Modeling Hsp70-Mediated Protein Folding

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ABSTRACT The Hsp70 chaperone system is the major molecular chaperone system that assists protein-folding processes in all cells. To understand these processes, we analyzed the kinetic characteristics of the *Escherichia coli* homologs of this chaperone system during folding of a denatured protein using computer simulations and compared the results with in vitro refolding experiments. Rate constants used for the model were derived from recent literature or were determined and scrutinized for their applicability to the refolding reaction. Our simulation results are consistent with reported laboratory experiments, not only simulating the refolding reaction of wild-type proteins but also the behavior of mutant variants. Variation of kinetic parameters and concentrations of components of the Hsp70 system demonstrate the robustness of the chaperone system in assisting protein folding. Furthermore, the importance of the synergistic stimulation of the ATPase activity of Hsp70 is demonstrated. The limitations of our kinetic model indicate sore spots in our understanding of this chaperone system. Our model provides a platform for further research on chaperone action and the mechanism of chaperone-assisted refolding of denatured proteins.

INTRODUCTION

Although the entire information for the precise three-dimensional structure of a protein is encoded in its amino acid sequence, in vivo, in the crowded environment of a cell, many proteins depend on the assistance by molecular chaperones such as Hsp70 and Hsp60 heat-shock proteins for folding from nascent state into their correct structure (1,2). Once in the native, active state, a protein is not safe but constantly endangered to loose its active conformation by denaturation and misfolding due to collisions with other cellular components as result of Brownian motion. This is particularly prevalent under stress conditions such as elevated temperature, which is counteracted by living organisms through the induction of a small set of proteins, called heat-shock proteins, that protect denatured proteins from aggregation and assist their refolding into the native state.

The *Escherichia coli* Hsp70 chaperone machinery, consisting of the Hsp70 homolog DnaK and the cochaperones DnaJ and GrpE, was shown to be the most efficient chaperone system in *E. coli* preventing aggregation of many proteins of different sizes in vivo at elevated temperatures and refolding them to the native state after return to optimal growth temperature (3,4).The DnaK system can efficiently repair denatured model proteins such as *Photinus pyralis* luciferase both in vivo and in vitro but cannot protect it from heat induced activity loss (5). Experimental findings suggest that this refolding process is achieved by ATP-dependent transient interaction between the DnaK chaperone with a short peptide stretch within the substrate polypeptide (S) (6). DnaJ and GrpE function as regulators in this system by stimulating DnaK's ATP hydrolysis activity and subsequent nucleotide exchange (7–10).

The kinetics of the DnaK chaperone system has been studied extensively in vitro. These studies elucidated that the nucleotide-regulated transition between two conformational states is crucial to the function of DnaK. These states are the ATP-bound state with high association and dissociation rates but low affinity for substrates and the ADP-bound state with two and three orders of magnitude lower association and dissociation rates but high affinity for substrates (Fig. 1 *A*) (11–13). The spontaneous transition between the two states is extremely slow but stimulated by substrates and DnaJ synergistically. Under physiological conditions of high ATP concentrations, nucleotide exchange is rate limiting for substrate release. However, nucleotide exchange of DnaK is slow but stimulated 5000-fold by the nucleotide exchange factor GrpE. Based on the results of a number of laboratories, Schröder et al. (5) suggested a chaperone folding cycle, which was further elaborated by McCarty et al. (8), Karzai and McMacken (14), and Laufen et al. (10) and is meanwhile widely accepted. In this proposed mechanism, an unfolded protein substrate (e.g., *P. pyralis* luciferase) first associates with DnaJ, which will present it to DnaK·ATP and induce the formation of a trimeric DnaK·ATP·DnaJ·substrate complex. DnaJ and substrate synergistically stimulate ATP hydrolysis by DnaK and thereby trigger the transition of DnaK from the ATP state with low affinities for substrates to the high-affinity ADP state. GrpE binds to the latter complex and catalyzes the release of ADP. Subsequent ATP binding induces conformational changes in the ATPase domain and substrate-binding domain leading to a rapid dissociation of GrpE and substrate from the complex. These steps form the cycle of DnaK-assisted protein folding (Fig. 1 *A*). With enough ATP and all the chaperone molecules, after many cycles, the substrate can be refolded back to its active state (Fig. 2 *A*; see also (5)).

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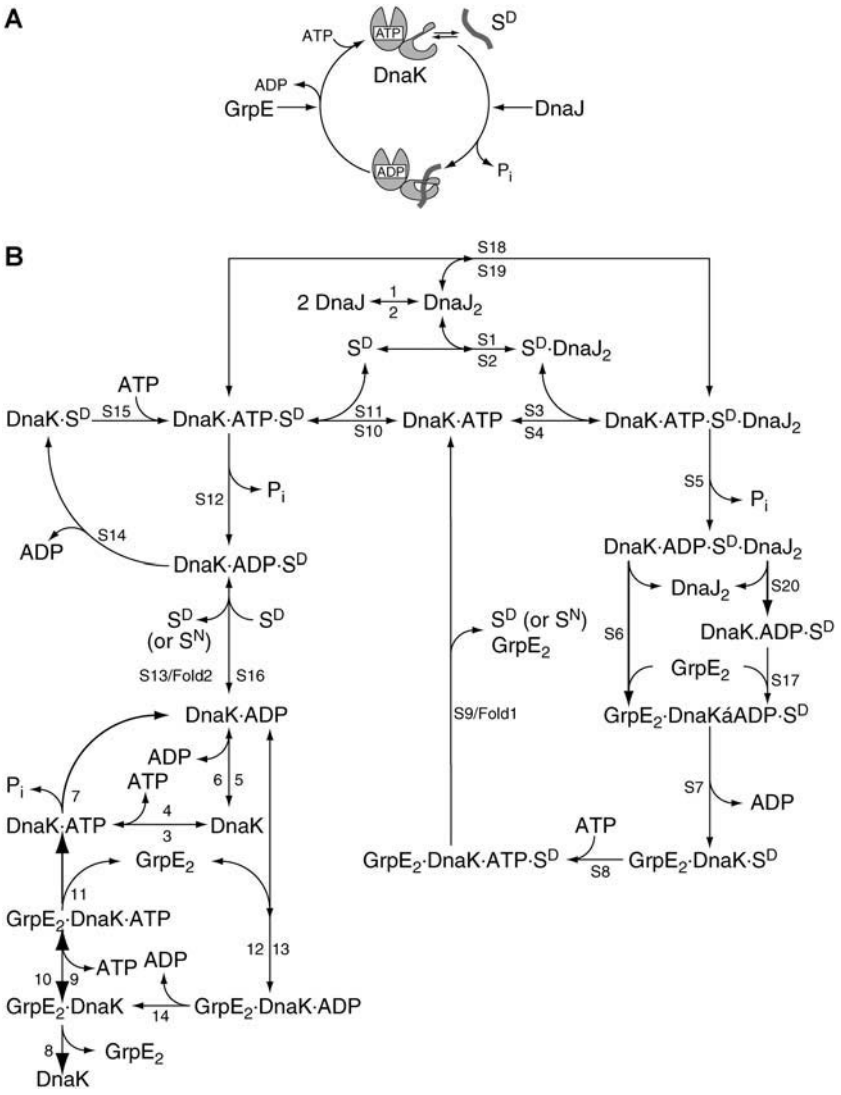

FIGURE 1 DnaK chaperone cycle. (*A*) Schematic ATPase cycle of the DnaK system. (*B*) Elemental chemical equations for the refolding of a denatured protein substrate S^D by the DnaK system. With a certain probability the substrate S^D can be refolded to the native state S^N within any given cycle of binding, ATP hydrolysis, and release simulated as alternative reactions at the indicated positions. Numbers at the arrows indicate the reaction number in Table 1, where by, at horizontal arrows, the top number indicates the reaction from left to right, and the bottom number indicates the reaction from right to left; at vertical arrows, the left number indicates the reaction from top to bottom, and the right number indicates the reverse reaction.

Here, we describe a kinetic model for the chaperone action of *E. coli* DnaK in protein refolding based on the different interactions of the five components of the systems DnaK, DnaJ, GrpE, substrate, and nucleotide reported in the literature (Fig. 1 *B*). Due to a lack of experimental data, this model is currently restricted to the simulation of the in vitro situation excluding effects of crowding and diffusion inside the cell. The rate constants were derived from the literature or completed by our experiments. Our model correctly simulates the behavior of DnaK's chaperone action including the behavior of DnaK mutant proteins with altered kinetic parameters. The sensitivity of refolding productivity to alterations in activity and concentration of the chaperones and ATP consumption are discussed.

MATERIALS AND METHODS

Reaction and parameters

All of the reactions and parameters used in this computer model were either based on published literature or measured in the laboratory of M.P.M. The list of reaction equations, including parameters and references, are given in Table 1, together with the buffer conditions used in the measurements. Parameters that were determined in different laboratories using buffers that were different from the buffer used for firefly luciferase were checked exemplarily in our laboratory in the appropriate buffer and found to be similar. For reactions that start with the same reactants but have alternative products depending on a probability factor, rate constants are assigned to accommodate the desired probability. The reaction volume is set to a typical volume of an *E. coli* cytoplasm.

Simulation

The simulations are based on an improved version of Gillespie's exact stochastic simulation algorithm (15) by Gibson and Bruck (16), as implemented in E-CELL version 3, an open source computer software package for simulation of large-scale cellular events developed at Keio University (http://www.e-cell.org) (17,18).

RESULTS

Model construction and validation

To construct a kinetic model for the chaperone-assisted refolding of a denatured protein, the refolding process was