Effect of Nucleotides, Peptides, and Unfolded Proteins on the Self-association of the Molecular Chaperone HSC70*

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In a previous study, we showed that the molecular chaperone HSC70 self-associates in solution in a reversible and likely unlimited fashion. Here, we examine the influence of nucleotides, nucleotide analogs, peptides, and unfolded proteins on the self-association properties of this protein. Whereas in the presence of ADP, HSC70 exists as a slow, concentration- and temperature-dependent monomer-oligomer equilibrium, in the presence of ATP, the protein is essentially monomeric, indicating that ATP shifts this equilibrium toward the monomer by stabilizing the monomer. Dissociation of oligomers into monomers is also obtained with the slowly hydrolyzable ATP analogs, adenosine 5’-O-(thiotriphosphate) and 5’-adenylyl-β,γ-imidodiphosphate, or the complex between ADP and the phosphate analog, BeF3, indicating that binding but not hydrolysis of ATP is necessary and sufficient for the stabilization of HSC70 monomer. Furthermore, binding of short peptides or permanently unfolded proteins to the peptide binding site of HSC70 promotes the dissociation of oligomers into monomers, suggesting that protein substrates are able to compete with HSC70 for the same binding site. Because the release of peptides or unfolded proteins from HSC70 has also been shown to require ATP binding, these results indicate that dissociation of oligomers is controlled by a mechanism similar to that of release of protein substrates and suggest that binding of HSC70 to itself occurs via the peptide binding site and mimics binding of HSC70 to protein substrates.

The correct folding of proteins is thought to be the result of a kinetic competition between a productive “on-pathway” leading to the native, biologically active, state and an abortive “off-pathway” leading to aggregation of incompletely folded intermediates (1–4). In vivo, the efficiency of the folding process depends on the presence of cellular factors such as molecular chaperones, which by transiently binding to unfolded or partially folded polypeptide chains prevent incorrect intra- and intermolecular interactions, thus favoring the productive pathway over aggregation (4–6). Among these factors, the 70-kDa heat shock cognate protein (HSC70), a constitutively expressed member of the highly conserved 70-kDa heat shock protein (HSP70) family, plays an essential role not only in protein folding but also in protein biosynthesis, assembly, transport, degradation, and signal transduction (for reviews see Refs. 6–9).

HSC70, purified originally as an ATPase that uncoats clathrin-coated vesicles (10–12), has a weak intrinsic ATPase activity (13, 14) but binds tightly to ATP and ADP (15–18). Upon binding to clathrin light chains, synthetic peptides, unfolded proteins, or other heat shock proteins such as DnaK homologs, HSC70 ATPase activity is stimulated 2–5-fold (13, 16, 19–28).

Limited proteolysis and mutagenesis studies have shown that the protein is made of two domains, a 44-kDa amino-terminal ATPase domain that binds and hydrolyzes ATP and a 30-kDa carboxyl-terminal domain involved in the binding of the protein substrates (29–33). Although the isolated amino-terminal fragment has been crystallized and its structure has been solved to a resolution of 2.2 Å (34), the three-dimensional structure of the entire protein is still unknown. Recently, the secondary structure topology of a subdomain (residues 385–543) belonging to the carboxyl-terminal domain, which has been shown to bind peptides (30), has been determined by NMR methods (35).

Previous studies have shown that HSC70 self-associates in solution to form dimers and trimers (10, 15, 16, 26, 36, 37). Consistent with these observations, oligomers of various sizes have been detected by electron microscopy (38). These studies, together with the fact that most other members of HSP70 family, such as the bacterial DnaK, plant HSC70, the bovine endoplasmic reticulum resident BiP, or the human heat shock-inducible HSP70, are also able to self-associate (39–46), suggest that self-association is related to function.

We therefore began to examine this general, well conserved, structural feature of the HSP70 protein family and in a first step analyzed the thermodynamic properties and mechanism of self-association by analytical ultracentrifugation. It appeared that HSC70 self-associates in solution in a reversible and likely unlimited fashion with a dissociation constant of about 5–10 μM (47). This finding was since corroborated by studies indicating that HSC70 is able to polymerize in the presence of yeast DnaJ and ATP (48). Although these studies reinforced the hypothesis of an involvement of self-association in HSC70 chaperone function, the relationship between self-association and central aspects of this function, such as ATP hydrolysis and protein substrate binding, has not been specifically addressed. In the present work, we investigate the effects of ATP and ATP analogs, as well as peptides and permanently unfolded proteins, on the monomer-oligomer equilibrium.

EXPERIMENTAL PROCEDURES

Materials—ATP was purchased from Sigma, and ADP, ATP-γ-S, and AMP-PNP were from Boehringer Mannheim. Beryllium sulfate...
Effect of Nucleotides and Peptides on HSC70 Self-association

Anion Chromatography and Analytical Ultracentrifugation—HSC70 was expressed and purified to homogeneity as described previously (26, 47). Reduced and carboxyl-methylated lactalbumin (RCMLA) as well as native lactalbumin were from Sigma. Peptide C with the carboxyl-terminal lysine replaced by a serine (KLIGVLSLFRPC) was synthesized and purified to more than 95% by Eurogentec (Seraing, Belgium).

Analysis of HSC70 Nucleotide Content by HPLC—Samples of pure HSC70 were treated with 0.6 M perchloric acid as described by Gao et al. (17). After centrifugation, the supernatant was loaded using a 50-μl sample loop onto an anion exchange synchropak AX300 column (synchro) equilibrated with 0.35 M KH₂PO₄, pH 3.5, 1.3 M NaCl. The column was run using a HPLC system from Beckman at a flow rate of 0.5 ml/min for a total elution time of 30 min, and absorbance at 260 nm was recorded.

Size Exclusion Chromatography—Size exclusion chromatography was carried out at room temperature, using an FPLC system (Pharmacia), on a Superose 12 (preparative grade) 10/30 column equilibrated with 20 mM Tris-HCl, pH 7.5, 100 mM KCl, and 1 mM β-mercaptoethanol. Elution was performed using the same buffer, fractions of 0.5 ml were collected at a flow rate of 0.2 ml/min, and absorbance was measured at 280 nm. Experiments in the presence of NaCl were performed using the same buffer except with 100 mM NaCl instead of KCl. The column was calibrated with high and low molecular weight calibration kit from Pharmacia. Chromatography in the presence of nucleotides or nucleotide analogs was performed with the same buffer, supplemented with the indicated nucleotides or nucleotide analogs at the indicated concentrations (see figure legends). For the experiments using BeF₃, the buffers were complemented with 5 mM NaF and 1 mM BeSO₄.

Evaluation of the proportion of each species relative to the total species was determined by deconvolution of the chromatographic profile and measure of the relative area under each peak, using the “Peak Fitting Module” included in “MicroCal Origin” software.

Sedimentation Velocity—Sedimentation velocity experiments were performed at 20 °C on a Beckman Optima XL-A analytical ultracentrifuge equipped with a Ti 60 titanium four-hole rotor with two-channel 12-mm path length centerpieces as described previously (47). Data analysis was performed using the computer programs XLAVEL and XLA-VELOC supplied by Beckman and Svedberg (49) provided by John Philo.

Sedimentation Equilibrium—Sedimentation equilibrium experiments were carried out at 4 °C using samples from various FPLC fractions at 11000 rpm. Protein concentration in each fraction was estimated to be 0.07 mg/ml for Fraction 1, 0.16 mg/ml for Fraction 2, and 0.13 mg/ml for Fraction 3. Radial scans of absorbance at 280 nm were taken at 2-h intervals, and samples were judged to be at equilibrium by the absence of systematic deviations in overlaid successive scans and when a constant weight average molecular weight was obtained in plots of molecular weight versus centrifugation time. Data was analyzed using the appropriate functions by nonlinear least-squares procedures provided in the Beckman Optima XL-A software package as described previously (47). A monomer molecular mass of 70,870 Da and a partial specific volume of 0.729 ml/g at 4 °C were calculated from amino acid composition.

RESULTS

Analysis of the Nucleotide Content of HSC70—The usual method for HSC70 purification involves an ATP elution step from ATP-agarose affinity columns. Previous studies have shown that after this step, bovine brain HSC70 is not nucleotide-free but has an ADP tightly bound to its active site and is consequently in the ADP form (15, 17, 51). Because the present study deals with the effects of nucleotides on the self-association properties of HSC70, the nucleotide content of the protein has been determined. The nucleotide extracted from HSC70 purified as described previously (26, 47) corresponds to ADP, and no trace of ATP was found (data not shown), indicating that the ATP bound to HSC70 during the affinity purification step has been entirely hydrolyzed, leaving the protein in the ADP form.

Analysis of the ADP-bound Form of HSC70 by Size Exclusion Chromatography and Analytical Ultracentrifugation—An FPLC profile of the ADP-bound form of HSC70 is shown in Fig. 1A. Two overlapping peaks are eluted with the included volumes of the column and correspond to species having the molecular mass of the monomer (about 70 kDa) and dimer and/or trimer (about 200–300 kDa). An additional peak, eluting with the exclusion volume of the column and corresponding to species having a molecular mass higher than 440 kDa, is also obtained. Analysis of relatively high and low protein concentrations also gives three peaks (data not shown). However, with high protein concentrations, an increase in the proportion of the high molecular mass species accompanied by almost a disappearance of the monomer peak is observed, and progressive dilution of the protein results in an increase in the monomeric species at the expense of the oligomeric species (47),

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indicating a concentration-dependent chemical equilibrium.

Next, the nature of the species present in each one of the three different peaks was assessed by analyzing fractions isolated from each peak and corresponding to elution volumes of 9.5 (Fraction 1), 11 (Fraction 2), and 13 ml (Fraction 3) (Fig. 1A). Each fraction was separated into two half-fractions. One half-fraction was immediately re-injected onto the column, whereas the other half was submitted to sedimentation velocity and sedimentation equilibrium. As shown in Fig. 1B, Fraction 1 elutes with the excluded volume of the column as a single sharp peak and thus contains species with molecular mass higher than 440 kDa. Fraction 2 elutes as a wide peak including a shoulder, corresponding to heterogeneous species with molecular masses of about 280 and 70 kDa (Fig. 1C). Fraction 3 elutes essentially as a single peak containing species of molecular mass of about 70 kDa (Fig. 1D). Even though the oligomeric species present in Fractions 1 and 2 were significantly diluted (Fig. 1, B and C), no dissociation into monomers was observed, indicating that interconversion between species in the equilibrium is very slow.

The average sedimentation coefficient as well as the weight average molecular weight for each of the fractions have been obtained by analytical ultracentrifugation. As shown in Table I, Fraction 1 gives an average sedimentation coefficient of $>20\, \text{S}$, whereas Fractions 2 and 3 sediment with coefficients of 6.73 and 4.46 $\text{S}$, respectively. Using bovine serum albumin as a molecular mass of about 67 kDa, and the relation ($S = 0.012 \, \text{M}^{1/2}$) the molecular masses given were obtained by fitting the equilibrium sedimentation data to a single ideal species.

Average sedimentation coefficient and weight average molecular weight of different FPLC fractions

| Fraction | Sedimentation Coefficient ($S$) | Weight Average Molecular Weight (Daltons) |
|----------|---------------------------------|-----------------------------------------|
| Fraction 1 | $>20$                            | 148,970 ± 3.066                          |
| Fraction 2 | 6.73 ± 0.02                      | 79,253 ± 1,244                           |
| Fraction 3 | 4.46 ± 0.01                      |                                          |

Effect of Nucleotides and Peptides on HSC70 Self-association Properties. HSC70 at 1 mg/ml (14 $\mu$M) was incubated for 30 min at 37°C without ATP (A, dotted line) or with 100 $\mu$M ATP (A, solid line), 100 $\mu$M AMP-PNP (B, solid line), 100 $\mu$M ATP-$\gamma$S (B, dotted line), or 1 mM ADP, BeF$_3$ (1 mM ADP, 5 mM NaF, 1 mM BeSO$_4$) (B, dashed line) and then immediately loaded on a Superose 12 column as described under "Experimental Procedures" except that the elution buffer contained the indicated nucleotide or analog. The molecular mass standards used are the same as those described in the legend to Fig. 1.

The slow nature of the ADP-HSC70 dimers into monomers is a rather slow phenomenon and suggest that these species are separated by a high energy barrier.

Effect of ATP and ATP Analogs on the Self-association Properties of HSC70—After analyzing the self-association properties of the protein in ADP, we turned to the study of the influence of ATP on the quaternary structure of the protein. This is of great importance in view of the fact that this nucleotide plays a crucial role in the chaperone function of HSC70, because the release of bound protein substrates during protein folding, assembly, or transport occurs only in its presence (6). As shown in Fig. 2A, in the presence of ATP, the protein elutes as a single sharp peak corresponding to monomeric species, the small peak eluting with the exclusion volume of the column being aggregated HSC70, as indicated by SDS-polyacrylamide gel electrophoresis (results not shown). Therefore, the presence of ATP is not only necessary for the release of bound protein substrates but also for complete dissociation of HSC70 oligomers into monomers. In view of the fact that ATP binding is sufficient for the dissociation of the polypeptide substrate from HSC70 (53), it was then of interest to know whether binding or hydrolysis of ATP is involved in the dissociation of oligomers into monomers. As shown in Fig. 2B, in the presence of ATP-$\gamma$S or AMP-PNP, HSC70 is essentially monomeric, indicating that ATP binding is sufficient for the dissociation of oligomers. Because ATP-$\gamma$S can still be hydrolyzed, albeit very slowly, a complex of ADP and a phosphate analog, BeF$_3$ (54), was used. As shown in Fig. 2B, a result identical to those reported for ATP-$\gamma$S or AMP-PNP is obtained, ruling out the possibility of slow hydrolysis of ATP analogs in the stabilization of HSC70 monomer. In fact, because HSC70 is already in the ADP-bound form, the use of BeF$_3$ alone is capable of monomer stabilization (Table II). Thus, exchange of the tightly bound ADP for ATP is sufficient for the dissociation of oligomers into monomers just as it is sufficient for the dissociation of bound polypeptides.
Effects of Monovalent Cations on HSC70 Self-association Properties—Dissociation of protein substrates from HSC70 upon ATP binding has been shown to occur only in the presence of K⁺ ions (53) but not with Na⁺. In the case of the dissociation of HSC70 oligomers into monomers, however, K⁺ ions do not appear to be absolutely required, because ATP binding is able to stabilize HSC70 monomer whether in the presence of K⁺ or Na⁺ (Table II). Nevertheless, in the presence of ATP analogs, dissociation of oligomers is complete only in the presence of K⁺ ions but not with Na⁺ (Table II), suggesting that ATP-K⁺ binding must be more efficient than ATP-Na⁺ to induce dissociation of oligomers.

Effects of Peptides and Unfolded Proteins on HSC70 Self-association Properties—As shown in Fig. 3, peptide C, a 13-residue peptide from vesicular stomatitis virus glycoprotein, which has been shown to bind to HSC70 with a dissociation constant of about 5–10 μM (21, 55), is able to induce the dissociation of HSC70 oligomers into monomers at a peptide/C:HSC70 concentrations ratio of 50:1. Binding of higher concentrations of peptide to obtain complete dissociation of oligomers was precluded by the low solubility of peptide C. However, a lower concentration of peptide resulted in a smaller effect, confirming the trend toward progressive oligomer dissociation upon the addition of increasing concentrations of peptide.

Replacement of peptide C by a polypeptide, RCMLA, a 14-kDa permanently unfolded protein, which is known to bind to HSC70 with high affinity (16), confirms the above results. As shown in Fig. 4, in the presence of RCMLA, a single peak eluting between the monomeric and dimeric peak of HSC70 (Fig. 4, compare A and B) and corresponding to the complex between HSC70 and RCMLA is obtained. Analysis of the collected fractions by SDS-polyacrylamide gel electrophoresis confirmed the tendency of HSC70 oligomers to dissociate into monomers in the presence of RCMLA (results not shown). Thus, RCMLA appears to promote the dissociation of HSC70 oligomers into monomers by forming a complex with the monomeric species. By contrast, native lactalbumin has no effect on the chromatographic profile (Fig. 4C).

**DISCUSSION**

In a previous investigation, the self-association properties of HSC70 have been studied by analytical ultracentrifugation and modeled as a monomer-dimer-trimer equilibrium with a dissociation constant of about 5–10 μM (47). The present work extends these studies by further characterizing this equilibrium and analyzing the effects of nucleotides and protein substrates.

The slow nature of the monomer-oligomer equilibrium described previously (47) gave an opportunity to isolate the respective HSC70 species by size exclusion chromatography and to characterize those species by analytical ultracentrifugation. The analysis of the isolated chromatographic peaks indicated the presence of species having average sedimentation coefficients (s) of 4.4, 6.7, and 20 S. The fact that these species could not be completely resolved by chromatography precluded further analysis to obtain hydrodynamical parameters for each of them, because each species in a given peak is slightly "contaminated" by the others, and only average s values could be determined. However the s values of 4.4 and 6.7 S are very close to the authentic s values for HSC70 monomer and dimer reported previously (47). These results are corroborated by sedimentation equilibrium analysis, which gives weight average molecular weights of 79,200 and 148,900 for the 4.4 and 6.7 S species, respectively, indicating the presence of a monomer and a dimer of HSC70. Thus, analysis of the nature of the HSC70 species, based on isolated chromatographic peaks as made in this work or on HSC70 preparation as a whole as presented in previous work (47), gives similar results and indicates that the first step in HSC70 self-association is dimerization.

Although in our previous study HSC70 was thought to be in the ADP-bound form, the nature of the nucleotide that was effectively bound to the protein was not ascertained. We show here that the protein exhibiting the self-association properties is indeed in the ADP-bound form, in agreement with reports by Schmid et al. (15) and Gao et al. (17, 51), indicating that after purification on ATP-agarose affinity columns, an ADP molecule remains tightly bound to the active site. This ADP-bound form of HSC70 exists as a slow, concentration- and temperature-dependent monomer-oligomer equilibrium. However, self-association does not seem to be the appanage of this form only, because ADP-free HSC70 (51, 56) as well as the isolated carboxyl-terminal domain, which has no ATP binding site, do self-associate in solution in a fashion similar to that of ADP-HSC70. Thus, the strong tendency of HSC70 monomer to oligomerize is not the result of a conformational change induced by ADP binding but rather the consequence of natural interactions between self-recognizing structural features that preexist ADP binding and that partition the protein into monomeric and oligomeric species in a concentration-dependent fashion. Nevertheless, ADP binding is able to shift the equilibrium toward the oligomeric species by stabilizing the oligomeric forms (40).

Likewise, dissociation of the HSC70 oligomers into monomers is not obtained solely in the presence of ATP but also in its absence, because dilution of the protein or increase in temper-

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2 N. Benaroudj, B. Fouchaq, and M. M. Ladjimi, manuscript in preparation.
nature lead to the dissociation of oligomers, indicating that this process is not due to an ATP-induced conformational change but rather to a stabilization of the monomeric structure that exists otherwise in a slow equilibrium with the oligomeric species.

Altogether, these results suggest that whether nucleotide-bound or nucleotide-free, HSC70 should exist as an equilibrium between monomeric and oligomeric species, the role of nucleotides being the stabilization of alternative “quaternary” structures, shifting the equilibrium toward one structure or the other.

Stabilization of the monomeric form by ATP is not due to hydrolysis but to binding of this nucleotide, because all the ATP analogs tested were able to stabilize the monomeric form. Most importantly, the fact that stabilization of HSC70 monomer occurs also in the presence a complex between ADP and a phosphate analog, BeF$_3$ (54), strongly suggests that oligomerization should occur after phosphate release and conversion of the protein into the ADP form.

The dissociation of HSC70 oligomers following ATP binding as shown in this study is reminiscent of the dissociation of HSC70 from target proteins upon ATP binding. It is now established indeed that release of unfolded protein substrates from the chaperone requires ATP binding but not hydrolysis (53). Therefore, dissociation of HSC70 from itself or from a protein substrate could be viewed as being the two sides of the same coin. By mimicking a substrate protein, HSC70 would bind to itself, as if it was an unfolded protein, and self-assemble. Binding of ATP would lead to the release of HSC70 bound just like it leads to the release of an unfolded protein substrate.

From this work, it also appears that HSC70 self-association is reversed by the binding of peptide or unfolded protein. The fact that both peptide C as well as RCMLA, which bind to the peptide binding site of HSC70, are able to promote dissociation of HSC70 oligomers, is suggestive of a competition between HSC70 and protein substrates for the same binding site. Moreover, the observation that dissociation of HSC70 oligomers occurs only upon specific binding of unfolded RCMLA but not native lactalbumin indicates that RCMLA induced dissociation of HSC70 oligomers is mediated via the peptide binding site of HSC70.

An important aspect of the chaperone function of the HSP70 protein family is the relationship between cycles of ADP/ATP binding and hydrolysis and cycles of unfolded protein binding and release. In most of the studies, HSP70 have been dealt with as a monomer, and in fact, HSP70 monomer was deliberately separated from oligomers in a last “purification” step and used within hours (53, 56, 57). Thus, the current paradigm of the chaperone function of the HSP70 protein family takes only the monomer into account and is based upon the existence of two monomeric forms differing in terms of conformation and the nature of the bound nucleotide: an ADP-bound form having high affinity for protein substrates but also characterized by slow association/dissociation rates and an ATP-bound form with low affinity for substrates and high on and off rates (55, 57, 58). Binding of substrates, thought to occur with the ATP form, triggers ATP hydrolysis and converts the protein to the ADP form, which has a high affinity for substrates (55, 58). Exchange of ADP for ATP switches back the protein to the low affinity form, thus leading to the release of the substrate protein and making it ready for another binding and release cycle (53, 55).

However, in this functional cycle, the intrinsic structural properties of the protein and its strong tendency to self-associate as shown in this work and previously (47) are not taken into account. Incorporating these data leads to the following model: HSP70 proteins exist as an equilibrium between two quaternary states, an oligomeric one, in which the protein binds to itself through the peptide binding site, and a monomeric one, where this site is either free or bound to another region of the protein. This equilibrium is regulated by nucleotides, protein substrates, and co-chaperones (Fig. 5). Because HSP70 binds to itself as if it were an unfolded protein, ADP should stabilize
this interaction, thereby shifting monomer-oligomer equilibrium toward the oligomeric state. Stabilization of the oligomeric state by ADP, as postulated in this model, has been shown to occur in the case of DnaK (40). By contrast, ATP, which decreases the stability of the chaperone-unfolded protein complex, would decrease the stability of the oligomeric state of HSP70, thus leading to dissociation of oligomers into monomers as shown in the present work. Unfolded protein substrates, in competition with HSP70 for the same binding site, would reverse the oligomerizing effect of HSP70 and shift the equilibrium toward the monomer. Furthermore, DnaJ homologs, which are known to stimulate HSP70 ATPase activity, would shift the equilibrium toward the ADP-bound state, which, as demonstrated in this work, is able to self-associate. In fact, King et al. (48) have recently shown that indeed HSC70 polymerizes in the presence of ATP and DnaJ. Likewise, GrpE homologs, which stimulate the ADP to ATP exchange, should shift the equilibrium toward the monomer. This has also recently been shown to be the case for DnaK by Schoenfeld et al. (45).

Thus, in contrast to previous functional models, the model presented in this paper emphasizes the importance of self-association in the regulation of the HSP70 chaperone function and postulates that the chaperone activity of HSP70 protein family is regulated through stabilization of alternative quaternary states not only by nucleotides but also by HSP70 itself and the co-chaperones.

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REFERENCES

1. Orsini, G., and Goldberg, M. E. (1978) J. Biol. Chem. 253, 3453–3458
2. Zettlmeissl, G., Rudolph, R., and Janicke, R. (1979) Biochemistry 18, 5567–5571
3. Kiefhaber, T., Rudolph, R., Kohler, H. H., and Buchner, J. (1991) Bio/Technology 9, 825–829
4. Randall, L. L., and Hardly, J. S. (1995) Trends Biochem. Sc. 20, 65–69
5. Buchner, J., and Kiefhaber, T. (1991) Biochemistry 30, 1586–1591
6. Hendrick, J. P., and Hartl, F. U. (1991) Annu. Rev. Biochem. 60, 349–384
7. Craig, E. A., Baxter, B. K., Becker, J., Halladay, J., and Ziegelhoffer, T. (1994) in ‘The Biology of Heat Shock Proteins and Molecular Chaperones’ (Morimoto, R. I., ed.) pp. 31–52, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
8. Rutherford, S. L., and Zucker, C. S. (1994) Cell 79, 1129–1132
9. Bohen, S. P., Kral, A., and Yamamoto, K. R. (1995) Science 268, 1303–1304
10. Schlossman, D. M., Schmid, S. L., Braell, W. A., and Rothman, J. E. (1984) J. Cell Biol. 99, 723–733
11. Ungewickell, E. (1985) EMBO J. 4, 3385–3391
12. Chappell, T. G., Welch, W. J., Schlossman, D. M., Palter, K. B., Schlesinger, M. J., and Rothman, J. E. (1986) Cell 45, 3–13
13. Sadis, S. E., and Hightower, L. C. (1992) Biochemistry 31, 9406–9412
14. Willanks, S. M., DeLuca-Flaherty, C., and McKay, D. B. (1994) J. Biol. Chem. 269, 12893–12898
15. Schmid, S. L., Braell, W. A., and Rothman, J. E. (1985) J. Biol. Chem. 260, 10057–10062
16. Palleros, D. R., Welch, W. J., and Fink, A. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5719–5723
17. Gao, B., Emoto, Y., Greene, L., and Eisenberg, E. (1993) J. Biol. Chem. 268, 8507–8513