Coexistence of native and denatured phases in a single protein-like molecule

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In order to understand the nuclei which develop during the course of protein folding and unfolding, we examine phase segregation of a single heteropolymer chain which occurs in equilibrium. These segregated conformations are characterized by a nucleus of monomers which are superimposable upon the native conformation. We computationally generate the phase segregation by applying a “folding pressure,” or adding an energetic bonus for native monomer-monomer contacts. The computer models reveal a fundamental difference in the nucleation process between heteropolymers and the more familiar vapor-liquid systems: in a polymer system, some nuclei hinder folding via topological constraints and must be partially destroyed in order for folding to proceed. To illustrate this finding, we examine the kinetics of protein unfolding in the long chain limit through scaling arguments. We find that because of the topological constraints, the critical nucleus size is of the order of the entire chain size so that unfolding time scales as exp \( c N^2/3 \), where \( N \) and \( c \) are the chain length and a constant.

Proteins fold and unfold cooperatively through the transition accompanied by a large latent heat and other signs of discontinuity. As long as a single protein molecule can be described in statistical terms, folding and unfolding should be identified as first order phase transitions. According to sophisticated equilibrium statistical mechanics models, this sharpness, or cooperativity, of the transition is a direct manifestation of protein nonrandomness. Indeed, proteins are heteropolymers, but with nonrandom sequences that have been selected, presumably in the course of evolution. It is now an outstanding challenge to understand why and how this selection causes proteins to meet the kinetic requirements of rapid (milliseconds to seconds) and reliable folding.

Since the first order phase transition nature of folding, with its connection to the evolutionary selection of sequences, was realized, much attention has been paid to nucleation as a natural scenario of folding. The straightforward implementation of the nucleation idea, however appealing, faces difficulties, as evidenced by the recent heated debates. In hindsight, these difficulties are hardly surprising as chain connectivity must significantly modify the very concept of nucleation in ways which we do not yet have the proper insight for.

Our goal in the present paper is to gain such an insight by reexamining the foundations of the nucleation concept for proteins and computationally generating conformational selection and phase coexistence in a protein-like molecule. We will systematically employ the analogy with other first order phase transitions, such as the liquid-gas transition, where the kinetic concept of nucleation is ultimately related to the phase segregated states in equilibrium. Indeed, the nucleus is nothing more than a piece of a “new” equilibrium phase which tentatively coexists with the surrounding sea of the “old” phase. Of course, the nucleus is not really in equilibrium since it grows. However, and we view that as the single major lesson to be learned from a liquid-gas type system, the nucleus is, in the proper sense, close to equilibrium. Indeed, a nucleus grows slowly - in the sense that all other degrees of freedom have sufficient time to relax while the nucleus size does not change appreciably. The nucleus size can thus be said to be a “good reaction coordinate.” In terms of landscape theory, the relevant profile is then that of the free energy taken as a function of the nucleus size. The important part of that picture is, again, that the nucleus is fairly close to being in equilibrium.

Thus, we have to address equilibrium phase segregation and phase coexistence in a protein-like molecule. Note that we are referring to the equilibrium coexistence of two distinct phases within a single protein chain which should not be confused with the coexistence of folded and unfolded molecules in solution. In order to address the phase segregated (macro)states, we resort again to the analogy with a liquid-gas system for which there are two ways to bring the system into the phase segregated state: one is to bring it exactly to the transition temperature, and then add (or remove) some heat; the other involves a range of temperatures and the control of pressure and volume. The former approach is not usable in Monte Carlo simulations since they are done at constant temperature and heat transfer cannot be controlled in canonical ensembles. As for the latter approach, the analogues of pressure and volume have not been defined for protein-like systems. This is precisely what we will do.

Recall that the equilibrium folding theory for nonrandom (designed) heteropolymers recognizes the native overlap \( Q \) as an order parameter (for any given conformation, \( Q \) is the number of native monomer-monomer bonds). This quantity is thermodynamically additive and is thus similar to volume in a liquid-gas system. It is then straightforward to define the analogue of pressure, which we call folding pressure \( P_Q \). It is the quantity conjugate to \( Q \) given the Hamiltonian \( H_0 \) of our protein, applying folding pressure \( P_Q \) means taking the Hamiltonian \( H = H_0 - P_Q Q \). In other words, folding pressure is an additional energy bonus for every correct (native) bond. The \( -P_Q Q \) term can also be thought of as a perturbation.
of the original Hamiltonian $H_0$ by Go interactions $[12]$. Although it may not be easy to directly realize pure folding pressure in real experiments, changing experimentally controlled environmental parameters, such as pH or denaturant concentration, affects the folding pressure as well. In this sense, examining folding pressure in the theoretical model is just as relevant as studying temperature. Besides, and even more importantly, the very simple idea of folding pressure allows us to exercise physical intuition in a new fruitful way.

To perform Monte Carlo simulations of the folding transition, the polymer is modeled as a self-avoiding chain of 27 or 48 monomers on a cubic lattice. The Hamiltonian is given by $H_0 = \sum_{I,J} B_{s_is_j} \Delta(r_I - r_J)$ where $I, J$ label monomers along the chain, $B_{s_is_j}$ is the interaction between species $s_I$ and $s_J$, $\Delta(r_I - r_J) = 1$ if $I$ and $J$ are nearest neighbors and $\Delta(r_I - r_J) = 0$ otherwise. We employ the model $[13]$ in which the energies $B_{ij}$ are chosen independently from a Gaussian distribution. The sequence of species along the chain was obtained through simulated annealing so that the ground state of the polymer is the native conformation $[14,3]$.

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![FIG. 1. The distribution of a 27-mer over $Q$ at various $P_Q$ at $T = 4.0$. Both $P_Q$ and $T$ are measured in the units of $\beta \theta$, the variance of the interaction matrix. At each $P_Q$, the normalized distribution is given by the gray level: darker places correspond to higher probability densities. Triangles indicate the peaks of the distributions. The Van der Waals isotherm in the inset shows close qualitative similarity.](image)

The $P_Q - Q$ isotherms which are obtained by performing long Monte Carlo runs at various temperatures and folding pressures are strikingly similar to the $P - V$ isotherms for a liquid-gas system (see Figure 1). The folding pressure appears as the $-P_Q Q$ term in addition to the usual energy $H_0$ in the standard Metropolis criteria. For 27-mers, runs are at least $2 \times 10^9$ (and up to $5 \times 10^{10}$) iterations, i.e. until the distribution of conformations over $Q$ has reached equilibrium. As we vary the pressure at each temperature, the distribution of $Q$ changes from a monomodal distribution to a bimodal distribution near the transition pressure and back again to a monomodal distribution (Figure 1). The bimodal distribution is characterized by two maxima at $Q = Q_{\text{max}}$ and $Q = Q_u$ and a wide minimum in between. For 27-mers, $Q_{\text{max}} = 28$, $Q_u$ varies from 2 to 8, and the minimum is centered around $Q = 18$. $Q_{\text{max}}$ corresponds to the folded state and $Q$’s near $Q_u$ correspond to the unfolded states. The bimodal distribution occurs over a range of pressures, thus manifesting the metastable states.

The immediate (trivial) insight following from Figure 1 is that there should be both regimes of nucleation and of spinodal decomposition. Indeed, if the system is initially equilibrated, say, in the unfolded phase (lower left corner in Figure 1) and folding is then caused by an instant folding pressure quench at constant temperature, the kinetics will proceed differently depending on whether the system is quenched to the region where the original state is metastable or totally unstable. In particular, we have observed both nucleation and spinodal decomposition by examining the time evolution of the native contacts close to or far away from the transition temperature, respectively $[13]$. Close to the transition point the polymer remains folded or unfolded for an extended period of time until a particular group of native contacts which form the “nucleus” or “critical loop” are formed or broken, after which the polymer rapidly folds or unfolds, as is characteristic of nucleation. Far away from the transition temperature, there is no longer a free energy barrier to the (un)folded phase during (un)folding, that is, all domains of the (un)folded phase are unstable. This can be seen as a gradual increase of the (un)folded phase. The spinodal decomposition scenario implies nonspecific “homopolymer” collapse as the first stage of folding $[16]$.

By applying folding pressure, we can bring the system to the transition point at any given temperature. Once we are at the transition point, the stage of the transition can be controlled by varying $Q$. This is analogous to controlling the volume in a liquid-gas system by moving the piston when the pressure is equal to the transition pressure. This naive “piston” model is particularly useful due to the finite size of the system. Indeed, for macroscopic systems, the critical nucleus size is always much smaller than the system size. Accordingly, an infinitesimally small change in volume or displacement of a piston, would be needed to produce an equilibrium phase segregated state in which the size of, say, the liquid phase would be approximately that of a critical nucleus. Since the polymers of interest are of moderate size, the expected critical nucleus is not negligibly small compared to the entire system. This is why the interesting stages of the transition are seen when $Q$ is significantly different from the values of any of the phases.

We computationally generated the phase segregated
macrostates of a heteropolymer by running simulations at the transition point \((T = 1.7, P_Q = 0.5\) for 27-mers and \(T = 2.28, P_Q = 1.0\) for 48-mers; both \(T\) and \(P_Q\) are given in units of \(\delta B\), the variance of the interaction matrix \([3]\)). To mimic a fixed “piston” position, we restrict the value of \(Q\) to some chosen level \(Q\). We first obtain conformations at \(Q = \overline{Q}\) by doing a Monte Carlo run with unconstrained \(Q\) and collecting conformations every time the system passes through the \(Q\) “surface.” Each of the collected conformations with \(Q = \overline{Q}\) can be used to initiate a new restricted run in which \(Q = \overline{Q}\). The set of microstates (individual conformations) encountered in the restricted run will form a macrostate at the transition temperature \(T\) characterized by the given values of \(P_Q\), and \(Q = \overline{Q}\). The phase segregation is clearly seen in Figure 2 where orientation and position are chosen for each microstate with maximum superposition with the native state. When the microstates are subsequently superimposed upon one another, we see that there is a set of “core” monomers, mostly superimposed upon the native state, which do not fluctuate in position (Figure 2). These monomers can be interpreted as the “native” phase, while the monomers which fluctuate belong to the “denatured” phase.

Based on the analogy with the liquid-gas system, we expect that phase segregated macrostates obtained at subsequent values of \(\overline{Q}\) (“piston positions”) would be very similar to those encountered subsequently in time during a real kinetic event. To test this expectation, we employ the general method suggested in the work \([17]\) and measured the folding probability \(p\) for each of the microstates belonging to the phase segregated macrostate. We found that the distribution of \(p\)'s is indeed very narrow for most of the macrostates. The three examples shown in Figure 2, the variance of \(p\) is as low as \(\langle \Delta p \rangle \approx 0.05\). This is to be compared with the very broad distributions of \(p\)'s for the ensemble of all states with the given \(Q = \overline{Q}\), where \(\langle \Delta p \rangle\) can be of order unity \([5]\). This means that by “moving the piston” in the way described above, we indeed drag the system along its natural kinetic path. However, as we mentioned, this is only valid for a majority of phase segregated macrostates. There are important exclusions from the rule which represent the fundamental difference between (hetero)polymeric system and the more familiar liquid-gas one.

To understand the problem, consider Figure 3. It demonstrates one of the examples in which “pushing the piston” brings the system to a dead end instead of dragging it along its path from one phase to the other. The reason for the deadlock is purely topological: while the amount of the folded phase is growing at the expense of the denatured phase, a topological constraint has been formed in the latter. As a result, the system arrives at a conformation which cannot fold without first destroying a significant part of its correctly folded “native phase.”

The insight we gained from considering phase segregated macrostates and the role of topological constraints can be now used to produce a scaling analysis for very long \((N \to \infty)\) chains, thus approaching the problem formulated in the work \([15]\). Here, we restrict ourselves to the scaling of unfolding time, which is more instructive.
in terms of the role of topological constraints. We begin with a correctly folded native globule, and then quench the temperature and/or folding pressure such that native state becomes (globally) unstable. The nucleus of the unfolded denatured phase, which sooner or later will appear in this system can be imagined as a “bubble” of polymer melt (or solution; Figure 3). This polymer liquid consists of one or several loops. It is important to note that on the time scale of interest, while the nucleus remains unchanged in size, the ends of all those loops are firmly quenched at the corresponding “root points” on the surface of native phase which is frozen.

Since all ends are fixed, the topology of the loops is well-defined and quenched. The crucial point here is not the classification of the given state as “entangled” or “unentangled” but that the mutual positions of the loops are quenched in the same topological class to which it belonged in the native state (unless a chain end belongs to the nucleus, which is improbable in the \( N \to \infty \) limit). This fact has profound consequences in terms of the critical nucleus size and, accordingly, the unfolding barrier height. Indeed, normally, in a vapor-liquid system, nucleation free energy can be schematically written as \( -\alpha \Delta T N + \sigma N^\Delta \), where the volume part \( (N) \), which is proportional to the deviation from the transition point \( \Delta T \) (degree of overheating or overcooling in the initial quench) is negative (favorable) and the surface part \( (N^\Delta) \) is positive (unfavorable). For a polymer, melting of the nucleus does not release all of the volume free energy \( -\alpha \Delta T N \) because the melted part remains topologically constrained. Thus, there appears an additional positive contribution to the nucleus free energy. As long as the nucleus remains small compared to the entire globule, this new term can be estimated as \( +T \ln \mathcal{M} \), where \( \mathcal{M} \) is the number of topologically different classes. Since \( \mathcal{M} \) grows exponentially with the number of monomers involved, we end up with an extra volume term which is always positive and independent of \( \Delta T \): \( -\alpha \Delta T N + \beta TN + \sigma N^\Delta \). To estimate \( \beta \), we performed an exhaustive enumeration of all two-loops conformations with fixed ends within a \( 3 \times 3 \times 3 \) cube \( (N = 27, \text{see Figure 3}) \) and found that \( \beta = 0.45 \) is of order unity and by no means small (contrary to an early estimate \([19]\)). Thus, topological constraints significantly increase the height of the barrier, in complete agreement with our simulations (Figure 3). As long as temperature jumps causing unfolding, \( \Delta T \), remain finite and \( N \)-independent, the critical nucleus is of the order of the entire globule, and thus the unfolding time scales as \( \text{exp} \left[ c \cdot N^{2/3} \right] \), as opposed to some \( N \)-independent time for the phantom polymer, which is allowed to freely pass through itself. As for \( c \), it is a constant, and we see no grounds to assume that it is significantly different from unity. Our result agrees very well with the original estimate of folding time under equilibrium conditions given by Finkelstein, but sharply contradicts to its latest improvement \([19]\).

To conclude, we found that study of the phase segregation occurring in an equilibrium proteinlike heteropolymer sheds light on the possible and impossible nuclei configurations relevant for folding and unfolding kinetics. We found in particular that topological constraints play an important role in determining the critical nucleus. In the case of unfolding, topological constraints dramatically increase the size of the critical nucleus, causing the unfolding time to scale exponentially with the chain length.

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\[ \text{FIG. 4. Schematic representation of unfolding nucleus inside a large folded globule and its lattice model.} \]

\[ \text{[1]} \text{ Privalov, P. J. Chem. Therm., 29, 447 (1997).} \]
\[ \text{[2]} \text{ Lifshitz, I. Sov. Phys. JETP, 28, 1280 (1968).} \]
\[ \text{[3]} \text{ Pande, V., Grosberg, A., Tanaka, T. Rev. Mod. Phys., to be published (1999).} \]
\[ \text{[4]} \text{ Fersht, A. Curr. Opin. Struct. Biol., 5, 79 (1995).} \]
\[ \text{[5]} \text{ Abkevich, V., Gutin, A., and Shakhnovich, E. Biochemistry, 33, 10026 (1994).} \]
\[ \text{[6]} \text{ Klimov, D. and Thirumalai, D. J. Mol. Biol., 282, 471 (1998).} \]
\[ \text{[7]} \text{ Wolynes, P. Folding & Design, 3, R107 (1998).} \]
\[ \text{[8]} \text{ Shakhnovich, E. Folding & Design, 3, R108 (1998).} \]
\[ \text{[9]} \text{ Thirumalai, D. and Klimov, D. Folding & Design, 3, R112 (1998).} \]
\[ \text{[10]} \text{ Lifshitz, E. and Pitaevskii, L. Physical Kinetics. Pergamon, New York, 1981.} \]
\[ \text{[11]} \text{ Bryngelson, J., Onuchic, J., Socci, N., and Wolynes, P. Proteins, 21, 167 (1995).} \]
\[ \text{[12]} \text{ Go, N. Ann. Rev. Biophys. and Bioeng., 12, 183 (1983).} \]
\[ \text{[13]} \text{ Shakhnovich, E. and Gutin, A. Biophys. Chem, 34, 187 (1989).} \]
\[ \text{[14]} \text{ Shakhnovich, E. and Gutin, A. Proc. Nat. Acad. Sci., 90, 7195 (1993).} \]
\[ \text{[15]} \text{ Du, R. MIT Thesis (1999).} \]
\[ \text{[16]} \text{ De Gennes, P. J. Phys. Lett., 46, L639 (1985).} \]
\[ \text{[17]} \text{ Fersht, A. and Goldbart, P. M. in preparation and references therein.} \]
\[ \text{[18]} \text{ Du, R., Pande, V., Grosberg, A., Tanaka, T., and Shakhnovich, E. J. Chem. Phys., 108, 334 (1998).} \]
\[ \text{[19]} \text{ Gutin, A., Abkevich, V., and Shakhnovich, E. Phys. Rev. Lett., 77, 5433 (1996).} \]
\[ \text{[20]} \text{ Finkelstein, A. and Badretdinov, A. Folding & Design, 2, 115 (1997); 3, 67 (1998).} \]