Theory of Kinetic Partitioning in Protein Folding

(With Application to Prions)

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Abstract

In this paper we study the phenomenon of kinetic partitioning when a polypeptide chain has two ground state conformations one of which is more kinetically reachable than the other. This question is relevant to understand the phenomenology of prions, proteins which exist in the cell in non-pathogenic \( \alpha \)-helical conformation but under certain circumstances may transform into pathogenic \( PrP^{Sc} \) state featuring increased \( \beta \)-sheet content. We designed sequences for lattice model proteins having two different conformations of equal energy corresponding to the global energy minimum. Folding simulations revealed that one of these conformations was indeed much more kinetically accessible than the other. We found that the number and strength of local contacts in the ground state conformation is the major factor which determines which conformation is reached faster: The greater the number of local contacts the more kinetically reachable a conformation is. We present simple statistical-mechanical arguments to explain these findings. The presented results are in a clear agreement with experimental data on prions and other proteins exhibiting kinetic partitioning.
INTRODUCTION

The study of prion biology and diseases is a new emerging area of biomedical investigation (reviewed by Prusiner, 1992). Prions are proteinaceous infectious particles that are composed largely, if not entirely, of an abnormal form of the prion protein (PrP) designated, in the case of scrapie, PrP$^{Sc}$ (Prusiner, 1991). The term “prion” was introduced by Prusiner (1982, 1991), who has shown that prions are unique among all infectious pathogenesis may be both inherited and transmissible. Six diseases of animals and four of humans are caused by prions (Prusiner, 1992). The initial hypothesis was that PrP$^{Sc}$ was derived from normal cellular prion protein (PrP$^{C}$) by a post-translational process (Borchelt et al., 1990, 1992; Caughey & Raymond, 1991; Taraboulos et al., 1992). Attempts to identify a post-translational chemical modification have been unsuccessful (Stahl et al., 1993). Recent structural studies demonstrate that PrP$^{Sc}$ and PrP$^{C}$ have the same chemical structure but dramatically different conformations (Pan et al., 1993). This suggests that prions have two low free energy states in both of which they can be stable during the lifetime of a protein, with pathogenic form PrP$^{Sc}$ being much less soluble than than cellular PrP$^{C}$. The prion puzzle has two aspects. First, it is the reason and the character of transition from cellular to the pathogenic form. The most intriguing aspect of it is unusual infectivity by PrP$^{Sc}$ which clearly suggests that intermolecular interactions are likely to play an important role here. A possible mechanism of transition from PrP$^{C}$ to PrP$^{Sc}$ has been suggested recently (Cohen et al., 1994) and its implications for prion pathogenesis were discussed.

The focus of this paper, however, is on another important aspect of prion puzzle, namely: Why after (or concomitantly with) the synthesis of their primary structure, prions do not
fold into the conformation of PrP$^{Sc}$, at the first hand?

One (trivial) explanation may be that PrP$^{C}$ is much more thermodynamically stable than monomeric PrP$^{Sc}$. However, the experimental data do not seem to support this view. One (indirect) evidence is that PrP$^{C}$ is much more susceptible to proteolysis than PrP$^{Sc}$. Pan et al (1993) suggested that PrP$^{C}$ is a kinetic trap.

If PrP$^{C}$ and monomeric PrP$^{Sc}$ have comparable thermodynamic stabilities and the partitioning into normal and pathogenic conformations was driven by thermodynamic rules, the yield of pathogenic species after synthesis of primary structure should be large. However, this is not so, and dominant form in normal, uninfected cells is PrP$^{C}$. Apparently, this may be so because PrP$^{C}$ conformation is more reachable kinetically. This links prion phenomenology to the most fundamental aspects of protein folding since they provide a clear (but not unique, see below) example of kinetic partitioning when a conformation becomes dominantly populated for kinetic, rather than thermodynamic, reasons.

In this paper, we, intrigued by the mystery of prions, study the phenomenon of kinetic partitioning in protein folding. Currently, folding simulations are feasible only in the realm of simplified lattice and off-lattice models, which already provided useful, experimentally testable, insights into such general principles of folding as nucleation mechanism (Abkevich et al., 1994b; Shakhnovich et al., 1996; Fersht, 1995; Itzhaki et al., 1995) and folding intermediates (Gutin et al., 1995a; Mirny et al., 1996; Fersht, 1995).

Our approach to study kinetic partitioning, relevant to prion folding, aims to simulate it in a simple, yet, nontrivial, model and seek full understanding of this phenomenon within the framework of the model. Then using the model results as an example where kinetic partitioning is understood, one can apply this as a lead to thinking about real systems and
as a tool to understand existing and plan future experiments. Of course such approach may be useful only if there is a generic reason for kinetic partitioning, which may be applicable not only to prions, but to a wider range of systems. In this paper we reveal a possible physical mechanism of kinetic partitioning which is due to global structural properties of native conformations and we argue that it may explain certain observed features of prion folding as well as other proteins where kinetic partitioning is likely to be important.

In our model study we would like to focus on the kinetic aspect of prion folding. Therefore, while it is not known which conformation, PrP$^C$ or PrP$^{Sc}$, has lower free energy, in the model, we assume that two “native” conformations have equal (free) energies and seek the reason why one of them is more reachable than the other.

Specifically, in this work we design sequences having two different conformations of global energy minimum and study their folding. Our results provide structural clues to kinetic partitioning explaining, why in a system with two ground state conformations, one of them can be more kinetically reachable than the other.

We compare the results of our study with phenomenology of prions and other proteins having kinetic partitioning.

THE MODEL

We represent proteins as self-avoiding chains on an infinite cubic lattice, such that covalently linked residues occupy neighboring lattice sites. The energy of a conformation is the sum of energies of pairwise contacts between monomers. Two monomers are defined to be in a contact if they are neighbors on the lattice and not connected by a covalent bond. The energy of a contact depends only on the identity of the two amino acids involved. The
interaction energies for amino acid pairs are determined from the statistical distribution of contacts in real proteins (Miyazawa & Jernigan, 1985, Table VI).

Our approach to folding simulations requires first to choose a target conformation to be the native and design amino acid sequences that fold to, and are stable in, this conformation. It was shown previously (Goldstein et al., 1992; Shakhnovich & Gutin, 1993; Gutin et al., 1995b) that such a sequence should render the target native conformation as a pronounced global energy minimum. This is the criterion used in our design algorithm (a Monte Carlo optimization in sequence space). The details of this algorithm have been published elsewhere (Abkevich et al., 1995). The quantity that is minimized in this design is relative value of native energy comparatively to the non-native conformations (Bowie et al., 1991; Gutin et al., 1995b):

$$Z = \frac{E_{nat} - E_{av}}{\sigma}$$

(1)

where $E_{nat}$ is energy of the native conformation, $E_{av}$ is the average energy of compact non-native conformations with corresponding dispersion $\sigma$.

Our aim in this work is to design sequences that exhibit prion-like behavior, i.e. which have more than one conformation as a global energy minimum. Correspondingly, the design procedure should be modified to achieve that goal. To this end we choose two different conformations and design such amino acid sequences that have low energy in both conformations. The essential parameter which is minimized in this design is

$$Z = Z_1 + Z_2 + (Z_1 - Z_2)^2$$

(2)

where $Z_1$ and $Z_2$ are relative energies of the model polypeptide in the first and second target conformations, respectfully. The last term in the equation (2) is introduced to ensure that
designed sequence have close energies in target conformations.

RESULTS

The first model protein we studied is a 36-mer chain which has equally low energy in two target conformations (Fig.1) arbitrarily chosen out of more than 84 million possible fully compact conformations (Pande et al., 1994). The sequence was designed to have low energy in both of these conformations (Fig.1c). In order to simulate folding, we used the standard Monte Carlo method (Hilhorst & Deutch, 1975). Different simulation runs begin from different random coil conformations. In the process of simulation designed protein always reached each of the conformations shown in Fig.1. Further, in a long Monte Carlo folding run (10⁹ steps) we did not observe any conformations having energy lower than these two which is indicative that these conformations are the lowest in energy.

The mean first passage time (MFPT)¹ into the structure shown in Fig.1b is found at least 20 times longer than into the one shown Fig.1a in a wide range of temperatures. To verify this result we measured MFPT for ten nonhomologous designed sequences that have close low energies in both conformations shown in Fig.1 and we consistently observed much

¹One may notice that sequences shown on Fig.1c and Fig.2c have unusually large content of W and M. This is due to the fact that our design program does not take into account geometrical properties of amino acids and their natural occurrence but only energy of interactions between them, which is determined only approximately. The strongest hydrophobic interactions in the energy set used in this work (Miyazawa & Jernigan, 1985, Table VI) is a one between tryptophan and methionine. That is why these amino acids dominate in our designed sequences.

²MFPT in all experiments reported in this paper was calculated by averaging over 50 folding runs each starting from different random conformations.
faster folding into the conformation shown in Fig.1a. This result was unexpected, and the first guess was that one of the conformations shown in Fig.1 is more kinetically reachable for geometric, or algorithmic reasons. To test this hypothesis we designed sequences were designed having each of the structures shown on Fig.1 as their unique native conformation. MFPT for these sequences at their folding transition temperature into their respective native conformations were approximately equal (data not shown). This ruled out the simple, but mostly artificial, possibility mentioned above.

Difference in folding time into conformations with completely different structure but close energy was also observed for random model polypeptides. Studying folding of ten random 27-mers with the same amino acid composition we found that nine of them fold into their lowest energy conformation in about $10^6$ Monte Carlo steps. However, the tenth sequence folded into the conformation with the lowest energy in more then $10^8$ steps. However folding time at the same temperature into a completely different conformation with only slightly higher energy is 20 times faster. Again such a difference in folding times appeared to be not just due to geometrical inaccessibility of the conformation with the lowest energy. Folding of the sequence which was designed to have this conformation as the global energy minimum was fast.

It seems that when random or specially designed sequence have close energy in two conformations folding time into these conformations is often quite different. This can be similar to what is found for prions which fold into their native conformation and stay in it during life time of a protein being digested by a protease before reaching the alternative stable conformation.

How can the existence of one low energy state influence folding into another? The
assertion that one conformation can play a role of a kinetica l trap (Abkevich et al., 1994a) is not sufficient to explain the observed behavior. At studied temperatures random 27-mer is not stable in its low energy conformation, and polypeptide spends most of its time in the unfolded state. Since the difference in folding times remains even when the ground state is unstable one should seek the reason of this difference in the propoerties of the unfolded state. It is known that in unfolded state strong local contacts prevail (DeGennes, 1979; Grosberg & Khohlov, 1994). The ground state conformations are the lowest in energy and thus ground state contacts are the strongest on average. So among contacts even in a unfolded state those which are the same as in the ground state, especially local ones, will dominate. Comparing the structure shown in Fig.1b with the one shown in Fig.1a we found significant difference in the number of local contacts (contacts between monomers \( i \) and \( i+3 \)). There are six local contacts in the structure shown on Fig.1b and eleven such contacts in the structure shown on Fig.1a.

When we compared the lowest energy state of a random 27-mer, for which kinetic partitioning was observed, with the alternative low-energy conformation, which was reached much faster, we found that both conformations have the same number of local contacts. However, average energy of local contacts was lower in the conformation faster reachable conformation (-0.27) than in the native one (-0.17).

Thus one can suggest that the number and strength of local contacts are the major factors determining which conformation will be reached first. To pursue this lead further, we chose another couple of structures for design: the one with many (seventeen) local contacts (Fig.2a) and another one without local contacts (Fig.2b). A sequence designed to Fig.2 have equally low energy in these structures is shown on Fig.2c. If our hypothesis is correct,
this sequence will fold into the structure shown on Fig.2a faster than into the one shown on Fig.2b, and the ratio of MFPT should be greater than for structures shown on Fig.1. The results of simulations fully confirm this prediction: At temperature when folding is the fastest, the MFPT from random coil into the conformation shown on Fig.2b is equal to $5.6 \cdot 10^6$ Monte Carlo steps, whereas MFPT into the conformation shown on Fig.2b is equal to $7.8 \cdot 10^8$: more than 100 times longer.

To show that this result is generic and does not depend on the parameter set, we extended analysis using so called Go model (Taketomi et al., 1975) in which all native interactions are set equal and attractive and all other interactions are set to zero. Apparently, such model does not consider any interactions except the native ones, and hence it is somewhat unphysical. However the Go model has some important advantages for the problem under investigation. Firstly, all contacts in both ground states have the same energy, and thus total energy of ground state contacts is simply proportional to their number. Secondly, many factors which influence folding rate in more realistic sequence models (dispersion of energy of native contacts (Abkevich et al., 1996), stability of the specific nucleus (Abkevich et al., 1994b; Fersht, 1995) and so on) are not important for the Go model. Further, the MFPT at the optimum temperature for the Go model is substantially less than for a sequence model. This allows one to overcome considerable computational difficulties of MFPT determination.

We compared MFPT for the Go model into the conformations shown in Fig.2 at different temperatures (Fig.3). We found that folding into the structure shown in Fig.2a is at least 100 times faster than into the one shown on Fig.2b.

The analysis of a typical folding trajectory for a long Monte Carlo run at folding transition temperature (Fig.4) provides further insight into the origin of such a pronounced difference
in folding rates into two different ground state structures.

For convenience of subsequent discussion we denote the structure with many local contacts (Fig.1a, Fig.2a) as $N_1$ and the structure with a few local contacts (Fig.1b, Fig.2b) as $N_2$. It can be seen from Fig.4 that properties of unfolded state give rise to the differences in folding into $N_1$ and $N_2$. The chain rapidly transforms into the set of (unfolded) states with significant structural similarity with $N_1$, having about 50% of the contacts in common with this ground state conformation. In contrast, the unfolded conformations bear very little structural similarity with $N_2$ and only after almost $3 \cdot 10^7$ MC steps it reaches conformations with sufficient number (and location) of $N_2$ contacts to enable nucleation and subsequent rapid folding into that ground state conformation. This is due to the fact that local contacts, numerous in $N_1$, are more favorable than other possible contacts. After these contacts are formed, structural similarity with $N_1$ is enforced. This facilitates faster folding into $N_1$. In order to nucleate folding into $N_2$, the most stable local contacts in the unfolded state must be broken. This process is uphill in free energy and therefore requires longer time.

This qualitative picture can be represented by the following scheme:

$$U_1 \leftrightarrow N_1$$

$$\upharpoonright$$

$$U_2 \leftrightarrow N_2$$

Here $U_1$ and $U_2$ - unfolded states in which dominate contacts from the corresponding ground states. $U_1$ might be similar to $PrP^*$ state (Cohen et al., 1994). As was noted earlier, folding from the state $U_1$ into the state $N_2$ firstly requires to tear contacts in $U_1$ which are common with $N_1$ but not with $N_2$. This corresponds to the transition from $U_1$ into $U_2$. The same is also true for the states $U_2$ and $N_1$. The essential features of the free energy
landscape of prions are summarized on a schematic diagram shown on Fig.5.

Even if the rate of folding from $U_1$ to $N_1$ is equal to such from $U_2$ to $N_2$, the folding time from the random coil state into states $N_1$ and $N_2$ will be different if equilibrium constant $K = [U_1]/[U_2]$ between states $U_1$ and $U_2$ is not unity. If $K \ll 1$ it can be easily shown that MFPT into the state $N_1$ is $K \cdot (K_1 + 1)$ times faster then MFPT into the state $N_2$. Here $K_1 = [N_1]/[U_1]$ is the equilibrium constant between states $N_1$ and $U_1$. When the ground state is stable ($K \gg 1$) the ratio of folding rates into states $N_1$ and $N_2$ is greater than in the case when ground state is unstable. This is due to the fact that at such conditions the state $N_1$ should be considered as a kinetic trap for folding into the state $N_2$.

This conclusion is qualitatively consistent with our numeric results which suggest (Fig.3) that the ratio of MFPT into ground state conformations becomes greater as temperature decreases, i.e. both ground states become thermodynamically stable. To test this prediction quantitatively, we estimated the temperature dependence of the equilibrium constant $K_1$ using histogram technique (Ferrenberg and Swendsen, 1989; Sali et al., 1994; Socci and Onuchic, 1995; Abkevich et al., 1995). A long Monte Carlo simulation was performed ($2 \cdot 10^7$ steps) in which the conformation shown in Fig.2a was folded and unfolded many times ($\sim 100$) but the conformation shown on Fig.2b was not yet reached. The statistics of occurrence of different states were collected. The two important parameters were taken into account: the energy of the chain $E$ and similarity parameter $Q_1$. The quantity of interest is the logarithm of the density of states $\nu(E, Q_1)$. Once calculated, it makes it possible to calculate population of states at different temperatures. This provided the estimate for the

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3 This is a reasonable assumption because folding rates of sequences, having only one of the conformations shown in Figs.1 and 2 as native are close to each other.
temperature dependence of equilibrium constant $K_1$. Fig.6 shows that the ratios of MFPT into states $N_1$ and $N_2$ at different temperatures fit theoretical curve quite well.

We have shown that number and strength of local contacts is an important factor which determines which of the two global minimum conformations is reached faster. This effect was observed for the model with designed and random sequences and for the Go model. However the question remains: whether strength and number of local contacts is the only factor which determines the ratio of MFPT into ground state conformations. To address this issue, we randomly selected two maximally compact conformations with equal number of local contacts (six). To ensure that energy of local ground state contacts is the same for both structures, we studied their folding using the Go model. We found that folding into one these structures is somewhat faster than into another. However, the ratio of MFPT into ground state conformations is significantly smaller than what we observed for the structures shown on Figs.1 and 2 (the data not shown). This ratio never exceeds a factor of three and at some temperatures is as small as 30%. Such numbers are typical scatter of MFPT for model proteins with different but unique native states.

This result suggests that for the studied model the number and strength of local contacts in the ground state is the only major factor which determines which of the ground state conformations is reached faster.

**DISCUSSION**

It is important to compare the properties of natural prions with model polypeptides with degenerate ground state which we studied in the present work. Structural studies demonstrate that PrP$^C$ and PrP$^{Sc}$ differ in conformation (Pan et al., 1993). PrP$^C$ was
found to have high content of α-helix (42%) and essentially no β-sheet (3%), whereas PrP$^{Sc}$ had a β-sheet content 43% and an α-helix content 30%. This is consistent with our findings because in a β-sheet non-local contacts dominate, whereas in an α-helix local contacts dominate.

The fact that PrP$^{Sc}$ is much less soluble than PrP$^{C}$ has been well established (Pan et al., 1993, Kocisko et al., 1994). As pointed out in the Introduction, one can suggest a “thermodynamic” explanation of prion behavior, namely that the stability of monomeric PrP$^{Sc}$ is vanishingly low. The implication of this possibility will be that under no circumstances, including any dilution or mildly denaturing conditions can monomeric PrP$^{Sc}$ be observed. This seems to contradict the experimental finding that dilution of prion solution from 3M of denaturant preserves infectivity while dilution from 6M of denaturant eliminates it (Kocisko et al., 1994). However interpretation of these experiments is not entirely clear (e.g. whether dilution from 6M of GuHCl leads to folding into PrP$^{C}$ or not) and we cannot completely rule out the “thermodynamic” explanation though we find it less likely. Perhaps further experiments including the stopped flow folding of prions upon rapid dilution from 6M GuHCl with subsequent monitoring of secondary structure formation can be useful in clarifying this very important question.

Prions are not the only proteins which can spontaneously undergo global structural changes and transform into another stable state. The theory described here predicts that in such transitions fraction of local contacts should decrease. What can be observed experimentally is the decrease of content of α-helixes accompanied by increase of content of β-sheets.

It is important to mention that conformational flips from β-sheet to α-helix are also
observed for some proteins (Reed & Kinzel, 1993). However these transitions occur when protein environment is altered (change of the solvent or addition of an agent which stabilizes \(\alpha\)-helical conformation). In this case relative stability of native state can change significantly, and the theory presented above is not applicable. We should concentrate on the cases when proteins are (metha)stable during their life time in different states under the same conditions. For example, upon exposure to bright illumination photosystem \(II\) irreversibly transforms into a stable inactive state. At the same time content of \(\alpha\)-helix drops from 67% to 24% whereas content of \(\beta\)-sheet increases from 9% to 41% (He et al., 1991). Another example is a human plasminogen activator inhibitor-1 (PAI-1) which also spontaneously folds into a stable inactive state without cleavage (Katagiri et al., 1992). In agreement with the presented theory, inhibitory activity of this protein can be restored through denaturation and renaturation (Hekman & Loskutoff, 1985; Katagiri et al., 1992). It was suggested that transition from active into the latent state is due to transformation of a surface helix into a \(\beta\)-sheet (Mottonen et al., 1992).

Another interesting question is the evolutionary origin of prions. It was estimated that the probability to randomly synthesize a protein sequence with degenerate stable ground states by chance is low (Gutin & Shakhnovich, 1993). This may imply that even if some properties of the “abnormal”, patogenic, state seem now useless and even harmful, proteins could have been specially designed during evolution to have “abnormal” state as well as the native one. Finding the possible biological role of the “abnormal” state can be important for understanding of protein’s properties. Alternatively we can assume that prion proteins have only weakly optimized sequences, so that they are intrinsically unstable in their ground state conformations at physiological temperature. Then probability of accidental synthesis
of a protein with degenerate native state is sufficiently high (Gutin & Shakhnovich, 1993).

Finally we would like to point out to limitations of the present analysis. In order to simulate the effect of kinetic partitioning, we had to use a simplified model. Such simplification comes at a price. One limitation is that we studied folding of model proteins much shorter than typical experimental systems exhibiting kinetic partitioning. E.g. prions are approximately 200 aminoacids long, while in the present study we simulated folding of 27-mers and 36-mers. While much longer model proteins can be folded on a lattice (successful folding simulations of model proteins of up to 100 aminoacids long have been reported by several groups (Kolinski et al, 1993, Shakhnovich, 1994), the detailed analysis presented in this paper required thousands of runs to collect sufficient statistics. This is feasible only for relatively short model proteins. However, we do not think that this is a crucial limitation since comparison of our results with experimental situation suggests that gross structural features leading to kinetic partitioning may well be reproduced by simulations of shorter chains.

Another limitation of the present study is that it did not include intermolecular interactions which are important for transition from PrP$^C$ to PrP$^S$. Here we should emphasize once again that our results are aimed to explain why prion proteins fold into the conformation of PrP$^C$ after synthesis of their primary structure. Certainly, the presented simulations and analysis do not address the mechanism of the conformational change of PrP$^C$ into PrP$^S$. It is possible to simulate small ensemble of lattice chains, or study template-mediated folding and address the issue of prion infectivity and conformational transitions (Cohen et al, 1994). We are planning to do so in the near future.
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FIGURE CAPTIONS

Fig.1 (a), (b) Randomly chosen maximally compact 36-mers on a cubic lattice and (c) a sequence for which these conformations have equally low energy.

Fig.2 Compact 36-mers on a cubic lattice with (a) many local contacts, and (b) without local contacts, and (c) a sequence for which these conformations have equally low energy. How these structures were found is described in our previous work (Abkevich et al., 1995).

Fig.3 Dependence of MFPT for 36-mers shown on Fig.2 on the inverse temperature (Go model). Gray points correspond to the folding into conformation shown on Fig.2a, and black points correspond to the folding into conformation shown on Fig.2b.

Fig.4 Monte-Carlo folding trajectory at folding transition temperature ($T = 0.6$) for the chain which has two ground state conformations shown in Fig.3 (Go model). a: the MC-Step dependence of the structural similarity $Q_1$ to the conformation $N_1$ shown in Fig.2a. This parameter was defined as the number of common contacts in the current and the ground state conformation $N_1$ divided by the total number of contacts in $N_1$. b: The same plot but for the conformation $N_2$ shown in Fig.2b.

Fig.5 Schematic representation of energy landscape in a case of degenerate ground state. $N_1$ and $N_2$ are the ground state conformations, $U_1$ and $U_2$ are unfolded conformations in which dominates contacts from the corresponding ground state.

Fig.6 Temperature dependence of ratio of MFPT into conformation shown in Fig.2b ($MFPT_2$) to MFPT into conformation shown in Fig.2a ($MFPT_1$). Experimentally observed data (grey dots) is taken from Fig.3. Theoretical curve is calculated with a help of histogram technique (Sali et al., 1994; Abkevich et al., 1995). The only fitting parameter was
equilibrium constant $K$ which was taken to be equal 81.