Minireview

Enzymes as chaperones and chaperones as enzymes

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Abstract Chaperones and foldases are two groups of accessory proteins which assist maturation of nascent peptides into functional proteins in cells. Protein disulfide isomerase, a foldase, and ATP-dependent proteases, responsible for degradation of misfolded proteins in cells, both have intrinsic chaperone activities. Trigger factor and DnaJ, well known *Escherichia coli* chaperones, show peptidyl prolyl isomerase and protein disulfide isomerase activities respectively. It is suggested that the combination of chaperone and enzyme activities in one molecule is the result of evolution to increase molecular efficiency.
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1. From spontaneous to assisted folding

It is now widely accepted that a large number of proteins do not fold and assemble spontaneously in vivo to form the biologically active molecules but require the help of other proteins. This results in a conceptional transition for protein folding: from the classical 'self-assembly' to the new 'assisted assembly' principle [1]. Most in vitro experiments employed conditions considerably different from the in vivo situation of high protein concentrations with a high risk of aggregation [2] and relatively high temperature for warm-blooded animals. The evolution of higher organisms must create a mechanism to overcome problems resulting from the crowded protein solution and the elevated temperature.

Two groups of proteins have been found to function as accessory proteins for folding: molecular chaperones and foldases which catalyze the necessary covalent reactions directly involved in protein folding [3]. Only two foldases, protein disulfide isomerase (PDI) and peptidyl prolyl *cis-trans* isomerase (PPI), have so far been characterized as foldases. Based on the definition of molecular chaperones as proteins assisting correct folding without covalent changes the foldases were therefore excluded as chaperones [1].

2. Chaperone activity of protein disulfide isomerase

PDI, a multifunctional protein present in the endoplasmic reticulum at high concentration [4], is remarkable in its capacity of non-specific peptide binding, an important feature for a protein to be a molecular chaperone. In addition, for PDI to promote the joining of thiol groups distantly situated in the peptide sequence to form correct disulfides the peptide chain has to be folded at least to some extent to bring the thiol groups together. The spontaneous folding of the peptide chain and the oxidation of thiol groups in vitro are often slow processes and the PDI-catalyzed folding does not need the presence of other chaperones. We therefore put forward the hypothesis that PDI is both an enzyme and a molecular chaperone [5]. Many in vitro and in vivo data have now accumulated to support the hypothesis that in addition to its isomerase activity in the catalysis of native disulfide formation PDI does have intrinsic activity as a chaperone [6–13].

The folding of a peptide chain and the formation of its native disulfide(s) are two intimately connected processes and work in cooperation. In order to distinguish explicitly the possible chaperone activity of PDI from its isomerase activity in assisting protein folding, proteins with no disulfide, D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [7] and rhodanese [8], were employed as target proteins. The presence of PDI in the folding system at near stoichiometric instead of catalytic amounts indeed increases greatly the reactivation yield of both guanidine hydrochloride denatured enzymes upon dilution, and suppresses their aggregation during refolding without being a part of the final functional structure. In addition, PDI suppresses aggregation of rhodanese during thermal denaturation. The above properties are entirely consistent with the definition by Ellis of chaperones [1] and fully meet the four criteria proposed by Jakob and Buchner [14] for characterization of a protein as a molecular chaperone.

The two thioredoxin-like -CGHC- sequences and the peptide segment 451–476 in the C-terminal region of PDI have been characterized to be the active sites for its isomerase activity [15] and the peptide binding site [16] respectively. PDI alkylated at the active site thiols shows little isomerase activity but retains nearly full ability for increasing reactivation and decreasing aggregation during refolding of denatured GAPDH as that of native PDI [17], suggesting that the chaperone activity of PDI is independent of its -CGHC- active sites. It has also been found that mutant PDI with no isomerase activity is still in many in vivo cases functional for cell viability [18], assisting folding of other proteins [11], and assembly of prolyl-4-hydroxylase [12] and microsomal triglyceride transfer protein complex [13] as essential subunits.

The mutant PDI, with its C-terminal 51 amino acid residues responsible for peptide binding deleted, shows neither peptide binding ability nor chaperone activity in assisting the refolding of denatured GAPDH, but keeps most of its catalytic activities [19]. The above provides a straightforward demonstration that the peptide binding site is directly responsible for

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its chaperone activity. In vitro, non-specific peptides compete with the target proteins for binding to PDI to prevent the PDI-assisted folding and the suppression of the aggregation of target proteins, indicating the role of the peptide binding site on its chaperone activity [17].

Both the chaperone and isomerase activities of PDI are necessary for its function as a foldase. For the maximal refolding and reactivation of denatured and reduced acidic phospholipase A_2, a protein composed of 124 amino acid residues and seven disulfide bonds, 90% of the native PDI in the refolding buffer can be replaced by the alkylated PDI with only chaperone but no isomerase activity [17], indicating that the in vitro action of PDI as a foldase consists of both its isomerase and chaperone activities [10]. The simultaneous presence of the C-terminal truncated mutant PDI with no peptide binding site [19] and the alkylated PDI with a peptide binding site but no isomerase activity [17] also shows a collaborative action to promote the reactivation of acidic phospholipase A_2 [19].

It has been proposed that PDI, like other chaperones, recognizes and binds with the non-native structure of unfolded or partially folded intermediates of nascent chains or denatured proteins during folding through its peptide binding site(s), thus prevents incorrect interactions and aggregation of its substrate. Unlike most of the ATP-dependent chaperones, such as GroEL [20] and Hsp70 [21], but like some other chaperones, such as Hsp90 [22], the binding of PDI with its substrates could be transient and the dissociation of the complex is ATP-independent. In such a way PDI prevents nonproductive interactions of peptide chains leading to aggregation and thus promotes their correct folding to a native-like conformation so as to bring the corresponding thiol groups into proximity to be joined up by oxidative formation of native disulfides. This latter reaction is the generally recognized function of PDI. The two intrinsic activities of PDI, the chaperone and isomerase activities, are, in a way, independent of each other but function in collaboration, possibly at consecutive stages of the folding process of the target protein.

3. Chaperone activities of ATP-dependent proteases

Is it an exceptional case for PDI to have both isomerase and chaperone activities in the same molecule? It has recently been demonstrated that some ATP-dependent proteases and closely related proteins also have intrinsic chaperone activities and the protease-associated chaperone activities have been named charonins [23]. Three different families of ATP-dependent proteases, ubiquitous in living organisms, show chaperone activities. For the CIp family, the peptide bond cleavage activity and the substrate recognition site are situated in separate peptide chains. The substrates are first bound with and remodeled by the ATPase component in a way similar to the action of a chaperone and then degraded by the peptidase activity. The two processes are both ATP-dependent. In the absence of the chain with peptidase activity, the substrate can be released in a refolded and active form. For the FtsH family, the two activities are in the same peptide chain. The protease active site mutants retain the ability to promote assembly of the ATP synthase, demonstrating an in vivo chaperone-like activity independent of protease activity. The homo-oligomeric Lon protease family shows the same behavior that a

mutation in the protease active site of the overproduced Lon also promotes membrane protein assembly. The chaperone activity of proteases is important for proteolysis, as the peptide binding domains can interact with substrates and promote chaperone-like remodeling/refolding of proteins. For Lon, unfolding is a necessary part of the proteolysis with high efficiency. As a matter of fact, classical chaperones do participate in proteolysis [23]. Thus in vivo, the combined action of chaperone and protease, situated either in different chains or on the same chain, functions for quality control of proteins in removing misfolded chains by degradation. Structural differences of the complex formed might be responsible for recognition either by proteases for degradation or by chaperones for folding to its native form.

4. Chaperones with enzymatic activities

The *Escherichia coli* chaperone, trigger factor, has recently been shown to be a member of FK506-binding proteins of the PPI family [24]. The trigger factor has a peptide binding site well separated from the isomerase active site and responsible for its high efficiency to promote protein folding with peptidyl-proline isomerization as the rate limiting step [25]. The small PPIs catalyze peptidyl-proline isomerization in short unstructured peptides much better than in the folding of proteins, while the trigger factor is much more effective in catalyzing the folding of large proteins than all known small PPIs, undoubtedly because in addition to the prolyl isomerization reaction, protein folding involves other complicated folding events, which can be handled by the chaperone part of the trigger factor molecule. An excised central fragment from the trigger factor molecule corresponding to the FK506 binding protein remains fully active as a prolyl isomerase with a short peptide as the substrate, but its activity is reduced about 1000-fold in assisting protein folding [25].

DnaJ in *E. coli* is well known as a chaperone involved in protein folding and renaturation after stress. It possesses thioredoxin-like active sites, a cysteine-rich region with four repeats of the sequence -CXXCXGXG-, in which the -CPHC- sequence is the same as that in DsbA, a member of the PDI family. Recently DnaJ was found to be able to catalyze the oxidative formation, reduction and isomerization of disulfide bonds of proteins as does PDI [26]. It has been suggested that DnaJ is involved in protein export by keeping exported proteins in a reduced state prior to membrane translocation and control of the redox state of inner membrane proteins before or during membrane insertion. In addition to its role in protein folding and protection against stress, DnaJ could be involved as chloroplast thioredoxin in the redox controls of several cellular enzymatic activities [27].

5. General discussion

It is interesting to note that substrate binding in a chaperone-like mode is a smart way for improving efficiency of several different classes of enzymes, PDI, ATP-dependent proteases, trigger factor and DnaJ. The chaperone-assisted unfolding and folding of substrates for these enzymes provides a very efficient enzymatic mechanism. Compared to thioredoxin, PDI acquired an additional -CXYC- containing domain, as well as a domain for peptide binding during evolution so as to function more efficiently as a foldase with

both isomerase and chaperone activities, while thioredoxin, showing neither peptide binding ability nor chaperone activity [16,17], has a much lower isomerase activity than PDI [28]. Similarly, the trigger factor is more effective for protein substrates as compared with the small PPIs. Recently, a new group of small PPIs, the parvulins, were discovered to be unrelated with the cyclophilins or the FK-506 binding proteins, and much more active for small peptides than protein substrates [29]. It seems reasonable to assume that addition of a peptide binding domain or subunit to enzyme molecules during evolution improves the efficiency of the enzymes involved in protein folding or unfolding, especially for large protein substrates. The evolutionary relationship between chaperonin and proteins of the ATPase families has been demonstrated by the finding that antibodies against F_1 ATPase α-subunit recognize mitochondrial chaperones [30]. The manifestation of a molecule with both chaperone and enzyme activities provides the protein with either higher efficiency or new functions, such as quality control in the selective removal of misfolded proteins in the case of the ATP-dependent proteases.

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