changes in cross-linking pattern, as demonstrated by κ-casein.

The ratios of the f_i calculated by matrix methods to those calculated by random flight statistics cluster much closer to unity than do the corresponding ratios involving g, provided attention is confined to cross-linked proteins in which both polypeptide chains are of the same amino acid sequence. This conclusion is apparent from Figures 2 and 3 or from comparison of the last two columns of Table II. A similar statement cannot be made if the cross-linked protein consists of polypeptide chains which differ in amino acid sequence. For example, the ratio of $\langle s^2 \rangle_0$ for the A chain of thrombin to $\langle s^2 \rangle_0$ for this chain cross-linked to the B chain lies between 0.75 and 0.78. We conclude that f_1 for cross-linked proteins is likely to be well approximated (to within about 6%) by eq 2[11] provided both polypeptide chains are of identical amino acid sequence.

$$f_1 = (n_1 + n_2)N^2[\sum(3Nn_j^2 - 2n_j^3)]^{-1} \quad (2)$$

Branches 1 and 2 constitute polypeptide chain 1.

The square root of f_i gives the ratio of a linear dimension of the ith polypeptide chain and the cross-linked polypeptide. This information can be used to assess the effect of the reduction of an interchain disulfide bond on the gel chromatography of disordered proteins,[23] provided the expansion factors are comparable for both molecules. For those cases where the cross-linked polypeptide chains are identical in amino acid sequence, $f_i^{1/2}$ ranges from 0.73 to 0.90. Consequently the cross-linked molecule will pass through the gel permeation column more quickly than the uncross-linked molecules, which is in accord with expectation.

Thrombin presents an interesting case in which f_i may exceed unity. The $f_i^{1/2}$ for the A chain are 0.36–0.39, depending upon the mode of cross-linking to the B chain. In contrast, the $f_i^{1/2}$ for the B chain are 0.96–1.03, indicating that cross-linked thrombin and the individual B chains will virtually coellute.

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Structural Properties of Double-Stranded Helical Poly(γ-benzyl D-L-glutamate) in Solution. Comparison with Some Solution Properties of Linear Gramicidin

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ABSTRACT: The double-stranded helical conformations of alternating PBD-LG found in the solid state have been studied by infrared, circular dichroism, and NMR techniques in solution in methylene chloride, chloroform, dioxane, and collidine. The infrared and CD properties of the solutions, transconformations between single- and double-stranded helices and transconformations within the family of double helices, support the hypothesis that the conformation in solution is the same as that found after evaporation of the solvent, namely the $\pi\pi_{DL}^{7.2}$, $\pi\pi_{DL}^{9.0}$, and $\pi\pi_{DL}^{10.8}$ helices, depending on the solvent. An attempt to identify the conformations of linear gramicidin is made on the basis of the CD spectra and the infrared frequencies conformation relationship established for PBD-LG. However, owing to the great number of different conformations observed for the antibiotic, no firm conclusion can yet be drawn, except for the probable existence of the double antiparallel helical structure.

Two types of conformations have been proposed for gramicidin A, a channel forming natural alternating D-L-peptide;[1] these are a family of π_{DL} helices[2-5] and a family of double stranded helical conformations.[6,7] However, these structures were only postulated on the basis of spectroscopic results. They have not yet been firmly established owing to a lack of crystallographic data. In order to test the possible existence of these conformations, strictly alternating poly(γ-benzyl D-L-glutamate) (PBD-LG)[8] has been examined and its structure studied by fibre crytallography. In the solid state, this synthetic poly(D-L-peptide) has now been shown to exist in several helical conformations, among which are the α_{DL} helix, the $\pi_{DL}^{4.4}$ helix,[9,10] a member of the π_{DL} family, and four double-stranded helical conformations: the $\pi\pi_{DL}^{5.6}$, $\pi\pi_{DL}^{7.2}$,

Table I
Infrared, Optical Rotatory, and NMR Characteristics of PBD-LG in Its Various Conformations[a]

Inferred conformational state	Solvent	Amide A, cm^{-1}	Amide I, cm^{-1}	Amide II, cm^{-1}	$[\alpha]_{546}$	$\lambda_{\theta extr}$, nm	$\delta_{\alpha\text{-}CH}$, ppm
Random coil	HFIP	3292	1665	1530	0.0	No	
	TFA				0.0	extr.	4.40
β_{DL}	Chloroform	3280	1625 +1690 sh	1540			
α	Chloroform	3289	1665	1552	−16.0		6.65 + 3.82
	Dioxane	3289	1665	1552	−25.0	222	(0.5% TFA)
$\pi_{DL}^{4.4}$	Dioxane 90°	3289	1648	1548	−10.0	228	4.45
$\pi\pi_{DL}^{5.6}$	(Solid state)	3280	1632	1536			
$\pi\pi_{DL}^{7.2}$	Methylene chloride	3280	1628 +1690 sh	1540	+28.0	220	$4.75 < \delta < 5.60$
$\pi\pi_{DL}^{9.0}$	Chloroform	3284	1628	1533	+14.0	217.5	5.40
	Dioxane	3284	+1691 sh	1533	+12.0	217.5	
$\pi\pi_{DL}^{10.8}$	Collidine	3288	1628 +1690 sh	1529	+10.0		

[a] Sample $LD_{Cat}II$ except for the β_{DL} conformation which is found only with a sample of low molecular weight. All measurements were recorded at 25 °C except when mentioned (sh = shoulder).

$\pi\pi_{DL}^{9.0}$, and $\pi\pi_{DL}^{10.8}$ helices.[11,12] The $\pi\pi_{DL}^{5.6}$ helix is obtained in the solid state by heating a sample of PBD-LG in the α_{DL} or $\pi_{DL}^{4.4}$ helical form. When dissolved and recast it gives rise to the $\pi\pi_{DL}^{7.2}$ helix from methylene chloride, the $\pi\pi_{DL}^{9.0}$ helix from dioxane or chloroform, and the $\pi\pi_{DL}^{10.8}$ helix from collidine. As pointed out,[12] it appears that the dimensions of the core of these helices are controlled by the smallest dimensions of the solvent molecules, and thus it is tempting to assume that the same conformation exists in solution as that observed in the solid state after removal of the solvent. We will report in this paper solution properties of PBD-LG which are consistent with this hypothesis, although no direct proof can at present be given. Indeed, using infrared, optical rotatory, and NMR techniques, our results indicate that different structures exist in the various solvents or groups of solvents and that they are able to transconform. Therefore, in the following, the names $\pi\pi_{DL}^{7.2}$, $\pi\pi_{DL}^{9.0}$, or $\pi\pi_{DL}^{10.8}$ helix stand for double helices formed in methylene chloride, dioxane or chloroform, and collidine, respectively. These results will be compared to the solution properties of the two single-stranded helices, the α and π_{DL} (most probably the $\pi_{DL}^{4.4}$), which have already been described in detail.[13,14] Finally, in the light of the spectroscopic characteristics of PBD-LG, a preliminary interpretation of the solution properties of linear gramicidin will be attempted.

Experimental Section

All the experiments reported in this paper were made on a PBD-LG sample called $LD_{Cat}II$[8] (its weight average molecular weight is 31 000 daltons and its *N*-terminal residue has a L configuration) although some of them were duplicated with other samples. Gramicidin was kindly provided by the "Société Rapidase" and is a mixture of gramicidin A, B, and C. All solvents were spectrograde and were used without further purification, except for the dioxane which was distilled over sodium.

Circular dichroism (CD) experiments were performed on a Roussel-Jouan dichrograph II using 1, 0.1, and 0.01 mm thick cells according to the region observed, and optical rotatory dispersion (ORD) on a spectropolarimeter Perkin-Elmer 141M. Infrared (IR) spectra were recorded on a Perkin-Elmer spectrophotometer Model 257 or, when more precision was required, on a Beckman IR 11.

Results and Discussion

(I) Characterization of the Double Helices of PBD-LG.
(1) Infrared Spectroscopy. A PBD-LG sample in the $\pi\pi_{DL}^{5.6}$ conformation or any other double helical conformation found in the solid has, at room temperature, when dissolved in methylene chloride, dioxane, chloroform, or collidine an infrared spectrum which does not depend on the concentration in the range studied ($0.5 < c < 100$ mg/mL) and shows amide I and II bands lying at wavelengths usually attributed to β structures with antiparallel chains (Figure 1 and Table I). The spectra in chloroform and dioxane are identical, but otherwise all spectra show slight differences in the position of amide A and II bands (see Table I). They can also be distinguished from the spectra of the other helical forms of the same sample, i.e., the α and π_{DL} structure, or from the β_{DL} conformation[15] of a low molecular weight fraction of the same polymer (see Table I).

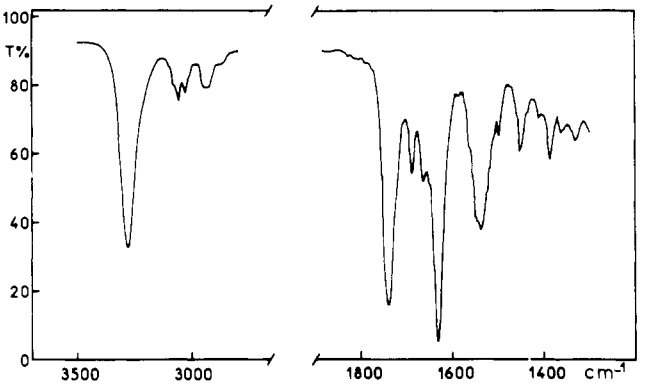


Figure 1. Infrared spectrum of double-helical PBD-LG in chloroform, c = 50 mg/mL. The small band near 1665 cm^{-1} may be due to a remainder of α helix.

These observations, together with the fact that the spectra in solution are very similar to those observed for the polymer after removal of the solvent,[12] strongly suggest that similar structures exist in the solid state and in the mother liquor.

(2) Circular Dichroism and Optical Rotatory Dispersion. When dissolved in the four above-mentioned solvents, PBD-LG in the double-helical conformation displays optical activity and circular dichroism absorption arising from a favored helical screw sense (owing to its ultraviolet spectroscopic properties, investigations in collidine were limited to ORD).

The CD spectra obtained for solutions in methylene chloride and dioxane or chloroform are reported on Figure 2, together with those of the α and π_{DL} helices. As expected, the spectra of double helices in dioxane and chloroform are identical, but all other spectra are different. Those obtained

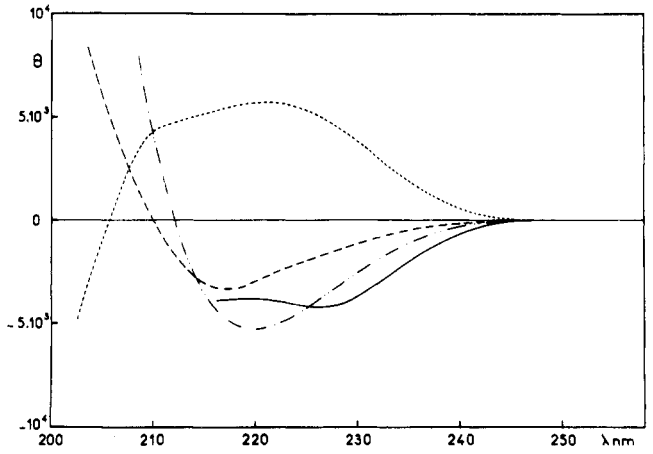


Figure 2. CD spectra of PBD-LG (sample $LD_{Cat}II^8$) in various helical conformations, c = 0.3 mg/mL: (- - -) α helix in dioxane at 25 °C; (—) π_{DL} helix in dioxane at 90 °C; (- · · -)$\pi\pi_{DL}^{7.2}$ helix in methylene chloride at 25 °C; (– – –) $\pi\pi_{DL}^{9.0}$ helix in chloroform or dioxane at 25 °C.

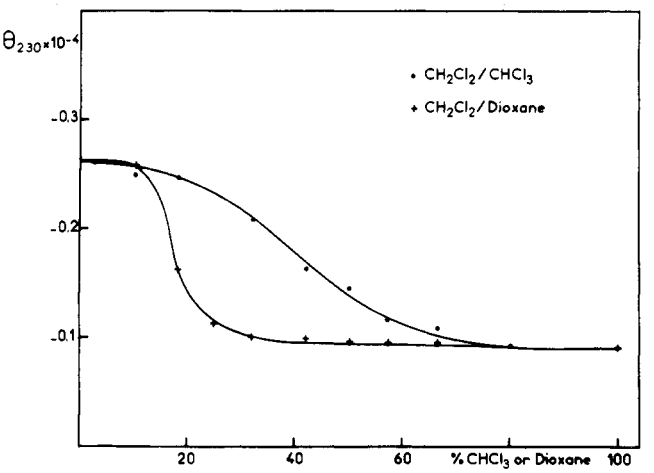


Figure 3. Variation of the ellipticity at 230 nm for double-helical PBD-LG (sample $LD_{Cat}II^8$) in solvent mixtures (v/v), c = 0.3 mg/mL.

for double helices in methylene chloride and chloroform (or dioxane) show strong similarities. They are, however, distinguishable by the intensity of the ellipticity and by a small difference of position of the extremum corresponding to the n–π^* transition (Table I). These two spectra also resemble that of poly(L-lysine) in a β-pleated sheat structure with antiparallel chains[16] (extremum at 215–216 nm). Assuming that the difference in position of the extrema does not arise from different overlappings of the dichroic bands, it can be concluded that the structure found in chloroform or dioxane is closer to that of an extended β structure than that found in methylene chloride. This is consistent with an increase of the diameter of the double helix when going from methylene chloride to chloroform, as observed in the solid state, and thus supports the hypothesis of different double-helical conformations in the two solvents.

No conclusion can be reached with regard to the relative intensities of the CD spectra as these depend on the unknown ratio of right to left handedness for each helical structure. However, as to the favored screw sense, one notes that the dichroism of the double helices has the same sign as the π_{DL} helix, but opposite to that of the α-helical conformation of the same sample.[13] Previously, it was concluded from experimental and theoretical considerations[13] that the α helix and the π_{DL} of a PBD-LG of finite length with a N-terminal L residue (as in the case of our sample) were preferentially left handed. It was also shown that the sign of the dichroism is opposite, being positive for the α helix and negative for the π_{DL} helix. Owing to the structural similarities between the π_{DL} and the $\pi\pi_{DL}$-type helices (the latter can be described as two intertwinned π_{DL} helices) it is likely that the preferred screw sense of the double-stranded helices is also left handed.

(3) ^{1}H NMR. The existence of different structures depending on the solvent has also been confirmed by an NMR study.[17] Indeed, the spectrum observed in methylene chloride differs in many respects from those obtained in chloroform or dioxane. The unusual low-field resonance of the α-C*H* peaks (see Table I) which characterizes all these helices has been attributed to the position of the α-CH proton relative to the backbone, and to side-chains effects, as revealed by the unusual resonances of the protons of the phenyl ring and benzylic-CH_2. This matter is discussed in more detail in the accompanying paper.[17]

To summarize this section, it appears that the metastable structures of PBD-LG which are obtained by dissolving the $\pi\pi_{DL}^{5.6}$ helix in methylene chloride, chloroform (or dioxane) and collidine are different from the already described single-stranded α and π_{DL} helices. The new structures exhibit spectroscopic properties in good agreement with an antiparallel β-type structure and, when comparison is possible, with the $\pi\pi_{DL}$ double-stranded helices observed in the solid state. The small differences in their spectroscopic properties cannot be attributed to a unique structure interacting in different ways with the solvent, as will be examined below.

(II) Helical Transconformations. Transconformation experiments between the various double-stranded helices and between one of the single-stranded helices and any double helix were performed. The former transconformations were followed mainly by ORD and, when possible, checked by CD and NMR. The latter could be followed in addition by infrared techniques.

$\pi\pi_{DL}^{7.2}$–$\pi\pi_{DL}^{9.0}$ Helix Transition. Examination of the optical activity (θ_{230} or $[\alpha]_{365}$) of double-helical PBD-LG in methylene chloride/dioxane mixtures reveals a sharp transition when the dioxane content reaches 19% in volume. In chloroform/methylene chloride this transition is smoother and occurs around 40% chloroform (Figure 3). This conclusion is corroborated by NMR investigations.[17] The existence of a sigmoidal variation of the optical activity with solvent composition strongly suggests that, in both cases, a transconformation between two double-stranded helices occurs, following our starting hypothesis, between the $\pi\pi_{DL}^{7.2}$ and $\pi\pi_{DL}^{9.0}$ helices. The difference in behavior for the two solvent mixtures, namely the fact that the $\pi\pi_{DL}^{7.2}$–$\pi\pi_{DL}^{9.0}$ helical transition does not occur at a constant volume ratio, indicates that, besides the geometry of the solvent molecules, the relative stability of the different double helices is also related to other properties of the solvent (polarity, ability to solvate the backbone, etc.).

$\pi\pi_{DL}^{7.2}$–$\pi\pi_{DL}^{10.8}$ and $\pi\pi_{DL}^{9.0}$–$\pi\pi_{DL}^{10.8}$ Transitions. These transconformations were performed in methylene chloride/collidine and chloroform/collidine mixtures and followed by ORD measurements only. In the first case, a transition occurred when the collidine content reached 60% (v/v) whereas in the second case, the transition, although difficult to observe in view of the close $[\alpha]_{365}$ values in both solvents, was obtained at 10% collidine.

It is worth mentioning that the transconformation between double-stranded helices can also be achieved through the dry state. For instance, the $\pi\pi_{DL}^{9.0}$ helix, obtained by drying a dioxane solution, yields, after redissolution in methylene chloride, the CD spectrum with a minimum at 220 nm typical

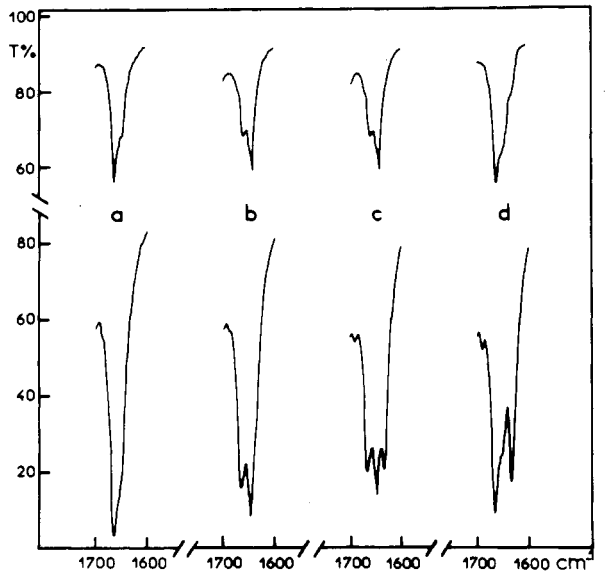


Figure 4. Infrared spectra of PBD-LG (sample $LD_{Cat}II^8$) showing the concentration effect when temperature is varied. Upper part, 13 mg/mL; lower part, 130 mg/mL: (a) 21 °C, (b) 90 °C, (c) 90 °C after 1 h and 30 min, (d) after cooling back to 21 °C.

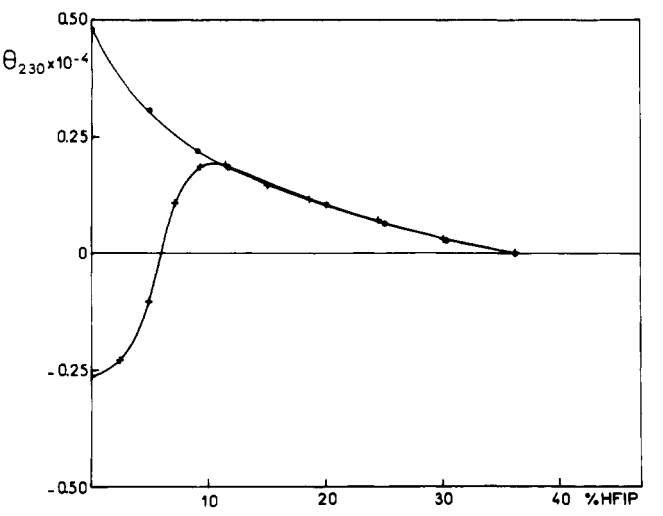


Figure 5. Variation of the ellipticity at 230 nm from double-helical PBD-LG (sample $LD_{Cat}II^8$) during the $\pi\pi^{7.2} \rightarrow \alpha \rightarrow$ random coil (+) transition compared to the $\alpha \rightarrow$ random coil (●) transition, both transitions in CH_2Cl_2/HFIP mixtures (v/v), c = 0.3 mg/mL.

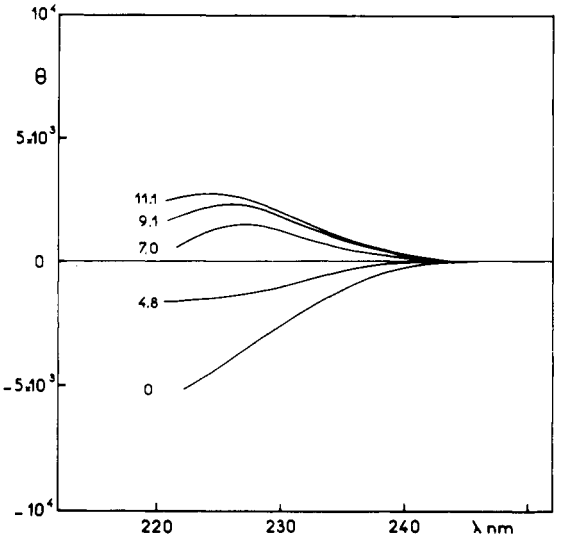


Figure 6. CD spectra showing the effect of addition of HFIP to a solution of double-helical PBD-LG (sample $LD_{Cat}II^8$) in CH_2Cl_2, c = 0.3 mg/mL. Concentrations of HFIP are given in v/v.

of the $\pi\pi_{DL}{}^{7.2}$ helix. On further drying the $\pi\pi_{DL}{}^{7.2}$ helix is maintained, as evidenced by diffraction techniques.

$\alpha \rightarrow$ Double-Helix Transition in Solution. It was shown previously[13] that the α to π_{DL} transition in dioxane is characterized, at 90 °C, by the existence of two amide I bands. The main band at 1648 cm^{-1} corresponds to the π_{DL} helix while the second one, located at 1665 cm^{-1}, indicates a remainder of α-helical structure, both conformations being in equilibrium (Figure 4, upper part). This transconformation is reversible if concentration is low enough (c < 30 mg/mL) and if the solution is not kept for prolonged periods at high temperature. However, when a 100 mg/mL solution is heated 2 h at 90 °C, the spectrum shows a gradual increase of a band centered around 1630 cm^{-1} while the intensity of the other amide I bands decreases (Figure 4 lower part). Further, when the temperature is lowered, the 1630-cm^{-1} band coexits with the α peak, indicating that the corresponding conformation is metastable and that perhaps a part of a hysteresis loop is generated.

Double-Helix $\rightarrow$ α-Helix Transition. Addition of hexafluoro-2-propanol (HFIP) to a solution of double-helical PBD-LG in methylene chloride generates first (at 6% concentration v/v) a change of sign of the ellipticity at 230 mm which, after reaching a maximum (at 11% HFIP), shows a continuous decrease, following the behavior of α-helical PBD-LG undergoing a helix to coil transition under the same conditions. Finally the ellipticity reaches zero at 35% HFIP (Figure 5). The CD spectra recorded in the 220–250-nm region are shown in Figure 6 for HFIP concentrations up to 11%. Their evolution indicates a transconformation from the $\pi\pi_{DL}{}^{7.2}$ to the α helix. However, the exact conformational state of PBD-LG in the 11% HFIP/solvent mixture is difficult to assess since HFIP may alter two equilibria, left–right handed α helices and α helix random coil. When trifluoroacetic acid (TFA) is used instead of HFIP, the same behavior is observed but for smaller amounts of TFA. Again the sign of θ_{230} is first reversed and then tends to zero. In that case, although the maximum θ_{230} value ($\theta = 0.30 \times 10^4$) is lower than that obtained in pure methylene chloride ($\theta = 0.50 \times 10^4$), the sample is fully α helical as revealed by NMR.[17] A similar behavior is observed when starting from chloroform solutions to which HFIP or TFA are added.

To sum up therefore, it appears clearly that transconformations occur between the double-stranded helices. This further strengthens our starting hypothesis, namely that the double-stranded helical conformations are the same in solution as those found after evaporation of the solvents. Therefore, dissolution of the $\pi\pi_{DL}{}^{5.6}$ or any of the double helices in methylene chloride gives rise to the $\pi\pi_{DL}{}^{7.2}$ double helix, in chloroform or dioxane to the $\pi\pi_{DL}{}^{9.0}$, and in collidine to the $\pi\pi_{DL}{}^{10.8}$ double helices. These double-helical structures are unstable in nonaggregating solvents such as DMF or chloroform/TFA mixtures. Thus no molecular weight determination of these structures could be made in order to check their state of dimerization or polymerization.

(III) Linear Gramicidin. As yet, the ion transport properties of linear gramacidins have been related to the existence of either the $\pi_{DL}{}^6$ helical conformation[3,4] or of different double-stranded helical conformations.[6,7] Urry et al.[3,4] proposed the π_{DL} conformation mainly on the basis of NMR measurements in DMSO and DMSO/acetone mixtures whereas Veatch et al.[6,7] suggested the double-helical confor-

Table II
Infrared Frequencies Observed for Linear Gramicidin[a]

	Amide I, cm^{-1}	Amide II, cm^{-1}
Solid state recasted from		
DMSO	1665	1550
DMSO + traces of water	1645	1545
DMSO simultaneously precipitated with water	1635	1530
HFIP or TFE on a NaCl crystal	1628	1545
HFIP or TFE in a glass flask (in KBr pellet)	1650	1540
In solution		
CH_2Cl_2 (freshly dissolved) or $CHCl_3$	1635 +1682 sh	1540
Dioxane	1635 +1684 sh	1537
DMSO 20 °C (Figure 7)	1663–1665	1550
DMSO 85 °C (Figure 7)	1665	1530–1535
DMSO/acetone (1:1, v/v) or HFIP or TFE	1665	1530
Dioxane (after evaporation of HFIP in a glass flask) (Figure 9)	1650 +1630 +1635	

[a] Except when mentioned, the starting material was commercial gramicidin which has, in KBr pellets, an infrared spectrum identical with that of species 3 (sh = shoulder).

mations with either parallel or antiparallel strands mainly on infrared evidence.

It appeared interesting to compare our IR results obtained with PBD-LG, related to the knowledge of precise structures, with the main results obtained so far with gramicidin A or its hydrogenated derivative and to reexamine their interpretation. Such a comparison, using PBD-LG as a standard, has obvious limitations. Rather than proving the existence of a structure, it is possible to reject a postulated one if its spectral characteristics are too far from those of the corresponding PBD-LG structure. For instance, the location of the amide infrared absorption bands of a given structure would not be expected outside a ± 10 cm^{-1} deviation from the standard.

We were led to make new experiments with gramicidin in order to have similar experimental conditions. Our starting material had an infrared spectrum in KBr pellet and a CD spectrum in dioxane which put it in the same category as species 3 of Veatch et al.[6] The results of these experiments and of others, which were duplications, are reported in Table II. Where comparison is possible (i.e., recorded in the same conditions) all our IR or CD spectra are in agreement with those published by other authors.

Solid State. When linear gramicidin is dissolved in HFIP, the infrared spectrum depends on the conditions of elimination of the solvent. If evaporation is done in a glass flask, amide I and II bands are found at 1650 and 1540 cm^{-1} respectively while they lie at 1628 and 1545 cm^{-1} if the solvent is evaporated on a NaCl surface (see Table II). Such a behavior is reminiscent of a poly(D-L-methionine) sample made up in the same conditions.[18]

An analogous behavior is observed when starting from a solution in DMSO. If the solvent is evaporated quickly, the infrared spectrum is close to that of α-helical PBD-LG, while it resembles that of the π_{DL} helix when evaporated slowly, i.e., the solvent is allowed to absorb some traces of water. If the amount of water is increased and thus gramicidin is precipitated, then the spectrum is very close to that of double helices.

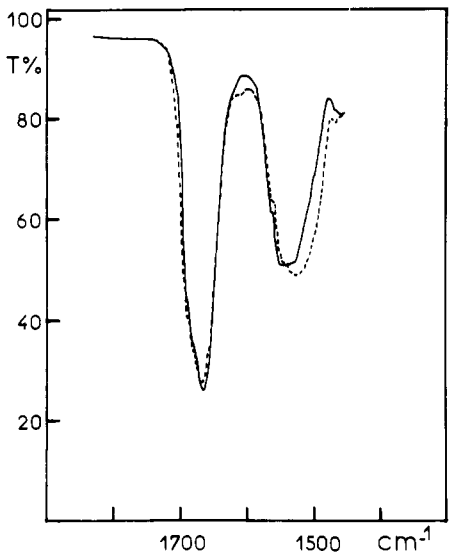


Figure 7. Infrared spectra of linear Gramicidin in DMSO, c = 80 mg/mL: (—) 20 °C; (- - -) 85 °C.

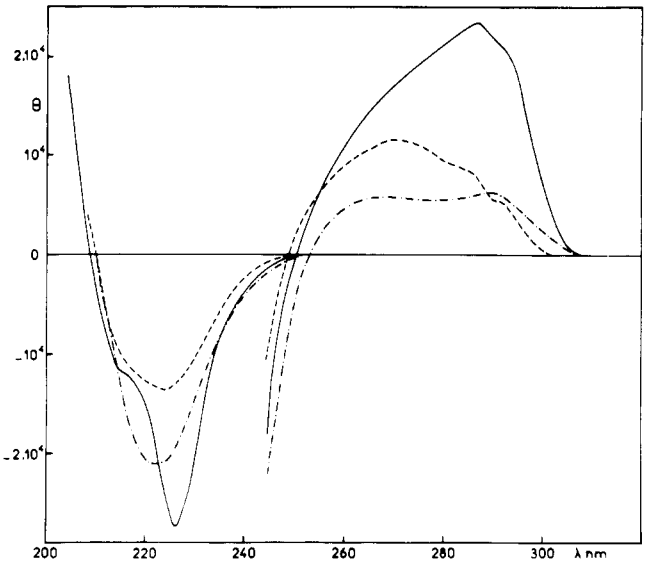


Figure 8. CD spectra of linear gramicidin in (—) dioxane, (- · -) $CHCl_3$, (- - -) CH_2Cl_2, $1 < c < 1.5$ mg/mL. Below 250 nm θ is the residue ellipticity and over 250 nm it is the molar ellipticity.

In Solution. The CD spectra of linear gramicidin solutions in trifluoroethanol (TFE) and HFIP are almost identical. Comparison of the IR spectrum with that of PBD-LG leads to the conclusion that the antibiotic is in the random coil form in both solvents. The same seems true for a solution in DMSO at 85 °C (see Table II and Figure 7).

As from Urry's results,[4,5] which will be discussed later, the hydrogenated derivative has a helical conformation in TFE and we are led to the curious conclusion that linear gramicidin and its hydrogenated derivative have not the same conformation in TFE.

In chloroform and dioxane as well as for a freshly prepared solution in methylene chloride, the infrared spectra are identical and in agreement with that of species 3 described by Veatch et al.[6] This is the expected β-type spectrum of double antiparallel helices although there are some differences in the positions of amide I bands as compared with double-helical

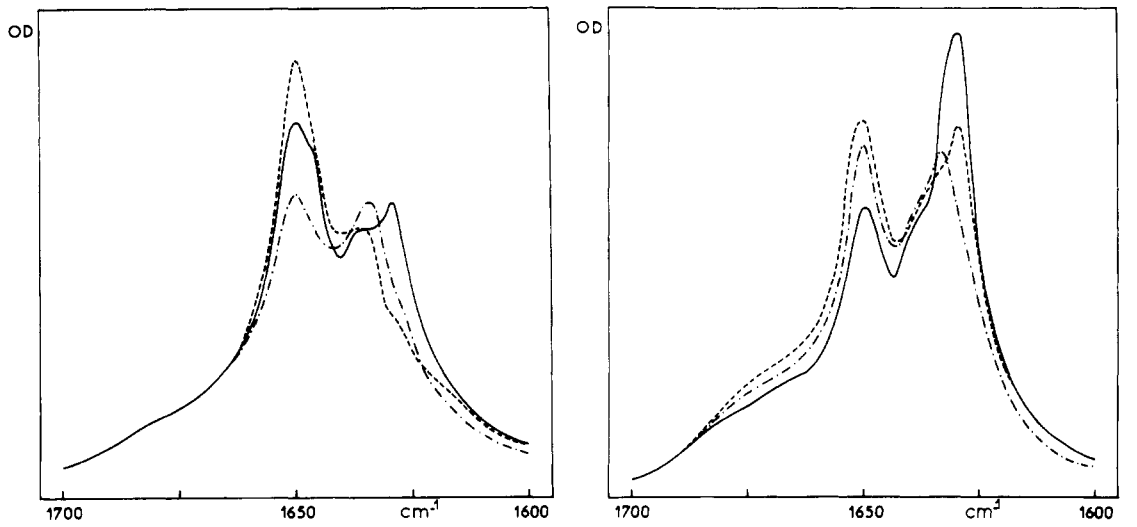


Figure 9. Infrared spectra in dioxane of linear Gramicidin previously dissolved and recast from HFIP: (—) after 0.5 h, (- - -) 2 h and 30 min (— • —) 24 h; (left, a) c = 1.6 mg/mL, (right, b) c = 4.8 mg/mL.

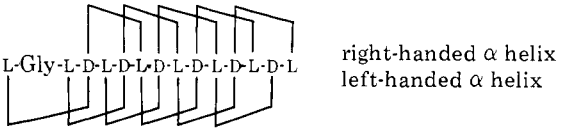
PBD-LG (compare Tables I and II). It should, however, be noticed that in these three solvents the CD spectra of linear gramicidin are all different (Figure 8) although these differences may arise only from different chromophoric side-chain interactions. As PBD-LG shows a $\pi\pi_{DL}^{7.2}$–$\pi\pi_{DL}^{9.0}$ transition in methylene chloride/chloroform or dioxane mixtures, the same experiments were reproduced with gramicidin in order to test a possible transconformation. However no sharp transition was observed in these conditions. Thus no conclusion can be made on the existence of different double helical conformations for gramicidin.

It is noteworthy to mention that, in methylene chloride, there is a transconformation with time as revealed by the appearance of a shoulder at 1630 cm^{-1}. The peptide precipitates slowly even if the concentrations used are as low as 1 mg/1 mL. The reliability of Byrn's[19] experiments in which ion transport measurements were made on gramicidin A dissolved in methylene chloride may therefore be questioned.

In connection with the experiments described by Isbell et al.,[20] who observed that the infrared and CD spectra of gramicidin lyophilized from acetic acid[21] are concentration dependent in dioxane, a similar behavior was observed with gramicidin previously dissolved in HFIP and the solvent evaporated in a glass flask. In fact, this experience clearly shows that the peptide is not at the conformational equilibrium which is reached only after several hours or days depending on the concentration (Figures 9a and 9b). Moreover, the infrared spectra reveal that the initial conformation gives rise to that obtained at equilibrium (probably species 3[6]) through an intermediary step not yet described (Figure 9) with an amide I band at 1630 cm^{-1}.

Let us now concentrate on the conformation of linear gramicidin when dissolved in DMSO/acetone mixtures (1:1, v/v). The infrared spectrum of gramicidin in this mixture at 30 °C is identical with that obtained in DMSO at 85 °C (see Table II) and can be interpreted on the basis of a random coil conformation. On the other side, in pure DMSO at 20 °C, amide I and II bands are found at 1664 and 1550 cm^{-1} respectively and would thus indicate a α-helical conformation. This is in contradiction to Urry's[4] conclusion that, from NMR observations in these solvents (the mixture at 10 °C and pure DMSO at 20 °C), a π_{DL} helical conformation was present.

In connection with the above interpretation, Urry et al.[5] concluded that hydrogenated gramicidin A is also in a π_{DL} helical conformation in TFE. However, the CD spectrum in this solvent resembles that of sample $LD_{Cat}II$ of PBD-LG in the α-helical conformation and is very close to that of a left-handed α helix, in spite of a small red shift of the extrema. Such a screw sense is, a priori, surprising when considering the stereochemical composition of the peptide. Indeed, one would expect for a peptide made of an excess of L over D residues (8 L and 6 D residues, the 15th residue, glycine, is optically inactive) a right-handed screw sense. However, as shown for PBD-LG[13] the interactions between the side chains determine the favored screw sense. In linear gramicidin, 12 side chains are interacting in the left-handed screw sense and only 10 for the other as illustrated on the following scheme.



Thus the left-handed screw sense may be favored for α-helical gramicidin.

Thus, the main discrepancy between our interpretation and that of Urry is found for the structure of gramicidin in DMSO or TFE. Urry concluded that, in these solvents, there was present a member of the π_{DL} helical family, whereas the comparison of infrared spectroscopic parameters with those of PBD-LG led us to postulate an α helix (DMSO) or a random coil (DMSO/acetone mixtures or TFE). It must be noticed that the coupling constant values found by Urry and interpreted, on the basis of Ramachandran's Karplus equation,[22] to reflect the presence of a π_{DL} helix can also be interpreted on the basis of Bystrov's curve[23] to reflect the presence of L residues engaged in a left-handed α helix and thus would be in favor of our interpretation.

Conclusion

In this paper we have described some solution properties of double-stranded helices of PBD-LG and compared them with those of the single-stranded helices of the same polymer. This model polypeptide of linear gramicidin has thus been shown to adopt three different double-helical conformations in solution and it is very likely that these helices are those identified in the solid state after dissolution of the mother helix ($\pi\pi_{DL}^{5.6}$) and recasting from the solvent, i.e., the $\pi\pi_{DL}^{7.2}$, $\pi\pi_{DL}^{9.0}$, and $\pi\pi_{DL}^{10.8}$ helices.

On the basis of the PBD-LG behavior a preliminary attempt has been made to identify the conformations of linear gramicidin. This antibiotic exists in solution under a great number of conformations which depend on the history of the sample. In the solid state, the support seems to play an important role. At present, except for species 3,[6] which can be assimilated to antiparallel double helices, it is not possible to draw any firm conclusion about these conformations by reference to the infrared frequencies–conformation relationship established for PBD-LG.

Thus other models have to be prepared and other techniques have to be used to determine the states of the natural product.

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High-Resolution NMR Studies at 270 MHz of Alternating Poly(γ-benzyl D-L-glutamate) in Double-Stranded Helical Conformations

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ABSTRACT: NMR spectra (270 MHz) are presented of strictly alternating poly(γ-benzyl D-L-glutamate) in dioxane, chloroform, and methylene chloride solutions from which cast films have been demonstrated to have a molecular conformation in the $\pi\pi_{DL}^{9.0}$ and $\pi\pi_{DL}^{7.2}$ double-helical forms. Unusually low chemical shift values are observed for the α-C*H* (~5.5 ppm) and N*H* (~9.0 ppm) resonances, well separated from those of the α and π_{DL} single helices. The aromatic protons and benzyl-CH_2 resonances are found slightly upfield of those of the single helices. It is proposed that these shift values are characteristic of the double helices and that the conformation of the PBD-LG sample in these solvents is the same as in the film obtained therefrom. This conclusion is supported by NMR spectra of a solvent-induced $\pi\pi_{DL}^{7.2} \rightarrow \pi\pi_{DL}^{9.0}$ double helical transconformation and a similar TFA-induced double helix $\rightarrow$ single helix conformational transition.

Alternating poly(γ-benzyl D-L-glutamate) (PBD-LG), a stereochemical model of Gramicidin A, has been shown to adopt a great number of helical conformations in the solid state. These are the α and $\pi_{DL}^{4.4}$ [2] single-stranded helices, and a family of double-stranded helices designated as $\pi\pi_{DL}^{5.6}$, $\pi\pi_{DL}^{7.2}$, $\pi\pi_{DL}^{9.0}$, and $\pi\pi_{DL}^{10.8}$ with probably antiparallel arrangement of the polypeptide chains.[3,4] The first member of the double-helix series, $\pi\pi_{DL}^{5.6}$, has been found only after heating in the solid state the α or π_{DL} conformations. When dissolved and recast it transconforms into a different double helix, the helix obtained being dependent on the dimensions of the solvent molecules. Thus the $\pi\pi_{DL}^{7.2}$ helix is found after recasting from methylene chloride, while the $\pi\pi_{DL}^{9.0}$ and $\pi\pi_{DL}^{10.8}$ helices are found from chloroform (or dioxane) and collidine, respectively, and the same conformations are believed to exist in the mother solvent.[4] Both the π_{DL} helix and the double helices are structures so far found only in a strictly alternating poly(D-L-peptide); they are specific for this sequence.

The solution properties of the α and π_{DL} (most probably the $\pi_{DL}^{4.4}$) helices of PBD-LG have been studied using ORD, CD, infrared spectroscopies,[5] and NMR.[6] The previous paper[7] describes ORD, CD, and infrared studies of solutions of the last three members of the double-helix family and their transconformations. We present here NMR spectra of the same materials, except for the $\pi\pi_{DL}^{10.8}$ double helix, which had to be omitted owing to the lack of deuterated collidine. The present data therefore involve the $\pi\pi_{DL}^{7.2}$ and $\pi\pi_{DL}^{9.0}$ double helices and compare their spectra with those of the α and π_{DL} single helices of the same sample. Transconformations have also been studied including the TFA-induced $\pi\pi_{DL}$(double) $\rightarrow$ α(single) helix–helix transitions. The results show that the double helices have α-C*H* peaks at unusually low field and far from that of either the α or the π_{DL} single helices.

Experimental Procedure

Sample $LD_{Cat}II$[8] of PBD-LG (weight average molecular weight = 31 000 daltons), heated for 3 h at 200 °C under vacuum to give the $\pi\pi_{DL}^{5.6}$ double helical conformation, was used in this study. All