barrier; so the forward rates are

$$\alpha(g_i s_i, g_j s_j) = \alpha_0 \mu(g_j) \exp[-\beta(g_j \epsilon_1 + g_i \epsilon_2 + s_j \epsilon_3)] p(s_j|s_i) \exp(-\beta Q_f) \quad (A.1)$$

while the backward rate constants are

$$\beta(g_i s_i, g_j s_j) = \alpha_0 \exp(-\beta n \Delta F_0) \quad (A.2)$$

This model leads to a simpler solution than in the article. If we write the λ's in the form

$$\lambda(g_j s_j) = \alpha_0 \exp[-\beta(n\Delta F_0 + g_j \epsilon_2)] u \quad (A.3)$$

then the set of eq 4.3 reduces to a single equation for u as a function of n

$$1 = \exp[\beta(n\Delta F_0 - Q_f)] J_n(u, \epsilon_2, \epsilon) \quad (A.4)$$

with

$$J_n(u, x, y) = \sum_{m=0}^{n} \frac{\mu(m) e^{-\beta y m}}{1 + u e^{-\beta x m}} \quad (A.5)$$

$$\epsilon = \epsilon_1 + \epsilon_2 + \epsilon_3 \quad (A.6)$$

There is a positive solution for u only when the crystal is thick enough to be stable, $n > n^*$.

We omit the details of the derivation of eq A.4 and subsequent results. A key step is the proof that $p(s_j|s_i)$ is a function of s_j only.

The current, for a fixed n, is found to be

$$S_{Tn} = N_0 \alpha_0 u e^{-\beta n \Omega} \{1 - x + x \exp[-\beta(\epsilon_1 + \epsilon_2)]\}^n \frac{J_n(u, \epsilon_2, \epsilon)}{J_n(u, \epsilon_2, \epsilon_1 + \epsilon_2)} \quad (A.7)$$

This flux turns out to behave in similar ways to the flux in the principle model.

The B content in the crystalline region, for a given n, is

$$\langle m \rangle_n = n x e^{-\beta \epsilon} \frac{J_{n-1}[u \exp(-\beta \epsilon_2), \epsilon_2, \epsilon]}{J_n(u, \epsilon_2, \epsilon)} \quad (A.8)$$

The concentration c may be determined by weighted summing of eq A.8 over n, according to eq 4.26. While this summation cannot be performed analytically, one can show that c is bounded by

$$\frac{x \exp[-\beta(\epsilon_1 + \epsilon_3)]}{1 - x + x \exp[-\beta(\epsilon_1 + \epsilon_3)]} > c > \frac{x e^{-\beta \epsilon}}{1 - x + x e^{-\beta \epsilon}} \quad (A.9)$$

The lower limit is the equilibrium value achieved at small supercoolings in this model, and the main model, when growth is slow. The upper value is that determined by the forward rate constants alone and is achieved when the flux is so rapid as to prevent any detachment. For the previous model the upper limit was the amorphous composition, x. The placement of the concentration between upper and lower limits in both models is effected in similar ways by variation of physical parameters, because in both cases it is a question of the extent of kinetic control *vs.* equilibration.

Nuclear Magnetic Resonance Relaxation Study of Poly(γ-benzyl L-glutamate) Side-Chain Mobility in Helix-Coil Transition[1a]

Mingjien Chien,[1b] E. T. Samulski,[1c] and Charles G. Wade*

Department of Chemistry, The University of Texas, Austin, Texas 78712.
Received January 3, 1973

ABSTRACT: Nmr spin-lattice relaxation times T_1 of F_3CCOOH proton and the side-chain phenyl protons of poly(γ-benzyl L-glutamate) ($(BzlGlu)_n$) were measured in $(BzlGlu)_n$-F_3CCOOH-$CDCl_3$. T_1 was affected by the solvent-induced helix-coil transition. F_3CCOOH proton T_1 behavior was interpreted by a two-site model, *i.e.*, F_3CCOOH molecules bind to the $(BzlGlu)_n$ or are free in solution. The T_1 values for the phenyl ring terminating the $(BzlGlu)_n$ side-chain increase before the onset of the helix-coil transition. This increase in side-chain mobility is discussed in terms of the effects of polydispersity and the concept of a side-chain secondary structure.

Studies with synthetic polypeptides have provided new insights into the understanding of protein structure. In the last 25 years a major part of the concentrated and sustained investigation of synthetic polypeptides has concerned the helix-random-coil transition in dilute solution. The literature contains numerous mechanisms describing how helix-breaking solvents might induce the helix-coil transition. Explanations of helix disruption by halogenated acids range from protonation of[2a,b] and hydrogen bonding with[3] the polypeptide amide group to less specific acid-polymer interactions involving the periphery of the polypeptide,[4] *e.g.*, solvation of side chains destroying helix-stabilizing side-chain–side-chain interactions.

The importance of the outer portions of polypeptide side chains in determining both the stability and precise conformation of the α helix has been recognized for some time. The concept of side-chain secondary structure has evolved; this term implies a regular arrangement of side chains to promote energetically favorable interactions between neighboring side chains and/or to minimize the occurrence of sterically hindered side-chain conformations.

(1) (a) This research is supported in part by the Public Health Service (NIH Grant HE-12528), The Robert A. Welch Foundation, and the Biomedical Sciences Support Grant of the University of Texas. (b) Robert A. Welch Foundation Predoctoral Fellow. (c) Robert A. Welch Foundation Postdoctoral Fellow; Department of Chemistry, University of Connecticut.

(2) (a) S. Hanlon, S. F. Russo, and I. M. Klotz, *J. Amer. Chem. Soc.*, **85**, 2024 (1963). (b) J. H. Bradbury and M. D. Fenn, *Aust. J. Chem.*, **22**, 357 (1969).

(3) W. E. Stewart, L. Mandelkern, and R. E. Glick, *Biochemistry*, **6**, 143 (1967).

(4) "Poly-α-amino Acids," G. D. Fasman, Ed., Marcel-Dekker, New York, N. Y., 1967, Chapter 11.

A number of anomalies in a variety of experimental studies on polypeptides have been ascribed to perturbations of the side-chain secondary structure: optical rotatory dispersion,[5,6] circular dichroism,[7] viscosity,[8,9] dielectric relaxation,[10] electric dichroism,[11] high-resolution nmr,[12,13] and X-ray diffraction in both the liquid crystalline state[14] and solid state.[15,16]

In addition to the above studies, the large effect on helix stability caused by minor structural changes in the side chain[17] indirectly implies a form of side-chain secondary structure. The ester derivatives of poly(L-aspartic acid)[18,19] and N^{ϵ}-substituted polylysines[20] provide striking examples of how minor changes in the outer portion of the side chains produce a major conformational change of the helix backbone. Furthermore, the cooperative specificity of side-chain–side-chain interactions may be inferred from differences in helix stability between copolypeptides with a block sequence and random sequence.[21]

The present contribution describes the results of an nmr study designed to monitor the mobility of the phenyl ring terminating the side chain of poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$) during a solvent-induced helix-coil transition. Nmr relaxation processes are extremely sensitive to variations in the molecular environment. Spin-lattice relaxation in particular is useful for detecting changes in the rotational mobility of a molecule or changes in intramolecular reorientation. The intramolecular dipolar contribution to the spin-lattice relaxation time, T_1, of the i nuclei resulting from rotational diffusion is given by the familiar formula

$$T_1^{-1} = (3/2)\gamma^4\hbar^2\sum_j \tau_c\langle r_{ij}^{-6}\rangle \qquad (1)$$

where γ is the magnetogyric ratio, $\hbar = h/2\pi$, where h is Planck's constant, $\langle r_{ij}\rangle$ is the average inter-proton distance to the neighbors, and τ_c is the correlation time for reorientation of r_{ij}. In the case of macromolecules in solution where the correlation time for molecular rotation is very long (10^{-6} sec), it has been shown that the predominant contribution to T_1 of protons located on the macromolecule mainchain or pendant groups comes from the more rapid intramolecular segmental or sidechain reorientations.[22] We report measured values of T_1 for both the phenyl protons of (BzlGlu)$_n$ and the acid proton of F_3CCOOH in F_3CCOOH–chloroform solutions.

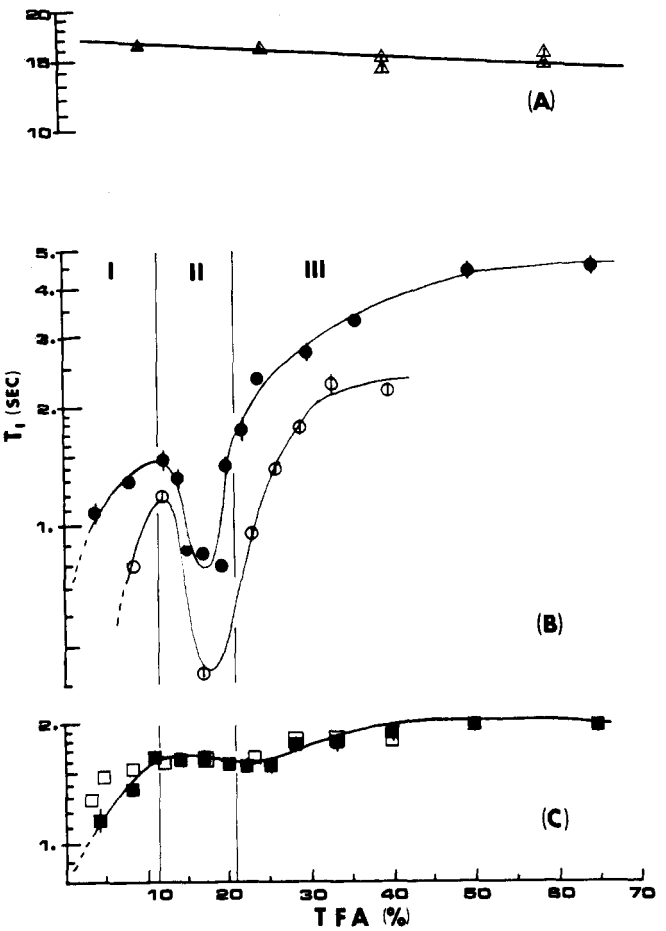

Figure 1. Spin-lattice relaxation times *vs.* per cent F_3CCOOH (TFA): (A) F_3CCOOH–$CDCl_3$ mixture, F_3CCOOH proton T_1; (B) (BzlGlu)$_n$–F_3CCOOH–$CDCl_3$ solution, F_3CCOOH proton T_1; (C) (BzlGlu)$_n$–F_3CCOOH–$CDCl_3$ solution, (BzlGlu)$_n$ phenyl proton T_1. In B and C ●, ■ correspond to solutions with 3.6% (BzlGlu)$_n$ (w/v); ○, □ correspond to solutions with 6.3% (BzlGlu)$_n$ (w/v). At low per cent F_3CCOOH (extrapolated dashed line) T_1 was less than 0.5 sec.

Experimental Section

The sample of (BzlGlu)$_n$ (sample R10, ref 23) has been extensively characterized.[23] The molecular weight determined by viscosity measurements was reported as 25,100. F_3CCOOH purchased from Matheson, Coleman & Bell was distilled once before use. Deuteriochloroform (99.8%) purchased from Stohler Isotope Chemicals was used without further purification.

A special effort was made to remove oxygen from solutions used in the T_1 measurements. Weighed samples of (BzlGlu)$_n$ were degassed in deuteriochloroform solution and then dried. Both F_3CCOOH and deuteriochloroform were degassed separately and transferred to the (BzlGlu)$_n$ under vacuum (10^{-6} Torr). The F_3CCOOH–deuteriochloroform ratio was determined by controlling the quantity of solvent transferred under vacuum from calibrated capillaries.

The T_1 measurements were made using a frequency sweep adiabatic fast-passage technique[24] on a Varian HA-100. Improvements in our external attentuation system allowed us to measure T_1 values as low as 700 msec. It was necessary to signal average polypeptide resonances in the dilute solutions. High-resolution nmr spectra were recorded on a Perkin-Elmer R-12. All measurements were made at 30°.

The helix-coil transition was determined by observing the chemical shift of the C^{α}–H resonance as the F_3CCOOH concentration was increased from 1 to 30% in the (BzlGlu)$_n$–choloroform solution. The chemical shift data indicated the transition oc-

(5) G. D. Fasman in "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., Univ of Wisconsin Press, Madison, Wis., 1962, p 221.
(6) Reference 4, p 516.
(7) D. F. Bradley, M. Goodman, A. Felix, and R. Records, *Biopolymers*, 4, 607 (1966).
(8) P. Doty and J. T. Yang, *J. Amer. Chem. Soc.*, 78, 498 (1956).
(9) J. T. Yang, *Tetrahedron*, 13, 143 (1961).
(10) Reference 4, pp 382-385.
(11) J. B. Milstein and E. Charney, *Biopolymers*, 9, 991 (1970).
(12) D. I. Marlborough, K. G. Orrell, and H. N. Rydon, *Chem. Commun.* 518 (1965).
(13) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. Goldman, *Nature (London)*, 225, 64 (1970).
(14) J. M. Squire and A. Elliott, *Mol. Cryst. Liquid Cryst.*, 7, 457 (1969); *J. Mol. Biol.*, 65, 291 (1972).
(15) R. B. D. Fraser, B. S. Harrap, R. Ledger, T. P. MacRae, F. H. C. Stewart, and E. Suzuki, *Biopolymers*, 5, 797 (1967).
(16) E. T. Samulski and A. V. Tobolsky, *Biopolymers*, 10, 1013 (1971).
(17) H. Obata and H. Kanetsuna, *J. Polym. Sci., Part A-2*, 9, 1977 (1971).
(18) E. H. Erenrich, R. H. Andreatta, and H. A. Scheraga, *J. Amer. Chem. Soc.*, 92, 1116 (1970).
(19) M. Hashimoto, *Bull. Chem. Soc. Jap.*, 39, 2713 (1966).
(20) N. Anand, N. S. R. K. Murthy, F. Naider, and M. Goodman, *Macromolecules*, 4, 564 (1971).
(21) Reference 4, p 530.
(22) J. E. Anderson, K.-J. Liu, and R. Ullman, *Discuss. Faraday Soc.*, 49, 257 (1970).
(23) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, 11, 277 (1970).
(24) M. Chien, E. T. Samulski, and C. G. Wade, *Rev. Sci. Instrum.*, in press.

curred over a range of 12–20% F_3CCOOH. At the midpoint of the helix-coil transition (≈15% F_3CCOOH) two resonances for the C^α–H proton were observed (see Discussion). The F_3CCOOH proton T_1 values as a function of per cent F_3CCOOH are given in Figure 1: pure F_3CCOOH-$CDCl_3$ mixture (Figure 1A) and polymer solutions (Figure 1B). In Figure 1C the $(BzlGlu)_n$ side-chain phenyl proton T_1 values are given; the latter appear insensitive to polymer concentration in the range examined in this study (3–6% $(BzlGlu)_n$ w/v).

Discussion

F_3CCOOH Spin-Lattice Relaxation. The difference between F_3CCOOH proton T_1 values in chloroform and in $(BzlGlu)_n$-chloroform solution can be attributed to interactions between F_3CCOOH and the polypeptide. A general description of these interactions in terms of the F_3CCOOH molecule can be formulated by assuming that the F_3CCOOH spends part of the time free in the liquid phase and the remainder of time bound to "sites" on the polypeptide. Possible sites include the side-chain ester group as well as the peptide amide and carbonyl. The observed spin–lattice relaxation time might then be given by

$$(T_1^{obsd})^{-1} = P_f(T_1^f)^{-1} + P_b(T_1^b)^{-1} \quad (2)$$

where f and b signify the free and bound states, respectively, and $P_f = 1 - P_b$ is the fraction of F_3CCOOH in the free liquid state. In eq 1 $\tau_c^{-1} = (\tau_c^x)^{-1} + (\tau_x)^{-1}$, where x = f, b; τ_x is the lifetime of F_3CCOOH in state x. From eq 2 and the data in Figure 1B we find that $T_1^b < T_1^f$ or from eq 1

$$1/[(\tau_c^f)^{-1} + (\tau_f)^{-1}] < 1/[(\tau_c^b)^{-1} + (\tau_b)^{-1}] \quad (3)$$

With the reasonable assumption that $\tau_c^f \ll \tau_f$ eq 3 implies $(\tau_c^f)^{-1} > (\tau_c^b)^{-1} + (\tau_b)^{-1}$ or $\tau_c^f < \tau_c^b, \tau_b$. This relationship between correlation times would be expected in the case of a strong bond between the F_3CCOOH and polypeptide reducing the F_3CCOOH mobility to that of the mobility of the site on the polymer. The decrease in the F_3CCOOH T_1 values on increasing the $(BzlGlu)_n$ concentration (Figure 1B) is consistent with this idea.

Brussau and Sillescu have presented a quantitative treatment of solvation dynamics in macromolecular solutions.[25] For solutions very dilute in polymer with $P_b \ll P_f$ where the fraction of bound solvent becomes $P_b = NX_M/(1 - X_M)$, where X_M is the molar fraction of monomer units and N is the number of sites for binding solvent per monomer unit, they are able to calculate $(\tau_c^b)^{-1}$ and $(\tau_b)^{-1}$ assuming the relaxation mechanism for the solvent is the same in the free and bound states, *e.g.*

$$\sum_j \langle r_{ij}^{-6} \rangle_b = \sum_j \langle r_{ij}^{-6} \rangle_f$$

We are hesitant to apply this theory to the F_3CCOOH-$(BzlGlu)_n$-chloroform system; the polymer solution is not dilute with respect to F_3CCOOH, the interacting portion of the solvent. Furthermore, P_b will depend on both the association constant for forming the F_3CCOOH-$(BzlGlu)_n$ complex and the self-association constant for F_3CCOOH. From the concentration dependence of the F_3CCOOH proton chemical shift in deuteriochloroform mixtures we find the latter effect important at low concentrations of F_3CCOOH (<5%). Also, it is not obvious that the relaxation mechanism of F_3CCOOH would be the same in the free and bound states.

The abrupt drop in T_1^{obsd} for the F_3CCOOH proton at the helix–coil transition does, however, indicate a substantial change in the F_3CCOOH-$(BzlGlu)_n$ interaction. The minimum in T_1^{obsd} in region II can be accounted for in the following manner. Consider the equilibrium between random-coil polypeptide (P) and F_3CCOOH (A)

$$P + A \overset{K}{\rightleftharpoons} P\cdots A$$

At equilibrium

$$K = \frac{x}{(C_P - x)(C_A - x)}$$

where C_P and C_A are the initial concentrations of polypeptide residues and F_3CCOOH, respectively. The fractions of free and bound F_3CCOOH used in eq 2 are given by $P_f = (C_A - x)/C_A$ and $P_b = x/C_A$. In region III, decreasing C_A causes P_b/P_f to increase, in which case T_1^{obsd} decreases as we find. Across the helix–coil region (region II), the cooperative nature of the transition causes an abrupt decrease in K. Accordingly, as one goes to lower F_3CCOOH concentrations in region II, the fraction of bound F_3CCOOH also abruptly decreases, and T_1^{obsd} rises. The subsequent drop in T_1^{obsd} at very low F_3CCOOH concentrations (region I) is again attributed to an increase in P_b/P_f with decreasing C_A for the second equilibrium between helix and acid which describes F_3CCOOH binding to sites on the helix (side-chain ester group and/or peptide moities of lower helix content near the chain ends).

The exact location of the binding site is uncertain. Hence the presence of a F_3CCOOH molecule at the site may or may not contribute to the observed chemical shift differences of the NH and C^α–H protons for helical and random-coil polymers. In any case, the equilibrium between polypeptide and F_3CCOOH suggested above should not be used to account for the appearance of two NH or C^α–H resonances in the helix–coil transition region (see region II discussion).

Side-Chain Spin–Lattice Relaxation. There is evidence that $(BzlGlu)_n$ helices will aggregate together (end to end and laterally) even at low concentrations in helicogenic solvents. Aggregation can be prevented by adding very small amounts of F_3CCOOH (<2%) to the solution. All of our measurements on the α helix were in this latter type of solution in which the $(BzlGlu)_n$ was presumably in the form of isolated, discrete helices.

The rotational correlation time for tumbling of a rodlike helical molecule in solution may be estimated from hydrodynamic measurements.[26] For an idealized rigid helix with diameter of 15 Å and length 170 Å = $n \times 1.5$ Å, where n is the degree of polymerization and 1.5 Å is the axial translation per residue, we calculate for the correlation time for reorientation of the long molecular axis, $\tau_{rot} \cong 5 \times 10^{-4}$ sec. Using the T_1 data in Figure 1C and eq 1 we find $\tau_c \cong 1 \times 10^{-10}$ sec when nearest-neighbor interactions only are considered to contribute to relaxation of the phenyl ring (r_{ij} = 2.47 Å). Previous studies with high molecular weight poly(ethylene oxide) gave T_1 values which were independent of the rotational diffusion of the entire molecule indicating that intramolecular segmental motion produces the relaxation.[22] Similarly, our findings of $\tau_c \ll \tau_{rot}$ may be interpreted as follows. Rapid side-chain reorientation uncoupled to helix tumbling is the dominant contribution to spin–lattice relaxation of the side-chain protons.

Region I. Referring to Figure 1C in region I where the polypeptide is α helical, T_1 for the phenyl group increases

(25) R. G. Brussau and H. Sillescu, *Ber. Bunsenges. Phys. Chem.*, 76, 31 (1972).

(26) Reference 4, Chapter 3.

with increasing F_3CCOOH concentration. Polymer T_1's increase as the solvent viscosity is decreased.[22] However, the viscosity of F_3CCOOH–$CDCl_3$ solutions is almost constant (actually increases slightly) in region I as the F_3CCOOH concentration is increased;[27] thus viscosity change cannot explain the observed increase in T_1. Disruption of a sidechain secondary structure by F_3CCOOH solvation of or binding to the side chains could increase side-chain mobility and account for the increase in T_1 as F_3CCOOH is added to the solution. A specific low-energy side-chain conformation for $(BzlGlu)_n$ has been suggested by theoretical calculations.[28] Unlike poly(β-benzyl L-aspartate) a high-resolution nmr study of the benzyl protons of $(BzlGlu)_n$ was unsuccessful in showing the existence of a specific side-chain conformation.[13] However, attempts focussing on chemical shift differences of the benzyl protons in the helix and random-coil conformation of $(BzlGlu)_n$ suggest the presence of a secondary side-chain conformation.[29] These latter chemical shift differences are small but are consistent with our results. That is, our T_1 measurements even at low F_3CCOOH concentration suggest a large amount of side-chain mobility which could average anisotropic shielding effects to unobservably small values.

Below 12% F_3CCOOH at 30° we find no evidence for the presence of random-coil polypeptide in the high-resolution nmr spectrum of the C^α–H resonance. However, we must consider the possibility that polydispersity is a source for the increase in T_1 in region I (See discussion below).

Region II. In the helix–coil transition region T_1 for the phenyl ring is nearly independent of F_3CCOOH concentration. This particular sample of $(BzlGlu)_n$ (R10 ref 23) has a wide molecular weight distribution and one might anticipate contributions to T_1 in this region from polydispersity. The effect of molecular weight distributions on nmr observations in the helix–coil transition region is a subject of considerable study. Discussion has centered about reconciling the simultaneous observation of two resonances for the C^α–H proton in the helix–coil transition region, one designated "random-coil" and the other "helix." The chemical shift data lead to a value of $\geq 10^{-1}$ sec[30] for the lifetimes of the C^α–H proton in the two environments helix and random coil while chemical relaxation experiments indicate a time scale of the order of 10^{-6} sec associated with the kinetics of the helix–coil transformation.[31] Very recently a 220-MHz nmr study of monodisperse $(BzlGlu)_n$ indicates that polydispersity is the sole origin of two resonances for the C^α–H proton in the helix–coil transition; an upper limit of 10^{-3} sec is derived from the chemical shifts for the time associated with the kinetics of the helix–coil transition.[32]

A theoretical treatment by Ullman consistent with this recent high-resolution data considers only the fast kinetic times associated with the helix–coil transition rate and ascribes the multiple peaks in the nmr spectrum to polydispersity and differences in helicity of peptide residues near the ends of the chain by comparison with residues near the middle.[33] Bradbury *et al.* interpret experimental spectra with similar concepts and suggest that in the ideal case of a very wide molecular weight distribution the two-peak behavior could be interpreted in terms of the fraction of molecules which are mainly helical or mainly random coil, relatively few molecules having partly helical conformations.[23] Accordingly for a polydisperse sample one would expect the fraction of random-coil polymer to increase across the transition range with increasing F_3CCOOH concentration. Assigning a characteristic relaxation time to the phenyl group in the random coil polymer, T_1^{rc}, and one for the helix, T_1^h, with $T_1^h < T_1^{rc}$ (see Figure 1C), T_1^{obsd} would be a weighted average of the two. A monotonic increase in T_1^{obsd} with increasing F_3CCOOH concentration would be expected across the transition region as the fraction of helical molecules is depleted. This type of behavior for T_1^{obsd} is not found in region II (see Conclusion).

The helix–coil state of the section of the polypeptide chain to which a specific sidechain is attached is time dependent, and even if a particular section of the molecule exists predominantly in the helical form, it may allow more rapid reorientation if it is in a coil form sufficiently often. In such a case, the onset of higher phenyl T_1 values could occur when the helix content is still high.[33] Although the wide molecular weight distribution and intermediate degree of polymerization of this $(BzlGlu)_n$ sample preclude definitive interpretation, this line of reasoning suggests that the increase in T_1^{obsd} in region I which was tentatively attributed to disruption of a side-chain secondary structure may have an alternate origin—pretransition effects arising from sample polydispersity and increases in side-chain reorientational mobility in partly helical molecules.

Region III. At higher F_3CCOOH concentrations T_1 for the side-chain phenyl group again increases approaching a constant value for the random-coil conformation. The equilibrium value for T_1^{rc} occurs well after the completion of the helix–coil transition as determined by high-resolution nmr. The fact that T_1^{rc} is independent of F_3CCOOH concentration between 40 and 100% F_3CCOOH shows that F_3CCOOH protons do not play any significant role in the relaxation of the phenyl group.

Concluding Remarks

The F_3CCOOH proton spin–lattice relaxation time, like numerous other observable parameters, exhibits dramatic changes in the helix–coil transition region. Unfortunately the behavior of the F_3CCOOH T_1 does not provide an unambiguous answer to the question of whether peptide protonation or solvation is responsible for the solvent-induced helix–oil transition. On the other hand, T_1 data on the polypeptide side chain do provide indirect information on the mechanism of the helix–coil transformation.

Spin–lattice relaxation of side-chain protons is achieved by segmental rotation of the side chain, translation of the side chain with respect to neighboring side chains and solvent molecules, and motion of the molecular backbone to which the side chain is attached. These experimental results indicate that the speeding up to side-chain mobility occurs prior to the helix–coil transition. If the concept of a side-chain secondary structure in the helical polypeptide is accepted, then we can further speculate and arrive at the stepwise physical picture of polypeptide denaturation, schematically depicted in Figure 2. In region I increasing the F_3CCOOH concentration disrupts a side-chain secondary structure imparting greater reorientational freedom to the side chain. Calculated correlation times for phenyl reorientation indicates that the side-chain secondary structure is not static but dynamic in nature permitting a

(27) At 25° we find η increases from 0.542 to 0.558 cP in the concentrate range of 0–50% F_3CCOOH in chloroform.
(28) J. F. Yan, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **9**, 2713 (1968).
(29) D. N. Silverman and H. A. Scheraga, *Biochemistry*, **10**, 1340 (1971).
(30) J. A. Ferretti and B. W. Ninham, *Macromolecules*, **3**, 30 (1970).
(31) G. Schwarz and J. Seelig, *Biopolymers*, **6**, 1263 (1968).
(32) K. Nagayama and A. Wada, *Chem. Phys. Lett.*, **16**, 50 (1972).
(33) R. Ullman, *Biopolymers*, **9**, 471 (1970); private communication.

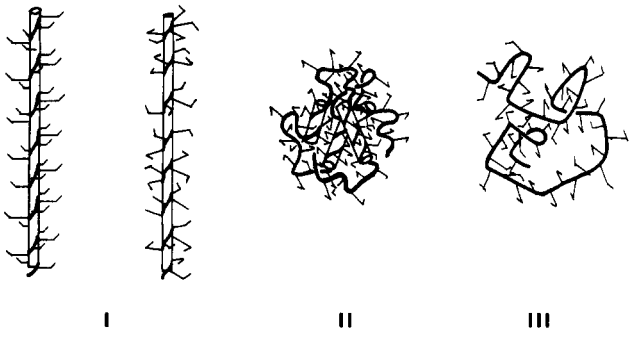

Figure 2. Schematic representation of polypeptide denaturation: region I, disruption of a secondary side-chain structure; region II, intermediate polypeptide conformation; region III, random coil.

high degree of side-chain mobility. Correspondingly, correlating these observations with specific calculated side-chain conformations does not seem relevant. Throughout the helix–coil transition (region II) an intermediate polypeptide conformation is postulated. In Figure 2 (II) this conformation is depicted as a broken helix resembling in some ways a native protein conformation. The structural details of the proposed intermediate conformation are not certain. However, the suggestion of a protein-like configuration may not be unreasonable. Somewhat analogous to hydrophobic interactions in proteins, polypeptide molecules having partial helix content might in the mixed-solvent system tend toward configurations in which the helical segments aggregate on the interior of the configuration because of preferential solvation of the random-coil residues by F_3CCOOH. In region III a posttransitional increase in the phenyl T_1 implies that the ideal, unrestricted, random-coil configuration is not attained until well after the helix–coil transition. This apparent increase in side-chain mobility occurring at high F_3CCOOH concentrations further supports the idea of a constricted intermediate conformation in the preceding helix–coil transition region.

Although the experimental data do not enable us to distinguish between the effects of polydispersity and a model utilizing a side-chain secondary structure, these preliminary findings do suggest that nmr relaxation studies may, in addition to elucidating the role side chains play in stabilizing the α helix, provide a more thorough understanding of the mechanism of the helix–coil transtion.

Acknowledgments. We are grateful to E. M. Bradbury for providing us with the $(BzlGlu)_n$ sample. The contributions of Dr. Ben Shoulders and Howard Johnson toward experimental techniques are gratefully acknowledged. We thank Dr. R. Ullman for stimulating comments on the F_3CCOOH relaxation data and on the effects of polydispersity.

Rayleigh Scattering from Solutions of Critically Branched Polycondensates

W. Burchard,[1a] K. Kajiwara,[1a] M. Gordon,*[1b] J. Kálal,[1b,c] and J. W. Kennedy[1b]

Institut für Makromolekulare Chemie, 78 Freiburg i.BR., West Germany, and the Chemistry Department, University of Essex, Colchester, England. *Received April 26, 1972*

ABSTRACT: The polycondensation system decamethylene glycol–benzene-1,3,5-triacetic acid has been shown previously to adhere very closely to the postulates of the classical random f-functional model, and to be capable of permanent stabilization in the critically branched state (*i.e.*, near the gel point). Such samples are here used for light-scattering studies. Combinatorial theories for interpreting such measurements are presented as generalizations of those deduced earlier using similar graph-theoretical methods (especially the formalism of cascades). It is shown that the situation is unusually favorable for critically and randomly branched materials: the Zimm plots are essentially rectilinear and the two parameters (the conversion α and the basic molecular length parameter b) of the model are obtained from the intercept and slope of a Zimm plot, respectively. Here α/α_{crit} is shown to be measurable to about 0.0001, and b is constant over a nearly 15-fold range of $(MW)_w$. The value of b reveals high chain extension. Systematic departures of the Zimm plots from rectilinearity, serious only at low $(MW)_w$, are ascribed to association by H bonding.

Recent analysis of the effects, on radiation scattering, due to stiffness and finite cross-section,[2a] and of the branching[2b] of molecular chains, have increased the usefulness of scattering techniques. Such methods hold great promise for polymer science. New model calculations have helped to shed light on the structure of natural glycogen, by applying them to the scattering behavior observable after the glycogen molecules have been chemically transformed to star-shaped assemblies bearing grafts of linear amylose chains.[2b] Statistical theories often take simple forms for completely random structures. Thus, it was shown[3] that random f-functional polycondensates in solution should exhibit rectilinear scattering envelopes in conventional experimental Zimm plots. Besides glycogen in the animal kingdom, branched covalent polymer systems occur in plants, *e.g.*, dextran and amylopectin. These polymers are made under the very specific action of two enzymes. Although the reactions imply some restrictions to the randomness, the statistical problem is still easy to handle by the mentioned theories.[4,5] The natural branched materials are of high weight-average molecular

(1) (a) Institut für Makromolekulare Chemie, Freiburg, West Germany. (b) Chemistry Department, University of Essex, Colchester, England. (c) Department of Macromolecular Chemistry, Institute of Chemical Technology, Prague, Czechoslovakia.

(2) (a) W. Burchard and K. Kajiwara, *Proc. Roy. Soc., Ser. A*, **316**, 185 (1970). (b) W. Burchard, I. Kratz, and B. Pfannemüller, *Makromol. Chem.*, **150**, 63 (1971).

(3) K. Kajiwara, W. Burchard, and M. Gordon, *Brit. Polym. J.*, **2**, 110, (1970).

(4) W. Burchard, K. Kajiwara, and B. Pfannemüller, *Angew. Chem., Int. Ed. Engl.*, **9, No. 7**, 532 (1970).

(5) W. Burchard, *Macromolecules*, **5**, 604 (1972).