Chaperonin-Mediated Protein Folding at the Surface of groEL Through a "Molten Globule"-Like Intermediate

Research by J. Martin, T. Langer, R. Boteva, A. Schramel, A.L. Horwich, and F.-U. Hartl, Nature ***1991,*** 352, *36*

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CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To investigate the role of the chaperonin protein groE in protein folding

RESEARCHERS' APPROACH

Using the *Escherichia coli* groEL (14-mer with subunits of 549 residues) and groES (7-mer of 97 residues each) components of the groE protein, the authors reconstituted in vitro the chaperonin-mediated folding of the two enzymes: dihydrofolate reductase (DHFR) and rhodanese. They monitored the refolding of the monomeric enzymes by measuring intrinsic tryptophan fluorescence (neither groEL nor groES contain tryptophans), adsorption of the fluorescent dye anilino-naphtalene-sulfonate (ANS), protease sensitivity, and enzyme activity. Effects of the activation of the ATPase in groEL were also investigated.

OBSERVATIONS

Spontaneous refolding of denatured DHFR was inhibited by the 14-mer groEL, which is constituted by two stacked 7-mer rings. DHFR could be released from groEL under ATP hydrolysis with a sigmoidal reactivation curve, suggesting folding intermediates. DHFR could rebind to groEL, but casein, having properties of a partially unfolded protein, competed with the rebinding. groES added in 1:1 amounts to groEL prevented rebinding. Denatured rhodanese did not refold spontaneously but formed aggregates. Complex formation with groEL prevented aggregate formation. Rhodanese released under ATP addition (quantitative release requires competition with casein) remained inactive. Efficient reactivation of rhodanese required the additional presence of groES, suggesting a specific function for groES.

A first intermediate both for DHFR and rhodanese bound to groEL was seen in tryptophan fluorescence; however, a 50% redshift towards the denatured state

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and a higher intensity both pointed to a lack of complete tertiary structural formation. Since both intermediates had high protease sensitivity and ANS fluorescence showed apolar binding sites neither present in the folded native or completely denatured state, a "molten globule" state is suggested for the protein bound to groEL. The molten globule shows some elements of tertiary formation as association of secondary structures; however, the fluctuating hydrophobic core still displays water contact. After addition of ATP, a second intermediate DHFR bound to groEL was observed with less tryptophan and ANS fluorescence and less protease sensitivity. A similar second rhodanese intermediate bound to groEL also required the presence of groES.

Reactivation of rhodanese from a groEL/groES complex, formed before addition of ATP, could not be stopped by casein competition; thus, a single interaction in the complex seems to be sufficient for reactivation. Unfolded rhodanese boosted the ATP hydrolysis rate of groEL and was released again without reactivation. GroES suppressed both, and the gradually decreasing ATPase activity was coupled to the reactivation of rhodanese using about 130 ATPs per rhodanese molecule.

COMMENTARY ON THE RESEARCH

The authors chose the best characterized molecular chaperone, the essential groE proteins from bacteria. Chaperones prevent partially folded and unassembled protein subunits from aggregating and precipitating. They (groEL) recognize, refold, and release (groEL/groES, ATP hydrolysis) protein subunits. The authors take earlier results[1] much further by investigating the changes of the denatured protein directly while it is bound to groEL and groEL/groES. They investigate three general protein refolding types: a protein that stays partially unfolded (casein, used as a competitor); one that refolds to the active state spontaneously (DHFR); and a protein that requires a chaperone for reactivation (rhodanese).

Quite strikingly the authors show for the first time clear indications for intermediate states in the refolding of the groEL-bound proteins. The first intermediate is more unfolded. Also, the ANS fluorescence shows appearance of apolar binding sites present only in this intermediate. These observations suggest a molten globule state for the intermediate. Part of the effect could be due to shielding of hydrophobic regions of the bound protein by groEL; however, a similar intermediate has recently been described also for free rhodanese.[2] A second, more compact intermediate, bound to the groEL and groES complex, could also be identified after ATP addition. Thus in this and in free protein folding studies,[3] defined folding intermediates emerge.

Creighton has speculated[4] on a model for these and other observations on the groE proteins. groEL may be a sandwich composed of two 7-mer rings and provides only one binding pocket (probably in the central channel in the stacked rings) for a not completely folded protein. Seven monomers would thus act in concert to attach proteins. The binding of an unfolded protein and the groES 7-mer would occur first to one and then to the other 7-mer in the groEL 14-mer, with the transfer within the central channel requiring ATP hydrolysis and a quaternary structural change of groEL. The groES function would keep the unfolded protein sequestered from the bulk solution. The protein would tend to fold to a more stable state during the transfer; without groES it would dissociate into the bulk solvent. Once refolded, the protein would no

longer bind to groEL, ATP hydrolysis would stop, groES would dissociate, and the more stable protein could be released. Further studies are necessary to test this and alternative models.

The understanding of the observed[5,6] "molten globule" states in protein folding remains a puzzle.[7] Also basic questions as to whether groEL recognizes unfolded proteins through hydrophobic patches normally buried in the native state or through contiguous sequence segments, as is the case[8] for hsp70, remain to be answered. It is also not clear if a completely unfolded protein binds groE or if it must be already largely folded as in a molten globule state before binding groE. Furthermore, do the chaperonin folding catalysts simply prevent pathways resulting in misfolded and aggregated states or do they actually participate in the folding process by lowering pathway activation energies.[9] Martin et al. could not show if the bound molten globules were achieved before or after groE binding. The solution to this important issue will be central for theorists trying to crack the protein folding problem, i.e., predicting tertiary structure from only a knowledge of the primary sequence. If proteins rely heavily on chaperonin interaction for the proper folding environment, then the folding riddle will not be easily solved since the elemental interactions found in the pathway will have little connection with the final tertiary structure.

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