α and π_{DL} Helical States of Alternating Poly(γ-benzyl D-L-glutamate) in Solution

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ABSTRACT: As in the solid state, strictly alternating poly(γ-benzyl D-L-glutamate) in solution can adopt two different helical conformations. Besides the α helix, a second helical conformation is found at higher temperatures in dioxane and chloroform, the properties of which correspond to that of the $\pi_{DL}{}^4$ helix. As the molecules have a finite length a screw sense is favored for both helical forms thus giving rise to optical activity allowing the study of the transconformation by optical rotatory dispersion and circular dichroism besides infrared and dielectric measurements. Thus, as the temperature is raised the equilibria right-left handed α helices and α–π_{DL} helical forms can be followed. The favored screw senses are determined by the number of interacting side chains for the α helix and by the number of hydrogen bonds which are formed in the π_{DL} helical conformation. The side chain-side chain interactions in the α helix are experimentally shown to be attractive.

Energy calculations on the conformation of alternating poly(D-L-peptides) have endorsed the existence of the α helix,[1] which was already established experimentally for random and nearly alternating poly(D-L-peptides).[2–4] Besides, a family of helices, specific for alternating poly(D-L-peptides), the π_{DL} helices, have been described.[1,5–7]

In the solid state, diffraction patterns, obtained on fibers or films, brought experimental evidences for the existence of the $\pi_{DL}{}^4$ helix for poly(γ-benzyl D-L-glutamate), a metastable form at room temperature.[8] In addition, a variant of the α helix, the ω helix and a special type of extended structure, called β_{DL},[9] have also been described. The same polymer has now been studied by infrared, NMR,[10] and dielectric measurements in solution. The α and π_{DL} helices have been found to exist and to transconform with temperature. As could be expected for molecules of finite length, both forms are optically active so that right-left handed α helical transition as well as α–π_{DL} transition could be followed by optical activity measurements. A preliminary report has already been published elsewhere.[11]

Material and Methods[12]

Samples. These samples were prepared through a racemization-free method previously described.[13] No measurable optical activity was found in DCA or TFA. This means that less than ca. 1 residue in 200, i.e., less than 1 per molecule in the average, is inverted. Sample $DL_{cat}I$ has a D residue at the N-terminal position and its molecular weight is intermediate between that of samples $LD_{cat}II$ and $LD_{cat}III$ (see Table I) which are fractions issued from the same polycondensation run; their N-terminal residue has the L configuration. Since the samples were prepared by a polycondensation method, the molecular weight distribution must be rather large, but may have been reduced by the elimination of a fraction of low molecular weight.[13]

Other samples mentioned in this work are DL-5 and DL-7 which were prepared by polycondensation of the D-L-dipeptide pentachlorophenyl ester. The optical activity of these nearly alternating polymers in TFA indicated pronounced racemization occurring during the polycondensation. This reaction leads to an excess of D residues, favoring a left-handed α-helical conformation in solvents such as chloroform, DMF, dioxane, TMP, and HFIP.[4]

Stepwise Additions of γ-Benzyl L- (or D) -Glutamate Residues at the N-Terminal Extremity of a PBD-LG Sample. HCL,H-D-Glu(OBzl)-L-Glu(OBzl)-D-Glu(OBzl)-Glu(OBzl)-OC_6H_4OH (300 mg) was polymerized during 24 hr as previously described.[13] In order to eliminate the β fraction, the mixture was taken up in DCA and the polymer precipitated with methanol and then dried under vacuum. To stabilize the N-terminal amine function the polymer was dissolved in chloroform and worked up by a solution of anhydrous HCl in ether and then precipitated again with methanol.

To a solution of 50 mg of this polymer $DL_{cat}IV$ in 7.5 ml of a mixture DMF–chloroform (6.5–1) 150 mg of Nps-L- (or D) -Glu(OBzl)-OPcp[14] (i.e., about 100 equivalents excess considering a molecular weight of 20000 for the polymer) and 10 μl of TEA were added. After 4 hr of stirring the solution was filtered and the Nps-N-protected polypeptide was precipitated with a mixture of methanol and ether. Removal of the Nps group was carried out by a solution of HCl in ether and the polymer was recovered by precipitation with methanol (yield 80–85%).

Five successive additions of L (or D) residues were thus made. Uv controls in the absorption bands of the Nps group (λ = 375 nm) gave an estimate of the apparent number molecular weight averages $\bar{M}_n$ which were found successively to be 28000, 53000, 82000, 105000, and 150000. However, viscosities in DCA of the elongated polymers are nearly the same as that of the initial polymer. The increase of $\bar{M}_n$ is therefore attributable to the disappearance of reactive amino extremities during each step of additions.

Instruments. Infrared spectra were recorded on a Perkin-Elmer spectrophotometer Model 257 using thermostated cells. ORD measurements were run on a Perkin-Elmer polarimeter Model 141 M equipped with a 1 or 5 cm long thermostated cell. CD spectra were recorded on a Roussel-Jouan dichrograph II equipped with a thermostated cell holder using 1 mm thick cells. Dielectric absorption measurements were made in the 200 Hz–2 MHz range using ethylene-glycol jacketed stainless steel capacitors of 89 pF. The impedence bridges used have been described previously.[15] The temperatures were measured on the thermostating liquid.

Solvents used in spectroscopic measurements were distilled before use and dioxane was redistilled over sodium.

Concentrations used were 0.03% for CD measurements, 0.5% for ORD and dielectric measurements, and 3% for infrared spectroscopy.

Results

Most results will refer to solution of PBD-LG in dioxane and chloroform because the polymer showed its more characteristic behavior only in these two solvents. However, when needed, some results obtained in other solvents (DMF, TMP, pyridine, or benzene) will also be given for comparison.

Table I
Optical Rotatory Properties of the Different PBD-LG or PBL-DG Samples
Prepared by two Different Methods and Their Limiting Viscosity Number Measured in DCA at 25°C[a]

		Dioxane		CHCl$_3$		DMF		Pyridine		Benzene	
	[η], ml g^{-1}	[α]^{25}546	b_0	[α]^{25}546	b_0	[α]^{25}546	b_0	[α]^{25}546	b_0	[α]^{25}546	b_0
DL$_{cat}$I	12.0	+19.0	−160	+24.2	−125	+10	−90				
LD$_{cat}$II	10.0	−15.8	+80	−25.0	+135	−13.1	+105	−31.2	+140	−27.4	+175
LD$_{cat}$III	14.4	−15.4	+130	−24.8	+180	−9.4	+100			−24.1	+130
DL$_{cat}$IV	16.6 (c = 1)			+31.0							
LD$_{cat}$V				−23							
DL-5	17.4			+38.9		−30.8	+310				
DL-7	15.5	−39.0	+400								

[a] The weight average molecular weight of sample LD$_{cat}$II determined by light scattering in DMF is 31000 daltons.

Table II
Infrared Characteristics (cm^{-1}) of Various PBG in Different Conformations in Dioxane Solution Except when Indicated

	Amide I	Amide II
PBLG	1652	1545
"Random" PBD, LG A and B form[16]	1660–1662	1550
DL-7[4]	1660	1554
α-helical PBD-LG	1665	1552
π_{DL}-helical PBD-LG	1648	1548
Random coil PBD-LG in HFIP	1662	1530
α_{DL}-helical PBD-LG[8] in the solid state	1664	1550
π_{DL}-helical PBD-LG[8] in the solid state	1645	1540

(I) Study at Room Temperature. Evidences for the α-Helical Conformation. The infrared spectra of strictly alternating PBD-LG samples in helicogenic solvents such as dioxane, chloroform, trimethylphosphate, pyridine, or benzene are very similar to that observed in the solid state for the α_{DL} helix[8] (Table II). The amide I band lies at higher wavenumber than that observed for right-handed α-helical PBLG but in the same range as "random" or nearly alternating PBD,LG which are α helical. The amide II band position corroborates the existence of an α helix as no absorption at 1535 cm^{-1}, characteristic of random coil,[17] is found.

CD measurements in chloroform and TMP (Figure 1) and dioxane (Figure 5) reveal an extremum at 222 nm, in agreement with an α-helical conformation.[18] From ORD measurements, the Moffitt coefficient b_0 calculated in different solvents (Table I) also indicates that the polymer is at least partially left (or right) handed. As a NMR study in dioxane and CDCl$_3$–0.5% TFA, despite multiple α CH peaks, did not reveal a fraction of polymer in the random coil form (see the following paper[10]), these results can be interpreted on the basis of a mixture of right- and left-handed α-type helices.

Origin of the Optical Activity. As the optical rotations of DL$_{cat}$I and that of its mirror images LD$_{cat}$II or LD$_{cat}$III (Table I) are opposite in sign and of the same order, optical activity seems to be a true property of the polymers. This phenomenon cannot arise from an excess of one helical sense due to the excess of one of the enantiomers resulting from a racemization reaction occurring during the synthesis. Indeed, in that case the sign of the rotation for DL$_{cat}$I should be the same as for samples DL-5 or DL-7,[4], whereas the reverse is true (Figure 1, Table I). Thus, if for samples DL-5 and DL-7 the favored screw sense can be deduced from the configuration of the enantiomer in excess, this is no longer possible for strictly alternating samples. In that case the favored helical sense can be explained as resulting from side chain–side chain interactions occurring in a α-helical poly(D,L-peptide). For a left-handed helix, the nearest neighbor of the β-carbon atom of the nth L residue is the C$_\beta$ atom of the (n + 3)th residue if this one has a D configuration. For the other screw sense, this neighboring occurs between the nth L residue and the (n − 3)th D residue. Thus, in the case of an alternating poly(D-L-peptide), all the β-carbon atoms are involved in these interactions which, depending on the authors, are considered to be attractive[19] or repulsive.[20] Recently, Hesselink and Scheraga[1] have shown by energy calculations that the interactions between the side chains of poly(D-L-valine) and poly(D-L-alanine) are attractive and stronger than the same interactions in the corresponding poly(L-peptides). Therefore, for a poly(D-L-peptide) with $2n$ residues, the N-terminal residue of which has a D configuration, the right-handed helical sense should be favored because there are $2n$ −2 interacting side chains as compared with $2n$ − 4 in the other screw sense (see Scheme I). This is in agreement with the observations made on samples prepared by a racemization-free method.

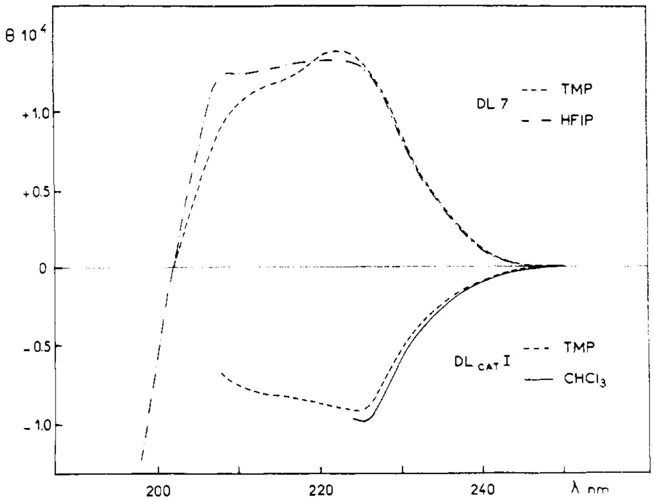
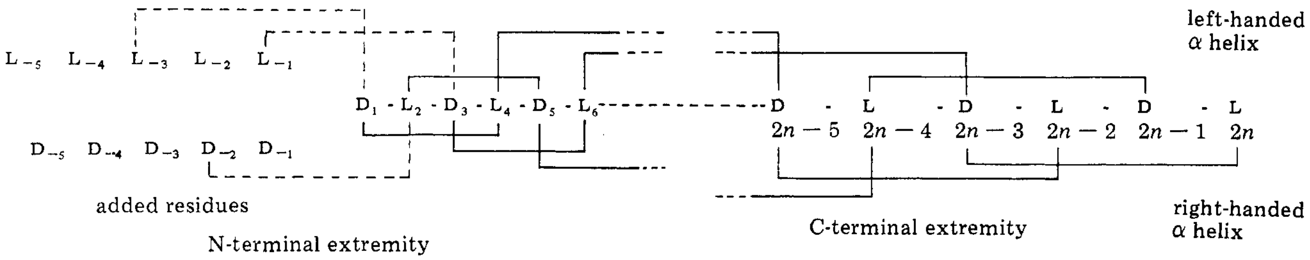


Figure 1. CD spectra of samples DL-7 and DL$_{cat}$I in different solvents; θ in deg cm^2 dmol^{-1}. For both samples the N-terminal residue has a D configuration (c = 0.03%).

In order to have more experimental support on the attractive nature of the side chain–side chain interactions in the case of PBD-LG, D or L residues were grafted at the N-terminal end of sample DL$_{cat}$IV.

In the initial polymer, depending on its screw sense, some side chains are not involved in interactions (at the N extremity, residue L$_2$ for the right-handed helix and residues D$_1$ and D$_3$ for the left-handed helix; see Scheme I). Successive additions of γ-benzyl L-glutamate (BLG) or γ-benzyl D-glutamate (BDG) will alter the ratios D over L and also modify the pattern of interactions, hence the favored helical sense. Five residues (BLG in one case and

Scheme I
Side Chain—Side Chain Interactions between D and L Residues in an α-Helical Poly(D-L-peptide)



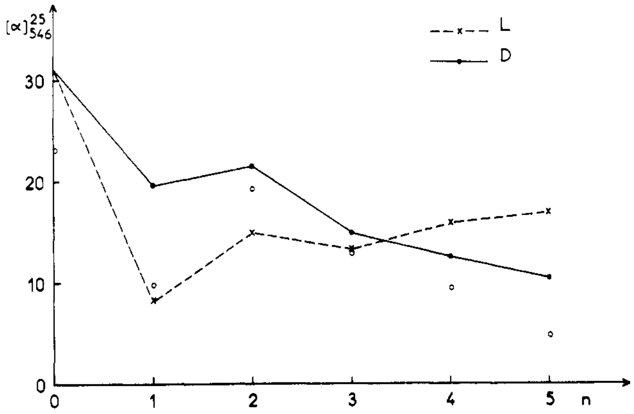


Figure 2. Optical rotation $[\alpha]^{25}546$ in chloroform of elongated samples; n is the number of added residues: (—) addition of D residue on sample DL$_{cat}$IV; (- - -) addition of L residues on sample DL$_{cat}$IV; (O) $-[\alpha]^{25}546$ on addition of L residues on sample LD$_{cat}$V. Notice that the result of additions of L residues on a LD$_{cat}$ sample is the mirror image of addition of D residues on a DL$_{cat}$ sample.

BDG in the other) were grafted stepwise and the optical rotation in chloroform $[\alpha]^{25}546$ (or $[\alpha]^{25}436$) reported on Figure 2 was measured after each addition. The initial positive rotation in chloroform indicated a favored right-handed helical sense. Addition of one BDG residue reduces the excess of right-handed helical sense, probably because there is now a slight excess of D residues over L residues. However, this trend is reversed after the addition of a second D residue which can now interact favorably with the L$_2$ residue of the original chain in the right-handed conformation (see Scheme I). Addition of more D residues does not introduce more interactions and the optical rotation decreases continuously.

The same reasoning can be applied to the variation of optical rotation following the addition of L residues. When the added residues cannot interact with residues of the initial chain the trend is given by the excess of L residues (that is the right-handed sense is more and more favored). This trend is reversed when interactions are possible (with residues D$_1$ and D$_3$; see Scheme I).

It is not necessary to consider the cases of a right- (or a left-) handed helix when BLG (or BDG) residues are added as no D (or L) residue of the initial polymer can then form D,L side chain interactions with the added residues. It should be noted that the observed variations of optical rotation are smoothed by the fact that after each addition some reactive extremities are lost as explained in the experimental part.

This experiment was repeated with the same conclusions by addition of five BLG residues at the N extremity of sample LD$_{cat}$V (Figure 2). Thus the theoretical predictions on the attractive nature of side chain–side chain interactions occurring in poly(D,L-peptides) seem to be supported by these experiments.

Behavior in Hexafluoro-2-propanol. When dissolved in HFIP, samples DL-5 and DL-7 prepared through the pentachlorophenyl ester method have an optical activity araising mainly from the existence of an excess of one helical screw sense[4] (Figure 1). For strictly alternating PBD-LG samples, no optical activity could be detected in this solvent, neither by ORD nor by CD measurements. As will be explained later, this cannot result from an equal amount of right- and left-handed helices which still should display optical activity. Infrared observations (Table II) in the 1500–1800 cm^{-1} region indicate that all DL$_{cat}$ samples adopt a random coil conformation in this solvent. Indeed, a significant difference is found for the amide II band which lies at 1530 cm^{-1}, a position more compatible with the disordered form,[17] compared with 1550 cm^{-1} found for DL-7. The lesser stability of the α-helical conformation of DL$_{cat}$ samples in HFIP may be due to the solvation of the side chains disturbing their interactions.

Discussion. Although the possible existence of α-helical structure for copoly(D,L-peptides) has been sometimes disputed,[21,22] it appears from the experiments just described that such a structure can be observed in helicogenic solvents for alternating PBD-LG. However, the fine details of the helix are not known. From infrared and NMR[10] measurements it appears to be a so-called distorded α helix, perhaps of the same kind as that found in the solid state,[8] i.e., a 3.8$_{13}$ helix with a rise per unit of 1.47 Å.

The relative stability of the α-helical structures of alternating poly(D-L-peptides) and random poly(D,L-peptides) containing an equal amount of D and L residues deserves some comments. For an infinite chain, in the first case, the attractive side chain–side chain interactions affect all the side chains whereas only one-half is concerned if the residues are randomly distributed. On the other hand, the repulsive interactions between the peptide carbonyl groups and the C_β atoms[23] affect half of the residues whatever the distribution is. Thus, the α-helical conformation of an alternating poly(D-L-peptide) should be more stable than that of the random copolymer. However, there is a lack of comparative material as no truly random poly(D,L-peptides) have been prepared, especially with benzyl glutamate.

We have found that poly(benzyl D,L-glutamates) prepared by copolymerization of the corresponding *N*-carboxyanhydrides (samples described as PBD,LG A and B in ref 3) are mainly helical in HFIP as revealed by their amide II band position around 1550 cm^{-1} (with a shoulder at 1535 cm^{-1} for form B). This result seems to contradict the above-mentioned conclusion. However, it should be kept in mind that these samples are not truly random copolymers but rather block copolymers.

Finally all these results show that the kinetic model proposed by Wada[24] is a first approximation. Indeed it is clear that it is not necessary that a sequence containing four residues of the same configuration should be formed to induce a helical conformation.

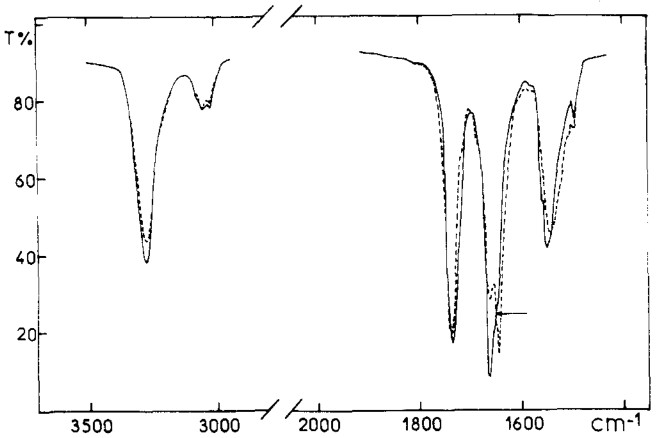


Figure 3. Infrared spectra of sample $DL_{cat}II$ in dioxane (c = 3%): (—) 25°C; (- - -) 95°C. The isobestic point is shown by an arrow.

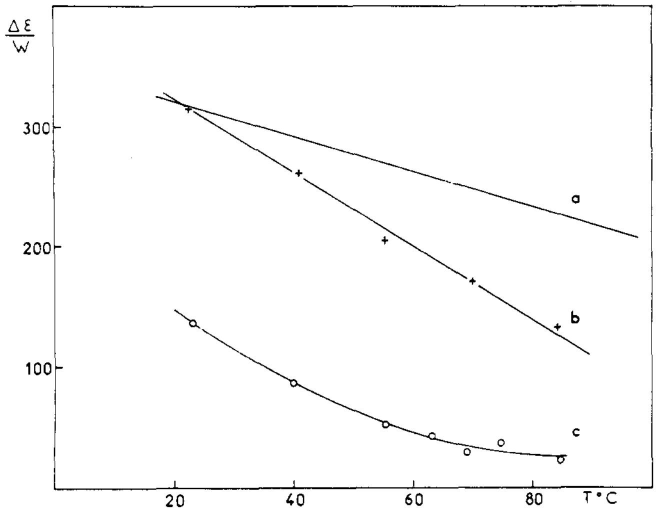


Figure 4. Variation of $\Delta\epsilon/w$ in dioxane with temperature (c = 0.5%): (a) PBLG;[25] (b) $LD_{cat}III$; (c) $LD_{cat}II$. μ^2 is proportional to $\Delta\epsilon/w$.

(II) High-Temperature Study. π_{DL} Helical Conformation. Important modifications of the infrared spectrum of all DL_{cat} samples were observed when a solution in dioxane (and to a lesser extent in chloroform) was heated from 25 to 95°C. As the behavior of sample $LD_{cat}II$ is the most characteristic, most of the following experiments refer to it (Figure 3 and Table II).

At the highest temperature the positions of amide I and amide II bands are comparable to those observed in the solid state for PBD-LG in the π_{DL} helical conformation.[8] This suggests that the same conformation is formed in solution.

In this structure, successive peptide carbonyl groups are approximately parallel to the helical axis and have opposite directions; therefore, during the α–π_{DL} transconformation, the dipole moment μ of a solution of PBD-LG should decrease asymptotically to a minimum value and thus dipole moment measurements provide a tool to test this transconformation.

The dielectric behavior of $LD_{cat}II$ and $LD_{cat}III$ in dioxane is compared to that of PBLG[25] (M_w = 59000) on Figure 4. Although these results cannot be interpreted quantitatively due to aggregation and flexibility of the polypeptide chains, they show for $LD_{cat}II$ the expected trend and are in agreement with the existence of a π_{DL} helical conformation at high temperature. The decrease of μ does not have its origin in the formation of aggregates as shown by the behavior of the critical frequency which indicated a lowering of the length of the molecules. The slower decrease of μ for sample $LD_{cat}III$ is in agreement with the infrared observations which revealed that the α–π_{DL} transconformation occurred to a lesser extent than for $LD_{cat}II$. The amount of π_{DL} structure as measured by infrared spectroscopy is about the same in the $LD_{cat}II$ sample at 25°C as for $LD_{cat}III$ at 95°C.

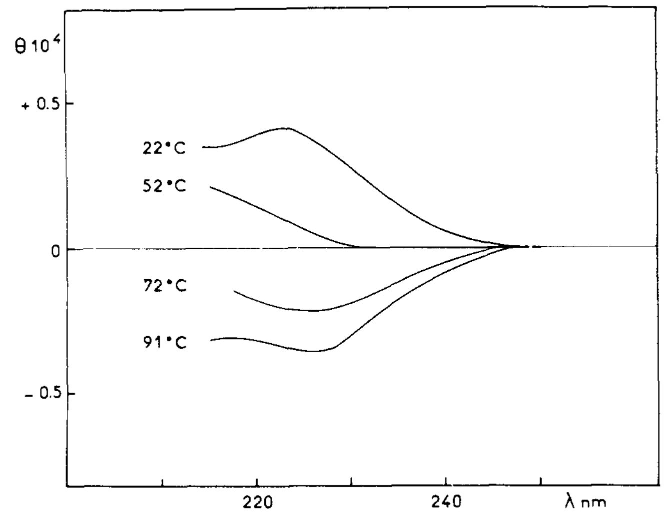


Figure 5. CD spectra of sample $LD_{cat}II$ in dioxane at different temperatures; θ in deg cm^2 dmol^{-1} (c = 0.03%).

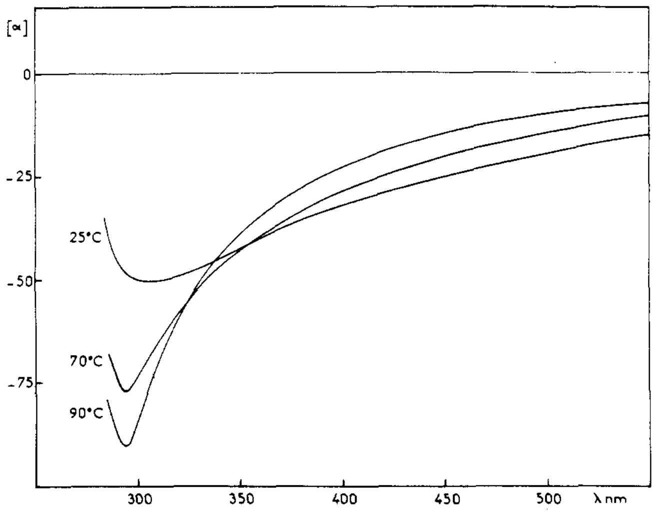


Figure 6. Temperature effect on the ORD curves of sample $LD_{cat}II$ in dioxane (c = 0.5%). For the sake of clarity, only three dispersion curves are shown.

Dissymetry also exists for the π_{DL} structure as well as for the α helix. The α–π_{DL} transconformation generates a change of the sign of the circular dichroism while the extremum corresponding to the peptide n–π^* transition is shifted from 222 to 227 nm (Figure 5). Also the optical rotatory dispersion is modified (Figure 6). The change of the sign of the circular dichroism does not necessarily imply that during the transconformation the favored helical sense has changed. Indeed, Bayley[26] has found by calculation that the CD spectrum of a left-handed $\pi_{DL}{}^4$ helix (4,4 amino acid residues per turn of Gramicidin A) shows in the 200–230 nm region a negative dichroism. There are, however, discrepancies between the observed and calculated positions of the minimum which may be attributed to differences in the torsional angles characterizing the theoretical and observed π_{DL} helices ($\varphi_L = -125°$, $\psi_L = +85°$, $\varphi_D = +85°$, $\psi_D = -125°$ were used by Bayley whereas the π_{DL} helix found for PBD-LG is defined by $\varphi_L = -135°$, $\psi_L = +95°$, $\varphi_D = +84°$, $\psi_D = -101°$ and the peptide groups are nonplanar). Note that the values indicated in ref 8 are mistaken.

Origin of the Dissymetry in the π_{DL} Helical Sense. Optical activity in the π_{DL} helix is probably due, as for the α helix, to a favored helical sense. But in a π_{DL} helix as no side chain–side chain interactions of the same type as in the α helix can occur, the favored helical sense should be looked for in the number of hydrogen bonds which can be formed considering one helical sense or the other. In a PBL-DG sample, in which the N-terminal residue has the L configuration, the left-handed π_{DL} helix will have $2n - 3$ hydrogen bonds including NH_2 and COOH end groups, whereas only $2n - 5$ hydrogen bonds are formed for the right-handed screw sense. Thus for PBL-DG the left-handed π_{DL} helix is more stable than the right-handed one as for the α helix.

Discussion. When dissolved in dioxane or chloroform PBD-LG samples can undergo an α to π_{DL} type helix transconformation. At this stage of the experiments it is not possible to decide which type of π_{DL} helix is found in solution. However, the comparison with the infrared properties in the solid state is a strong argument in favor of the $\pi_{DL}{}^4$ helix, i.e., 2.2 repeat units per turn, although the CD spectrum is somehow comparable to that of Gramicidin A[6,27] (negative extremum at 228 nm) in dioxane, for which on the basis of NMR measurements and ion transport phenomenon[7,28] a left-handed $\pi_{DL}{}^6$ helix was proposed.

The extent of the α–π_{DL} transconformation depends, other things being equal, on the samples and seems to be related to the molecular weight: the higher the molecular weight, the higher is the transition temperature. This observation can be explained by a cooperative process.

***(III)* Equilibria between Helical Forms.** It results from the preceeding experiments that four different helices of PBD-LG can exist in solutions, i.e., the right- and left-handed α and π_{DL} helices, and that the equilibria between these species can be followed by optical rotatory measurements. A comparative study of samples LD_{cat}II and LD_{cat}III in chloroform and dioxane has been made and the two extreme cases will be discussed: sample LD_{cat}III in chloroform undergoing no apparent transition by infrared and sample LD_{cat}II undergoing large α to π_{DL} transconformation in dioxane by heating.

Sample LD_{cat}III in Chlorofrom. When dissolved in chloroform, sample LD_{cat}III does not show any change in the infrared spectrum when temperature is varied between 22 and 55°C. ORD examinations in the same conditions show a decrease of the optical rotation in the visible range whereas an isobestic point at 294 nm ($[\alpha]294 = -27°$) is formed (Figure 7). One would not expect infrared spectroscopy to be able to distinguish between right- and left-handed α helices of a poly(D,L-peptide), but the existence of an isobestic point in ORD is consistent with an equilibrium between these two species. As the optical rotation at the isobestic point has a value different from zero, the dispersion curves of right- and left-handed α helices of PBD-LG cannot be deduced one from the other by a change of sign. This behavior reflects the fact that the mirror image of the right-handed helix of a poly(D-L-peptide) of finite length is not the left-handed helix of the same polymer, but it is that of the poly(L-D-peptide).

As this phenomenon has its origin in chain-end effects, the value of the optical rotation at the isobestic point should increase when the molecular weight is lowered. Indeed, this is checked by the study of sample DL_{cat}I, the infrared behavior of which is similar to that of LD_{cat}III but has a lower molecular weight (see Table I). In that case the isobestic point is found for $[\alpha]310 = +47°$.

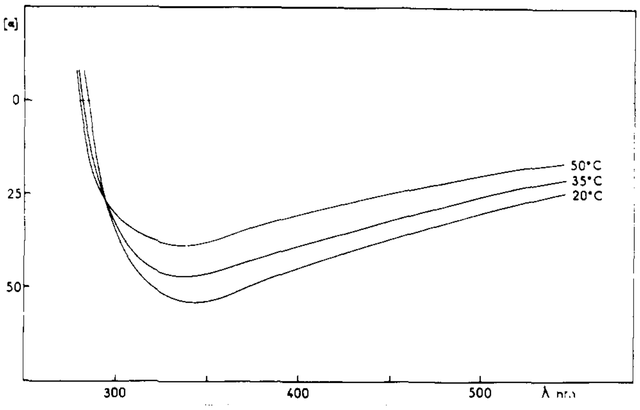


Figure 7. Temperature effect on the ORD curves of sample LD_{cat}III in chloroform showing an isobestic point (c = 0.5%).

Sample LD_{cat}II in Dioxane. As already described, when dissolved in dioxane, sample LD_{cat}II undergoes by increasing temperature in α to π_{DL} helical transconformation. Simultaneously an isobestic point appears in the infrared spectrum at 1652 cm^{-1} (Figure 3). Thus, from the point of view of infrared spectroscopy, two kinds of molecules are in equilibrium, α helices and π_{DL} helices. In turn, no isobestic point is detected by ORD measurements in the same conditions (Figure 6) as there are at least three and probably four kinds of molecules, right- and left-handed α and π_{DL} helices, in equilibrium.

Discussion. The equilibria between the different helical forms of PBD-LG can be summarized in the following scheme where the favored helical sense is underlined.

$$\begin{tikzcd}
\underline{\text{right}}\text{–handed } \alpha \text{ helix} \arrow[r, shift left, "2"] \arrow[d, shift right, "1"'] & \underline{\text{right}}\text{–handed } \pi_{DL} \text{ helix} \arrow[l, shift left] \arrow[d, shift left, "3"] \\
\text{left–handed } \alpha \text{ helix} \arrow[u, shift right] \arrow[r, shift left, "2'"] & \text{left–handed } \pi_{DL} \text{ helix} \arrow[l, shift left] \arrow[u, shift left]
\end{tikzcd}$$

The equilibria 2 and 2′ are detected undiscernably by infrared spectroscopy while equilibrium 1 can be followed by ORD measurements. For equilibrium 3, the temperature range was too short to allow information but it may be followed also by ORD.

Thus for a strictly alternating noninfinite α-helical chain composed of chemically identical D and L residues, even if right- and left-handed helices are present in exactly the same amount, there is necessarily an optical rotation as all the ORD curves pass through the isobestic point. This conclusion explains the behavior of PBD-LG samples in DMF which reveals that an increase of temperature leads to a decrease of the optical rotation at any observed wavelength over 300 nm whereas by NMR a shoulder which could correspond to random coil appears in the α C*H* region at high temperature.

Conclusion

The study of strictly alternating poly(γ-benzyl D,L-glutamate) in dioxane or chloroform solutions has revealed that two helical structures of different type exist and transconform with temperature. These are probably the same helices identified in the solid state: a distorted α helix and the $\pi_{DL}{}^4$ helix.[8]

For both helices, there is a favored helical sense. In the α helix, it is determined by the side chain–side chain interactions which are experimentally shown to be attractive. For the π_{DL} helix the favored helical sense is related to the 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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References and Notes

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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-