number of hydrogen bonds which can be formed. The dissymetry in the secondary structure should vanish with increasing molecular weight.

It must be mentioned that no α to π_{DL} transconformation could be detected in other solvents than dioxane or chloroform. Indeed the α-helical structure is stable with temperature in TMP, pyridine, or benzene and is partially destroyed into random coil in DMF. Addition of 0.5% of formamide to dioxane inhibits the transconformation. No clear relation with the nature of the solvent can be established.

The solution obtained by dissolving at room temperature the π_{DL} form, which is metastable in the solid state, shows identical properties to that obtained by dissolution of the α_{DL} form.

Due to its stereochemical structure, PBD-LG is a model for Gramicidin A. The above-mentioned proposed π_{DL}[4] helix for PBD-LG belongs to the same family as that proposed by Urry[6,7,27,28] for Gramicidin A, i.e., the π_{DL}[6] helix. No evidence for this latter structure has yet been found for PBD-LG.

In another connection, from the point of view of infrared spectroscopy, the α to π_{DL} transconformation is reversible if the solution is not too concentrated ($c < 3\%$) and not kept too much time at high temperature, otherwise new conformations, based on double helices analogous to those proposed by Blout et al.,[29,30] are found.[31]

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(12) Abbreviations used: Glu, glutamic acid; PBLG, poly(γ-benzyl L-glutamate); PBD-LG or PBL-DG to indicate the configuration of the C- and N-terminal residues, alternating poly(γ-benzyl D-L- or L-D-glutamate); PBD,LG, random poly(γ-benzyl D,L-glutamate); Bzl, benzyl; BDG, γ-benzyl D-glutamate; BLG, γ-benzyl L-glutamate; DCA, dichloroacetic acid; DMF, dimethylformamide; HFIP, hexafluoro-2-propanol; TEA, triethylamine; TFA, trifluoroacetic acid; TMP, trimethylphosphate.
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High-Resolution Nuclear Magnetic Resonance Studies at 270 MHz of Alternating and Random Poly(benzyl D,L-Glutamates)

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ABSTRACT: The solution conformations of several D,L copoly benzyl glutamates both random and alternating are studied by comparing their NMR spectra in chloroform and also in dioxane and dimethylformamide. The α *CH* chemical shifts characteristic of the α helix of strictly alternating D-L copolymers in chloroform/0.5% TFA are established (3.65 and 3.82 ppm) and differ from that of the regular α helix (3.92 ppm). It is concluded that alternating copolymers prepared by an essentially racemization-free method are completely in the α-helical conformation which is characteristic of strictly alternating D-L copolymers, whereas random copolymers are largely regular α. The $\alpha \rightarrow \pi_{DL}$ helix/helix transition of an alternating copolymer in dioxane has been monitored and the α *CH* resonance characteristic of the π_{DL} helix is found to be at the unusually low chemical shift of 4.45 ppm.

Since ORD and CD are of limited applicability to the study of polypeptides containing randomly distributed D and L residues of the same amino acid, NMR spectroscopy together with infrared and hydrodynamic measurements are the most useful methods for studying the conformation of D,L copolymers in solution. Random poly(γ-benzyl glutamates) (PBG) have been the most studied poly(D,L-peptides) to date and for high molecular weight samples the existence of a α-helical conformation is now recognized. However, for alternating D-L copolymers questions still remain on their conformation. Although for the latter polymers information can sometimes be obtained by other techniques such as ORD, a comparative NMR study of random and alternating poly(D,L-peptides) can be very infor-

mative about the local difference which exists between the two kinds of polymers.

Bovey et al.[2a] showed by 100-MHz spectroscopy that upon addition of trifluoroacetic acid (TFA) to solutions in chloroform ($CDCl_3$), statistical PBD,LG appeared to behave identically with helical PBLG. The same was found for an alternating sample (called DL-7). In a recent work on random copolymers, Paolillo et al.[2b] reached the same conclusion but they observed in the α C*H* region an unusual secondary peak at 3.65 ppm which was not clearly assigned but seemed to be related to the increase of D residue amount in a right-handed L-polymer helix. This peak suggested the existence of a distorted helix. The same authors observed that in dimethylformamide (DMF) no corresponding upfield peak can be detected, and that the chemical shift of the α C*H* proton of helical PBD,LG is 0.04 ppm upfield of that of PBLG. This shows that the D,L-copolymer conformation is not identical with that of the homopolymer in DMF and this shift difference might also be due to a distortion of α helices.

In their study of alternating poly(D-L-peptides), Hardy et al.[3] did not reach the same conclusion as Bovey. By examination of the N*H* and α C*H* regions they concluded that the conformation of their polymers differed from the α helix as regards the environment of the N*H* and α C*H* protons, i.e., the main chain conformation, and supported their conclusions by ORD measurements.

It should be noted, however, that during the synthesis of the alternating polymers cited above (DL-7 and the samples of Hardy et al.) some racemization occurred, thus creating sequences of three successive residues having the same chirality (see Table I) and thus leaving some uncertainty as to whether the results are a true reflection of perfectly alternating D–L sequences.

Since we have at our disposal alternating PBD-LG[4] prepared by a racemization-free method,[5] we undertook further investigation by NMR spectroscopy in order to clarify the conformational question. Since it has been shown that when dissolved in dioxane these samples can undergo a temperature induced $\alpha \rightarrow \pi_{DL}$ transition between two different types of helices,[6] this transition was also monitored. In addition, several samples of PBD,LG with different ratios of D/D + L and samples prepared by different synthetic methods were also studied.

Materials and Methods

When studied by infrared spectroscopy[6] in chloroform solution it is concluded that all the samples described here are fully α helical, except $LD_{cat}II$ which reveals at room temperature the presence of a small fraction in the π_{DL} helical conformation. Except DL-A, all samples are optically active in this solvent and the coefficients b_0 of the Moffitt equation are given below in order to estimate the right handed/left handed ratio (RH/LH). For all alternating samples $0 < |b_0| < 600°$ indicating that both helix senses are present.

Experimental Section

PBLG (CBM 0) $\bar{M}_w$ = 20,000 was prepared by polymerization in benzene of the corresponding *N*-carboxyanhydride.

DL-A: the same sample as used by Bovey et al;[2a] prepared by polymerization in benzene of an equimolar mixture of D and L *N*-carboxyanhydrides. It has the conformation of a broken rod in DMF and each helical part seems to be formed by nearly homopolymer blocks.[7,8]

DL-7: the same sample as used by Bovey et al;[2a] prepared through the active ester method by polycondensation of the dipeptide pentachlorophenyl ester.[9] The ratio D/L + D was calculated as 0.54 from ORD measurements in TFA but is probably underestimated (0.66 if compared to the optical rotation of sample LDL in the same solvent);[10] b_0 = +400° in $CHCl_3$ and +365° in DMF. In the solid state it has a slightly distorted α-helical conformation from X-ray diffraction: rise per residue = 1.49 Å; $\bar{M}_w$ = 21000.

Table I
Distribution of D and L Residues in the Different PBD,LG Samples

Samples	Sequences
PBLG	$(\text{-L-L-L-L-})_n$
DL-A	$-(\text{L} + \epsilon\text{D})_n - (\text{D} + \epsilon\text{L})_m - (\text{L} + \epsilon\text{D})_p -$ $\epsilon < 20\%$
DL-7	$\cdots (\text{D} - \text{L})_n - \text{D} - \text{D} - (\text{D-L})_m \cdots$
LD_{cat}	$(\text{L-D-L-D-L-D})_n$
LDL	$(\text{LDLLDLLDL})_n$
LD 80/20	$\cdots \text{L}_n - \text{D}_m - \text{L}_p \cdots$ $m < n$ and p

LD 80/20 (series 375[11]) prepared by polymerization of the corresponding mixture of *N*-carboxyanhydrides in dioxane; b_0 = −540° in $CHCl_3$/0.5% TFA.

$LD_{cat}II$: prepared by polycondensation of the tetrapeptide 2-hydroxyphenyl ester.[4] No racemization could be detected by optical rotation measurements on the polymer dissolved in TFA. Its conformation has been identified as the α helix when dissolved in chloroform but a small fraction in the π_{DL} helical conformation is detectable by infrared spectroscopy.[6] Heating solutions in DMF up to 60°C leads to a slight decrease of the optical rotation at any wavelength over 300 nm probably due to the formation of random coil.[6] This sample shows an α to π_{DL} helix transition in dioxane when the temperature is raised from 20 to 85°C. In the solid state, after evaporation of chloroform at room temperature, it has an α_{DL} helical conformation (3.8 residues per turn, rise per unit = 1.47 Å) and after heating at 120°C under vacuum it has the π_{DL} helical conformation (2.2 dipeptide units per turn, rise per dipeptide unit = 2.33 Å):[12] $\bar{M}_w$ = 31000; $[\eta]^{25}$DCA = 10.0 ml g^{-1}.

$LD_{cat}III$: prepared in the same polycondensation run as $LD_{cat}II$ but is a higher molecular weight fraction. No π_{DL} helix exists at room temperature in solution in chloroform or in dioxane and the α to π_{DL} transition occurs to a smaller extent than with $LD_{cat}II$ when the temperature is raised up to 85°C. In the solid state it has the same behavior as $LD_{cat}II$, $[\eta]^{25}$DCA = 14.4 ml g^{-1}.

LDL: prepared by the same method as the two last samples:[4] $\bar{M}_w$ = 20000; b_0 = −345° in DMF and −410° in $CHCl_3$/0.5% or 1% TFA.

The primary structures of the samples are summarized in Table I.

NMR. All spectra were recorded at 270 MHz on a Bruker WH 270 working in the F.T. Mode. All spectra in $CDCl_3$–TFA mixtures were recorded at 18°C. Spectra obtained at higher temperatures in DMF and dioxane were calibrated to 1°C using a propane diol sample.

Results and Discussion

In $CDCl_3$ Containing 0.5% TFA. Under these conditions all the samples were established as being in a α-helical conformation on the basis of infrared measurements (amide I at ~1660 cm^{-1} and II at ~1550 cm^{-1}).[6] Since all alternating samples have an optical activity due to a favored helical sense which has its origin in the presence of an excess of one enantiomer (LDL and DL-7[9]) or in the existence of side chain–side chain interactions ($LD_{cat}II$ and $LD_{cat}III$) (see preceding paper) the α-helical conformation could be confirmed by ORD and CD measurements.

It is important to appreciate that for all the copolymer samples, both the right- and left-handed helical sense exist. The exception to this is the fully right-handed LD 80/20.

The NMR spectra obtained are compared in Figure 1, and the chemical shifts of the N*H* and α C*H* protons are listed in Table II.

As expected from the block model proposed earlier for DL-A,[7] its spectrum is similar to that of PBLG although the α C*H* and N*H* peaks are not quite as sharp as those of PBLG. The side chain spectrum of DL-A is, however, sig-

Table II
Chemical Shifts of the N*H* and α C*H* Protons of PBD,LG's in $CDCl_3$/0.5% TFA

Samples	δ, ppm	
	N*H*	α C*H*
PBLG	8.23	3.92
DL-A	~8.1	3.94
LD 80/20	8.25	3.91 + 3.66
LDL	8.71 + 8.31	3.91 + 3.83 + 3.70
DL-7	8.50	(3.9_2) + 3.83 + 3.655
LD_{cat} II	8.56	3.82 + 3.655
LD_{cat} III	8.56	3.82 + 3.655

nificantly different from that of PBLG and clearly there is some conformational heterogeneity in DL-A, probably due to the fact that the rigid helical blocks are connected by more flexible parts. The spectra of DL-A and PBLG differ from that observed for random PBD,LG which showed the main α C*H* peak at 3.95 ppm but also a secondary upfield peak at 3.65 ppm.[2b] This peak is clearly seen (Figure 1) in the spectrum of LD 80/20, which closely resembles that of random PBD,LG. These spectral differences are good evidence for different primary structures and support the idea that the 3.65 ppm peak has its origin in the introduction of D residues into an L polymer (or vice versa). Thus the sample 375 (1) 52/48 described by Paolillo et al.[2b] is the B form type of PBD,LG as expected from the solvent in which the polymerization was done (dioxane). The A form (DL-A) is obtained by polymerization in benzene.[7,8] An exact interpretation of the peak at 3.65 ppm does not follow immediately from a measurement of the relative intensities of the two α C*H* peaks at 3.95 and 3.65 ppm. This ratio is nearly the same in DL 80/20 and the 52/48 sample of Paolillo et al. (the upfield peak constitutes about 15% of the total α C*H* area for the latter polymer). More data relevant to the origin of the 3.65 ppm peak can be obtained by the study of the periodic copolymers. Samples LDL, DL-7, and LD_{cat} show a gradual increase in the intensity of the 3.65 ppm peak as D (or L) residues are introduced up to 50% into a chain initially formed by L (or D) residues. This behavior of the 3.65 ppm peak indicates clearly that it has its origin in the introduction of D (or L) residues into an L (or D) chain while the conformation remains helical. At the same time the 3.95 ppm peak is shifted to 3.82–3.83 ppm. Examination of the N*H* region also shows drastic changes characterized by a down-field shift of the resonance and by the appearance of two N*H* peaks in the case of LDL.

The assignment of α C*H* peaks in the spectra of Figure 1 follows from the fact that while the 3.65 ppm peak is absent in PBLG its intensity increases as the amount of regularly alternating sequence increases, until in LD_{cat}III it constitutes just half the total α C*H* area. Since the LD_{cat} polymers were prepared without any detectable racemization occurring during the synthesis, the higher molecular weight sample LD_{cat}III should be conformationally homogeneous. As both right- and left-helical senses of the distorted α helix are present for this polymer,[6] the 3.65 ppm peak must be due both to D residues on right-handed helices (RH) and L residues on left-handed helices (LH) (i.e., on the "wrong" sense) and the 3.83 ppm peak must be due to L residues on RH helices and D residues on LH helices.

Thus in a perfectly nonracemized sample the two areas would be exactly the same (as observed) whatever proportion of right- and left-handed helices in the fully helical polymer. In a previous paper[12] it was shown that alternating PBD-LG prepared through the catechol ester method (the LD_{cat} copolymers) has a distorted α-helical conformation in the solid state (rise per residue = 1.47 Å and 3.8 residues per turn, instead of 1.50 Å and 3.6 residues per turn for the classical α helix). It is possible that this deformation of the classical α helix still exists in solution for samples LD_{cat}II and LD_{cat}III but it might be that on dissolution the helix reverts to something closer to the regular α. Since the DL-7 polymer contains some D-D-D sequences (due to some racemization) it will be pushed toward a greater proportion of LH helices than RH helices and the additional D residues would be largely incorporated into helices of the "correct" sense thereby contributing to the 3.83 ppm peak. DL-7 also shows a small assymetry in the region of 3.95 ppm which might be assigned to regions of the chain in which two or more D-D-D sequences are adjacent. The LDL polymer again has a very low degree of racemization and probably therefore a very regular conformation. Its α C*H* spectrum consists of three peaks. The component at 3.70 ppm is very close to the 3.65 ppm component of the LD_{cat} samples and can likewise be assigned to residues on the "wrong" helix sense (i.e., largely the D residues since the polymer is largely right handed). The two low-field components would then be assigned to the L residues which are not exactly equivalent because there is a small local and regular perturbation of the main chain or side chain arrangement in the region of one of the L residues. These assignments on LDL are supported by the observation that the relative intensity R = summed area of low-field peaks (3.91 + 3.83)/area of the high-field peak (3.70 ppm) is close to 2 and a similar ratio is found for the two N*H* peaks.

In the case of random D,L copolymers the 3.65 ppm peak would be assigned to "wrong helix sense" residues also, but only to those taking up locally the specific helix characteristic of nonracemized alternating L–D copolymers. On the basis of the present assignment therefore, α C*H* peaks at 3.65 and 3.82 ppm for D,L copoly benzyl glutamates in $CDCl_3$/0.5% TFA are diagnostic of the α helix taken up by a regular alternation of L and D residues. We therefore conclude that both the LD_{cat} copolymers are essentially all in this helix in this solvent.

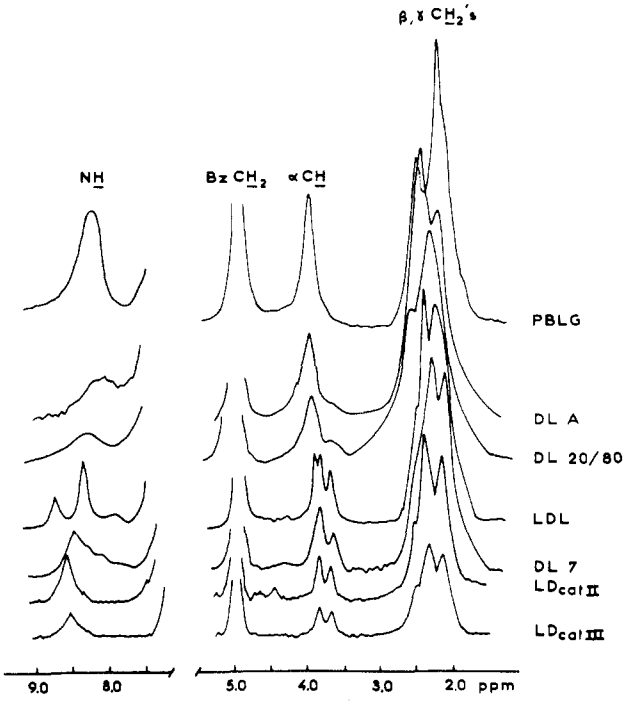

Figure 1. NMR spectra of several PBD,LG samples at room temperature in $CDCl_3$/0.5% TFA.

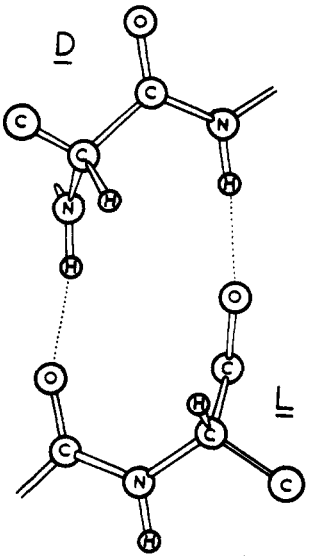

Figure 2. Relative orientation of the α C*H* protons of an L residue in position i and a D residue in position $i + 3$ in a right-handed α helix.

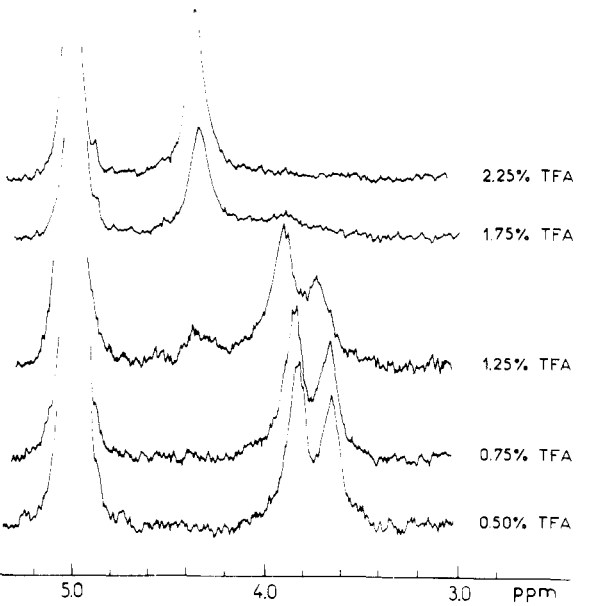

Figure 3. Behavior of the α C*H* peaks during the TFA induced helix–coil transition of sample $LD_{cat}III$ in chloroform.

The existence of multiple α C*H* peaks is not however surprising for copolymers built from both D and L residues. Earlier results obtained on copolymers containing L-aspartate esters revealed that the chemical shift of the α C*H* proton of the aspartyl residue depends on the helical sense. It lies at 4.30 ppm when in a LH helix and at 4.40 ppm in a RH helix.[13,14] The case of D,L copolymers is similar since residues of one chiralty are engaged in both right- and left-handed helices. Examination of molecular models shows that for a right-handed helix (Figure 2) the α C*H* hydrogens of D and L residues do not have the same environment; the D residue has its α C*H* close to two NH groups, while it is close to two carboxyl groups in the L residue.

It is also conceivable, but less likely, that different side chain conformations are responsible for the double α C*H* resonances of the LD_{cat} copolymers, rather than the main chain conformation. It is known for example that the α C*H* shift of RH helical poly(β-ethyl L-aspartate) (4.40 ppm) and poly(L-phenylalanine) (4.20 ppm) differ from that of poly(γ-benzyl L-glutamate) (3.95 ppm) in the same solvent. Thus the nature of the side chain can influence the α C*H* shift (particularly when there is an anisotropic grouping on the β carbon atom), though there is no evidence to date that the conformations of a given side chain can influence the α C*H* shift. As shown in the preceding paper[6] in a poly(D,L-peptide), the C_β atom of an L residue in position i interacts with the same atom of the $(i + 3)$th residue if this one has a D configuration. These interactions could modify the conformation of the side chains as compared to that of the all-L polymer and thus modify the magnetic environment of the α C*H* atoms whatever the configuration of the residue to which they belong. However, if the side chain conformations were the major influence on the chemical shift then the assignment of the two α C*H* peaks to "correct" and "wrong" sense residues would still be as above but the shift values observed with benzyl glutamate polymers might not be the same if the side chains were changed.

Since the basis of the α C*H* chemical shift cannot be determined absolutely it follows that from the NMR spectrum one cannot decide whether the helical conformation in solution is α_{DL} (the solid state conformation[12]) or some other α type helix. For this reason the solution conformation is referred to here simply as α.

A peak at 4.45 ppm which can be seen on Figure 1 for sample $LD_{cat}II$ can probably be assigned to the π_{DL} helix and will be discussed below.

The Helix–Coil Transition of the LD_{cat} Copolymers in $CDCl_3$/TFA. For $LD_{cat}III$ the α C*H* spectra are shown in Figure 3 (the spectra recorded for $LD_{cat}II$ were the same except that a slightly lower amount of TFA was needed to effect the transition). As TFA is added to $LD_{cat}III$ a peak characteristic of coil appears at 4.4 ppm and increases in intensity while the two "helical" peaks decrease in intensity. This is the typical "double-peak" spectrum[15] characteristic of a polydisperse sample undergoing the helix–coil transition and it demonstrates that neither the 3.65 ppm nor the 3.83 ppm peak in $CDCl_3$/0.5% TFA can be assigned to coil; i.e., $LD_{cat}III$ is fully helical under these conditions.

α–π_{DL} Helix Transition. In the previous paper[6] it was shown that, when dissolved in dioxane sample $LD_{cat}II$ undergoes an α to π_{DL} helix conformational transition when the temperature is raised to 85°C. This transition which occurs between two helices of different hydrogen bonding patterns has been monitored in solution for $LD_{cat}II$ by different techniques. In the infrared spectrum one observes a shift from 1665 to 1648 cm^{-1} for the amide I band, while in the ORD this transconformation leads to an amplification of the optical rotation below 300 nm and a decrease at higher wavelengths. In the CD the π_{DL} helix is characterized by an n–π^* transition lying at 228 nm instead of 222 nm for the α helix.[6] Since in the π_{DL} helix the hydrogen bonds are alternatively antiparallel, the α to π_{DL} transition has also been followed by dipole moment measurements. In the solid state both helices were described on the basis of X-ray, electron diffraction, and infrared data.[12] For sample $LD_{cat}III$ the π_{DL} helix was characterized only in the solid state; in dioxane solution the helix–helix transition occurs only to a small extent as shown in the infrared by the appearance of a shoulder on the amide I band near 1650 cm^{-1}. The amount of π_{DL} form is nearly the same for $LD_{cat}III$ at 85°C as for $LD_{cat}II$ at room temperature. However, neither in the solid state nor in solution could an intermediate form between the α and π_{DL} helices be detected. The NMR behavior of these two samples in dioxane-d_8 will now be described and compared to that of PBLG which does not undergo any temperature induced conformational transition in this solvent. The spectrum of PBLG in dioxane (see Figure 4) is almost identical with that in

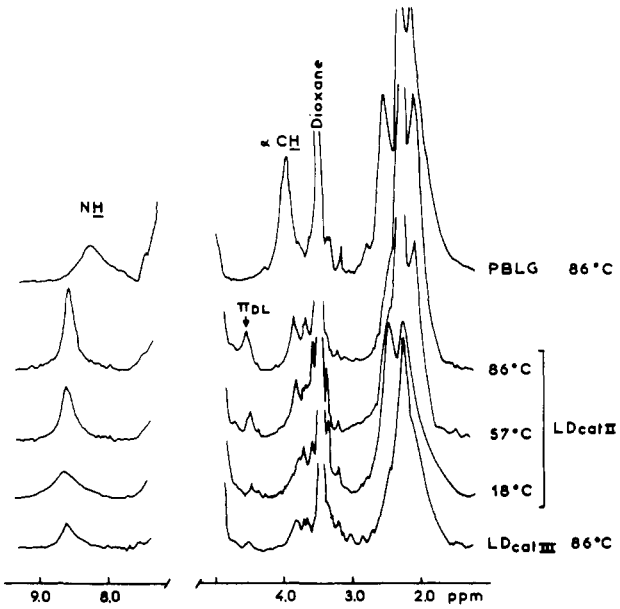

Figure 4. NMR spectra in dioxane-d_8 of PBLG at 86°C and $LD_{cat}II$ at 18, 57, and 86°C.

$CDCl_3$/0.5% TFA; it is concluded that the conformation is the regular α.

At 20°C the spectrum of the α C*H* region is obscured by the resonance of undeuterated dioxane but for $LD_{cat}II$ a small peak at 4.45 ppm is observed, the area of which increases with temperature (Figure 4). For $LD_{cat}III$ no 4.45 ppm peak is observed at 20°C but it exists at 85°C having about the same intensity as for the low molecular weight sample $LD_{cat}II$ at 20°C. No such peak at 4.45 ppm is observed for PBLG either at 20 or 86°C. As the temperature of the $LD_{cat}II$ sample is increased to 86°C the N*H* peak moves upfield from 8.55 to 8.45 ppm. We attribute resonances at about 8.45 ppm (N*H* proton) and at 4.45 ppm (α C*H* proton) to the existence of the π_{DL} helix.

From infrared observations it is noted that the addition of TFA to dioxane inhibits formation of the π_{DL} helix and we have observed that the 4.45 ppm peak decreases on addition of small amounts of TFA while resonance in the region of 3.8 ppm increases. This indicates that the new peak at 4.45 ppm is not due to random coil conformation. It will be recalled that $LD_{cat}II$ in $CDCl_3$/0.5% TFA shows a peak at 4.50 ppm that was not observed for the other polymers and was assigned to a small fraction of π_{DL} helix in this solvent mixture. In the 3.8–4.0 ppm region it is difficult to make any firm assignments due to the residual dioxane resonance. However, transition to the π_{DL} helix is seen to be incomplete at 86° (confirming the infrared results[6]) and the remaining α helix gives rise to two peaks of approximately equal intensity, as with the LD_{cat} polymers in chloroform (see Figure 1) and having the same chemical shifts as in chloroform. A chemical shift as low as 4.45 ppm is unprecedented for a helical structure but by no means impossible. Inspection of molecular models shows that in the π_{DL} helix (see Figure 2 in ref 12) the α CH bond lies between an adjacent C=O and an NH bond. In the α helix the α CH bonds lie alternatively between the C=O bonds and between two NH bonds (see Figure 2).

Spectra in Dimethylformamide-d_7. Samples run in DMF-d_7 at 18°C were found to contain a water impurity peak at ~3.8 ppm, severely overlapping the α C*H* resonance, and the spectra were therefore repeated at 57°C. At this temperature the water resonance had moved upfield, clear of the α C*H* to ~3.6 ppm. No differences were apparent between spectra at the two temperatures and Figure 5

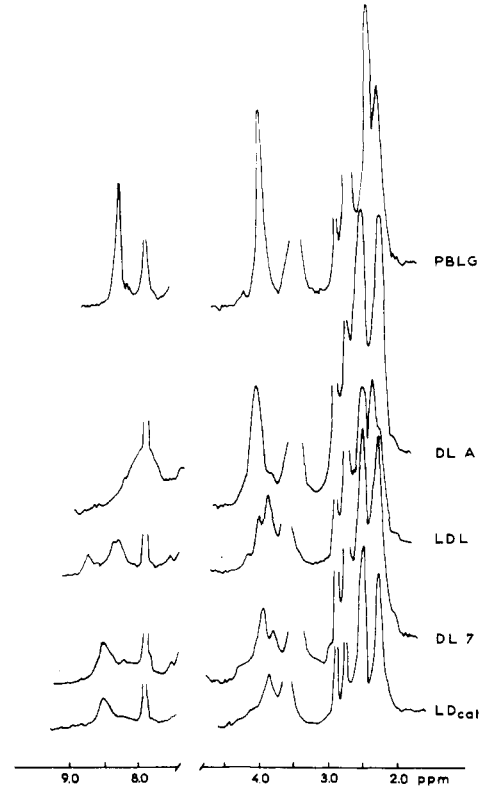

Figure 5. NMR spectra of several PBD,LG samples in DMF-d_7 at 57°C.

Table III
Chemical Shifts of the N*H* and α C*H* Protons of PBD,LG's in DMF-d_7

Samples	δ, ppm	
	N*H*	α C*H*
PLGB	8.31	4.01
DLA	~8.0	4.06
DL-7	8.53	3.95 + 3.79
LDL	8.76 + 8.38 + 8.27	4.03 + 3.89
$LD_{cat}II$ $LD_{cat}III$	8.54	3.88

presents those obtained at 57°C. Comparison with published α C*H* spectra[2b] shows that all samples are very largely helical under these conditions since no major peaks are observed in the region of 4.4 ppm, the shift of coil residues in DMF-d_7. The α helix of PBLG shows sharp α C*H* and N*H* peaks that lie 0.17 ppm upfield of those previously reported;[2b] this difference is due to the temperature difference between the measurements. The α C*H* and N*H* spectra of the remaining copolymers in DMF-d_7 are complex, as in $CDCl_3$. However, close comparison between Figures 5 and 1 shows that the same peak multiplicities probably exist in both solvents and therefore that similar conformational conclusions can be drawn. The apparent differences between the spectra arise since the same conformation of the same residue does not necessarily have an identical shift in the two solvents (DMF is expected to be more strongly solvating than either chloroform or dioxane). DL-A shows an α C*H* spectrum similar to, although somewhat broader than, that of PBLG as expected from its block-like nature (as in $CDCl_3$). The N*H* resonance of DL-A is broad and upfield of that in PBLG (likewise as in $CDCl_3$). It is concluded that in DL-A most residues have a conformation close to that of the homopolymers, although the increased breadth of the peaks indicates that DL-A is not as conformationally homogeneous as PBLG. $LD_{cat}II$ (and $LD_{cat}III$

which gave a similar spectrum) shows only a single α C*H* peak despite two of equal intensity being observed in $CDCl_3$. It follows that both the "correct" and "wrong" sense residues have the same or a similar chemical shift. The slightly racemized alternating DL-7 shows two α C*H* peaks of unequal intensity (very much as in $CDCl_3$) equal to high field and low field of the single maximum of $LD_{cat}II$. By analogy with the spectra in $CDCl_3$ it is concluded that the α C*H* of $LD_{cat}II$ is an unresolved symmetrical doublet, the components of which are at the shifts shown by DL-7 (3.95 and 3.79 ppm) and can be assigned to residues on their "correct" and "wrong" α helical sense. The spectrum of LDL in DMF-d_7 also shows a strong resemblance to that in $CDCl_3$: there are two N*H* peaks of very different shift which have an intensity ratio of 2:1 and the α C*H* spectrum shows a peak (4.03 ppm) not observed in $LD_{cat}II$ or DL-7 that has a shift close to that found for PBLG. The general conclusion to be drawn is therefore that the conformations taken up by the polymers in DMF-d_7 do not differ greatly from those in $CDCl_3$, i.e., all the polymers are α helical. Furthermore, there is no sign at 57°C of a low-field α C*H* resonance that would indicate a transconformational change to the π_{DL} helix.

Conclusion

It has been previously demonstrated that benzyl D,L-glutamates both random and alternating are largely helical in chloroform solution and it was proposed that they form a distorted helix. By the use of alternating copolymers having an almost negligible degree of racemization (LD_{cat}) it has been possible to interpret the complex α C*H* spectrum obtained in chloroform/0.5% TFA and thereby establish the α C*H* spectral features characteristic of the α helix built with D and L residues: a spectrum with two peaks assignable to residues on the "correct (3.82 ppm)" and "wrong (3.65 ppm)" helix sense. These two peaks cannot be assigned to the coexistence of helix and coil as demonstrated by the addition of TFA to promote the helix–coil transition. Since the LD_{cat} copolymers show no α C*H* resonance characteristic of the normal PBLG helix (3.95 ppm), it is concluded that in $CDCl_3$/0.5% TFA they are entirely in the form of the characteristic α helix of poly(D-L-peptides). The presence of some racemization in an alternating copolymer (DL-7) leads to a greater proportion of residues being on the "correct" sense α helix and to the occurrence of some regular α helix as seen from the presence of a weak overlapped component at about 3.9 ppm. In the case of random D,L copolymers, the proportion of the poly(D-L-peptide) α helix is much less as seen for example from the low intensity of the 3.65 ppm component in the LD 80/20 copolymer.

The spectra of the LD_{cat} polymers in dioxane (Figure 4) reveal the presence of the π_{DL} helix by a new peak at 4.45 ppm (a very low shift for helix) and demonstrate that the temperature induced $\alpha \rightarrow \pi_{DL}$ transition of $LD_{cat}II$ is not complete at 86°C. The spectra in dimethylformamide show strong similarities to those in chloroform and it is concluded that the conformations in the two solvents are similar. In particular there is no evidence in either solvent for the presence of the π_{DL} helix.

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Use of a Symmetry Condition to Compute the Conformation of Gramicidin S[1]

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ABSTRACT: Using an improved method for computing conformations of closed rings with symmetry, in conjunction with an improved empirical energy function, the conformational space of Gramicidin S is reexamined. The search for minimum energy conformations is confined to the subspace containing closed symmetric rings. A large number of initial conformations selected from that subspace is subjected to energy minimization or is eliminated in a sequence of steps designed to locate the global minimum-energy conformation. One conformation having distinctly low energy is found and is judged to be the global minimum-energy conformation. This conformation is of the β-pleated sheet type and is in complete agreement with experimental data. Similar structures with β-pleated sheet-type conformations have been proposed previously on the basis of less extensive examinations of the conformational space; the condition of exact ring closure, and the extensive examination of conformational space, used here, establish this structure on a firm basis.

The conformation of Gramicidin S (Gr-S), a cyclosymmetric decapeptide antibiotic, has been studied extensively in recent years both experimentally and by conformational energy calculations. The cyclosymmetric nature of Gr-S makes it an ideal system for conformational energy calculations since the cyclic structure imposes constraints on the