But at higher concentrations (>0.8 μM), PDI inhibits aggregation under the same conditions. With denatured citrate synthase, PDI does not facilitate aggregation, but higher concentrations do inhibit aggregation. Anti-chaperone behavior is associated with the appearance of both PDI and substrate proteins in insoluble complexes, while chaperone behavior results in the formation of large (>500 kDa) but soluble complexes that contain both proteins. Physiological concentrations of calcium and magnesium specifically increase the apparent rate of PDI-dependent aggregation and shift the chaperone activity to higher PDI concentrations. However, calcium has no effect on the K_m or V_max for PDI-catalyzed oxidative folding, suggesting that the interactions that lead to chaperone/anti-chaperone behavior are distinct from those required for catalytic activity. To account for this unusual behavior of a folding catalyst, a model with analogy to classic immunoprecipitation is proposed; multivalent interactions between PDI and a partially aggregated protein stimulate further aggregate formation by noncovalently cross-linking smaller aggregates. However, at high ratios of PDI to substrate, cross-linking may be inhibited by saturation of the sites with PDI. The effects of PDI concentration on substrate aggregation and the modulation of the behavior by physiological levels of calcium may have implications for the involvement of PDI in protein folding, aggregation, and retention in the endoplasmic reticulum.

* This work was supported by National Institutes of Health Grant GM40379 (to H. F. G.). 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. Tel.: 713-798-5880; Fax: 713-796-9438; E-mail: hgilbert@bcm.tmc.edu.
1 The abbreviations used are: ER, endoplasmic reticulum; PDI, protein disulfide-isomerase; BiP, immunoglobulin heavy chain-binding protein (grp78); DTT, dithiothreitol; ADH, alcohol dehydrogenase; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.
one-associated aggregates that are retained in the ER. When permissive folding conditions are restored, these ER-retained aggregates are rapidly cross-linked by disulfides and then released and folded into native structures (20, 21). A similar observation has been made in HepG2 cells upon induction of thyroglobulin synthesis by thyroid hormones; the protein initially aggregates in the ER in association with specific chaperones before productive folding and secretion occur (22).

Some of the effects of chaperone overexpression on protein secretion are quite puzzling. Overexpression of BiP or PDI in either mammalian cells (23) or yeast (24) can result in increased or decreased secretion levels of coexpressed proteins. Intermediate levels of the yeast chaperone hsp104 are essential for transmission of the Ψ factor in yeast through a complex mechanism that may involve aggregation similar to that found with β-amyloid formation. Disruption of the hsp104 gene or its overexpression inhibits the process (25).

A better understanding of the protein aggregation problem is of obvious importance in the development of predictable high level expression systems for recombinant proteins (26). In addition, protein aggregation has been associated with the development of specific pathologies such as Alzheimer’s disease, prion-mediated disease, and a host of others (27). The unusual behavior of PDI and BiP in facilitating in vitro aggregation and the observation of very large, chaperone-associated aggregates in cells raise the intriguing possibility that aggregation might actually be facilitated by chaperones under certain conditions.

To begin to define the mechanisms by which PDI can behave as a chaperone under some conditions and as an anti-chaperone under others, we have examined the effects of PDI and other proteins on the aggregation of thermally and chemically denatured liver alcohol dehydrogenase (ADH) and citrate synthase, two proteins that do not require disulfides for folding. As in the oxidative refolding of lysozyme, PDI displays chaperone and anti-chaperone activity with ADH, but behaves only as a chaperone with citrate synthase. We also find that physiological levels of calcium increase the rate and extent of PDI-dependent aggregation and decrease the chaperone activity. A model, based on analogy to classical immunoprecipitation, is proposed that provides a basis for understanding the complex interactions of PDI with unfolded proteins and its behavior in both inhibiting and stimulating aggregation.

EXPERIMENTAL PROCEDURES

Materials—Recombinant rat PDI was expressed in Escherichia coli and purified as described previously (28). Bovine PDI was isolated from liver by the method of Hillson et al. (6). DTT, equine liver alcohol dehydrogenase, porcine heart citrate synthase, magnesium chloride, and HEPES were purchased from Sigma. Calcium chloride dihydrate and 1% (50 mM) was incubated in a spectrophotometer cell thermostatted at 40 °C in 50 mM HEPES, pH 7.0, with (○) or without (□) 10 mM DTT. Various concentrations of PDI were added as indicated. Aggregation was monitored by measuring apparent light scattering (A 360) every 30 s. The rate of ADH aggregation (Aa/min) after a short lag was normalized to the rate observed in the presence of DTT and absence of PDI. Error bars indicate the standard deviation from two to four independent measurements. B, samples were taken from the spectrophotometer and microcentrifuged, and the pellet (P) and supernatant (S) fractions were subjected to reducing SDS-PAGE. ADH was incubated alone at 40 °C for 0 (lanes 11 and 12), 10 (lanes 1 and 2), or 20 min (lanes 3 and 4). Two μM PDI was heated alone for 20 min (lanes 5 and 6). ADH was heated for 20 min in the presence of an anti-chaperone concentration (0.3 μM) (lanes 7 and 8) or a chaperone concentration (2 μM) of PDI (lanes 9 and 10). SDS-PAGE denotes molecular mass standards (lane 13). All incubations contained 10 mM DTT.

Gel Filtration—Analytical gel filtration was performed on a 600 × 7.5-mm Bio-Sil 250-10 column (Bio-Rad) connected to a Beckman 110A HPLC system. The solvent was 100 mM NaCl, 50 mM HEPES, pH 7.0. ADH was denatured in 6 M guanidine HCl for 1 h at 25 °C and diluted to 5.5 μM in 50 mM HEPES in a siliconized microcentrifuge tube (carry-over guanidine HCl was 50 μM). Following incubation at room temperature or 37 °C and centrifugation in a microcentrifuge for 2 min to remove insoluble aggregates, the solution was applied to the column. The flow rate was 1 ml/min, and the effluent was monitored by absorbance at 280 nm.

FIG. 1. PDI demonstrates chaperone and anti-chaperone activity during the thermal aggregation of ADH. A, native ADH dimer (11 μM) was incubated in a spectrophotometer cell thermostatted at 40 °C in 50 mM HEPES, pH 7.0, with (○) or without (□) 10 mM DTT. Various concentrations of PDI were added as indicated. Aggregation was monitored by measuring apparent light scattering (A 360) every 30 s. The rate of ADH aggregation (Aa/min) after a short lag was normalized to the rate observed in the presence of DTT and absence of PDI. Error bars indicate the standard deviation from two to four independent measurements. B, samples were taken from the spectrophotometer and microcentrifuged, and the pellet (P) and supernatant (S) fractions were subjected to reducing SDS-PAGE. ADH was incubated alone at 40 °C for 0 (lanes 11 and 12), 10 (lanes 1 and 2), or 20 min (lanes 3 and 4). Two μM PDI was heated alone for 20 min (lanes 5 and 6). ADH was heated for 20 min in the presence of an anti-chaperone concentration (0.3 μM) (lanes 7 and 8) or a chaperone concentration (2 μM) of PDI (lanes 9 and 10). SDS-PAGE denotes molecular mass standards (lane 13). All incubations contained 10 mM DTT.

RESULTS

Effects of PDI on the Aggregation of Thermally Denatured ADH—When native ADH is heated to 40 °C, it unfolds and aggregates into particles that are sufficiently large to scatter 360-nm wavelength light (Fig. 1A). Low concentrations of recombinant rat PDI accelerate ADH aggregation (anti-chaperone behavior), but at higher concentrations, PDI prevents aggregation (chaperone behavior). Similar results are observed with bovine liver PDI. Neither of these activities is dependent
Sodium chloride (5 mM) does not (Fig. 4).

HassimilareffectsonPDI-dependentaggregation(Fig.3).Activity of PDI with reduced, denatured RNase A as substrate exerted on PDI rather than the substrate. Magnesium (5 mM) (data not shown), suggesting that the effects of calcium are little effect on the aggregation of ADH in the presence of BSA higher PDI concentrations (Fig. 3). By contrast, calcium has the transition between chaperone/anti-chaperone behavior to levels of calcium increase PDI-dependent aggregation and shift the chaperone/anti-chaperone behavior of PDI. Physiological 55°C. 2 ATP (upto 1 mM) has no effect on PDI-dependent ADH change in the secondary structure of PDI at temperatures up to 55 °C. ATP (up to 1 mM) has no effect on PDI-dependent ADH aggregation in the presence of 5 mM MgCl₂ (data not shown), nor does it affect the isomerase activity of PDI with RNase A (35).

As observed for PDI- and BiP-dependent lysozyme aggregation (9, 18), the insoluble aggregates induced by low anti-chaperone concentrations of PDI contain both PDI and ADH (Fig. 1B, lane 8 versus lane 4). At higher PDI concentrations, where chaperone activity is observed, a significant decrease is noted in the amounts of both PDI and ADH that are found in the insoluble pellet (Fig. 1B, lane 10).

Specificity—PDI was replaced in the ADH aggregation assay by several control proteins (Fig. 2). Neither native RNase A nor native lysozyme significantly affects ADH aggregation. BSA, a protein that is known to have multiple hydrophobic sites that could interact with an unfolded protein (36, 37), also stimulates aggregation at low concentrations and inhibits aggregation at higher concentrations. Chaperone-like activity has been previously reported for BSA (38). In the absence of ADH, there is no aggregation of PDI, RNase, lysozyme, or BSA.

Calcium Effects on PDI-dependent Aggregation—The thermal denaturation and aggregation of ADH are slightly inhibited by 5 mM calcium; however, calcium has a dramatic effect on the chaperone/anti-chaperone behavior of PDI. Physiological levels of calcium increase PDI-dependent aggregation and shift the transition between chaperone/anti-chaperone behavior to higher PDI concentrations (Fig. 3). By contrast, calcium has little effect on the aggregation of ADH in the presence of BSA (data not shown), suggesting that the effects of calcium are exerted on PDI rather than the substrate. Magnesium (5 mM) has similar effects on PDI-dependent aggregation (Fig. 3A), but sodium chloride (5 mM) does not (Fig. 4).

Calcium (5 mM) has a negligible effect on the isomerase activity of PDI with reduced, denatured RNase A as substrate (data not shown). Both $K_m$ and $k_{cat}$ values are unaffected (<3% difference), consistent with a previous report (39). UV difference spectroscopy suggests the possibility of an effect of calcium on the environment of tryptophan residues (13); however, 5 mM calcium has insignificant effects on the circular dichroism spectra of PDI (data not shown). Although sodium chloride at concentrations as high as 10 mM has only a small effect on the PDI-dependent aggregation of ADH, the presence of sodium chloride (5 mM) partially suppresses the effects of 5 mM calcium (Fig. 4), consistent with the reported inhibition of calcium binding by potassium chloride (13).

Soluble Complex Formation between PDI and ADH—The effects of PDI on the rate of aggregation of chemically denatured ADH are similar to those observed during thermal dena-
When guanidine-denatured ADH (5.5 mM final concentration) is diluted into buffer, microcentrifuged to remove large aggregates, and then analyzed by gel filtration HPLC, no peak representing native ADH is detected (Fig. 5 A) because the ADH is in large aggregates that are removed by low speed centrifugation. At higher concentrations (2 mM), PDI can solubilize ADH as a high molecular mass complex ($\approx 500$ kDa). SDS-PAGE of this complex (Fig. 5 B) shows the presence of both ADH and PDI. The isolated complex is very stable at room temperature ($t_{1/2} \approx 24$ h) and does not dissociate as judged by reapplication onto HPLC. Calcium prevents formation of this complex (Fig. 5A); however, when calcium is added after isolation of the complex, it has no effect on the stability. Under conditions where 10–15% activity is recovered during the refolding of chemically denatured ADH (40), PDI has little effect on the recovery of activity (data not shown). While PDI can maintain ADH solubility, it does not assist in the recovery of ADH activity.

Calcium Effects on the Aggregation of Thermally Denatured Citrate Synthase—With thermally denatured citrate synthase as substrate, PDI acts only as a chaperone, and there is no significant acceleration of aggregation (Fig. 6). As with ADH, 5 mM calcium decreases the chaperone activity of PDI, demonstrating that the calcium effects observed with ADH are not unique to that substrate. Controls show that the thermal denaturation and aggregation of citrate synthase are not significantly affected by calcium (data not shown).

**DISCUSSION**

**A Working Model**—Several unusual experimental observations must be accommodated by a working model for facilitated aggregation. 1) Low concentrations of chaperones increase aggregation, but higher concentrations inhibit aggregation (Fig. 1). 2) The behavior is “specific.” Not all proteins affect aggregation (Fig. 2). Specific substrates are required; aggregation is accelerated for some unfolded substrate proteins, but not for others (Figs. 1 and 6). In addition, there is competition between different anti-chaperones for incorporation into aggregates at a limited number of sites (18). 3) PDI is associated with unfolded protein substrates under both anti-chaperone and chaperone conditions (Figs. 1 and 5). 4) Calcium, through an effect on PDI, affects both the chaperone and anti-chaperone activity (Fig. 3A).

A working model that accounts for the ability of PDI and other chaperones/anti-chaperones to stimulate aggregation at low concentrations but inhibit aggregation at high concentrations is shown in Fig. 7. Analogous to immunoprecipitation by polyclonal antibodies, the model suggests that PDI accelerates aggregation by cross-linking smaller aggregates into much larger assemblies containing PDI. This would account for both the increase in aggregation rate and the incorporation of PDI into the insoluble aggregates. Aggregation would be stimulated when PDI-dependent growth of the insoluble aggregates (Fig. 7, $k_{p1}$ and $k_{chap}$) is faster than the self-association of the unfolded protein (or its aggregates) ($k_{self}$).

According to this working model, the switch to chaperone behavior at high PDI concentration results from a partitioning of the soluble, unfolded protein aggregates (UU) between two alternative fates, one leading to insoluble aggregates cross-linked by PDI (UU-P-UU) and the other leading to a soluble complex (P-UU-P). At low concentrations of PDI, the soluble UUP species would be more likely to encounter another UU.
Facilitated Aggregation

![Diagram](image)

**Fig. 7. A working model for the chaperone/anti-chaperone activity of PDI.** The anti-chaperone activity, like immunoprecipitation by a bivalent antibody, results from PDI cross-linking smaller aggregates and promoting their growth until they become insoluble. Aggregation will be faster in the presence of PDI ($k_{chap}$), when PDI-dependent cross-linking is faster, than in the absence of PDI ($k_{self}$). Chaperone activity ($k_{chap}$) is observed when the PDI concentration is sufficiently high to make the interaction of PDI with the unfolded substrate faster than the interaction of this same intermediate (UU-P) with another molecule of UU.

species than PDI, leading to increased formation of insoluble aggregates (Fig. 7, $k_{chap}$). However, at higher PDI concentrations, UU-P species would be more likely to associate with additional PDI to produce soluble P-UU-P complexes (Fig. 7, $k_{chap}(P)$). The situation at high PDI concentrations would be analogous to the inhibition of immunoprecipitation in regions of antibody excess. Because aggregation in these model systems is irreversible, it is not necessary to invoke strong interactions between PDI and the unfolded proteins, consistent with observations of relatively weak interactions ($>\mu M$) between PDI and unfolded proteins and peptides (41).

Although it shows anti-chaperone activity toward lysozyme (9) and ADH (Fig. 1), PDI behaves only as a chaperone with citrate synthase, glyceraldehyde-3-phosphate dehydrogenase (10), and rhodanese (11). The model of Fig. 7 provides for this behavior as well. If self-aggregation of the protein is sufficiently fast (Fig. 7, $k_{self}$), it would take a correspondingly high concentration of PDI before association with PDI could compete with self-association of the substrate. At the higher PDI concentrations required to compete with this faster self-association, partitioning of the UU-P intermediate could be predominantly toward further PDI association, leading only to soluble chaperone complexes.

**Experimental Evidence in Support of the Model—**Several lines of evidence are consistent with the proposed model. To “cross-link” aggregates, PDI (and other chaperones/anti-chaperones) must have at least two sites to interact with the unfolded protein. So far, all proteins that have shown anti-chaperone activity are capable of interacting with multiple hydrophobic substrates. PDI interacts with a surprisingly large array of hydrophobic molecules. A nonspecific peptide/protein-binding site appears essential to PDI-catalyzed oxidative folding (41, 42). PDI also binds thyroid hormone (43) and estrogen (44), serves as the $\beta$-subunit of prolyl 4-hydroxylase (45), and is part of the microsomal triglyceride-transfer protein complex (46). Experiments to identify the binding sites involved in these interactions are in progress. BSA, which also exhibits chaperone/anti-chaperone behavior, can also bind at least three hydrophobic substrates (36, 37), while BiP exists in monomeric and multimeric forms (47).

Anti-chaperone behavior is specific. With unfolded lysozyme as substrate, PDI and BiP are incorporated into aggregates, and the two chaperones compete for a limited number of sites on the unfolded protein. Other soluble, folded proteins are not incorporated nonspecifically into lysozyme aggregates (9, 18). PDI is also associated with chaperone complexes. Although these complexes are soluble, they may be very large (>500 kDa) (Fig. 5). Thus, as suggested by our working model, both chaperone behavior and anti-chaperone behavior are associated with the formation of unfolded protein-PDI complexes. Neither soluble nor insoluble complexes contain active ADH (data not shown), suggesting that the chaperone complexes involve unfolded substrate and PDI.

The working model proposed for the chaperone/anti-chaperone behavior of PDI cannot be conclusively distinguished at this point from an alternative model in which the PDI-dependent increase in aggregation rate might be due to PDI stabilizing an aggregation-prone intermediate, effectively facilitating (catalyzing?) the association process (25). However, a "catalytic" mechanism for anti-chaperone activity would not require the appearance of PDI in insoluble aggregates, contrary to experimental observations.

**Effects of Calcium on Chaperone/ Anti-chaperone Activity—**Calcium, at total concentrations near those estimated in the ER, shifts the transition between anti-chaperone and chaperone behavior to higher PDI concentrations. This is the only effect on PDI function that has been found for calcium. A calcium-dependent change in the partitioning of the unfolded protein between the formation of soluble (P-UU-P) and insoluble (UU-P-UU) complexes could account for this behavior. An increase in the rate constant for association of UU-P with more UU or a decrease in the rate constant for association of PDI with UU-P could produce the shift of the transition to higher PDI concentration. Given the complex nature of the aggregation, our results, so far, do not distinguish between the two alternatives.

**Facilitated Aggregation in the Cell—**An increasing amount of experimental data suggest that aggregation may represent a normal response of the ER to misfolded proteins. If disulfide formation is inhibited by including dithiothreitol in the growth medium, unfolded proteins accumulate and are retained in the ER as large aggregates. Upon restoring conditions to permit folding, these aggregates are rapidly disulfide-cross-linked and with time are solubilized from the aggregates and productively folded (20, 21). Similar aggregation has been noted during the normal folding of thyroglobulin when expression is induced by thyroid hormone (22). Thus, in contrast to the irreversible aggregates we find in vitro, the eukaryotic ER provides a mechanism to retain and recover these aggregates. The stoichiometry observed for the incorporation of PDI (9) and BiP (18) into aggregates suggests that one anti-chaperone molecule is capable of associating, directly or indirectly, with multiple molecules of unfolded protein. Thus, one molecule of a multivalent chaperone might significantly increase the retention capacity of the ER under stress conditions by anti-chaperone-like mechanisms (18). ER chaperones such as BiP have been found associated with large aggregates formed in the ER (20–22), and PDI has been cross-linked to large lysozyme aggregates that accumulate when lysozyme folding is altered by mutation of a disulfide bond (19).

The switch from anti-chaperone to chaperone behavior might also explain several paradoxical observations. Overexpression of the chaperone BiP in Chinese hamster ovary cells decreases the secretion of overexpressed proteins such as tissue plasminogen activator (48), a finding that is consistent with the behavior expected if the system were operating on the unfolded protein-access side of the chaperone/anti-chaperone response curve. In yeast, a careful examination of the effects of the constitutive overexpression of heterologous proteins revealed that the ER was depleted of soluble PDI and BiP (24) and that these two proteins were present in large, insoluble aggregates. Overexpression of BiP did not increase the soluble levels of BiP,
although pulse-chase analysis indicated that BiP expression and translocation into the ER were not impaired. However, overexpression of PDI increased the secretion of platelet-derived growth factor and yeast acid phosphatase (49).

When calcium concentrations are lowered in the ER, the calcium-dependent chaperones, such as calnexin, lose their chaperone activity. Based on the effects of calcium on the concentration would tend to increase the chaperone buffer capacity.

REFERENCES

1. Hwang, C., Sinskey, A. J., and Lodish, H. F. (1992) Science 257, 1496–1502
2. Getting, M. J., and Sambrook, J. (1992) Nature 355, 55–56
3. Freedman, R. B., Hirsh, T. R., and Tuite, M. F. (1994) Trends Biochem. Sci. 19, 331–336
4. Noiva, R. (1994) Protein Expression Purif. 5, 1–13
5. Gilbert, H. F. (1994) in Protein Folding (Pain, R., ed) pp. 104–136, IRL Press Ltd., Oxford
6. Hillson, D. A., Lambert, N., and Freedman, R. B. (1984) Methods Enzymol. 107, 281–292
7. Lyles, M. A., and Gilbert, H. F. (1991) Biochemistry 30, 613–619
8. Hartl, F. U., Martin, J., and Neupert, W. (1992) Eur. J. Biochem. 212, 293–322
9. Puig, A., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 7764–7771
10. Cai, H., Wang, C., and Tsou, C. (1994) J. Biol. Chem. 269, 24550–24552
11. Song, J. L., and Wang, C. C. (1995) Eur. J. Biochem. 232, 312–316
12. Maser, D., and Koch, G. (1986) J. Cell Sci. 81, 61–70
13. Lebeche, D., Lucere, H. A., and Kaminer, B. (1994) Biochem. Biophys. Res. Commun. 202, 556–561
14. Lodish, H. F., Kong, N., and Wikstrom, L. (1992) J. Biol. Chem. 267, 12753–12760
15. Booth, C., and Koch, G. L. E. (1989) Cell 59, 729–737
16. Zapun, A., Creighton, T. E., Rowling, P. J. E., and Freedman, R. B. (1995) Proteins Struct. Funct. Genet. 14, 10–15
17. Puig, A., Lyles, M. A., Noiva, R., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 19128–19135
18. Puig, A., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 25889–25896
19. Otsu, M., Omura, F., Yoshimori, T., and Kikuchi, M. (1994) J. Biol. Chem. 269, 6874–6877
20. Tatu, U., Braakman, I., and Helenius, A. (1993) EMBO J. 12, 2151–2157
21. Braakman, I., Helenius, J., and Helenius, A. (1992) EMBO J. 11, 1717–1722
22. Kim, P. S., and Arvan, P. (1993) J. Biol. Chem. 268, 4873–4879
23. Dorner, A. J., Krane, M., and Kaufmann, R. J. (1988) J. Cell Biol. 123, 1355–1363
24. Robinson, A. S., and Wittrup, K. D. (1995) Biotechnol. Prog. 11, 171–177
25. Cherruff, Y. O., Lindquist, S. L., Oono, B., Inge-Vechtomov, S. G., and Lieberman, S. W. (1995) Science 268, 880–884
26. King, J., Haase-Pettingell, C., Robinson, A. S., Speed, M., and Mitraki, A. (1996) FASEB J. 10, 57–66
27. Thomas, P. J., Qu, B. H., and Pederson, P. L. (1995) Trends Biochem Sci. 20, 456–459
28. Gilbert, H. F., Kruzel, M. L., Lyles, M. M., and Harper, J. W. (1991) Protein Expression Purif. 2, 194–196
29. Jakob, U., Meyer, I., Bugli, H., Andre, S., Bardwell, J. C. A., and Buchner, J. (1995) J. Biol. Chem. 270, 14412–14419
30. Gilbert, H. F. (1989) Biochemistry 28, 7286–7305
31. Cannon, D. J., and McKay, R. H. (1989) Biochem. Biophys. Res. Commun. 35, 403–409
32. West, M. W., Kelly, S. M., and Price, N. C. (1990) Biochim. Biophys. Acta 1057, 332–336
33. Laemmli, U. K. (1970) Nature 227, 680–685
34. Ramaswamy, S., Eklund, H., and Plapp, B. V. (1994) Biochemistry 33, 5230–5237
35. Guthapfel, R., Gueguen, P., and Quemeneur, E. (1996) J. Biol. Chem. 271, 2663–2666
36. Pal, M. K., and Patra, S. K. (1994) Indian J. Biochem. Biophys. 31, 109–114
37. Wilson, D. C. (1980) Biochem. J. 270, 163–166
38. Zhi, W., Landry, S. J., Gierasch, L. M., and Srere, P. A. (1992) Protein Sci. 1, 522–529
39. Schwaller, M. D., and Noiva, R. (1994) FASEB J. 8, 741 (abstr.)
40. Gerschutz, J., Rudolph, R., and Jaenicke, R. J. (1978) Eur. J. Biochem. 87, 591–599
41. Noiva, R., Freedman, R. B., and Lennarz, W. J. (1993) J. Biol. Chem. 268, 19210–19217
42. Morjana, N. A., and Gilbert, H. F. (1991) Biochemistry 30, 4985–4990
43. Cheng, S., Gong, Q., Parkinson, C., Robinson, E. A., Appela, E., Merline, G. T., and Pastan, I. (1987) J. Biol. Chem. 262, 11221–11227
44. Tsibris, J. C., Hurst, L. T., Ballejo, G., Barker, W. C., Toney, L. J., and Spellacy, W. N. (1989) J. Biol. Chem. 264, 13967–13970
45. Koivu, J., Myllyla, R., Helaakoski, T., Pihlajaniemi, T., Tasanen, K., and Kivirikko, K. I. (1987) J. Biol. Chem. 262, 6447–6449
46. Wetterau, J. R., Combs, K. A., McLean, L. R., Spinner, S. N., and Aggerbeck, W. N. (1989) Biochemistry 28, 880–884
47. Blond-Elguindi, S., Fourie, A. M., Sambrook, J. F., and Gething, M.-J. H. (1989) EMBO J. 8, 741 (abstr.)
48. Dorner, A. J., Bole, D. G., and Kaufman, R. J. (1987) J. Cell Biol. 105, 2665–2674
49. Robinson, A. S., Hines, V., and Wittrup, K. D. (1994) Bio/Technology 12, 381–384