Conformations of "Alternating" Sequential Polypeptides in Solution

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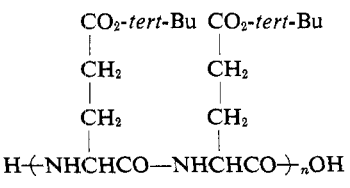
ABSTRACT: Spectroscopic studies of alternating polypeptides, prepared by polymerizing dipeptides containing one L and one D residue, indicate that in helicogenic solvents they adopt conformations with properties different from those of the normal α helix. The properties of such polypeptides in which polymerization has been accompanied by appreciable racemization are similar to those of poly(L-aspartic esters), and a distorted type of α helix is suggested; when racemization is negligible a new conformation, the nature of which is uncertain, appears to be formed.

This paper is concerned primarily with the conformations adopted in solution by "alternating" sequential polypeptides of the type $H\text{-}(AB)_nOH$, where A and B are amino acid residues which give rise to poly(amino acids), HA_nOH and HB_nOH, with α-helical conformations of opposite sense in helicogenic solvents. Much earlier work[1] has been devoted to the study of the conformations of polypeptides of the type $H\text{-}(A_x,B_y)_nOH$, prepared by copolymerization of mixtures of the *N*-carboxy anhydrides

The interpretation of the results is, however, complicated by the uncertain sequence of A and B residues in the products. This uncertainty does not arise in the present work, in which the alternating polypeptides were prepared by polymerization of the dipeptides HABOH or their active esters.

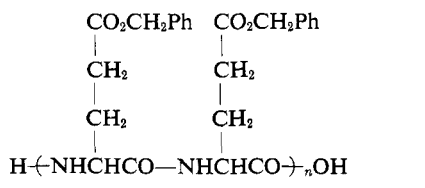
Some years ago, two of us[2] reported studies of the ^1H nmr spectra and optical rotatory dispersion (ORD) of the alternating polypeptide poly(γ-*tert*-butyl-L-glutamyl-*tert*-butyl-D-glutamate) (I) and its optical antipode; these products contained a small excess of L and D residues, respectively, arising from racemization of the C-terminal residue in the polymerization of the dipeptide monomer used to make the polypeptides. We concluded that the two polypeptides adopted helical conformations in chloroform solution which were very easily



I (L/D = 56/44)

disrupted by addition of the helicoclastic solvent dichloroacetic acid; from the observed values of the Moffitt parameter b_0 (see Table I) it was concluded that I formed right-handed helices, the sense of the helix being determined by the configuration (L in this case) of those residues in excess in the polypeptide. Later, Spach, Brack, and Heitz[3] reached a similar conclusion in the case of the analogous benzyl esters.

In continuation of our earlier work we have now synthesized poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II) by an improved procedure causing less racemization and giving a product containing 52% L and 48% D residues. The ORD of this product in chloroform is shown in Figure 1 (curve B),



II L D (L/D = 52/48)

III D L (L/D = 43/57)

IV L L (L/D = 100/0)

together with that of the all-L compound, poly(γ-benzyl L-glutamate) (IV) (curve A); the Moffitt parameters for the two compounds are given in Table I. Taken at its face value, the negative sign of b_0 for II suggests that the polypeptide forms a right-handed α helix, and the magnitude of b_0 that the solution contains about 17% α helix and 83% random coil. This simple interpretation is, however, ruled out by the ^1H nmr spectra of II in $CDCl_3$–trifluoroacetic acid (TFA) mixtures (see Figure 3). In $CDCl_3$ alone the NH and α-CH peaks are so broad as to be undetectable in a single pass, indicating a wholly ordered structure and ruling out the presence of a large amount of random-coil conformation.[4-7] Separate "ordered" and random-coil peaks[7-10] are observable in accumulated spectra (Figure 4) in mixtures containing small amounts of TFA.

At this stage in our work, Dr. Spach kindly sent us a specimen of the D,L polypeptide III, which we found to contain 57% D and 43% L residues on the basis of its ORD in TFA solution. The ORD of this material in chloroform solution is shown in Figure 1 (curve C). The peaks at 360 nm in the ORD curves for II and III might be thought to be due to an

(1) For references, see G. D. Fasman in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., p 533.
(2) D. I. Marlborough and H. N. Rydon in "Some Newer Physical Methods in Structural Chemistry," R. Bonnett and J. G. Davis, Ed., United Trade Press, London, 1967, p 211.
(3) G. Spach, A. Brack, and F. Heitz, *C. R. Acad. Sci., Ser. C*, **267**, 809 (1968).
(4) F. A. Bovey, G. V. D. Tiers, and G. Filipovich, *J. Polym. Sci.*, **38**, 73 (1959).
(5) M. Goodman and Y. Masuda, *Biopolymers*, **2**, 107 (1964).
(6) D. I. Marlborough, D. G. Orrell, and H. N. Rydon, *Chem. Commun.*, 518 (1965).
(7) H. N. Rydon, *Polym. Prepr., Amer. Chem. Soc., Div. Polym. Chem.*, **10**, 25 (1969).
(8) J. A. Ferretti, *Chem. Commun.*, 1030 (1967).
(9) J. C. Haylock and H. N. Rydon, "Peptides," Proceedings of the 9th European Peptide Symposium, 1968, p 19.
(10) E. M. Bradbury, C. Crane-Robinson, H. Goldman, and H. W. E. Rattle, *Nature (London)*, **217**, 812 (1968).

TABLE I
ORD PARAMETERS FOR SOME POLYPEPTIDES

Polypeptide	Moffitt equation[a] a_0	b_0	Two-term Drude equation $A_{(\alpha,\rho)(193)}$	$A_{(\alpha,\rho)(225)}$
I	+68	−214	+907	−618
II	+106	−115	+546	−306
III	−576	+539	−2603	+1401
IV	+260	−665	+2911	−1910
V	+191	+225	−813	+760
OCH_2Ph | $HAsp_nOH$	−1593	+630	−3543	−1189

[a] For $\lambda_c = 212$ nm.

optically active absorption band at too high a wavelength to permit the safe use of the Moffitt or two-term Drude equations; however, circular dichroism measurements in hexafluoro-2-propanol (kindly carried out for us by Dr. F. A. Bovey) show that this is not so, since III shows a positive CD maximum at about 218 nm and II a very weak negative maximum at about 225 nm, which is the position of the longest wavelength maximum for poly(γ-benzyl L-glutamate) (IV), to which the Moffitt and Drude equations are certainly applicable. The Moffitt parameters for III are given in Table I; the positive sign of b_0 suggests the presence of a left-handed helical conformation, in agreement with the excess of D residues, while the magnitude of b_0 would indicate an α-helix content of 81%, with 19% random coil. However, the reduced mean residue rotation, [m'], of the material is very high (see Figure 1), being three to four times that of the all-L compound IV in the visible range of the spectrum, and the shape of the ORD curve, like that for II, is quite different from that for IV. It seems quite impossible to interpret these results on the basis of the alternating polypeptides II and III having the same, α-helical, conformation as the all-L polypeptide IV, whether mixed with random-coil material or not, and we are forced to the conclusion that the alternating polypeptides must adopt some other conformation.

In Table I are also shown the values of the two-term Drude

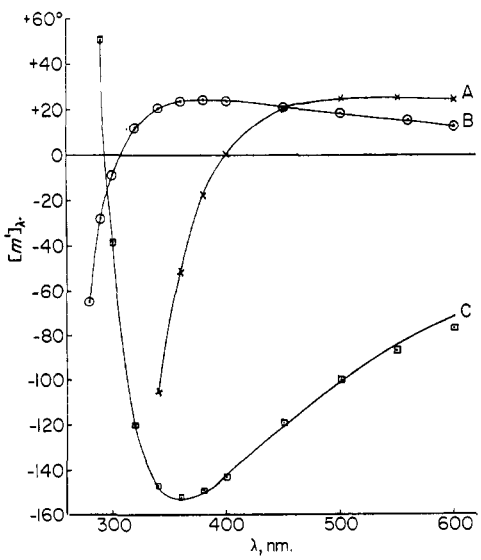


Figure 1. Optical rotatory dispersion of solutions in chloroform at 33°: A, poly(γ-benzyl L-glutamate) (IV); B, poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II); C, poly(γ-benzyl-D-glutamyl-γ-benzyl-L-glutamate) (III).

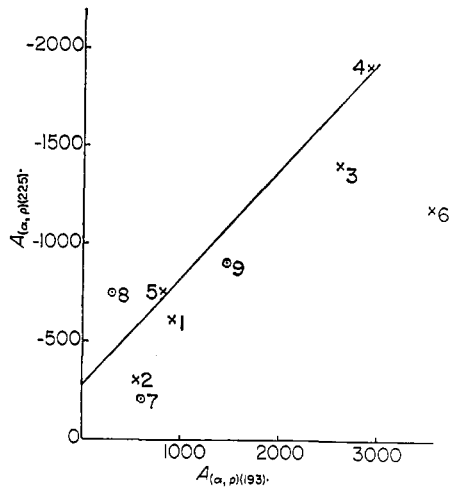
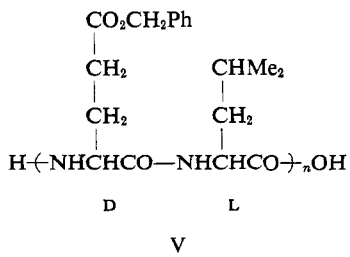


Figure 2. Two-term Drude parameters. The numbers identify the polypeptides: × (present work) 1, poly(γ-*tert*-butyl-L-glutamyl-γ-*tert*-butyl-D-glutamate) (I); 2, poly(γ-benzyl L-glutamyl-γ-benzyl-D-glutamate) (II); 3, poly(γ-benzyl-D-glutamyl-γ-benzyl-L-glutamate) (III) (signs reversed); 4, poly(γ-benzyl L-glutamate) (IV); 5, poly(γ-benzyl-D-glutamyl-L-leucine) (V) (signs reversed); 6, poly(β-benzyl L-aspartate); ⊙ (ref 11, polypeptides known not to be α helical); 7, poly(*O*-acetyl-L-serine); 8, poly(*S*-methyl-L-cysteine); 9, poly(L-proline) I (signs reversed).

parameters,[11] $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)(225)}$, for the alternating polypeptides I, II, and III and for poly(γ-benzyl L-glutamate) (IV). As will be seen from Figure 2, the parameters for the alternating polypeptides depart from the relationship[12] connecting these parameters for α helix ⇄ random coil transitions to as great an extent as do those for polypeptides in which it is well established that the ordered form is not the α helix. This evidence, too, indicates that the ordered form in the alternating polypeptides is not the α helix.

Optical titration shows that the ordered structure present in chloroform solutions of these polypeptides is much weaker than the undoubted α helix present in similar solutions of poly(γ-benzyl L-glutamate) (IV); it is necessary to add 30% TFA to reach the midpoint of the conformational transition in the latter case,[6] but only 3% is required in the case of the alternating polypeptide II.

Similar, but less clear-cut, results have been obtained with poly(γ-benzyl-D-glutamyl-L-leucine) (V), in which alternate residues again tend to give rise to α helices of opposite sense. The optical parameters for this polypeptide, in which the



V

polymerization process has caused the inversion of 1 in 50 of the L-leucine residues, are given in Table I. In this case the divergence of the two-term Drude parameters from the Schechter–Blout relationship is very small; this is probably fortuitous, since there is other evidence (see below) which indicates that the very weak ordered structure present in

(11) E. Schechter and E. R. Blout, *Proc. Nat. Acad. Sci. U. S.*, **51**, 695 (1964).
(12) E. Schechter and E. R. Blout, *ibid.*, **51**, 794 (1964).

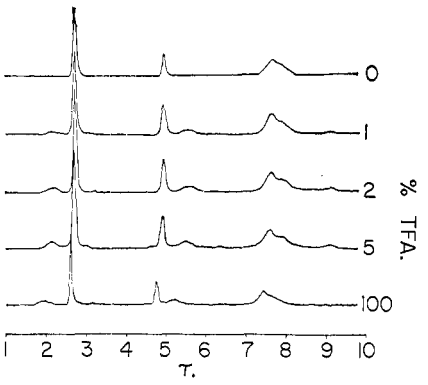


Figure 3. ^{1}H nmr spectra (60 MHz) of poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II) in $CDCl_3$–TFA at 33°.

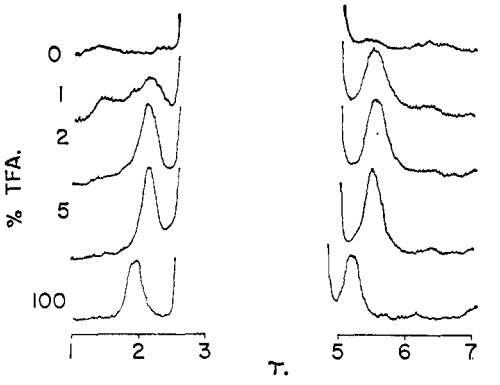


Figure 4. ^{1}H nmr spectra (60 MHz) of poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II) in $CDCl_3$–TFA at 33°; NH and α-CH regions; 150 accumulations.

chloroform solutions, which needs only 2% TFA for its half-destruction, is not the α helix.

The 60-MHz ^{1}H nmr spectra of the alternating polypeptide II in $CDCl_3$–TFA mixtures[13] are shown in Figures 3 and 4. It will be seen from Figure 3 that, as in our earlier work,[2,6] there is a progressive "unfreezing" of the molecule from the periphery inward, indicating the presence of an ordered structure in $CDCl_3$ which passes with the addition of TFA into the random-coil form. Well-separated peaks can be observed in accumulated spectra (Figure 4) for the ordered and random-coil forms in both the NH and α-CH regions; the separation of the peaks is much greater than it is for all-L polypeptides such as IV, for which the chemical shift difference is too small ($\delta = \tau$ 0.2–0.3) to allow completely separated peaks to be observed at 60 MHz.[9] Analysis of the NH region of the ^{1}H nmr spectrum of II in 1% TFA–$CDCl_3$ (Figure 4) shows the presence of three Lorentzian peaks (τ 1.50, 1.85, and 2.20; relative areas 2:1:7); the published ^{1}H nmr spectrum[14] of poly(γ-benzyl L-glutamate) (IV) shows only two NH peaks, at τ 1.85 and 2.20, corresponding to α helix and random coil, respectively. Attention is drawn to the presence in the spectrum of II of an ordered-structure NH peak downfield by τ 0.35 from the position of the α-helix peak in the spectrum of IV; we return later to the nature of the third NH peak in the spectrum of II. Although there are presumably also three peaks in the α-CH region of the ^{1}H nmr spectrum of II, only two can be resolved, at τ 5.5 and 6.3 in 1% TFA–$CDCl_3$; the spectrum[14] of IV in 4–12% TFA shows random-coil and α-helix peaks at τ 5.5 and 6.0, respectively. In this region, too, the ordered-structure peak in the spectrum of II is displaced, upfield this time, by τ 0.3 from the position of the α-helix peak in the spectrum of IV. We know of no macromolecular polypeptide or poly(α-amino acid), for which the α-helical structure has been established, in which the peak for the ordered NH proton appears as far downfield (τ 1.50) and that for the ordered α-CH proton as far upfield (τ 6.3) as they do in the nmr spectra of the alternating polypeptide II. We regard these considerable differences between the chemical shifts for the NH and α-CH protons in the ordered structure of II and those for the corresponding protons in the known α-helical structure of IV as strong evidence that the ordered structure of II differs from the α helix in the nature of the environment of the NH and α-CH protons, *i.e.*, in the conformation of the peptide chain.

The ^{1}H nmr spectra of the alternating polypeptide V are complicated by the presence of two different side chains, and interpretation is made difficult by the lack of the spectra of the corresponding L,L compound for comparison. However, the spectra clearly indicate the presence of an ordered structure in $CDCl_3$ solution. In the NH region in 1% TFA–$CDCl_3$ the ordered peak, at τ 1.3, is 0.5–0.6 downfield from the random-coil peak, while in the α-CH region there are two ordered peaks, one 0.3 downfield and one 0.7 upfield from the random-coil peak at τ 5.5. Again, as with II, we have ordered peaks in both the NH and α-CH regions abnormally widely separated from the random-coil peaks, suggesting that here, too, we have a conformation which is different from the α helix. The third α-CH peak will be discussed later.

A further spectroscopic difference between the alternating polypeptides and stereochemically homogeneous poly(amino acids) is found in their infrared spectra, the principal frequencies in which are recorded in Table II. It will be seen that both the N–H stretch (amide A) and amide I bands are shifted to higher frequencies, by about 10 cm^{-1} in each case, in the alternating polypeptides; there is no such clearly defined shift in the ester C=O, amide II, and amide II′ bands. It will also be seen from Table II that similar frequency shifts are observed in the ir spectrum of poly(D,L-methionine), made by polymerization of D,L-methionine *N*-carboxy anhydride (*cf.* the earlier observations of Tsuboi, *et al.*,[15] on poly(γ-benzyl D,L-glutamate).

Others[16–18] have observed similar frequency shifts in the N–H stretch and amide I bands on passing from right-handed to left-handed helical conformations in esters of poly(L-aspartic acid); for the left-handed helices the bands are at 3302 ± 3 and 1666 ± 2 cm^{-1}, respectively, whereas for the right-handed helices they are at 3296 ± 3 and 1659 ± 2 cm^{-1}. In this respect, the ir spectra of our alternating polypeptides are very similar to those of the left-handed poly(L-aspartic esters); we do not, however, observe the smaller shifts found in the ester C=O and amide II bands in the latter compounds.

The ORD parameters for poly(β-benzyl L-aspartate) given

(13) *Cf.* F. A. Bovey, J. J. Ryan, G. Spach, and F. Heitz, *Macromolecules*, **4**, 433 (1971).
(14) J. A. Ferretti and B. W. Ninham, *ibid.*, **3**, 30 (1970); *cf.* F. A. Bovey, *Pure Appl. Chem.*, **16**, 417 (1968).
(15) M. Tsuboi, Y. Mitsui, A. Wada, T. Miyazawa, and N. Nagashima, *Biopolymers*, **1**, 297 (1963).
(16) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, and W. E. Hanby, *J. Mol. Biol.*, **5**, 230 (1962).
(17) M. Hashimoto and S. Arakawa, *Bull. Chem. Soc. Jap.*, **40**, 1968 (1967).
(18) E. M. Bradbury, B. G. Carpenter, and R. M. Stephens, *Biopolymers*, **6**, 905 (1968).

TABLE II
PRINCIPAL INFRARED ABSORPTIONS OF SOME POLYPEPTIDES[a]

Polypeptide	N—H	Ester C=O	Amide I	Amide II	Amide II′
I	3310	1722[b]	1661	1550	1456
II	3300	1738	1665 (1650)	1549	1457
III	3303	1733	1662	1553	1457
V	3290	1736	1663 (1641)	1549	1454
H-DL-Met$_n$OH	3304		1662	1550	*c*
Mean	3303	1736	1663	1550	1456
OCH_2Ph | HGlu$_n$OH	3293	1734	1653	1552	1454
HMet$_n$OH	3292		1653	1550	*c*
OCH_2Ph | H(Glu,Met)$_n$OH	3292	1737	1652	1550	*c*
Mean	3292	1736	1653	1551	1454

[a] Wave numbers in cm^{-1}; 1% solutions in chloroform. [b] Excluded from mean. [c] Obscured by $-SCH_3$ at 1441 cm^{-1}.

in Table I are calculated from the data of Karlson, *et al.*;[19] these authors concluded, from the positive sign of b_0, that this polymer formed a left-handed helix, but pointed out that the change in $[m']$ in passing from poly(γ-benzyl L-glutamate) to poly(β-benzyl L-aspartate) was much greater than would be expected for simply passing from a right-handed to a left-handed α helix. The values of the two-term Drude parameters for poly(β-benzyl L-aspartate) fall far off the Schechter–Blout line for α helices (see Figure 2).

We have studied the nmr spectra of poly(β-benzyl L-aspartate) in $CDCl_3$–trichloroacetic acid and $CDCl_3$–dimethyl sulfoxide mixtures.[7] At low concentrations of the helicoclastic solvent (3% TCA; 10% DMSO) the peak for the NH proton in the ordered component is at abnormally low field (τ 1.15 and 1.23, respectively), although the position of the random-coil peak is normal (τ 2.05 and 1.90); Ferretti[20] records, without comment, a similar abnormal position (τ 1.3; 0.7 downfield of the random-coil peak) for the NH proton in the ordered component of the corresponding methyl ester, which is also known to form a left-handed helix.

The evidence presented above demonstrates that in their spectroscopic properties (ir, ORD, nmr) the alternating polypeptides I, II, III, and V resemble the left-handed poly(L-aspartic esters) much more closely than right-handed α-helical polypeptides such as poly(γ-benzyl L-glutamate). It is tempting to ascribe this resemblance to a similarity in conformation and to suggest that both types of polypeptides share a common conformation which is not the normal α helix. The left-handedness of the poly(L-aspartic esters) first studied was ascribed[21,22] to interactions between the side-chain ester group and the peptide backbone. However, as Bradbury and his colleagues[23] have pointed out, the realization that poly(L-aspartic esters) can adopt left-handed or right-handed conformations according to the nature of the ester group, the solvent, and the temperature makes it clear that there is in fact a delicate balance between many factors. The poly(L-aspartic ester) ordered structure is very much weaker than well-authenticated α helices,[19,24] and it seems not unreasonable to suggest that it is not in fact a true α helix; this view finds further support in the recently observed profound difference in the electric dichroism of poly(γ-benzyl L-glutamate) and poly(β-benzyl L-aspartate).[25]

We suggest that both the alternating polypeptides we have studied and the left-handed poly(L-aspartic esters) have a much distorted α-helical conformation, the degree of distortion being such as to effect the energetically best compromise between the avoidance of unfavorable side chain–side chain interactions, *e.g.*, between successive L and D residues in I, II, and III, or unfavorable side chain–backbone interactions in left-handed poly(L-aspartic esters), and the weakening of intrachain hydrogen bonds which such distortions will inevitably cause. An extreme conformation of this kind is the α′ helix of Némethy, *et al.*[26–28] As these authors point out, "the entire region between the α- and the α′-helix on the conformational map is of low potential energy" and many intermediate conformations are possible; it is this range of conformations which we suggest as likely structures for alternating polypeptides and left-handed poly(L-aspartic esters). A similar, but less definite, suggestion was made by Tsuboi, *et al.*,[15] for poly(γ-benzyl D,L-glutamate).

In these distorted structures the NH groups are tilted inward and the CO groups outward relative to the axis of the helical framework. This tilting makes it easier for the structure to accommodate D residues in a right-handed helix, and *vice versa*, and it is for this reason that we find the α′ helix, or some conformation intermediate between this and the α helix, so attractive for our alternating polypeptides. It is obvious that the changed orientation of the amide groups, resulting in a change in the angle between the transition moment and the axis of the helix, will have a marked effect on the ORD of the molecule.[29] The change in orientation of the NH groups will affect the position of the N–H stretch

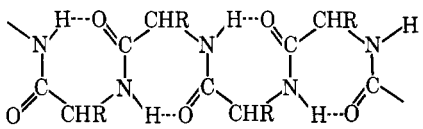
(19) R. H. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2268 (1960).
(20) J. A. Ferretti, *Polym. Prepr., Amer. Chem. Soc., Div. Polym. Chem.*, **10**, 29 (1969).
(21) M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *Biopolymers*, **1**, 371 (1963).
(22) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).
(23) E. M. Bradbury, B. G. Carpenter, and H. Goldman, *Biopolymers*, **6**, 837 (1968).
(24) E. M. Bradbury, A. R. Downie, A. Elliott, and W. E. Hanby, *Proc. Roy. Soc., Ser. A*, **259**, 110 (1960).
(25) E. Charney, J. B. Milstien, and K. Yamaoka, *J. Amer. Chem. Soc.*, **92**, 2657 (1970).
(26) G. Némethy, D. C. Phillips, S. J. Leach, and H. A. Scheraga, *Nature (London)*, **214**, 363 (1967).
(27) Némethy, *et al.*,[26] designated their structure the α_{II} helix but, following Ramachandran and Sasisekharan,[28a] we prefer the designation α′, since α_{II} has been preempted[28b] for another structure.
(28) (a) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 283 (1968); (b) M. L. Huggins, *Chem. Rev.*, **32**, 195 (1943).
(29) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956); *Proc. Nat. Acad. Sci. U. S.*, **42**, 736 (1956).

ir band, weakening the hydrogen bonds owing to their departure from linearity and thus causing a shift to higher frequency; it will also affect the position of the amide I band by decreasing the strength of the $i/(i + 3)$ interactions, again leading to a shift to higher frequency.[30,31] The effect on the ^{1}H nmr spectrum is less easy to predict, since both hydrogen bonding of the NH of the ith peptide group to the CO of the $(i + 3)$ group and shielding by other nearby atoms and groups will be involved; it seems, from the nature of the shifts actually observed, that the predominant effect on the NH proton is decreased shielding rather than weakened hydrogen bonding, while the α-CH proton experiences a net increase in shielding.

It seems, both from our present and earlier[2] studies and those of Spach and his colleagues,[3,32] that the helical sense adopted by an imperfect alternating polypeptide is determined by the nature of the amino acid residue in excess. In II, for example, each "imperfection" resulting from racemization of the C-terminal residue in the monomer gives rise to a sequence of three L residues; there is, of course, likely to be more than one imperfection in each molecule and all will be of the L–L–L type. Each such sequence will tend to generate a right-handed helix and, once a sufficient number of such sequences is present, it is not unreasonable to suppose that the polymer will adopt a conformation, within the α–α' range, as close to a right-handed α helix as the various interactions permit.

There remains one anomaly, *viz.*, the origin of the low-frequency amide I bands in the ir spectra of II and V, at 1650 and 1641 cm^{-1}, respectively (Table II); these have areas relative to the 1633-cm^{-1} bands of 0.7 and 2.0, respectively. Such bands are not seen in the ir spectra of the other alternating polypeptides I and III. In the case of II, the relative area of the low-frequency amide I band correlates as well as can be expected with the area of the second ordered NH ^{1}H nmr peak at τ 1.85, which is half that of the other ordered NH peak at τ 1.50. In V there is a similar correlation between the intensity of the ir band at 1641 cm^{-1} and the area of the ordered α-CH ^{1}H nmr peak at τ 5.2, which is much greater than that of the other ordered α-CH peak at τ 6.2. We ascribe these low-frequency ir bands and second ordered ^{1}H nmr peaks to a nonhelical conformation taken up by those molecules which have no imperfections arising from racemization during the polymerization process. It is easy to show that such "perfect" molecules can form an appreciable proportion of the polymer only when very little racemization accompanies polymerization. The fractions of molecules H–(–AB–)$_n$–OH containing 0, 1, 2, ... imperfections are given by the first, second, third, ... terms of the binomial expansion of $(p + q)^n$, where p and q are the fractions of unchanged and inverted B residues in the whole polymeric product and n is the degree of polymerization. The results of such calculations for the four alternating polypeptides we have studied are given in Table III; the values of p (and $q = 1 - p$) were calculated from the experimentally determined extent of racemization of the B residues in the four polypeptides. It will be seen that perfect alternating molecules are likely to be found to a much greater extent in just those two polypeptides, II and V, for which the low-frequency amide I band is observed.

Whatever the conformation of these perfect alternating molecules is, it must be capable of accomodating alternate L and D residues with equal facility. Since the NH stretch band at 3300 cm^{-1} shows no sign of moving to yet higher frequency on dilution, it is also probable that all the NH groups are intramolecularly hydrogen bonded. A structure which would fulfill these requirements is the α_{II} structure (VI) of Huggins[28b] and it may be that this conformation, long ago[33] suggested for poly(γ-benzyl L-glutamate), is that adopted by



VI

our perfect alternating polypeptides; much more evidence is needed, however, before this suggestion can be accepted.

TABLE III
IMPERFECTIONS IN SOME POLYPEPTIDES

Polypeptide	p	q	n	Fraction containing given number of imperfections 0	1	2
I	0.88	0.12	25	0.04	0.16	0.31
II	0.96	0.04	50	0.13	0.27	0.28
III	0.86	0.14	50	0.0005	0.004	0.02
V	0.98	0.02	50	0.36	0.37	0.19

Experimental Section

Materials. Full details of the preparation of the polypeptides used will be reported elsewhere, an outline only being given below.

(a) Poly(γ-*tert*-butyl-L-glutamyl-γ-*tert*-butyl-D-glutamate) (I) was prepared in 69% yield by polymerization of γ-di-*tert*-butyl-L-glutamyl-D-glutamate with dicyclohexylcarbodiimide in acetonitrile; low-molecular-weight material was removed by shaking a solution in dimethylformamide with G15-Sephadex. The degree of racemization was determined by comparing optical rotation, $[\alpha]^{21}_{300}$, of the product of complete hydrolysis by hydrochloric acid at 110° with that of a control using L-glutamic acid. The molecular weight, which is a minimum value, was determined by gel filtration (G75-Sephadex) of the poly(glutamic acid) obtained from the polypeptide ester I by treatment with 90% aqueous trifluoroacetic acid.

(b) Poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II) was prepared in 42% yield by polymerization of α-1-succinimidyl-γ-benzyl-L-glutamyl-D-γ-benzyl-glutamate in chloroform; low-molecular-weight material was removed by Soxhlet extraction with methanol and by precipitation from chloroform with methanol. The degree of racemization was determined by comparing the optical rotation (simple dispersion) of a solution in trifluoroacetic acid with that of a solution of poly(γ-benzyl L-glutamate) in the same solvent between 280 and 600 nm. The molecular weight (minimal) was determined by gel filtration (G150-Sephadex) of the poly(glutamic acid) obtained from the polypeptide ester II by treatment with 50% hydrogen bromide in acetic acid.

(c) Poly(γ-benzyl-D-glutamyl-L-leucine) (V) was prepared in 51% yield by polymerization of α-1-succinimidyl-γ-benzyl-D-glutamyl-L-leucine in tetrahydrofuran; low-molecular-weight material was removed by Soxhlet extraction with methanol and by precipitation from chloroform with methanol. The degree of racemization was ascertained by determination of residual D-leucine in an acid hydrolysate after removal of D-glutamic acid by chromatography and L-leucine by L-amino acid oxidase. The molecular weight (minimal) was determined as for II.

Poly(γ-benzyl L-glutamate) (IV) was prepared by polymerization of the recrystallized *N*-carboxy anhydride in 5% solution in freshly redistilled dioxane with diethylamine (0.1 equiv) as initiator and isolated as described by Blout and Karlson.[34]

(30) T. Miyazawa, *J. Chem. Phys.*, **32**, 1647 (1960).
(31) T. Miyazawa and E. R. Blout, *J. Amer. Chem. Soc.*, **83**, 712 (1961).
(32) F. Heitz and G. Spach, *Macromolecules*, **4**, 429 (1971).
(33) C. H. Bamford, W. E. Hanby, and F. Happey, *Proc. Roy. Soc., Ser. A*, **205**, 30 (1951).
(34) E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, **78**, 941 (1956).

Poly(β-benzyl L-aspartate) was prepared by polymerization of the recrystallized *N*-carboxy anhydride in 2% solution in freshly redistilled dichloromethane with sodium methoxide (0.005 equiv) as initiator and isolated by precipitation with petroleum ether (40–60°), followed by repeated washing with ethanol, mol wt (osmotic pressure in chloroform) 22,000.

Physical Measurements. Optical rotatory dispersion measurements were made on a Bellingham and Stanley Pepol spectropolarimeter with a 4-cm cell or on a Bendix-Ericsson Polarmatic 62 recording polarimeter, with a 1-mm cell. Nuclear magnetic resonance spectra were recorded on a Perkin-Elmer R10 60-MHz nmr spectrometer fitted with a Digico Digiac computer for averaging accumulated spectra. Infrared spectra were recorded, at ambient temperature, on a Hilger and Watts Infrascan H900 recording spectrophotometer.

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Poly(L-proline) II Ring Conformations Determined by Nuclear Magnetic Resonance

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ABSTRACT: From a computer simulation of the 220-MHz nmr spectrum of poly(L-proline) II, ring vicinal couplings have been obtained. These couplings are related to ring rotation angles φ, χ_i, i = 1–4, using a Karplus type function. Owing to uncertainties in couplings involving the approximately equivalent γ protons, the Karplus analysis indicates that a number of ring conformations are consistent with the nmr data; however, it is found that the ring conformation proposed for poly(L-proline) II in the solid state is inconsistent with the couplings determined from the simulation. From the nmr analysis and previously reported energy calculations, it is proposed that in form II the proline ring rapidly interconverts between two equally populated conformations, (C^+) and (C^-). C_γ is exo in (C^+) and endo in (C^-), being *ca.* 0.5 Å out of the plane of the remaining four atoms in each conformation.

The solid-state conformations of poly(L-proline) I and II have been determined by X-ray analysis.[1-3] It has been established[4,5] that in solvents such as water and acetic acid the polypeptide backbone structure is similar to that found in the solid state for form II; that is, $\omega = 0°$ (trans peptide bonds),[6] $\psi \approx 300°$ (trans' C_α—C=O bonds), and $\varphi \approx 120°$ (as required to form the pyrrolidine ring). The form II ring conformation obtained from the X-ray analysis[2] has N, C_α, C_γ, and C_δ nearly coplanar, with C_β-exo[7] *ca.* 0.4 Å out of this plane (*i.e.*, $\chi_1 \approx 25°$). Since intermolecular (interchain) interactions present in the solid lattice are absent in solution, the ring conformation in solution may be different from that in the solid state. Calculations[8] suggest that in solution, where intramolecular interactions are expected to predominate, two puckered conformations may be present, one having C_γ exo and the other with C_γ endo.

High-resolution nmr has been widely applied[9] to the study of molecular conformation in solution. Recently, the Hyp ring conformation of cyclo(L-Pro-L-Pro-L-Hyp) benzoate and the Pro ring conformations of cyclo(tri-L-prolyl) have been deduced[10] from a Karplus type analysis[11-13] of vicinal couplings obtained from direct measurement and computer simulation of 220-MHz nmr spectra of these compounds. In this paper a similar type of analysis is made of the 220-MHz nmr spectrum of poly(L-proline) II in D_2O. Ring conformations are proposed on the basis of the nmr analysis and energy calculations.[8]

Experimental Section

Materials. The synthesis and characterization of the low-molecular-weight (DP ≈ 25) sample of poly(L-proline) used in this report have appeared in the literature.[14,15] D_2O ("100.0%") was purchased from Diaprep, Inc. and *tert*-butyl alcohol-d_1 was obtained from Merck Sharp and Dohme.

Methods. Nmr spectra were obtained using a Varian HR-220 spectrometer, and homonuclear spin decoupling was accomplished using a General Radio 1107-A audio oscillator. The *tert*-butyl resonance at τ 8.77 (relative to DSS in D_2O) of *tert*-butyl alcohol-d_1 was used as internal reference. The poly(L-proline) II solution was prepared by first equilibrating a form I sample (7 mg) in D_2O (2 ml). This solution was lyophylized, the sample dissolved in 100.0% D_2O (0.4 ml), and the spectrum obtained at 20°. It was not possible to reduce the line widths by raising the sample temperature, since the polymer precipitates at elevated temperatures ($T \gtrsim 60°$). A sharp methyl singlet[15] at τ 6.27 and the HDO side bands have been edited from the spectrum shown.

(1) P. M. Cowan and S. McGavin, *Nature (London)*, **176**, 501 (1955).
(2) V. Sasisekharan, *Acta Crystallogr.*, **12**, 897 (1959).
(3) W. Traub and U. Shmueli, *Nature (London)*, **198**, 1165 (1963).
(4) J. P. Carver and E. R. Blout in "Treatise on Collagen," Vol. I, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, p 441, and references therein.
(5) L. Mandelkern in "Biological Macromolecules," Vol. I, G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 675, and references therein.
(6) For explanation of conventions used in dihedral angle nomenclature: J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Némethy, G. N. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).
(7) An out-of-plane ring atom is endo if it lies on the same side of ring plane as the carbonyl carbon. An exo ring atom lies on the opposite side of the ring.
(8) G. N. Ramachandran, A. V. Lakshmenarayanan, R. Balasubramanian, and G. Tegoni, *Biochim. Biophys. Acta*, **221**, 165 (1970).
(9) H. Booth, *Progr. Nucl. Magn. Resonance Spectrosc.*, **5**, 149 (1969), and references therein.
(10) C. M. Deber, D. A. Torchia, and E. R. Blout, *J. Amer. Chem. Soc.*, in press.
(11) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); **33**, 1842 (1960).
(12) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).
(13) M. Barfield and M. Karplus, *ibid.*, **91**, 1 (1969).
(14) C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, *ibid.*, **92**, 6191 (1970).
(15) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).