ed here. For example, knowledge of the variety of random-coil configurations indicates that a better theory should include multiple random-coil states and rates for transitions among them. A general kinetic description of helix–random-coil transitions for an isolated polypeptide is not available. Any general formulation must also include the slower rate for initiation of helix in a random-coil region and perhaps the rate for the process of initiation of coil within a helix sequence.

We conclude that no long-lived helix-containing configurations are likely to exist, at least not for polypeptides comprising less than a few hundred residues. Existence of multiple helix regions will shorten average lifetimes; thus, lifetimes calculated for long chains with one helix sequence would serve as an upper bound for helix lifetimes in all longer chains in which multiple helix regions are important. The distinction between transitions occurring in two regions of time does, however, permit simple conclusions to be drawn. By nmr, a long transition time, as in the transformation of the all-random-coil conformation to any helix-containing conformation, is independent of the short times characteristic of the transitions of all conformations containing helix. Lifetimes of the order of Δt_1 represent rapidly exchanging species; whereas Δt_2 corresponds to slow exchange on the nmr time scale. Qualitatively we would expect two nmr peaks, one for the long-lived totally random-coil conformation and another which arises collectively from all other short-lived, helix-containing, conformations. These conclusions are in agreement with the experimental results.[2a,3]

Conformational Lifetimes in the Helix–Random-Coil Transition Region by Nuclear Magnetic Resonance with Application to Poly(γ-benzyl L-glutamate)[1]

J. A. Ferretti* and R. L. Jernigan

Physical Sciences Laboratory, Division of Computer Research and Technology, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Maryland 20014.
Received March 26, 1973

ABSTRACT: Nuclear magnetic resonance spectra of three poly(γ-benzyl L-glutamate) samples of $\overline{DP}_w$ of 55,280 and 910 have been studied in the helix–random-coil transition region. Double peaks whose behavior is chain-length dependent are observed for the α-CH proton resonance. A two-site model for systems undergoing chemical exchange is used to obtain lifetimes and to fit the behavior of the two peaks throughout the transition region. These results are in good agreement with theoretical spectra calculated on the basis of a description of the time-dependent behavior of the transition.

High-resolution nuclear magnetic resonance (nmr) spectroscopy is often used to study the helix–random-coil transition in synthetic polypeptides.[2a,b,3] In a number of examples such as poly(γ-benzyl L-glutamate,[4] poly(L-alanine,[5] and poly(L-arginine),[6] the α-CH and peptide NH protons each show two peaks in the transition region. The detailed behavior of these separate peaks depends on the polypeptide, solvent conditions, and the chain length of the polymer. A variety of explanations have been suggested for this observation of separate peaks. J. H. Bradbury *et al.*[7] and Tam and Klotz[8] considered slow protonation of amide residues; Scheraga *et al.*[9] proposed slow solvation; Nagayama and Wada[10], E. M. Bradbury *et al.*,[11] and Ullman[12] suggest that polydispersity together with a variation in helicity as a function of monomer placement is the cause of the separate peaks. Goodman *et al.*[13] indicate that the double peaks arise from the presence of low molecular weight polypeptide oligomers. Previous investigations in our laboratory have led us instead to conclude that the two peaks are a result of slow nucleation of helix from randomly coiled peptide units.[14,15]

The observation of two separate nmr peaks for a given proton implies that only two conformations or two groups of conformations exist for times as long or longer than 10^{-3}–10^{-1} sec;[16] all other exchanges occur more rapidly. This slow rate determined by nmr differs from relaxation times measured by other techniques. Kinetic measurements such as temperature jump and ultrasonic relaxation techniques utilized in the transition region yield times in the range 10^{-5}–10^{-8} sec.[17-19] Theoretical investigations have been presented,[14,15,20] one in the previous paper,[15] to explain the kinetic and nmr experiments. These models of the transition demonstrate that the observation of two different time scales is not an inconsistency. In terms of these theories the experimentally measured fast times are related to the time for adding to or melting one helix unit

(1) J. A. Ferretti and R. L. Jernigan, paper presented to the Division of Polymer Chemistry, 164th National Meeting of the American Chemical Society, New York, N. Y., Sept 1972; *Polym. Prepr., Amer. Chem. Soc., Div. Polym. Chem.*, 13, 946 (1972).
(2) (a) J. A. Ferretti, *Chem. Commun.*, 1030 (1967). (b) E. M. Bradbury, C. Crane-Robinson, H. Goldman, and H. W. E. Rattle, *Nature (London)*, 217, 812 (1968).
(3) L. Paolillo, P. A. Temussi, E. M. Bradbury, and C. Crane-Robinson, *Biopolymers*, 11, 2043 (1972).
(4) J. A. Ferretti and B. W. Ninham, *Macromolecules* 3, 30 (1970).
(5) J. A. Ferretti and L. Paolillo, *Biopolymers*, 7, 155 (1969).
(6) M. Boublik, E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Eur. J. Biochem.*, 12, 258 (1970).
(7) J. H. Bradbury and M. D. Fenn, *Aust. J. Chem.*, 22, 357 (1969).
(8) J. W. O. Tam and I. M. Klotz, *J. Amer. Chem. Soc.*, 93, 1313 (1971).
(9) F. J. Joubert, N. Lotan, and H. A. Scheraga, *Biochemistry*, 9, 2197 (1970).
(10) K. Nagayama and A. Wada, *Chem. Phys. Lett.*, 16, 50 (1972).
(11) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, 11, 277 (1970).
(12) R. Ullman, *Biopolymers*, 9, 471 (1970).
(13) M. Goodman, F. Toda, and N. Ueyama, *Proc. Nat. Acad. Sci. U. S.*, 70, 331 (1973).
(14) J. A. Ferretti, B. W. Ninham, and V. A. Parsegian, *Macromolecules*, 3, 34 (1970).
(15) R. L. Jernigan, J. A. Ferretti, and G. H. Weiss, *Macromolecules*, 6, 684 (1973).
(16) J. W. Emsley, J. Feeney, and H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1967, p 481.
(17) R. Lumry, R. Legare, and W. G. Miller, *Biopolymers*, 2, 434 (1964).
(18) G. G. Hammes and P. B. Roberts, *J. Amer. Chem. Soc.*, 91, 1812 (1969).
(19) A. Wada, T. Tanaka, and H. Kihara, *Biopolymers*, 11, 587 (1972).
(20) W. G. Miller, *Macromolecules*, 6, 100 (1973).

from an existing helical sequence; whereas the nmr data give the times required for the formation of an initial helical sequence from a random-coil segment of the polypeptide. On this basis the predicted nmr spectra agree well with experiment.

Although the separate peaks have been reported for a number of polypeptides, the related lifetimes, τ, have only been reported to be greater than 10^{-2} sec. If the magnitudes of τ are in the range 10^{-3}–10^{-1} sec, then their values may be determined by the total line-shape analysis (tls) of the spectra. In this paper we evaluate the lifetimes associated with the separate peaks of the α-CH proton resonance of poly(γ-benzyl L-glutamate) $(BzlGlu)_n$ in the helix-random-coil transition region. Nmr spectra were measured under various conditions for $(BzlGlu)_n$ with degrees of polymerization, $\overline{DP}_w$, of 55, 280, and 910. Lifetimes were evaluated by the tls method using a modified Gutowsky–Holm equation[16] for exchange between two sites with unequal spin–spin relaxation times. The results are compared with predictions based on the model calculations for the transition, wherein these sites are identified with (A) the totally random-coil conformation and (B) all other polypeptide conformations which contain at least one helix unit.

Experimental Section

The $(BzlGlu)_n$ samples were purchased from Miles-Yeda Ltd. The weight-average molecular weights (M_w) were determined from viscosity measurements in dichloroacetic acid using a Cannon-Ubbelohde D-552 dilution viscometer.[21] The values for $\overline{M}_w$ were found to be 12,000 63,000, and 200,000 and correspond to degrees of polymerization of 55, 290, and 910, respectively. A separate investigation has shown the sample of DP $\simeq$ 55 to be relatively monodisperse.[21] The trifluoroacetic acid used in the nmr experiments was from a fresh bottle stored in a dry box. Spectroscopic grade deuteriochloroform was used. Solutions of $(BzlGlu)_n$ were 4% by weight.

The spectra were obtained on an HR-220 Varian nmr spectrometer which was equipped with a variable-temperature probe. The ambient temperature at the receiver coil was about 21°. Spectra were gathered at 37 and 67° as well as at ambient temperature.

Computations were performed on a Digital Equipment Corp. PDP-10 with a digital display.

Results

Exchange Model. Only if the exchange rates were less than the chemical shift differences between the two peaks, would fitting with two Lorentzian lines be appropriate. Initial attempts to analyze the $(BzlGlu)_n$ spectra with the assumption of very slow exchange rates (*i.e.*, fitting the data to two Lorentzian lines gave poor agreement with experiment). When the lifetimes τ (reciprocal exchange rates) are of the order of the inverse of the chemical shift difference ($\tau \simeq \Delta\omega^{-1}$), the lineshape function is complicated. However, the values of τ may be evaluated by the tls method. The behavior exhibited by the $(BzlGlu)_n$ spectra (Figures 1–5) suggests lifetimes similar to the chemical shift difference between the two peaks. First we consider the results on the 12,000 molecular weight $(BzlGlu)_n$.

The two-site model of Gutowsky and Holm is the simplest method available for describing the nmr spectra of systems undergoing chemical exchange. The two sites will be identified with (A) the completely random-coil conformation and (B) all other conformations which contain at least one helix unit (*vide infra*). Gutowsky and Saika[22] have given the frequency dependence of the intensity of the absorption spectrum. For the case in which the line widths in the absence of exchange for the two sites A and B are unequal, the intensity $I(\omega)$ is given by

$$I(\omega) = \frac{\omega_1 M_0\{[1 + \tau(P_A T_{2A}^{-1} + P_B T_{2B}^{-1})]L + QN\}}{L^2 + N^2}$$

where

$$L = \tau\{T_{2A}^{-1}T_{2B}^{-1} - (\omega - \omega_A)(\omega - \omega_B)\} + P_A T_{2A}^{-1} + P_B T_{2B}^{-1}$$

$$Q = \tau[(1/2)(\omega_A + \omega_B) - \omega - (1/2)(P_A - P_B)(\omega_A - \omega_B)]$$

$$N = (\omega_A T_{2B}^{-1} + \omega_B T_{2A}^{-1})\tau - \omega[1 + \tau(T_{2A}^{-1} + T_{2B}^{-1})] + P_A\omega_A + P_B\omega_B \quad (1)$$

The lifetime τ is defined in terms of the lifetimes τ_A and τ_B of the molecules in sites A and B such that $1/\tau = 1/\tau_A + 1/\tau_B$. The chemical shifts for molecules in sites A and B in the absence of exchange are given by ω_A and ω_B. The parameters P_A and P_B refer to the fractional populations of sites A and B, respectively. The values T_{2A} and T_{2B} are the nuclear spin relaxation times associated with A and B. The factor $\omega_1 M_0$ is a measure of the induced magnetization.

Nmr Spectra. In Figure 1a are the α-CH proton nmr spectra at ambient temperature over the helix–random-coil transition region for the $(BzlGlu)_n$ sample with 12,000 molecular weight ($\overline{DP}_w \simeq 55$). Similar spectra at 67° for the same sample are shown in Figure 2a. The transition from helix to random coil was induced by increasing the CF_3COOH concentration in the $CDCl_3$–CF_3COOH mixed-solvent pair. In the manner which has been demonstrated to be typical of low molecular weight polypeptides (*e.g.*, $\overline{DP}_w \leq 100$), the lower field peak increases in intensity with a corresponding decrease in the intensity of the higher field peak. Also the chemical shift difference, $\Delta\omega$ ($\Delta\omega = \omega_A - \omega_B$), does not change appreciably over the transition region.

Careful examination of these nmr spectra for the 12,000 molecular weight $(BzlGlu)_n$ suggests an exchange rate of the order of magnitude of $\Delta\omega$. Analysis of the spectra according to eq 1 for appropriate values of P_A and P_B gave good agreement with experiment. Computed spectra (Figures 1b and 2b) were generated for the two sets of data with $T_{2A} = 0.020$ sec and $T_{2B} = 0.016$ sec (which correspond to 16 and 20 Hz, respectively, for line widths in the absence of exchange). These values of T_{2A} and T_{2B} were chosen by measuring the line widths at the two extremes of the transition (*i.e.*, completely helical and completely random coil). Values for the average lifetime τ and the chemical shift difference $\Delta\omega$ were chosen to give computed curves which resembled qualitatively the experimental spectra. The agreement between the experimental results and the computed spectra is good. The magnitude of τ is in the range of 10^{-2} sec. Values obtained for τ, P_A, and $\Delta\omega$ for each spectrum are shown with the figures.

In Figure 3 the experimental and computed spectra for nearly equal peak heights are shown for 21, 37, and 67°. Because the helix content in $(BzlGlu)_n$ increases with increasing temperature, it is possible to maintain a constant helix content with increasing temperature by also increasing the CF_3COOH concentration in the CF_3COOH–$CDCl_3$ mixed solvent. The agreement with experiment is good and the results show a small but significant decrease in τ with increased temperature.

Theoretical spectra were calculated on the basis of a model description of the time-dependent behavior of the helix–random-coil transition.[15] The premise there is that nucleation times for helix formation within completely random-coil molecules are longer than lifetimes of any

(21) J. B. Milstien and J. A. Ferretti, *Biopolymers*, in press.
(22) H. S. Gutowsky and A. Saika, *J. Chem. Phys.*, 21, 1688 (1953).

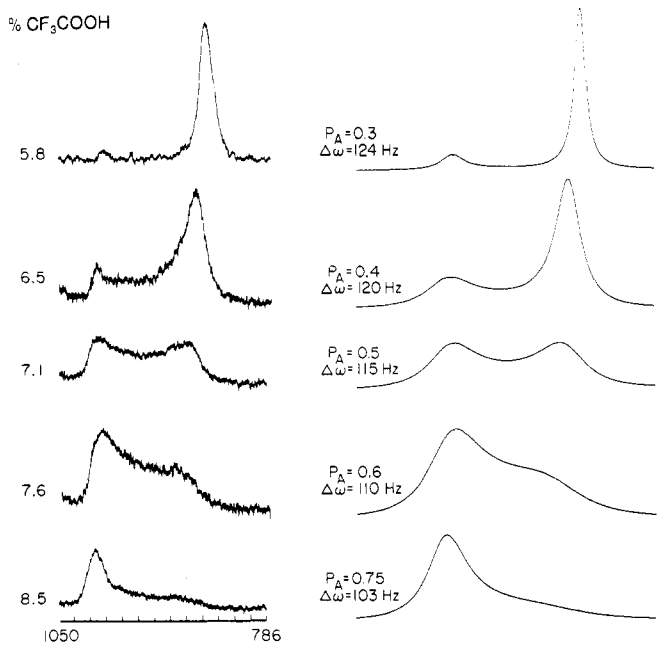

Figure 1. Experimental nmr spectra (left) at 19° of the α-CH proton resonance of $(BzlGlu)_n$ ($\overline{DP}_w$ = 55) and the computed fits (right) with eq 1. The values of the average lifetime τ were from top to bottom 0.015, 0.013, 0.009, 0.008, and 0.007 sec.

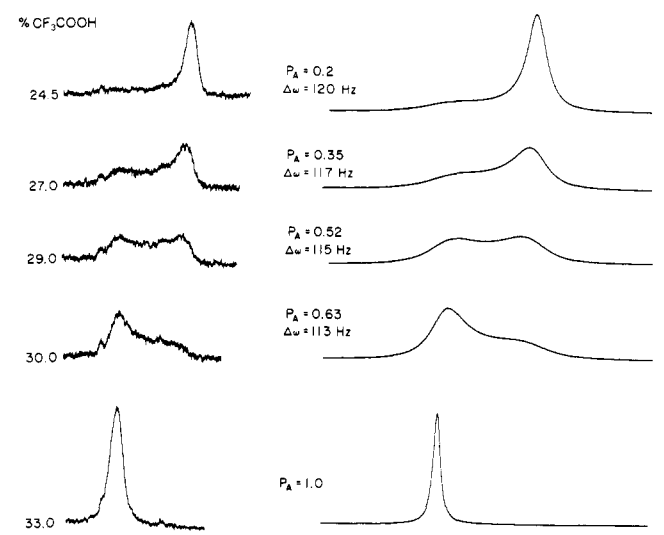

Figure 2. On the left are the experimental nmr spectra of $(BzlGlu)_n$ ($\overline{DP}_w$ = 55) at 67°, and on the right are the fitted results with eq 1 and a single value of τ = 0.005 sec.

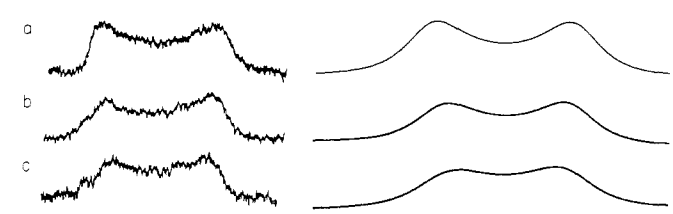

Figure 3. Temperature dependence of the line shape at the midpoint of the helix-random-coil transition for $\overline{DP}_w$ = 55 for $(BzlGlu)_n$. From top to bottom the results are τ = 0.009 sec at 19°, τ = 0.007 at 37°, and τ = 0.005 sec at 67°.

peptide units in a helix state. If the time for uncoiling or adding one helix unit to an existing helical sequence is less than 10^{-6} sec,[20] those calculations imply that lifetimes as helix for all units in the chain are too short to allow detection of any specific helix-containing configuration by nmr (*i.e.*, their lifetimes are short on the nmr time scale). Our conclusions are that nucleation from random-coil molecules is the rate which is interpreted from the line-shape analysis and the helix peak represents an average over all molecules containing one or more helix units. For a given helix content the chemical shift of this helix peak will be a function of chain length by virtue of the equilibrium statistics.[15] Although this model considers only a single helical sequence, the effect of multiple sequences will be to further shorten the helix unit lifetimes. Also the qualitative effect on the chemical shift for different chain lengths will be similar for either a single sequence or multiple sequences.

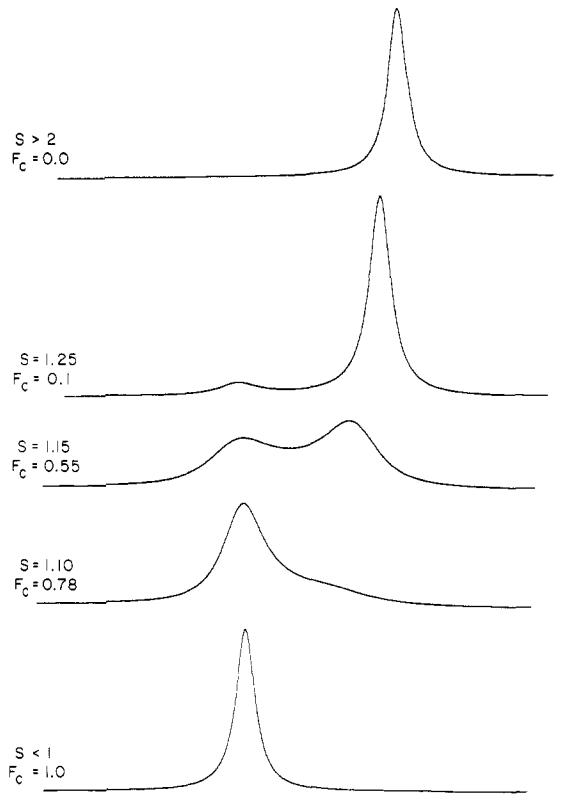

Figure 4. Theoretical spectra of the α-CH proton resonance for n = 55 and $\sigma = 10^{-4}$ with the fractions of helix and random coil computed using eq 2-4. The spectra were generated with the exchange model of eq 1 for a value of τ = 0.009 sec.

For a single sequence of helix the fraction of completely random-coil molecules for a chain of length n with $n - 2$ hydrogen bonds is given by F_c where

$$F_c = [1 + \sigma\sum_{j=1}^{n}(n - j + 1)s^j]^{-1} \qquad (2)$$

Here σs is the statistical weight for a first helix unit and s is the statistical weight for additional helix units. The chemical shift of the random coil is always ω_c. For those molecules which contain some helix the chemical shift $\omega(n,s)$ is given by

$$\omega(n,s) = \omega_H P_H + \omega_c P_c \qquad (3)$$

where

$$P_H = [\sum_{j=1}^{n} j(n - j + 1)s^j]/[n\sum_{j=1}^{n}(n - j + 1)s^j] \qquad (4)$$

ω_H is the chemical shift of a helical unit, P_H is the fraction of helix in molecules which contain at least one helix unit, and $P_c = 1 - P_H$. Theoretical spectra where eq 2-4 were used to determine the fraction of helix and the chemical shifts of the helix peak are shown in Figure 4. Equation 1 was used to generate the spectra where F_c was taken as P_A, $1 - F_c$ as P_B, ω_A as ω_c, and $\omega(n,s)$ as ω_B. Values of τ were chosen to be similar to those found by

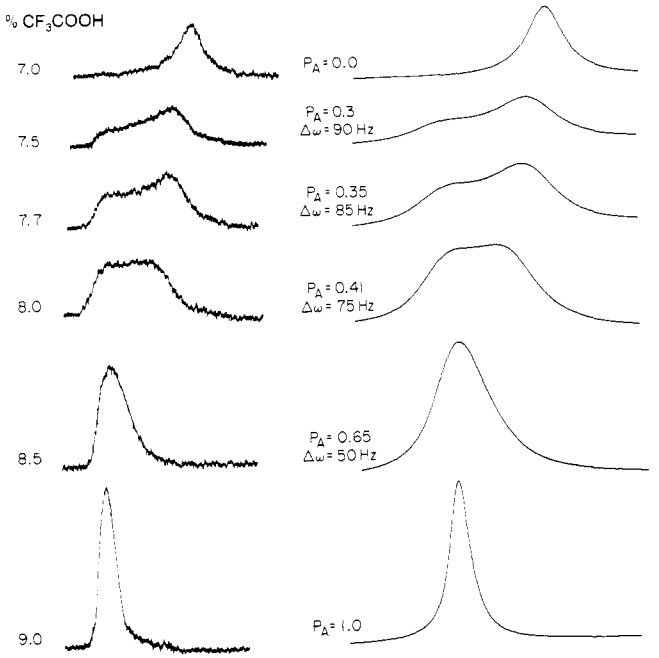

Figure 5 Experimental (left) and fitted spectra (right) of the α-CH proton resonance of $\overline{DP}_w$ = 280 for $(BzlGlu)_n$. A single value of = 0.008 sec was used to fit these data (eq 1).

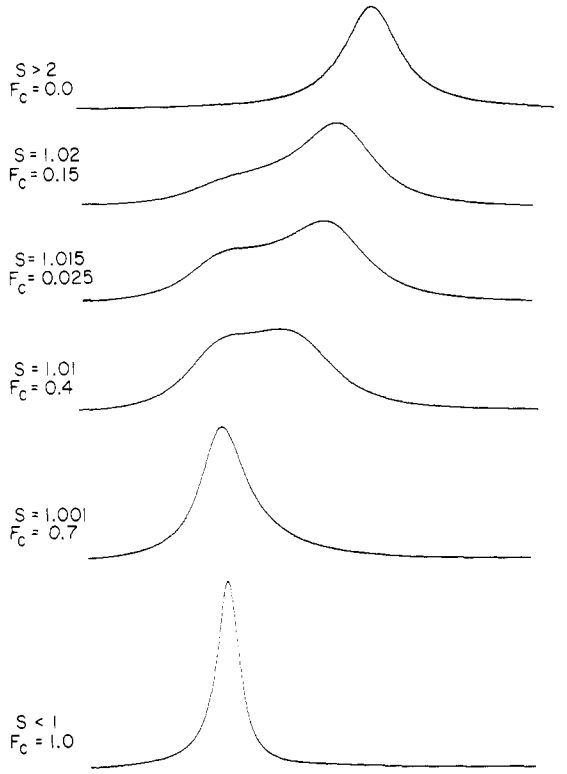

Figure 6. Theoretical spectra of the α-CH proton resonance computed throughout the helix–random-coil transition region (eq 2–4) for n = 280 and $\sigma = 10^{-4}$. Spectra were generated with eq 1 and τ = 0.009 sec.

fitting the experimental results, although no attempts were made to obtain quantitative agreement with the data. The major purpose of this calculation of spectra is to demonstrate the qualitative fit between the experimental spectra and the theoretical ones.

In Figure 5 are displayed the experimental spectra of the α-CH proton resonance for 63,000 ($\overline{DP}_w \simeq 280$) molecular weight $(BzlGlu)_n$ together with the spectral fits using eq 1. As in the case for the 12,000 molecular weight material, values of τ, ω_A, and ω_B were chosen to give computed curves which qualitatively simulate the experimental data. For these results, T_{2A} and T_{2B} were chosen from the line widths observed at the two extremes of the transition. The respective values were 0.016 and 0.008 sec. The random-coil peak does not shift appreciably in going through the transition region but merely changes intensity. The helix peak on the other hand shifts toward the random-coil peak and decreases in intensity as the acid concentration is increased.

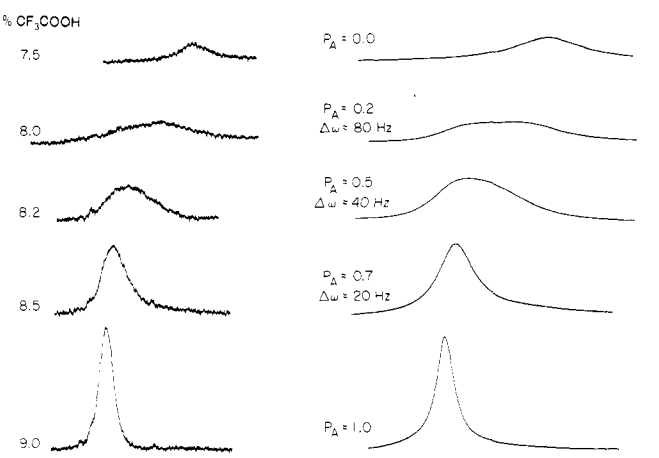

Figure 7. On the left are the experimental spectra at 19° of $\overline{DP}_w$ = 910 for $(BzlGlu)_n$ over the helix–random-coil transition region. The results obtained by fitting these spectra with eq 1 and τ = 0.008 are shown on the right.

Theoretical spectra (Figure 6) for various values of F_c and ω_{obsd} were generated for representative values of s over the helix–random-coil transition region. Comparison of these results of 63,000 molecular weight material with those on 12,000 molecular weight $(BzlGlu)_n$ shows that the shift of the helix peak through the transition region is predicted by the model (eq 3 and 4). In terms of the model this shift of the helix peak is more pronounced for longer chains because the transition is sharper and the midpoint is closer to s = 1 than for the shorter chain.

The results for $(BzlGlu)_n$ of mol wt 200,000 ($\overline{DP}_w$ 910) are shown in Figure 7. Careful examination of the spectra in the transition region shows a noticeable and reproducible asymmetry in the line shape, although two separate peaks are not resolved there. Using the same procedure as above for $\overline{DP}_w$ = 55 and 280 for $(BzlGlu)_n$, these spectra were fit with the exchange model of eq 1 for values of T_{2A} and T_{2B} equal 0.016 and 0.004 sec, respectively. (These values represent line widths of 16 and 80 Hz for the random coil and the helix.) The separate peaks are not resolved because the line widths of the helix peak are large and, in the transition region, the shift of the helix peak toward the random-coil peak is quite pronounced for this long-chain polypeptide. Nevertheless, it was not possible to obtain a good fit of these data with any simpler model with only a single Lorentzian line. On the basis of the goodness of the fit over a wide range of molecular weights, we conclude that the exchange model is appropriate to explain our data.

The theoretical spectra of $\overline{DP}_w$ = 910 for $(BzlGlu)_n$ (Figure 8) agree well with the experimental data (Figure 7). The theory predicts that the shift of the helix peak toward the random-coil peak in going through the transition is more pronounced for longer chain lengths. Although the model of eq 2–4 is not strictly valid for chains of length 910, it is still interesting to compare the predictions of this model with experiments. For all chain lengths the model predicts that the frequency of the random-coil peak remains constant. For long-chain polypeptides the contribution to the random-coil peak over most of the

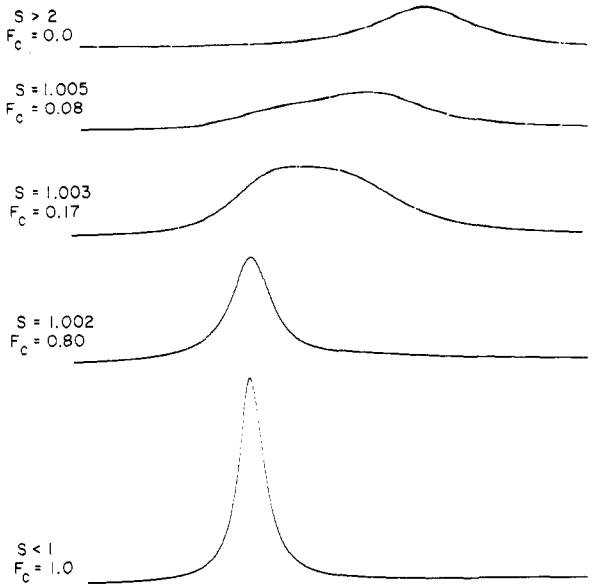

Figure 8. For n = 910 and $\sigma = 10^{-4}$, theoretical spectra were computed using eq 2-4 to evaluate the fraction of helix and random coil for the α-CH proton resonance over the helix–random-coil transition region. Spectra were generated with eq 1 and τ = 0.009 sec.

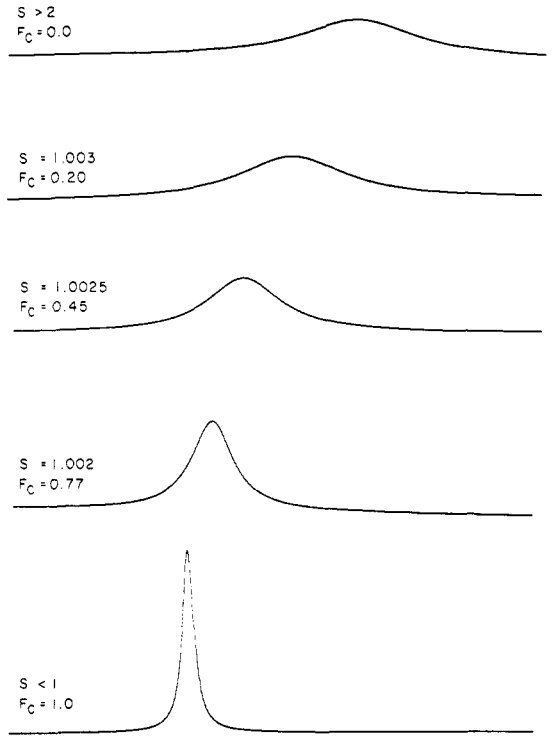

Figure 9. For $\sigma = 10^{-3}$, n = 1000, and τ = 0.001 sec, theoretical spectra were computed for the α-CH proton resonance over the helix–random-coil transition region using eq 1–4.

helix–random-coil transition region may be quite small. Under these conditions it might not be possible to resolve the separate peaks.

Discussion

Lifetimes and Nmr Spectra. Experimental nmr spectra were presented for $(BzlGlu)_n$ samples of three different average chain lengths. For these samples it was possible to fit the spectra to a two-site Gutowsky–Holm equation which was modified to take into account unequal linewidths. This analysis of the data showed that the chemical shift of the "helix" peak is dependent on chain length in the helix–random-coil transition region. This chemical shift behavior is in agreement with that predicted by model calculations of the time-dependent conformational properties of polypeptides in the transition region. The helix peak shifts toward the random-coil peak for higher molecular weights during the transition because of the equilibrium statistics and the variation of the transition region in s with chain length. For short chains where $s > 1$ over most of the transition region, the all-random-coil form contributes significantly to the population. Consequently the helix-containing conformations (*i.e.*, those which make up the "helix" peak) do not change appreciably in the transition region. However for longer chains where $s \simeq 1$ in the transition region, the contribution of the all-random-coil conformation is less significant. Here the distribution of helix-containing conformations changes with s with a resultant shift of the "helix" peak.

An important feature of this study of $(BzlGlu)_n$ is the measurement, at equilibrium, of lifetimes of the order of 10^{-2} sec. These times are interpreted in terms of the rate of nucleation of helix from completely random-coil molecules. Lifetime (or rates) of this magnitude are longer than have been previously expected for the helix–random-coil transition. Nevertheless, the measurement of such slow rates (long lifetimes) does not contradict the results obtained from relaxation experiments where shorter times ($\sim 10^{-5}$ to 10^{-8} sec) are measured. Propagation times or step times for the formation of additional helical units (and also for the opposite process of helix units changing to random-coil units) may be derived from these faster rates. Helix propagation times are expected to differ from nucleation times by a factor of the order of σ (*e.g.*, 10^{-4}). Therefore, for step times in the range of 10^{-6} sec, nucleation times of 10^{-2} sec would be expected.

To show acceptable agreement between the computed and experimental $(BzlGlu)_n$ spectra, it is necessary to treat the all-random-coil conformation as a separate contribution (eq 2) with a lifetime longer than the longest lived helical unit. The observation of separate peaks appears to be more common for polypeptides dissolved in mixed organic solvents such as $CDCl_3$ and CF_3COOH than in water solutions. Separate peaks are generally not observed for water-soluble polypeptides. This would be the case if $\tau \leq 0.1/\Delta\omega$ (eq 1). For example, in aqueous solutions of poly(L-glutamic acid)[23] where $\sigma = 10^{-3}$, separate peaks have never been observed.[24] Theoretical spectra (Figure 9) computed for n = 1000 and using a value of τ (eq 1) of 10^{-3} show only a single peak over the entire helix–random-coil transition region. These results demonstrate that the nmr spectra are quite sensitive to the values of both n and τ.

Comparison with Other Models. The measurement by nmr of rates different from those obtained with other relaxation techniques has intrigued many investigators. The arguments of J. H. Bradbury *et al.*[7] and also Tam and Klotz,[8] which rely on protonation of amide residues to induce the helix–random-coil transition, are inconsistent with CD and ORD data.[25,26] However, there is some evidence[27] that some small amount of protonation may occur. By the CD and ORD techniques it has been demonstrated that the majority of amide groups are not protonated during the transition. Furthermore, if the amide group were protonated one would expect the peptide NH resonance chemical shift to be sensitive to acid concentra-

(23) R. L. Snipp, W. G. Miller, and R. E. Nylund, *J. Amer. Chem. Soc.*, 87, 3547 (1965).
(24) E. M. Bradbury, C. Crane-Robinson, H. Goldman, and H. W. E. Rattle, *Biopolymers*, 6, 851 (1968).
(25) F. Quadrifoglio and D. Urry, *J. Amer. Chem. Soc.*, 90, 2755 (1967).
(26) F. A. Bovey, *Rev. Pure Appl. Chem.*, 16, 417 (1968).
(27) J. H. Bradbury and H. H. H. Yuan, *Biopolymers*, 11, 661 (1972).

tion because the chemical shift of a protonated amide group should differ from that of an unprotonated amide group by several parts per million. No such chemical shift dependence has been reported.

The proposal of slow solvation as the cause of the separate peak by Scheraga *et al.*[9] has been shown by E. M. Bradbury *et al.*[28] to be inconsistent with the nmr data. He pointed out that for a slow solvation mechanism the relative area of the "solvated coil" peak should increase with addition of acid because all the random-coil residues will eventually contribute. In fact, the integrated areas of the two peaks remain constant.

Goodman *et al.*[13] in a recent study suggested that the separate peaks arise from the presence of oligomers in the polypeptide samples. However, extension of Goodman's explanation would require invocation of reversible change in oligomer content during transition.

One explanation which has gained some credence is that of Ullman,[12] E. M. Bradbury *et al.*, and Nagayama and Wada,[10] who indicate that the nmr results might arise from polydispersity. We have examined this hypothesis in detail in a recent paper[21] and determined that fractionation of the polypeptide samples does not significantly alter the nmr spectra. The mutliple peaks are present even for samples with very narrow molecular weight distributions. These results indicate that polydispersity cannot be used to explain the nmr data. However, similar experiments by Nagayama and Wada led to one broad peak near the transition midpoint. The origin of this discrepancy is unknown at present. One possible explanation is that both polydispersity and exchange broadening (*i.e.*, slow nucleation) contribute to the line shape in the transition region. To envision this particular possibility it is helpful to consider the exchange broadening and polydispersity effects separately. Neglecting polydispersity, one might conceive of the situation wherein the line width near the transition midpoint would be exchange broadened but not split into two peaks (*i.e.*, above coalescence). Then the effect of polydispersity on this broadened line could be the cause of peak doubling, depending on the nature of the molecular weight distribution. The precise form of the lineshape would be dependent on the distribution and could explain the differences between the results of ours and Wada's experiments. In this case the magnitude of τ determined in the previous section might be overestimated by as much as a factor of two.

The explanation in this paper requires a helix nucleation time τ long enough to lead to two separate nmr peaks. The alternative explanation based on polydispersity also gives satisfactory agreement with experiment for the same chain-length polypeptide. However, the polydispersity advocates have not explained the observed behavior for the longer chain lengths. An independent determination of τ is therefore of the greatest importance in distinguishing between these two alternate interpretations of the nmr double peak behavior.

(28) E. M. Bradbury, P. Cary, C. Crane-Robinson, L. Paolillo, T. Tancredi, and P. A. Temussi, *J. Amer. Chem. Soc.*, **93**, 5916 (1971).

Dynamics of Polymers as Structurally Disordered Systems. Vibrational Spectrum and Structure of Poly(tetrafluoroethylene)[1]

Giuseppe Zerbi* and Mario Sacchi

Istituto di Chimica delle Macromolecole del CNR, Milan 20133, Italy. *Received March 21, 1973*

ABSTRACT: The problem of the structure and vibrational spectrum (infrared, Raman, neutron scattering) of poly(tetrafluoroethylene) (PTFE) considered as a solid containing structurally ordered and disordered regions is treated in two works, one following the other. In paper I the theoretical aspects of the problem are considered and the calculation of the vibrational spectrum is carried out first using the classical lattice dynamical approach on a perfect system. In these calculations phonon dispersion curves $\omega(k)$, density of states $g(\omega)$ and $k = 0$ phonon frequencies are calculated for several models of PTFE chain which should be most stable ones on the basis of theoretical considerations. Lattice dynamical calculations are then performed on the polymer chain considered as a disordered system. Predictions are derived where to look in the vibrational spectrum for absorption in infrared or scattering in the Raman originating from geometrical defects. The results from calculations discussed in paper I, together with the experimental verification discussed in paper II, provide new information for a more comprehensive understanding of the experimental data from X-ray scattering, nmr broad line, dielectric and mechanical experiments. A detailed interpretation of the vibrational spectrum is suggested.

In a previous paper[2a,b] it has been shown that the analysis of the vibrational spectrum of a polymer as a geometrically disordered system based on a calculation of the density of states $g(\omega)$ by numerical methods seems to provide a more satisfactory interpretation of the observed spectral features. From this type of analysis structural information was derived.[2a]

In this paper (paper I) we study with the same technique and with standard methods the lattice dynamics of poly(tetrafluoroethylene) [$(CF_2)_\infty$, PTFE], which, from the available literature, seems to contain disordered regions. The theoretical predictions derived in paper I are compared with the experimental findings in the following paper (paper II). The purpose of our work is twofold: (i) to further analyze the validity of the method we have proposed and complete the interpretation of the spectrum of PTFE; (ii) to derive information on the structure of PTFE as seen from the vibrational spectrum.

The conclusions seem to us quite gratifying. Conclusive evidence is collected for the existence of chain structures predicted theoretically but never experimentally verified. A mechanism for the phase transition at 19°C is proposed. Information is collected for a further reanalysis of recent nmr and dielectric experiments. An alternative explana-

(1) This work was partly presented at the IUPAC International Symposium on Macromolecules. Helsinki, July 1972.

(2) (a) G. Zerbi, L. Piseri, and F. Cabassi, *Mol. Phys.*, **22**, 241 (1971). (b) G. Zerbi, *Pure Appl. Chem.*, **26**, 499 (1971).