Proton Nuclear Magnetic Resonance Studies of Model Polypeptides. Aspects of the Helix–Random Coil Interconversion*

W. E. Stewart, L. Mandelkern, and R. E. Glick

ABSTRACT: Proton high-resolution nuclear magnetic resonance (nmr) spectra have been obtained for poly-L-alanine and poly-DL-alanine in trifluoroacetic acid–chloroform-*d* solutions, a solvent system postulated to support the helical–random coil or interconverting configuration of poly-L-alanine, but only the random coil configuration of the DL polymer. Pertinent nmr spectral features vary in a way analogous to the variation of the optical rotatory related Moffitt parameter, b_0, but appear to afford additional information indicating variation in the polymer–solvent interaction as the solvent composition is changed. It is shown that the conformational purity of the polymer is such that it is very probably all helix in low acid, that the helix may be loosened as acid is added, and that in 100% acid no helical content is present. Further, it is demonstrated that the polypeptide–acid interaction involves hydrogen bond formation without proton transfer; this interaction is postulated, in high acid, to effect conformational conversion. Poly-L-leucine was also examined as indicated above. Nmr spectral features in this case paralleled those of poly-L-alanine.

We recently reported proton high-resolution nuclear magnetic resonance spectra of the model polypeptide poly-L-alanine in dilute solution (Glick *et al.*, 1966). In addition to the demonstration of characteristic spectra for both helical and random coil configurations of the polymer, evidence was presented that in trifluoroacetic acid–chloroform-*d* solutions, amide–proton exchange with the acid proton occurred with equal facility for either the ordered or disordered configuration. This study presents results of further proton nmr[1] investigations on poly-L-alanine and other model polypeptides in the indicated solvent system that afford information on the structure and interconversion of the two pertinent species as well as on details of the interaction between the polymer and the solvent component seemingly responsible for the interconversion. For the systems studied, proton high-resolution nmr has been demonstrated to be a very sensitive method for the study of the helix–coil transition.

Experimental Section

The poly-L-alanine, poly-DL-alanine, and poly-L-leucine used in these studies were obtained from Mann Research Laboratories and the poly-γ-ethyl-L-glutamate was obtained from Pilot Chemical Co. The molecular weights of poly-L-alanine and poly-DL-alanine were reported by the manufacturer as 50,000 (DP 700) and 6500 (DP 91), respectively. Before use, all polypeptide samples were dried overnight over P_2O_5 at 100° in an Abderhalden and stored in a desiccator over anhydrous calcium sulfate.

The trifluoroacetic acid, obtained from Matheson Coleman and Bell, was distilled at atmospheric pressure before use and stored in a dry box in an all-glass container. The deuterated chloroform was obtained from Merck Sharp and Dohme of Canada Ltd. and used without further treatment.

The solutions were prepared by dissolving a weighed amount of the polypeptide in a weighed amount of trifluoroacetic acid and then adding the appropriate weight of $CDCl_3$. To the solutions used for the nmr measurements, about 1% of tetramethylsilane was added to serve as the internal standard. All measurements were made immediately after mixing. For the nuclear magnetic resonance determinations, a Varian Associates high-resolution nmr spectrometer (HR 60) with Model V-4311 R_F unit and probe for operation at 60,000 Mcycles was used.

Chemical shifts were measured from tetramethylsilane as internal standard by the side-band technique. The side bands were produced by modulating the magnetic sweep field with a Hewlett-Packard Model 200 CD audiooscillator. The frequencies of the audiooscillator were monitored with a Hewlett-Packard Model 523C frequency counter. For each peak calibration six to ten sweeps were averaged. The measured peak positions are estimated to be accurate to ±0.017 ppm.

Refractive indices of the trifluoroacetic–deuterated chloroform mixtures were measured on a Bausch

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[1] Abbreviations used: $CDCl_3$, chloroform-*d*; nmr, nuclear magnetic resonance; PDLA, poly-DL-alanine; PELG, poly-γ-ethyl-L-glutamate; PLA, poly-L-alanine; PLL, poly-L-leucine; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

TABLE I: Chemical Shifts of PLA Protons in $CDCl_3$–TFA Mixtures (Shifts in Parts per Million from Tetramethylsilane).

Wt % TFA	PLA Concn (mg/ml)	$-b_0$	δ_{CH_3}	δ_{CH}	δ_{NH}	δ_{TFA}	$\delta_{CH} - \delta_{CH_3}$
9.76	15.2		1.557	4.283	7.933	11.000	2.726
18.16	26.4	341[a]	1.557	4.300	7.867	11.300	2.743
25.2	26.3		1.562	4.323	7.850	11.450	2.761
37.6	26.1		1.588	4.372	7.850	11.605	2.784
45.0	26.2		1.608	4.402	7.867	—	2.794
49.8	25.5	328.1	—	—	—	—	2.794
52.9	25.8	328.2	1.617	4.423	7.867	—	2.806
65.6	24.7		1.625	4.467	7.867	11.617	2.842
69.7	26.0	307.4	—	—	—	—	2.842
76.0	26.0		1.648	4.505	7.867	11.617	2.857
87.3	24.5	214.5	1.608	4.617	7.817	11.733	3.009
89.1	25.1	193.3	1.603	4.640	7.817	11.717	3.037
100.0	26.4	124.3	1.590	4.707	7.783	—	3.117

[a] b_0 measured on a solution containing 2.20 mg of PLA/ml.

and Lomb Abbe-3L refractometer using the sodium D line. The solvent refractive index at the sodium D line was used throughout, with no corrections being made for dispersion of the refractive index.

The optical rotations were measured on a Rudolph Model 80 photoelectric spectropolarimeter equipped with Rudolph Model 600 sodium and mercury lamps. All-glass polarimeter tubes of 10-dm path lengths were used. Rotations were obtained at six wavelengths for each sample. The b_0 values were then determined from the Moffitt equation (Urnes and Doty, 1961).

Results

The proton magnetic resonance spectrum of a dilute solution of poly-L-alanine (PLA) in trifluoroacetic acid (TFA), as previously reported, consists of three main absorption peaks arising from the amide proton, the methyne proton, and the side-chain methyl protons The methyl absorption (CH_3) appears as a symmetrical doublet with a separation between components of about 0.113 ppm and a half-width of about 0.250 ppm; the methyne absorption (CH) is split into a broad symmetrical triplet with component separations of about 0.100 ppm and half-width of about 0.500 ppm, while the amide proton (NH) absorption is split into a narrow symmetrical doublet with component separation of about 0 100 ppm.

The nmr frequencies of the various protons are displaced as TFA is mixed with chloroform-*d* ($CDCl_3$). The results of measurements from 9.8 to 100% acid are given in Table I. Displacements of CH_3 and CH increase in a linear fashion as the TFA concentration is increased until about 75% acid when both shifts show a rather sharp change; that of the CH_3 decreasing and that of the CH increasing. The NH chemical shift undergoes a 0.067-ppm decrease as the solvent is varied from 9.8 to 18% TFA, remains constant through the region 18–75%, and then shows a 0.083-ppm decrease from 75–100% acid. These results are summarized in Figures 1–3.

Table I also provides b_0, the Moffitt parameter (Urnes and Doty, 1961), for poly-L-alanine at various TFA concentrations in $CDCl_3$; in Figure 4, b_0 is plotted against weight per cent TFA. Figure 4, virtually identical with that obtained with nondeuterated (Fasman, 1963; Hanlon and Klotz, 1965) rather than deuterated chloroform, shows that $-b_0$ slowly decreases from 341 in 10% to 315 at 70% TFA after which the quantity drops sharply to the terminal value of 124 in 100% acid. This precipitous variation in b_0 occurs in the same range of TFA in which the sharp changes in chemical shifts are observed. Figures 2 and 3 also display chemical shifts for appropriate protons of poly-L-leucine (PLL). These displacements and the corresponding b_0 variation (Figure 4) again exhibit strongly similar behavior. Figures 1–3 additionally present chemical shifts for appropriate protons of poly-DL-alanine (PDLA); displacements in this case are linear functions of added acid.

Discussion

Nmr and b_0 Criteria for Helix–Random Coil Configurations. Of the many physical properties of dilute solutions of polypeptides that have been interpreted in terms of a helical, random coil, or interconverting structure for the polymer, the b_0 criterion has been most extensively employed for this purpose (Fasman, 1963; Yang and Doty, 1957). The values of b_0 found in Figure 4 are generally interpreted in terms of predominance by a particular configuration: low acid,

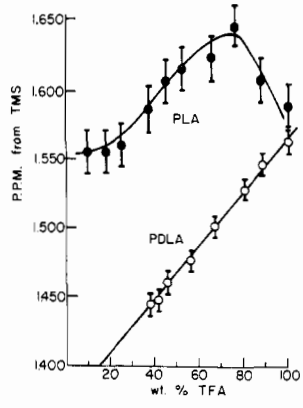


FIGURE 1: Chemical shifts of the methyl protons in poly-L-alanine (PLA) and poly-DL-alanine (PDLA) as a function of TFA concentration in TFA–CDCl mixtures.

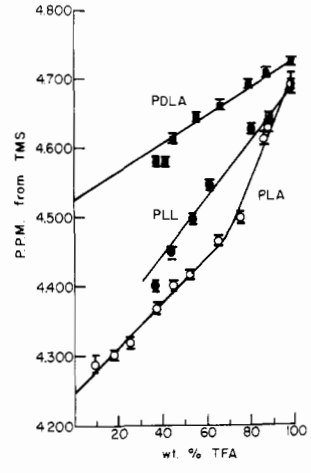


FIGURE 2: Chemical shifts of the α-CH protons in poly-L-alanine (PLA), poly-L-leucine (PLL), and poly-DL-alanine (PDLA) as a function of TFA concentrations in TFA–$CDCl_3$ mixtures.

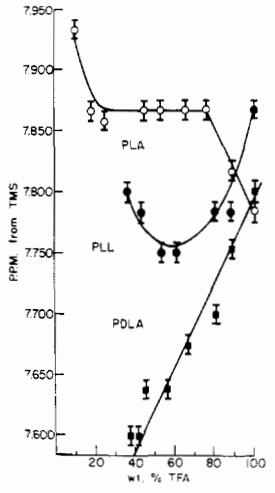


FIGURE 3: Chemical shifts of the NH protons in poly-L-alanine (PLA), poly-L-leucine (PLL), and poly-DL-alanine (PDLA) as a function of TFA concentrations in TFA–$CDCl_3$ mixtures.

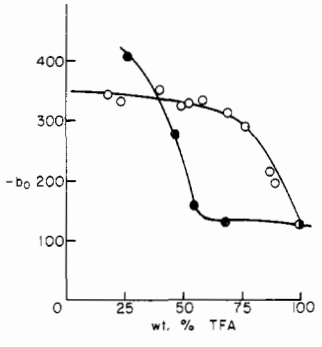


FIGURE 4: Plot of the Moffit parameter $-b_o$ *vs.* weight per cent trifluoroacetic acid (TFA) for poly-L-alanine (○) and poly-L-leucine (●) in TFA–$CDCl_3$ mixtures.

helix; highest acid, random coil; and interconversion of helix to random coil, region of sharply varying b_0. Figures 1–3 justify a similar interpretation, PLA and PLL exhibiting nmr chemical shift features that parallel precisely the corresponding b_0 variation.

The contrast in the nmr features of poly-DL-alanine with those of poly-L-alanine also attest to the structural conclusions inasmuch as poly-DL-alanine has the same chemical structure as PLA, but is in the random coil form at every acid concentration (Downie *et al.*, 1957; Gratzer and Doty, 1963; Bryan and Nielsen, 1960; Elliot, 1963). The chemical shift differences (Figures 1–3) between corresponding groups in helical PLA and random coil PDLA at the same TFA concentrations are interpreted, therefore, as substantial evidence for predominance of the indicated configurations.

The difference in nmr frequencies for protons in the helical as compared to those in the random coil polypeptide can derive from anisotropic shielding effects. In the relatively rigid helix, the positions of the various groups are held fixed with respect to each other and anisotropic effects associated with the carbonyl bond, and to a lesser extent that for other bonds, may make a fairly large contribution to the shielding constant of a proton held near the bond axis; the direction and magnitude of the shift depending upon the relative orientation of the group and the other bond axes (Pople, 1962; Narasimhan and Rogers, 1959). In the random coil form of the polypeptide, motion of the various groups with respect to each other can occur more freely resulting in a greatly decreased anisotropic contribution. In the α-helical structure of PLA, the methyne proton is *cis* to the carbonyl bond, the methyl group is *trans*, and the amide proton is *trans* to the

carbonyl bond (Schellman and Schellman, 1964); previous work (Kowalewski, 1960; Hatton and Richards, 1962; LaPlanche and Rogers, 1963) has shown that the resonance of an N-methyl group in dimethylamides or the methyl of an N-ethyl group in diethylamides occurs at higher field when the group is *cis* to the carbonyl oxygen than when it is *trans*. Thus, comparison of our chemical shifts for the methyne, methyl, and amide protons in PLA and PDLA at a given acid concentration show that the differences are not only consistent with the suggested structures, but are also in the proper direction.

In addition to expected chemical shift differences for the helical and random coil configurations, polypeptide chemical shifts and b_0 values can be further interpreted structurally as follows; in the low acid region PLA and PDLA nmr frequencies differ but converge in 100% TFA as is seen in Figures 1–3; we conclude that PLA and PDLA have the same configurations in 100% TFA. An examination of Figure 3 reveals that the initial slope for PLL is parallel to that for PLA in the intermediate interconversion region, based on the b_0 criterion, PLL in that range of acid is undergoing transition, while at high acid concentration PLL is in the random coil form and the slope parallels that for the corresponding PDLA proton; this spectral behavior strongly suggests that both PLL and PLA are in the random coil configuration in 100% TFA. It will be noted in Figure 4, however, that the 100% acid value for each b_0 is significantly different from zero. Although nonzero-terminal b_0 values have been found for other substances (Fasman, 1963; Urnes and Doty, 1961; Blout, 1960; Bradbury *et al.*, 1960; Karlson *et al.*, 1960), a conventional interpretation would indicate a residual helical content for each polypeptide. The nmr behavior, however, confirms the fact that PLA is random coil in 100% TFA. This conclusion is also confirmed by intrinsic viscosity studies of this system (A. Takahashi, L. Mandelkern, and R. E. Glick, to be published). Therefore, we can conclude that the empirical assignment of helical content through b_0 values cannot be made for PLA or PLL.

The magnitude of b_0 in the helical region has been treated by the further empirical relation (Urnes and Doty, 1961); per cent helix $= (-b_0/630) \times 100$. Applying this, a value of 54% is obtained for the maximum helical content of PLA. Although we have no evidence to contradict this result, we believe that no reliance can be placed upon this quantitative assessment. The b_0 plot given in Figure 4 does indeed indicate that a cooperative transition occurs at about 80% TFA; this is now confirmed by nmr and viscosity studies (A. Takahashi, L. Mandelkern, and R. E. Glick, to be published).

Nmr Spectra of Helical Biopolymers. An important aspect of this study is that proton peaks for PLA are observable over the entire range of TFA concentrations studied. This is the first reported instance in which nmr features due to protons attached to the backbone have been observed when the polypeptide is purported to be in a helical conformation. Previous studies have indicated that several polynucleic acids exhibit nmr absorption only when the polymer possesses a disordered structure (McTague *et al.*, 1964; McDonald and Phillips, 1964). Additionally, in a study similar to ours, Goodman and Masuda (1964), examining the nmr spectra of poly-γ-ethyl-L-glutamate (PELG) in TFA, trifluoroethanol (TFE), and TFA–TFE mixtures, reported that the NH resonance was not observable in pure TFE or in the regions of low TFA concentrations where the helical conformation predominates. The absence of a helical NH resonance was attributed to extreme broadening due to the amide being held rigidly in an interpeptide hydrogen bond and to its not being solvated. The NH absorption was supposed to appear as the polypeptide was transformed to the random coil conformation.

In order to directly compare PELG with PLA, a solution of 48 mg of PELG in 1 ml of a 8.37% TFA–91.63% $CDCl_3$ solution was prepared. At room temperature, the solution was very viscous and cloudy, and it was necessary to raise the temperature to 32° before the solution became clear. ORD data was obtained at this temperature and a b_0 of −400, which indicates a helical content of 63%, was obtained. When the nmr spectrum was run at 32°, no NH absorption was apparent, but when the temperature was lowered to 29°, PELG precipitated out and the NH signal appeared with the intensity ratio for CH_3:NH of 3.

Our interpretation of this behavior is that at 32° the solution was sufficiently concentrated to cause aggregation to occur. When the temperature was lowered to 29°, solution conditions appear to be such that the concentration of PELG was below that corresponding to the critical point for aggregation. Aggregation would be expected to lead to a small T, and, consequently, broadening of the peaks (Pople *et al.*, 1959). Further, aggregation would also greatly hinder solvation of the peptide groups (Bensusan and Nielsen, 1964) also leading to broadening of the NH peak. Admittedly, Goodman and Masuda used a different solvent system, but in view of the fact that their solutions were 15% in PELG, aggregation effects could have been even larger in their case.

In our studies, the polymer concentration was kept low (about 2%) with no visible evidence of aggregation. Further, to make certain that we were quantitatively observing the polypeptide resonance signal, we employed an internal standard of known concentration and verified that, in the range of TFA concentration in which the helical form predominates, absolute polypeptide proton intensities were obtained. We conclude, therefore, that the effects observed by Goodman and Masuda are not of general validity but depend specifically upon the nature of the polypeptide, the solvent system, the polypeptide concentration, and the temperature.

As has been noted only one absorption is observed for each proton *i.e.*, separate peaks are not observed for a given proton in a helical region and one in a random coil region. Therefore, the frequency for protons in what is presumed to be the helical region can be

the weighted average of the shifts for helical and random coil species, if in low acid PLA is not all helix, or the frequency could be that owing to a proton in pure helix. In either case, comparing the chemical shifts of CH of poly-L-alanine with those of poly-DL-alanine, in, for example, a 38% TFA solution, one can obtain an upper limit to the lifetime of a proton in a particular configuration. If the polymer is very largely in the helical configuration, the lifetime, will be given by $\tau \lesssim 1/2\pi\delta$, where δ is the chemical shift between a proton in helical configuration and the same proton in a random coil configuration in the same solvent composition. Under these conditions, τ will be greater than the quantity on the right. If the helical region contains random coil sections, τ will be less than the indicated quantity. It will then be the difference between the frequency for a proton in the pure helix and that for a proton in the random coil configuration. In this example the value of the right-hand quantity is of the order of 10^{-2} sec. Although we have no direct evidence to indicate what the "configurational purity" of the helical regions are, the exchange studies previously reported (Glick *et al.*, 1966) indicate that the helical polymer contains some random coil segments.

TABLE II: Chemical Shifts of PDLA Protons in $CDCl_3$–TFA Mixtures (Shifts in Parts per Million) from Tetramethylsilane).

Wt % TFA	PDLA (mg/ml)	CH_3	CH	NH
38.1	25.5	1.443	4.587	7.592
42.0	24.8	1.447	4.578	7.595
45.7	30.3	1.460	4.610	7.638
56.4	25.4	1.477	4.642	7.638
67.1	26.9	1.502	4.668	7.675
80.5	26.0	1.528	4.693	7.700
88.3	32.3	1.547	4.715	7.753
100.0	40.6	1.563	4.730	7.805

Nature of Amide–Proton Nmr Absorption. Of further interest is that the NH doublet persists and is relatively narrow throughout the entire range of TFA–$CDCl_3$; the NH half-width varies from 0.200 in 100% TFA to about 0.333 ppm in the region of high $CDCl_3$ concentration. The small line width of the NH peak indicates that the nitrogen nucleus and the proton are effectively decoupled, *i.e.*, the quadrupolar broadening of the nmr absorption of a proton directly attached to an ^{14}N nucleus is not observed. This narrowing of the NH peak is believed to arise from the bulkiness and unique structural features of the polypeptide.

Since the nitrogen nucleus has a spin of unity, it might be expected that the nmr absorption of a proton attached to nitrogen would be split into a triplet by spin–spin interaction with the three nitrogen spin states (Grunwald *et al.*, 1957; Roberts, 1956; Ogg, 1954a,b). If, however, fairly rapid exchange of the NH between two or more sites can occur, or if the electric quadrupole moment of the ^{14}N nucleus is strongly coupled to the tumbling motions of the molecule (Roberts, 1956; Pople, 1958), the triplet will collapse to a singlet. The NH absorption of simple amides at room temperature consists of a broad (about 1.2 ppm) singlet because of the latter effect. If the quadrupole relaxation rate of the nitrogen nucleus becomes very fast, then effective decoupling of the nitrogen nucleus and the proton occurs and the proton peak will sharpen to a single narrow line (Pople, 1958). The relaxation time, T_1, is given by

$$\frac{1}{T_1} = \frac{3}{8}\left(1 + \frac{n}{3}\right)^2\left(\frac{e^2qQ}{\hbar}\right)\tau_q \qquad (1)$$

where τ_q is the correlation time characterizing the reorientation, $(e^2qQ/\hbar)$ is 2π times the quadrupole coupling constant in cycles per second, 3×10^7 rads sec^{-1} for ^{14}N, and n is the asymmetry parameter; τ_q is about 10^{-10} to 10^{-13} sec for simple monomeric amides. Pople (1958) has calculated the theoretical line shapes for protons attached to ^{14}N atoms as a function of the quantity $(10\pi T_1 J)$, where J is the spin–spin coupling constant in cycles per second and these line shapes indicate that the proton absorption will appear as a narrow single peak when $(10\pi T_1 J)$ approaches unity or when T_1 approaches 6×10^{-2} sec. According to eq 1 the relaxation time, T_1, will decrease when τ_q increases or when the electric field gradient, q, increases. The motion of a long polypeptide chain would be expected to be slow, τ_q would be larger than that for a simple monomeric amide under the same environmental conditions, and therefore, one might expect a faster nitrogen relaxation and resultant narrowing of the NH peak in the polypeptide case. That the effect may be appreciable is indicated by the fact that for an aqueous solution of polymethacrylamide the peaks due to the amide protons are very narrow (Bovey and Tiers,

TABLE III: Chemical Shifts of PLL Protons in $CDCl_3$–TFA Mixtures (Shifts in Parts per Million from Tetramethylsilane).

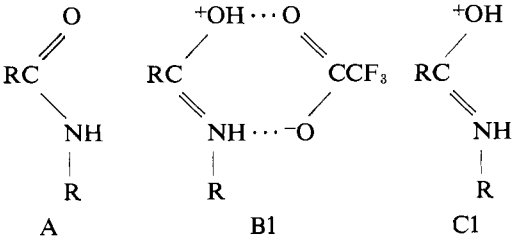
Wt % TFA	$\delta_{(CH_3)_2}$	$\delta_{(CH_2CH)}$	δ_{CH}	δ_{NH}	$\delta_{CH} - \delta_{(CH_3)_2}$
36.4	0.950	1.683	4.400	7.800	3.450
43.6	0.950	1.667	4.450	7.783	3.500
53.7	0.950	1.667	4.500	7.750	3.550
61.0	0.967	1.700	4.550	7.750	3.583
80.2	0.967	1.683	4.633	7.783	3.666
87.9	0.967	1.683	4.650	7.783	3.683
100.0	1.000	1.733	4.700	7.867	3.700

1963). Further support comes from the observation that when about 50% by volume of glycerol is added to an aqueous solution of acetamide, the NH peak changes from a very broad singlet to a sharp doublet (Bovey and Tiers, 1963).

Another means of decreasing T_1 is by increasing the electric field gradient at the nitrogen nucleus. Although no predictions can be made as to the effects of various substituent groups upon the field gradient, Tiers and Bovey (1959) have observed narrowing effects which they ascribe to this mechanism. In the model peptides acetylglycine, glycylglycine, triglycine, acetylalanine, and other similarly substituted acetamides, the line width of the NH peak decreased to about 0.10–0.17 ppm. Exchange narrowing of the NH peak was ruled out by the multiplet structure of the NH peak and of the adjacent methylene proton peaks. Since the polypeptide is made up of repeating units substituted similarly, this effect would be expected to make an appreciable contribution to the NH line narrowing observed in poly-L-alanine.

Thus, owing to a combination of the two effects discussed above, the ^{14}N nucleus and the attached proton may be effectively decoupled in poly-L-alanine and any changes in NH line width or position may be detailed in terms of configurational changes of the polypeptide chain or the specific interactions with the solvent and not to any anomalous effects arising from interaction with the quadrupolar ^{14}N nucleus.

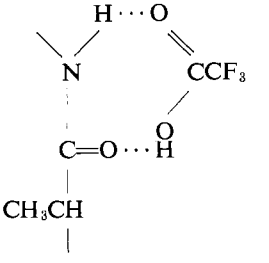
Polypeptide–Solvent Interactions. In a companion proton nmr study on amide monomers (Stewart *et al.*, 1967) in TFA–$CDCl_3$ solutions, it was concluded that the following species were present under appropri-



ate conditions. In the absence of acid, the unsolvated amide A was present. B1 in equilibrium with A was postulated as acid was added until A was removed as the amide–acid ratio exceeded 1:1; then C1 in equilibrium with B1 was proposed as more acid was added until, in high acid, the only amide species present was C1. Although these conclusions are based primarily on the very large chemical shift of the TFA acid proton, the chemical shifts found for the remaining protons confirmed the proposed structures. This concurs with the proposed structure suggested as arising upon amide–acid interaction (Hanlon *et al.*, 1963; Stake and Klotz, 1966; Hanlon, 1966).

Analogs of the several structures proposed above have been suggested as representing the interaction between TFA-type solvents and polypeptides (Hanlon *et al.*, 1963; Stake and Klotz, 1966; Hanlon, 1966). Although the chemical shifts of the various peptide protons (Figures 1–3) indicate that there is some interaction between solvent and polypeptide, a more detailed examination of our results seems to indicate that the interaction is not protonation of the peptide units in a manner analogous to that for the simple amides. This conclusion follows from the fact that the chemical shift for the TFA proton undergoes no downfield shift indicative of protonation as it did in the amide solutions. As may be seen in Table I, the TFA acid proton shows a total shift of about 0.267 ppm in going from 25% TFA to 100% TFA. On the other hand, simple amides induce more than an order of magnitude displacement in this proton (Stewart *et al.*, 1967); if protonation of the polypeptides occurs, no more than 10% of the peptide would be protonated. Furthermore, the magnitude of the NH chemical shift in PDLA in the range of 38–100% TFA is one-third of that for the analogous group in *N*-methylacetamides.

The most general interaction between the PLA or PDLA and TFA probably is a hydrogen bonding interaction of the following type



The acid proton is not completely transferred to the peptide carbonyl oxygen to form a protonated peptide group and an associated TFA anion. Rather, the acid proton remains primarily bonded to the acid as in the hydrogen bonded structure shown above.

We can also note from Figure 4 that b_0 slowly decreases in the region preceding that of helix–coil interconversion. The question has arisen as to whether the gradual b_0 change represents a loosening of the helix or whether it is due to solvent or other effects upon b_0 (Fasman, 1963; Hanlon and Klotz, 1965). Thus far, no method used to study the helix–coil transitions of polypeptides has been able to answer this question, but the results can provide a partial answer. The fact that, for example, CH chemical shift is larger in helical PLA than in the corresponding case in PDLA indicates that some additional mechanism is occurring in PLA that is not occurring in PDLA. This additional mechanism could be a loosening of the helix with a consequent decrease of magnetic anisotropy caused by increased motion. (Loosening of the helix is interpreted as a slight departure of the space coordinates of the various groups from their equilibrium positions.) The loosening of the helix could arise from a partial disruption of the dipole–dipole interactions stabilizing the helical configuration (Arridge

and Canon, 1964; Brant and Flory, 1965) caused by the proposed hydrogen bonds formed by TFA and the helical polymer. Electrostatic repulsion between the peptide groups could then distort the helix sufficiently to allow partial rotation about the CC bonds of the backbone, when this effect dominates, the polypeptide converts to the random coil configuration.

Conclusion

Nmr spectral data on model polypeptides in a helical–coil interconverting media have been demonstrated not only to be obtainable throughout the anticipated range of structural polymer forms, but also to be interpretable in terms of detailed structural considerations. Although the general features of the nmr spectra of the two model polypeptides, poly-L-alanine and poly-L-leucine, follow the helix to random coil variation anticipated from the change of b_0 in the transition region, it has been concluded that the conventional utilization of b_0 is not a reliable index of either the maximum or minimum per cent helix. We further conclude that, for the particular solvent pair, trifluoroacetic acid–chloroform-*d*, polymer–solvent interaction differs significantly from that previously postulated (Hanlon and Klotz, 1965; Hanlon *et al.*, 1963; Stake and Klotz, 1966; Hanlon, 1966). We propose instead that the principal acid–polymer interaction is hydrogen bonding without proton transfer. As the acid concentration is increased, intramolecular hydrogen bonding is surpressed in favor of intermolecular hydrogen bonding between TFA and polypeptide. This then is the driving force converting the polypeptide from the helical to the random coil form. Additional intrinsic viscosity measurements support this conclusion (A. Takahashi, L. Mandelkern, and R. E. Glick, to be published.)

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