Chaperone Properties of Bacterial Elongation Factor EF-G and Initiation Factor IF2*

(Received for publication, June 7, 1999, and in revised form, October 14, 1999)

Teresa Caldas, Soumaya Laalami, and Gilbert Richarme

From the Biochimie Génétique, Institut Jacques Monod, Université Paris 7, 2 place Jussieu, 75005 Paris, and ESA031 du CNRS, Institut de Biologie Moléculaire et d'Ingénierie Génétique, Université de Poitiers, 40 ave. du Recteur Pineau, 86022 Poitiers, France

The Journal of Biological Chemistry Vol. 275, No. 2, Issue of January 14, pp. 855–860, 2000

The elongation phase of protein synthesis is promoted by two G proteins, elongation factor EF-Tu, which delivers aminoacyl tRNAs to the ribosome, and EF-G, which catalyzes the translocation step, during which the A- and P-site tRNAs move to the P and E sites of the elongating ribosome, respectively, and mRNA is advanced by one codon (1–3). EF-G binds to the ribosome in its GTP form, hydrolyzes GTP to drive tRNA movement on the ribosome (4), and is released in its GDP form. The functional cycle is completed upon GDP release and reactivation of the empty factor by binding of a GTP molecule (1–3).

EF-G and IF2 are involved in protein folding and protein renaturation after stress. They prevent the aggregation of citrate synthase under heat shock conditions, and they form stable complexes with unfolded proteins such as reduced carboxymethyl α-lactalbumin. Furthermore, the EF-G and IF2-dependent renaturations of citrate synthase are stimulated by GTP, and the GTPase activity of EF-G and IF2 is stimulated by the permanently unfolded protein, reduced carboxymethyl α-lactalbumin. The concentrations at which these chaperone-like functions occur are lower than the cellular concentrations of EF-G and IF2. These results suggest that EF-G and IF2, in addition to their role in translation, might be implicated in protein folding and protection from stress.

*This work was supported by Grant 96N88/0006 "Physique et Chimie du Vivant" from the CNRS (to G. R.), by the "Programme de recherches fondamentales en microbiologie et maladies infectieuses et parasitaires," Ministère de l’Éducation Nationale, de la Recherche et de la Technologie, and by Fundação da ciência e tecnologia Grant PRAXIS/BD/13898/97 (to T. C.). 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.: 33 01 44 27 50 98; Fax: 33 01 44 27 35 80; E-mail: richarme@ccr.jussieu.fr.

‡ From the Biochimie Génétique, Institut Jacques Monod, Université Paris 7, 2 place Jussieu, 75005 Paris, and ESA031 du CNRS, Institut de Biologie Moléculaire et d’Ingénierie Génétique, Université de Poitiers, 40 ave. du Recteur Pineau, 86022 Poitiers, France

§ To whom correspondence should be addressed. Tel.: 33 01 44 27 50 98; Fax: 33 01 44 27 35 80; E-mail: richarme@ccr.jussieu.fr.

§ This work was supported by Grant 96N88/0006 "Physique et Chimie du Vivant" from the CNRS (to G. R.), by the "Programme de recherches fondamentales en microbiologie et maladies infectieuses et parasitaires," Ministère de l’Éducation Nationale, de la Recherche et de la Technologie, and by Fundação da ciência e tecnologia Grant PRAXIS/BD/13898/97 (to T. C.). 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.

Viewpoint Citation

EXPERIMENTAL PROCEDURES

Materials—Citrate synthase (from porcine heart), α-glucosidase (from yeast), BPTI, R-CMLA, bovine serum albumin, ovalbumin, lysozyme, and all other chemicals, including GTP, GDP, and GTPγS, were from Sigma and were reagent grade. DEAE-Sepharose and thiol-Sepharose resins were from Amersham Pharmacia Biotech, and hydroxylapatite (Bio-Gel HTP) was from Bio-Rad.

Purification of EF-G, IF2, and DnaK—EF-G was purified by covalent chromatography on thiol Sepharose (24). Crude extracts from the E. coli K12 strain C600 (leuB6 thi-1 thr-1 supE44) were prepared by a lysozyme/EDTA method (25). EF-G was purified by DEAE-Sepharose chromatography with column buffer (20 mM Tris, pH 8.0, 0.2 mM EDTA, 1 mM dithiothreitol, 10% glycerol) and elution with a linear 0–0.35 M

This paper is available on line at http://www.jbc.org
Purified protein was dialyzed against 20 mM Tris, pH 7.4, 100 mM KCl, and concentrated by ultrafiltration. EF-G was more than 98% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Initiation factor 2 was purified according to Luchin et al. (8). The purified protein was dialyzed against 20 mM Tris, pH 7.4, 100 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol. DnaK was prepared as described previously (26) from an overproducing strain of E. coli bearing plasmid pLNA2 derived from plasmid pDM38 (27) (a gift from Dr. O. Fayet, Microbiology et Génétique, Microbiologie CNRS, Toulouse, France).

Refolding of Citrate Synthase and α-Glucosidase—Denaturation and renaturation reactions were carried out at 25 °C. For both proteins, renaturation was initiated by pouring the renaturation solution onto the unfolded protein, under vortex agitation, in Eppendorf polyethylene tubes. Citrate synthase was denatured at a concentration of 10 μM in 8 M urea, 50 mM Tris-HCl, 2 mM dithiothreitol, 100 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol. DnaK was prepared as described and diluted 100-fold in 40 mM Hepes, 50 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM potassium acetate, pH 8.0. The enzymatic activity of citrate synthase was measured as described (28). α-Glucosidase was denatured at a concentration of 3 μM in 8 M urea, 0.1 mM potassium phosphate, 2 mM dithiothreitol, 20 mM dithiothreitol, pH 7.0 for 15 min. Renaturation was initiated by a 30-fold dilution in 40 mM Hepes-KOH, pH 7.5 at 20 °C. The enzymatic activity of α-glucosidase was measured as described (28). Citrate synthase renaturation in the presence of nucleotides was done as described above (unless otherwise indicated) in the presence of 150 μM GDP, GTP, or GTPyS and 200 μM MgCl₂. The effect of nucleotides alone on citrate synthase renaturation was low (GTP and GTPyS) or negligible (GDP) and was subtracted.

Thermal Aggregation of Citrate Synthase—The native enzyme (80 μM) was diluted 100-fold in 40 mM Hepes, 50 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM potassium acetate, pH 8.0 at 43 °C in the absence of added proteins or in the presence of DnaK, EF-G, or IF2. Citrate synthase aggregation was monitored by measuring the absorbance at 650 nm as described in Ref. 28.

Size Exclusion Chromatography—For binding assays of R-CMLA and unfolded BPTI and EF-G, IF2, and DnaK, gel permeation columns (Bio-Gel P-200 from Bio-Rad for studies with R-CMLA or Sephadex G-75 from Amersham Pharmacia Biotech for studies with BPTI) were equilibrated with column buffer containing 50 mM Tris-HCl (pH 8.2 for studies with R-CMLA and pH 7.4 for studies with BPTI), 50 mM KCl, 1 mM dithiothreitol, 100 μg/ml bovine serum albumin. Reaction mixtures containing EF-G, IF2, or DnaK and radio-labeled native BPTI, unfolded BPTI, or R-CMLA at indicated concentrations were incubated for 20 min at 23 °C in column buffer without serum albumin and applied to the column at room temperature. Fractions were collected at a flow rate of 1 drop/fraction or 30 s and counted for radioactivity. DNAK was incubated for 3 h at 37 °C before use. Unfolded BPTI was prepared as described previously from native BPTI (29). Unfolded BPTI, native BPTI, and R-CMLA were [H]-labeled by reductive methylation (30).

EF-G and IF2 GTpase Assays—4 pmol of purified EF-G in a reaction mixture (2.5-μl volume) containing 20 mM Tris-HCl, pH 7.8, 10 mM MgCl₂, 75 mM NH₄Cl, 1 mM dithiothreitol was incubated for 50 min at 25 °C with 0.5 μl of 750 μM [3H]GTP and 1.5 μM of R-CMLA at the final concentrations indicated in Fig. 3 (31). 1 pmol of purified IF2 in a reaction mixture (2.5-μl volume) containing 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 100 mM NH₄Cl, 10 mM 2-mercaptoethanol was incubated for 20 min at 25 °C with 0.5 μl of 750 μM [3H]GTP and 1.5 μM of R-CMLA at the final concentrations indicated in Fig. 7 (8). The reactions were terminated by applying 3 μl of each sample to a polyethyleneimine cellulose thin layer chromatography plate (29). [3H]-labeled GTP was from Amersham Pharmacia Biotech and was used at 1 Ci/nmol. The specific activities of EF-G and of IF2 were similar to those reported in Refs. 8 and 31.

RESULTS

EF-G Increases the Amount of Correctly Folded Citrate Synthase and α-Glucosidase—We first investigated whether EF-G acts as a molecular chaperone in the folding of proteins. Citrate synthase and α-glucosidase, whose refolding is facilitated by several chaperones such as GroEL, DnaK, hsp90, and small hsp's (28, 32–34), were chosen as substrates for this reaction. They were unfolded in the presence of 8 M urea, and allowed to refold upon dilution of the denaturant, in the absence or in the presence of EF-G (protein folding in the presence of DnaK was studied in parallel). Under our experimental conditions, the refolding yield of 0.1 μM citrate synthase was increased from 7% in the absence of added proteins to 21% in the presence of 5 μM EF-G and 31% in the presence of 3 μM DnaK (Fig. 1A). The dependence of citrate synthase refolding on the concentration of EF-G is shown in Fig. 1B. The maximal recovery of citrate synthase activity reaches 24% in the presence of 10 μM EF-G, and half-maximal refolding occurs at 2 μM EF-G, a concentration slightly higher than that of DnaK (around 1 μM) required for half-maximal refolding of citrate synthase in similar conditions (not shown and Ref. 34). The EF-G concentration required for half-maximal refolding of citrate synthase is somewhat higher than the concentration of citrate synthase but is lower than the concentrations of EF-G in the cytoplasm (around 20 μM) (3, 35). As reported previously (34), other proteins such as ovalbumin and lysozyme were unable to stimulate citrate renaturation, whereas serum albumin could stimulate it to some extent (not shown). In similar experiments, the refolding of 0.1 μM α-glucosidase was increased from 6% in the absence of added protein to 18% in the presence of 2 μM EF-G and 26% in the presence of 2 μM DnaK (not shown). These results suggest that, like molecular chaperones, EF-G interacts with unfolded proteins and increases their productive folding.

Effect of Nucleotides on the EF-G-dependent Refolding of Citrate Synthase—Elongation factor G belongs to the GTPase superfamily of proteins whose functional cycle includes at least four major conformational states, the nucleotide-free state, the
The GTPase activity of EF-G (Fig. 3), whose $k_{cat}$ at 25 °C (calculated from the results shown in Fig. 3) rises from 0.002 min$^{-1}$ in the absence of added protein, to 0.02 min$^{-1}$ in the presence of 15 μM R-CMLA. The $K_m$ of the stimulation of the EF-G GTPase by R-CMLA is around 5 μM. The stimulation of the EF-G GTPase by an unfolded protein is reminiscent of that of the DnaK ATPase, whose $k_{cat}$ rises from 0.04 min$^{-1}$ in the absence of a peptide substrate to 0.16 min$^{-1}$ in its presence (37).

**EF-G Protects Citrate Synthase from Irreversible Aggregation during Thermal Stress**—We investigated the function of EF-G under heat shock conditions. As reported previously (28, 32, 34), citrate synthase loses its native conformation and undergoes aggregation during incubation at 43 °C. The addition of EF-G (3 μM) or DnaK (2 μM) partially reduces citrate synthase (0.8 μM) aggregation, whereas both 5 μM DnaK and 8 μM EF-G suppress citrate synthase aggregation (Fig. 4). In contrast, the addition of up to 35 μM bovine serum albumin (Fig. 4), ovalbumin, or lysozyme (not shown and Ref. 34) does not protect citrate synthase from thermal aggregation. Thus, EF-G is nearly as efficient as DnaK and other chaperones (28, 34) in protecting citrate synthase from thermal denaturation and is much more efficient than other proteins such as bovine serum albumin, ovalbumin, or lysozyme. Furthermore, the concentration of EF-G required for an efficient thermal protection of citrate synthase (around 5 μM) is several fold lower than its cellular concentration (around 20 μM).

**Interaction between EF-G and Unfolded Proteins**—One characteristic of molecular chaperones is their preferential interaction with unfolded proteins (21–23). R-CMLA, a permanently unfolded protein that maintains an extended conformation without any stable secondary structure in the absence of denaturant, strongly interacts with several chaperones, including DnaK (34, 36). Complex formation between R-CMLA (20,000 Da) and EF-G (82,000 Da) was analyzed by gel filtration on a Bio-Gel P-200 column. When R-CMLA (2 μM) is filtered in the presence of EF-G (10 μM), 14% of R-CMLA fractionates as a higher molecular weight complex than R-CMLA alone (Fig. 5). The interaction between EF-G and R-CMLA is not significantly different from that observed between R-CMLA and DnaK, 10 μM DnaK binds 33% of R-CMLA (not shown). Thus, EF-G seems to interact strongly with R-CMLA in a manner similar to that of DnaK. Unfolded BPTI is known to interact with chaperones, including DnaK (34, 38). Complex formation between 1 μM unfolded BPTI (6,000 Da) and 10 μM EF-G (82,000 Da) was studied by gel filtration on a Sephadex G-75 column; a significant percentage (39%) of unfolded BPTI fractionates as a higher molecular weight complex than unfolded BPTI alone (Fig. 6).

In similar conditions 4 μM DnaK retained 27% of unfolded BPTI (Fig. 6). In contrast, when 1 μM native BPTI and 10 μM EF-G were loaded on the gel permutation column, native BPTI did not elute as a high molecular weight complex (Fig. 6). When similar experiments were carried out with bovine serum albumin (30 μM) or ovalbumin (30 μM), unfolded BPTI did not elute as a high molecular weight complex (not shown). Thus, EF-G,

**Stimulation of the GTPase Activity of EF-G by an Unfolded Protein**—In the absence of ribosomes, EF-G promotes very little GTP hydrolysis ($k_{cat}$ lower than 0.002 min$^{-1}$ at 30 °C). The EF-G GTPase is efficiently stimulated by ribosomal particles ($k_{cat} = 20$ min$^{-1}$) and, to a lesser extent, by several aliphatic alcohols such as 2-propanol ($k_{cat} = 0.009$ min$^{-1}$ in the presence of 20% 2-propanol) (31). R-CMLA is a permanently unfolded protein that maintains an extended conformation without any stable secondary structure in the absence of denaturant (36). It interacts with chaperones, and it stimulates their ATPase activity (23, 34, 36). R-CMLA stimulates 10-fold the GTPase activity of EF-G (Fig. 3), whose $k_{cat}$ at 25 °C (calculated from the results shown in Fig. 3) rises from 0.002 min$^{-1}$ in the presence of 20% 2-propanol) (31). R-CMLA is a permanently unfolded protein that maintains an extended conformation without any stable secondary structure in the absence of denaturant (36). It interacts with chaperones, and it stimulates their ATPase activity (23, 34, 36). R-CMLA stimulates 10-fold the GTPase activity of EF-G (Fig. 3), whose $k_{cat}$ at 25 °C (calculated from the results shown in Fig. 3) rises from 0.002

**Chaperone Properties of EF-G and IF2**

**Fig. 2. Influence of GTP, GDP, and GTPγS on the EF-G-dependent refolding of citrate synthase.** Citrate synthase was denatured in urea and subsequently renatured for 20 min by dilution of the denaturant, as described under “Experimental Procedures,” in the presence of nucleotides. A, 0.1 μM citrate synthase was renatured in the presence of 2 μM EF-G, 200 μM MgCl$_2$, and 150 μM GDP or GTPγS (GTP-gamma-S). B, 0.1 μM citrate synthase was renatured in the presence of 0.1 μM EF-G and 200 μM MgCl$_2$ and in the absence or presence of 150 μM GTP or GDP.

In similar conditions 4 μM DnaK retained 27% of unfolded BPTI (Fig. 6). In contrast, when 1 μM native BPTI and 10 μM EF-G were loaded on the gel permutation column, native BPTI did not elute as a high molecular weight complex (Fig. 6). When similar experiments were carried out with bovine serum albumin (30 μM) or ovalbumin (30 μM), unfolded BPTI did not elute as a high molecular weight complex (not shown). Thus, EF-G,
like molecular chaperones, interacts preferentially with unfolded proteins.

**Chaperone Properties of IF2**—Citrate synthase was unfolded in the presence of 8 M urea and allowed to refold upon dilution of the denaturant in the absence or in the presence of IF2 (under conditions similar to those described for the experiment represented by Fig. 1 for EF-G). The refolding yield of 0.1 mM citrate synthase was increased from 6% in the absence of IF2 to 20% in the presence of 1 mM IF2 (Fig. 7A). Half-maximal reactivation of citrate synthase occurs at 0.15 μM IF2 (Fig. 7A). Thus, IF2 stimulates the refolding of citrate synthase with a similar efficiency as EF-G and is efficient at lower concentrations. In similar experiments, the refolding of 0.1 mM α-glucosidase was increased from 7% in the absence of added protein to 15% in the presence of 1 mM IF2 (not shown).

We investigated the ability of IF2 to protect citrate synthase from irreversible aggregation during thermal stress under con-
ditions similar to those described for the experiment represented by Fig. 4 for EF-G. IF2 (3 μM) reduces citrate synthase (0.8 μM) aggregation at 43 °C with an efficiency that is similar to that of 3 μM EF-G (compare Figs. 7B and 4).

We also studied the interaction of IF2 with unfolded proteins. The interaction of IF2 with unfolded BPTI is shown in Fig. 7C. Complex formation between 1 μM unfolded BPTI (6,000 Da) and 5 μM IF2 (97,000 Da) was studied by gel filtration on a Sephadex G-75 column, under conditions similar to those described for the experiment represented by Fig. 6. A significant percentage (32%) of unfolded BPTI fractionates as higher molecular weight material than unfolded BPTI alone (Fig. 7C). In contrast, when 1 μM native BPTI and 5 μM IF2 were loaded on the gel permeation column, native BPTI did not elute as a high molecular weight complex (not shown). Similar experiments with IF2 and the permanently unfolded protein R-CMLA showed a strong interaction between the initiation factor and R-CMLA (not shown). Thus, IF2, like EF-G and molecular chaperones, interacts preferentially with unfolded proteins.

We have shown that R-CMLA can stimulate the GTPase activity of EF-G. IF2 possesses a GTPase activity that has been reported to be totally dependent on the presence of the ribosome (5, 8). We measured the GTPase activity of IF2 in the presence of several concentrations of R-CMLA (in the absence of ribosomes). The IF2 GTPase activity, which is undetectable in the absence of ribosomes, as reported by others, is stimulated by R-CMLA with a Kₐ of around 15 μM and a kₐ of 0.4 min⁻¹ at 25 °C (calculated from the results shown in Fig. 7D). This activity is similar to that of IF2 in the presence of ribosomes (kₐ = 0.8 min⁻¹ at 37 °C). In accordance with the stimulation of the IF2 GTPase by an unfolded protein, the renaturation of 0.1 μM unfolded citrate synthase by 0.1 μM IF2 was stimulated 1.7-fold by 1 mM GTP (not shown).

**DISCUSSION**

We present biochemical evidence suggesting that EF-G and IF2 play a chaperone-like function in protein folding, protection against thermal denaturation, and interaction with unfolded proteins. EF-G and IF2 increase approximately 3-fold the yield of citrate synthase and α-glucosidase renaturation, as do molecular chaperones. The stimulation factors of protein renaturation (more than 3-fold) and EF-G or IF2 concentrations required for half-maximal protein renaturation (2 μM and 0.15 μM, respectively) are not significantly different from those obtained with DnaK, hsp90, or small hsps (this study and Refs. 28, 34). They are in the same range as the cytoplasmic small hsp (expressed as monomers) required for a similar protection (28, 34). The efficient protection of citrate synthase from thermal denaturation afforded by EF-G and IF2 suggests that during heat shock these translation factors might contribute to a reservoir of chaperones and chaperone-like molecules that serve as a chaperone-buffer in preventing the aggregation of non-native proteins until permissive renaturation conditions are restored. Finally, the chaperone properties of EF-Tu (17, 18), EF-G, and IF2 (this study) and the protein disulfide isomerase activity of EF-Tu (19) suggest that translation factors are ancestral protein folding factors that appeared before dedicated chaperones and protein disulfide isomerases.

**Acknowledgments**—The authors thank Dr. M. Kohiyama for constant support throughout this work, Dr. O. Fayet (Laboratoire de Microbiologie et Génétique Moléculaire, CNRS, Toulouse, France) for the DnaK/DnaJ hyperproducing strain, Dr. A. Parmeggiani (Ecole Polytechnique, Palaiseau, France) for critical reading of the manuscript, and A. Kropfner for corrections of the English language.

**REFERENCES**

1. Miller, D. L., and Weisbech, H. (1977) in Molecular Mechanisms of Protein Biosynthesis (Pestka, S., and Weisbech, H., eds) pp. 323–373, Academic Press, New York.
2. Brut, N. (1977) in Molecular Mechanisms of Protein Biosynthesis (Pestka, S., and Weisbech, H., eds) pp. 375–411, Academic Press, New York.
3. Kurland, C. G., Hughes, D., and Ehrenberg, H. (1995) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., ed.) pp. 979–1004, American Society for Microbiology, Washington, D. C.
4. Rodnina, M. V., Savelieberg, A., Katunin, V. I., and Wintermeyer, W. (1997) Nature 385, 37–41.
5. Hershey, J. W. B. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 613–647, American Society for Microbiology, Washington, D. C.
6. Grunberg-Manago, M. (1996) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., and Beckwith, W. S. Riley, M., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 1429–1457, American Society for Microbiology, Washington, D. C.
7. Harty, D., McPeethers, D. S., and Geld, L. (1990) Genes Dev. 3, 1899–1912.
8. Luchin, S., Putzer, H., Hershey, J. W. B., Cenatiempo, Y., Grunberg-Manago, M., and Laalami, S. (1999) J. Biol. Chem. 274, 6774–6779.
9. Wool, I. G. (1996) Trends Biochem. Sci. 21, 164–165.
10. Travers, A. A., Debenham, P. G., and Pongs, O. (1980) Biochemistry 19, 1651–1656.
14. Shiina, N., Gotoh, Y., Kubomura, N., Iwamatsu, A., and Nishida, E. (1994) Science 266, 282–285
15. Bektas, M., Nurten, R., Gürel, Z., Sayers, Z., Bernek, E. (1994) FEBS Lett. 356, 89–93
16. Gonen, H., Smith, C. E., Siegel, N. R., Kahana, C., Merrick, W. C., Chakraburty, K., Schwartz, A. L., and Ciechanover, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7648–7652
17. Kudlicki, W., Coffman, A., Kramer, G., and Hardesty, B. (1997) J. Biol. Chem. 272, 32206–32210
18. Caldás, T. D., El Yaagoubi, A., and Richarme, G. (1998) J. Biol. Chem. 273, 11478–11482
19. Richarme, G. (1998) Biochem. Biophys. Res. Commun. 252, 156–161
20. Kudlicki, W., Coffman, A., Kramer, G., and Hardesty, B. (1997) Fold. Des. 2, 101–108
21. Ellis, R. J., and Hemmingsen, S. M. (1989) Trends Biochem. Sci. 14, 339–342
22. Georgopoulos, C., Liberek, K., Zylicz, M., and Ang, D. (1994) in The Biology of the Heat Shock Proteins and Molecular Chaperones (R. I. Morimoto, A. Tissieres, and C. Georgopoulos, eds) pp. 209–250, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Hendrick, J. P., and Hartl, F. U. (1993) Annu. Rev. Biochem. 62, 349–384
24. Dantas Caldás, T., El Yaagoubi, A., Kohiyama, M., and Richarme, G. (1998) Protein Expression Purif. 14, 65–70
25. Cull, M., and McHenry, C. S. (1990) Methods Enzymol. 182, 147–153
26. Zylicz, M., and Georgopoulos, C. (1984) J. Biol. Chem. 259, 8820–8825
27. Missiakas, D., Georgopoulos, C., and Raina, S. (1993) J. Bacteriol. 175, 2616–2624
28. Jakob, U., Gaestel, M., Engel, K., and Buchner, J. (1993) J. Biol. Chem. 268, 1517–1520
29. Richarme, G., and Kohiyama, M. (1993) J. Biol. Chem. 268, 24074–24077
30. Langer, T., Pfeifer, G., Martin, J., Staubeinstein, W., and Hartl, F. U. (1992) EMBO J. 11, 4757–4765
31. De Vendittis, E., Masullo, M., and Bocchini, V. (1986) J. Biol. Chem. 261, 4445–4450
32. Wiech, H., Buchner, J., Zimmermann, R., and Jakob, U. (1992) Nature 358, 169–170
33. Buchner, J., Schmidt, M., Fuchs, M., Jaenicke, R., Rudolph, R., Schmid, F., and Kiehhaber, T. (1991) Biochemistry 30, 1586–1591
34. Richarme, G., and Caldás, T. D. (1997) J. Biol. Chem. 272, 15607–15612
35. Weijland, A., Harmark, K., Cool, R. H., Anborgh, P. H., and Parmeggiani, A. (1992) Mol. Microbiol. 6, 683–688
36. Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer M. K., and Hartl, F. U. (1992) Nature 358, 383–392
37. Burkholder, W. F., Panagiotidis, C. A., Silverstein, S. J., Cegielska, A., Gottesman, M. E., and Gattanaris, G. A. (1994) J. Mol. Biol. 242, 374–367
38. Liberek, K., Skowyra, D., Zylicz, M., Johnson, C., and Georgopoulos, C. (1991) Proc. Natl. Acad. Sci. U. S. A. 22, 14491–14496
39. Arai, N., Arai, K., and Kaziro, Y. (1975) J. Biochem. (Tokyo) 78, 243–246
40. Hendrick, J. P., Langer, T., Davis, T. A., Hartl, F. U., and Wiedman, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10216–10220
41. Valent, Q. A., Kendall, D. A., High, S., Kusters, R., Oudega, B., and Luijink, J. (1995) EMBO J. 14, 5404–5505
42. Valent, Q. A., de Gier, J. W. L., von Heijne, G., ten Hagenjogman, C. M., Oudega, B., and Luijink, J. (1997) Mol. Microbiol. 25, 53–64
43. Hesterkamp, T., Hauser, S., Lütke, H., and Bukau, B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4437–4441
44. Shibata, T., Fujii, Y., Nakamura, Y., Nakamura, K., and Yamane, K. (1996) J. Biol. Chem. 271, 13162–13168
45. Hardesty, B., Tsalkova, T., and Kramer, G. (1999) Curr. Opin. Struct. Biol. 9, 111–114

Chaperone Properties of EF-G and IF2