Conformational Studies of Polymers and Copolymers of L-Aspartate Esters. III. Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: High-resolution pmr spectra have been obtained in chloroform–trifluoroacetic acid of left-handed helical poly(β-benzyl and β-methyl L-aspartates) and right-handed helical poly(β-ethyl L-aspartate) and copolymers of β-benzyl L-aspartate with L-alanine, L-leucine, and γ-benzyl L-glutamate. A dependence of the chemical shift of the main-chain protons on the sense of the helix has been established for poly(L-aspartates) as follows: right-handed helix, amide N*H* 8.2 ppm, α-C*H* 4.40 ppm; left-handed helix, amide N*H* 8.7 ppm, α-C*H* 4.30 ppm. Random-coil shifts in chloroform–5% trifluoroacetic acid are amide N*H* 8.0 ppm, α-C*H* 4.85 ppm. Poly(L-aspartate esters) taking up the right-handed helix in chloroform have been found to reverse helix sense on the addition of small quantities of haloacetic acids before undergoing transition to the random coil. A study of the coupling constants of the β and benzyl CH_2 groups in left-handed poly(β-benzyl L-aspartate) in chloroform has indicated considerable conformational mobility in the side chain of this polymer.

The conformations of ester derivatives of poly(L-aspartic acid) have been shown to be dependent on the precise nature of the side chain and on the solution conditions. Thus, whereas poly(β-benzyl L-aspartate) and poly(β-methyl L-aspartate) take up the left-handed (LH) α-helical conformation in chloroform solution,[1–4] poly(β-ethyl L-aspartate), poly(β-propyl L-aspartate), poly(β-isopropyl L-aspartate), and poly(β-phenethyl L-aspartate) are in the right-handed (RH) form.[1] Further, it has been demonstrated that para substitution of the benzene ring of poly(β-benzyl L-aspartate) with a nitro,[5–7] methyl, chloro, or cyano[8,9] group causes a reversal of the helix sense to the RH form, while the ortho and meta chloro derivatives of poly(β-benzyl L-aspartate) remain in the LH helical form.[10] Many of the experimental studies were possible only through the availability of copolymers of poly(β-benzyl L-aspartate) with the other aspartate ester under investigation. The concept of utilizing copolymers for the conformational analysis of poly(aspartate esters) is not new; Bradbury, *et al.*,[2] and Karlson, *et al.*,[3] established the LH helix sense of poly(β-benzyl L-aspartate) by using copolymers of (β-benzyl L-aspartate) with L-alanine or with γ-benzyl L-glutamate. As regards the effects of solvents, it has been shown that a copolymer of (β-benzyl L-aspartate) with (β-*p*-methylbenzyl L-aspartate) in a 1:1 molar ratio undergoes an LH → RH helix transition in chloroform–dimethylformamide as the composition of the mixed solvent is varied,[9] the transition occurring at 90% chloroform–10% dimethylformamide. Furthermore, copolymers of ethyl and benzyl L-aspartate have been found to undergo a conformational transition from the RH helical form to the LH helical form as the temperature of the chloroform solution is raised.[1] It can be seen, therefore, that aspartate copolymers provide a system in which it is frequently possible to obtain a particular copolypeptide in either the left- or right-handed helical form by appropriate variation of the temperature of the chloroform solution.

In the case of LH α-helical forms of L-aspartate polymers, in particular poly(benzyl L-aspartate), it is possible to induce a transition to the RH conformation by the inclusion of small proportions of residues which themselves have a strong preference for the RH helix; thus, random copolymers of β-benzyl L-aspartate with either 10% L-alanine or 20% L leucine or γ-benzyl L-glutamate are in the RH helical form in chloroform solution,[2] and these materials provide a useful system for the observation of RH helical poly(β-benzyl L-aspartate) with only a minimum inclusion of foreign residues. The delicate balance of energy which is seen to exist between the right- and left-handed helical forms of poly- and copoly(aspartate esters) makes them particularly suitable for experimental observation of conformational changes and also for theoretical calculation of the energetically favorable conformations of both the main chain and the side chain. Indeed, the interest in the conformations of poly(aspartate esters) shown by experimental workers is shared also by theoretical workers in this field.

Scheraga, *et al.*,[11] have calculated that the dipole–dipole interaction between the side-chain ester group and the main-chain amide group is repulsive for poly(β-benzyl L-aspartate) for left- and right-handed α helices, but less so for the left-handed form, resulting in a preference for this sense. It is interesting that although the electrostatic energy has been calculated to be a minimum in the left-handed form of all poly(aspartate esters), the values of the torsional and nonbonded energies are calculated to outweigh this factor in all but the benzyl, methyl, and ethyl esters, whose senses are thus predicted as left handed; the RH helix sense has been predicted for all the longer side chain poly(aspartate esters) and the para-substituted benzyl esters. The discrepancy between the theoretically predicted helix sense of the ethyl ester and the experimentally determined RH sense may be corrected

(1) E. M. Bradbury, B. G. Carpenter, and H. Goldman, *Biopolymers*, **6**, 837 (1968).
(2) E. M. Bradbury, A. R. Downie, A. Elliott, and W. E. Hanby, *Proc. Roy. Soc., Ser. A*, **259**, 110 (1960).
(3) R. H. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2268 (1960).
(4) M. Goodman, F. Boardman, and L. Litowsky, *ibid.*, **85**, 2491 (1963).
(5) M. Goodman, C. M. Deber, and A. M. Felix, *ibid.*, **84**, 3771 (1962).
(6) D. F. Bradley, M. Goodman, A. M. Felix, and R. Records, *Biopolymers*, **4**, 607 (1966).
(7) M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *ibid.*, **1**, 371 (1963).
(8) M. Hashimoto and J. Aritomi, *Bull. Chem. Soc. Jap.*, **39**, 2707 (1966).
(9) M. Hashimoto, *ibid.*, **39**, 2713 (1966).
(10) E. H. Erenrich, R. H. Andreatta, and H. A. Scheraga, *J. Amer. Chem. Soc.*, **92**, 1116 (1970).
(11) J. F. Yan, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **49**, 2713 (1968).

by small modifications of the parameters used in the calculations. In addition to predicting the helix senses of many poly(aspartate esters), Scheraga and coworkers[11] have also calculated, for helical poly(aspartate esters) *in vacuo*, that there are four possible preferred side-chain conformations of minimum energy, two longitudinal and two transverse, the longer side chain apparently showing a preference for the transverse orientation while the shorter side chain esters have minimum energy for the longitudinal orientation. They have calculated that for poly(β-benzyl L-aspartate) the side-chain conformations should be transverse in a counterclockwise direction for both helix senses viewed from the N-terminal end of the helical molecule. Nuclear magnetic resonance studies have indicated[12] that the side chains of poly(aspartate esters) might be oriented for the helical forms in solution, and there is some indication that the orientation of the side chain of RH helical poly(β-ethyl L-aspartate) is different from that of LH poly(β-benzyl L-aspartate) and several other LH helical copoly(aspartate esters).

The work presented in this paper extends the study of preferred conformations of poly(aspartate ester) side chains to copolymers of β-benzyl L-aspartate with nonaspartate residues, and utilizes chemical shift and coupling constant data for the characterization of right- and left-handed helical forms. The first part of the paper deals with the chemical shift of the α-C*H* and N*H* resonances and demonstrates that their precise values can be used to characterize the two helix senses of poly(L-aspartate esters), while the second part is concerned with the spectrum of the side-chain hydrogens, particularly their coupling constants.

Experimental Section

Nuclear Magnetic Resonance Spectroscopy. Nmr spectra were recorded on a Jeolco 100-MHz JNM-4H-100 spectrometer, the temperatures other than ambient being achieved by use of the standard variable-temperature accessory which was calibrated from the chemical shift of the propane diol O*H* resonance. Additional measurements were made using the Varian 220-MHz spectrometer belonging to the Science Research Council and situated at the ICI Research Laboratories, Runcorn, and also with a Varian 300-MHz spectrometer temporarily sited at the laboratories of Varian Associates, Walton-on-Thames. The polypeptide solutions were of concentration 3% (w/v), 5-mm tubes being used throughout the measurements. Peak positions were measured relative to internal tetramethylsilane.

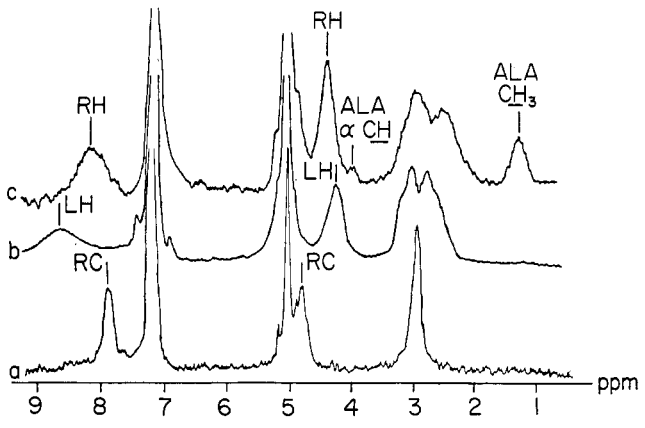

Figure 1. Spectra (100 MHz) of poly(β-benzyl L-aspartate): (a) the random-coil (RC) form in $CDCl_3$–5% TFA, (b) the left-handed (LH) helical form in $CDCl_3$–0.5% TFA, (c) the right-handed (RH) helical form of poly(90% β-benzyl L-aspartate-*co*-10% L-alanine] in $CDCl_3$–0.5% TFA.

Optical Rotatory Dispersion. ORD curves were measured over the spectral range 278–555 nm on a Bendix Polarmatic 62. The solutions, being the same as those used for the nmr experiments, were contained in an optical jacketed fused silica cell of 3-mm path length. The solution temperatures were varied by circulating water from a Haake thermostat bath type F through the outer jacket of the cells. The value of the parameter b_0 of the Moffitt–Yang equation was obtained for each solution by plotting $[R'][(\lambda^2 - \lambda_0^2)/(\lambda_0^2)]$ against $\lambda_0^2/(\lambda^2 - \lambda_0^2)$ using a value of $\lambda_0 = 212$ nm.

Solvents. All solvents except absolute alcohol (Burroughs), diethyl ether, and sulfuric acid (both BDH analytical grade) were dried and distilled before use. Chloroform was washed with concentrated sulfuric acid and water, dried over calcium chloride, and then distilled. Deuterated chloroform of isotopic purity 99.7% was obtained from Stohler Isotope Chemicals Ltd. and was used without further purification. Trifluoroacetic acid (Aldrich Chemicals) was distilled twice before use to remove all trace of impurity peaks in the nmr spectrum.

Materials. One sample of poly(β-benzyl L-aspartate) (DP 700) and one sample of poly(β-ethyl L-aspartate) were purchased from Pilot Chemicals. Other samples of poly(β-benzyl L-aspartate) of DP ranging between 100 and 300 were synthesised by W. E. Hanby at Courtaulds Ltd., and further samples of DP between 7 and 100 were synthesized in this laboratory. Copolymers of β-benzyl L-aspartate with other aspartate esters were prepared as described in paper 1 of this series. The series of copolymers of β-benzyl L-aspartate with L-alanine and with γ-benzyl L-glutamate were synthesized by W. E. Hanby (described by Bradbury, *et al.*[2]) and a series of copolymers of β-benzyl L-aspartate with L-leucine was synthesized in this laboratory (by B. G. Carpenter). The DP's of all these copolymers ranged from 100 to 400. All determinations of degree of polymerization were made with a capillary viscometer, and the polypeptides were dissolved in DCA at 0.5% (w/v). The calibration of Doty, *et al.*,[13] for poly(γ-benzyl L-glutamate) was used on the assumption that in the random-coil form discrepancies would not be large.

Results

The inclusion of 10% L-alanine residues in a polymer consisting largely of β-benzyl L-aspartate residues results in a helix transition from the LH form favored by the β-benzyl L-aspartate residues to the RH form dictated by the L-alanine residues. With this series of copolymers, it is possible, therefore, to study the effect of helix sense on the proton magnetic resonance spectrum of β-benzyl L-aspartate. Figure 1 shows the 100-MHz spectra of poly(β-benzyl L-aspartate) in the LH helical and random-coil forms and poly(10% L-alanine-*co*-90% β-benzyl L-aspartate) in the RH helical form. There are marked spectral differences between these three conformations; in particular, the chemical shift of the aspartate amide N*H* for the LH form is 8.75 ppm, for the RH form 8.20 ppm, and for the random-coil form 8.00 ppm. The aspartate α-C*H* peak is also sensitive to conformation, being at 4.30 ppm for the LH helix, 4.40 ppm for the RH helix, and at 4.85 ppm for the random coil. Other changes to note are those for the β-CH_2 resonance at 3 ppm, which shows a single peak for the random-coil form and different multiplets for the two helix forms. These changes can now be considered in more detail. The first question is whether they are general for the different poly(L-aspartate esters). Considering first the helix sense dependence of the chemical shift of the amide N*H*, Figure 2 shows the low-field 220-MHz spectra of the LH form of poly(β-benzyl L-aspartate) and the

(12) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. Goldman, *Nature (London)*, **225**, 64 (1970).

(13) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Amer. Chem. Soc.*, **78**, 947 (1956).

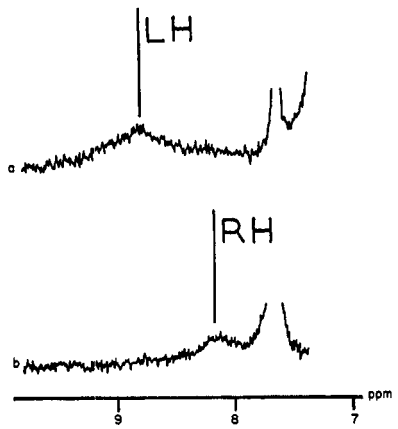

Figure 2. Amide N*H* region of the 220-MHz spectra of: (a) poly(β-benzyl L-aspartate) (LH) in $CDCl_3$–0.5% TFA and (b) poly(β-ethyl L-aspartate) (RH) in $CDCl_3$–1% TFA.

RH form of poly(β-ethyl L-aspartate). For the latter, the amide N*H* chemical shift is very close (8.16 ppm) to that found for the RH form of poly(90% β-benzyl L-aspartate-*co*-10% L-alanine) shown in Figure 1. Furthermore, poly(β-methyl L-aspartate) in the LH helical form in chloroform–0.5% TFA shows an amide N*H* resonance at 8.73 ppm. Thus a chemical shift difference of between 0.5 and 0.6 ppm between the two helix senses appears general for the N*H* resonance of poly(aspartate esters). This result differs from that of Ferretti,[14] who reported a shift of 7.95 ppm for the N*H* peak of LH poly(β-methyl L-aspartate); we have no explanation for this discrepancy.

The differences observed for the chemical shifts of the

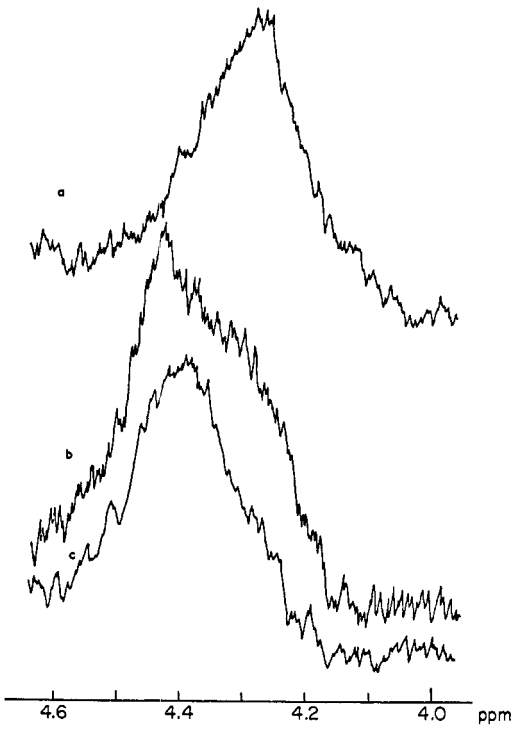

Figure 3. Spectra (220 MHz) of the α-C*H* resonance of β-benzyl L-aspartate residues in: (a) poly(β-benzyl L-aspartate), (b) poly(5% L-alanine-*co*-95% β-benzyl L-aspartate), (c) poly(10% L-alanine-*co*-90% β-benzyl L-aspartate) in $CDCl_3$–1% TFA at room temperature.

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

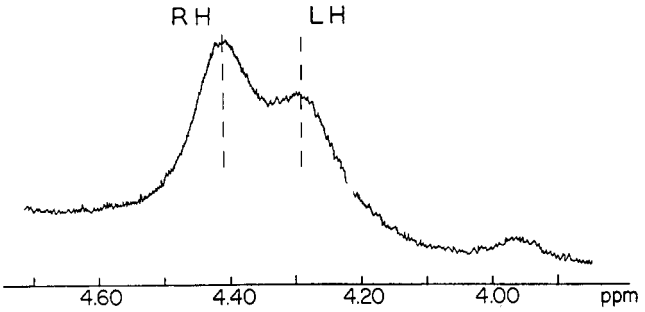

Figure 4. Spectrum (300 MHz) of the α-C*H* resonance of helical poly(95% β-benzyl L-aspartate-*co*-5% L-alanine) in $CDCl_3$–0.5% TFA.

aspartate α-C*H* were much smaller than for the amide N*H* and, although detected in our earlier work,[15] the correlation was not established owing to the restricted range of samples studied. Figure 3 shows the 220-MHz spectra of homopoly(β-benzyl L-aspartate) and copolymers with 5 and 10% L-alanine. The polymers were dissolved in the mixed solvent 99% $CDCl_3$–1% TFA and were observed at room temperature. The 1% TFA was added to avoid aggregation broadening. In spectrum a of the LH homopoly(β-benzyl L-aspartate) the α-C*H* resonance is situated at 4.3 ppm, whereas in spectrum c of RH poly(90% β-benzyl L-aspartate-*co*-10% L-alanine) the resonance from the same proton is situated at 4.4 ppm. Spectrum b for poly(95% β-benzyl L-aspartate-*co*-5% L-alanine) displays two α-C*H* peaks situated at 4.4 and 4.3 ppm. Figure 4 shows a spectrum of this polymer at 300 MHz, and a more clearly defined separation of the peaks is obtained. In $CHCl_3$–1% TFA this 5% L-alanine copolymer shows a b_0 value close to zero, consistent either with a random-coil conformation or with a mixture of the two helix senses. The presence of these two peaks at the characteristic chemical shift values for the helical forms and the absence of a resonance at the poly(β-benzyl L-aspartate) α-C*H* random-coil chemical shift of 4.80 ppm indicates that the copolymer is helical. The α-C*H* resonance of the 5% L-alanine is situated at about 4.0 ppm, a shift characteristic of RH L-polypeptides in chloroform–TFA, *i.e.*, with the exception of polymers of L-aspartate esters. Figure 5 shows the behavior of the chemical shift and b_0 values for copolymers of β-benzyl L-aspartate with increasing amounts of L-alanine. It is clear that the sharp transition in chemical shift is paralleled by the change in helix sense.

Series of random copolymers of β-benzyl L-aspartate have also been prepared with γ-benzyl L-glutamate and with L-

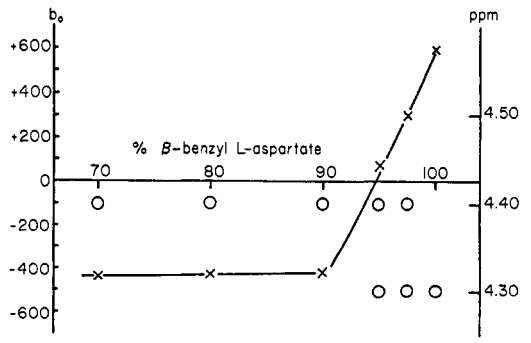

Figure 5. α-C*H* shift (100 MHz) of benzyl L-aspartate residues (O) and b_0 values (—) in the series poly(β-benzyl L-aspartate-*co*-L-alanine) dissolved in $CDCl_3$–TFA.

(15) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. W. E. Rattle, *Nature (London)*, **220**, 69 (1968).

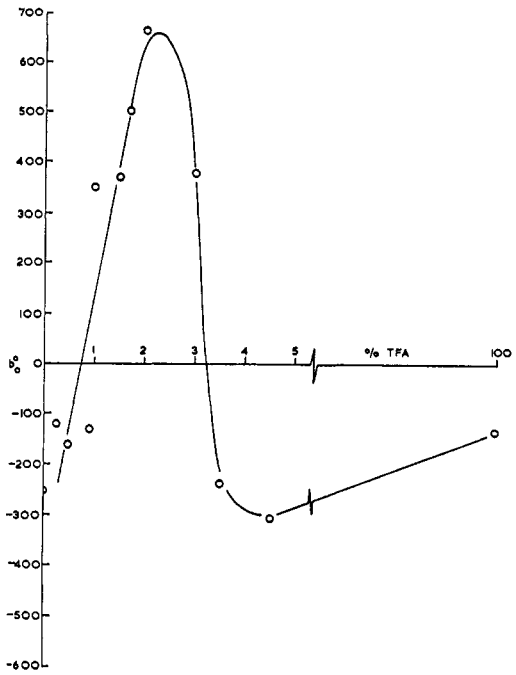

Figure 6. b_0 values of poly(45% ethyl L-aspartate-*co*-55% benzyl L-aspartate) in chloroform–TFA mixtures at room temperature.

leucine, both residues strongly favoring the RH helix sense. Poly(80% β-benzyl L-aspartate-*co*-20% L-leucine) was found to be in the RH conformation, and the aspartate α-C*H* chemical shift was found to be 4.41 ppm; similarly, for the RH form of poly(80% β-benzyl L-aspartate-*co*-20% γ-benzyl L-glutamate), the aspartate α-C*H* chemical shift was 4.42 ppm. A helix sense dependence of the α-C*H* resonance of β-benzyl L-aspartate is thus clearly demonstrated. Sternlicht and Wilson[16] have calculated that the chemical shift of the peak from the α-C*H* proton in a RH α helix would be 0.4 ppm downfield of the same proton in a LH α helix, while Conti[17]

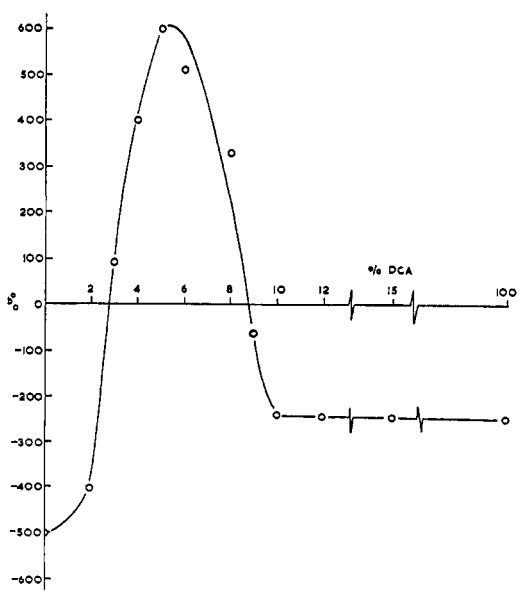

Figure 7. b_0 values of poly(60% ethyl L-aspartate-*co*-40% benzyl L-aspartate) in chloroform–DCA mixtures at room temperature.

predicts the reverse. The observed shift difference is at variance with both of these predictions.

As an extension of this study of the effect of the conformation of poly(L-aspartate esters) on the chemical shift of the proton groups, it was intended to study also the temperature-induced RH → LH helix transition of copolypeptides of L-aspartate esters, *e.g.*, it is known that poly(49% ethyl L-aspartate-*co*-51% benzyl L-aspartate) is in the right-handed form at 10° and in the left-handed form at 60°.[1] The higher temperature form, *i.e.*, the LH helix, has been studied and chemical shift values characteristic of this form have been obtained and found to coincide with those already given. Unfortunately, at the lower temperature and even at room temperature, the sum effects of aggregation and reduced molecular motion result in line widths too great for measurement of α-C*H* or amide N*H* chemical shifts. In an attempt to overcome the effects of aggregation, small amounts of TFA, up to 1%, were added to the $CDCl_3$ solution. This led to the unexpected result that TFA addition induced a RH → LH transition, and this is shown in Figure 6 for poly(45% ethyl L-aspartate-*co*-55% benzyl L-aspartate). The b_0 value in pure chloroform solution, −250°, is a result of the polypeptide composition and the temperature of measurement (see ref 1, Figure 4), and in this case the sample is rather more RH helical than LH helical in chloroform solution. Addition of 2% TFA to the solution causes a reversal of the sign of b_0 to +650°, indicating the LH helical form. Upon further addition of TFA, the polypeptide undergoes a LH helical to random-coil transition, the center of the transition occurring at ~3% TFA–97% chloroform. Copolymers of β-benzyl L-aspartate and β-phenyl L-aspartate also undergo the RH helix to LH helix transition on addition of TFA to the chloroform solution, the LH form being supported by 2% TFA–98% chloroform solvent. The substitution of dichloroacetic acid (DCA) for TFA produces a very similar effect, except that the LH form is supported in a solvent of composition 5% DCA–95% chloroform. A typical transition for DCA addition is shown in Figure 7. Poly(60% β-ethyl L-aspartate-*co*-40% β-benzyl L-aspartate), measured at room temperature, shows a transition from almost fully RH helical (b_0 = −500°) in pure chloroform to LH helical (b_0 = +600°) in 5% DCA–95% chloroform. 10% DCA is required for transition to the random-coil form. In all cases the random-coil form of the copoly(aspartate esters) yielded the usual anomalous value of $b_0 \approx -240°$. The stability of these helical copoly(L-aspartate esters) to breakdown by acid to the random coil is thus seen to be similar to that found[1] for homopoly(aspartate esters), *viz.*, about 3% TFA and 8% DCA are required.

It is of interest to note that the addition of acid to the chloroform solution of a copoly(L-aspartate ester) has the same effect upon the polypeptide as increasing the temperature of such a solution. Lotan, *et al.*,[18] have formulated a mechanism to explain the temperature-induced helix sense reversal. They have calculated that an increase in the radius of the hydrogen atom from 1.275 to 1.300 Å (to take account of increased librational motion at elevated temperatures) would reverse the result of their calculation of the preferred room-temperature RH helix sense of poly(β-*n*-propyl L-aspartate) to the LH form. This postulated increase in the hydrogen radius has a marked effect upon the side-chain nonbonded and torsional energies, rather than the main-chain energy, and this is the cause of the helix sense reversal.

(16) H. Sternlicht and D. Wilson, *Biochemistry*, **6**, 2881 (1967).
(17) F. Conti in "International Conference on Magnetic Resonance in Biological Research," C. Franconi, Ed., Gordon and Breach, New York, N. Y., 1971.
(18) N. Lotan, F. A. Momany, J. F. Yan, G. Vanderkooi, and H. A. Scheraga, *Biopolymers*, **8**, 21 (1969).

Small quantities of haloacetic acids could have an effect similar to temperature increase by virtue of their ability to radically alter these energies as a consequence of hydrogen bonding to the side-chain ester group. Such hydrogen bonding could also influence the electrostatic interaction energy between the side chain and the main chain.

The practical result of these acid-induced right-handed → left-handed helix transitions was that in the range of molecular weights used we were unable to observe sufficiently sharp backbone proton peaks of the right-handed forms to measure their chemical shift.

The Nmr Spectrum of the Aspartate Side-Chain Protons. According to the calculation of Scheraga, *et al.*,[11] the helix sense of poly(aspartate esters) depends on the conformation adopted by the side chains and a close study has therefore been made of the spectrum from the β-CH_2 and the benzyl CH_2 protons in the case of poly(β-benzyl L-aspartate) in the LH helical form.

The Random-Coil Spectrum. Figure 8 shows the upfield region of the spectrum of poly(β-benzyl L-aspartate) in the random-coil form in TFA.

The resonance centered at 5.0 ppm is that due to the α-C*H* proton. The spectrum of the N*H* proton in the random coil is a symmetrical doublet indicating J_{NC} = 7.0 Hz (in agreement with the value predicted by Gibbons, *et al.*[19]). When the polymer is dissolved in TFA-d_1 and the N*H* proton thereby exchanged for deuterium, the α-C*H* resonance becomes a broad symmetrical 1:2:1 triplet of separation ~6 Hz. The approximate 1:3:3:1 quartet nature of the α-C*H* resonance in TFA shown in Figure 8 is thus readily understood.

The β-CH_2 centered at 3.05 ppm is the AB part of the ABX system with the α-C*H* and shows Δ ≈0.07 ppm and J = 17 ± 1.5 Hz, broadened in part by the $J_{\alpha\beta}$ coupling. The spectrum is thus in fact an ABX octet. The shift difference of 0.07 ppm is a not unexpected consequence of the adjacent asymmetric α-carbon atom.

The benzyl CH_2 resonance centered at 5.15 ppm is an AB quartet of Δ 0.05 ppm and J = 12.5 Hz. There is no coupling to other protons, and the group is very mobile; the spectrum is therefore quite sharp. The benzyl CH_2 group of poly(γ-benzyl L-glutamate) shows a similar J_{gem} value, although it cannot be measured accurately owing to the very low Δ value.[12] Furthermore, the monomer β-benzyl L-aspartate in TFA shows Δ ≈0.03 ppm and J_{gem} = 12 ± 0.5 Hz. A J_{gem} value around 12 Hz is that expected for free rotation;[20] however, the shift difference of 0.05 ppm is unusual, the group being distant from the asymmetric center. Neither the planar benzene ring nor the planar ester group would be expected to generate a shift difference between these two protons; however, small shift differences have been noted in groups as far as seven bonds removed from an asymmetric center.[21]

Helical Structure. Poly(β-benzyl L-aspartate) in the LH helical form in chloroform at 100° has previously[12] been reported to yield a multiplet for both the benzyl CH_2 resonance (Δ 0.13 ppm) and the β-CH_2 resonance (Δ 0.37 ppm). The increases in the Δ values on forming the helix from the random coil reflect the formation of the asymmetric α helix, the effect being added to that due to the other asymmetries seen in the random-coil form.

Although these shift differences are readily measured and

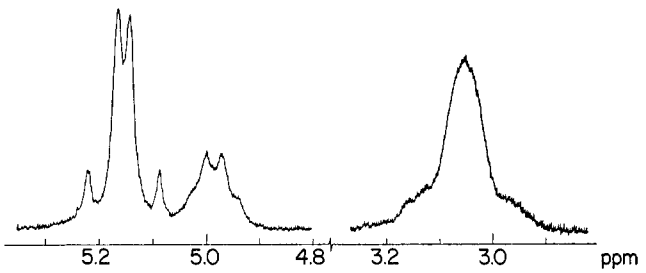

Figure 8. Spectrum (220 MHz) of poly(β-benzyl L-aspartate) in TFA.

are clearly very susceptible to conformational changes, it is nevertheless true that it is extremely difficult to make unambiguous interpretations in structural terms based on Δ values. Coupling constants, on the other hand, are in principle capable of precise interpretation, for example in terms of side-chain conformations, and this we have made an attempt to do. A study of coupling constants in polypeptides is also of interest in view of the potential of J values for defining the amino acid side-chain conformations of proteins in solution. However, the large size of globular proteins, and even the polypeptides used here, results in line widths comparable with J values and thus makes accurate measurement difficult. The small polypeptide antibiotics, hormones, and ion-transfer molecules can represent an exception to this general problem.

The β-CH_2 Resonance. Figure 9 shows a 100-MHz spectrum of the β-CH_2 resonance of LH poly(β-benzyl L-aspartate) together with a curve-resolver readout and analysis. It is the AB part of an ABX system, the α-C*H* being the X proton (this has been verified by decoupling the α-C*H* proton). It can be seen that the coupling constant of the A (low field) proton, $J_{\alpha A}$, is smaller than $J_{\alpha B}$, the values being 4 ± 1 and 7.4 ± 0.3 Hz, respectively. The peak numbered 9 in the figure is assigned to a small quantity of random-coil material and must in reality be a multiplet as seen in Figure 8. An attempt has been made to use these α–β vicinal coupling constants to obtain information on the allowed conformations about the α–β bond. These conformations can be most simply represented in terms of the three staggered rotamers shown in Figure 10. Assuming that no other conformations about this bond play a significant role, they may be assigned the frac-

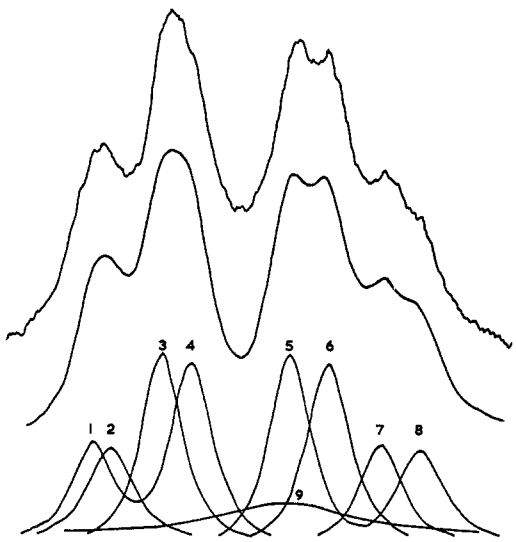

Figure 9. Spectrum (100 MHz) of the β-CH_2 group of poly(β-benzyl L-aspartate) in chloroform at 100° together with a curve resolver readout and analysis.

(19) W. A. Gibbons, G. Nemethy, A. Stern, and L. Craig, *Proc. Nat. Acad. Sci. U. S.*, **67**, 239 (1970).
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(21) G. M. Whitesides, D. Holtz, and J. D. Roberts, *J. Amer. Chem. Soc.*, **86**, 2628 (1964).

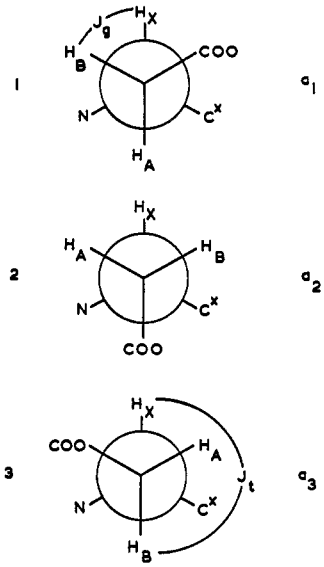

Figure 10. The three possible staggered conformations about the α-β bond of poly(aspartate esters).

tional lifetimes a_1, a_2, and a_3. Analysis in terms of these conformers then requires values for the coupling constants between protons in the gauche arrangement (J_g) and in the trans arrangement (J_t).

Paschler[22] has studied a number of amino acids and concludes that the values J_t = 13.6 Hz and J_g = 2.6 Hz are appropriate for the molecular grouping C–CH_2–CH–(NH_2)-CO_2^-. Although it is now apparent[23] that the vicinal coupling constant for the gauche arrangement may not be precisely identical in all three rotamers, it is not feasible at present to derive more precise values for these constants, and it is also difficult to assess the possible error in these quantities. However, since the anionic form of L-aspartic acid shows two $J_{\alpha\beta}$ coupling constants of 4.0 and 10.0 Hz,[24] it follows that $J_t > 10$ Hz and $J_g < 4$ Hz.

If the rotamer lifetimes are then calculated from the equations

$$a_1 = J_{AX} - J_g/J_t - J_g$$
$$a_3 = J_{BX} - J_g/J_t - J_g$$
$$a_2 = 1 - (a_1 + a_3)$$

using J_t = 13.6 Hz and J_g = 2.6 Hz, one obtains a_1 = 0.14, a_2 = 0.39, and a_3 = 0.47, although, since it is not possible to unequivocally assign the two β protons, there is an alternative solution with the a_1 and a_3 lifetimes interchanged. Space-filling models can be used, albeit very imprecisely, to determine whether each of the rotamers found for an amino acid is equally allowable for the same residue included in an α helix. Inspection of a space-filling model of poly(β-benzyl L-aspartate) in the LH α-helical form shows that there is considerable steric hindrance between the side-chain carboxyl group and the helical backbone which would largely prevent the formation of rotamer **2** (χ_1 = 60°). Using Courtauld models, this rotamer cannot be made. In terms of the above analysis using the staggered 60° rotamers shown in Figure 10, this implies that $J_{AX} + J_{BX} = J_g + J_t$, and this equality can only hold if J_g and J_t are considerably reduced from the values given above. One is led to the conclusion that the experimentally observed coupling constants cannot be interpreted in terms of the 60° rotamers unless exceptionally low values of J_g and J_t are used or one disregards the steric factors which hinder the formation of rotamer **2**. Since the α-C atom is included in a rigid asymmetric helix, it is possible that the minima in the potential energy function of rotation about the C_α–C_β bond do not lie at the 60° staggered conformations. Inspection of the model indicates that for rotamer **1** the $C_\alpha H_X$–$C_\beta H_B$ angle might increase from 60 to 70° and for rotamer **3** the $C_\alpha H_X$–$C_\beta H_A$ angle might increase to 75°. Imprecise though these figures are, it is of interest to see what effect they would have on the coupling constants. The variation in coupling constant with dihedral angle can be represented by a Karplus-type function, and we have used an approximate $\cos^2$ relationship arranged to have a value of 13.6 Hz at 180°, 2.6 Hz at 60°, and 0 at 90°. This curve yields, for the distorted rotamer **1**, $J_{H_X-H_B} \simeq 0.5$ Hz, $J_{H_X-H_A} \simeq 11$ Hz and for the distorted rotamer **3**, $J_{H_X-H_A} \simeq 1.0$ Hz and $J_{H_X-H_B} \simeq 12.0$ Hz. It follows from this that the observed J values cannot be explained in terms of either of these rotamers alone. Furthermore, inspection of this postulated variation of J with dihedral angle indicates that there is no single position of the β-CH_2 group possible such that one proton would show $J \approx 4$ Hz and the other $J \approx 7.5$ Hz. Thus no single rotamer, distorted or not, can account for the observed couplings.

Scheraga, *et al.*,[11] have predicted two possible side-chain conformations for LH poly(β-benzyl L-aspartate) having low energy. These are designated Lt(−) (having a transverse side-chain arrangement) and Ll(+) (having a longitudinal side-chain arrangement), the former being of lower energy. In both situations the β-CH_2 conformation is close to that of rotamer **1** in Figure 9 ($\chi_1 \approx 180°$) and the precise $C_\alpha H_X$–$C_\beta H_\beta$ angles are 54° for Lt(−) and 66° for Ll(+). One β proton should thus show a vicinal coupling of 12–13 Hz and the other 1–3.5 Hz, but this is not observed. Thus neither of these rotamers alone, nor a combination of just these two, can explain the observed results. The most reasonable conclusion is that under the conditions of measurement the β-CH_2 is in rapid motion between two rotamers that approximate **1** and **3**. It might be assumed that the effective values of the coupling constants are an average of those in the postulated rotamers, *i.e.*, J_g = (1.0 + 0.5)/2 = 0.7 Hz and J_t = (11 + 12)/2 = 11.5 Hz, since they do not differ greatly from each other. It is seen that $J_g + J_t$ = 12.2 Hz, which does not differ greatly from the sum of the observed J values. The observed couplings are therefore consistent with rapid motion between the postulated rotamers, and their values suggest about one-third of one rotamer and two-thirds of the other. It is not of course possible, as stated earlier, to distinguish which of **1** or **3** predominates without unambiguous assignment of the A and B protons on the β carbon. In this context, however, it is worth noting that infrared dichroic studies[25] of films of LH poly(β-benzyl L-aspartate) show strongly parallel dichroism in the β-CH_2 bending mode at 1416 cm^{-1}. Assuming the presence of a single rotamer in films, this data is compatible only with rotamer **3**.

The geminal coupling of the β-CH_2 protons is observed to be 16.5 ± 0.5 Hz in both the LH helical form and the random-coil form. Moreover, aspartic acid itself shows a value of J_{gem} between 16.3 and 17.8 Hz,[26] depending on the pH, and we have found that poly(95% L-aspartic acid-*co*-5% L-alanine)

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in TFA shows $J_{gem} = 18 \pm 0.5$ Hz when dissolved in TFA. This coupling constant is expected to be dependent on the orientation of the β-CH_2 group with respect to the π orbitals of the γ-carboxyl group. This has been discussed theoretically by Barfield and Grant,[27] and the results have been summarized by Cookson, *et al.*[28] A J_{gem} value between 16 and 18 Hz is not consistent with free rotation about the C^{β}—C^{γ} bond and indicates that the preferred conformations are those in which the direction of the C^{γ}=O bond lies close to the bisector of the H_A—C^{β}—H_B angle when both are projected onto a plane at right angles to the C^{β}—C^{γ} bond direction. Thus, although there is restriction to free rotation about this bond, there is no evidence that the preferred conformations are different in the LH helical form from those in the random-coil form or in the amino acid. In the two conformations postulated by Scheraga, *et al.*,[11] the angles between the C^{γ}=O direction and the β-CH_2 bisector are Lt(−), 21°, and Ll(+), 58°. The observed J_{gem} values thus exclude the Ll(+) conformation but are not inconsistent with the Lt(−).

The RH form of poly(β-benzyl L-aspartate) can be induced by incorporation of 10% L-alanine as described previously. We have not been able to observe a very sharp β-CH_2 spectrum from this polymer, though the shift difference Δ between these two protons lies between 0.5 and 0.6 ppm, a value larger than observed for the LH form and in rough agreement with the value of 0.6 ppm observed for RH poly(β-ethyl L-aspartate).[1]

The Benzyl CH_2 Spectrum. The shift difference between the two benzyl CH_2 protons of LH poly(β-benzyl L-aspartate) has previously been reported as 0.13 ppm and, as stated above, is 0.06 ppm in the random-coil form. This has been further studied by examining the benzyl CH_2 resonance of the series of random copolymers of benzyl L-aspartate and L-alanine and plotting Δ for each copolymer (dissolved in $CDCl_3$ and the minimum quantity of TFA for dissolution) against composition and b_0.

The results are shown in Figure 11, and it is seen that the change in conformation from LH to predominantly RH (as indicated by the b_0 change) is accompanied by an increase of Δ from 0.13 to 0.28 ppm. The value of Δ in both helical conformations, particularly the RH, is much in excess of that in the random coil. The 220-MHz spectrum of the benzyl CH_2 group of poly(γ-benzyl L-glutamate)[12] shows no measurable splitting, and it is clear that the asymmetry of the benzyl L-aspartate helix, both RH and LH, is strongly felt at the benzyl CH_2 group. However, as indicated previously, the interpretation of Δ values in terms of rotamer populations is extremely difficult and, while in the present case unequal rotamer populations clearly occur and are moreover dependent on the helix sense, it is nevertheless very difficult from the shift data given to obtain any real estimate of the degree to which the side chain is immobilized. Restriction to free rotation about the bond between the benzyl CH_2 group and the benzene ring could readily give rise to Δ values of the observed order. However, the J_{gem} value observed for the benzyl group of poly(β-benzyl L-aspartate) in the LH form is the same as those measured for the random-coil form and for the monomer in TFA (12 ± 0.5 Hz). These data do not suggest any change in the conformation about this bond on forming the helix.

The change of Δ in the RH helix sense as more than 20% L-alanine is added (see Figure 11) is unexpected and must be

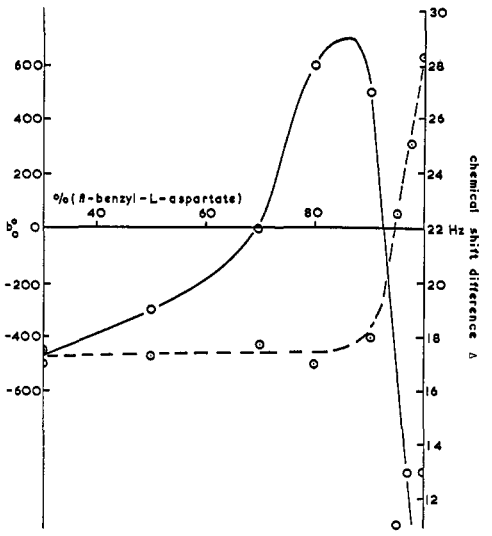

Figure 11. The shift difference Δ (100 MHz) between the benzyl CH_2 protons of β-benzyl L-aspartate residues and b_0 for the series poly(benzyl L-aspartate-*co*-L-alanine) in chloroform–TFA at room temperature: —, shift difference; – – –, b_0.

due to changes in the rotamer populations of the benzyl L-aspartate side chains as the polymer composition changes. It is reasonable to suppose that while the L-aspartate side-chain conformations would remain essentially unchanged by the presence of a small amount of "foreign" residue, they would be radically altered by a large proportion. Similar experiments have been performed by copolymerizing benzyl L-aspartate with L-leucine and also with benzyl L-glutamate. In both series of copolymers, Δ changes monotonically, without any maximum, from 0.13 ppm in pure LH poly(β-benzyl L-aspartate) to 0.18 ppm in the RH copolymers containing 20–25% of the added foreign residues. This difference between the L-alanine containing copolymers and those with L-leucine or benzyl L-glutamate must be a result of the fact that the side-chain conformations characteristic of LH poly(β-benzyl L-aspartate) can accommodate 20% alanine residues but not a similar quantity of the much larger L-leucine or benzyl L-glutamate side chains.

Conclusions

Studies of copolymers of L-aspartate esters have clearly demonstrated a dependence of the chemical shift of the backbone protons, the N*H* and α-C*H*, on the helix sense. The chemical shifts of the N*H* resonance are 8.20 and 8.75 ppm for the RH and LH helix senses, respectively, while for the α-C*H* resonance they are 4.40 ppm for the RH helix and 4.30 ppm for the LH helix. Since there are no detailed data at hand on the coupling constants of the RH form of poly(L-aspartate esters), it is not possible to say from the experimental results whether these changes are due solely to differences in the main-chain anisotropies or whether differences in side-chain conformations also contribute. That the side-chain conformations are different in the solid state for the two helix senses of poly(β-benzyl L-aspartate) has been noted in polarized infrared spectroscopic studies of films of oriented polymers.[29] The conformational dependence of the backbone proton chemical shift values of poly(L-aspartate esters) can be used for the conformational analysis of L-aspartate copolymers, and

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this has been done for a series of block copolymers with γ-benzyl L-glutamate.[30]

The predicted[11] helix sense of poly(β-benzyl L-aspartate) [as with that of other esters of poly(L-aspartic acid)] rests on a small free energy difference between two conformations having opposite helix sense and specified side-chain conformation. For the four low-energy conformations considered[11] (two RH, two LH), the conformational dependence of the side-chain energy terms (torsional, nonbonded, and electrostatic) is much in excess of the final energy difference between the total energy of the lowest energy RH and lowest energy LH form, *i.e.*, the predicted helix sense is critically dependent on the assumed side-chain conformations. The present results indicate considerable motion in the β-CH_2 group in chloroform solution, and this does not seem compatible with the degree of side-chain immobilization envisaged in the free energy calculations of ref 11. If the side chain possessed a dominant conformation, this would have been readily apparent from the nmr spectra. The helix sense of poly(aspartate esters) is clearly dependent on the statistical sum of a wide range of side-chain conformations.

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Conformational Aspects of Polypeptide Structure. XXXIV. Amino Acid Substituted Poly-L-lysines

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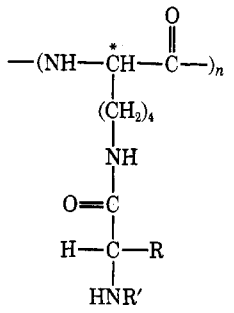
ABSTRACT: An investigation was carried out on several poly-L-lysines containing amino acid substituents on the N^{ϵ} position. The conformations of these polypeptides were examined using circular dichroism and 220-MHz nuclear magnetic resonance techniques in both aqueous and organic media. The results suggest that amino acid side chains can have important influences on the backbone conformation of a polypeptide. Specifically, we observed that whereas poly-L-lysine hydrobromide and poly(N^{ϵ}-glycyl-L-lysine hydrobromide) exist in a "random coil" conformation in distilled water, both poly(N^{ϵ}-L-phenylalanyl-L-lysine hydrobromide) and poly(N^{ϵ}-L-leucyl-L-lysine hydrobromide) are α helical.

The use of poly(α-amino acids) as models for proteins has yielded significant but often simplified information on the various factors affecting conformational stability. In an attempt to gain more detailed information concerning the stereochemical influence of peptide branches in proteins and cell membranes, we have undertaken a conformational investigation of a series of N^{ϵ}-substituted polylysines of the following form.



R = H, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$

R′ = H(HBr), $COOCH_2C_6H_5$

Thus, derivatives of polylysines containing glycine, phenylalanine, and leucine residues on the N^{ϵ} position were examined. The class of compounds having R′ = Z will be referred to as "blocked" polymers, whereas when R′ = H(HBr) the compounds are usually called salts. In this paper we discuss the conformational analysis of these polypeptides using circular dichroism (CD) and 220-MHz nuclear magnetic resonance (nmr) techniques.

Experimental Section

(a) Preparation of Materials. Poly(L-lysine hydrobromide). Poly(L-lysine hydrobromide) was prepared according to the method of Katchalski.[2] The benzyloxycarbonyl protecting group was removed using hydrogen bromide in trifluoroacetic acid.

Poly(N^{ϵ}-*N*-benzyloxycarbonyl-glycyl-L-lysine) (I). Poly(L-lysine hydrobromide) (627 mg, 3 mmol) was dissolved in 4.5 ml of water and triethylamine (0.33 g, 3.3 mmol) was added. The resulting solution was stirred at 5–10° and a solution of *p*-nitrophenyl *N*-benzyloxycarbonylglycinate (1.38 g, 4.5 mmol) in 10 ml of dioxane was added dropwise. An additional 110 ml of dioxane was added in small portions, and the reaction was allowed to stir at room temperature for 40 hr. The resulting mixture was concentrated *in vacuo*. The residue so obtained was washed with ether, dried, and finally washed with water. After drying *in vacuo* over phosphorus pentoxide, a white amorphous powder (0.85 g, 89%) was obtained. The intrinsic viscosity determined in dichloroacetic acid at 25° was 0.19. This corresponds to a molecular weight of approximately 20,000.

Poly(N^{ϵ}-glycyl-L-lysine hydrobromide) (II). The blocked polymer (300 mg) was dissolved in 5 ml of a 45% solution of hydrogen bromide in acetic acid and allowed to stand at 20° for 30 min. Addition of a large excess of ether resulted in the precipitation of the

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