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Helix–Coil, Liquid Crystal, and Spin Glass Transitions of a Collapsed Heteropolymer

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Using simple statistical mechanical models, we investigate secondary structure formation in the molten globule state of a helix-forming heteropolymer. The theory based on the helix–coil model of Bragg and Zimm correctly treats the one-dimensional aspect of the helix–coil transition but has been modified to include the randomness effects of the tertiary interactions in a heteropolymer. Rough approximations to the effects of confinement, excluded volume, and packing order of the helices are used to give the resulting generalization of the random energy model. In this model the packing effects give rise to a liquid crystalline order in the molten globule state. For reasonable values of parameters at the spin glass transition, we estimate that the heteropolymer is about 80% helical with helix lengths of order 20. Analysis of this simple model allows an estimate of the effective Levinthal entropy and determines that about 10^{11} configurations would have to be searched at the glass transition for a 100-mer.

Introduction

Even before it was realized that enzymes and other functional proteins have an organized and fairly precise three-dimensional structure, it was appreciated from the X-ray diffraction study of fibrous proteins that proteins can possess a repeating local structure characteristic of helices.¹ As Pauling pointed out, this repetitive helical structure is to be expected on the grounds of translational symmetry for a biomolecule with a repeating sequence.² It is then perhaps remarkable that this secondary structure persists, at least to some extent, in the three-dimensional structures of globular proteins which, after all, do not have such simple repeating sequential patterns. In the intervening years, two rather different explanations for the persistence of secondary structure in globular proteins have been invoked. The first of these is the idea that the local hydrogen bonding is of paramount importance in determining these secondary structures.³ More recently, attention has focused on the possibility that steric constraints caused by the close packing of protein interiors drives the formation of secondary structures.^{4a} Since both effects are present both in macroscopic fibrous proteins and globular proteins, it is difficult a priori to rule either explanation entirely out. These two differing views have also come to inform most discussions of the mechanism of protein folding. Some models emphasize the prior formation of secondary structural elements followed by their assembly,⁵ while other models put the emphasis on the collapse to a compact structure driving local organization.⁶ Dill has recently presented studies on small 2-dimensional model lattice proteins with explicit hydrogen bonding, but it still seems worthwhile to construct a simple theory applicable to three dimensions.^{4b}

In recent years, it has come to be realized that the search for a unique structure in a globular protein involves the discrimination between different overall folded structures and that the collapse into a globule with some secondary structure present does not by itself solve the problem of search for a unique three-dimensional organization. The most striking evidence for this is the existence of the so-called molten globule phase of

proteins.^{6,7} Molten globules generally have collapsed to a compact structure with a good deal of residual secondary structural content but are not completely folded. The search for a unique structure depends crucially on the heteropolymeric nature of natural proteins. This is clear since artificial homopolymers, such as polyethylene, even when they crystallize have many structures which depend on the kinetics of annealing.⁸ Alongside the qualitative structural and mechanistic discussion of secondary structure formation, collapse, and unique three-dimensional folding has stood a body of statistical mechanical theory which strives to quantitatively understand these phenomena. Starting in the 1950s, the simplest sort of secondary structure formation, the helix–coil transition, has been studied intensively by theoreticians. A plethora of models for the simple one-dimensional phase transition giving rise to helical structures were invented and used to quantitatively understand the helix–coil transitions of soluble homopolymers as well as noncollapsed heteropolymers.⁹ The simplest, and for our purpose the most useful of these models, are those due to Zimm and Bragg^{10a} and to Peller.^{10b} The statistical mechanics of polymer collapse has primarily entered the protein folding field through the study of lattice models.¹¹ Such models have forcefully made the point that the secondary structure of globular proteins may be largely due to excluded volume effects. An important class of homopolymer models coupling secondary structure and collapse have been studied by Garel and Orland.^{12a,b} The problem of the unique three-dimensional structure of heteropolymer proteins has been addressed by statistical mechanicians through lattice models but, also, by borrowing the techniques from the statistical mechanics of disordered systems used to describe spin glasses.¹³ The spin glass approach to heteropolymer folding has allowed some understanding of the possible collapsed phases of heteropolymers,¹⁴ the kinetics of search for unique conformations,¹⁵ and the probability of achieving thermodynamic foldability with a randomly chosen sequence.¹⁶

Both the secondary structure formation and collapse have been discussed in the context of spin glass theories of protein folding before. As first pointed out by one of us¹³ and later by others,^{16c} the previous spin glass approaches do not deal appropriately with the one-dimensional aspects of the helix–

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coil transition itself. Indeed, the early treatments and some later ones¹⁷ still treat the secondary structure interactions in a mean field manner which has long been known to break down for purely one-dimensional problems. Since the collapse and three-dimensional structure formation are themselves truly three-dimensional transitions, it is not clear how terrible the use of the mean field approximation really is. Nevertheless, it is desirable to treat the spin glass statistical mechanics of protein folding for understanding the phase diagram in a way fully consistent with our knowledge of the one-dimensional statistical mechanics of ordinary helix-coil transitions. In this paper we present a calculation which does precisely this.

The model we describe uses only the simplest statistical mechanics of the helix-coil transition, a simple estimate of the excluded volume and confinement effects of collapse and the random energy model description of heteropolymer folding. The analysis begins with a combinatorial approach to the helix-coil transition appropriate for the microcanonical description needed to characterize the spin glass transition. The tertiary structure effects coming from the heteropolymeric randomness of the sequences are taken into account, using the random energy approximation. Putting these features together leads to a random energy model which depends on two order parameters characterizing the helix-coil transition, namely the number of helical segments and the number of interfaces. We also incorporate into the model some polymer physics effects connected with confinement and the steric constraints in the collapsed globule. There are two competing effects. First, confinement to a collapsed structure leads to an inevitable frustration of the formation of helical order. This frustration arises since generally a few very long helices cannot form the tertiary contacts needed for spherical collapse. An estimate of the entropy loss by confinement can easily be made and leads to a constraint on the amount of helical order. Second, as witnessed by the lattice studies, the steric constraints of packing encourage the formation of linear structures which can pack efficiently. The packing induced formation of secondary structure is very much analogous to Onsager's model of nematic liquid crystal ordering for rigid rods^{18a} as studied by Grover and Zwanzig.^{18b} This effect has been much studied for bulk nematic polymers^{18c} and has been described by DeGennes and Pincus^{18d} as "induced rigidity". These effects also exist in the homopolymer models of protein studied by Garel and Orland but are more simply treated here. We discuss such a simple way of taking this liquid crystal ordering into account using a single orientational order parameter.^{19,35a} At least two styles of liquid crystalline order are possible. One is characteristic of helical bundles, while a weaker form of steric constraint would lead to an ordering like that seen in polyhedral globular structures analogous to myoglobin. Such multiple transitions are quite familiar in theory of cholesteric liquid crystals such as those exhibiting "blue phases". Again, liquid crystallinity is a familiar feature of fibrous proteins such as collagen.²⁰ In fact, these liquid crystal orderings are certainly coupled to overall shape transitions of the globular proteins from spherical to ellipsoidal. This effect is not explicitly taken into account in this paper.

We note that Pitsyn has long characterized protein molten globules as liquid crystals.²¹ Our work is a quantitative but perhaps overly precise incarnation of that notion. It will be interesting to see if in fact some molten globule phases do have orientational but not translational order. The most striking NMR investigations on molten globule like phases due to Wand have been interpreted as showing specific translationally ordered structure, but we must bear in mind this would be a natural result from the usual methods of data analysis.²² Liquid crystalline

phases of polymer globules are known in the context of the collapsed phases of DNA. Grosberg has developed a theory for such a phase treating DNA as a wormlike chain.²³ Such phases may be relevant to the nucleosome.²⁴

After discussing the statistical mechanical treatment of these problems, we turn to the details of numerical solution for several cases. We discuss the nature of the helix-coil transition in the collapsed confined globule, for models containing explicit strong hydrogen bond energies. An important result is that the coupling of the liquid-crystal transition with the helix-coil transition makes the latter appear as a first order phase transition jump. This jump arises from a different physical mechanism but is mathematically quite analogous to a model of helix-coil transitions coupled with collapse due to Grosberg.²⁵ Obviously the observation of a latent heat for a transition in a polymer globule does not by itself imply a completely three-dimensionally ordered protein. In models with such local helical biases, we discuss the configurational entropy relevant to the glass transition and are thereby able to address the question of the extent to which the Levinthal problem for search is reduced by the formation of helical secondary structures. We also compare the statistics of helices in frozen globules with the protein database.

The theory described here is directly appropriate for and has implications for random polypeptides like those studied by LaBean et al.^{26a} and Sauer's group^{26b} and perhaps partially random proteins studied by Hecht.²⁷ We believe natural proteins that have evolved to fold rapidly are not completely random but obey a "principle of minimal frustration"^{14a} in which interactions stabilizing the contacts and local aspects of the native structure contribute more energy than would be expected by chance. As in earlier treatments such biased energy landscapes possess a folding transition distinct from those studied here. This transition needs to be described by at least one additional order parameter. In any event the folding transition for minimally frustrated heteropolymers is coupled to those discussed here. The low entropy of a liquid crystalline molten globule allows much weaker biases to affect the structure profoundly. Thus local biases such as helix start-stop signals or weak tertiary packing patterns may be much more efficient than initially expected. We postpone quantitative discussion of such minimally frustrated heteropolymers with helical order to later work.

Statistical Mechanical Model

A most efficient route to the thermodynamics of random and glassy systems is through the random energy approximation.²⁸ This approximation is most easily carried out using the microcanonical ensemble. Thus for the problem of helix-coil and spin glass transitions of heteropolymers, we must separately evaluate the combinatorial problems of secondary structure formation, collapse, and ordering so as to yield an entropy. We must also find the probability distribution of the energy of a given configuration. We separately consider various contributions to each of these quantities. Although many of the features of our analysis above the glass transition can be carried out using a canonical ensemble analysis, the crucial entropic features that are important in the final search are most easily seen in the microcanonical formulation. A unified treatment with the glassy aspects is thus desirable.

To set the stage, we must understand how the ordinary helix-coil transition would be treated in the microcanonical ensemble. The most straightforward treatment of the helix-coil transition is based on the transfer matrix method which is most easily carried out in the canonical ensemble. However, an alternate

combinatorial approach to the one-dimensional Ising model lends itself to the microcanonical treatment. Before we consider the effects of confinement, the helix-coil combinatorial problem can be solved if we are given the number of helical units N_h (monomers) and the number of interfaces N_i between helices and coils in a given configuration of a protein with N residues. The degeneracy of arrangements Ω_c is given by

$$\Omega_c = C(N_h, N_i, N) (Z_c)^{N_c} (Z_i)^{\alpha N_i/2} (Z_h)^{N_h - N_i/2} \quad (1)$$

with

$$C(N_h, N_i, N) = \frac{N_h! (N - N_h)!}{(N_h - N_i/2)! (N - N_h - N_i/2)! [(N_i/2)!]^2}$$

where N_c is the number of residues in coils,

$$N_c = N - N_h$$

In this equation, Z_c is the number of conformational states available to a coil segment, and Z_i reflects the configurational entropy of each helical run-coil junction. This depends on the orientational ordering of the helical runs. This ordering is reflected in the parameter α to be discussed in detail later. The helical segment is taken to be in a single conformational state so that $Z_h = 1$. The combinatorial factor $C(N_h, N_i, N)$ comes from the number of ways of arranging helical segments and interfaces.^{10b,29} We remind the reader that the combinatorial factor in eq 1 is misleadingly reminiscent of the combinatorial factor in Bryngelson and Wolynes's (BW) theory of the minimally frustrated heteropolymer. In their theory one counts the number of correctly versus incorrectly configured residues not the number which are helical vs coil. The BW combinatorics would refer to an additional order parameter which must be added to the present model to describe proteins which have evolved to fold rapidly.

On the other hand for a homopolymer the energy can be expressed as

$$\bar{E} = +2\epsilon_{hb}N_h - 4\epsilon_{hb}N_i \quad (2)$$

where ϵ_{hb} is the energy of forming a hydrogen bond. We see that both the entropy and the energy are expressed in terms of two order parameters, N_h and N_i . In real life the effective hydrogen bond free energy may have hydrophobic and electrostatic contributions from local compaction. This complication need not concern us in understanding the physics of the model but may play a role in fitting parameters to laboratory experiments.

For the heteropolymer to complete the specification of the random energy approximation, we must find the probability distribution for the energy of a given configurational state as a function of these order parameters for the helix-coil transition. We also simplify the analysis here by using these probability distributions only for the completely collapsed structures. Thus the mean energy for collapse is left out of the calculation.

For simplicity we will here neglect the randomness effects on the secondary structure energies and consider only the randomness effects coming from tertiary structural terms. As usual, then, we expect this probability distribution to be a Gaussian around a mean which depends on the fraction of helical content and the number of interfaces. Aside from the collapse contribution the mean energy should be the same as in the homopolymer problem. The variance ΔE^2 depends on the number of pair contacts in the molten globule, a result due to Bryngelson and Wolynes (BW)^{14a,b}:

$$\Delta E^2 = N_n \Delta K^2 \quad (3)$$

ΔK^2 measures the variance of interaction energy between individual pair terms in the tertiary structure and N_n is the typical number of pair contacts in the molten globule, which is itself proportional to N . For collapsed structures the variance parameter ΔK is related to the energy of the typical hydrophobic interaction. In calculations we take ΔK comparable to the hydrogen bond energy.

The probability distribution of the energy for a given configuration depends only on its number of helices and interfaces and should have the Gaussian form

$$P(E) = e^{-(E-\bar{E})^2/2\Delta E^2} / \sqrt{2\pi\Delta E^2} \quad (4)$$

With these two ingredients, the statistical mechanical calculation of the random heteropolymer with helical structure can be carried out in a way totally analogous to the random energy approximation for heteropolymer collapse discussed earlier by Bryngelson and Wolynes. We recapitulate their analysis here for the present model.

The microcanonical analysis of the REM is straightforward. One proceeds through the evaluation of the average number of energy levels at a given energy. This number $\Omega(E)$, dependent on N_h and N_i , is given by

$$\Omega(E, N_h, N_i, N) = \Omega_c e^{-(E-\bar{E})^2/2\Delta E^2} / \sqrt{2\pi\Delta E^2} = C(N_h, N_i, N) (Z_c)^{N_c} (Z_i)^{\alpha N_i/2} e^{-(E-\bar{E})^2/2\Delta E^2} / \sqrt{2\pi\Delta E^2} \quad (5)$$

This combines then the entropy considerations above as well as the probability distribution of the energies. The entropy corresponding to this average density of state is also easily computed as a function E , N_h , and N_i :

$$S(E, N_h, N_i) = N_h \log N_h + (N - N_h) \log(N - N_h) - (N_h - N_i/2) \log(N_h - N_i/2) - (N - N_h - N_i/2) \log(N - N_h - N_i/2) - N_i \log(N_i/2) - \frac{(E - \bar{E})^2}{2\Delta E^2} + (N - N_h) \log Z_c + 1/2 \alpha N_i \log Z_i \quad (6)$$

This entropy must be maximized as a function of the parameters N_h and N_i , giving the variational equations

$$0 = \frac{\partial S}{\partial N_h} = \log \left[\frac{N_h(N - N_h - N_i/2)}{(N_h - N_i/2)(N - N_h)} \right] + 2\epsilon_{hb} \frac{(E - \bar{E})}{\Delta E^2} - \log Z_c \quad (7)$$

$$0 = \frac{\partial S}{\partial N_i} = 1/2 \log(N - N_i/2)(N - N_h - N_i/2) - \log(N_i/2) - 4\epsilon_{hb}(E - \bar{E})/\Delta E^2 + 1/2 \alpha \log Z_i \quad (8)$$

In addition, the temperature is determined through the usual thermodynamic relation giving

$$\frac{1}{T} = \frac{\partial S}{\partial E} = \frac{-(E - \bar{E})}{\Delta E^2} \quad (9)$$

When these equations are combined, they yield the Zimm/Bragg description of the helix coil transition if we make the identifica-

$$s = \frac{1}{Z_c} \exp(-2\epsilon_{hb}/T) \quad \sigma/s^2 = Z_i \exp(8\epsilon_{hb}/T) \quad (10)$$

We see the introduction of these two order parameters gives a kind of mean field theory which is *exact* for the one-dimensional problem. If no other phase transitions occur, the REM analysis would give no difference from the traditional theory. The helix-coil transition is however modified by other transitions which depend on polymer configurational statistics.

One of the important polymer transitions that affects the helix-coil transition is collapse. The free energetics of heteropolymer collapse are quite delicate. Collapse can occur from a general hydrophobic attraction³⁰ or by means of adventitious random contacts even when the mean attraction is zero as described by many workers (see ref 13b for a review). For small proteins surface forces can play a role. For a minimally frustrated heteropolymer, the partial ordering due to biases can also contribute to collapse. The energetics of the collapse phase also strongly depends on whether the globule is above its glass transition or below it. If the globule is below T_g , collapse itself will be non-self-averaging. We finesse these issues by dealing only with that part of the phase diagram in which collapse is already known to have taken place. This reduces our considerations to ones focusing on geometric effects and entropy. Thus we do not introduce explicit energy terms for collapse, but they are decidedly present in the model.

The first step in the analysis of these transitions within the collapsed part of the phase diagram is the evaluation of the entropy as a function of the order parameters and a fixed confinement radius. Thus we use these order parameters to also carry out the random energy approximation for our model of helix-coil transitions in collapsed heteropolymers:

$$S(E, N_h, N_i, R) = S_{BZ} + \Delta S_{\text{confine}} + \Delta S_{\text{steric}} \quad (11)$$

S_{BZ} is the entropy that the chain would have with this number of helices and interfaces if it were not confined to a globule. It is thus the same as expression 6 used above. The other two terms $\Delta S_{\text{confine}}$ and ΔS_{steric} are approximations to the entropy loss caused by confining the chain to a small volume of radius R and the entropy loss caused by the avoidance of overlap of the chain within that volume. In fact, these two terms are coupled in a complete analysis, but we approximately separate them here in a manner analogous to that done by Bryngelson and Wolynes^{14b} earlier for chains without secondary structure.

The confinement entropy loss, ΔS_{conf} , is taken to be the change in the logarithm of the number of configurations of a chain without excluded volume when it is placed in a sphere of radius D . This entropy loss depends upon the natural radius of gyration which in turn is a function of the chain's helical content. A free-flight chain made up of helices and random coils would have a mean square displacement given by R_0 :

$$R_0^2 = (N - N_h)R_c^2 + \frac{2N_h^2}{N_i + 2}R_h^2 \quad (12)$$

where R_h is the contribution to the rmsd of a helical rod due to a single helical segment. R_c is the persistence length per residue of a coil. We have assumed here that the helices have an average sequence length given by $2N_h/N_i$, where $N_i/2$ is the number of helices. Because helices are straight, their contribution varies like N_h^2 . We see that if a chain became entirely helical, it would have a very large radius of gyration if it were not confined. Consequently, the confinement entropy loss is greater if there is a considerable amount of secondary structure

formed without many interfaces. For the confinement entropy as a function of R_0 , we use the leading term in the expression of Cassasa³¹ for the confinement of a free-flight chain to a sphere of radius D :

$$\Delta S_{\text{confine}} = -\frac{\pi^2 R_0^2}{6D^2} + \log \frac{6}{\pi^2} \quad (13)$$

This expression differs from that used by Bryngelson and Wolynes in their previous analysis and earlier used by DeGennes³² and Post and Zimm³³ for DNA collapse and many others. As pointed out by Birshstein and Pryamitsyn,³⁴ the result used previously was incorrect for strongly collapsed chains. The error did not play an important qualitative role for many problems since the primary restoring force for stabilizing the collapsed phase is the excluded volume which we will discuss momentarily. On the other hand, for the present problem, that of quantifying the frustration caused by the conflict between the local tendency to form long helices and the necessity to remain collapsed, this confinement term is quite crucial.

A second point is that the result we have shown here assumes that the globule is spherical. In fact, the formation of secondary structure can give rise to ellipsoidal volumes, or even needlelike ones for some sequences, depending on the surface forces. An analysis of these possible shape transitions is potentially extremely interesting. It can probably be dealt with within the same kind of framework discussed here through the addition of terms representing surface energies and taking into account the coupling of helical ordering and the shape.

We next come to the evaluation of the steric and excluded volume effects on the entropy. An initial estimate of this entropy loss can be obtained via the Flory theory of excluded volume for a bulk flexible polymer, as was done earlier by Bryngelson and Wolynes. This leads to an expression for the steric entropy loss:

$$\Delta S_{\text{steric}} = -N \left[\frac{1 - \eta(R)}{\eta(R)} \log(1 - \eta(R)) + 1 \right] \quad (14)$$

where $\eta(R)$ is the packing fraction and N the total number of residues. In the BW analysis, this entropy acted as a restoring force to prevent the collapse of the protein to a point under the influence of the general attractive forces. In fact, collapse should occur to a maximal density near to close packing, $\eta \sim 0.9$, in the simple theory. If complete close packing is achieved, $\eta = 1$, we see that the effect of this is a diminution of the total number of configurations by a factor $1/e^N$. Since this excluded volume effect on the entropy in this approximation depends only on the total amount of free volume, it would be independent of the fractional helical content and, therefore, would not affect the helix coil transition, while it would play a role in the glass transitions of the model as discussed by Bryngelson and Wolynes. This simple isotropic estimate is a reasonable one if we assume that helices are placed nearly at random in the collapsed structure. It is clear, however, that in a dense structure, the helices would rather align just as rods do in Onsager's famous treatment of the liquid crystal transition caused by steric effects.¹⁸ An effect of this type on helix-coil transitions was previously studied in detail by Grover and Zwanzig in an analysis which is quite appropriate at low-packing densities.^{18b} They showed that at low densities, the liquid crystal ordering which is possible for the helical rods gives them an excluded volume advantage, thus allowing the helix-coil transition to be encouraged when there is a finite concentration of polymers. Kim and Pincus also developed a canonical ensemble based mean field theory of this induced rigidity

appropriate for the bulk liquid-crystal helix-coil transition.^{18c} This theory uses transfer matrix formalism and allows the orientational order parameters to be computed. A high density theory due to Matheson and Flory³⁵ treats the transition combinatorially using the explicit helical length distribution as an order parameter too.

These detailed analyses of induced rigidity may be used for collapsed heteropolymer globules, but we believe a very simple model of the same effect is sufficient to illustrate the points. We simply use the fact that if the helices are perfectly aligned, their contribution to the excluded volume is extremely small through interactions with each other while, on the other hand, they still represent obstacles for the coil segments. Thus for the case of perfect alignment in which such nematic order has been established, then the excluded volume contribution to the entropy depends only on N_c and the bulk steric diminution of the entropy is

$$\Delta S_{\text{steric}} = -N_c \left[\frac{1 - \eta(R)}{\eta(R)} \log(1 - \eta(R)) + 1 \right] \quad (15)$$

Intermediate degrees of liquid crystalline order can be treated roughly with the expression

$$\Delta S_{\text{steric}} = -(N_c + \alpha N_h) \left[\frac{1 - \eta(R)}{\eta(R)} \log(1 - \eta(R)) + 1 \right] \quad (16)$$

in which the coefficient, α , is ~ 0 for aligned helices (nematic phase). α should be ~ 1 for entirely jumbled helices (disordered phase) and some intermediate value perhaps of the order 0.25, for helices arranged at the crossing angles typical of polyhedra helical globules. A more complete treatment of dense liquid crystalline alignment due to Flory gives essentially these same limiting results for the disordered and aligned cases.¹⁹ Further elaborations also exist. Since helices are chiral, the liquid crystalline order is actually cholesteric rather than simple nematic as described here, but this should not affect the excluded volume much.

The coefficient α also appears in the combinatorial entropy eq 1. For jumbled helices each helical rod has several directions in which to point, but in the liquid crystalline phases the correlation of the rods reduces that degree of freedom. For a helical bundle, once one rod's direction is set the others must follow, thus again the value $\alpha \approx 0$.

While the simple entropy estimate is rough, we see that it immediately gets some very simple physics right. The effective entropy of a coil is reduced by an amount $\log e$, while the number of configurations of a helix remains the same at 1. Thus even in the absence of hydrogen bonds favoring helical structures, we would expect the fraction of helical content in a collapsed globule to be $1/(1 + Z_c/e)$. The lattice estimate of $Z_c = 6$ thus gives a fractional helical content of approximately 30%. This is in reasonable agreement with the lattice enumeration studies of Dill when one appreciates that the finite size effects are neglected here.

We believe these estimates for the confinement effects are crude but reasonable. In any case they can be extended or tested by studies on lattice models of the type already undertaken by Dill, but of course it would be nice to study rather longer chains. A direct experimental test could be carried out by studying the phase transitions of heteropolymers in porous media such as sol-gel glasses.³⁶

Combining these results gives a complete expression for the entropy as a function of the order parameters N_i , N_h , and radius of the collapsed structure:

$$S(N_h, N_i, E, D) = N_h \log N_h + (N - N_h) \log(N - N_h) - (N_h - N_i/2) \log(N_h - N_i/2) - (N - N_h - N_i/2) \log(N - N_h - N_i/2) - N_i \log(N_i/2) - \frac{(E - \bar{E})^2}{2\Delta E^2} + \frac{1}{2} \alpha N_i \log Z_i + (N - N_h) \log Z_c - (N_c + \alpha N_h) \left[\frac{1 - \eta}{\eta} \log(1 - \eta) + 1 \right] - \frac{\pi^2 R_0^2}{6D^2} + \log \frac{6}{\pi^2} \quad (17)$$

As mentioned earlier, we will not consider here the delicate questions of the overall energetics of collapse, so we will take D to be a parameter that scales with the size of the system in the usual way for a sphere, $D = (3/4\pi Q)^{1/3}$. This entropy must be maximized as a function of the parameters, N_h and N_i , to find their most probable values at each temperature:

$$0 = \frac{\partial S}{\partial N_h} = \log \left[\frac{N_h(N - N_h - N_i/2)}{(N_h - N_i/2)(N - N_h)} \right] + 2\epsilon_{\text{hb}} \frac{(E - \bar{E})}{\Delta E^2} - \log Z_c + (1 - \alpha) \left[\frac{1 - \eta}{\eta} \log(1 - \eta) + 1 \right] - \frac{\pi^2}{6D^2} \left[-R_c^2 + \frac{4N_h}{N_i + 2} R_h^2 \right] \quad (18)$$

$$0 = \frac{\partial S}{\partial N_i} = \frac{1}{2} \log(N - N_i/2)(N - N_h - N_i/2) - \log(N_i/2) - 4\epsilon_{\text{hb}} \frac{(E - \bar{E})}{\Delta E^2} + \frac{1}{2} \alpha \log Z_i + \frac{\pi^2}{6D^2} \left[\frac{2N_h^2}{(N_i + 2)^2} R_h^2 \right] \quad (19)$$

We will discuss the numerical solution of this equation and the resulting entropy and free energy in the following section. It is easy to see that if the confinement effects are turned off while the randomness is still included, there is no effect on the Zimm/Bragg theory above the glass transition temperature. We will see, however, that the confinement terms do indeed frustrate the formation of complete helical order and that on the other hand, the liquid crystal ordering encourages the formation of secondary structure. Thus the effective s and σ for collapsed heteropolymer globules are quite different from those of the uncollapsed phase. In fact, we shall see the effective σ in the combined globule changes in the opposite manner from the bulk steric effects considered by others.

The analysis which just been presented is valid when the estimate of the average number of energy levels is correct. When this average number of energy levels as a function of the mean energy falls to a value of the order of 1, freezing into the spin glass phase occurs. Just as in the random heteropolymer studied previously, this frozen phase consists of a small number of configurations which are near to the ground state in energy. This spin glass transition then occurs when the configurational entropy of the system vanishes. The transition temperature T_g and the corresponding energy are found from the equations

$$S(N_h, N_i, T_g, D) = 0, \quad E = \bar{E} - \Delta E^2/T_g \quad (20)$$

The free energy follows straightforwardly from

$$F = E - TS \quad (21)$$

This should be a good estimate of the ground-state energy of collapsed structures that have secondary structure present. Whatever secondary structure is present just above this transition

will be frozen in. Thus we can make estimates of the amount of secondary structure in energy minima using this theory.

Another interesting quantity to calculate at the glass transition is the effective Levinthal entropy at the glass transition. This quantity is the entropy of the system if we were to neglect the ruggedness of the energy landscape at the glass transition temperature. This purely geometrical entropy is given as a function of temperature by

$$S_L = S(N_h, N_i, E, D) + \Delta E^2/2T^2 \quad (22)$$

At the glass transition $S_L = \Delta E^2/2T_g^2$. While a complete dynamical analysis would be desirable for this model in which secondary structure forms, it is reasonable to assume, following the previous analysis of Bryngelson and Wolynes, that it is this remnant configurational entropy which would actually determine the degree of difficulty of the conformational search for random heteropolymers cooled to the glass transition temperature. The early formation of helices essentially would reduce the effective number of elements in the chain and thus makes the configurational search easier. (This is of course also the idea behind many structure prediction schemes^{37a}). Below the glass transition there are only energetic contributions to the free energy. The order 1 contributions to the free energy are, however, non-self-averaging and are relevant to determining the probability of a thermodynamic two-state-like behavior below T_g as discussed by Shakhnovich and Gutin.

We discuss the numerical solution of the variational equations in the next section and evaluate the relevant entropies. We then use these results to describe the phase diagram for random heteropolymers with helical propensity and the quantitative features of the problem.

Numerical Results of the Model

For concreteness we fix some of the parameters of the model in order to mimic actual three-dimensional proteins. We choose the number of configurations of a random coil segment to be $Z_c = 10$, following the estimate of Flory for backbone configurations only. We also neglect the energetic contribution to the interface entropy, $Z_i = 10$. The persistence length of helices and of coil segments are given by $r_H = 1.5 \text{ \AA}$, $r_C = 3.8 \text{ \AA}$, again using Flory's estimates from the conformational isomeric state model. A back of the envelope calculation using the experimental packing density of completely folded proteins fixes the confinement radius for a 100-mer to be $D = 18 \text{ \AA}$.

If we choose a value of $0.6 \text{ kcal mol}^{-1}$ for the energy of breaking a hydrogen bond at room temperature, then the Zimm/Bragg parameters have the conventional values of $s = 0.74$ and $\sigma = 1.8 \times 10^{-3}$. The energy of the hydrogen bond in our units is $\epsilon_{hb} = -1$ and the creation of a helical monomer unit decreases the energy by $2\epsilon_{hb}$ ($1.2 \text{ kcal mol}^{-1}$) according to eq 2. The parameter s determines the position of the helix-coil transition and σ the steepness of the effective sigmoidal curve.

In Figure 1 we plot the order parameters of the helix-coil transition for various models. The temperature $T = 1$ corresponds to room temperature. In these plots we take ϵ_{hb} to be independent of temperature and set by the considerations of the helix-coil parameters measured on free chains. We must bear in mind that the hydrogen bond strength can be renormalized in a temperature-dependent fashion by collapse. Honig et al. have shown that the effective hydrogen bond strength involves both electrostatics and solvent exclusion forces.^{37b} The models shown are (a) the confined globule with liquid crystalline ordering characteristic of the helical bundle ($\alpha = 0$), (b) the confined globule without liquid crystalline ordering ($\alpha = 1$),

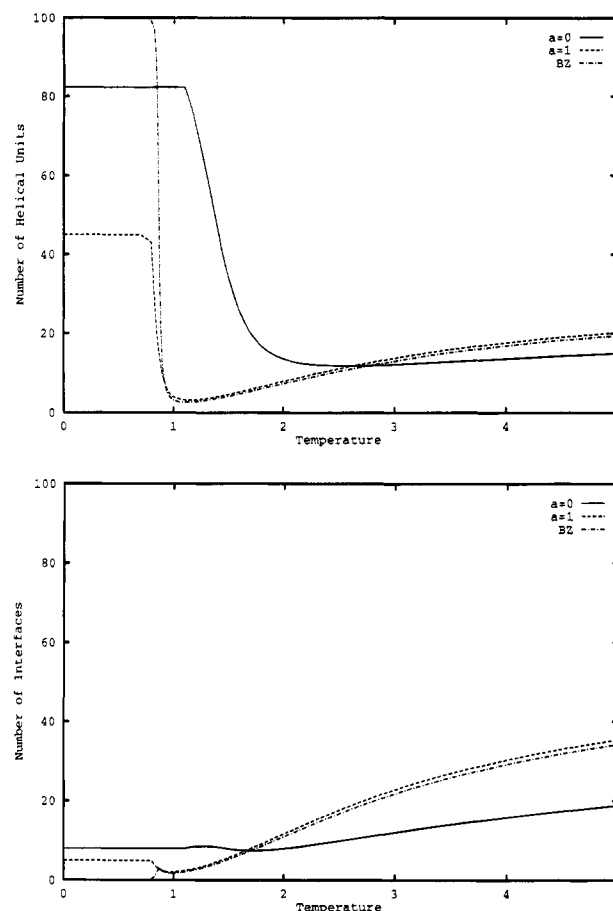


Figure 1. Temperature dependence of the two order parameters of the helix coil transition: (a, top) the number helical units N_h and (b, bottom) the number interfaces N_i . The solid line gives the results for the confined liquid crystalline case ($\alpha = 0$). The dashed line describes the confined disorder case ($\alpha = 1$), and the dot-dashed line the Bragg/Zimm model. In the confined cases, N_h and N_i are set equal to their values at the glass transition for $T < T_g$. T_g is obtained from Figure 3 from $S(T_g) = 0$. The temperature is defined such that $T = 1$ corresponds to room temperature 298 K.

(c) the free chain without confinement or steric effects. The effects of confinement are visible by comparing the fraction of helix and number of interfaces for the unconfined chain and for the chain without liquid crystalline ordering. While for the free chain, the fraction of helix rapidly and sharply grows as the temperature is lowered below the transition, confinement causes the transition to be considerably broader. The transition still occurs at roughly the same temperature but is much less steep. The liquid crystal phase shows the same frustration but the ordering temperature is higher because of the steric effects favoring helices. The effective value of the Zimm/Bragg cooperativity parameter σ which would fit this confined ordered phase is approximately $\sigma = 10^{-1}$ at the transition temperature $T_t = 1.35$. The cooperativity parameter σ is considerably larger than the free value ($\sigma = 10^{-3}$ at $T_t = 0.86$) and, indeed, is consistent to the diminished cooperativity inferred by free energy based neural network approaches taken by Bryngelson et al.³⁸ This diminished cooperativity is the result of the frustration of helix formation by the collapse. We find that at the glass transition the typical length of a helix is 22, which is very close to the values found for helical proteins of this size. We note that the theories of bulk-phase-induced rigidity essentially give a σ_{eff} close to zero.^{18c,d,35}

The two different confined phases differ in free energy, as illustrated in Figure 2. Here the free energies predicted are plotted. The liquid crystalline phase is higher in free energy at

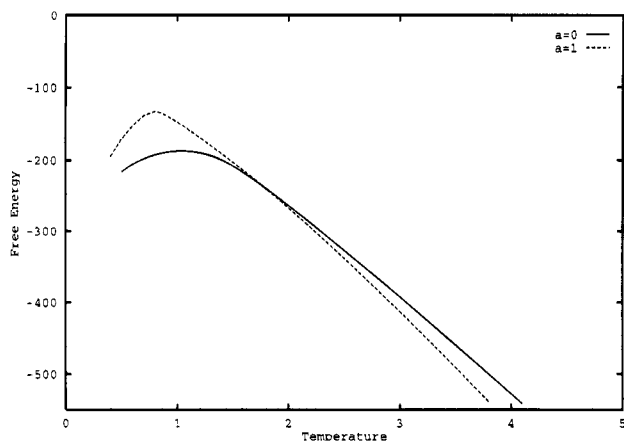


Figure 2. Temperature dependence of the free energy: The solid line is the free energy for the confined liquid crystalline case ($\alpha = 0$), and the dashed line the confined disordered case ($\alpha = 1$) using eqs 17–21. A first-order phase transition from the disordered to the ordered packing of helices in the confined phase occurs at $T_1 = 1.8$.

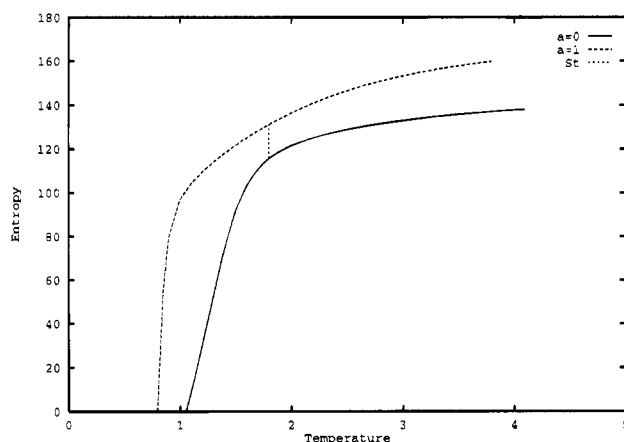


Figure 3. Temperature dependence of the entropy $S(N_h, N_i, E, D)$: The solid line is the entropy for the liquid crystalline case ($\alpha = 0$), and the dashed line the confined disordered case ($\alpha = 1$) using eq 17. The curves are stopped at the glass transition temperature $S(T_g) = 0$ since for $T < T_g$ no further conformational changes are possible. The dashed vertical line indicates the discontinuity in the entropy at the first-order phase transition exhibited in Figure 2. The entropy is in units of k_B .

high temperature. Thus without the effects of hydrogen bonding, steric effects alone do not cause long helices to form. At a temperature $T_1 = 1.8$, however, the free energy of the liquid crystalline helical bundle phase drops below that of the disordered phase. A first-order transition occurs at this point. Notice this transition is strongly driven by the emerging formation of helical structures in the ordered phase with decreasing temperature.

Differentiating the free energy curves in Figure 2 with respect to temperature gives the entropies of the two collapsed phases as shown in Figure 3. At the first-order transition there is a discontinuity in the entropy of $15k_B$. This reflects the latent heat of the ordering transition. Notice each of the free energy curves from eq 21 has a maximum with temperature. This maximum then signals the vanishing of the entropy. The position of the maximum depends on ΔE^2 from eq 3. Using our earlier estimates of the strength ($\Delta K \sim 0.5$) of tertiary contacts based on hydrophobic forces and assuming a packing fraction close to 1, we approximate ΔE^2 to be $\Delta E^2 = 0.5N$ which is numerically equal to 50 for a 100mer. The glass transition occurs at the temperature of this maximum and no significant further conformational changes can occur on cooling. The glass transition in the liquid crystalline ordered globule with helical

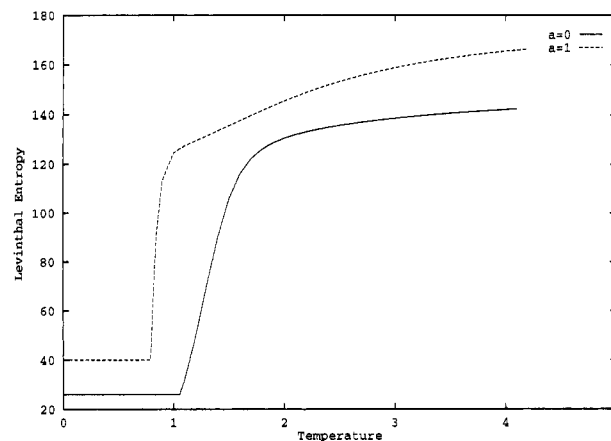


Figure 4. Levinthal entropy S_L , according to eq 22: the solid line is the Levinthal entropy for the confined liquid crystalline case ($\alpha = 0$) and the dashed line the confined disordered case ($\alpha = 1$). For $T < T_g$, S_L is set to its value at the glass transition temperatures (defined in Figure 3) since no further conformational changes are possible.

structure occurs at a temperature $T_g = 1.05$. This is somewhat above room temperature and suggests that the simple hydrophobic estimate of ruggedness is too large. In any event, the glass transition for the model is strongly affected by secondary structure formation. If no secondary structure formation were to occur, one obtains from the REM $T_g = 0.44$. This latter estimate is the temperature at which the configurational entropy assumed to be the high temperature form $S_C = k_B \ln Z_C/e$ just compensates the entropic contribution arising from the random energy approximation $\Delta E^2/2T_g^2$.

At the glass transition conformations become frozen in. Thus we see that the assumed value of ΔK gives a helical fraction at the glass transition of 80% as indicated in Figure 1. This is somewhat larger than that seen in most helical proteins again arguing for a somewhat smaller ΔK . The fraction of interfaces also freezes in. Combining these results we get the typical helix length discussed in the previous paragraphs.

We can estimate the configurational entropy the system would have if the number of helices and interfaces are constrained to have been preformed. This plot is useful because it allows one to separately consider the effects of varying ΔE^2 on the search problem and glass transition. This effective Levinthal entropy is plotted as a function of temperature in Figure 4. Notice that at the glass transition temperature this entropy is $26k_B$. We see that the local hydrogen bond formation can diminish the Levinthal problem.^{39a,b} The number of effective configurations to be searched through at the glass transition is 10^{11} for a highly helical 100-mer. Using the conventional 10^{-9} /s estimate for conformational transition time carrying over the Bryngelson–Wolynes kinetic theory would give a maximal search time of a minute. Thus strongly helical proteins of length 100 can find their global minimum or short laboratory time scales. Helical proteins much longer than this, however, would become considerably more sluggish in their search.

Conclusions

In summary we see that it is possible to develop a theory for helix–coil transitions and spin glass transitions within the collapsed globule phase of a random heteropolymer. This theory does justice to the particular features of one-dimensional ordering characteristic of helix–coil transitions. We see that above the glass transition there is a frustration between the confinement effects and secondary structure formation. At the same time, the steric effects do indeed encourage the formation of secondary structure. Within the approximations of our model,

the effective σ for collapsed phases agrees rather well with known data about protein globules and is quite different from the data for uncollapsed polyamino acids.

We see that the theory shows that secondary structure formation can indeed resolve a large fraction of the Levinthal paradox for highly helical proteins of length 100 but not for proteins much longer than this. Prediction schemes based on packing preformed helical elements in an exhaustive fashion thus may be quite viable for short helical proteins.³⁴ In addition the raising of the glass transition temperature due to helix formation should have significant effects on the dynamics and may explain the effective ruggedness of the energy surface which is seen in laboratory experiments. Further detailed analysis of the model will allow predictions of the probability of thermodynamic folding as a function of composition since one can evaluate the effects of changes of the fraction of hydrophobic amino acids and changes in the degree of helix forming tendency, due to side-chain branching.

There are, of course, many things which can be done further. First the model for the transition we have discussed here for heteropolymers has much in common with homopolymer models of helical structure formation in collapse phases due to Orland and co-workers.¹² Their theory takes into account many of the excluded volume effects we have discussed, although in a somewhat more elaborate way. Their estimate of the length of helices in a homopolymer are quite different from those we find for a frozen heteropolymer. The exact relationships of the theories needs further exploration.

An important concept which comes about from this analysis is the importance of partially frustrated liquid crystalline order in collapsed phases of helical polymers. This is doubtless also coupled with transitions between different shapes of the overall globule, i.e., spherical vs prolate or oblate ellipsoid. These effects can be taken into account. In addition the similarity of the combinatorial problems here to those encountered in β -sheet polymers suggest that a similar analysis could be done for β -sheet transitions in such systems. Indeed this will resemble calculations already undertaken by Caliri et al.,⁴⁰ although several other effects must still be included beyond those studied by them. The idea of global shape transition coupled to internal liquid crystal transition suggests that only a few parameters determine structural class at this level of description. Perhaps this explains the success of simple compositional prediction schemes of protein class. Such "soapy liquid crystalline drop" models deserve further study.

The study of phase behavior of the type we have discussed along with the concomitant partial ordering in the molten globule allows the possibility of examining partial random designs of proteins in which a few compositional biases are used to tune helicity and tune the glass transitions. The emergence of a first-order transition without complete three-dimensional ordering means that evidence for two-state-like behavior in random polypeptides with large helical tendency does not imply a *truly* folded protein. Sauer's recent experiments on random polypeptides of glutamic acid, asparagine, and leucine may be seeing this liquid crystal helix-coil ordering transition. If however the ruggedness of the landscape is sufficient to make the helical ordered state glassy at room temperature, for some sequences the detailed configurations may be unique in the frozen state as suggested by spin glass theory.^{16a} For strongly helical proteins kinetic considerations do not entirely rule this scenario. The considerations here are, of course, somewhat different from the partial random design of Hecht, but it is clear something in the same spirit could be done once surface forces are taken into account. It is clear from that study that periodicity of

hydrophobicity pattern will be very important in the understanding of the shape transitions of protein globules.

Another consequence of the partial ordering of the molten globule is that its low entropy allows very weak biases to tip the balance for complete folding. The analysis of models with minimal frustration and associated order parameters will be very interesting.

The possible complexity of order of the molten globule phase greatly enriches the questions that experimentalists can address in folding. As in the field of metallurgy, nothing would be as helpful to understanding these complex transitions as direct imaging. Even low-resolution scanning tunneling or atomic force microscopy might help resolve the nature of the phase diagram, vis-à-vis orientational order. Interestingly there are tantalizing hints of liquid crystalline ordering in images of denatured glucose oxidase.⁴¹

Finally, we hope that the analysis of simple models of this type will make more accessible the ideas of the spin glass theory of proteins and allow experimentalists to look at further aspects of folding kinetics which can be tuned through the changes of secondary structure.

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