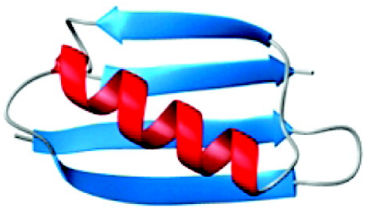
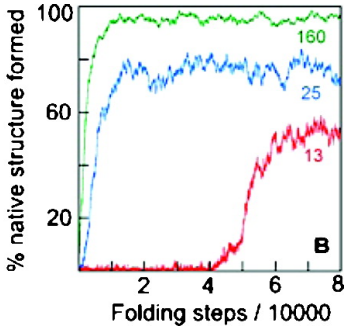
Article

Protein Folding through Kinetic Discrimination

Sara Linse, and Bjrn Linse

J. Am. Chem. Soc., **2007**, 129 (27), 8481-8486• DOI: 10.1021/ja070386e • Publication Date (Web): 12 June 2007





More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 06/12/2007

Protein Folding through Kinetic Discrimination

Sara Linse* and Björn Linse

Contribution from the Biophysical Chemistry, Lund University, Chemical Centre, P.O. Box 124, SE-221 00 Lund, Sweden

Received January 18, 2007; E-mail: sara.linse@bpc.lu.se

Abstract: Proteins fold on a μs−ms time scale. However, the number of possible conformations of the polypeptide backbone is so large that random sampling would not allow the protein to fold within the lifetime of the universe, the Levinthal paradox. We show here that a protein chain can fold efficiently with high fidelity if on average native contacts survive longer than non-native ones, that is, if the dissociation rate constant for breakage of a contact is lower for native than for non-native interactions. An important consequence of this finding is that no pathway needs to be specified for a protein to fold. Instead, kinetic discrimination among formed contacts is a sufficient criterion for folding to proceed to the native state. Successful protein folding requires that productive contacts survive long enough to obtain a certain level of probability that other native contacts form before the first interacting unit dissociates. If native contacts survive longer than non-native ones, this prevents misfolding and provides the folding process with directionality toward the native state. If on average all contacts survive equally long, the protein chain is deemed to fold through random search through all possible conformations (i.e., the Levinthal paradox). A modest degree of cooperativity among the native contacts, that is, decreased dissociation rate next to neighboring contacts, shifts the required ratio of dissociation rates into a realistic regime and makes folding a stochastic process with a nucleation step. No kinetic discrimination needs to be invoked in regards to the association process, which is modeled as dependent on the diffusion rate of chain segments.

Introduction

Many proteins fold rapidly and spontaneously to the native state. This has puzzled investigators for decades as the process would not be completed within any reasonable time scale if the protein was deemed to randomly search through all possible conformations. We show here that no pathway needs to be specified and that kinetic discrimination among intra-protein contacts provides the folding process with directionality. Prolonged survival of native relative to non-native contacts is a sufficient requirement for folding to proceed rapidly and with high fidelity toward the native state. Cooperativity among the formed contacts limits the required ratio of the average lifetime of native over non-native contacts to realistic values.

Many small proteins fold spontaneously to their native states without the help of other cellular components and also refold spontaneously after denaturation. This would not happen within any reasonable time frame if folding of the protein chain had to operate via a random search through all possible conformations. According to the Levinthal paradox, the number of possible conformations of the polypeptide backbone is so large that random sampling will not allow the protein to fold within the lifetime of the universe.[1] Yet, proteins fold on a μs−ms time scale[2−5] implying a high degree of directionality of the process. Levinthal interpreted this as an evidence for pathways that direct the search,[1] and spontaneous folding is often taken as an evidence that there are one or a few obligatory intermediate structures that the chain must adopt on its way from unfolded to the native state. However, for many proteins no intermediates have been detected and they are classified as two-state folders. The "new view" invokes parallel routes for ensembles of proteins.[6,7] Although the so-called funnel model[7] has shed light on the process it does not resolve the Levinthal paradox in a readily comprehensible way.

A number of protein folding models have been discussed over the last decades and two major groups of models emerge.[6,8−11] In the hydrophobic collapse model,[12] the initial event is the condensation of hydrophobic side chains, which is followed by the formation of secondary structure, and adjustment of the tertiary structure. The other group of models are called hierarchic or building-block models, as well as framework or diffusion-collision models.[13,14] These models propose that the simplest structures are built first and are assembled to larger and larger units, with the secondary structures formed first and assembled

(1) Levinthal, C. *J. Chim. Phys.* **1968**, *65*, 44.
(2) Anfinsen, C. B.; Haber, E.; Sela, M.; White, F. H., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 1309−1314.
(3) Hagen, S. J.; Hofrichter, J.; Szabo, A.; Eaton, W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11615−11617.
(4) Maxwell, K. L.; et al. *Protein Sci.* **2006**, *14*, 602−616.
(5) Fulton, K. F.; Devlin, G. L.; Jodun, R. A.; Silvestri, L.; Bottomley, S. P.; Fersht, A. R.; Buckle, A. M. *Nucleic Acids Res.* **2005**, *33*, D279−D283.
(6) Baldwin, R. L. *J. Biomol. NMR* **1995**, *5*, 103−109.
(7) Dill, K. A.; Chan, H. S. *Nat. Struct. Biol.* **1997**, *4*, 4−9.
(8) Daggett, V.; Fersht, A. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 497−502.
(9) Daggett, V.; Fersht, A. *Trends Biochem. Sci.* **2003**, *28*, 18−25.
(10) Baldwin, R. L.; Rose, G. *Trends Biochem. Sci.* **1999**, *24*, 26−33.
(11) Baldwin, R. L.; Rose, G. *Trends Biochem. Sci.* **1999**, *24*, 77−83.
(12) Ptitsyn, O. B. *J. Protein Chem.* **1987**, *6*, 273−293.
(13) Kim, P. S.; Baldwin, R. L. *Ann. Rev. Biochem.* **1990**, *59*, 631−660.
(14) Tsai, C.-J.; Nussinov, R. *Protein Eng.* **2001**, *14*, 723−733.

10.1021/ja070386e CCC: $37.00

into subdomains or supersecondary structure motifs before they associate into the native structure. From experimental studies it is clear that while many proteins display intermediates along the folding pathway,[5,13,15,16] a large number of small proteins have no detected intermediates and are termed fast two-state folders. This suggests that for many proteins, the folding of secondary and tertiary structure are simultaneous events and cannot be separated from one another.

Rate measurements on model peptides show that a short (21 residue) α-helix helix can fold in a few hundred ns,[18,19] while the folding rate is approximately a factor of 30 lower for a 16 residue β-hairpin[20] because of the longer range contacts. Experimental techniques for protein folding involve rapid mixing or laser-pulsing or other methods to obtain ultrafast changes in the solution conditions.[3,18,21−23] Kinetic studies show that for the fastest folding proteins, the folding time is on the order of 3−100 μs.[4,17] Clearly therefore, folding cannot occur via a random search over all possible conformations. However, this does not necessarily mean that folding needs to proceed in any special order or by following any obligatory pathway, and for many of the very fast folders no pathway has been experimentally verified. Among the more successful theoretical approaches to protein folding are models based on energy landscape theory and principles of minimal frustration that use only a small set of tunable parameters.[24−26] These efforts explain the dependence of folding kinetics on protein topology in terms of the funnel-shaped energy landscape.[27,28]

In the present work, we have produced a folding algorithm with reduced dimensionality that can perform protein folding simulations in very limited CPU time. We address whether kinetic discrimination among the formed contacts can promote folding and find that this is a sufficient requirement for folding to proceed rapidly and with high fidelity toward the native state. We find that no pathway needs to be specified for a protein to fold. We investigate the required ratio of the average lifetime of native over non-native contacts and show that this ratio is reduced to a realistic value when a modest degree of cooperativity is invoked among the formed contacts. The algorithm is capable of correctly ordering the folding rates of a set of small proteins.

Results and Discussion

Folding Algorithm. The algorithm is based on a contact diagram, a two-dimensional representation of the three-dimensional structure, and on kinetic discrimination between native and non-native contacts. The starting configuration is a fully unfolded chain without any noncovalent inter-residue contacts. Two residues are randomly chosen and the association rate between them is governed by their effective contact order (ECO; cf. Figure 1) at that particular step in the simulation. In other words, native and non-native contacts are equally probable to associate. However, the algorithm discriminates between native and non-native contacts by assigning them different dissociation rate constants. The user specifies time constants for native (attractive) and non-native (weakly attractive) contacts in units of simulation cycles. The actual lifetime for each newly formed contact is pulled from a random distribution that corresponds to an exponential decay based on the time constant. Hence only the average lifetime is specified and gives the probability that a contact will dissociate during any given simulation cycle, and the dissociation process is assumed to follow first-order kinetics. The user can choose to display the updated contact diagram at a chosen interval of simulation steps to watch the progression of the folding process. While all protein structures can be represented by a contact diagram, every possible contact diagram does not correspond to a physically realisable structure. To deal with this problem we only allow each residue to have maximum 12 contacts, a typical maximum number seen for proteins in the structure databank. The algorithm is described in more detail in the Materials and Methods section.

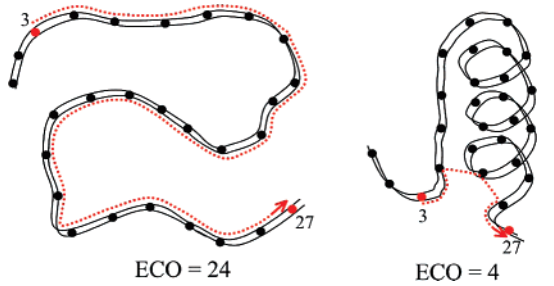


Figure 1. Effective contact order. Cartoon illustrating how the effective contact order (ECO) is calculated as the smallest number of residue contacts (covalent and noncovalent) that connect two randomly selected residues through a continuous path.

Prolonged Survival of Native Contacts Provides the Folding Process with Directionality. The algorithm is capable of folding proteins to their target structure in limited CPU time (about 1 s on a 1 GHz laptop computer), as here illustrated for the 56-residue B1 domain of protein G (PGB1, Figure 2A). Examples of simulations of PGB1 with three different dissociation time constants for native contacts are shown in Figure 2B. The number of simulation steps it takes to reach equilibrium is only weakly dependent on the dissociation time constant. However, the level of success (or stability of the protein) measured as the % of the native contacts formed at equilibrium clearly depends on the time constant for native contacts (Figure 2B). This shows that, although the process is random in the sense that no pathway is specified, prolonged survival of native contacts directs the search. It is more probable to build a new contact next to a pre-existing one because of the reduced distance in space and thereby shorter diffusion time of the chain segments to come into contact, as taken into account via the dependence of association probability on $1/\mathrm{ECO}^{1.5}$. With too short lifetime, this benefit is never gained and the protein does not fold, because the folding process has to start over and over

(15) Laity, J. H.; Montelione, G. T.; Scheraga, H. A. *Biochemistry* **1999**, *38*, 16432−16442.
(16) Teilum, K.; Maki, K.; Kragelund, B. B.; Poulsen, F. M.; Roder, H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9807−9812.
(17) Kubelka, J.; Hofrichter, J.; Eaton, W. A. *Curr. Opin. Struct. Biol.* **2004**, *14*, 76−88.
(18) Williams, S.; Causgrove, T. P.; Gilmanshin, R.; Fang, K. S.; Callender, R. H.; Woodruff, W. H.; Dryer, R. B. *Biochemistry* **1996**, *35*, 691−697.
(19) Thompson, P. A.; Eaton, W. A.; Hofrichter, J. *Biochemistry* **1997**, *36*, 9200−9210.
(20) Muños, V.; Thompson, P. A.; Hofrichter, J.; Eaton, W. A. *Nature* **1997**, *390*, 196−697.
(21) Udgaonkar, J. B.; Baldwin, R. L. *Nature* **1988**, *335*, 694−699.
(22) Roder, H.; Elove, G. A.; Englander, S. W. *Nature* **1988**, *335*, 700−704.
(23) Otzen, D. E.; Oliveberg, M. *J. Mol. Biol.* **2001**, *313*, 479−483.
(24) Wolynes, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6170−6175.
(25) Socci, N. D.; Onuchic, J. N. V.; Wolynes, P. G. *Proteins* **1998**, *32*, 136−158.
(26) Levy, Y.; Onuchi, J. N. *Acc. Chem. Res.* **2006**, *39*, 135−142.
(27) Chavez, L. L.; Goasavi, S.; Jennings, P.; Onuchic, J. N. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10254−10258.
(28) Go, N.; Taketomi, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 559−563.

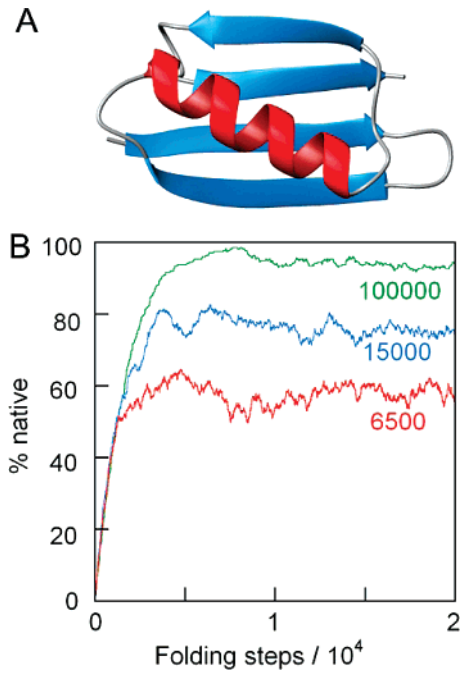


Figure 2. Folding of PGB1: Effect of native contact lifetime. (A) Ribbon structure of PGB1 (pdb code 3gb1. The figure was prepared using Molmol.[35] (B) Single folding traces shown as the % of the native contacts that are formed as a function of the number of simulation steps performed. The time constant for the exponential dissociation process for native contacts is given next to each curve in units of simulation cycles (6500 cycles, red; 15000 cycles, blue; 100000 cycles, green). The time constant for non-native contacts was 2 cycles.

again and each association event becomes more or less independent. Successful protein folding hence requires that productive contacts survive long enough to obtain a certain level of probability that other native contacts form before the first interacting unit dissociates. Thus, it is the prolonged survival of native contacts that provides the folding process with directionality toward the native state.

The assignment of a longer time constant for native compared to non-native contacts is in analogy with the Go model which assigns high stability to native contacts and close to zero stability of non-native contacts.[29] This reflects the fact that the interactions are on average better in the native state as this state utilizes a higher ratio of favorable over non-favorable interactions compared to the non-native state. However, our model differs from an extreme Go model in that we consider partial rather than absolute native versus non-native discrimination, and we use our model to investigate the folding behavior at different levels of discrimination. The finding that proteins do not need residual structure, or intermediates, or pathways to fold does not mean that proteins do not have them, and there is a large literature of elegant experimental exploration of these phenomena (as reviewed in refs 30 and 31). In some cases it is clearly documented that a protein folds slower because it has an intermediate.[32]

Fidelity of Folding. The algorithm was also used to investigate the fidelity of folding by counting, at equilibrium,

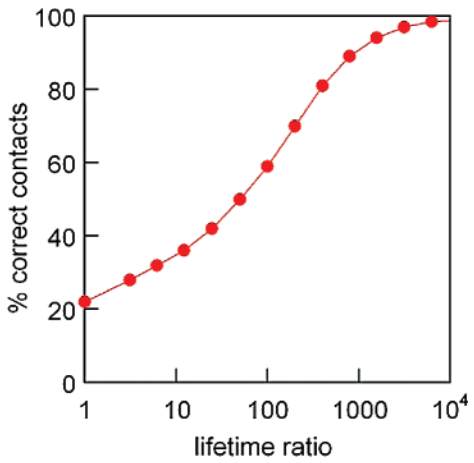


Figure 3. Fidelity of folding. Panel A shows the % of the formed contacts that are native as a function of the ratio of the time constants for native over non-native contacts using a native time constant of 100000 cycles.

how many of the formed contacts are native and non-native, respectively. For this exercise the dissociation time constant for native contact was set to 100000, and the folding process from extended chain was performed for a number of non-native dissociation time constants ranging from 2 to 100000 (Figure 3). We find high fidelity of folding (over 95% of the formed contacts are native ones) for a ratio of native over non-native dissociation time constants above 2000. Since the association kinetics are the same for both types of contacts, this means that the free energy difference between native and non-native contacts is -19 kJ/mol ($-RT$ ln 2000) for 95% fidelity. When the time constant of non-native contacts is approaching that of native ones, the protein becomes more likely to misfold. Only half of the formed contacts are correct when the lifetime ratio is 40 (corresponding to a -9 kJ/mol free energy difference), and when both lifetimes are the same, only 20% of the formed contacts are correct. This corresponds to the situation described by the Levinthal paradox. In the absence of kinetic discrimination among the formed contacts, all configurations are equally probable and the protein chain is deemed to random walk. In this limit, the native structure is not formed within any reasonable time, and in fact the chain is never even close to the native structure. In other words, it is important that non-native contacts dissociate quickly to avoid forming non-wanted "seeds" for more false contacts and thus allow the chain to form the productive contacts instead. A similar resolution of the Levinthal paradox has been presented by Zwanzig who showed the requirement for a bias toward native contacts.[33]

Cooperativity. The dependence of association rate on effective contact order provides cooperativity in the formation of new contacts, because it is more likely to form a contact next to pre-existing ones than between distant chain segments.[34] One would also expect that after it is formed, a contact that is stabilized by surrounding contacts is less likely to dissociate than a lone contact. Our algorithm therefore allows the user to add cooperativity by extending the lifetime of a newly formed native contact by a factor that depends on the number of pre-existing contacts involving any one of the two residues that form

(29) Finke, J. M.; Onuchic, J. N. *Biophys. J.* **2005**, *89*, 488−505.
(30) Ferreira, S. T.; De Felice, F. G.; Chapeaurouge, A. *Cell. Biochem. Biophys.* **2006**, *44*, 539−548.
(31) Englander, S. W. *Ann. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 213−238.
(32) Schultz, D. A.; Schmid, F. X.; Baldwin, R. L. *Protein Sci.* **1992**, *1*, 917−924.
(33) Zwanzig, R.; Szabo, A.; Bagchi, B. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 20−22.
(34) Dill, K. A.; Fiebig, K. M.; Chan, H. S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1942−1946.

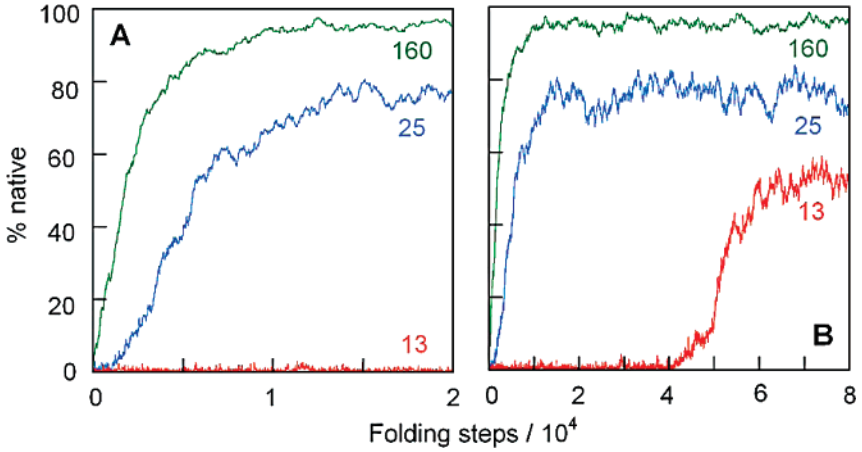


Figure 4. Cooperative folding. Single folding traces shown as % of the native contacts formed as a function of the number of simulation steps performed. The time constant for native contacts in units of simulation cycles is given next to each curve, and the time-constant for non-native contacts was 2. The cooperativity factor is 3.0, meaning that if a contact forms next to a pre-existing one, its lifetime is prolonged by a factor of 3. Note that panel B covers 4 times as many simulation steps as panel A.

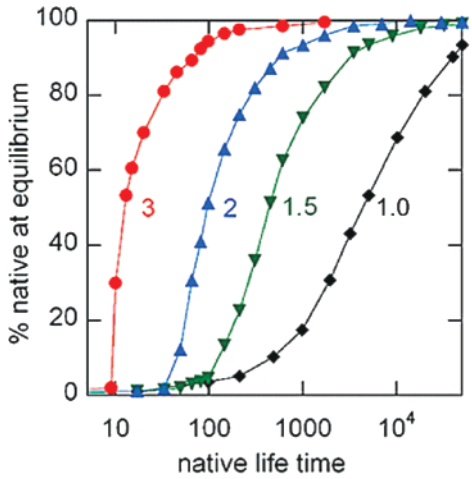


Figure 5. Effect of cooperativity factor. The % of the native contacts formed at equilibrium is shown as a function of the time constant for native contacts. The label next to each curve is the cooperativity factor used to multiply the lifetime for a newly formed contact for each pre-existing neighboring contact. The time constant for non-native contacts was set to 2 cycles.

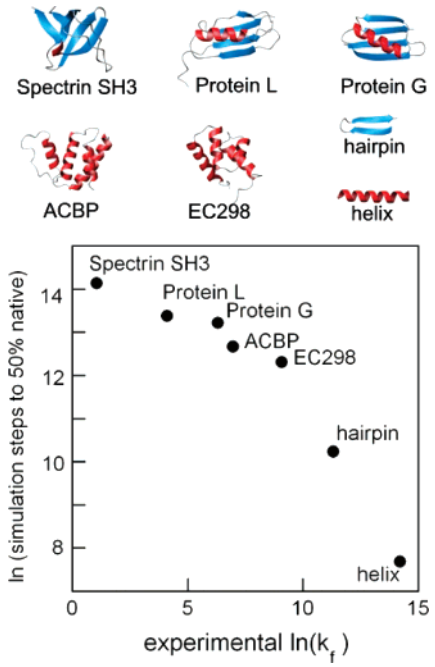


Figure 6. Folding rates. The natural logarithm of the number of simulation steps needed to reach 50% of the native contacts is plotted versus ln(k_f) where k_f is the experimental folding rate in s^{-1} from references 4 and 18−20.

the contact, or their sequential neighbors. Only a modest degree of cooperativity needs to be invoked to drastically lower the required time constant for native contacts (Figure 4, 5) and its ratio over non-native contacts. A cooperativity factor of 3 was used in Figure 4, and for the data described by the red curve in Figure 5, meaning that the average lifetime is increased by a factor of 3 next to one pre-existing contact, a factor of 6 next to two, and a factor of 9 with three pre-existing neighboring contacts, and so on. This corresponds to a very reasonable number (−2.5 kJ/mol) for the free-energy coupling between two neighboring contacts. With this level of cooperativity and a very short non-native lifetime (2 cycles), the required lifetime ratio of native over non-native contacts for successful folding is below 100 corresponding to a −11 kJ/mol free energy difference between native and non-native contacts. If the cooperativity factor among native contacts is increased to 4 or above (< −3.5 kJ/mol), it is possible to achieve successful folding even when the native and non-native lifetimes are the same (e.g., when both are set to 10 cycles, zero free energy difference).

Because of the lower intrinsic lifetime, a cooperative folder takes longer time to fold than a non-cooperative one, if we compare cases that yield the same fraction of native contacts at equilibrium (Figures 4 and 2B). When the native time constant approaches the lower limit for folding to occur, the folding process becomes a stochastic event with a nucleation step. This is because in the beginning of the folding process there are none or few contacts to build onto, and they dissociate quickly as long as they are isolated. Once a nucleus is formed the folding process continues rapidly toward the native state (Figure 4B). This behavior is in analogy with the funnel model and folding of the remaining structure becomes easier and easier. There seems to be a sharp limit of native lifetime above which the probability to form more than isolated contacts becomes nonvanishing, seen as the steep beginning of the red curve in Figure 5.

Correlation with Experimental Folding Rates. The presented algorithm is of Monte Carlo type, hence no time

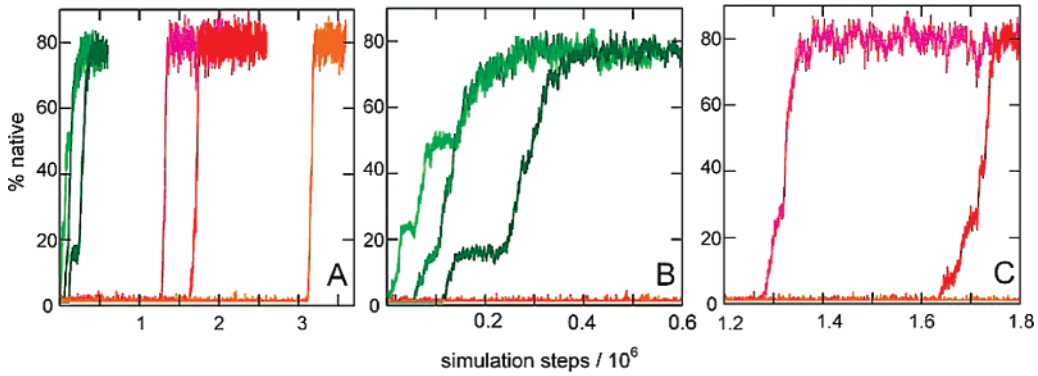


Figure 7. Folding of EC298 and spectrin SH3 domain. Examples of single folding traces for the helical protein EC298 (green curves) and the β-sheet rich spectrin SH3 domain (pink, red, orange curves) shown as % of the native contacts formed as a function of the number of simulation steps performed: (A) data up to 3.6×10^6 simulation steps; (B) data up to 0.6×10^6 simulation steps; (C) data beween 1.2 and 1.8×10^6 simulation steps.

information can be extracted from the simulations. However, it may still be of interest to compare the number of simulation steps it takes to fold proteins with different contact order and reported variations in folding rate. For this purpose, simulations were performed for a model 20-residue α-helix, a model 16-residue β-hairpin, and five different proteins with variation in the reported folding rates and topologies.[4,18−20] For each of these seven cases, five simulations were performed, and the number of steps needed to form 50% of the native contacts was averaged over the five simulations (Figure 6). Clearly, the algorithm is able to reproduce the progressive change in folding rate between the seven cases. Examples of single simulations are shown in Figure 7 for the helical protein EC298 and the β-sheet rich SH3 domain from spectrin, and the structural progression of individual folding attempts was monitored through the continuously updated contact diagram (data not shown). Clearly, the SH3 domain requires more steps to fold and also displays the largest variation in the number of folding steps between individual attempts (Figure 7A). This appears to be due to the low probability of nucleation of the β-sheet structure. However, once a nucleus has formed, the rest of the structure follows on rapidly, and the process is more or less all-or-nothing (Figure 7C). The helical protein appears much easier to nucleate; however, folding is less cooperative (Figure 7B). All helices are equally likely to nucleate and nucleation can occur anywhere along the helices. The next step is then either an independent nucleation of one or more additional helices followed by long-range contacts between helices or the first formed helix is seen to form long-range contacts with residues belonging to another helix and that helix is then observed to fold onto the first one. Although no preferred pathway appears for the ensemble of EC298 molecules (as represented by the individual attempts), each individual polypeptide chain may "rest" for a while in an intermediate structure after completion of one or more substructures. Note that the here discussed folding behavior arises from topological constraints only, as all native contacts are treated equally, and the model cannot account for differences in folding rates of two protein variants with exactly the same structure. The correlation between experimentally determined folding rate and contact order was previously demonstrated by Plaxco et al.,[36] who also found using a simple algorithm that the key feature is contact order and not protein size. While the goal of the current work was to investigate the role of kinetic discrimination in protein folding, the algorithm may be developed toward protein structure prediction using amino acid-pair potentials instead of a target structure.

(35) Koradi, R.; Billeter, M.; Wüthrich, K. *J. Mol. Graphics* **1996**, *14*, 51−55.
(36) Plaxco, K. W.; Simons, K. T.; Baker, D. *J. Mol. Biol.* **1998**, *277*, 985−994.

Conclusions

We have shown that kinetic discrimination leads to successful protein folding and no pathway needs to be specified. Instead a sufficient condition for the chain to fold is a longer lifetime for native noncovalent interactions compared to non-native ones. Native contacts need to survive long enough to obtain a certain level of probability that other productive contacts form before the first interacting unit dissociates. The fact that they live longer than non-native ones provides the folding process with directionality. If non-native contacts do not dissociate fast enough the chain is deemed to misfold. A modest degree of cooperativity shifts the required ratio into a realistic regime, and makes folding a stochastic event with a nucleation step. The algorithm can reproduce the order of experimentally observed differences in folding rates.

Materials and Methods

The folding algorithm is written in c in a Linux environment. A protein chain of N residues is represented by an $N \times N$ contact diagram. The starting configuration is a fully unfolded chain with no noncovalent contacts between any residues. Interactions are classified either as attractive (contacts found in the native structure) or weakly attractive (non-native contacts). The program distinguishes between native and non-native interactions by assigning different time constants to them, the values of which can be chosen by the user and optimized.

At each step of the folding process, the algorithm selects two residues at random. If these two residues are not the same or are not already engaged in a contact with one another, the program proceeds to generate the survival time, otherwise a new pair of residues is generated. The association rate between the two randomly chosen residues is governed by their effective contact order (ECO) at that particular step in the simulation. ECO is taken as the number of intervening connections along the closest path of covalent connections and already existing contacts, yielding a number between 1 and $N - 1$, where N is the number of residues in the protein. For example, if residues 3 and 27 are selected when contacts already exist between residues 4 and 22, and between 22 and 26, then ECO is 4 and it is equally likely to form a new contact between residues 3 and 27 as between residues 3 and 7 (Figure 1). The contact is formed if $ECO^{-1.5}$ is larger than a number that is pulled from a linear random distribution between $N^{-1.5}$ and 1. As an additional criterion to avoid unrealistic structures, the contact is only formed if each of the residues has less than 12 pre-existing contacts.

If the newly formed contact is a native contact its survival time is set to the time constant for native (attractive) interactions times a random number that is pulled from an exponentially decaying distribution between 1 and 0.067. If instead the pair represents a non-native contact, the time constant for non-native (weakly attractive) interactions is used times the random number from the exponentially decaying distribution.

The program also provides the possibility to add cooperativity in the dissociation kinetics. If a native contact is formed and one of the two residues that form the contact, or their sequential neighbors, are already engaged in contacts, the survival time is multiplied by a cooperativity factor that depends on the number of pre-existing contacts. The user can specify the cooperativity factor or decide not to use it (i.e., set its value at 1.0).

The algorithm finalizes each simulation step by removing all contacts whose survival time expires after that step.

In the initial simulations of PGB1, a number of different combinations of lifetimes, and with and without cooperativity at different levels were tested. In the simulations to compare the seven selected cases (model 20 residue α-helix, model 16 residue β-sheet, EC298 (1ryk.pdb), ACPB (1nti.pdb), Protein G (3gb1.pdb), Protein L (2ptl.pdb) and spectrin SH3 domain (1shg.pdb)), the cooperativity factor was set to 3.0, and the native lifetime was chosen so as to allow the slowest folder (spectrin SH3) to nucleate. Since the algorithm tests one possible connection at a time, and the number of possible connections scales with the square of the number of residues, the native lifetime was also scaled with the square of the number of residues to provide a fair comparison of proteins of different length.

Acknowledgment. The present work was supported by the Swedish Research Council, VR (S.L.)

Supporting Information Available: Complete ref 4. This material is available free of charge via the Internet at http://pubs.acs.org.

JA070386E