Successive Action of DnaK, DnaJ, and GroEL Along the Pathway of Chaperone-Mediated Protein Folding

Research by T. Langer, C. Lu, H. Echols, J. Flanagan, M.K. Hayer, and F.-U. Hartl, Nature ***1992,*** 356, *683*

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CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To investigate a chaperone-mediated protein folding pathway

RESEARCHERS' APPROACH

The single and combined effect of the *Escherichia coli* heat shock proteins DnaK (a prokaryotic hsp70 homologue), DnaJ, GrpE, and GroEL/ES (hsp 60 homologues), as well as Mg-ATP, on refolding of rhodanese was studied in vitro by stochiometry, gel chromatography, intrinsic tryptophan fluorescence (only rhodanese and DnaK contain tryptophans), absorption of the dye anilino-naphtalene-sulfonate (ANS), protease sensitivity, and time courses.

OBSERVATIONS

Rhodanese aggregation was suppressed by a high molar excess of DnaK (>20:1) or DnaJ (5:1) while GrpE at 10-fold excess had no effect. Sub-stochiometric concentrations of DnaJ markedly increased the potency of DnaK to bind to and prevent displacement of rhodanese, albeit rhodanese remained inactive even in the presence of Mg-ATP. Addition of GrpE stimulated the ATPase activity of the DnaK/J complex and allowed slow refolding. Different relative concentrations of GrpE/DnaK/DnaJ did not improve the final yield of 30% refolding. Though the combination of DnaK, DnaJ, Mg-ATP, and GroEL/ES did not lead to reactivation either, the addition of GrpE did lead to rapid folding to the native enzyme. Optimal yield was achieved at a 2–3 molar excess of DnaK over DnaJ with higher concentrations of DnaK reducing the yield, especially when its concentration exceeded that of GrpE.

Left in buffer alone for 10 min rhodanese could not be refolded by any combination of heat shock proteins; nonetheless, the presence of DnaK/J maintained rhodanese competent for refolding for more than 1 hour. Rhodanese bound to DnaJ alone could be reactivated by GroEL/ES with a yield of 20%,

CHEMTRACTS—BIOCHEMISTRY AND MOLECULAR BIOLOGY 3:149–151 (1992)
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with or without the addition of GrpE. GroEL alone binds to unfolded rhodanese but not to its aggregated state. The transfer from DnaK/J to GroEL with Mg-ATP but without GrpE yielded only 20% of the total rhodanese as folded. If Mg-ATP was omitted, however, transfer became GrpE independent as the DnaK/J complex was less stable, but its reactivation by GroEL/ES still required GrpE. GroEL/ES refolds repeatedly unfolded rhodanese where four to five rounds of reactivation could be observed as compared to only one round in the presence of GroEL/ES with DnaK/J and GrpE missing.

Gel chromatography showed the presence of a DnaK/J/rhodanese complex in the presence of ATP and magnesium. DnaK binding alone to rhodanese was unstable, but it did bind the extended polypeptide of reduced carboxy methylated α-lactalbumin (R-CMLA) that GroEL or DnaJ alone or in the presence of ATP did not bind. In contrast, casein or partially unfolded lactalbumin, model proteins for the "molten globule" state,[1] were only recognized by GroEL and DnaJ. A weak three-protein interaction between DnaJ, DnaK, and R-CMLA could be detected.

Intrinsic fluorescence of tryptophan in rhodanese occurs at 295 nm. Binding DnaJ to rhodanese led to a red shift halfway (343 nm) to the unfolded state and to ANS-fluorescence (after correcting for the ligand-free fluorescence of DnaJ) indicative of tryptophan in a hydrophobic, collapsed environment. ANS fluorescence is absent in native or fully unfolded rhodanese but the triple complex DnaJ/DnaK/rhodanese reaches fluorescence intensities found when rhodanese is in the presence of GroEL.[2] Chaperone-bound rhodanese was very sensitive to digestion by proteinase K, while the native and aggregated forms were highly resistant.

COMMENTARY ON THE RESEARCH

Using a refined in vitro system, the authors dissected the action of *E.coli* heat shock proteins on rhodanese. Some protein folding intermediates exhibit a strong tendency to aggregate, a notoriously difficult feature in refolding rhodanese. The authors could show that DnaK together with substoichiometric amounts of DnaJ bound and stabilized rhodanese much more effectively than either alone; the effect was even more pronounced when ATP was added. The modulation of DnaK by DnaJ is reminiscent of the interaction of GroEL and GroES. The authors demonstrated further that transfer of the DnaK/DnaJ complex, which prevents aggregation of the rhodanese folding intermediate, to GroEL/ES in the presence of Mg-ATP required yet another chaperonin, GrpE. GroEL allowed the partially folded pool to achieve the native, folded state in a "catalytic" manner as it could be multiply recycled. This detailed analysis strongly supports the idea that a chaperone pathway exists in which DnaK recognizes first extended forms of incompletely synthesized proteins. This complex is stabilized by DnaJ. Subsequent transfer to GroEL requires the coupling factor GrpE. In the presence of GroEL/GroES, accompanied by ATP hydrolysis, the final folded state is achieved.

How general is this pathway? In vitro studies show examples that productive refolding requires the presence of a complete peptide or folding domain.[3] The authors emphasize that for in vivo synthesis extended peptides are produced first and lack stabilizing tertiary interactions. This suggests a general role in protein synthesis for the hsp70[4] family; namely, DnaK, DnaJ, and GrpE.

In contrast to rhodanese, the requirement of chaperones for proteins that can spontaneously refold from the denatured state is less evident. Perhaps the emerging N-terminus with its inherent secondary structure might direct the remaining fold. Even for the proteins that fold without chaperones, there is always the necessity for heat shock proteins to prevent denaturing in adverse conditions. As biological systems exhibit a very high level of complexity, it should not be too surprising if further experiments show that several modes are indeed exploited for protein folding. Moreover, true catalysis of protein folding is known for disulfide bonds and proline isomerizations by the enzymes disulfide isomerase and peptidyl-prolyl *cis-trans* isomerase.

The results of Langer et al. show now that there is a complete set of chaperones available to bind proteins in a wide range of unfolded states with DnaK utilized for most unfolded, extended states and GroES triggering the final activation necessary to reach the native state. Even for the most skeptic observer this underlines that chaperones have the potential to interact with proteins in all states of unfolding and that transfer between different folding states and complexes is possible.

It would be expected that there is also functional cooperation of hsp 70s in eukaryotes as found in *E.coli* and thus that eukaryotic proteins like DnaJ and GrpE exist. Nonetheless, the intriguing question still remains if chaperonins and their assisting factors can actually induce specific secondary structures and tertiary interactions upon the folding protein.[5-7]

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