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should be, if **Ia**(sr) is correctly calculated, the fraction (probability) observed for these residues in the data base *irrespective* of the protein. And so for all calculated fractions and the four predicted conformations, this is effectively observed. Besides an unlikely compensation between **Ia**(sr) and **Ia**(lr|sr) for all the calculated fractions, **Ia**(lr|sr) being zero implies that there is no correlation between the long-range interactions, acting as "noise", and the residues having the same value of **Ia**(sr) in the data base. This should be evident for unrelated proteins, but the data base contains a certain number of homologous proteins that could bring such a correlation. However, in previous simulation, Gibrat et al. (1987) found that the *average* accuracy of the prediction was not significantly modified by the presence of homologous proteins in the data base. This is probably because they are small in number and percentage of identity, and they are overweighted by nonhomologous proteins in the data base. On the other hand, further extension of the short-range interactions over ± eight amino acids does not improve the prediction, suggesting that the long-range interactions really act as noise. Then the average accuracy of prediction, 65%, reflects correctly only the short-range interactions.

**Registry No.** Bovine trypsin inhibitor, 12407-79-3.

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# GroE Facilitates Refolding of Citrate Synthase by Suppressing Aggregation


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ABSTRACT: The molecular chaperone GroE facilitates correct protein folding in vivo and in vitro. The mode of action of GroE was investigated by using refolding of citrate synthase as a model system. In vitro denaturation of this dimeric protein is almost irreversible, since the refolding polypeptide chains aggregate rapidly, as shown directly by a strong, concentration-dependent increase in light scattering. The yields of reactivated citrate synthase were strongly increased upon addition of GroE and MgATP. GroE inhibits aggregation reactions that compete with correct protein folding, as indicated by specific suppression of light scattering. GroEL rapidly forms a complex with unfolded or partially folded citrate synthase molecules. In this complex the refolding protein is protected from aggregation. Addition of GroES and ATP hydrolysis is required to release the polypeptide chain bound to GroEL and to allow further folding to its final, active state.


Correct in vivo folding and assembly of newly formed polypeptide chains appears to be dependent on the presence of several cellular proteins. These "molecular chaperones" (Laskey et al. 1978; Ellis, 1987, 1990), which belong to the group of heat-shock proteins, can interact with nonnative or partially folded polypeptide chains in an ATP-dependent manner (Ellis & van der Vies, 1988; Roy et al., 1988; Bochkareva et al., 1988; Ostermann et al., 1989). GroEL (also called cpn60[1]) from *Escherichia coli* is a prominent member


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of the molecular chaperone family. It forms a complex of two heptamers (subunit $M_r$ = 57000; Hendrix, 1979) under native conditions and interacts with one heptamer of GroES (or cpn10; subunit $M_r$ = 10000; Chandrasekhar et al., 1986). Both proteins are encoded by the *groE* operon (Fayet et al., 1986; Georgopoulus & Ang, 1990). Proteins homologous to GroEL have also been detected in mitochondria (Hsp60; McMullin & Hallberg, 1988; Hallberg, 1990) and chloroplasts (Rubisco subunit binding protein; Hemmingsen et al., 1988; Hemmingsen, 1990). GroEL is a weak, potassium-dependent ATPase (Viitanen et al., 1990). In the absence of nonnative polypeptide chains, this ATPase activity is partly inhibited by GroES. Rubisco subunit binding protein is required for the correct assembly of active ribulose bisphosphate carboxylase (Rubisco) complex in chloroplasts (Roy et al., 1988). Expression of Rubisco from *Rhodospirillum rubrum* in *E. coli* requires concomitant overexpression of GroE to yield active protein (Goloubinoff et al., 1989a). GroE also facilitates the reconstitution of active Rubisco from the urea-induced unfolded state in vitro (Goloubinqff et al., 1989b; Viitanen et al., 1990). GroEL (cpn60), GroES (cpn10), and MgATP are necessary for the successful in vitro reactivation of this protein (Goloubinoff et al., 1989b).

The way in which GroE assists protein folding is not known. Two alternative models have been proposed for the mechanism of action of molecular chaperones such as GroE. The first model is based on specific binding of chaperones to unfolded or partially folded proteins. According to this mechanism, chaperones could determine the folding pathway and thus actively direct the folding polypeptide chain to the correct native conformation and assembly state (Ostermann et al., 1989; Rothman, 1989; Hartl & Neupert, 1990). In the second model, transient association of folding chains with chaperone molecules is assumed not to modulate the folding process itself but to protect partially folded intermediates from aggregation reactions with each other or with other cellular components (Ellis, 1990; Viitanen et al., 1990).

Here we use the in vitro refolding of citrate synthase (CS) to investigate the effect of GroE on protein folding. Citrate synthase, a dimeric protein of $M_r$ = 50000, was selected as a model system because its unfolding appears to be irreversible (West et al., 1990). To probe the effect of GroE, we measured reactivation (by enzymatic assays) as well as aggregation (by light scattering) of CS, both in the absence and in the presence of GroE. Our results indicate that GroE promotes reactivation of CS. It effectively suppresses aggregation of refolding CS molecules and thus facilitates refolding in a specific, ATP-dependent fashion.

## Experimental Procedures

GroEL and GroES were purified from an overexpressing *E. coli* strain (Fayet et al. 1986). Cells were grown overnight in LB broth containing 50 μg/mL ampicillin. After being pelleted at 10000*g* for 1 h, the cells were resuspended in a buffer of 50 mM Tris/HCl, pH 7.5, and 2 mM EDTA (10 mL/g of pellet). A French press was used for cell lysis. After centrifugation at 12000*g* for 1 h, DNase (0.1 mg/mL) and $MgCl_2$ (2 mM) were added to the supernatant. The solution was stirred for 30 min at room temperature and then applied to a size exclusion column (Sephacryl S 300 HR, equilibrated with 50 mM Tris/HCl, pH 7.5, and 2 mM EDTA buffer). GroEL eluted at the void volume of the column. GroES was found in the second peak together with other *E. coli* proteins as analyzed by SDS–PAGE. Fractions containing GroEL or GroES were then applied to an anion-exchange column (Q-Sepharose, equilibrated with 50 mM Tris/HCl, pH 7.5, and 2 mM EDTA buffer). Elution was achieved with a linear NaCl gradient from 0 to 2 M. GroEL was eluted at about 0.4 M NaCl; GroES, at about 0.2 M NaCl. The GroES-containing fraction was further purified by repetition of the anion-exchange chromatography step.

CS from pig heart (E.C. 4.1.3.7 from Boehringer Mannheim) was denatured in a buffer of 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE for at least 1 h at room temperature. Renaturation was initiated by diluting the denatured CS 100-fold into a buffer containing 0.1 M Tris/HCl, pH 8.0, 10 mM $MgCL_2$, 10 mM KCl, and 2 mM ATP at 25 °C. In order to ensure rapid mixing, the refolding solution was stirred vigorously during addition of the unfolded protein.

CS activity was assayed in 0.1 M Tris/HCl, pH 8.0, and 2 mM EDTA buffer by using oxaloacetate and acetyl-CoA as substrates (Srere et al., 1963). The condensing reaction was monitored by binding of Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] to the free SH group of the released CoA. Residual concentrations of 40 μM ATP had no influence on CS activity in the assay.

Light scattering was measured with a Hitachi F4000 fluorometer with excitation and emissiosn at 500 nm. The spectral band width was 1.5 nm for both excitation and emission.

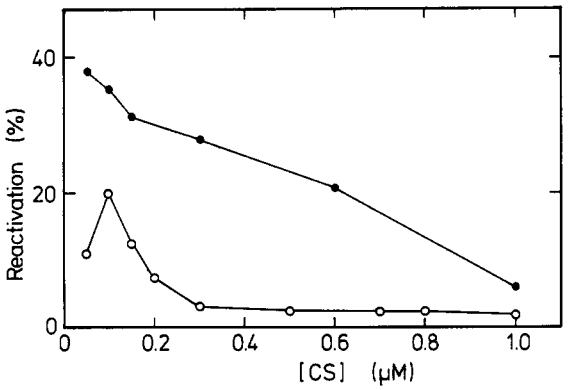


FIGURE 1: Concentration dependence of the yield of reactivation of CS in the presence (●) and absence (○) of GroE at 25 °C. CS was completely denatured in a buffer containing 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE at various protein concentrations. Refolding was initiated by 100-fold dilution of the unfolded protein into a buffer of 0.1 M Tris/HCl, 10 mM $MgCl_2$, 10 mM KCl, and 2 mM ATP, pH 8.0, to the indicated CS concentration. For refolding in the presence of GroE, a 6-fold molar excess of GroE complex over CS was added to the refolding solution. The extent of reactivation was measured after 2 h of refolding.

## Results

*The Yield of Reactivated CS Is Concentration Dependent.* West et al. (1990) have attempted the refolding of CS, showing that unfolding is irreversible under the given experimental conditions. Since reactivation of oligomeric proteins frequently exhibits a strong concentration dependence (Zettlmeissl et al., 1979), we varied the CS concentration in the refolding step. Figure 1 shows that the yield of reactivated enzyme becomes almost zero at protein concentrations beyond 0.3 μM, which is close to the concentration employed by West et al. (1990). The yield can be increased significantly when the final protein concentration is lowered. A maximum amount of about 20% of the original activity is recovered at 0.1 μM CS. The de-

[1] Abbreviations: cpn60, chaperonin60; cpn10, chaperonin10; Rubisco, ribulose 1,5-bisphosphate carboxylase; CS, citrate synthase; GdmCl, guanidinium chloride; Hsp70, heat shock protein with a $M_r \approx 70000$; BiP, immunoglobulin heavy-chain binding protein; BSA, bovine serum albumin; DTE, dithioerythritol.

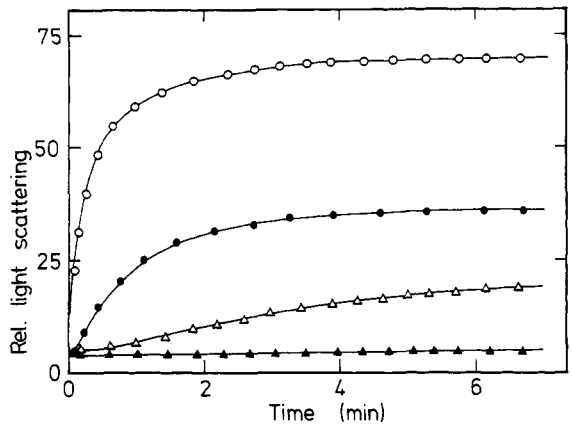


FIGURE 2: Concentration dependence of the increase in light scattering in the time course or refolding of CS. Completely denatured CS (in a buffer of 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE) was diluted 100-fold into 0.1 M Tris/HCl, 10 mM $MgCl_2$, 10 mM KCl, and 2 mM ATP buffer, pH 8.0, at 25 °C. CS concentrations in the refolding solution were 300 nM (○), 225 nM (●), 150 nM (△), and 100 nM (▲). Light scattering was measured at 500 nm.

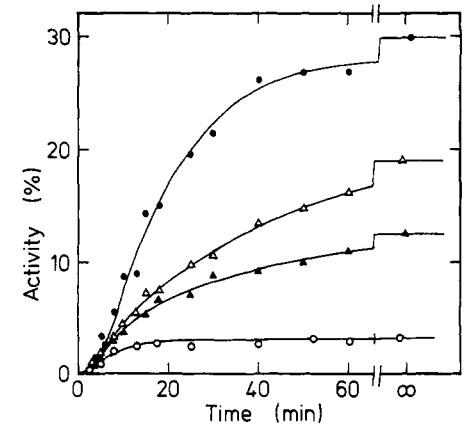


FIGURE 3: Time course of reactivation of CS in the presence of 1.93 μM (●), 0.71 μM (△), and 0.14 μM (▲) GroE complex and in the absence of GroE (○). Unfolded CS (in a buffer of 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE) was diluted 100-fold into 0.1 M Tris/HCl, 10 mM $MgCl_2$, 10 mM KCl, and 2 mM ATP buffer, pH 8.0, at 25 °C. The concentration of CS in the refolding solution was 0.3 μM. Activity assays were performed after various times of refolding. The activity of the refolding CS is given as percentage relative to a control sample of 300 nM native CS. The final values were measured after 2 and 3 h of reactivation.

crease in the extent of reactivation at very low concentrations is probably due to unspecific adsorption to the surface of the glass cuvette. Similar effects have been observed during in vitro renaturation of other proteins (Rudolph & Jaenicke, 1976; Gerschitz et al., 1977).

*Aggregation Competes with Folding of CS.* The foregoing results raise the question whether the concentration-dependent decrease in the yield of active CS during in vitro refolding is caused by aggregation of unfolded or partially folded molecules that competes with correct folding. As illustrated in Figure 2, aggregation during refolding of CS does occur. It leads to a strong time- and concentration-dependent increase in light scattering. At 0.3 μM CS aggregation occurs with a half-time of less than 15 s. At lower protein concentrations, where partial reactivation is observed (Figure 1), the increase in light scattering is slowed down to the minutes range and shows only a small amplitude. The increase in the extent of aggregation and the decrease in the yield of active enzyme (Figure 1) occur in the same concentration range, suggesting that the two phenomena are correlated. Since aggregation proceeds rapidly after dilution of the unfolded protein into native conditions, fast mixing is essential to obtain reproducible results in the reactivation and light scattering experiments. This suggests that aggregation, which predominates at high local concentrations of unfolded protein, occurs at a very early, transient stage in folding. Further quantitative analyses of the light scattering data are not warranted, since the observed signal depends not only on the extent of aggregation but also on the size distribution of the aggregates.

*GroE Increases the Amount of Correctly Folded CS.* To investigate whether the yields of reactivation could be increased by GroE, refolding of CS (0.3 μM) was performed in the presence of various concentrations of GroEL and GroES. The results in Figure 3 demonstrate that the extent of reactivation is strongly increased in the presence of GroE and MgATP in a concentration dependent manner. A 6-fold molar excess of GroE complex[2] over CS leads to a 10-fold increase in the yield of reactivated protein compared to renaturation in the absence of the complex. The kinetics of formation of active CS are not affected by GroE. The half-times of reactivation are independent of GroE concentration (Figure 3). An initial lag phase is observed in all reactivation experiments, which shows that folding of CS is a sequential process. Similar to the renaturation of other oligomeric proteins, inactive monomers may be produced in an early folding step. These monomers then have to undergo additional folding and/or association steps to form active dimers (Jaenicke, 1987).

The protective effect of GroE against nonproductive side reactions depends on the concentration of CS. When reactivation of CS is carried out in the presence of a constant 6-fold molar excess of GroE complex, the yield of active protein still depends on CS concentration. However, reactivation in the presence of GroE can be achieved at higher CS concentrations compared to reactivation in the absence of GroE (Figure 1).

*GroE Suppresses Aggregation but Does Not Dissolve Aggregates.* To determine whether the increase in the yield of reactivation in the presence of GroE (Figure 3) originates from a suppression of aggregation reactions, aggregation kinetics in the presence of various GroE concentrations were monitored directly by light scattering. The concentrations of CS and GroE were the same as in the reactivation experiments (Figure 3). The results in Figure 4A show that GroE suppresses aggregation in the same concentration-dependent way as it increases the yield of correctly refolded CS. At GroE concentrations where maximum yields of reactivated CS were obtained (Figure 3), aggregation is suppressed almost completely.

Next we examined whether GroE could solubilize previously aggregated polypeptide chains and subsequently allow reactivation. In these experiments the kinetic competition between folding and aggregation was initiated in the absence of GroE and the chaperone was then added after various times. The results in Figure 4B demonstrate that GroE is able to stop further aggregation at any stage of refolding. The intensity of light scattering, however, does not decrease after addition of GroE, indicating that already-formed aggregates cannot be dissolved by GroE.

*The Effect of GroE Is Specific.* To probe the function of GroE, refolding experiments with CS were performed in the presence of various proteins. Light scattering measurements show that GroEL alone is sufficient to prevent aggregation of CS during refolding (Figure 4C). The effect is even more pronounced in the absence of MgATP. In contrast, GroES

[2] In the following, the term "GroE complex" designates the complex of a 14-mer of GroEL with a 7-mer of GroES.

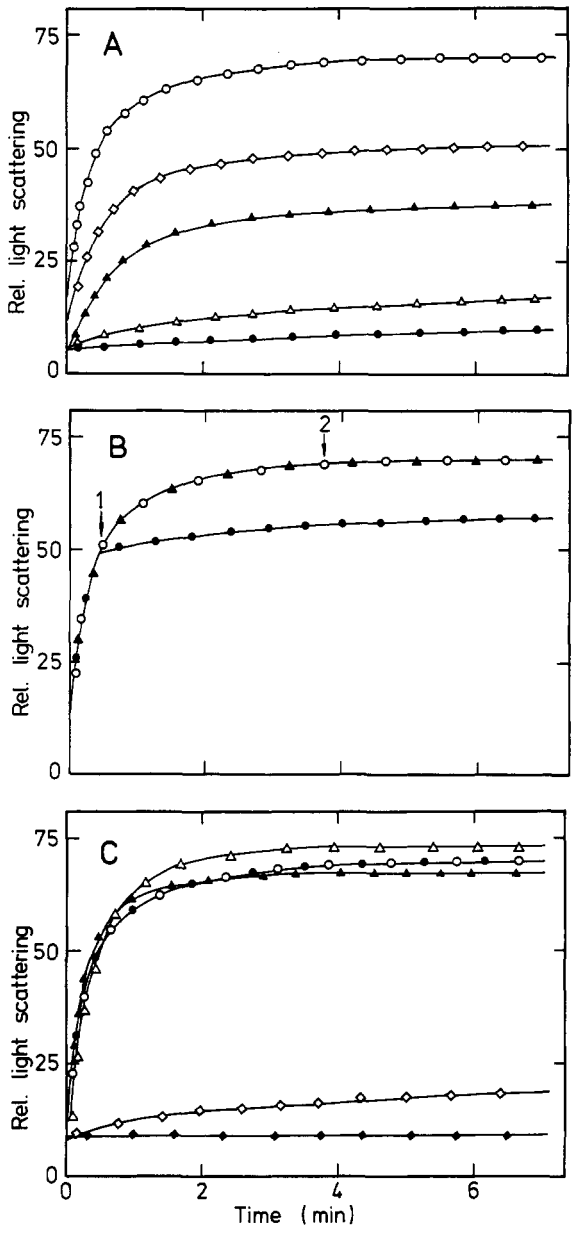


FIGURE 4: (A) Light scattering in the time course of refolding of CS in the presence of 1.93 μM (●), 0.71 μM (△), 0.14 μM (▲), and 0.07 μM (◇) GroE complex and in the absence of GroE (○). (B) Effect of GroE added after various times of refolding/aggregation of CS. (○) Time course of reactivation in the absence of GroE; 1.93 μM GroE complex was added 30 s (●, arrow 1) or 210 s (▲, arrow 2) after initiation of refolding. (C) Light scattering of refolding CS in the presence of 1.6 mg/mL BSA (▲), 1.6 mg/mL lysozyme (△), 1.93 μM GroES (●), 1.93 μM GroEL in the presence (◇) and absence (◆) of ATP, and of CS in the absence of additional proteins (○). Completely denatured CS (in a buffer of 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE) was diluted 100-fold into 0.1 M Tris/HCl, 10 mM $MgCl_2$, 10 mM KCl, and 2 mM ATP buffer, pH 8.0, at 25 °C. The concentration of CS in the refolding step was 0.3 μM.

alone has no influence on the appearance of aggregates in the time course of refolding. Also, high concentrations of other proteins such as bovine serum albumin (BSA) or lysozyme cannot suppress aggregation.

While GroEL alone is able to inhibit aggregation completely, it is not sufficient to allow significant reactivation. As shown in Table I, GroEL, GroES, and MgATP are required for a maximum recovery of CS activity. The same result is obtained when the GroEL·CS complex is formed first and GroES and MgATP are added after 45 min. While GroES alone does not promote reactivation, the presence of GroEL

Table I: Influence of Various Proteins on the Reactivation Yields of CS[a]

| added components | reactivation yield (%) |
|---|---|
| GroEL + GroES + ATP | 28 |
| GroEL + ATP | 16 |
| GroES + ATP | 2 |
| GroEL | 0 |
| addition of GroES + ATP after 45 min[b] | 28 |
| GroEL + GroES | 0 |
| addition of ATP after 45 min[c] | 29 |
| BiP + ATP | 0 |
| Hsp70 + ATP | 3 |
| BSA + ATP | 7 |
| lysozyme + ATP | 6 |
| ATP | 3 |
| none | 1 |

[a] Unfolded CS (in a buffer of 6.0 M GdmCl, 0.1 M Tris/HCl, pH 8.0, and 20 mM DTE) was diluted 100-fold into 0.1 M Tris/HCl, 10 mM $MgCl_2$, and 10 mM KCl buffer, pH 8.0, at 25 °C. Concentration of CS was 0.3 μM in the refolding step. Concentrations of BSA and lysozyme were 1.6 mg/mL. The concentrations of GroEL, GroES, and GroE complex were 1.93 μM. The concentrations of BiP and Hsp70 were 2 μM. Concentration of ATP was 2 mM. [b] Refolding was initiated in the presence of GroEL. GroES and ATP were added after 45 min. [c] Refolding was initiated in the presence of GroEL and GroES. ATP was added after 45 min.

in the absence of GroES leads to an increase in the yield of native CS. This effect, however, requires MgATP. In the absence of MgATP, no reactivation is observed, pointing to a stable binding of CS folding intermediates to GroEL when GroES and MgATP are absent.

BSA facilitates reactivation of several proteins in an unspecific way (Jaenicke & Rudolph, 1989). However, BSA, as well as lysozyme, does not exert a significant effect on the reconstituion of CS. The same holds true when Hsp70 or BiP is added at concentrations similar to GroE. These two heat-shock proteins have been proposed to play important roles in the folding and transport of various proteins [for reviews, see Pelham (1989) and Haas (1990)].

These results indicate that GroEL specifically interacts with unfolded or partially folded forms of CS and thus prevents them from aggregating, as well as from further folding. Interaction with GroES and MgATP is presumably required for the release of the protein from GroEL. When native CS is incubated with GroE, no change in enzyme activity is observed. This confirms that GroE can only act during the refolding process, when partially folded intermediates are present in high amounts. It does not affect the conformation of correctly folded CS.

## Discussion

In vitro refolding of CS gives poor yields of active enzyme since aggregation effectively competes with rapid steps on the correct folding pathway. Such a kinetic competition has previously been recognized as a major determinant for decreased yields or virtual irreversibility in refolding studies in vitro (Jaenicke & Rudolph, 1986; Jaenicke, 1987). Since aggregation is a second or higher order process (Zettlmeissl et al., 1979), it can be much faster than first-order folding and therefore outruns folding with increasing protein concentration (Rudolph et al., 1990). The strong differences in rate between reactivation (Figure 3) and aggregation (Figures 2 and 4) show that aggregation takes place well before the rate-limiting events of folding. Evidently the kinetic competition does not occur between the slow steps of reactivation and the aggregation process. Rather, aggregation competes with the rapid formation of a critical intermediate that is already protected

against these side reactions and can enter the slow folding steps.

The presence of a 6-fold excess of GroE during refolding virtually blocks aggregation of all CS molecules at a concentration of 0.3 $\mu$M. Concomitantly, the amount of reactivated protein is increased. While GroEL alone is sufficient to suppress aggregation, GroEL, GroES, and MgATP are required to yield high amounts of active protein, which suggests an ordered sequence of events. In the first step GroEL binds to a partially folded form of CS. This binding competes directly with aggregation. The extent to which the enzyme is protected from aggregation is determined by the concentrations of GroEL and of the refolding protein as well as by the refolding conditions. In a subsequent step, binding of GroES and ATP hydrolysis presumably leads to a release of the folding intermediates from GroEL. For this reaction the presence of GroES may not be essential since substantial amounts of reactivated CS (Table I) and pre-$\beta$-lactamase (Laminet et al., 1990) can be regained by adding ATP to the GroEL·CS complex. However, we cannot rule out that trace amounts of GroES are present in GroEL preparations. The released protein substrate can probably complete the final slow steps of folding in the absence of GroE. The observed arrest of reactivation in the presence of GroEL alone demonstrates that folding of CS cannot continue to completion when CS is bound to GroEL. We do not know at present whether CS molecules can fold from the aggregation-sensitive state to an aggregation-resistant form on the surface on GroEL. While aggregation is suppressed almost completely in the presence of GroE, the yield or reactivation does not exceed 40% under our best experimental conditions. The released protein may still be susceptible for some nonproductive side reactions such as adhesion to surfaces, since we observed that reactivation yields of CS do depend on the kind of vessels that are used in the experiments.

The presented model describing the interplay between GroEL, GroES, and MgATP is consistent with the findings by Goloubinoff et al. (1989b), who were able to show that both GroEL and GroES are required for the successful reactivation of Rubisco.

As pointed out, the effect of GroE on folding may be explained by a mechanism in which binding of GroEL to early folding intermediates competes directly with their irreversible aggregation. Therefore, under conditions where aggregation occurs extremely fast, e.g., at very high protein concentrations, aggregation is still predominant. Whether this simple model can explain the cellular role of GroE for de novo protein folding remains to be shown. Aggregation was also suggested to be the major reason for the irreversibility of unfolding of Rubisco (Viitanen et al., 1990). Since aggregation reactions are both temperature- and concentration-dependent, one of the cellular functions of GroE may be to provide protection from intracellular aggregation processes, particularly under stress conditions and/or at high rates of protein synthesis.

Once formed, CS aggregates cannot be redissolved by adding GroE. This suggests that GroE is not able to rescue aggregated proteins, as postulated for other heat-shock proteins (Pelham, 1986). The activity of native CS molecules is also not influenced by GroE. When the correct native state has been reached, GroE is not able to interact with CS molecules and unfold them. Rather, the results indicate that GroE is only active during early steps in protein folding, when intermediates prone to aggregation are present at high concentration.

Different heat-shock proteins do not have the same effect on the refolding of CS (cf. Table I). Only GroE facilitates reactivation; under our experimental conditions Hsp70 and BiP have no influence. BiP, in particular, completely prevents refolding of CS. This indicates that heat-shock proteins do not display a common function.

The rate of refolding of CS is not increased in the presence of GroE. It thus appears to differ in its role in protein folding from protein disulfide isomerase (Freedman, 1984; Freedman et al., 1989) and prolyl isomerase (Fischer et al., 1984; Lang et al., 1987). These two enzymes accelerate protein folding by catalyzing slow, rate-limiting steps on the refolding pathway [for a review see Fischer and Schmid (1990)], whereas GroE inhibits off-pathway reactions, such as aggregation. Two alternative mechanisms seem to be involved in facilitating correct protein folding in the cell. One is the catalysis of slow steps on the folding pathway and thus the reduction of the time of exposure of hydrophobic surfaces in folding intermediates (cf. prolyl isomerase and protein disulfide isomerase). The second process involves chaperones such as GroE that bind to folding intermediates and thus prevent unproductive aggregation reactions. In this case, transient binding of GroE to unfolded or partially folded polypeptide chains may substitute for unspecific self-aggregation. No evidence was found that GroE actively guides correct folding and assembly by "selecting" the correct folding pathway.

In summary, the mode of action proposed for GroE is consistent with the concept that protein folding is determined by the information encoded by the amino acid sequence and is driven by the difference in the Gibbs free energy between the native and the unfolded states (Anfinsen, 1973). Nevertheless, "folding helpers" may be essential to avoid nonproductive off-pathway reactions. As a matter of fact, the possible existence of cellular "folding helpers" has already been discussed by Anfinsen and co-workers many years ago (Epstein et al., 1963).

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# Effect of Amino Acid Ion Pairs on Peptide Helicity†

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ABSTRACT: The three ER ion pairs in the peptide acetyl-W(EAAAR)$_3$A-amide were replaced in turn with the ion pairs EK, EO, DR, DK, and DO, where O represents an ornithine residue. The far-ultraviolet circular dichroic spectra of the six peptides measured in 10 mM NaCl at pH 2 and 0 °C form a nested set having an isodichroic point at 203 nm of −17 000 deg cm$^2$ dmol$^{-1}$. The ellipticity values of the six peptides at 222 nm range from −31 600 to −7400 deg cm$^2$ dmol$^{-1}$ in the order listed. Changing the pH of each peptide solution from 2 to 13 also generates a nested set of dichroic spectra with the same isodichroic values. Increasing the pH from 2 to 7 differentially increases the ellipticity at 222 nm in a single transition having an apparent p$K$ of 4.1 for the E-containing peptides are 3.6 for the D-containing peptides. Increasing the pH beyond neutrality differentially decreases the ellipticity at 222 nm in a single transition having an apparent p$K$ of $\geq$13.2 for the R-containing peptides, 11.1 for the K-containing peptides, and 10.7 for the O-containing peptides. It is proposed that the difference in the ellipticity of the six peptides chiefly reflects the helix preferences for the variable residues supplemented by intrahelical electrostatic interactions in the neutral pH range.

Model helical peptides commonly contain acidic and basic residues to increase solubility in aqueous solution and to form favorable interactions with each other and with the helix macrodipole. Marqusee and Baldwin (1987) investigated the effect of the orientation and the spacing of three glutamate/lysine ion pairs on the helical content of a 17-residue monomeric peptide containing 11 alanine residues. They observed that the helical content was optimized at neutral pH when the glutamate/lysine ion pairs were separated by three alanine residues with the glutamate residue being N-terminal. These results can be interpreted to indicate the contribution of salt bridges and helix macrodipole/side-chain electrostatic interactions to helical stability. In this paper, the contribution of different paired acidic and basic residues to helical content is investigated. The peptides studied all have the sequence

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