A Kinetic Description of the Helix–Random-Coil Transition at Equilibrium†

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Abstract: Ising model conformational probabilities and a discrete, one-dimensional random walk were used to calculate mean conformational lifetimes for each unit in a helically nucleated polypeptide chain under the assumption of slow conformational nucleation. Calculations based on a model restricting the molecules to a single helical sequence predicts long helical unit lifetimes for long-chain molecules whereas short-chain molecules have relatively short conformational lifetimes under the same conditions. The single-helix restriction is shown to considerably overestimate helix lifetimes compared to the multiple-helical sequence Ising model under conditions of high helical content, but to give good estimates of the lifetimes in the neighborhood of the helix-coil transition. Application of the lifetimes to the calculation of nuclear magnetic resonance spectra suggests that the observed double maximum in short-chain polypeptides in the helix-coil transition region is most likely a result of independent contributions to the spectra from protons in completely random-coil molecules. As the occurrence of completely random-coil molecules is a function of the conformational thermodynamic parameters, a means of testing the applicability of the model to the nmr data is suggested.

The cooperative helix-random-coil transition in synthetic polypeptides has received much attention in terms of its statistical thermodynamic description.[1] The one-dimensional Ising model leads to a phenomenological equation which adequately describes all known thermodynamic data. The kinetics of this cooperative transition is not so well characterized. Experimental approach-to-equilibrium relaxation studies indicate that the relaxation time associated with growth or melting of units from the end of a helical sequence is a microsecond or less.[2-13] Several theoretical treatments have been given.[14-18]

Recently nuclear magnetic resonance data were interpreted to indicate that long conformational lifetimes (>0.1 sec) were observed even in short-chain polymers,[19-21] though this point of view has been challenged.[22-24] Shortly thereafter two theoretical models were presented to explain the apparent inconsistency between the two classes of observed lifetimes.[22,25] The model proposed by Ullman[16] assumes short conformational lifetimes for all residues and explains the experimental results in terms of molecular weight polydispersity. The model of Ferretti *et al.*[17] assumes slow random-coil initiation and an equilibrium conformational distribution which is considerably different from that given by the Ising model.

Inasmuch as the Ising model adequately desribes the thermodynamic data on the helix-random-coil transition a kinetic description of the transition has been derived[26] assuming Ising model conformational statistics. In this communication the model is discussed further and extended to include molecules containing more than one helical sequence.

Conformational Statistics

A matrix of statistical weights is constructed using the Zimm-Bragg σ and s parameters, indicating helix initiation and growth, respectively. The random-coil conformations are arbitrarily taken to have zero energy. Under a variety of attainable experimental conditions a single-helical sequence adequately describes the chain conformation.[1,27] Furthermore more elaborate description than nearest-neighbor interaction, even to the tenth neighbor, has little effect on the conformational statistics.[27] Under the restriction of a single-helical sequence with only nearest-neighbor interactions the configurational partition function for a chain of N units is given by

$$Q_s = [100]U_s^N[111]^T \tag{1}$$

where

$$U_s = \begin{bmatrix} 1 & \sigma s & 0 \\ 0 & s & 1 \\ 0 & 0 & 1 \end{bmatrix}$$

or by

$$Q_{sh} = [100]U_s^N[011]^T \tag{2}$$

if the all random-coil conformation is excluded from the population *(cf. seq.)*.

Considering only molecules containing one or more heli-

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cal residues the probability that the ith unit is random coil is

$$P_{c,i} = (1/Q_{sh})[100]U_s^{i-1}U_s'U_s^{N-i}[011]^T \tag{3}$$

where

$$U_s' = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{bmatrix}$$

If the all-random-coil conformation is included $P_{c,i}$ is given by

$$P_{c,i} = (1/Q_s)[100]U_s^{i-1}U_s'U_s^{N-i}[111]^T \tag{4}$$

The fraction of the molecules in the all-random-coil conformation is given by

$$P_{\text{all rc}} = 1/Q_s \tag{5}$$

Under some conditions there may be a significant number of molecules with two or more helical sequences. If the population statistics are restricted to no more than two helical sequences the partition function becomes

$$Q_d = [10000]U_d^N[11111]^T \tag{6}$$

where

$$U_d = \begin{bmatrix} 1 & \sigma s & 0 & 0 & 0 \\ 0 & s & 1 & 0 & 0 \\ 0 & 0 & 1 & \sigma s & 0 \\ 0 & 0 & 0 & s & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

or by

$$Q_{dh} = [10000]U_d^N[01111]^T \tag{7}$$

if the all-random-coil configuration is excluded.

Again considering molecules containing one or more helical residues the probability that the ith unit is random coil is

$$P_{c,i} = (1/Q_{dh})[10000]U_d^{i-1}U_d'U_d^{N-i}[01111]^T \tag{8}$$

where

$$U_d' = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

The fraction of the molecules in the all-random-coil conformation is now given by

$$P_{\text{all rc}} = 1/Q_d \tag{9}$$

The average fraction of residues in a helical conformation is given by

$$f_h = (1/N)\mathrm{d}\ln Q_{s \text{ or } d}/\mathrm{d}\ln s \tag{10}$$

where Q_s is used for restriction to a single-helical sequence and Q_d when up to two helical sequences are allowed. Equation 10 can be expressed in matrix formulation[28] for computational purposes.

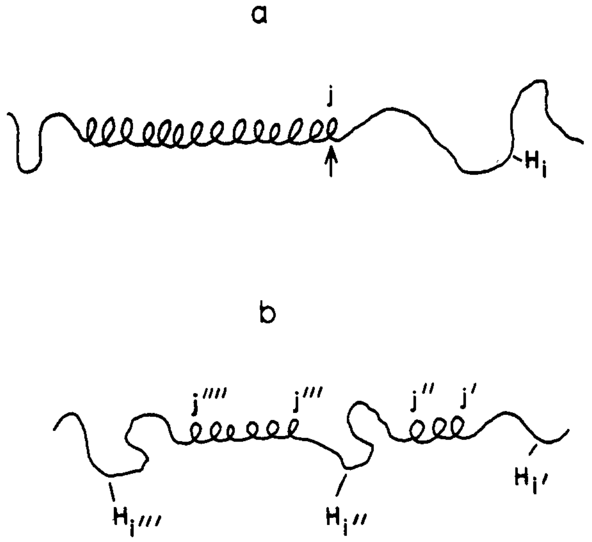

Figure 1. Molecule containing a single-helical sequence (a) or two-helical sequences (b).

Conformational Lifetimes

Single-Helical Sequence. If a molecule initially has the conformation shown in Figure 1a, proton H_i will belong to the random-coil population until the boundary between helical and random-coil residues moves from residue j to residue i. If we then consider position i to be an absorbing barrier and let the boundary, initially at j, take a random walk, the probability that it will reach i in exactly n steps is given[29] by

$$W(z,n) = (z/n)n!(1 + s)^{-n}s^{0.5(n+z)}/[\tfrac{1}{2}(n - z)]![\tfrac{1}{2}(n + z)]! \tag{11}$$

where $z = |j - i|$. If the time per step is Δt, the average lifetime for the ith proton as a random coil under these initial conditions is

$$<t_z> = \sum_n^{\infty} n\Delta t W(z,n) \tag{12}$$

where $n = z, z + 2, \ldots$. The expression given in eq 11 assumes the lattice is semiinfinite, *i.e.*, there is no bound on the number of steps which may be taken to the left. Inasmuch as the helix-coil boundary cannot move past the end of the chain, a reflecting barrier should be placed at the end of the chain. Although in principle this can be handled mathematically[30] it considerably increases the computational difficulties. If, however, eq 11 is applied under appropriate conditions, little error should result in ignoring the reflecting barrier at the end of the chain. The probability that the helix-coil boundary will move a step toward i is $s/(1 + s)$. So long as $s > 1$ the walk will be highly biased in favor of moving toward i. Only if $j - i$ is large and j is near the end of the chain will significant error result so long as $s > 1$. The bias also permits the left helix-coil junction to be ignored as it is biased to move away from i. Analysis of the distribution of first passage times *(cf. seq.)* will further justify the qualitative remarks made here.

The helix-coil boundary may occur at any place along the chain. If P_j is the probability that the boundary is initially at position j, the mean lifetime for proton i as a random coil is

$$\tau_{c,i} = \Sigma_{j \neq i} P_j \langle t_{j-i} \rangle \tag{13}$$

Equation 13 may be expressed in matrix form and is given by eq 14. The lifetime for a proton in residue i as a helix is

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(29) W. Fuller, "An Introduction to Probability Theory," Vol. 1, 2nd ed, John Wiley and Son, New York, N. Y., 1957, p 323.

(30) M. Kac, *Amer. Math. Monthly*, **54**, 369 (1947).

$$\tau_{c,i} = \frac{[100]\begin{bmatrix}1 & \sigma s & 0\\0 & s & 1\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}1 & \sigma s & 0\\0 & s & \langle t_{i-1}\rangle\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}1 & \sigma s & 0\\0 & s & \langle t_{i-2}\rangle\\0 & 0 & 1\end{bmatrix}\cdots\begin{bmatrix}1 & \sigma s & 0\\0 & s & \langle t_{2}\rangle\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}1 & 0 & 0\\0 & 0 & \langle t_{1}\rangle\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}1 & \sigma s\langle t_1\rangle & 0\\0 & s & 1\\0 & 0 & 1\end{bmatrix}\cdots\begin{bmatrix}1 & \sigma s\langle t_{N-i}\rangle & 0\\0 & s & 1\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}0\\1\\1\end{bmatrix}}{[100]\begin{bmatrix}1 & \sigma s & 0\\0 & s & 1\\0 & 0 & 1\end{bmatrix}^{i-1}\begin{bmatrix}1 & 0 & 0\\0 & 0 & 1\\0 & 0 & 1\end{bmatrix}\begin{bmatrix}1 & \sigma s & 0\\0 & s & 1\\0 & 0 & 1\end{bmatrix}^{N-i}\begin{bmatrix}0\\1\\1\end{bmatrix}} \qquad (14)$$

now determined, as

$$\tau_{\mathrm{h},i} = (P_{\mathrm{h},i}/P_{\mathrm{c},i})\tau_{\mathrm{c},i} \qquad (15)$$

where $P_{c,i}$ is given by eq 3 and $P_{\mathrm{h},i}$ by $1 - P_{c,i}$.

Implicit in eq 14 and 15 is slow helix and random-coil nucleation. Slow nucleation is also inherent in the Ferretti *et al.* model, but in an entirely different manner. In our model effects of nucleation on the conformational lifetime are neglected. In the Ferretti *et al.*,[25] model nucleation plays a central role.

No More Than Two Helical Sequences. If chains are allowed to have one or two helical sequences (Figure 1a,b), the computational problem even in the absence of reflecting barriers is greatly increased. First we assume that during the lifetime of a proton in a given conformation the number of helix-coil boundaries does not change, as in the previous section. The molecules may be classified according to the number of helical sequences they contain. Those having a single helical sequence may be handled in a manner similar to the previous section. When considering the ith proton as a random coil and occuring in molecules having two helical sequences a distinction must be made as to whether or not the proton is located between the two helical sequences. If it is not between, *e.g.*, at H_i' or H_i''', one need only calculate the mean time for j' or j'''' to reach H_i' or H_i''', respectively. If, however, the proton is at H_i'', two boundaries, j'' and j''', can independently move toward H_i''. Let $W''(z'', n)$ be the probability of j'' reaching i'' in exactly n steps, and $W'''(z''', n)$ be the probability of j''' reaching i'' in exactly n steps. Then

$$W''(z'',n)[1 - \sum_{n^*}^{n} W(z''',n^*)]$$

where $n^* = z''', z''' + 2, \ldots$, is the probability of j'' reaching i'' in exactly n steps, and before j''' gets there

$$W'''(z''',n)[1 - \sum_{n^*}^{n} W''(z'',n^*)]$$

where $n^* = z'', z'' + 2, \ldots$, is the probability of j''' reaching i'' in exactly n steps, and before j'' gets there, and

$$W''(z'', n)\,W'''(z''', n)$$

is the probability of j'' and j''' simultaneously reaching i'' in exactly n steps. The average lifetime of i'' as a random coil when junctions are initially $z''(= |j'' - i''|)$ and $z'''(= |j''' - i''|)$ units away is as shown in

$$\begin{aligned}\langle t_{z''',z''}\rangle = \Big\{&\sum_{n_\mathrm{a}}^{\infty}[W''(z'',n_\mathrm{a})(1 - \sum_{n_\mathrm{b}^*}^{n_\mathrm{a}} W'''(z''',n_\mathrm{a}^*))]n_\mathrm{a}\\ &+ \sum_{n_\mathrm{b}}^{\infty}[W'''(z''',n_\mathrm{b})(1 - \sum_{n_\mathrm{b}^*}^{n_\mathrm{b}} W''(z'',n_\mathrm{b}^*))]n_\mathrm{b}\\ &+ \sum_{n_\mathrm{c}}^{\infty} W''(z'',n_\mathrm{c})W'''(z''',n_\mathrm{c})n_\mathrm{c}\Big\}\,\Delta t\end{aligned} \qquad (16)$$

where $n_\mathrm{a} = z'', z'' + 2, \ldots$; $n_\mathrm{a}^* = z''', z''' + 2, \ldots$; $n_\mathrm{b} = z''', z''' + 2, \ldots$; $n_\mathrm{b}^* = z'', z'' + 2, \ldots$; and $n_\mathrm{c} = z'', z'' + 2, \ldots$ if $z'' > z'''$, or $z''', z''' + 2, \ldots$ if $z''' > z''$. The lifetime of a random-coil unit existing between two helical units is a one-dimensional analog of a chemical species which may be quenched by two competitive, independent diffusion processes.[31] The lifetime of the ith proton as a random coil becomes a sum of four terms

$$\tau_{c,i} = (A + B + C + D)/Q_{\mathrm{dc}i} \qquad (17)$$

where eq 18 holds true. The A term is given by eq 19. It

$$Q_{\mathrm{dc}i} = [10000]U_\mathrm{d}^{i-1}\begin{bmatrix}1&0&0&0&0\\0&0&1&0&0\\0&0&1&0&0\\0&0&0&0&1\\0&0&0&0&1\end{bmatrix}U_\mathrm{d}^{N-i}[01111]^T \qquad (18)$$

$$\begin{aligned}A = [10000]U_\mathrm{d}&\begin{bmatrix}1&\sigma s&0&0&0\\0&s&\langle t_{i-1}\rangle&0&0\\0&0&1&\sigma s&0\\0&0&0&s&1\\0&0&0&0&1\end{bmatrix}\cdots\\ &\begin{bmatrix}1&0&0&0&0\\0&0&\langle t_1\rangle&0&0\\0&0&1&0&0\\0&0&0&0&1\\0&0&0&0&1\end{bmatrix}\begin{bmatrix}1&\sigma s\langle t_1\rangle&0&0&0\\0&s&1&0&0\\0&0&1&\sigma s&0\\0&0&0&s&1\\0&0&0&0&1\end{bmatrix}\cdots\\ &\begin{bmatrix}1&\sigma s\langle t_{N-i}\rangle&0&0&0\\0&s&1&0&0\\0&0&1&\sigma s&0\\0&0&0&s&1\\0&0&0&0&1\end{bmatrix}\begin{bmatrix}0\\1\\1\\0\\0\end{bmatrix}\end{aligned} \qquad (19)$$

accounts for all chain conformations containing a single-helical sequence. The term B is given by eq 20 and term C

$$\begin{aligned}B = [10000]U_\mathrm{d}^{i-1}&\begin{bmatrix}1&0&0&0&0\\0&0&0&0&0\\0&0&0&0&0\\0&0&0&0&0\\0&0&0&0&0\end{bmatrix}\begin{bmatrix}1&\sigma s\langle t_1\rangle&0&0&0\\0&s&1&0&0\\0&0&1&\sigma s&0\\0&0&0&s&1\\0&0&0&0&1\end{bmatrix}\cdots\\ &\begin{bmatrix}1&\sigma s\langle t_{N-i}\rangle&0&0&0\\0&s&1&0&0\\0&0&1&\sigma s&0\\0&0&0&s&1\\0&0&0&0&1\end{bmatrix}\begin{bmatrix}0\\0\\0\\1\\1\end{bmatrix}\end{aligned} \qquad (20)$$

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(31) F. C. Collins and G. E. Kimball, *J. Colloid Sci.*, 53, 425 (1949).

by eq 21. Terms B and C account for the ith proton in a random-coil sequence at the right and left end of the molecule, respectively, in molecules containing two helical sequences. The term D accounts for the ith proton trapped between two helically sequences. It cannot be put into a simple matrix formulation. The statistical weight matrix for the ith unit must contain information describing the

$$C = [10000]U_d \begin{bmatrix} 1 & \sigma s & 0 & 0 & 0 \\ 0 & s & 1 & 0 & 0 \\ 0 & 0 & 1 & \sigma s & 0 \\ 0 & 0 & 0 & s & <t_{i-1}> \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \cdots \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & <t_1> \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} U_d^{N-i} \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{bmatrix} \quad (21)$$

position of junction j'' as well as j'''. It is analogous to the problem of trying to account for the random-coil loops in a double-stranded DNA. In a very laborious manner term D may be computed according to eq 22.

$$D = \sum_{j'''=1}^{i''-1} [10000]U_d^{j'''-1} \begin{bmatrix} 0 & \sigma s & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} U_d^{i''-j'''-1} \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} 1 & \sigma s & 0 & 0 & 0 \\ 0 & s & 1 & 0 & 0 \\ 0 & 0 & 1 & \sigma s <t_{z''',1}> & 0 \\ 0 & 0 & 0 & s & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & \sigma s & 0 & 0 & 0 \\ 0 & s & 1 & 0 & 0 \\ 0 & 0 & 1 & \sigma s <t_{z''',2}> & 0 \\ 0 & 0 & 0 & s & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \cdots \begin{bmatrix} 1 & \sigma s & 0 & 0 & 0 \\ 0 & s & 1 & 0 & 0 \\ 0 & 0 & 1 & \sigma s <t_{z''',N-i''}> & 0 \\ 0 & 0 & 0 & s & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{bmatrix} \quad (22)$$

Calculations; Molecules Containing a Single-Helical Sequence

Conformational Lifetimes. In order to calculate mean lifetimes the conformational thermodynamic parameters σ and s, and the random-walk-step interval Δt must be specified. In mixed organic solvents, the type of solvent system in which the apparent long relaxation time is generally observed, the cooperativity parameter is of the order of 10^{-4}.[32-35] When σ is this small, an s value only slightly greater than unity will ensure that the preponderance of chains contain one or less helical sequences so long as the chain length is less than 200-300 units. To simulate lifetimes relevant to polypeptides in mixed organic solvents eq 3, 14 and 15 were employed, using $\sigma = 1.0 \times 10^{-4}$ and varying s. The random-walk interval appropriate to mixed organic solvents is less certain. Although dielectric relaxation measurements on poly(benzyl glutamate) indicated[4] a relaxation time of about 10^{-8} sec, this value is open to question.[13] At present there appears to be no reliable value for the relaxation time associated with the melting or growth from the end of a helical sequence in mixed organic solvents. In aqueous solvents a value of about 10^{-6} sec has been observed,[11,12] though it may be as small as 10^{-8} sec.[2,5,8,9,15] In addition the approach-to-equilibrium relaxation time must be converted to a lifetime associated with changing the length of a helical sequence by one unit, which can only be done by resorting to a model such as triplet analysis.[7,17] For the sake of calculations we have taken Δt to be 1×10^{-6} sec. The resulting conformational lifetimes may be converted to any other step interval by scaling.

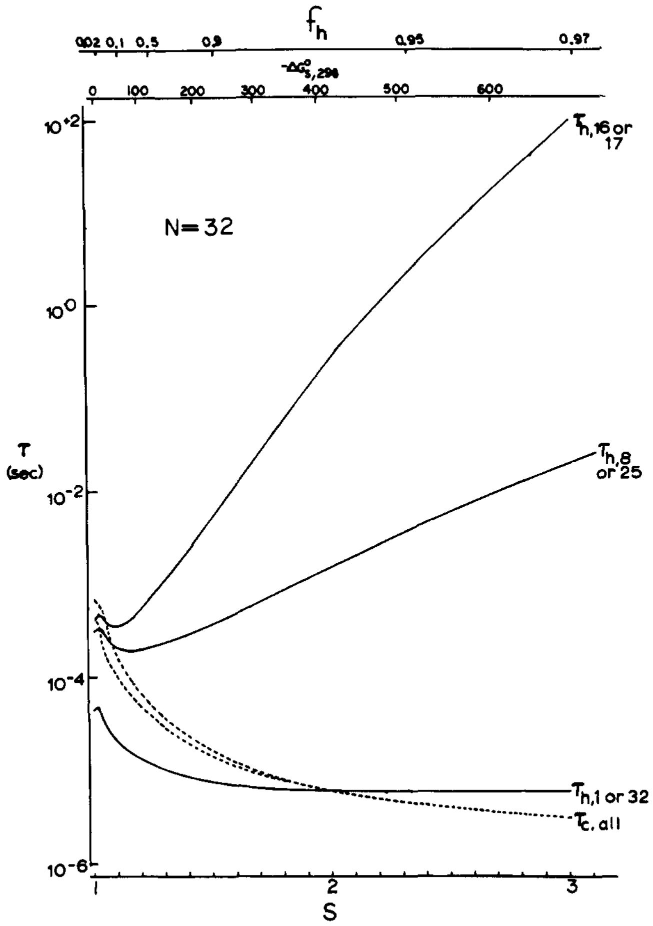

Figure 2. Lifetime as a function of s for several units in a chain of 32 units. $\tau_{h,i}$ is given by a solid line. $\tau_{c,i}$ is given by the dashed lines, the upper curve for $\tau_{c,1}$ and the lower for $\tau_{c,16}$. The chain is restricted to a single-helical sequence, Δt was 10^{-6} sec. and σ was 10^{-4}. The $\tau_{c,i}$ are mean lifetimes for protons in molecules containing helical units.

In Figure 2 the lifetime of several residues is shown as a function of s for a chain of 32 units. When the thermodynamic parameters strongly favor the helical conformation (large s) the random-coil lifetimes become short-lived and will eventually approach the step interval Δt. The random-coil lifetimes are only slightly position dependent in marked contrast to the helix lifetimes. In the center of the molecule the helix lifetimes have nearly an exponential dependence on s.

Extending the chain to 256 units results in lifetimes as shown in Figure 3. The lifetimes in the random-coil conformation are again position independent and comparable to those in the shorter chain. Interior units in the

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(32) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, 31, 526 (1959).

(33) J. Applequist, *J. Chem. Phys.*, 38, 934 (1963).

(34) T. Ackermann and E. Neumann, *Biopolymers*, 5, 649 (1967).

(35) F. E. Karasz, J. M. O'Reilly, and H. E. Bair, *Biopolymers*, 3, 241 (1965).

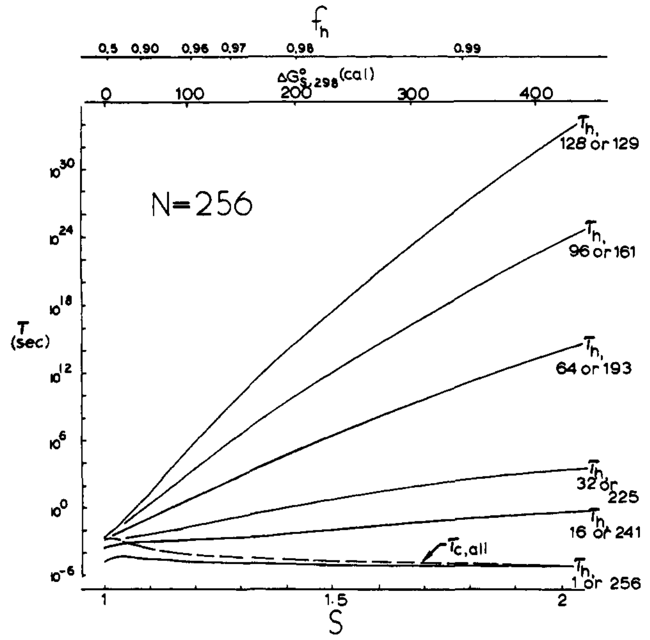

Figure 3. Lifetimes as a function of s in a chain of 256 units. Parameters same as in Figure 2.

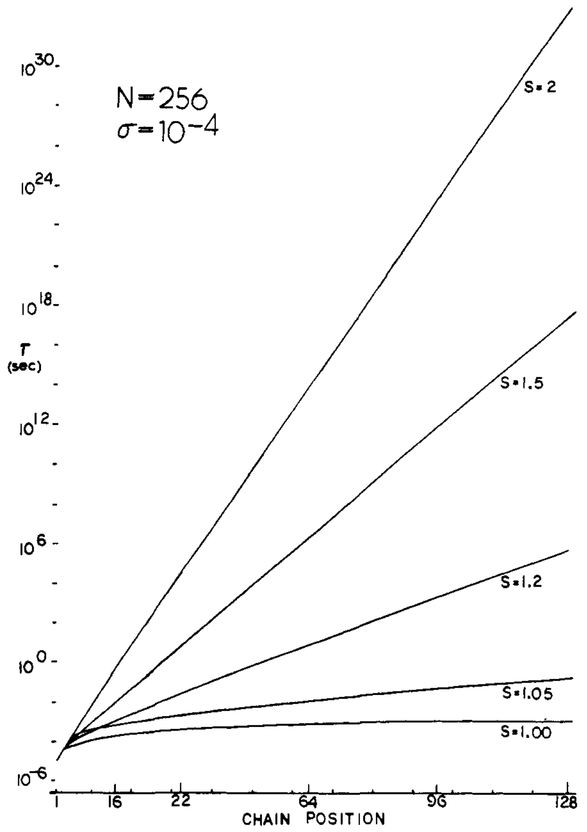

Figure 4. Helix lifetimes as a function of chain position.

chain have much longer lifetimes as helical units than interior units in short chains when s is large. They can indeed approach rather long lifetimes. The lifetime of helical units as a function of chain position is shown in Figure 4. Near the conformational transition midpoint the lifetime becomes almost position independent and is roughly 1000 times the step time, in contrast to the exponential position dependence for large s.

Allowing the conformational transition to be less cooperative, say by taking σ equal to 10^{-3}, appropriate for poly(glutamic acid) in aqueous solution,[36] gives little change in lifetimes unless one is very near the midpoint of the helix-coil transition ($s \approx 1$). Even then the change is never more than a factor of ten.

Under conditions of high helical content s is significantly greater than one and the step time for the helix moving one unit away from a proton in a random-coil position is s^{-1} times that for moving toward the proton. Alteration of eq 12 to account for this difference results in minor changes in the calculated lifetimes.

Inasmuch as the model presented in this section is that of a one-dimensional, semiinfinite random walk rather than a random walk with a reflecting barrier, it is of interest to investigate the error introduced by this approximation, particularly for short chains. To this end eq 14 was altered so that the distribution of first passage times would be investigated. By substituting $W(z,n)$ values from eq 11 term by term into eq 14, the distribution of first passage probabilities could be determined at each chain position. As the random-coil lifetimes were nearly chain position independent it was not surprising to find the first passage time distribution to be nearly independent of chain position. The cumulative first passage time probability as a function of the number of steps is shown in Figure 5. At high helical content the mean number of steps taken to terminate a unit as a random coil is exceedingly small, consistent with a random-coil lifetime of the order of Δt. As the transition midpoint is approached, the distribution becomes much broader. Thus one can see that the absence of a reflective barrier will have little effect on $\tau_{c,i}$ at high helical content, but may lead to an erroneously long relaxation time for small chains when $s \approx 1$. The first passage distribution for protons in helical conformations cannot easily be determined by this approach when $s > 1$.

Simulated Nuclear Magnetic Resonance Spectra. If the two conformations give rise to a chemical-shift difference, the spectrum for a given proton will depend on the population probability as well as on the lifetime in a given conformation. We assume the two-site model adequately describes a particular type of proton, *e.g.*, the α-CH proton, and that the chemical shifts are independent of chain position. The frequency dependence of the intensity of the ith proton will be proportional to the real part of the right-hand side of

$$I_i(\omega)\alpha Re\left\{\frac{\tau_{h,i}+\tau_{c,i}+\tau_{h,i}\tau_{c,i}(\alpha_c P_{c,i}+\alpha_h P_{h,i})}{(1+\alpha_c\tau_{c,i})(1+\alpha_h\tau_{h,i})-1}\right\} \quad (23)$$

where $\alpha_c = T_{2c}{}^{-1} - i(\omega_c - \omega)$ and $\alpha_h = T_{2h}{}^{-1} - i(\omega_h - \omega)$. Here T_{2c} and T_{2h} are the relaxation times in the absence of conformational exchange and ω_c and ω_h are the resonance frequencies for a proton in random-coil or helical environments, respectively. In the case of a single-helical sequence $P_{c,i}$, $\tau_{c,i}$, and $\tau_{h,i}$ are given by eq 3, 14, and 15, respectively. The spectrum for each of the N protons is computed and the results summed.

Calculated spectra for protons in molecules containing one or more helical units are shown in Figures 6-8 for chain lengths 32, 128, and 256, respectively, together with experimental spectra for comparable chain lengths. The chemical-shift difference was taken[20] to be 60 sec^{-1}, and T_{2h} and T_{2c} were assumed to be 1 sec. The σ parameter

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(36) R. L. Snipp, W. G. Miller, and R. E. Nylund, *J. Amer. Chem. Soc.*, 87, 3547 (1965).

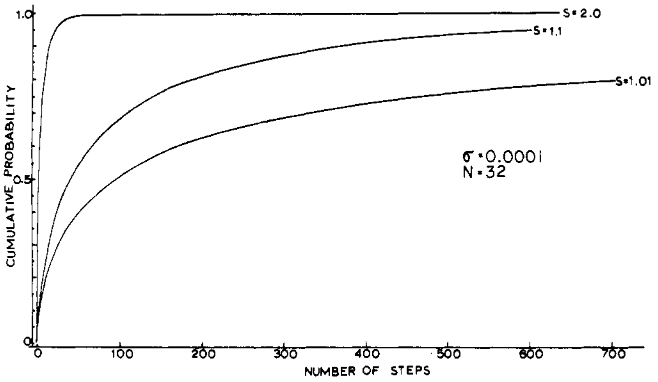

Figure 5. Cumulative random-coil first passage time probability as a function of the total number of steps taken.

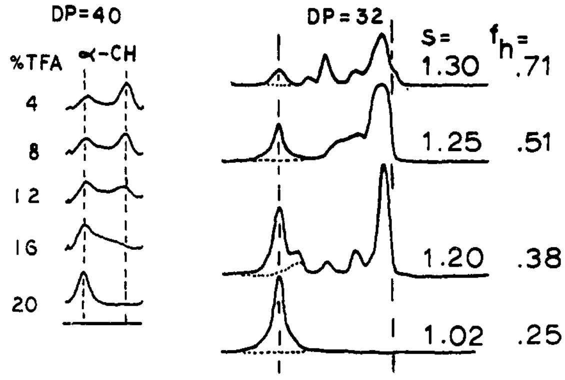

Figure 6. Calculated (right side) nmr spectrum for a chain of 32 units with $\sigma = 10^{-4}$, $T_{2h} = T_{2c} = 1$ sec (0.1 sec for protons in the all-random-coil molecules) and a chemical-shift difference of 60 $\sec^{-1}$. Dashed vertical lines indicate position of random-coil and helix resonances. Left-side spectra are the observed α-CH spectra of poly(benzyl glutamate) (DP = 40).[20] The dashed line in the random-coil region of the computed spectra is the contribution from molecules containing helical units. Outside the dashed-line region only helix-containing molecules contribute to the intensity.

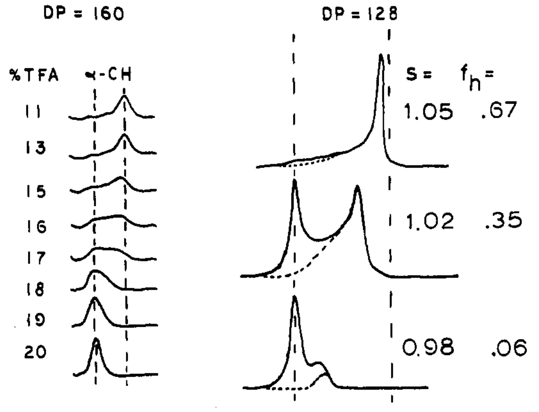

Figure 7. Similar to Figure 6 except for chain length.

was taken as 10^{-4}. The sharp and irregular features of the spectra, particularly in Figure 6, are a result of the rather long T_2.

When $s > 1$ and N is large the number of protons in the all-random-coil conformation, as given by eq 5, is insignificant. With shorter chains the all random-coil conformation may contribute significantly to the population. Little is known about the actual nucleation rates of the helix from the all-random-coil conformation. If we assume it is slow compared to the nmr time scale, the contribution to the spectrum from the all-random-coil conformation may be added on. Thus the total intensity would be

$$I(\omega) = P_{\text{all rc}} I_{\text{all rc}}(\omega) + (1 - P_{\text{all rc}}) \sum_{i=1}^{N} I_i(\omega) \tag{24}$$

where $I_i(\omega)$ is the spectrum for a proton in molecules containing a single-helical sequence. Inclusion of the all random-coil conformation results in increasing the intensity in the random-coil resonance region, as shown in Figures 6–8.

Effect of Restriction to a Single-Helical Sequence

There is a significant number of molecules containing more than one helical sequence when N becomes large, or the transition less cooperative. It was thus of interest to investigate through use of eq 17–22 this effect on the lifetimes. Initially choosing $N = 32$ in order to conserve computational time the contribution to the lifetimes from molecules containing a single-helical sequence (eq 19) dominated eq 18 and gave lifetimes in good agreement with eq 14 when $s \approx 1$. As s was allowed to take on increasingly larger values, where the conformational statistics more strongly disfavor multiple-helical sequences, the contribution to $\tau_{c,i}$ from eq 19 became of increasing variance with that calculated from eq 14. In addition, the random-coil probabilities calculated from eq 3 and 8 differed markedly. This in turn causes the ratio of helix-to-coil lifetimes to differ, as shown in Figure 9. There is little difference in these ratios between a two-helical restriction, and an arbitrary number of allowable sequences as given by the general Ising model.

Inasmuch as $\tau_{c,i}$ approaches Δt for large s, and $P_{h,i} \approx 1$, the helix lifetimes become

$$\tau_{h,i} \approx \Delta t / P_{c,i} \tag{25}$$

Thus the long helix lifetimes shown in Figures 2–4 result from random-coil probabilities in the single-helix model which are vastly different from the Ising model. The origin of this difference is easy to see. As s becomes large, the configurational partition function approaches σs^N in the Ising model or in any of the restricted sequence models presented herein. Thus in the single-helix model

$$\begin{aligned} P_{c,i} &\approx \frac{\sigma s^{i-1} 1^{N-i+1}}{\sigma s^N} \\ &\approx 1/s^{N-i+1} \quad (s \longrightarrow \infty) \end{aligned} \tag{26}$$

while by any multiple-helical sequence model

$$\begin{aligned} P_{c,i} &\approx \frac{\sigma s^{i-1} \cdot 1 \cdot \sigma s^{N-i}}{\sigma s^N} \\ &\approx \sigma / s \quad (s \longrightarrow \infty) \end{aligned} \tag{27}$$

So long as $s \approx 1$ the lifetime ratios are in reasonable agreement, as can be seen in Figure 9.

Discussion

Conformational Lifetimes. Even though the preponderance of molecules contain only a single-helical sequence when s is large in a nearest-neighbor Ising model, restricting the conformational lifetime calculations to a model allowing no more than one-helical sequence leads to helix lifetimes considerably too large. The overestimation is a direct result of the large underestimation of the random-coil probabilities for units in the interior of the chain. If the Ising model gives a good conformational de-

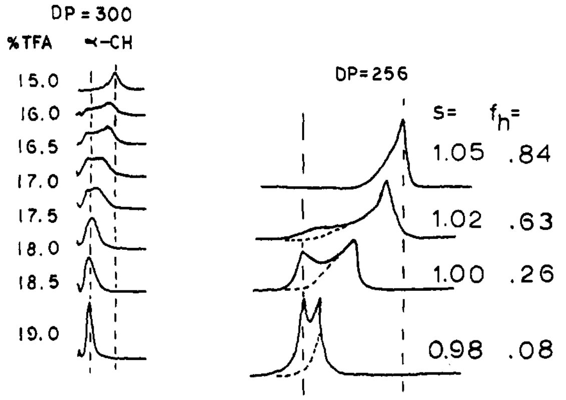

Figure 8. Similar to Figure 6 except for chain length.

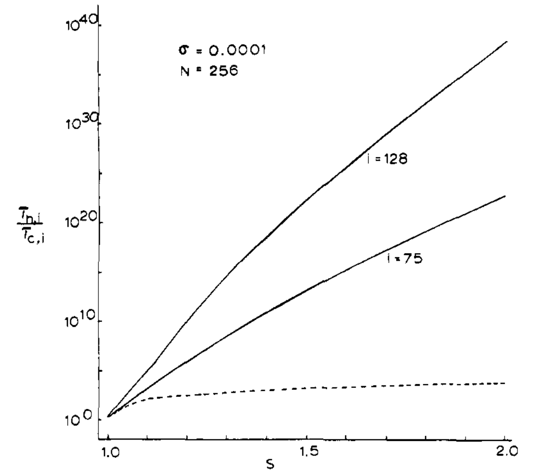

Figure 9. Ratio of helix-to-coil lifetimes as a function of s. Solid lines calculated from single-helical sequence model, chain positions as indicated. Dashed line calculated allowing multiple-helical sequences, and is independent of chain position except near the end of the chain.

scription of the helix-coil transition and if nucleation is slow compared to propagation, then eq 17–22 will give much more realistic Ising model lifetimes when s is large inasmuch as the random-coil probabilities calculated from eq 8 are nearly identical with those using the Zimm–Bragg Ising model statistical weight matrix. When s is approximately unity, the single-helix model will give lifetimes in sensible agreement with the Ising model. The range of s values in which the single-helix model is applicable will depend significantly on both N and σ and is best bounded by comparing lifetime ratios as in Figure 9. Even though eq 17–22 are the equations of choice, it is impractical to use them for anything other than short chains. This is a consequence of term D (eq 22). This term is important, particularly when s is large, and do to the laborious summation requires an uneconomical amount of computing time.

In the preceding paragraph the helix lifetime calculations were compared with what would be expected for a complete nearest neighbor Ising model. There is, however, scant information relating to the applicability of the Ising model other than it gives an adequate phenomenological description around the midpoint of the transition. It is unknown whether or not the Ising model gives the correct sequence distribution, or if it is applicable under conditions far removed from the transition midpoint.

The assumption of slow nucleation is also of unknown validity. If nucleation is sufficiently fast that it must be

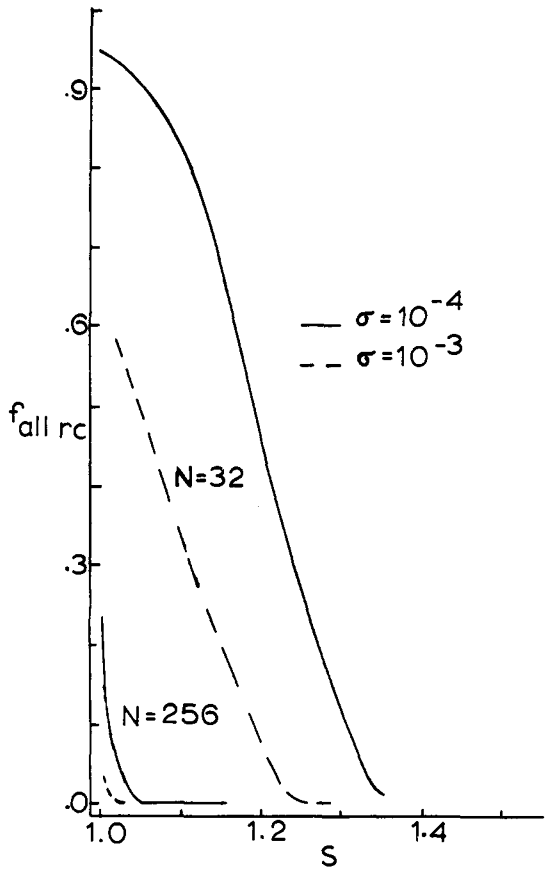

Figure 10. Fraction of molecules having the all-random-coil configuration as a function of s, σ, and N.

taken into account, its effect would be to lower both helix and random-coil lifetimes. If the Ising model is a good description of the conformational population, under conditions of high helical content the helix lifetimes for interior units will have as a maximum value

$$\lim(s \rightarrow \infty)\ \tau_{\mathrm{h,interior}} = (s/\sigma)\Delta t \qquad (28)$$

Similarly under conditions where the random coil is highly favored and the helical unit lifetime approaches Δt the lifetime of a random-coil unit must approach

$$\lim(s \rightarrow 0)\ \tau_{\mathrm{c},i} = \Delta t/\sigma s \qquad (29)$$

Nuclear Magnetic Resonance Spectra. In Figures 6–8 computed and experimental nmr spectra were presented for various chain lengths. If the all-random-coil contribution is included, the computed and experimental spectra show reasonable similarity. According to the model presented here the interpretation of the experimentally observed double peak for short-chain lengths is not related to any long helical lifetimes but to independent contributions from the completely random-coil and partially helical molecules. Although for short chains no long conformational lifetimes are involved, the computed spectra would still differ from those of Ullman who assumed all lifetimes were very short. This is partially but not totally a result of including the all-random-coil molecules as a separate contribution.

In the conformational transition region a change in Δt will not alter significantly the short-chain-length spectra unless Δt becomes very long. With longer chains only the spectrum of those protons with conformational lifetimes comparable to the reciprocal of the chemical-shift difference will be effected by a change in Δt.

In the observed nmr doublet, at least in poly(benzyl glutamate),[20] the random-coil peak does not shift significantly as one goes through the helix-random-coil transition, but merely changes intensity. If the doublet is not a result of molecular weight heterogenity, then the unshifting random-coil peak must mean that at least in short chains the partially helical and completely helical molecules contribute separately to the nmr spectrum. This is the best experimental evidence to date which indicates helix and random-coil nucleation is indeed slow.

It is of interest to note that nmr spectra of polypeptides in aqueous solution generally do not show the double peak in the helix-coil transition region. According to our model the double peak is likely to occur only when a significant amount of all random-coil molecules exist. For a given s value, the fraction of all random-coil molecules is a strong function N and σ, as shown in Figure 10. These values are roughly independent of whether a single-helix or multiple-helix partition function is used. It is obvious from Figure 10 that one would not expect to see the double maximum if the chain length is too large, or the cooperativity too small. Thus poly(glutamic acid), characterized[36] by a σ value of the order of 10^{-3}, should have a less pronounced double maximum at any chain length than poly(benzyl glutamate) in mixed organic solvents. In fact one way to decide if the model and assumptions presented here give a good description of the experimental systems would be to correlate the appearance of the double maximum with the cooperativity parameter in a series of polymers and solvents.

Isotopic Exchange. The isotopic exchange of the amide proton in the polypeptide backbone with solvent has been investigated, with particular interest in the effect of conformation on the exchange rate.[37-39] Previously we have shown by utilizing Ising model statistics and assuming that the rate-limiting step was not the conformational lifetimes, experimental and calculated exchange rates were in reasonable agreement for both homopolymers and copolymers. Inasmuch as the lifetime for isotopic exchange in small model compounds is of the order of seconds, the assumption of conformational lifetimes short in comparison to exchange now seems fully justified.

Recently, however, a much more detailed study of isotopic exchange in poly(glutamic acid) has appeared[40] which yielded information on the exchange rate as a function of chain position and chain length. Even though the largest chain length studied was less than 100 units their interpretation of the exchange data yielded a "fluctuation frequency" of helical structure varying by 10^{13} sec^{-1}. Considering the Ising model parameters for this system there is no way in which the usual Ising model statistics could give rise to this large variation in helical lifetimes.

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Separation of Atactic and Syndiotactic Methyl Methacrylate Polymers by Thin-Layer Chromatography

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ABSTRACT: Separation of atactic and syndiotactic methyl methacrylate polymers (PMMA) has been investigated with the thin-layer chromatographic technique (tlc). Mixtures of ethyl acetate (ETOAc) and isopropyl acetate (i-PrOAc) and of acetonitrile (MeCN) and methanol (MeOH) were found to be effective as developer. Values of R_f (normalized rate of flow of the polymeric species) obtained with the mixture (ETOAc + i-PrOAc) increased with decrease in both syndiotactic content (T_s) and molecular weight (M), whereas those with the mixture (MeCN + MeOH) increased with increase in T_s but with decrease in M. No solvent pair which allows the separation solely by the difference in T_s has been found. These development characteristics were discussed on the basis of separation mechanisms proposed previously. The R_f values determined with each mixture were expressed as a function of T_s and M. With the aid of the functions thus established, a procedure to estimate T_s of a given sample from values of R_f obtained by the use of both mixtures was described, and proved to be applicable. A PMMA sample, which has been artificially prepared so that it had appreciable heterogeneity in T_s as well as M, was chromatographed with the mixture (MeCN + MeOH), and into two components. Despite such success in the separation it is pointed out that the experimental procedure was too complicated to be applied generally to practical problems, this being indicative of the limitation of applicability of tlc to polymer fractionation.

When the chemical constitution of a substance is to be determined, one should first investigate whether or not it is of a single component. In the field of polymer chemistry, such investigations often encounter various difficulties for lack of knowledge of an adequate separation technique. This situation appears to be quite in strange contrast to the advanced developments being currently accomplished in instrumentation for the determination of chemical structure. At present only gel permeation chromatography (GPC) finds practical, heavy use for the purpose of separating polymeric substances and this is by molecular size.[1]

Recently we have demonstrated that thin-layer chromatography (tlc) allows mixtures of isotactic and syndiotac-

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