Theory of the Helix–Random Coil Transformation

James A. Ferretti, Barry W. Ninham,[1] and V. Adrian Parsegian

Physical Sciences Laboratory, Division of Computer Research and Technology,
National Institutes of Health, Department of Health, Education and Welfare,
Bethesda, Maryland 20014. *Received September 8, 1969*

ABSTRACT: A kinetic theory of the helix-random coil transformation which resolves the appparent contradiction between the times measured by kinetic and nmr techniques is presented. It is shown that kinetic times (*ca.* 10^{-6} sec) and nmr lifetimes ($\tau \geqslant 10^{-1}$ sec) observed in polypeptide systems are reconciled. The kinetic time emerges as a consequence of propagation effects whereas the nmr lifetimes are associated with nucleation. The model explicitly takes account of nucleation and finite chain length. A consequence is the correct (*i.e.*, in agreement with experiment) of qualitatively different "long" and "short" chain length behavior of the nmr spectra in the transition region. The theory also leads naturally to a picture of the transformation in terms of a whole-molecule collective excitation, rather than the usual two-state description.

The kinetics and equilibrium properties of the helix–random coil transformation in polypeptides are of considerable fundamental interest. Experimentally, the temperature-jump technique,[2a] the dielectric relaxation experiments of Schwarz and Seelig,[2b] and ultrasonic absorption methods[3] all measure times of the order of 10^{-6} sec presumably associated with the kinetics of the transformation. On the other hand, nuclear magnetic resonance (nmr) spectroscopy has been an extremely useful tool for studying the transformation under equilibrium conditions.[4–6] Lifetimes associated with the helix and random coil, as measured by nmr, are reported to be greater than *ca.* 10^{-1} sec,[6] a result which would appear to be in gross contradiction to the kinetic experiments by at least five orders of magnitude.

The purpose of this paper is to resolve the apparent conflict between the kinetic times and the nmr lifetimes. This has been done by examining an exactly solvable kinetic model of the transformation for finite N where nucleation (initiation) processes are included (N is the number of monomer units in the chain or, equivalently, the degree of polymerization). A consequence of this model is that the observed kinetic times are associated with propagation rate constants, whereas the nmr lifetimes are related to nucleation rate constants. Furthermore, solution of the model leads to a prediction of qualitative differences in the nmr behavior for different values of N. A detailed examination of the solution also suggests a reinterpretation of the model in terms of a collective mode picture of the transformation. On this basis it will be demonstrated that the kinetic and the nmr data are consistent, thus resolving any presumed conflict between the two techniques.

In order to understand the nmr data, it is necessary to examine the kinetics of the process, as well as the equilibrium behavior. The well-known equilibrium theories are equivalent to a generalization of the one-dimensional Ising lattice model[7–9] and appear to provide an adequate description of the transition curves.[10] However, with regard to the kinetic theories, the situation is less satisfactory. Schwarz[11] has presented the general formulation of a kinetic model which reduces, at equilibrium and in the thermodynamic limit, to the model of Zimm and Bragg.[7] An attempt to find explicit analytical solutions to the Schwarz model would appear to be quite impracticable since, in full generality, the theory contains at least five parameters. Therefore, in practice, this theory is limited to systems of large N in which limit parameters associated with end effects disappear. This is a severe restriction since the nmr experiments have been carried out on chains where $N \leq 1000$. Clearly, therefore, the effect of finite N is important. Pipkin and Gibbs,[12] on the other hand, considered the kinetics of conformational change explicitly for finite N, but their solution does not take account of any nucleation process. For these reasons resolution of the "conflict" between nmr and kinetic lifetimes has not been clear.

Nature of the Nmr Spectra

High-resolution nmr spectroscopy at 100 and 220 MHz on a number of polypeptides has shown that separate well-defined peaks, associated with the helix and random coil, are observed for both the α-CH and peptide NH protons over the region of the helix–random coil transformation.[4] As one proceeds through the transformation, the helix peak decreases and the random coil peak increases in intensity, with only a very small change in chemical shifts. An additional complicated effect has been reported for poly(γ-benzyl L-glutamate) (PBLG).[5,13,14] Here the situation

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(1) Department of Applied Mathematics, University of New South Wales, Kensington, N.S.W., Australia 2033.

(2) (a) R. Lumry, R. Legare, and W. G. Miller, *Biopolymers*, **2**, 434 (1964); (b) G. Schwarz and J. Seelig, *ibid.*, **6**, 1263 (1968).

(3) G. G. Hammes and P. B. Roberts, *J. Amer. Chem. Soc.*, **91**, 1812 (1969).

(4) J. A. Ferretti, *Chem. Commun.*, 1030 (1967).

(5) J. A. Ferretti and B. W. Ninham, *Macromolecules*, **2**, 30 (1969).

(6) J. A. Ferretti and L. Paolillo, *Biopolymers*, **7**, 155 (1969).

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

(8) J. H. Gibbs and E. A. DiMarzio, *ibid.*, **30**, 271 (1959).

(9) S. Lifson and A. Roig, *ibid.*, **34**, 1963 (1961).

(10) P. Doty and J. T. Yang, *J. Amer. Chem. Soc.*, **78**, 498 (1956).

(11) G. Schwarz, *Biopolymers*, **6**, 873 (1968).

(12) A. C. Pipkin and J. H. Gibbs, *ibid.*, **4**, 3 (1966).

(13) J. A. Ferretti, paper presented at the 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969.

(14) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Nature*, **217**, 812 (1968).

is as above for short chains (degree of polymerization, DP $\lesssim$ 100). However, for longer chains (DP $\gtrsim$ 250), the "helix" peak, in proceeding through the transformation, decreases in intensity but shifts toward and eventually coalesces with the random coil peak which, throughout the transformation, is increasing in intensity. On the assumption that each type of proton exists in one of two environments which are arbitrarily labeled "helix" and "random coil," the observation of separate peaks implies slow exchange (less than *ca.* 10 sec^{-1}) between the "helix" and the "random coil."

It is well known from the theory of magnetic resonance[15] that nmr spectra of systems composed of molecules which may exist in either of two well-defined states, α and β, will consist of two separate peaks if the exchange rate is sufficiently slow. On the other hand, if the exchange rate is rapid, a single appropriately averaged peak will be observed. For intermediate rates of exchange, the line shape is complex and may be obtained from expressions given by Gutowsky, McCall, and Slichter.[16] From their equations it follows that in the limit of slow exchange, a minimum limit for the average lifetime τ_1 of molecules in either state is given by

$$\tau_1 \geq \frac{1}{\omega_\alpha - \omega_\beta} \tag{1a}$$

where $\omega_\alpha - \omega_\beta$ is the chemical shift difference in hertz between protons in the two environments. The observation of separate "helix" and "random coil" peaks then permits the assignment of lower bounds to the average lifetimes of protons in either environment. If a single peak corresponding to a weighted average of the chemical shifts associated with state α and state β is observed, then one may assign only an upper bound to τ_1, given by

$$\tau_1 \leq \frac{1}{\omega_\alpha - \omega_\beta} \tag{1b}$$

In the nmr investigation of the helix–random coil transformation, the validity of a simple two site description is by no means readily apparent. An important purpose of this paper will be to show that a detailed consideration of the nature of the transition through an exact model calculation does, in fact, lead to a two-site picture for both short and long chains, wherein the line shape theory of Gutowsky, *et al.*,[16] becomes applicable.

Preliminary Remarks

The two-state models of the transformation use the linear Ising lattice model as a basis, where the spin states correspond to "helix" and "random coil," and only nearest neighbor interactions are considered. The elementary steps in the reaction are the transformation of individual segments, as in the process

$$C_j \underset{k_B}{\overset{k_F}{\rightleftarrows}} C_{j+1} \tag{2}$$

Here C_j represents the fraction of molecules with j random coil units and $N - j$ helical units, and k_F and k_B are the appropriate nucleation or propagation rate constants, which are related by a nucleation parameter, σ. Random coil nucleation both from the ends of the helix and from interior points within the helix, as well as helix nucleation of the random coil may be considered. Thus, if the fraction of triple sequences with three consecutive coil or three consecutive helix units are represented as ccc or hhh, respectively, then random coil nucleation may be written as

$$\text{hhh} \overset{k'_{\text{nuc}}}{\rightleftarrows} \text{hhc (exterior random coil nucleation)}$$

$$\text{hhh} \overset{k''_{\text{nuc}}}{\rightleftarrows} \text{hch (interior random coil nucleation)}$$

while helix nucleation is represented by

$$\text{ccc} \overset{k'''_{\text{nuc}}}{\rightleftarrows} \text{cch (exterior helix nucleation)}$$

$$\text{ccc} \overset{k''''_{\text{nuc}}}{\rightleftarrows} \text{chc (interior helix nucleation)}$$

The usual assumption, necessary to generate sigmoidal equilibrium curves, is that nucleation rate constants are characterized by much longer times than the corresponding propagation rate constants. Bixon and Lifson[17] have discussed in detail the effect of the different types of nucleation on the equilibrium statistical mechanics of the transformation.

Apart from the fact that, with five parameters, an analytic solution to Schwarz's model is a formidable problem, there is a more serious theoretical objection to this and similar models.[18] All such models have as an underlying physical basis a system which is three dimensional. The implicit assumption of all linear theories, that the three-dimensional degradation of the system may be mapped into a one-dimensional space with appropriate rate parameters, is equivalent to the assertion that forces which stabilize the helix are either "on" or "off;" that is, bonds are either "formed" or "broken." The model is then assumed to contain all of the important kinetic and equilibrium behavior of the real three-dimensional system. In this context no allowance is made for the fact that the twisting of a single bond could be shared over several sites and so produce further (lower lying) collective states of the system. For example, a long helix is known to support three kinds of elastic wave propagation[19] along the helix axis: longitudinal and transverse phonons, and torsional or twisting waves. Wave propagation in a helix is a consequence of its three-dimensional structure, and it could distort many of the properties required for the applicability of the simple one-dimensional system. If allowance is made for this (weak) perturbation, then lower-lying collective states may be generated.

An important motive of this paper is to gain some physical insight into the helix–random coil transformation process by examining in detail the explicit solution to the set of kinetic equations given in the next section.

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(15) J. W. Emsley, J. Feeney, and H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1967, and references cited therein.

(16) H. S. Gutowsky, D. W. McCall, and C. P. Slichter, *J. Chem. Phys.*, **21**, 1688 (1953).

(17) M. Bixon and S. Lifson, *Biopolymers*, **5**, 509 (1967).

(18) See, for example, H. A. Scheraga in "Poly-α-Amino Acids, Protein Models for Conformational Studies," G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., p 391.

(19) P. Bugl and S. Fujita, *J. Chem. Phys.*, **50**, 3137 (1969).

In fact, although the "one-dimensional" (in reaction state space) model has been solved exactly, it is specifically this explicit solution which permits a nonformal although logical extension to the real three-dimensional system. In the context of this paper the time behavior of the system is regarded as a stochastic process with the quantities of interest being probabilities (or probability densities) and averages rather than deterministic quantities.

The Model

In order to set up a solvable two-state model, it is convenient at first to make some simplifying assumptions. DeGennes[20] has shown for a long helical chain (*i.e.*, large N) that, even if interior random coil nucleation does occur, it cannot propagate easily. For a short helix (small N), the probability of interior random coil nucleation is probably very small, since three bonds must be broken simultaneously for nucleation to be complete. Therefore, the following scheme is proposed as a first approximation, one which should contain most of the features of the one-dimensional (in reaction state space) kinetic process.

$$\begin{array}{ccc} C_0(t) & \underset{B}{\overset{a}{\rightleftarrows}} & C_1(t) \\ \cdot & & \cdot \\ \cdot & & \cdot \\ C_j(t) & \underset{B}{\overset{A}{\rightleftarrows}} & C_{j+1}(t) \\ \cdot & & \cdot \\ \cdot & & \cdot \\ C_{N-1}(t) & \underset{b}{\overset{A}{\rightleftarrows}} & C_N(t) \end{array} \tag{3}$$

In these equations, each step corresponds to the formation or breakage of one helical unit. The $C_j(t)$'s are probabilities that a given molecule contains j coil units at time t, where $C_0(t)$ and $C_N(t)$ represent probabilities of completely helical and completely random coil molecules, respectively. It is to be emphasized that, like other kinetic schemes, the correspondence of the parameters of the model with the underlying three-dimensional physical structure of the system is yet to be established. The parameters A and B represent propagation rate constants, and b and a are "helix" and "random coil" nucleation rate constants, respectively. In terms of the two-state picture, a and b serve to define the sharpness or "cooperativity" of the transition curve. The helix nucleation rate constant, b, takes account of the difficulty associated with simultaneously forming three or more bonds to make a helix. This term is due to a reduction of the conformational phase space accessible to the system (a decrease in entropy) and, as pointed out by Flory,[21] also to a deficiency in dipolar energy associated with the end of the chain. The random coil nucleation rate constant, a, is somewhat more difficult to define for the helix–random coil transformation. It might best be viewed in terms of the amount of energy required to "break up" an aggregate of helical molecules, which are believed to exist in some polypeptide systems, in preparation for the formation of "random coil" units. For the helix nucleation rate constant, b, it will be assumed that $b \ll A,B$. The system of equations which describes the sequence of first-order reactions in eq 3 may be written as

$$\begin{aligned} \dot{C}_0 &= -aC_0(t) + BC_1(t) \\ \dot{C}_1 &= aC_0(t) - (A + B)C_1(t) + BC_2(t) \\ &\ \ \vdots \\ \dot{C}_j &= AC_{j-1}(t) - (A + B)C_j(t) + BC_{j+1}(t) \qquad (4) \\ &\ \ \vdots \\ \dot{C}_{N-1} &= AC_{N-2}(t) - (A + B)C_{N-1}(t) + bC_N(t) \\ \dot{C}_N &= AC_{N-1}(t) - bC_N(t) \end{aligned}$$

These equations may be written more simply as a master equation

$$\dot{\underset{\sim}{C}} = \underset{\approx}{M}\underset{\sim}{C} \tag{5}$$

where $\dot{\underset{\sim}{C}}$ and $\underset{\sim}{C}$ are column vectors and $\underset{\approx}{M}$ is a transition rate matrix. While the general properties of the master equation have been discussed by various authors[22] it is nevertheless true that the precise form of the relaxation spectrum associated with eq 5 depends very much on the details of the model, and must be investigated separately for each separate model.

In order to avoid cumbersome algebra, the initial detailed discussion will be restricted to the case where $a = A$. Physically, this is equivalent to the assumption that there is no special parameter which corresponds to random coil nucleation at the ends of an entirely helical molecule. The case where $a \neq A$ will be shown to lead to a more complicated relaxation spectrum, which in turn can yield more complex nmr spectra. This latter case ($a \neq A$) will be discussed separately after the solution for the simpler case ($a = A$) is described.

If $G(\infty)$ denotes the equilibrium number of helical units per molecule defined by

$$G(\infty) = \sum_{j=0}^{N} jC_j(\infty) \tag{6}$$

it is straightforward to show from eq 4 that this "mean helix content" is given by

$$G(\infty) = \frac{b/A[1 - (N + 1)(B/A)^N + N(B/A)^{N+1}]}{(1 - B/A)\{1 - B/A + b/A[1 - (B/A)^N]\}} \tag{7}$$

It should be noted that a more general model could be constructed in the event that the helix contains interior breaks or defects. In this case it suffices to define

$$\begin{gathered} G(\infty) = \mathop{\mathrm{E}}\left\{m_k \sum_{j=0}^{N^{(k)}} jC_j(\infty)\right\} \\ \sum_{j=0}^{N} m_k N^{(k)} = N \end{gathered} \tag{8}$$

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(20) P. DeGennes, *J. Chem. Phys.*, **65**, 962 (1968).

(21) P. Flory, "Statistical Mechanics of Chain Molecules," Interscience Publishers, New York, N. Y., 1969, p 288.

(22) I. Oppenheim, K. E. Shuler, and G. H. Weiss, *Advan. Mol. Relaxation Processes*, **1**, 13 (1967–1968), and references cited therein.

where E denotes an expectation value, m_k is the number of segments of length k, and $N^{(k)}$ is a random variable which denotes the effective number of monomer units per elementary segment. This formalism also permits one to handle molecular weight distributions as a straightforward generalization of the simpler model.

The behavior of $G(\infty)$ as a function of the ratio A/B is shown in Figure 1 for various values of N. It is important to note that the equilibrium solutions are of sigmoidal form, even though there is only a single nucleation rate constant. The effect of a second nucleation rate constant on $G(\infty)$ will, in general, be small and difficult to discern experimentally. For very large N, the transition is dominated by the term $(A/B)^N$ and becomes very sharp and occurs over a very narrow range of A/B. The midpoint of the transition (*i.e.*, the inflection point) occurs at $A = B$ only when $N \rightarrow \infty$. This behavior is seen clearly in graphical form in Figure 1. For smaller values of N, the transition broadens and the midpoint occurs at some value of the ratio A/B less than unity. For the case where $a = b$, the midpoint of the transition will occur at $A = B$ for all values of N, although the sharpness (cooperativity) will still be dictated largely by the value of N. This behavior is similar to that found in the equilibrium statistical mechanical theories, where the midpoint always occurs where the propagation equilibrium constant is unity. Clearly then, in the kinetic model of this paper, the relative magnitudes of both a and b in addition to N are very important in determining the nature of the process.

In order to interpret the nmr spectra, it will be necessary to examine the distribution of the $C_j(t)$ as the system relaxes to equilibrium, where $C_j(t)$ now represents the fraction of molecules in state j at a time t.

The distribution of $C_j(t)$ will be related to the values of b and N. The equilibrium distribution $C_j(\infty)$ is found from eq 4 by setting $\dot{C}_j(\infty) = 0$. One then obtains (for the case where $a = A$)

$$C_j(\infty) = (B/A)^{N-(j+1)}C_{N-1}(\infty) \qquad j \leq (N-2) \quad (9)$$

$$C_{N-1}(\infty) = (b/A)C_N(\infty) \quad (10)$$

Under the conservation condition

$$\sum_{j=0}^{N} C_j(t) = 1 \quad (11)$$

the equilibrium distribution is given by the expression

$$C_j(\infty) = \frac{(B/A)^{N-(j+1)}}{\left[A/b + \sum_{j=1}^{N} (B/A)^{j-1}\right]} \quad (12)$$

At the two extremes of the transition, $A/B \rightarrow 0$ and $A/B \rightarrow \infty$, the distribution is dominated by contributions from $C_0(\infty)$ and $C_N(\infty)$, respectively. In the region of the transition, the contribution from $C_N(\infty)$ will always be appreciable under the condition that $b \ll A,B$. For small values of N, the graphs in Figure 1 demonstrate that $B/A > 1$ over most of the transition region. Thus, the numerator in eq 12, $(B/A)^{N-(j+1)}$, will cause terms near $j = 0$ to be favored over terms near $j = N$. This will result in a distribution skewed toward $j = 0$, except for a subsidiary maximum at $j = N$. As N increases and $B/A \rightarrow 1$ in the region of the transition, the distribution will become more diffuse over all the C_j's.

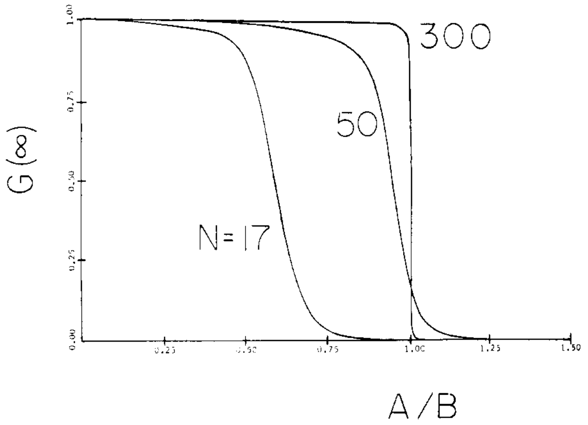

Figure 1. A plot of the mean number of helix units, $G(\infty)$ *vs.* the ratio A/B for $b/A = 10^{-4}$.

The change of the distribution of the $C_j(t)$ with time will be the important factor indicating the behavior of the nmr spectra, as will become apparent in the Discussion. Therefore, in the region of the transition, the time evolution of the system of equations, eq 4, is the appropriate quantity of interest. Although a formal and exact solution may be obtained for various initial conditions, it is more instructive to consider the solution for a single given initial condition. The specific initial condition considered in this paper is $C_j(0) = \delta_{j,0}$, where

$$\delta_{j,0} = \begin{cases} 1 \text{ for } j = 0 \\ 0 \text{ for } j \neq 0 \end{cases}$$

A corresponding experimental condition might be a temperature-jump study of the transformation, where the polypeptide is initially completely helical. This is also a good approximation to the actual equilibrium distribution on the helix side of the transition (*cf.* Figure 1). The solution of eq 4 for arbitrary initial conditions and the calculation of associated first passage times is a nontrivial problem. Solution of the first passage time problem would give a precise measure of the average time spent by any individual site in a given $C_j(t)$. However, most of the features of the general solution are contained in the solution for the above initial condition.

Solution of the Model

The general solution of the system of equations (4) subject to the initial condition $C_j(0) = \delta_{j,0}$ and arbitrary a and b has been described elsewhere by Ninham, Nossal, and Zwanzig.[23] These solutions provide an approximation to the process for any value of N in the presence of two nucleation rate constants. This model approximation holds throughout the entire region of the transition as the ratio A/B is varied. A special case of the general solution for the above initial condition and $a = A$ is of special interest in this paper.

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(23) B. W. Ninham, R. J. Nossal, and R. Zwanzig, *J. Chem. Phys.*, in press.

This specific initial condition is of particular interest because it readily lends itself to an explicit asymptotic solution.[23] A further motivation behind this choice of parameters and initial condition with regard to the nmr experiments will be considered in detail in the Discussion.

The time relaxation of the system to equilibrium for $C_j(0) = \delta_{j,0}$ and general a and b is given by the integral

$$\Delta G(t) = e^{-(A+B)t}(2\beta/2\pi i)\int_{c-i\infty}^{c+i\infty} \frac{e^{2\sqrt{AB}t\epsilon}\sum_{j=1}^{N} \gamma^j\,[U_{N-j}(\epsilon) - \xi' U_{N-j-1}(\epsilon)]\,\mathrm{d}\epsilon}{(2\epsilon - \gamma - 1/\gamma)[U_N(\epsilon) - (\xi + \xi')U_{N-1}(\epsilon) - \xi\xi' U_{N-2}(\epsilon)]} \tag{13}$$

where

$$\Delta G(t) = G(t) - G(\infty) \tag{14}$$

The contour is chosen to separate the poles at

$$\epsilon = \epsilon_0 = {}^1\!/_2(\gamma + 1/\gamma) \tag{15}$$

which corresponds to the solution at $t = \infty$, from those of the rest of the integral. The pole at $\epsilon = \epsilon_0$ gives the equilibrium solution.

In eq 13, $\beta = a/A$, $\xi = (A - a)/\sqrt{AB}$, and $\xi' = (B - b)/\sqrt{AB}$. The functions $U_N(\epsilon)$ are Tchebycheff polynomials[23] of the second kind, and $\gamma = (A/B)^{1/2}$. For the specific case of interest where $a = A$, the time relaxation is given by

$$\Delta G(t) = e^{-(A+B)t}\,\frac{2(1 - \xi')}{2\pi i} \times \int_{c-i\infty}^{c+i\infty} \frac{e^{2\sqrt{AB}t\epsilon}\sum_{j=1}^{N} \gamma^j U_{N-j}(\epsilon)\,\mathrm{d}\epsilon}{(2\epsilon - \gamma - 1/\gamma)[U_N(\epsilon) - \xi' U_{N-1}(\epsilon)]} \tag{16}$$

Equation 16 follows from eq 13 by straightforward substitution and the use of the recurrence relations for the Tchebycheff polynomials.

$$U_N(\epsilon) = 2\epsilon U_{N-1}(\epsilon) - U_{N-2}(\epsilon) \tag{17}$$

The actual relaxation spectra associated with eq 13 and 16 are fairly complicated. The details of the calculation and the evaluation of the integrals have already been presented elsewhere.[23] The results of those calculations which are of interest are merely summarized here for convenience. In eq 16 the poles of the integrand which describe the approach to equilibrium are due to the zeros of the polynomial $U_N(\epsilon) - \xi' U_{N-1}(\epsilon)$. Several cases of interest then arise (1 and 2).

$$(1) \qquad \xi' = \frac{B - b}{\sqrt{AB}} > 1 + 1/N \tag{18}$$

From the graph of the equilibrium solutions (Figure 1), it can be seen that this situation occurs when N is small and/or when $a = A$ and $b \ll B$. In this case two of the $N + 1$ eigenvalues of $\underset{\approx}{M}$ will lie outside the interval $-1 \leq \epsilon \leq 1$, as illustrated in Figure 2. (The eigenvalue at $\epsilon = \epsilon_0 = {}^1\!/_2(\gamma + 1/\gamma)$ gives the equilibrium solution.) The remaining $N - 1$ eigenvalues of $\underset{\approx}{M}$ lie inside the interval $-1 \leq \epsilon \leq 1$. If there exists an isolated largest eigenvalue, ϵ_1 which is distinct from the equilibrium eigenvalue and is not a limit point of the eigenvalues in $-1 \leq \epsilon \leq 1$ (*i.e.*, the continuum in the limit $N \rightarrow \infty$), then this eigenvalue dominates the relaxation to equilibrium in the limit of large times.

$$(2) \qquad -(1 + 1/N) < \xi' < (1 + 1/N) \tag{19}$$

This situation occurs when N is large, since the transition occurs for $A \cong B$. In this case, the eigenvalue spectrum forms a band which includes ϵ_1. For $\xi' = (1 + 1/N)$, ϵ_1 becomes a limit point of eigenvalues. This result holds for all values of a. If either $\xi' > (1 + 1/N)$ or $\xi > (1 + 1/N)$, one and only one eigenvalue will be distinct from the band (in addition to the equilibrium eigenvalue, ϵ_0). In the limit as $N \rightarrow \infty$ and $A = B$, all eigenvalues including ϵ_0 merge into a continuum and no exponential relaxation occurs.

The time dependence of $\Delta G(t)$ for large time may be exhibited explicitly by evaluation of the residues of eq 16 at the poles. Following Ninham,[23] *et al.*, one obtains

$$\Delta G(t) = d_1 e^{-b(1-1/\xi'^2)t} + d_2 e^{-(\sqrt{A}-\sqrt{B})^2 t} \tag{20}$$

where

$$d_1 = -\frac{(1 - \xi')(\xi'^2 - 1)[\gamma^N(\xi'^2 - 1) - (\gamma\xi' - 1)\xi'^N]}{(\xi' + 1/\xi' - \gamma - 1/\gamma)} \tag{21}$$

and

$$d_2 \simeq -\frac{(\xi' - 1) - e^{(-N^2/4\sqrt{AB}\,t)}}{2\pi(\sqrt{\gamma} - 1/\sqrt{\gamma})^2|1 - \xi'|} \times \left[\frac{1}{(\sqrt{AB}\,t)^{3/2}} - \frac{N^2}{2(\sqrt{AB}\,t)^{5/2}}\right] \tag{22}$$

For the case where $a \neq A$, one gets

$$\Delta G(t) = d_3 e^{-a(1-1/\xi^2)t} + d_4 e^{-(\sqrt{A}-\sqrt{B})^2 t} \tag{23}$$

$$\Delta G(t) = d_1' e^{-b(1-1/\xi'^2)t} + d_2' e^{-(\sqrt{A}-\sqrt{B})^2 t} \tag{24}$$

Here the amplitudes d_1', d_2', d_3, and d_4 are strongly N dependent and relatively weakly dependent on the other parameters. Their precise expressions are quite complicated and may be obtained from ref 23.

The eigenvalue spectra of eq 13 and 16 are not too surprising (*cf.* Figure 2). In fact, the set of equations, eq 13 and 16, in frequency space are equivalent to the set describing the vibration of a set of N coupled harmonic oscillators with a single defect.[24] The emergence of a single eigenvalue, which is separate from the band of eigenvalues, in the presence of a defect is a well-known phenomenon in solid state physics. This phenomenon has been studied in much detail.[24]

For small values of N the discrete eigenvalue $b(1 - 1/\xi'^2)$ dominates the relaxation behavior of the system. For much larger values of N, the relaxation behavior is

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(24) A. A. Maradudin, E. W. Montroll, and G. H. Weiss, "Theory of Lattice Dynamics in the Harmonic Approximation (Solid State Physics, Suppl. 3)," Academic Press, New York, N. Y., 1963.

dominated by the band of eigenvalues (*i.e.*, the terms in $(\sqrt{A} - \sqrt{B})^2$), since ϵ_1 becomes part of the continuum. In the systems of interest in this paper relative to the nmr data (*i.e.*, $N < 1000$), the eigenvalue $b(1 - 1/\xi'^2)$ will always be effectively distinct from the other eigenvalue, $(\sqrt{A} - \sqrt{B})^2$.

Discussion

It is now possible to use the formal results of the previous section to derive a physical picture of the molecular processes underlying both the nmr and the kinetic data. This involves a conceptual extrapolation to a three-dimensional picture of transition from the one-dimensional *ad hoc* formalism (*i.e.*, a one-dimensional two-state space—bonds "formed" or "broken") of eq 4. The several parameters employed—the chain length, N, the ratio of propagation rate constants A/B which is varied experimentally, and at least one nucleation rate constant, b—should suffice to contain the essential features of the real three-dimensional system. From the nmr and kinetic data it is known that there are at least two very different times which characterize the helix–random coil transformation. These times can be understood in terms of the rate constants and the eigenvalue spectra of the model. The correct qualitative behavior emerges even when $a = A$ is assumed in eq 4, so that the rate constants of interest are b, A, B. The requirement for observing separate peaks is that the molecule remains trapped in a given state (or a given set of states) for a time long compared to the resolution time of an nmr experiment (*i.e.*, the chemical shift difference, $(\omega_\alpha - \omega_\beta)$). Within the framework of the model the longest lifetime will be of the order of b^{-1}, so that to observe separate peaks one must have that $b \ll (\omega_\alpha - \omega_\beta)$, where b is a rate constant for nucleation. Nevertheless, the propagation rate constants A and B can be quite large compared to $\omega_\alpha - \omega_\beta$. While the solution of the model is derived only for the special initial condition $C_j(0) = \delta_{j,0}$, the form of the relaxation process is independent of this initial condition and is described essentially by the two eigenvalues, $b(1 - 1/\xi'^2)$ and $(\sqrt{A} - \sqrt{B})^2$. For the cases of interest here it will be true that $b(1 - 1/\xi'^2) \ll (\sqrt{A} - \sqrt{B})^2$. Therefore, there will be only two times which characterize the system where the long time is of the order of b^{-1} more than $1/(\omega_\alpha - \omega_\beta)$ and the short time is less than $1/(\omega_\alpha - \omega_\beta)$. The fact that b is less than $\omega_\alpha - \omega_\beta$ implies that the lifetimes of state N and of an average state which is a weighted sum over all other j states ($j \leq N - 1$) are long. The short time is too short to permit resolution of the individual states ($j \neq N$) by nmr.

It then seems plausible to visualize an "average helix" state seen as the "helix" peak in the nmr spectrum. This helical state is formally composed of all of the individual j ($j \neq N$) states of the stochastic representation and its properties can be calculated from the sum of the $C_j(\infty)$ ($j < N$). But since equilibration between the helical j states in terms of the model must be infinitely fast compared to resolution times, the helix state is indeed physically defined only in terms of a composite average. Further the rapid transition processes intrinsic to this average helical state may be better imagined in terms of *collective motion* of the whole molecule than as the stepwise process in which they were formally expressed. Presumably this motion will be torsional rather than vibrational (*cf.* the next section). The nature of the "average helical" state for different values of N can be understood by observing the distribution, $\{C_j(\infty)\}$, for different N as follows.

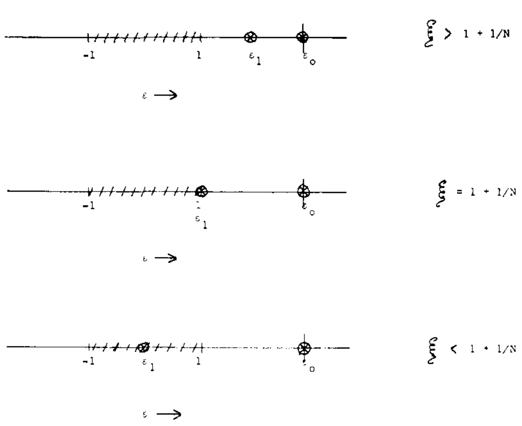

Figure 2. A plot of the eigenvalue spectra for various values of ξ'. The eigenvalue ϵ_0 represents the equilibrium solution.

(1) Short Chain Length Behavior. The equilibrium curve for $N = 17$ of Figure 1 implies that the transition for short N is relatively broad and that the ratio A/B is less than unity over essentially the entire transition region. It then follows from eq 12 that the distribution, $\{C_j(\infty)\}$, of states j ($j \neq N$) will be skewed toward $j = 0$. It also follows that the form of the distribution ($j \neq N$) will not change appreciably as the ratio A/B is varied through the transition region. Since to explain the data, A and B must be fast and b slow compared to the resolution time of the spectrometer, the helix–random coil exchange process is written as

$$\bar{C}(t) \underset{k_B}{\overset{k_F}{\rightleftarrows}} C_N(t) \qquad (25)$$

Equation 25 is very similar to eq 2 except that k_F and k_B are now the helix–random coil exchange rates and an average state whose probability is $\bar{C}(t)$ and state N are the two states observed by the nmr spectrometer. Since for small N, the distribution does not change appreciably in going through the transition, $\bar{C}(\infty)$ remains essentially constant. For this reason there is no change in the resonant frequency of the "average helix" peak in going through the transition (*cf.* Figure 3).

(2) Long Chain Length Behavior. For longer chains the transition region ($N = 300$ from Figure 1) is much sharper and the transition occurs about $A/B = 1$. Since $A/B \approx 1$ in the transition region, one sees from eq 12 that the distribution, $\{C_j(\infty)\}$, will strongly depend on the precise value of the ratio A/B. Therefore, the distribution of states j ($j < N$) will change appreciably in going through the transition. Nevertheless it is still valid to define an "average helix" state whose probability is given by $\bar{C}(t)$ (eq 25). As one proceeds through the transition from helix to random coil, the distribution will become increasingly weighted toward the states j of higher values of N (*cf.* Figure 4). Therefore the "average helix" state will become increasingly coil-like with increasing values of the ratio

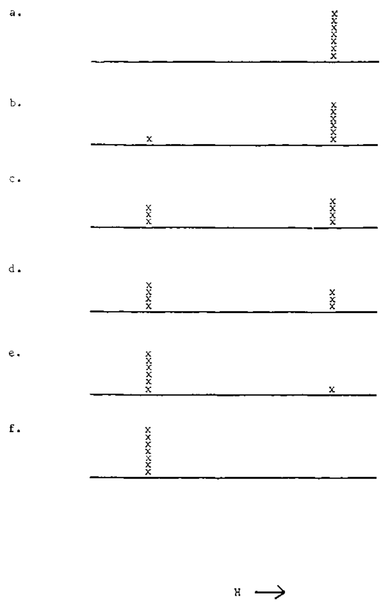

Figure 3. Theoretical spectra over the region of the transformation for small N and $a = A$. The high-filed peak represents the "average helix" and the low-field peak represents the "random coil."

A/B. Hence, one expects a shift of the "average helix" peak toward the "random coil" peak until eventually (when the helix is completely degraded) it becomes equivalent to a "random coil" peak (*cf.* Figure 5).

(3) Intermediate Chain Length Behavior. For the case where the distribution of the $C_j(t)$ probabilities is changing at an intermediate rate with the ratio A/B (*cf.* $N = 160$ in Figure 1 and intermediate N behavior of Figure 4), the change in the nature of the "average helix" state will not be as dramatic as in the long chain length situation. Consequently, a peak in the nmr spectrum which represents the "average helix" state will shift toward the "random coil" peak. However, the magnitude of the shift will be smaller than in the long chain case and, the "average helix" state for intermediate chain length systems will never become equivalent to a "random coil" state. Typical simulated nmr spectra over the region of the transition are presented in Figure 6.

For the intermediate chain length case, the effect of a molecular weight distribution on the spectra will be more pronounced than for either the short chain length or long chain length cases. In the intermediate chain length region, the magnitude of the shift will be dictated by the precise value of N. Therefore, should a molecular weight distribution exist, the "average helix" peak will become diffuse. The envelope of the peak will actually reflect the molecular weight distribution.

Consider now the condition $a \neq A$ in eq 4. The relaxation behavior is more complex and is given by eq 13. For the specific case $a = b$ the equilibrium curve will be symmetric about $A/B = 1$. This specific case parallels the results of the usual equilibrium theories. Here again, on one side of the midpoint there is effectively a relaxation process described by two eigenvalues, $b(1 - 1/\xi'^2)$ and $(\sqrt{A} - \sqrt{B})^2$, as before. At the midpoint the eigenvalues merge. On the other side of the midpoint, a further eigenvalue, $a(1 - 1/\xi'^2)$, emerges from the band. Precisely at the midpoint the reaction is diffusion controlled, and the model describes a symmetric one-dimensional random walk in reaction state space. The complication introduced by an additional helix nucleation parameter, a, introduces a further peak in the nmr spectra. The qualitative nature of the resulting nmr spectra is depicted over the region of the transition in Figure 7.

Remarks on Kinetic Experiments

On the basis of the previous sections it is possible to relate the model to the kinetic experiments mentioned in the first section. In the previous section it was shown that the effect of a single nucleation rate constant can be to yield separate "average helix" and "random coil" peaks in the nmr spectra of polypeptides in the region of the helix–random coil transformation.

Any kinetic process which alters the helix content may be viewed as a chemical relaxation process.

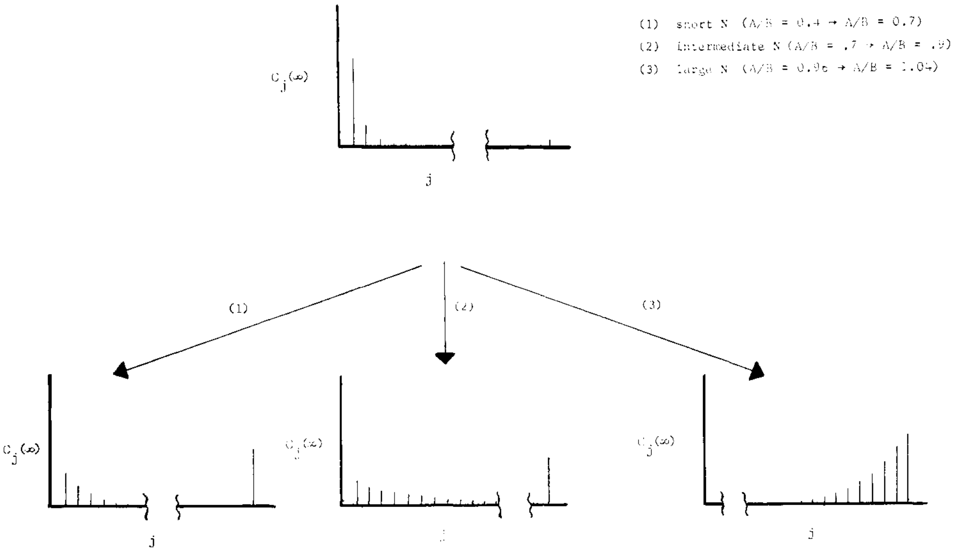

Figure 4. A plot of the distribution of the $C_j(t)$ probabilities for small, intermediate, and large values of N for $G(\infty)$ near unity and near 0.

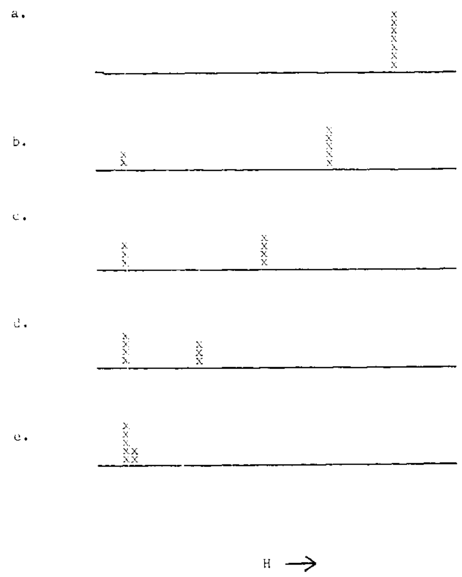

Figure 5. Theoretical spectra over the region of the transformation for large N and $a = A$. The high-field peak represents the "average helix" and the low-field peak represents the "random coil."

Therefore, eq 13 and 16 for the time relaxation of the system of 4 are applicable for sufficiently long times. As pointed out in the first section, a number of methods for measuring chemical relaxation times are known. Of these methods, temperature jump, ultrasonic absorption, and dielectric relaxation have been most extensively used. In contrast with nmr, these kinetic experiments have been carried out on polypeptides having very large values of N. In the temperature jump experiment a shift in the equilibrium, $G(\infty)$, is caused by a sudden change in temperature. In the context of the model, the magnitude of the change will strongly depend on the initial condition, in addition to values of the rate parameters. In the ultrasonic absorption experiment, the shift in equilibrium is caused by periodical changes in pressure and temperature resulting from an ultrasonic wave. These experiments are clearly in the nature of a relatively violent perturbation which can drastically alter the helix–random coil equilibrium. (The relaxation process associated with these experiments, which is determined by the eigenvalue $(\sqrt{A} - \sqrt{B})^2$, is characterized by very short times.) In the dielectric relaxation experiment, the application of a sinusoidal electric field shifts the chemical equilibrium of a dipolar reaction process, which causes dielectric polarization. Should chemical relaxation be faster than ordinary rotational reorientation, then the former can be observed by studying the frequency dependence of the dielectric loss at the higher frequencies. This is the basis of the Schwarz and Seelig[2b] experiments on poly(γ-benzyl L-glutamate).

In the temperature-jump experiment, for large N one may consider (in the original two-state picture) the distribution of the $C_j(t)$ probabilities to change quite sharply with the ratio A/B, since $A \simeq B$ over essentially the entire transition region. In the collective mode picture one would say that the relative population of j states changes rapidly in the region of the transition. The relaxation of the system is given by the eigenvalue, $(\sqrt{A} - \sqrt{B})^2$. This eigenvalue may be very large, even for $A \simeq B$, since A and B may be quite large. Consequently, the kinetic experiments may yield very short time behavior, although the nmr will still show separate peak long lifetime behavior. The same mixing of j states which simply causes a shift of the well-defined "average helix" peak for large N is seen as a rate in the kinetic measurements.

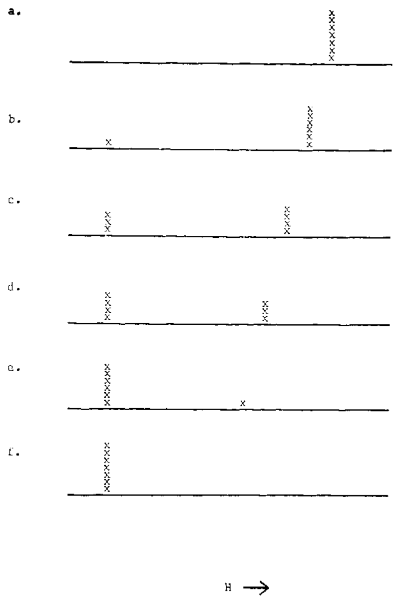

Figure 6. Theoretical spectra over the region of the transformation for intermediate N and $a = A$.

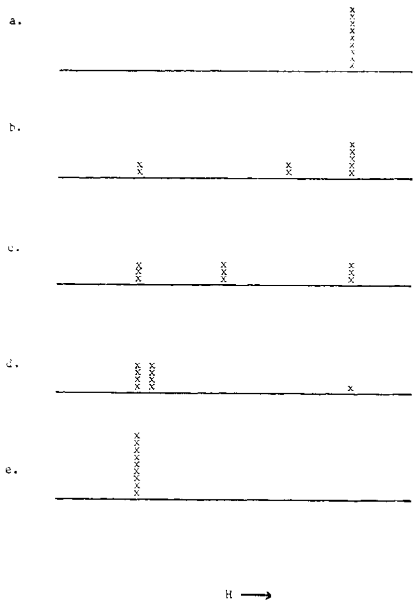

Figure 7. Theoretical spectra over the region of the transformation for a — A. The intensities are unnormalized.

In the dielectric relaxation experiments, Schwarz[2b] defines a mean relaxation time τ^* in terms of his general kinetic model. According to Schwarz,[2b] the maximum value of this mean relaxation time, $\tau^*_{\max}$, is a measure of the *initiation* time for the process, where

$$\frac{1}{\tau^*_{\max}} = \frac{1}{\Delta G}\frac{\mathrm{d}G}{\mathrm{d}t}\bigg]_{t=0} \qquad (26)$$

For the Schwarz model $\tau^*_{\max}$ is proportional to a nucleation rate constant which, in the nomenclature of his model, may be written as

$$\frac{1}{\tau^*_{\max}} = 4\sigma k_F \tag{27}$$

where σk_F is some nucleation rate constant and σ is a nucleation parameter. On the basis of the kinetic model of this paper, τ^* may be expressed as

$$\frac{1}{\tau^*} = \frac{aC_0(0) - bC_N(0) + (A - B)(1 - C_0(0) - C_N(0))}{G(\infty) - G(0)} \tag{28}$$

Equation 28 assumes that the relaxation behavior is of the form

$$\frac{G(t) - G(0)}{G(\infty) - G(0)} = 1 - e^{-t/\tau} \tag{29}$$

If the system is initially at equilibrium in the absence of an applied electric field, then $\dot{C}_j(0) = 0$ in eq 4. Upon application of a weak electric field, E

$$\dot{C}_j = \dot{C}_j(E,t) \tag{30}$$

One may then expand $\dot{C}_j$ in a power series to first order obtaining

$$\dot{C}_j = \dot{C}_{j(E=0)} + \left.\frac{\partial \dot{C}_j}{\partial E}\right]_{E=0} \mathrm{E} \tag{31}$$

Since $\dot{C}_j(0,t) = 0$, one may write for the rate of change of G (*cf.* eq 6)

$$\dot{G} = \sum_{j=0}^{N} j\dot{C}_j = E\left.\sum_{j=0}^{N} j\partial\dot{C}_j/\partial E\right]_{E=0} \tag{32}$$

Similarly, Schwarz and Seelig[2b] give an expression for the shift in equilibrium from 50% helix content upon application of an electric field

$$G(\infty) - G(0{,}50\%\ \text{helix}) = 0.5 \times \left[\frac{\alpha^2E^2}{16 + \alpha^2E^2}\right]^{1/2} \approx \frac{\alpha E}{8} \tag{33}$$

where α is constant.

After recognizing the dependence of the rate parameters on E, one can expand them as

$$P = P_0 + P'E \tag{34}$$

where P refers to A, B, a, and b. Substitution into eq 4 then yields

$$\dot{C}_j(E) = A_0C_{j-1} - (A_0 + B_0)C_j + B_0C_{j+1} + E[A'C_{j-1} - (A' + B')C_j + B'C_{j+1}] \tag{35}$$

where $1 < j \leq (N - 1)$. One gets similar expressions for $j = 1$ and $j = N$. Therefore one gets for τ^*

$$\frac{1}{\tau^*} = \frac{a'C_0(0) - b'C_N(0) + (A' - B')(1 - C_0(0) - C_N(0))}{\alpha} \tag{36}$$

This result is then sensitive only to that part of the rate constants which depends on E. Furthermore, other estimates of ΔG will change the denominator of eq 36 which suggests an even more puzzling dependence of measured relaxation times on the rate constants. Clearly then, the observation of a frequency *ca.* 10^6 sec^{-1} in the dielectric relaxation experiments on PBLG is not inconsistent with the long nmr lifetime behavior.

Several further remarks are in order. Should collective modes be present and should they be characterized by a frequency of the order of 10^6 sec^{-1} to correspond with the measurements of Schwarz and Seelig[2b] one would expect a fundamental wavelength of the order of two times the chain length (*i.e.*, $\lambda_0 = 5.25 \times 10^{-5}$ cm). This suggests a velocity of propagation along the helix axis of the order of 100 cm/sec, which is much less than the velocity of sound. Propagation velocities of this magnitude are on the scale of rotation times, which is consistent with the interpretation given above.

The following conjecture is made as a concluding remark. The concept of collective behavior applied to a polypeptide chain may suggest a similar phenomenon in biological polypeptides. Lumry[25] has noted that enzymes may act as energy-storage or energy-transfer units during catalysis. It is clear that any collective motion (torsion, rotation, vibration) in an enzyme will depend on more than chain length alone. Indeed the selection of amino acid sequence and size allows a great range of possible collective motion. It is of interest, therefore, to go beyond the resolution of static structure and identification of "active sites" for a template model of enzyme action, and to regard the enzyme amino acid sequence as the energy surface which allows the molecule to exhibit specific dynamic properties.

It is inferred from the present results that polypeptide chain length alone can change the time-dependent response of the molecule. A rather elementary kinetic model for this is still all that has been solved, and that model is capable of only a formal description. Solution of the analogous model for a nonhomogeneous three-dimensional polypeptide will unfortunately be still more formal. Nevertheless, there is a clue here which suggests a new line of reasoning about enzyme action.

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(25) R. Lumry, paper presented to the Division of Physical Chemistry, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968; Abstracts S157.