Mini-Review

Chaperonin-mediated Folding of Actin and Tubulin

Sally A. Lewis, Guoling Tian, Irina E. Vainberg, and Nicholas J. Cowan

Department of Biochemistry, New York University Medical Center, New York, New York 10016

The central dogma of molecular biology holds that proteins contain within their primary amino acid sequence all the information that is required to dictate their three-dimensional structure. In principle, therefore, proteins can fold spontaneously (2). It is now clear, however, that the proper folding of many proteins requires facilitation. The facilitation reaction is accomplished via interaction with chaperonins, a class of multisubunit toroidal complexes which hydrolyzes ATP as part of the mechanism whereby its members contribute to the productive folding of their target polypeptides (4, 5, 11, 12, 15, 16, 20). Chaperonins are both structurally and functionally distinct from a class of molecules (typified by the heat shock protein hsp70) that is also thought to participate in the overall protein folding pathway by maintaining polypeptides in a partially unfolded conformation pending their translocation or presentation to chaperonin (for a review see reference 15). Bacteria, chloroplasts, mitochondria, and the cytosol of eukaryotes each contain a single kind of chaperonin molecule. These are different from one other, but homologous. No chaperonin has so far been found in the endoplasmic reticulum. Here we present our view of how the eukaryotic cytosolic chaperonin functions, particularly in relation to what is known about the mechanism of facilitated folding by chaperonin from prokaryotes.

The Jack-in-the-Box Model

Much of what we know about chaperonin-mediated protein folding has been derived from extensive studies on the E. coli paradigm, GroEL. In common with other chaperonins, GroEL is a toroidal structure; each of its two stacked rings is assembled from seven identical 60 kD subunits (5). In conjunction with the cochaperonin GroES—itsel itself a single seven-membered ring of 10 kD subunits (6)— GroEL facilitates the folding of many E. coli proteins (17, 30).

The mechanism whereby GroEL/GroES facilitates protein folding was originally thought to involve sequestration of the bound target protein, providing a protected environment in which spontaneous folding could occur in the absence of intermolecular interactions that would otherwise lead to aggregation (the Jack-in-the-Box model) (1, 9, 11). Electron microscopic evidence (7, 19), and, more recently, crystallographic analysis (5) coupled with a study of site directed mutants that fail to bind target protein (10) point to the inside rim of the chaperonin as the site where target proteins bind, presumably via exposed hydrophobic surfaces. Upon ADP/ATP exchange and hydrolysis, the chaperonin was thought to release its bound polypeptide into the central cavity, giving the opportunity for correct folding to occur; any off-pathway reactions would result in rebinding, so that the native structure would eventually form as a result of sufficient rounds of ATP-dependent chaperonin-target protein interaction. Ultimately, since hydrophobic surfaces are typically buried in native proteins, the correctly folded protein would no longer bind to the chaperonin, and would be discharged into free solution (Fig. 1, cycle a).

Cytosolic Chaperonin and the Cycling of Target Proteins

Cytosolic chaperonin is the functional homolog of GroEL in the cytosol of eukaryotes (11, 12, 24). Unlike GroEL, to which it is only distantly related, cytosolic chaperonin is assembled from eight different (though homologous) polypeptides (18, 23). Both biochemical (11, 12, 24) and genetic studies (8, 29, 31) implicate the participation of cytosolic chaperonin in the facilitated folding of actin and tubulin, as well as actin and tubulin-related polypeptides (21). In the case of actin, the action of cytosolic chaperonin alone in the presence of ATP is sufficient for the generation of native product (12). In contrast, the facilitated folding of the GTP-binding proteins a- and b-tubulin requires cytosolic chaperonin, ATP, GTP, and additional protein cofactors (3, 13, 23). The mechanism of action of protein cofactors in the productive folding of a- and b-tubulin has yet to be established in detail. However, when a cytosolic chaperonin-mediated a- or b-tubulin folding reaction is done in the presence of ATP and GTP but without cofactors, the target protein is released in a nonnative form that can be captured by other chaperonin molecules: for example, the inclusion of excess mitochondrial chaperonin in such reactions results in the bulk transfer of labeled tubulin target protein to mitochondrial chaperonin (14).

The ATP-dependent discharge of nonnative target proteins is not peculiar to the facilitated folding of tubulin: in the case of GroEL, both isotope dilution experiments (28) and the use of mutant GroEL molecules that can act as traps for the capture of nonnative molecules generated
Chaperonins as Unfolders of Off-Pathway Intermediates

The demonstration of the phenomenon of target protein cycling has prompted a rethinking of how chaperonins might work. Since target molecules can be discharged in a nonnative state, an attractive idea seemed to be that the chaperonin functioned by unfoldng target molecules that were kinetically trapped, and discharging these unfolded intermediates so that they could fold spontaneously in bulk solution. Molecules that fail to partition to the native state and again become kinetically trapped would then rebind to another chaperonin molecule, become unfolded once more, and be released into solution where they would "try again" to reach the native state (28, 32) (Fig. 1, cycle b). This concept of chaperonin action is consistent with the notion that the chaperonin itself imparts no information to the target molecule—it merely unfolds it, without discriminating between native and nonnative contacts—and also with the well-established principle that target proteins themselves are endowed with all the necessary information required for them to reach the native state (2).

Chaperonin Specificity

If, as the above model suggests, the function of chaperonins is to act as unfolders of kinetically trapped intermediates, then it seems reasonable to suppose that chaperonins would operate indiscriminately, facilitating the folding of whatever misfolded target proteins they might encounter. This notion is consistent with the observation that GroEL facilitates the folding of a range of target proteins in E. coli (17), while cytosolic chaperonin, which behaves as a single functional molecular species, facilitates the folding of β-actin, the vertebrate actin-related protein RPV, and α-, β-, and γ-tubulin (21). However, experimental evidence shows that chaperonins cannot indiscriminately facilitate the folding of any target protein (25). When β-actin is presented to GroEL as an unfolded target protein, for example, a binary complex is formed; incubation with ATP results in the cycling of β-actin between GroEL molecules, but not in the production of correctly folded protein. Similarly, tubulin target proteins bound to GroEL do not reach the native state upon incubation with ATP and GTP, whether or not the appropriate cofactors are included in the reaction. These experiments demonstrate that, while GroEL is capable of the ATP-dependent cycling of actin and tubulin, the nonnative forms of target protein released by the prokaryotic chaperonin at each cycle cannot partition to the native state. Thus, distinct spectra of intermediates are produced by different kinds of chaperonin (25).

Stable Intermediates in Tubulin Folding

Clearly, a detailed understanding of the mechanism of chaperonin action depends on an analysis of the intermediates generated during folding reactions. This presents a major experimental challenge, since such intermediates are not only vastly heterogeneous, but also transient in existence and very prone to aggregation. Fortunately, the fact that cytosolic chaperonin-mediated tubulin folding reactions require the participation of protein cofactors presents a unique opportunity to study such intermediates, because in tubulin folding reactions, the action of cytosolic chaperonin (which is ATP dependent) can be uncoupled from the action of cofactors (which is ATP independent): the yield of native tubulin produced in in vitro folding reactions is essentially the same whether cofactors are present at the outset, or added after incubation with ATP and GTP and quenching of the ATP-dependent reaction with hexokinase and glucose. Thus, in the absence of cofactors, α-tubulin folding intermediates accumulate (26).

The α-tubulin folding intermediates that accumulate during cytosolic chaperonin-mediated folding are fairly stable, with a half-life of 50 min at 30°C, and are the substrates upon which cofactors act in order to generate native tubulin. These intermediates, defined as Intermediates Quasifolded (IQ), are chaperonin-associated, since
they comigrate with cytosolic chaperonin on sucrose gradients and on gel filtration columns. Moreover, though most α-tubulin intermediates do cycle in the cytosolic chaperonin-mediated folding reaction, I₂ intermediates do not; thus, I₂ intermediates represent an end-state of cytosolic α-tubulin intermediates that have acquired their native-like structure while bound to chaperonin.

Target Range of Cytosolic Chaperonin

If actin and tubulin folding occurs in part on the surface of cytosolic chaperonin, then the spectra of folding intermediates released would be determined by the details of the interaction between the cytosolic chaperonin and these two classes of target protein. Such interactions could reflect the evolution of cytosolic chaperonin in eukaryotes as the quasifolded state can occur while the target protein is cytosolic chaperonin bound (26).

A Model for Chaperonin Action

How might we envision the mechanism of cytosolic chaperonin action? There is evidence that folding intermediates bind to ADP/cytoplasmic chaperonin (22), presumably via exposed hydrophobic surfaces. ADP/ATP exchange then occurs, resulting in an altered conformation of chaperonin (7, 12) (Fig. 1). We propose that this combination of binding and flexing by cytosolic chaperonin causes a conformational change in bound actin or tubulin target proteins ("tweaking"), potentially lifting them over kinetic barriers to productive folding. By analogy with GroEL (28, 32), ATP hydrolysis then results in the release of these target proteins, which either reach the native state or become kinetically trapped and are recaptured by other cytosolic chaperonin molecules. This is essentially the folding-in-Solution model depicted in Fig. 1, cycle b. However, given the existence of I₂ intermediates and the target protein specificity of chaperonins, it is clear that the spectrum of states of target protein released from chaperonin (U in Fig. 1) must include intermediates that are far from fully unfolded, and that have acquired their native-like structure while bound to chaperonin.

Because of space limitations, we apologize to those whose work was not directly cited. The term "Jack-in-the-Box" as applied to certain models of chaperonin-mediated folding was originally coined by Dr. George Lorimer.

This work was supported by a grant from the National Institutes of Health.

Received for publication 19 September 1995 and in revised form 14 November 1995.

References


A Model for Chaperonin Action