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A Simple Model of Chaperonin-Mediated Protein Folding

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ABSTRACT **Chaperonins are oligomeric proteins that help other proteins fold. They act, according to the "Anfinsen cage" or "box of infinite dilution" model, to provide private space, protected from aggregation, where a protein can fold. Recent evidence indicates, however, that proteins are often ejected from the GroEL chaperonin in nonnative conformations, and repeated cycles of binding and ejection are needed for successful folding. Some experimental evidence suggests that GroEL chaperonins can act as folding "catalysts" in an ATP-dependent manner even when no aggregation takes place. This implies that chaperonins must somehow recognize the kinetically trapped intermediate states of a protein. A central puzzle is how a chaperonin can catalyze the folding reaction of a broad spectrum of different proteins. We propose a physical mechanism by which chaperonins can flatten the energy barriers to folding in a nonspecific way. Using a lattice model, we illustrate how a chaperonin could provide a sticky surface that helps pull apart an incorrectly folded protein so it can try again to fold. Depending on the relative sizes of the protein and the chaperonin cavity, folding can proceed both inside and outside the chaperonin. Consistent with experiments, we find that the folding rate and amount of native protein can be considerably enhanced, or sometimes reduced, depending on the amino acid sequence, the chaperonin size, and the binding and ejection rates from the chaperonin.**

Key words: energy landscape, kinetic traps, hydrophobic interaction, multiple folding pathways, chaperone action, lattice models

INTRODUCTION

Molecular chaperones may act by various mechanisms.[1–4] Hsp70-family chaperones appear to prevent protein aggregation during folding.[1–5] Here we consider the hsp60-family chaperonins, which can also act as folding catalysts,[5–11] in some cases even when there appears to be no significant aggregation.[9,11] The structure of the *E. coli* chaperonin GroEL is now known.[12,13] It has a double-doughnut shape, with enough room in the center to hold a compact collapsed protein. The inner walls of the doughnut are hydrophobic.

Experiments of Martin et al.[14] suggested that "globular proteins may generally be sequestered during folding at the surface of a chaperonin-type machinery."[14] This is sometimes referred to as the "Anfinsen cage"[15] or "box of infinte dilution"[16,17] model, in which the main role of the chaperonin is to protect the folding protein from aggregation. An alternative mechanism to this "caging" process was later proposed by Burston et al.,[6] in which the chaperonin plays a more active role. According to this hypothesis, the energy provided by adenosine triphosphate (ATP) hydrolysis is used to disrupt incorrect interactions in the kinetically trapped "misfolded" protein, allowing it to escape. This picture has gained support from more recent experiments.[7,9–11] Here we use a lattice model to study the energy barriers to protein folding in the presence of chaperonins.

Studies of the folding kinetics of lattice model proteins indicate that some proteins can fall into low-energy kinetic traps as they fold.[18–21] The trapped conformations are compact and hydrophobically clustered, and the subsequent slow steps in folding involve climbing up an energy hill out of the traps by breaking noncovalent bonds in a sort of "breathing" step by which the chain expands to reach its folding transition states. The nonspecific act of pulling apart hydrophobic contacts can bring the protein to a higher vista on the energy landscape, where it can try again to fold. We propose that the hydrophobic interiors of chaperonins may simply provide a hydrophobic surface that competes with intrachain hydrophobic collapse and helps to pull apart compact chain conformations. ATP is envisioned to provide the energy to unstick the protein from the chaperonin and release it to the exterior. In this way, the protein is given a series of opportunities to attempt folding inside and outside the chaperonin.

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HP LATTICE MODEL

We model a folding protein using the two-dimensional (2D) HP lattice model.[18,19] Simple lattice models have been useful for exploring principles of protein folding and addressing problems of conformational changes that are too large to be treated by more microscopic models.[5,18–26] Here a protein is represented as a short chain of a specific sequence of two types of monomers, hydrophobic (H) and polar (P), for which configurations are explored by exhaustive enumeration on 2D square lattices. We consider only HP sequences that fold to unique native states. Contacts between nonpolar units (HH contacts) are favorable by an energy $\epsilon < 0$. Despite the obvious simplifications, such models have been shown to resemble real proteins in their collapse transitions, development of secondary and tertiary structure, mutational properties, and folding kinetics.[19] The top section in Figure 1 shows a kinetically trapped configuration, **B**, followed by its transition states a, b, c and the corresponding part of its "energy landscape," on its way to the native state **N**.

We model the chaperonin as two hydrophobic surfaces separated by c lattice sites, so c is related to the radius of the doughnut hole. Only chain conformations that can fit into a $c \times c$ box can bind the chaperonin. Figure 1 shows how the chaperonin flattens the energy barriers. The energies are lowered for the conformations that are otherwise high-energy transitional states for the isolated protein along pathways of escape from the trap. The top of Figure 1 shows that, in the absence of the chaperonin, stepping from the trap to the transition state involves breaking two HH contacts, up an energy hill of two units. The middle of Figure 1 shows ways the hydrophobic residues of the protein can stick to the chaperonin instead of forming intrachain contacts. Thus the chaperonin pulls apart compact conformations.[7] In some cases this can involve only small local structural rearrangements, although extensive unfolding inside the chaperonin is also possible in the present model. Sticking to the chaperonin involves downhill energetic changes because the total energy is proportional to the total number of hydrophobic contacts, either from intrachain interactions or from protein/chaperonin interactions. The protein can undergo further conformational changes inside the chaperonin after initial binding (Fig. 1). Such changes need not involve large expansions. In this model, ATP then pays the price of the main uphill energetic step, which involves unsticking the protein from the chaperonin and ejecting the protein.

Upon ejection, the protein is likely to have fewer intrachain HH contacts than when it entered. The protein is now higher on its energy landscape, so a new attempt can be made to fold by moving energetically downhill outside the chaperonin. Because the process is nonspecific in the model, there is no guarantee that ejection and recollapse will necessarily lead to the correct native state. In general, the ejected chains are not necessarily "committed" to the native state, but some fraction of them will fold to the native state via "throughway paths,"[18,20] i.e., along smooth funnel-like downhill energetic routes. Others will fall into new kinetic traps[18,20] and the binding-ejection cycle is repeated.[6–10]

The chaperonin thus acts through a general mechanism of barrier flattening by providing alternate folding pathways, or, in the terminology of M.J. Todd, G.H. Lorimer, and D. Thirumalai,[27] an "iterative annealling" process. The model shows that the chaperonin does not merely reduce barrier heights, as catalysts reduce transition state barriers, rather the chaperonin completely changes a significant part of the energy landscape, the part corresponding to compact conformations. The landscape of the complex, chaperonin plus protein, bears no simple relation to the landscape of the protein alone. The chaperonin acts by iterative ejection of the proteins, but the distribution of conformations ejected from the chaperonin is in general different from that of the initial denatured conformations in refolding experiments. Therefore, the probability ϕ_c that the ejected protein folds along throughway paths could be different from the probability ϕ of throughway folding of the unchaperoned protein. It follows that if ϕ is the yield of an unchaperoned folding experiment, the additional yield measured after one cycle of chaperonin action could be different from $\phi(1 - \phi)$.

The bottom of Figure 1 shows a folding path of a chain that undergoes two cycles through a chaperonin before it reaches the native state. In all the HP sequences we studied, the chaperonin reduces the energy barrier for folding (although this is not sufficient to imply acceleration of folding; see below). Other theories[5,27–29] also propose that chaperonins create alternate folding pathways, but the present work is the first, as far as we know, to model the underlying physical mechanisms explicitly.

Can the protein fold inside the chaperonin? Not every conformational change inside the chaperonin necessarily involves further opening up of the protein. Hence some kinetic progress towards the native state is possible for some sequences. However, it can be difficult for a large protein to reach its native conformation inside a small chaperonin,[14–17] for two reasons. First, in a small hydrophobic cavity, the protein can adopt conformations for which the total energy is lower than the native state by reconfiguring to stick to the cavity walls. For a chaperonin of size $c = 4$, the most stable states of all the 13-mer HP sequences we studied are found when proteins are stuck to the chaperonin walls in otherwise nonnative states. Second, steric constraints of a small cavity make some conformational rearrangements impossible, so the protein cannot reach its native state from some compact states.

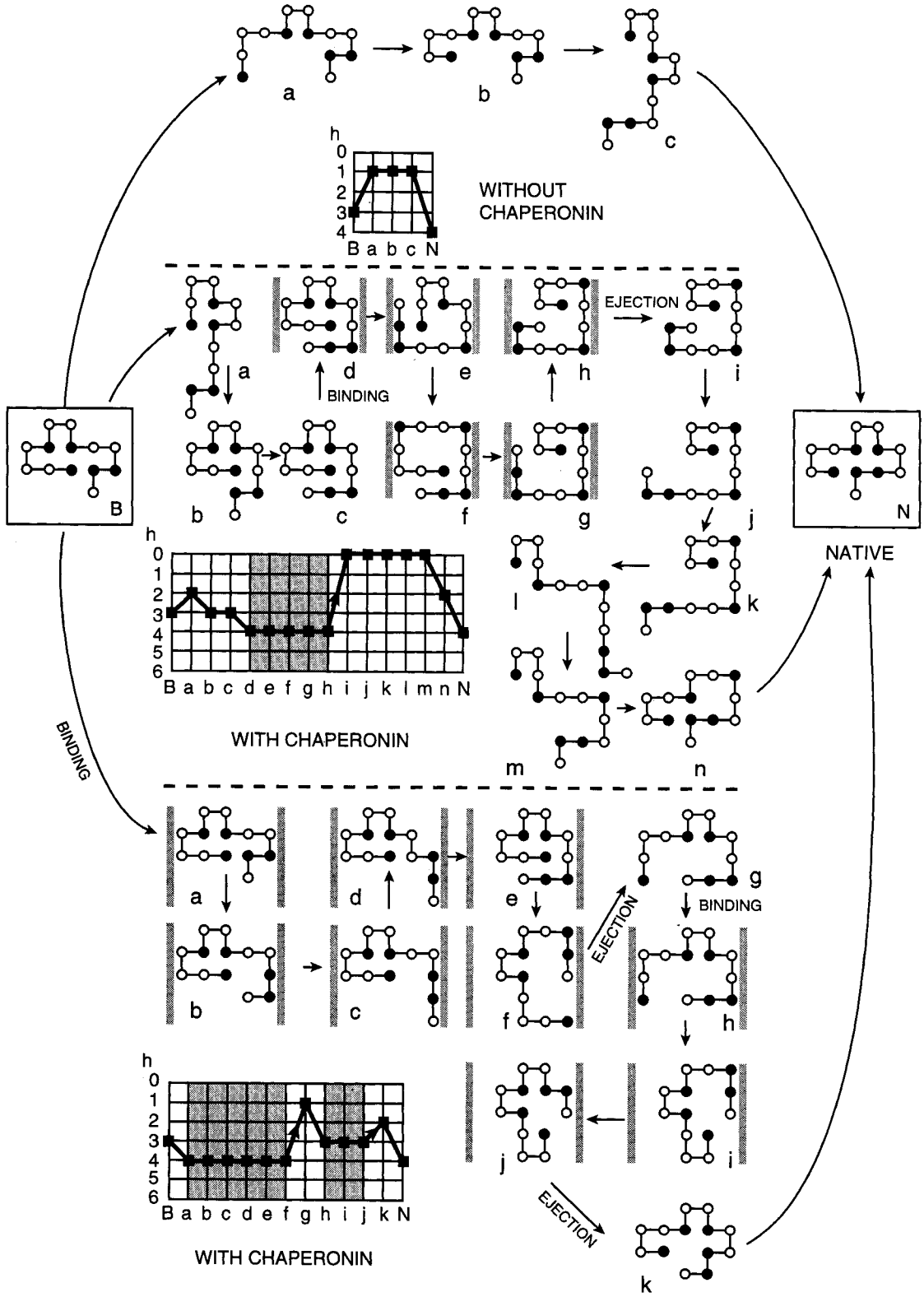

Fig. 1. Model of chaperonin action. **Top:** In the absence of chaperonins, **B** is a kinetically trapped conformation that requires breaking at least two HH contacts before it can reach the unique native structure **N.** The energy landscape gives the history of the number of HH contacts *h* along the path. **Middle:** The chaperonin (pair of hydrophobic walls, $c = 4$) is a sticky surface that competes with intrachain hydrophobic contacts and randomly unfolds parts of the protein. Now the conformations that were transition states for the unchaperoned protein are stabilized by the wall sticking energy. The energy cost of unsticking the partly unfolded protein from the chaperonin is paid by ATP hydrolysis. The partly unfolded ejected protein now makes a new attempt to refold. On the energy landscape, shaded area indicates binding of the protein to a chaperonin and arrows indicate ATP-assisted protein ejection. **Bottom:** The minimum barrier height to native from the same kinetic trap **B** is reduced to zero by a $c = 5$ model chaperonin. The model protein enters the chaperonin twice to fold along this path.

In this model, kinetic adjacency among unbound free conformations and bound states of different conformations are specified by move set MS2.[18,20] The different bound states of a given conformation, which correspond to different ways of binding the chaperonin, are kinetically adjacent to one another and are adjacent to the free state of the same conformation.[18,20]

On the other hand, inside a larger chaperonin ($c = 5$), folding or partial folding can sometimes occur inside the chaperonin[14] (bottom section of Fig. 1). The native structures of six sequences we studied are identical to the lowest energy states of the protein inside a $c = 5$ chaperonin. We do not model effects of GroES or possible cavity size changes caused by it.[12]

KINETICS

To model the kinetics, we use a transition matrix method, described elsewhere.[18,20] Three quantities describe the kinetics in the presence of chaperonins:

1. S is the free energy barrier to protein binding to the chaperonin. We use $S = \exp[3\epsilon/(2kT)]$ to ensure that binding is favorable only when more than one protein-chaperonin HH contact is formed upon binding. Although we do not model protein aggregation here, we note that protein conformations having a greater exposure of surface hydrophobic groups have a greater tendency both to aggregate and to be captured by the chaperonin. This may account for protection against protein aggregation by chaperonins.
2. B is the binding rate of encounter between proteins and chaperonin, dependent on the component concentrations.
3. E is the rate of ejection of the protein from the chaperonin, which depends on the ATP concentration. However, even at $E = 0$ the protein can drift out of the chaperonin in our model, often at a small rate, as specified below.

Transitions occur only between kinetically adjacent conformation states.[18,20] The transition matrix element between adjacent states a and b is given by

$$(\mathbf{T})_{ab} = \begin{cases} \min\{1,\exp[-\epsilon(h_b - h_a)/(kT)]\}/A_{\max} \\ \qquad \text{for } a \neq b, \text{ when both } a \text{ and } b \\ \qquad \text{are free or both are bound} \\ \min\{1,S\exp[-\epsilon(h_b - h_a)/(kT)]\}(B/A_{\max}) \\ \qquad \text{for free } a \text{ and bound } b \\ (\min\{1,\exp[-\epsilon(h_b - h_a)/(kT)]/S\} + E)/A_{\max} \\ \qquad \text{for bound } a \text{ and free } b \\ 1 - \Sigma_{b \neq a}(\mathbf{T})_{ab} \qquad \text{for } a = b \end{cases} \quad (1)$$

where h_a and h_b are the number of HH contacts in conformational states a and b, respectively. These include both intrachain and protein-chaperonin HH contacts. kT is Boltzmann constant times absolute temperature. $A_{\max}$ is a normalization constant such that all elements of $(\mathbf{T})$ are non-negative.[18,20]

We have studied the folding kinetics of this model for all 173 13-mer HP sequences that fold to unique native states. We have explored a wide range of values for the parameters B and E. We find that chaperonins can change either the folding rates or yields or both (Table I). The yield, which is defined in our model as the steady-state native population at large times, depends on the binding and ejection rates and is sequence dependent. Native structures with more exposed hydrophobic surfaces can form many contacts with the chaperonin. They are likely to have lower yields because they bind more tightly to the chaperonin. For example, for $B = 0.03$, $E = 0.001$, sequences with high yields (larger than 95%) on average have only 1.4 exposed hydrophobic surfaces that can bind to the chaperonin, whereas sequences have low yields (less than 95%) have on average 3.2 exposed hydrophobic surfaces that can bind to the chaperonin.

TABLE I. Average Folding Time Reduction Factors t_c/t_0 for Different Binding and Ejection Rates B and E for Chaperonin Sizes $c = 4$ and $c = 5$*

	B ($c = 4$)			B ($c = 5$)		
E	1.0	0.03	0.001	1.0	0.03	0.001
1.0	0.0076 (87/87)	0.075 (88/91)	0.57 (88/93)	0.017 (12/12)	0.088 (15/15)	0.27 (23/25)
0.03	0.049 (63/64)	0.11 (82/85)	0.61 (86/95)	0.073 (10/10)	0.049 (15/15)	0.094 (31/31)
0.001	1.0 (22/52)	0.70 (45/66)	0.75 (71/97)	0.93 (2/4)	0.059 (23/23)	0.12 (33/33)

*For given B and E, the top number is the geometric mean of t_c/t_0 over sequences with steady-state native population larger than 95%. The unchaperoned and chaperonin-assisted (90% native) folding times t_0 and t_c are defined in Figure 2, computed here with the same $\epsilon = -9.3kT$ and move set MS2[18,20] as that in Figures 2 and 3. A smaller t_c/t_0 implies that the folding rate is speeded up more by chaperonins. For given B and E, the numerator of the fraction in parentheses is the number of sequences whose foldings are speeded up, whereas the denominator is the number of sequences among the 173 $n = 13$ unique sequences[18,20] that have over 95% steady-state native population.

Chaperonins can either accelerate or decelerate folding. When both binding and ejection rates are fast, folding is accelerated for sequences that intrinsically fall into kinetic traps. Not surprisingly, the steady-state native population is reduced when ejection is slow and binding is fast. When ejection and binding are both slow, folding is slowed because the long waits to enter and exit the chaperonin now overcompensate for the folding acceleration the chaperonin provides. Larger E and B lead to faster overall kinetics, i.e., smaller folding times t_c/t_0 (Table I; compare circles and squares in Fig. 2 and see also Fig. 3a). Chaperonin size also affects folding rate. Model calculations using square chaperonin cavities of two different sizes show that larger chaperonin cavities speed folding more (Figs. 1, 2 and Table I), but they also lead to lower steady-state native populations (compare the $c = 4$ and $c = 5$ columns in Table I), because a larger fraction of conformations can stick to bigger chaperonin cavities. The prediction of a size dependence implies that there may be biological advantage to having chap-

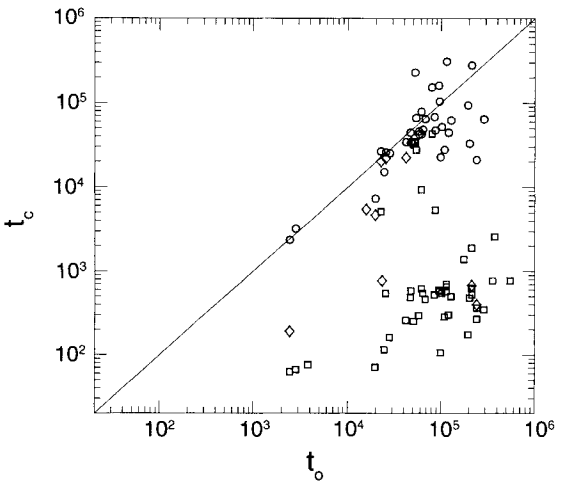

Fig. 2. Chaperonin-mediated folding times vs. unchaperoned folding times. Some lattice proteins are accelerated under these conditions, and others are decelerated. Different values of binding and ejection rates affect acceleration and deceleration. Folding time is the time required to achieve 90% native population (see below). t_0 is unchaperoned folding time and t_c is chaperonin-mediated folding time, which is computed for all sequences with larger than 95% steady-state native population (at time $\rightarrow \infty$). The number of such sequences is a function of B and E (Table I). Circles: $c = 4$, $B = 0.03$, and $E = 0.001$; squares: $c = 5$, $B = 0.03$, and $E = 0.001$; diamonds: $c = 4$, $B = E = 1.0$. Each point represents a single sequence. (Note that a sequence and its reverse sequence have exactly the same properties in this model and thus their points coincide.[37]) Points for different (B,E)s that have the same unchaperoned folding time t_0 describe the same sequence. They give the different chaperonin-mediated folding times t_cs for the given sequence under different (B,E) conditions. Points below the diagonal $t_c = t_0$ indicate chaperonin-mediated acceleration of folding; above the diagonal, folding is slowed down by the chaperonin under the given conditions. Larger chaperonins give faster folding (squares).

Kinetics is determined by a reduced transition matrix formulation.[18,20] Free conformations are classified into (h,d) states, as in ref. 18, whereas bound conformations are classified into (h,d_c) states. Here d is the minimum total number of HH contacts a conformation has to break, in the absence of chaperonins, before it can reach the native structure. The quantity d_c is the minimum number of HH contacts a conformation has to break in its approach to native in the presence of chaperonins, summing over every conformational transition that involves a decrease in h except the ejection steps from the chaperonin (Fig. 1). We determine d and d_c by exact enumeration.[18,20] The initial population at $t = 0$ is a uniformly distributed $h = 0$ ensemble of open free conformations. Folding time is given by $t = \tau/A_{max}$, where τ is the number for which $(\mathbf{T})^\tau$ gives a 90% native population. A_{max} in Eq. (1) is set to 120 (for MS2). The folding times t_0 and t_c are independent of A_{max} provided A_{max} is sufficiently large. We use $\epsilon = -9.3kT$ in Figures 2 and 3 so that the equilibrium native population in the absence of chaperonins is above 98% for all $n = 13$ unique sequences.[18]

eronins, as nature does, that are only slightly larger than the proteins that they help to fold.[13]

As has been pointed out before,[18,20] the kinetics obtained from this type of model calculations are dependent on the choice of allowed chain moves (i.e., the move set).[18,20] However, the general behavior observed above appears to be robust and is relatively independent of move set, as verified by the results in Table II obtained using a different move set.

Although our model postulates a nonspecific action for chaperonins, the folding rates are nevertheless predicted to be dependent on the amino acid sequence (Fig. 3) and the relative sizes of the protein and the chaperonin, because chaperonins perturb different energy landscapes differently. This may provide a partial explanation for the specificity in chaperonin-mediated folding.[30] In general, many slow-folding sequences can be accelerated but some fast-folding sequences are decelerated because fast-folding chains are repeatedly pulled apart even when they were otherwise on the right track for folding (Fig. 3b). We have also explored folding in the presence of the chaperonin without ATP, by setting the ejection rate to $E = 0$ (Fig. 3c,d). This leads to low (usually very low) steady-state native populations because then the chaperonin captures the protein and nearly terminates its folding attempts. For example, when $B = 1.0$ but $E = 0$, only 5 of the 173 sequences studied have yields in excess of 10%. Under these conditions, some sequences still fold at their *intrinsic* folding rates (i.e., their rates in the absence of chaperonins) (Fig. 3c), but others fold much more slowly (Fig. 3d). Slowed folding has been observed for barnase in an ATP-free solution of GroEL.[31]

TABLE II. MS1: Average Folding Time Reduction Factors t_c/t_0 for Different Binding and Ejection Rates B and E for Chaperonin Sizes c = 4 and c = 5*

	B (c = 4)			B (c = 5)		
E	1.0	0.03	0.001	1.0	0.03	0.001
1.0	0.0030 (73/73)	0.023 (74/74)	0.31 (80/82)	0.0055 (12/12)	0.025 (14/14)	0.070 (16/16)
0.03	0.020 (54/54)	0.044 (70/70)	0.43 (71/76)	0.017 (10/10)	0.061 (12/12)	0.011 (24/24)
0.001	0.43 (19/35)	0.28 (41/49)	0.51 (55/78)	0.13 (4/4)	0.0046 (23/23)	0.0063 (31/31)

*Same as Table I, but for move set MS1.[18,20]

CONCLUSIONS

This model appears to be consistent with recent experiments indicating that chaperonins 1) increase protein yields[2–4,6–11,32]; 2) enhance folding rates in some cases[9,11,32] and decrease them in other cases[31,32]; 3) require ATP for their catalytic action[6–11,32,33]; 4) act nonspecifically to help fold a variety of proteins by binding to their nonnative states[2–4,6–10,32,34–36]; and 5) sometimes act by repetitive folding attempts outside the chaperonin.[6–10] The present predictions regarding the dependence of chaperonin action on protein size and binding and ejection rates are testable. The present work provides a quantitative model for chaperonin action, showing how a nonspecific hydrophobic cavity, by pulling apart hydrophobic contacts in compact misfolded states of proteins, can often speed up their folding.

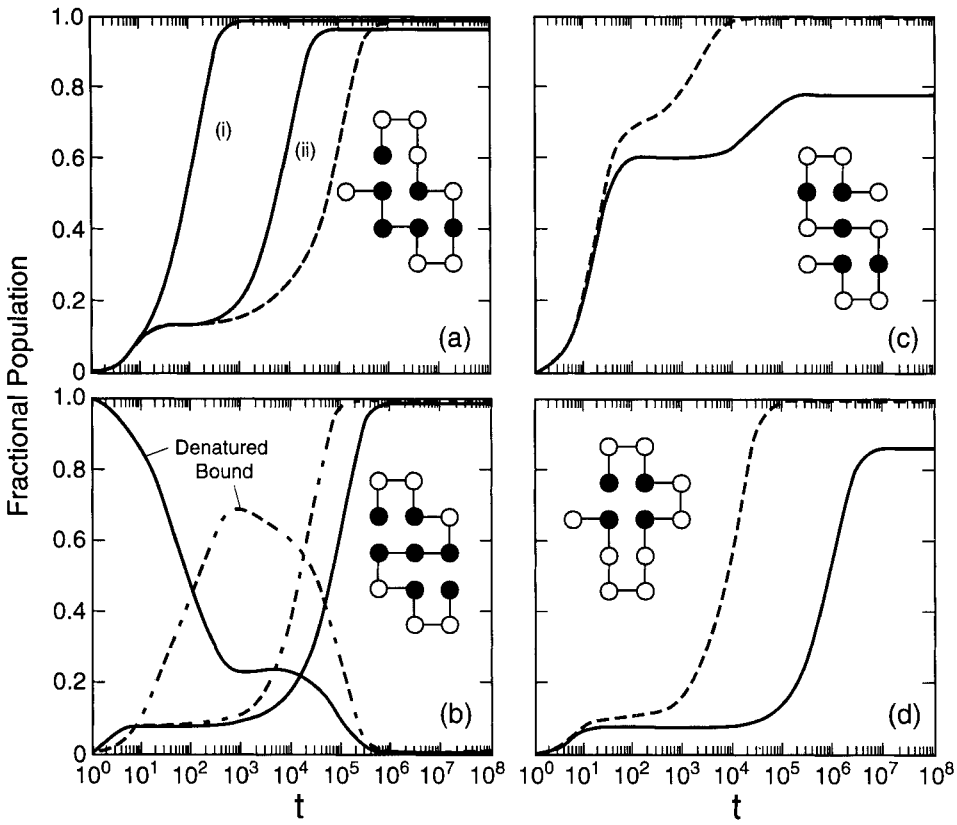

Fig. 3. Folding kinetics examples. Native (conformation shown) population is computed using reduced transition matrices, move set MS2,[18] and $c = 4$. Unless labeled otherwise, dashed and solid curves show native population vs. time t for unchaperoned and chaperoned kinetics, respectively. **a:** Chaperonin accelerates folding of this sequence. Solid curves show native population in the presence of chaperonins: (i) fast binding and ejection, $B = E = 1.0$, and (ii) slow binding and ejection, $B = 0.03$ and $E = 0.001$. **b:** Chaperonin decelerates folding of this sequence ($B = 0.03$, $E = 0.001$). Also shown are the denatured population kinetics of chains outside chaperonins and the bound population inside chaperonins. **c and d:** Fast binding and no ejection, $B = 1.0$ and $E = 0$, indicating that chaperoned folding without ATP gives reduced yields.

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