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Chaperonins and Protein Folding: Unity and Disunity of Mechanisms [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1993 **339**, 297-304
doi: 10.1098/rstb.1993.0028

Chaperonins and protein folding: unity and disunity of mechanisms

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SUMMARY

Chaperonin-facilitated folding of proteins involves two partial reactions. The first partial reaction, the formation of stable binary complexes between chaperonin-60 and non-native states of the target protein, is common to the chaperonin-facilitated folding of all target proteins investigated to date. The structural basis for this interaction is not presently understood. The second partial reaction, the dissociation of the target protein in a form committed to the native state, appears to proceed by a variety of mechanisms, dependent upon the nature of the target protein in question. Those target proteins (e.g. rubisco, rhodanese, citrate synthase) which require the presence of chaperonin-10, also appear to require the hydrolysis of ATP to bring about the dissociation of the target protein from chaperonin-60. With one exception (pre-β-lactamase) those target proteins which do not require the presence of chaperonin-10 to be released from chaperonin-60, also do not require the hydrolysis of ATP, since non-hydrolysable analogues of ATP support the release of the target protein in a state committed to the native state. The question of whether or not chaperonin-facilitated folding constitutes a catalysed event is addressed.

1. INTRODUCTION

Chaperonins are abundant, ubiquitous and seemingly indispensable proteins, which are believed to be involved *in vivo* in post-translational events leading to the appearance of biologically active proteins (Ellis, this symposium). Although chaperonins were initially implicated with the assembly of oligomeric structures (Hemmingsen *et al.* 1988), there exists no compelling *in vitro* evidence that they are directly involved in associative steps. On the other hand, a growing body of *in vitro* evidence suggests that the chaperonins passively or actively facilitate the folding of many monomeric polypeptides, as well as the subunits of oligomeric proteins, under conditions which more closely approximate the physiological conditions within the cell.

Much modern biological research, especially at the molecular level, is a search for unifying mechanisms. This guiding principle has served us well and it was with the expectation of uncovering a unique, generally applicable mechanism that we embarked upon *in vitro* studies of the chaperonins four years ago (Goloubinoff *et al.* 1989*a*,*b*). That this expectation has proven naive can be partly attributed to the unanticipated promiscuity of the chaperonins (Viitanen *et al.* 1992*a*) and to the multiple and varied structures they presumably encounter within the cell. Some common principles have emerged nevertheless, the most important of which is the formation of stable binary complexes between chaperonin-60 and the non-native states of many proteins. However, a common mechanism for the conversion of these stable binary complexes to native states is currently lacking and may not exist. The extent to which folding events occur while the target protein is associated with chaperonin-60, and the manner in which these events are (sometimes, but not always) coupled to the apparent hydrolysis of ATP and the presence of chaperonin-10, is hardly understood at all, so that it is currently not possible to write a unifying mechanism for the action of the chaperonins.

2. THE RUBISCO PARADIGM

The first clear-cut, *in vivo* and *in vitro* demonstration for the post-translational involvement of the chaperonin proteins in the synthesis of ribulosebisphosphate carboxylase (rubisco) was provided a decade ago (Barraclough & Ellis 1980; Roy *et al.* 1982). In a series of radiolabelling experiments these authors demonstrated the existence of a transient, binary complex between the large subunit of rubisco and a chloroplastic protein, then known as the rubisco subunit binding protein. This protein was subsequently shown to be substantially homologous to a prominent, heat-shock protein of *Escherichia coli* and other prokaryotes, GroEL (Hemmingsen *et al.* 1988). The GroEL protein, and its partner, the GroES protein, had previously been identified genetically as essential host proteins in the production of phage particles (Tilly & Georgopoulos 1982). (We will refer to the GroEL and GroES, and their eukaryotic and prokaryotic homologues by the generic terms, chaperonin-60 and

Phil. Trans. R. Soc. Lond. B (1993) **339,** 297–304
Printed in Great Britain

chaperonin-10.) The function of the chaperonins was unknown at that time. But we reasoned that if the synthesis of rubisco in chloroplasts involved the chaperonins, then so too would the synthesis of recombinant rubisco in *E. coli*. We obtained genetic evidence for this proposition, and further established that: (i) both chaperonin-60 and chaperonin-10 were necessary; and (ii) the chaperonins were involved post-translationally at some point between the synthesis of the nascent large subunit and the formation of the large subunit dimer, the smallest biologically active structure (Goloubinoff *et al.* 1989*a*).

We next sought to determine what influence purified chaperonins might have on the *in vitro* refolding of urea-denatured dimeric rubisco, using the classic dilution approach pioneered by Anfinsen (1973). Rubisco activity was reconstituted at 25°C in a reaction that depended upon chaperonin-60, chaperonin-10, MgATP and a monovalent cation, K^+ or NH_4^+ (Goloubinoff *et al.* 1989*b*; Viitanen *et al.* 1990). Under the chosen conditions no refolding of Rubisco occurred in the absence of any of the four components. In dissecting this reconstitution reaction several partial reactions were developed and these are discussed below.

3. PARTIAL REACTIONS

(*a*) *Interaction of chaperonin-60 with the non-native states of proteins: the formation of stable binary complexes, the suppression of aggregation but the inhibition of spontaneous folding*

A common property of the non-native state of many proteins is to undergo irreversible aggregation rather than isomerizing to the native state (Mitraki & King 1989). The commitment to aggregation is very rapid (Goldberg *et al.* 1991). Rubisco is no exception in this regard (van der Vies *et al.* 1992). When unfolded rubisco is diluted into solutions lacking chaperonin-60 at temperatures greater than 25°C, it rapidly forms a species which irreversibly aggregates. This species is moderately stable at 0°C and can be studied spectroscopically before it reverts spontaneously to the native state (van der Vies *et al.* 1992). It has the properties of a folding intermediate, rubisco-I. In the presence of chaperonin-60, at both 0°C and at 25°C, rubisco-I forms a very stable binary complex, which can be isolated by gel filtration chromatography (Viitanen *et al.* 1992*a*). This binary chaperonin-60–rubisco-I complex, observed *in vitro*, is the same complex as was observed in the *in vivo* studies of Roy *et al.* (1982) and in isolated chloroplasts by Barraclough and Ellis (1980). In its native state chaperonin-60 exists either as a single heptameric toroid (Viitanen *et al.* 1992*b*) or more commonly as a double toroid with seven subunits per ring (Hendrix, 1979). The stoichiometry of binary complex formation appears to be one rubisco per toroid (van der Vies *et al.* 1992). Thus, the first partial reaction can be written as follows:

[rubisco-I] + [chaperonin-60]7 → [chaperonin-60]7–[rubisco-I]

A significant consequence of binary complex formation is that both aggregation of rubisco-I and its spontaneous folding to the native state are arrested.

The formation of stable binary complexes involving the non-native states of proteins and chaperonin-60 is now regarded as a common, first step. The ability of chaperonin-60 to suppress aggregation has been clearly demonstrated by light scattering or kinetic techniques employing various urea- or guanidine-HCl-denatured proteins (Buchner *et al.* 1990; Martin *et al.* 1991; Mendoza *et al.* 1991) and using thermally denatured proteins (Höll–Neugebauer *et al.* 1991; Mendoza *et al.* 1992; Yoshida *et al.* 1992). The spontaneous folding of these proteins is also arrested upon formation of the binary complex.

Chaperonin-60 interacts with the non-native states of some proteins but not with the native states of these proteins. This observation implies that some structural feature is present and accessible in the non-native state but absent or inaccessible in the native state. Chaperonin-60 forms stable binary complexes *in vitro* with about half of the soluble proteins of *E. coli*, provided they are presented to the chaperonin in a non-native state (Viitanen *et al.* 1992*a*). This result suggests that this structural feature recognized by chaperonin-60 is a very common one. On the basis of NMR studies with small peptides Landry & Gierasch (1991) have suggested that chaperonin-60 may recognize and stabilize nascent α-helices. While this may be true, the demonstration that chaperonin-60 also interacts with the non-native state of an all β-protein (Schmidt & Buchner 1992) indicates that other interactions are possible.

The ability of chaperonin-60 to suppress aggregation accounts for the ability of plasmids overexpressing the chaperonins *in vivo* to suppress the formation of inclusion bodies and promote the formation of some soluble, biologically active, recombinant proteins (Goloubinoff *et al.* 1989*a*; Lee & Olins 1992; Wynn *et al.* 1992).

(*b*) *Requirements for recovering native proteins from binary chaperonin-60 complexes*

Following isolation of the binary chaperonin-60–rubisco-I complex it was possible to recover native rubisco merely by incubating the binary complex with chaperonin-10, MgATP and K^+ (Viitanen *et al.* 1990). The failure of non-hydrolysable analogues of ATP to support reconstitution suggested that the chaperonin-facilitated reconstitution of rubisco required the hydrolysis of ATP. The requirement of the reconstitution reaction for K^+ was attributed to the requirement of the chaperonin-60 ATPase for this cation (see below). The requirement for chaperonin-10 was rationalized in terms of it acting as a 'coupling factor', linking the K^+dependent hydrolysis of ATP by chaperonin-60 to the release of rubisco in a state

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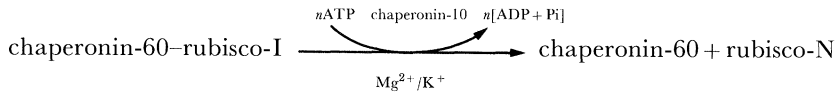
committed to forming the native state. Attempts to measure the stoichiometry of mols of ATP hydrolysed per mol of native rubisco have not yet yielded reliable values. Nevertheless, we felt justified in describing the second stage of chaperonin-facilitated refolding as follows:



As indicated in table 1, this mechanism could be applied to the chaperonin-facilitated folding of rhodanese (Mendoza *et al.* 1991) and citrate synthase (Buchner *et al.* 1991; Zhi *et al.* 1992). Thus in the presence of both chaperonin-10 and ATP, the target proteins are released in a form that is committed to the native state. The addition of ATP in the absence of chaperonin-10 induces the dissociation of both rhodanese (Martin *et al.* 1991) and Rubisco (Viitanen *et al.* 1992*a*) from chaperonin-60. However, in neither case is the native state of rhodanese or rubisco formed, implying that in the absence of chaperonin-10 the target proteins are released in an uncommitted state. An additional implication is that, in the presence of both ATP and chaperonin-10, the target protein is transformed from an uncommitted to a committed state while in association with chaperonin-60; i.e. some productive isomerization towards the native state occurs while the protein is in association with chaperonin-60.

The generality of this mechanism for the second stage of chaperonin-facilitated refolding was soon called into question. Examples (table 1) were discovered where chaperonin-10 is not strictly necessary (Laminet *et al.* 1990). In the case of pre-β-lactamase ATP continued to be necessary since non-hydrolysable analogues did not support the reaction. In still other cases, however, non-hydrolyzable analogues of ATP, or simply ADP alone, is sufficient to induce the formation of the native states of the target proteins from the chaperonin-60 binary complex (table 1). In those cases where there is no absolute requirement for chaperonin-10, its presence often accelerates the release of the target protein. It appears that in those cases where an absolute requirement for chaperonin-10 exists, there is also an absolute requirement for the hydrolysis of ATP. Thus, the pathway from the binary complex between chaperonin-60 and the target protein to the native state of the target protein is characterized by a notable lack of mechanistic unity. The pathway appears to be governed to a large extent by the nature of the target protein.

Recognizing that the pathway depends upon the physical properties of each protein, we have formulated a mechanism invoking the minimum number of *ad hoc* assumptions (figure 1). The central pathway (block letters) represents the classic case of spontaneous, unassisted folding. With very few exceptions all proteins are able to follow this pathway to a greater or lesser extent. We recognize two, kinetically defined, intermediate states, uncommitted states (Protein-I_{uc}), which possess some propensity to aggregate, and committed states (Protein-I_c), which are destined to become the native state. The population of uncommitted states may vary considerably. In the extreme case (i.e. for well-behaved proteins or at infinite dilution) the population of uncommitted states may approach zero, so that the native state is attained with 100% efficiency. The *in vitro* environmental conditions (e.g. temperature, ionic strength, protein concentration) that need to be imposed for this to occur are not necessarily physiologically relevant. Next we introduce chaperonin-60 which has the capacity to interact with these intermediate states. Because the extent of aggregation depends upon the concentration of uncommitted states, a reagent such as chaperonin-60, which has the ability to reversibly lower the concentration of free uncommitted states, will ultimately enhance the yield of the native state, merely by suppressing irreversible losses due to aggregation. Note that this will apply regardless of whether or not any folding events occur while the target protein is associated with chaperonin-60. It also applies regardless of whether or not a ligand, such as an adenine nucleotide, is needed to effect the release of the target protein from chaperonin-60. This mechanism predicts

Table 1. *Diverse requirements for recovering native proteins from the binary complex of chaperonin-60–protein-I*

protein	requirements	reference
I. rubisco[a]	chaperonin-10 + ATP	Goloubinoff *et al.* (1989*b*)
rhodanese[a]	chaperonin-10 + ATP	Mendoza *et al.* (1991); Martin *et al.* (1991)
citrate synthase	chaperonin-10 + ATP	Buchner *et al.* (1990); Zhi *et al.* (1992)
II. pre-β-lactamase	ATP	Laminet *et al.* (1990)
III. dihydrofolate reductase	ATP or ATP analogues[b]	Viitanen *et al.* (1991)
lactate dehydrogenase	ATP or ATP analogues	Badcoe *et al.* (1991)
glutamine synthetase	ATP or ATP analogues, but not ADP	Fisher (1992)
tryptophanase	ATP or ATP analogues, and ADP	Mizobata *et al.* (1992)

[a] ATP alone discharges the binary complex but the released protein is not committed to the native state.
[b] ATP analogues include 5′-adenyl imidodiphosphate (AMP-PNP), adenoside-5′-O-(3-thiotriphosphate) (ATP-γ-S).

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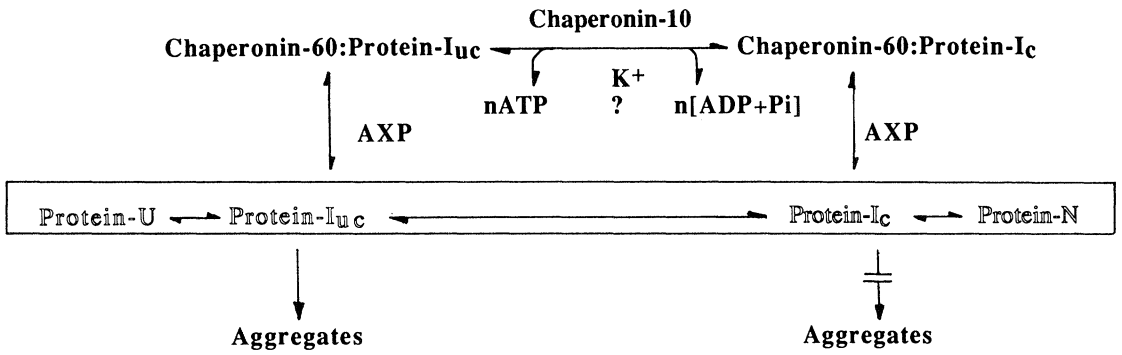

Figure 1. A general scheme for spontaneous and chaperonin-facilitated protein folding. Unfolded and native states of the target protein are designated as Protein-U and Protein-N, respectively. Intermediate states uncommitted and committed to proceeding to the native state are designated Protein-I_{uc} and Protein-I_c, respectively. AXP refers to adenine nucleotides of the hydrolysable and non-hydrolysable type.

that chaperonins will enhance the yield of folded protein rather than the rate of folding. Indeed, depending upon the rate with which the target protein is released from chaperonin-60, the rate at which the native state is reached may be significantly retarded, relative to the spontaneous rate of folding. In some cases the binary complex is so stable that folding to the native state is more or less completely arrested. Our ability to trap and isolate chromatographically binary complexes of chaperonin-60 with the non-native states about half of the soluble proteins of *Escherichia coli* is a reflection of this stability (Viitanen *et al.* 1992*a*).

Although it is possible to account for the chaperonin-60 enhanced folding of some target proteins that occurs independently of chaperonin-10 or ATP, in terms of the buffering action of chaperonin-60 on uncommitted states, this cannot entirely account for the enhanced folding that is chaperonin-10 and ATP-dependent. As noted above, it is possible to dissociate rubisco or rhodanese from chaperonin-60 merely by the addition of ATP. However, the species which are released are uncommitted. They do not proceed to the native state. That they do so in the presence of both chaperonin-10 and ATP implies that folding events occur while these target proteins are associated with chaperonin-60, events which convert the target protein from an uncommitted state to a committed state.

The notion that one protein or protein-domain may fold while in association with other proteins or other domains of the same protein is not radically new. There already are several examples of unfolded proteins being successfully refolded while associated with insoluble chromatographic matrices (Creighton 1985) to dispel the notion that proteins must be entirely free in solution to fold to their native states. Recent NMR evidence indicates that the domains of a protein fold independently of one another (Teuten *et al.* 1991), a result that is viewed as the folding of one domain while it is covalently tethered to other domain(s). Viewed in this perspective, the idea that target proteins might very well undergo some folding events while in association with chaperonin-60 hardly seems heretical. But does such chaperonin-facilitated folding constitute catalysis?

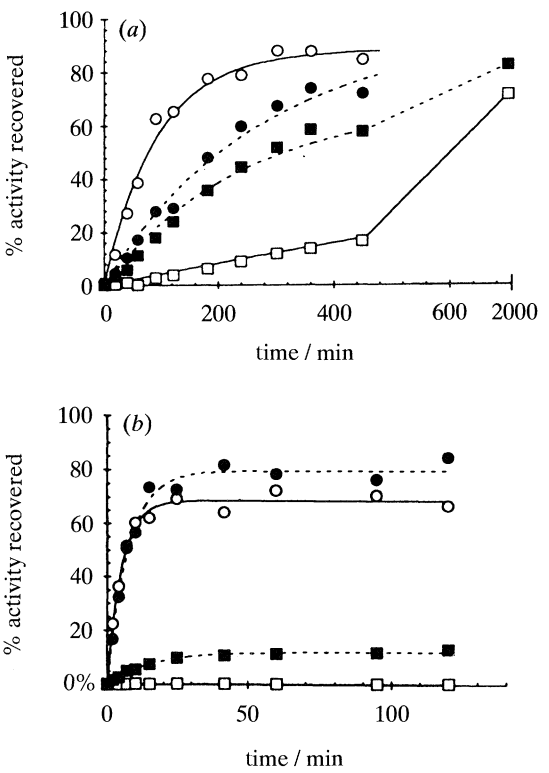

Figure 2. The influence of temperature and ionic strength on the spontaneous (squares) and chaperonin-facilitated (circles) folding of acid-denatured rubisco at low ionic strength (open symbols) and at high ionic strength (closed symbols) at (*a*) 0°C and (*b*) 25°C. Acid-denatured rubisco (final concentration, 250 nM) was diluted into 100 mM HEPES-NaOH, pH 7.8, 10 mM potassium acetate, 10 mM magnesium acetate, 2 mM dithiothreitol, 0.1 mM EDTA, 5 μM bovine serum albumin, containing ± 200 mM sodium chloride, ± 3.5 μM (chaperonin-60 plus chaperonin-10 protomers) at either 0°C or 25°C as indicated. The samples containing chaperonins contained additionally 2 mM ATP. The reactions were quenched with glucose or hexokinase at the indicated times and assayed for rubisco as described by Goloubinoff *et al.* (1989*b*).

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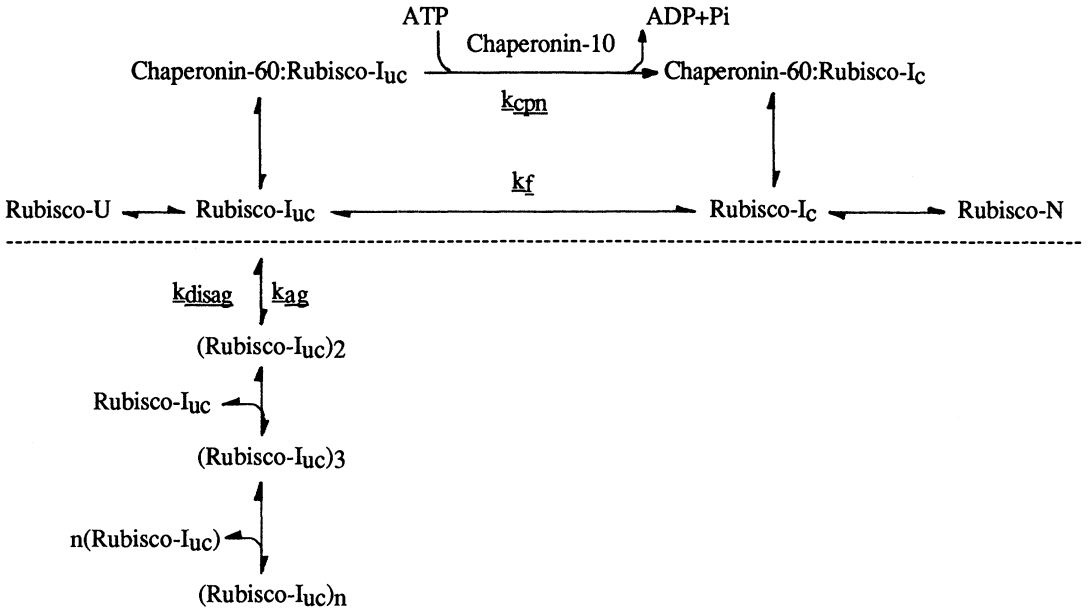

Figure 3. A scheme to account for the rate enhancement observed at 0°C and low ionic strength during the chaperonin-facilitated folding of rubisco. The rate enhancement (chaperonin-facilitated versus spontaneous) could arise if the chaperonins were to catalyse the folding process (i.e. $k_{cpn} \gg k_f$). This we consider improbable. The apparent rate enhancement could also arise if one takes into account the processes occurring below the broken line. If, during spontaneous folding at low ionic strength and at 0°C, there is rapid, but reversible, partitioning of uncommitted folding intermediates (I_{uc}) to aggregated forms which only very slowly revert to the monomeric state, the overall rate of appearance of the native state may become rate-determined by the rate of dissaggregation (i.e. $k_{cpn} = k_f \gg k_{disag}$). An apparent rate enhancement of folding will then be observed in the presence of the chaperonins, which merely prevent the uncommitted folding intermediates from undergoing the initial aggregation. In this case no catalysis of folding is involved.

4. CHAPERONINS AS CATALYSTS

Chaperonin-60 catalyses the K^+-dependent hydrolysis of ATP to ADP and Pi (Viitanen *et al.* 1990). It displays multiple turnovers, saturation kinetics and a sizeable rate enhancement above the spontaneous rate of hydrolysis. In this respect chaperonin-60 is unquestionably a catalyst, albeit a sluggish one. However, the question as to whether or not chaperonins catalyse protein folding is more perplexing. We do not doubt that chaperonins turn over *in vivo*; the large ratio of native rubisco to chaperonin-60 within the stroma of chloroplasts clearly establishes that point. However, nearly all *in vitro* studies with chaperonins have been single-turnover experiments on account of the instability of the non-native states of the target proteins (for an exception, see Langer *et al.* (1992)). Thus it has not proven possible to demonstrate saturation kinetics with chaperonin-facilitated protein folding. Moreover, in those cases where comparative measurements have been made, the rate of chaperonin-facilitated folding has usually been equal to or less than the rate of spontaneous folding, with one exception (see below). Thus, the weight of evidence suggests that the chaperonins do not catalyse protein folding†.

To date there has been only one report of a rate enhancement associated with chaperonin-facilitated folding. In that case a small (four- to fivefold) acceleration was associated with the folding of rubisco at 15°C (Viitanen *et al.* 1990). We have recently begun to explore the conditions necessary to demonstrate this apparent rate enhancement (figure 2). The ionic strengths of the refolding solutions are critical. At 0°C and low ionic strength, a 25-fold apparent rate-enhancement of chaperonin-facilitated folding versus spontaneous folding is evident (figure 2a). Note that, given sufficient time, the yield of spontaneously refolded rubisco approaches that of the chaperonin-facilitated reconstitution. At higher ionic strength, this apparent rate-enhancement of chaperonin-facilitated folding versus spontaneous folding is very much reduced. The chaperonin-facilitated reaction is slightly inhibited at higher ionic strength.

At 25°C an altogether different result is obtained (figure 2*b*). At low ionic strength no spontaneous refolding of rubisco occurs in the absence of the chaperonins. At higher ionic strength some spontaneous refolding occurs. However, chaperonin-facilitated folding proceeds at the same rate as does spontaneous folding; i.e. there is no rate enhancement at 25°C. While the rate enhancement observed with the chaperonins at low ionic strength and at 0°C, might be construed as evidence of folding catalysis,

† It is commonly believed that enzymes stabilize the transition states of the reactions they catalyse by forming structures that are complementary to those transition states (Pauling 1946). The crystal structures of a number of enzymes with so-called transition-state analogues bound at their active sites provide strong support for this notion (Lolis & Petsko 1990). Applied to chaperonin-facilitated protein folding, this concept would require the chaperonin protein to adopt conformations that are complementary to the transitions states for folding of a vast number of structurally unrelated proteins. This is absurd.

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alternative and trivial explanations (e.g. figure 3) must first be excluded.

In vitro complementation experiments (van der Vies *et al.*, 1992) indicated that the species, rubisco-I, is either monomeric or exists in equilibrium with the monomeric species. However, during spontaneous folding at 0°C, the possibility must be considered that higher order off-pathway aggregates are formed reversibly. If disaggregation of these higher-order aggregates were very slow, it could determine the apparent rate of spontaneous folding. In conditions which stabilize the monomeric species, such as in the presence of chaperonins or at higher ionic strength, folding would appear more rapid than that occurring spontaneously. But that would not constitute catalysis of folding, since in effect one would be comparing the rate of an off-pathway reaction with the rate of genuine folding event. We are currently seeking to verify this alternative explanation for the origin of the rate enhancement in the folding of rubisco at low ionic strength and at low temperature.

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Discussion

N. C. Price (*Department of Biological and Molecular Sciences, University of Stirling, U.K.*). Is there any evidence that the chaperonins can assist the assembly of oligomeric proteins or does each chaperonin deal with one polypeptide chain at a time?

G. H. Lorimer. I am not aware of any evidence that the chaperonins facilitate the association steps in the assembly of oligomeric proteins. The product of the chaperonin-mediated step in the case of bacterial rubisco appears to be monomeric; it is not possible to say whether this is the native state of the monomer or a state that is committed to folding to the native state. It is my belief that the transition state for folding occurs after the dissociation of the polypeptide from the chaperonin because in most instances the chaperonins do not enhance the rate of correct folding. We suspect that the rate enhancement we observe with rubisco is an *in vitro* artefact.

R. Jaenicke (*Department of Biophysics and Physical Biochemistry, University of Regensburg, F.R.G.*). Is it correct that the authors' observed stoichiometry of binding is two molecules of rubisco monomer per 14-mer of chaperonin 60?

G. H. Lorimer. This is true for the chaperonin 60 from *E. coli*, but in the case of the chaperonin 60 from beef mitochondria we assume that the stoichiometry is one molecule of rubisco monomer per 7-mer as this chaperonin functions as a 7-mer.

R. Jaenicke. Because the bacterial rubisco is active as a dimer it is tempting to speculate that the two monomers which will associate are joined together on the chaperonin surface. Have experiments been done with proteins which are monomeric, such as dihydrofolate reductase?

G. H. Lorimer. It is hard to exclude more complex scenarios but there are no reasons to consider these at present; the chaperonins will assist the refolding of monomeric proteins such as rhodanese and dihydrofolate reductase.

F.-U. Hartl (*Memorial Sloan-Kettering Cancer Center, New York, U.S.A.*). A recent study by A. Girshovich suggests that the stoichiometry of binding of rhodanese depends upon the conditions used. For example, two molecules of rhodanese are bound per 14-mer in some conditions, but in the presence of the chaperonin 10 only one molecule is bound.

I would like to comment on the observation that the *in vitro* refolding of certain proteins (e.g. dihydrofolate reductase and β-lactamase) by chaperonin 60 does not require either chaperonin 10 or ATP hydrolysis. These proteins can fold spontaneously very well, unlike proteins such as rhodanese or rubisco which have a strict requirement for both chaperonin 10 and ATP hydrolysis. However, it is likely that *in vivo* the assistance of folding of all proteins involves chaperonin 10 and ATP hydrolysis.

R. B. Freedman (*Biological Laboratory, University of Kent, U.K.*). In most of the chaperonin-assisted refolding studies the chaperonins are present in considerable molar excess over the proteins that are refolding. Presumably *in vivo* each chaperonin molecule is used more than once. What is the evidence that the chaperonins can be used more than once?

G. H. Lorimer. Dr Freedman is correct in pointing out that most of the *in vitro* experiments represent one turnover events as regards the chaperonins; it is possible to increase this beyond unity, but it is not easy because of the tendency of the folding protein to self-aggregate when not bound to the chaperonin 60.

F.-U. Hartl. We have managed to show a turnover of four to six with rhodanese by adding two other chaperones, DnaJ and GrpE, which we believe co-operate with the chaperonin system and stabilize the folding protein.

M. Yoshida (*Tokyo Institute of Technology, Yokohama, Japan*). In our studies we observed a stoichiometry of two chaperonin molecules per molecule of target polypeptide, but we are not sure if the chaperonin preparation is homogeneous, so I feel that discussions of stoichiometry are premature until chaperonin preparations are shown to be homogeneous, i.e. that all the molecules are active in protein folding.

I notice that the authors obtained better spontaneous refolding at 4°C than at higher temperatures. Is this because of the reduced rate of random collisions at low temperatures?

G. H. Lorimer. I suggest that it is due to the decreased hydrophobic effect at low temperature which I assume is the driving force behind the aggregation reaction.

W. J. Welch (*Department of Medicine and Physiology, University of California, San Francisco, U.S.A.*). I should like to raise again the question as to whether the chaperonins are involved in association events between separate chains, especially where these involve hydrophobic interactions. Are there any data to suggest that the chaperonins participate in higher order assembly?

G. H. Lorimer. I am not aware of any. To address

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that possibility you would need to study proteins where the appearance of the native state of a polypeptide shows second order kinetics. Studies by others on the assembly of the trimeric enzyme ornithine transcarbamylase suggest that the chaperonins assist the formation of the monomers, but that the association steps are dependent on random collisions between these monomers.

W. J. Welch. But the native state of a monomer as a monomer may be different from its native state when the monomer is associated with other polypeptides.

G. H. Lorimer. That is correct; the data for ornithine transcarbamylase simply say that the chaperonins do not facilitate the trimerization step. John Andrews showed many years ago that it is possible to remove the small subunits from the hexadecameric rubisco and then reassociate them with the large subunit core. This reassociation is very fast and proceeds in the absence of the chaperonins or any other chaperones.

B. O'Hara (*Birkbeck College, University of London, U.K.*). Are the chaperonins capable of recovering correctly folded molecules from aggregates?

G. H. Lorimer. The evidence about aggregates suggests that they are specific and have some secondary structure; this was shown by Jaenicke and Goldberg many years ago. The initial steps in aggregation may be reversible as there are cases where correctly folded molecules can be prepared from aggregates consisting of up to tetramers; however, where the aggregates are large enough to scatter light, it has not been possible to recover molecules which will fold correctly in the presence of the chaperonins.

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