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

Table I
Chemical Shifts of N*H*, C_6H_5, BzCH_2, and α-C*H* of PBD-LG (Sample $LD_{Cat}II^8$) in the Various Helical Conformations

Sample	Conformation	Solvent	δ, ppm: N*H*	C_6H_5	Bz-CH_2	α-C*H*
PBLG	α	$CDCl_3$ 0.5% TFA	8.23	7.30	5.08	3.92
PBD-LG	α	$CDCl_3$ 0.5% TFA	8.56	7.28	5.05	3.655 3.82
	π_{DL}	Dioxane-d_8 86 °C	8.45	7.28	5.05	4.45
	$\pi\pi_{DL}^{7.2}$	CD_2Cl_2	8.90 9.12	7.18	4.91	$4.75 < \delta < 5.60$
	$\pi\pi_{DL}^{9.0}$	$CDCl_3$	9.0	7.06[a] +7.00 sh[a]	4.81 +4.87 sh	5.40
	$\pi\pi_{DL}^{9.0}$	Dioxane-d_8	9.05	7.03 7.12	4.90	5.45

[a] sh = shoulder.

spectra were recorded at 20 or at 86 °C on a Bruker WH 270 working in the Fourier transform mode. Convolution difference spectra were obtained by the method of Campbell et al.[9]

Results and Discussion

Figure 1 shows spectra recorded for the $\pi\pi_{DL}$ helices in methylene-d_2 chloride, deuteriochloroform, and dioxane-d_8, together with spectra of the α and π_{DL} helices in deuteriochloroform/0.5% TFA and dioxane-d_8 at 86 °C, respectively.

The spectra of the sample in dioxane-d_8 at room temperature and in deuteriochloroform are very similar and support the proposal that the conformation is the same in both these solvents (i.e., the $\pi\pi_{DL}^{9.0}$ double helix). In particular the α-C*H* peak is double and centered at ~5.5 ppm, very much to the low field side of that observed for either the α or the π_{DL} helices (see upper spectra in Figure 1). The N*H* peak is also almost the same at room temperature in dioxane-d_8 and in deuteriochloroform, and likewise has an unusually low chemical shift (~9 ppm). The spectrum of the $\pi\pi_{DL}^{9.0}$ double helix is also characterized by small upfield displacements of both aromatic protons (~7 ppm) and the benzyl-CH_2 protons (~4.7 ppm) from the shift values found for all the single helices and the random coil. Similar differences may occur for the β- and γ-CH_2 protons but the complexity of the spectra makes characterization less easy. These unusual side-chain shift values observed for the $\pi\pi_{DL}^{9.0}$ helix probably imply a stronger interaction of side-chain and backbone than in the single helices, bearing in mind that the chemical shift values of the single helices side-chain protons are the same as for the random coil.

The $\pi\pi_{DL}^{7.2}$ double helix in methylene chloride shows a peptide N*H* spectrum that clearly distinguishes it from the single helices and the overall band shape also distinguishes it from the $\pi\pi_{DL}^{9.0}$ double helix N*H*. The α-C*H* resonance of the $\pi\pi_{DL}^{7.2}$ double helix is unfortunately obscured by residual solvent protons but clearly lies in the region of 5.3–5.5 ppm, close to that of the $\pi\pi_{DL}^{9.0}$ α-C*H*. The aromatic and benzyl-CH_2 proton resonances of the $\pi\pi_{DL}^{7.2}$ double helix are shifted slightly upfield of the positions characteristic of the single helices and random coil, but to a lesser extent than the $\pi\pi_{DL}^{9.0}$ double helix. As with the latter helix, this perturbation may imply a side-chain/backbone interaction.

Table I summarizes the chemical shift data of both the single and double helices, from which it is clear that the two double helices, $\pi\pi_{DL}^{9.0}$ and $\pi\pi_{DL}^{7.2}$, have rather similar spectra but can be differentiated on the basis of their N*H* signals. The double helical spectra are, however, very readily distinguished from the single α and π_{DL} helices, particularly using the somewhat sharper α-C*H* spectrum.

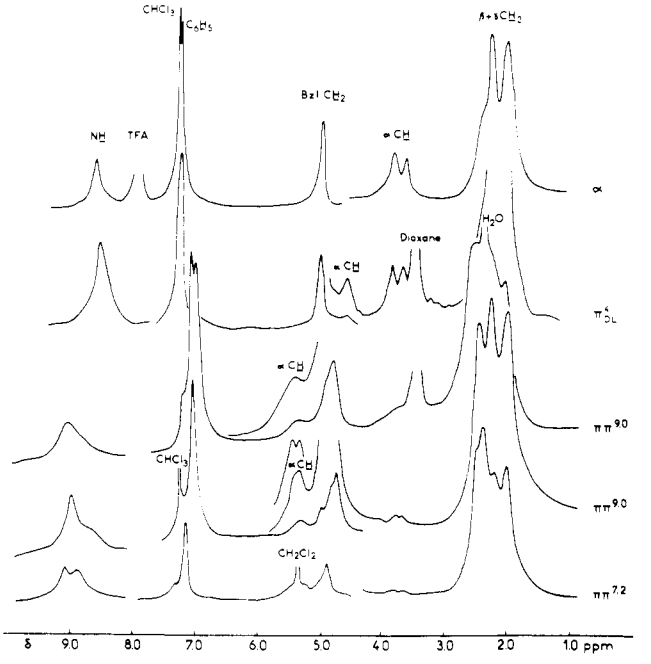

Figure 1. Spectra (270 MHz) of PBD-LG in various helical conformations: (α) single helix (20 °C, *c* 7 mg/mL, $CDCl_3$/0.5% TFA); (π_{DL}^4) single helix (86 °C, *c* 7 mg/mL, dioxane-d_8); ($\pi\pi_{DL}^{9.0}$) double helix; upper spectrum (20 °C, *c* 7 mg/mL, dioxane-d_8); ($\pi\pi_{DL}^{9.0}$) double helix, lower spectrum (20 °C, *c* 7 mg/mL, $CDCl_3$); ($\pi\pi_{DL}^{7.2}$) double helix (20 °C, *c* 7 mg/mL, CD_2Cl_2). Insets are vertical expansions except that of the doublet structure of the α-C*H* peak of the $\pi\pi_{DL}^{9.0}$ double helix which was revealed by convolution difference.

The two double helices studied (in a total of three solvents) have similar chemical shifts for the main resonances. It is thus probable that the unusually low-field shift values are, in large part, an intrinsic property of the double-helical structure. Inspection of models shows that the $C_\alpha H$ bonds are nearly parallel to the helix axis and the α-hydrogen atoms are situated between C=O and N—H bonds (see Figures 2a and 2b). The arrangement in the π_{DL} helix[6] is more akin to that in the double helices (see Figures 2c and 2d) than to the α helix since in the latter the C_α–H bonds point rather outside the helical core and lie between either two N—H or two C=O groups, depending on the chirality of the residue (see Figure 2 in ref 6).

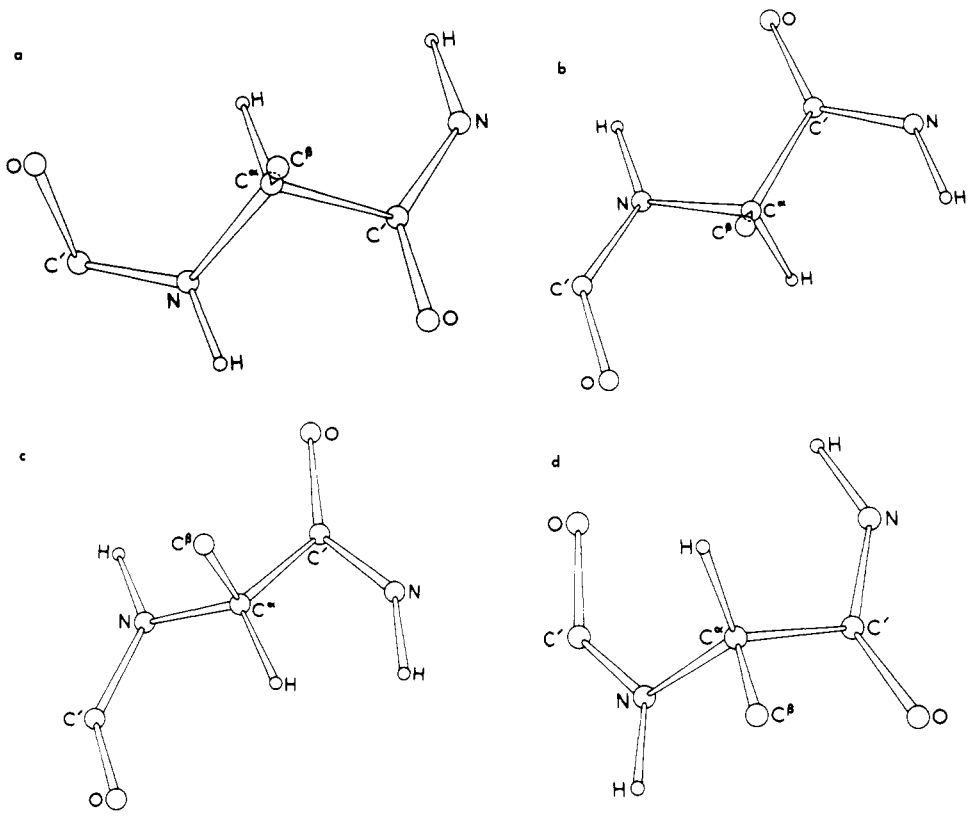

Figure 2. Representation using the atomic coordinates given in ref 4 of the respective positions of the α-CH protons (helical axis vertical): (a) L residue in a right-handed $\pi\pi_{DL}{}^{9.0}$ helix; (b) D residue in a right-handed $\pi\pi_{DL}{}^{9.0}$ helix; (c) L residue in a right-handed $\pi\pi_{DL}{}^{4}$ helix; (d) D residue in a right-handed $\pi_{DL}{}^{4}$ helix.

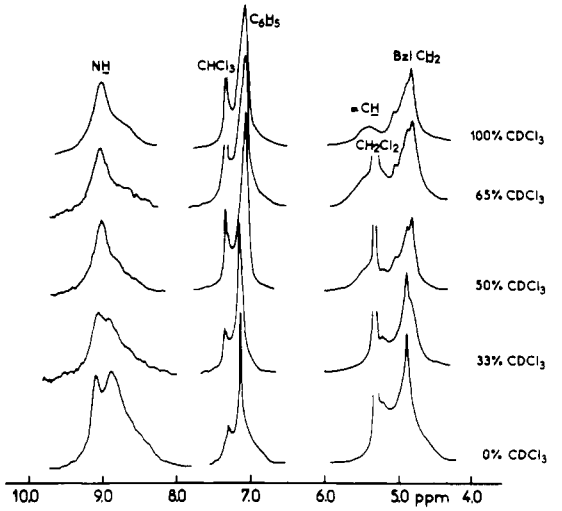

Figure 3. Spectra (270 MHz) at 20 °C of the double-helical transconformation $\pi\pi_{DL}{}^{7.2} \rightarrow \pi\pi_{DL}{}^{9.0}$ in methylene chloride/chloroform mixtures (v/v), *c* 7 mg/mL.

A very low field position for an α-C*H* resonance (5.4 ppm) has also been reported[10] for alternating poly(D-L-methionine) dissolved in chloroform. It could therefore be that an α-C*H* resonance in the region of 5.4–5.5 ppm is a true property of poly(D-L-peptides), although the conformation proposed for the latter polypeptide is of an intramolecularly folded β type.

Transconformations

(a) Double-Helical Transition. As pointed out in the preceding paper,[7] spectral differences observed in various solvents do not necessarily imply conformational differences since they might be due to the changing solvation of a single conformation. The rigorous demonstration of conformation in solution is, of course, always extremely difficult. However, observation of spectral changes in mixed solvents over a relatively narrow composition range is strongly suggestive of a conformational change. For this reason we have followed spectral changes in several solvent mixtures. Figure 3 shows spectra obtained when chloroform ($\pi\pi_{DL}{}^{9.0}$ double helix inducing) is added to a solution of $\pi\pi_{DL}{}^{7.2}$ double helix in methylene chloride. Several features of the spectra demonstrate a transition between 33 and 50% (v/v) chloroform with a midpoint at about 40% chloroform. This is in accord with the CD and ORD observations.[7] Both the aromatic (~7.1 ppm) and benzyl-C*H*$_2$ (~4.9 ppm) peaks move upfield with increasing chloroform concentration, showing a double peak type behavior, and there is a loss of N*H* resonant intensity at 8.9 ppm. NMR spectra were also obtained using dioxane instead of chloroform, where a transition centered at ~16% dioxane was observed. We conclude that a transconformation $\pi\pi_{DL}{}^{7.2} \rightarrow \pi\pi_{DL}{}^{9.0}$ between double helices in fact occurs in these solvent mixtures.

(b) Double Helix → Single Helix → Random Coil. It was shown that addition of TFA to a solution of double helical PBD-LG in either methylene chloride or chloroform ($\pi\pi_{DL}{}^{7.2}$ or $\pi\pi_{DL}{}^{9.0}$) induces first a change of sign of the optical activity and then a marked decrease.[7] An NMR study of the same system (see Figure 4 for methylene chloride/TFA mixtures) shows that the change of sign corresponds to a $\pi\pi_{DL}$ double helix → α single helix transition, with a midpoint at about 0.4% TFA for the polymer concentration used. Since the spectra of the single and double helices are very different, the conformational transition is clearly apparent in Figure 4. For example, as the characteristic double peak of the α helix at ~3.8 ppm increases in intensity there is a loss of intensity of

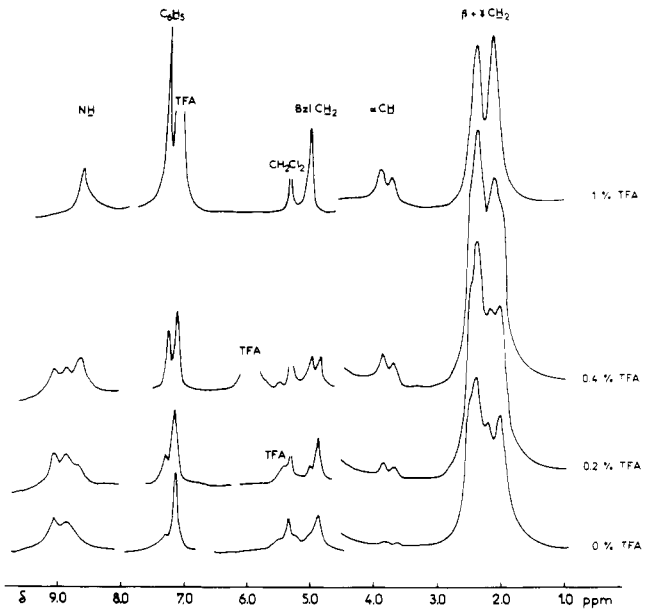

Figure 4. Spectra (270 MHz) at 20 °C of the double to single helical transconformation, $\pi\pi_{DL}^{7.2} \rightarrow \alpha$, induced by TFA addition to PBD-LG in methylene chloride, *c* 7 mg/mL.

double helix α-C*H* peak (~5.3 ppm); likewise the double N*H* peak at ~9.0 ppm characteristic of the double helix is replaced by the single peak at ~8.6 ppm corresponding to the single helix. Since both the aromatic and benzyl-CH_2 side-chain proton resonances of the double helix are markedly upfield of their positions in the single helix, these two resonances both clearly show the occurrence of a transition. At the midpoint, "double peak" spectra are observed (i.e., simultaneous observation of both conformers) which probably originate from the polydispersity, but might also be due to a genuinely slow rate of conformational transition. Addition of TFA beyond 1% leads to breakdown of the α helix to random coil, as documented in ref 6.

Conclusions

The distinctive spectra presented for heat-treated PBD-LG dissolved in dioxane, chloroform, and methylene chloride suggest that the polymer conformation in these solvents is indeed that found in solid films cast from these solvents, i.e., the $\pi\pi_{DL}^{7.2}$ and $\pi\pi_{DL}^{9.0}$ double helices. This conclusion is strongly supported by the double helix–double helix and double helix–single helix transition observed in the NMR spectra. The main-chain N*H* and α-C*H* resonances of the double helices lie at unusually low field. These shift values may have their origin in the existence of a β-type structure and in side-chain effects, as suggested by the displacement of the aromatic and benzyl-CH_2 proton resonances.

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Carbon-13 NMR Determination of Pentad Tacticity of Poly(vinyl alcohol)

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ABSTRACT: Carbon-13 Fourier transform spectra (22.6 and 67.9 MHz) of poly(vinyl alcohol), PVA, were obtained. By using the appropriate experimental conditions, we were able to resolve the methine carbon resonances into a triplet of triplets which are readily assignable to pentad tacticity. Quantitative analysis of the methine carbon spectra provided further support to the previous conclusion that stereoregularity of radical-initiated polymerization of vinyl acetate is almost ideally atactic. In addition, we found that the stereochemical sequence distribution in the isotactic PVA derived from cationic polymerization of vinyl trimethyl silylether conforms to first-order Markov statistics.

Previous work has clearly demonstrated the utility of high-resolution NMR spectroscopy for determining the stereochemistry of vinyl acetate polymerization.[1-5] Examination of the perdeuterated dimethyl sulfoxide (DMSO-d_6) solution of the derived poly(vinyl alcohol), PVA, revealed that both the hydroxyl proton[1,4] and methine carbon resonances[4] can be used for quantitative measurements of triad tacticity. In fact, the methylenes carbon spectra are attributable to tetrad sequence placements.[2,4] Moreover, the 220-MHz acetoxy methyl proton spectra of the corresponding poly(vinyl acetate) dissolved in nitromethane are resolved into six lines and provide a partial analysis of pentad tacticity.[5] From these results, we concluded that the configuration sequence distribution of radical polymerization of vinyl acetate is independent of polymerization conditions but describable by a Bernoulli-trial process.[4,5] Recently we further investigated the carbon-13 spectra of PVA; a method for quantitative analysis of pentad tacticity is reported in this paper.