These factors, *i.e.*, the more extended chain structure, the different orientation of backbone carbonyls, and the smaller size of the ring, less hydrophobic than the pyrrolidine ring, make more favorable the interactions with the solvent in *cis*-PLAze than in *cis*-poly(L-proline).

In conclusion we suggest that cis units of our polymer appear in water, whereas they do not for poly(L-proline), because they are, to a certain degree, stabilized by their capability of establishing polar interactions with this solvent.

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Side-Chain Interactions and Conformation in α-Helical Poly(γ-phenacyl L-glutamate). Aggregation in Dilute Solutions

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ABSTRACT: The behavior of α-helical poly(γ-phenacyl L-glutamate) (PPLG) has been studied by hydrodynamic, optical rotation, dielectric, and nmr measurements in different solvents. Special attention has been paid to side-chain interactions which depend on the ease of solvation of the phenacyl chromophore. Thus, in hexafluoro-2-propanol the circular dichroism spectrum is very similar to that usually observed for an α helix, and hydrogen bonding was shown to occur between the solvent and the phenacyl CO group. In other helicogenic solvents, side chain–side chain interactions occur. In dimethylformamide and pyridine they give rise at low temperature and low concentrations to an aggregation phenomenon whose molecular mechanism implies a molecular weight dependent folding of the molecules. Models for the side-chain conformation are proposed for the polymer in the solid state on the basis of infrared dichroism.

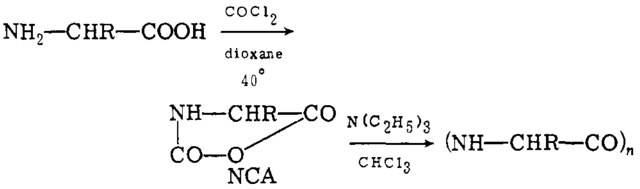
Polypeptide side chain–side chain interactions are detected in the circular dichroism (CD) spectra by the appearance of optically active bands located in the absorption region of the chromophores, although the chromophores themselves have no optically active center. These extrinsic Cotton effects are due to an asymmetrical ordering of the chromophores and to electronic interactions with the dissymmetrical field of the helix or of the asymmetrical carbon atoms. Thus, overlapping of the CD bands of the side chains and that of the peptide chromophore makes backbone conformational determination from optical rotation or CD measurements hazardous. Such side-chain interactions have been observed in particular with polymers composed of aromatic amino acids. In these cases, the chromophores are separated from the backbone by only one methylene group[2] (*e.g.*, polytyrosine, polytryptophan, and polyphenylalanine). When the nonaromatic part of the side chains becomes more important, these interactions can still be observed for some polypeptides such as polyaspartic acid nitrobenzyl esters.[3] In polyglutamic acid esters, which contain one more methylene group in the side chains, these would be expected to be more flexible and thus to give rise to less optical activity due to the side-chain chromophores. However, it was recently shown that polyglutamic acid nitrobenzyl esters in solution also display optical activity in the side-chain absorption bands.[4] Another ester of a strong chromophore ($\epsilon_{250} = 13300$), poly(γ-phenacyl L-glutamate) (PPLG), was also shown to display in some helicogenic solvents peculiar optical properties arising from side chain–side chain interactions competing with side chain–solvent interactions.[5,6] We have further studied the conformation of this polymer and the interactions and the conformation of its side chains.

We have now shown, from viscosity and light-scattering measurements, that PPLG adopts an α-helical conformation. From optical rotation measurements we conclude that the screw sense is right handed whatever the helicogenic solvent, but that in hexafluoro-2-propanol (HFIP) side chain–solvent interactions are predominant whereas side chain–side chain interactions are favored in other solvents. The aggregation phenomenon in dilute pyridine solutions was studied by dielectric measurements which revealed that it is accompanied by a folding of the molecules. Linear infrared dichroism on orientated films has provided information on the conformation of the side chain in the solid state.

Preparation and Characterization of the Samples

All samples of PPLG except one were prepared by polymerizing the corresponding *N*-carboxyanhydride (NCA) in 0.5% chloroform solution. Sample PPLG-1 was obtained by polycondensation in benzene (1 g/ml) of α-pentachlorophenyl γ-phenacyl L-glutamate. Poly(*p*-bromophenacyl L-glutamate) (PBrPLG) and random copolymers of phenacyl L-glutamate and benzyl L-glutamate (co(PLG-BLG)) or benzyl L-aspartate (co(PLG-BLA)) were prepared by polymerization of their NCA's. The syntheses are summarized in the scheme shown below. The polymers were recovered by precipitation with methanol. Low molecular weight PPLG is soluble in many solvents used throughout this work. The solubility is lower in trimethylphosphate (TMP), chloroform, and dichloromethane when the molecular weight reaches 60,000. PBrPLG dissolves only slightly in dimethylformamide (DMF) and TMP; it swells in dichloroacetic acid (DCA) and *m*-cresol. The infrared spectra of films of all samples cast from solutions in *m*-cresol show the characteristic bands of α-helical polypeptides (amide I and II near 1657 and 1553 cm^{-1}), as well as bands arising from side-chain groups (C=O ester and ketone at 1745 and 1705 cm^{-1}).

Scheme of the Synthesis of Poly(phenacyl glutamates). (i) Through *N*-carboxyanhydrides



$$NH_2{-}CHR{-}COOH \xrightarrow[\text{dioxane, }40^\circ]{COCl_2} \text{NCA} \xrightarrow[CHCl_3]{N(C_2H_5)_3} (NH{-}CHR{-}CO)_n$$

The NCA's were recrystallized from chloroform–heptane: R = $-CH_2-CH_2-CO_2-CH_2-CO-C_6H_4-R_1$; R_1 = H, γ-phenacyl L-glutamate–NCA, mp 87–88° dec; R_1 = Br, γ-*p*-bromophenacyl L-glutamate–NCA, mp 86° dec.

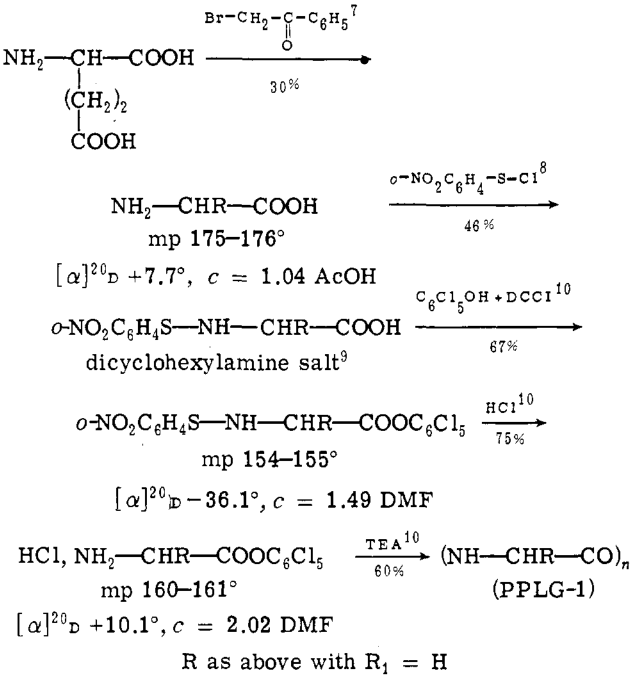
Table I
Light-Scattering Experiments on PPLG

	Sample			
	7	8	9	11
$\overline{M}_{w,\mathrm{DMF},60^\circ}$	309,000	26,000	200,000	165,000
$\overline{M}_w$[a]	270,000	26,000	210,000	150,000
$(\overline{R}^2)^{1/2}$, Å	562		407	378
$(\overline{L}^2)^{1/2}$, Å	1,950		1,400	1,310
L_w, Å	1,875		1,220	1,015

[a] Calculated from the limiting viscosity number in DCA. See text and ref 11.

Anal. Calcd for $C_{13}H_{13}NO_4$ (PPLG): C, 63.15; H, 5.26; N, 5.67. Found: C, 62.83; H, 5.36; N, 5.71. Calcd for $C_{13}H_{12}BrNO_4$ (PBrPLG): C, 47.85; H, 3.68; N, 4.29. Found: C, 47.41; H, 3.85; N, 4.48. $[\alpha]^{25}\mathrm{D}$ of PPLG = −24° (*c* = 1, dichloroacetic acid).

(ii) Through active ester



$$NH_2{-}CH(CH_2CH_2COOH){-}COOH \xrightarrow[30\%]{Br{-}CH_2{-}CO{-}C_6H_5\,^{7}} NH_2{-}CHR{-}COOH$$

mp 175–176°

$[\alpha]^{20}\mathrm{D}$ +7.7°, c = 1.04 AcOH

$$\xrightarrow[46\%]{o\text{-}NO_2C_6H_4{-}S{-}Cl\,^{8}} o\text{-}NO_2C_6H_4S{-}NH{-}CHR{-}COOH \text{ dicyclohexylamine salt}^{9} \xrightarrow[67\%]{C_6Cl_5OH + DCCI\,^{10}} o\text{-}NO_2C_6H_4S{-}NH{-}CHR{-}COOC_6Cl_5$$

mp 154–155°

$[\alpha]^{20}\mathrm{D}$ −36.1°, c = 1.49 DMF

$$\xrightarrow[75\%]{HCl\,^{10}} HCl,\ NH_2{-}CHR{-}COOC_6Cl_5 \xrightarrow[60\%]{TEA\,^{10}} (NH{-}CHR{-}CO)_n \ \text{(PPLG-1)}$$

mp 160–161°

$[\alpha]^{20}\mathrm{D}$ +10.1°, c = 2.02 DMF

R as above with R_1 = H

Conformation in Solution

Light-Scattering and Viscosity Measurements. The molecular weights of the PPLG samples were determined by light scattering in DMF using a refractive index increment value of 0.140 ml g^{-1} at 5460 Å and 60°. Below 40° the measurements are unreliable for high molecular weight samples because of the formation of a gelled solution.[6] Therefore the experiments were carried out at 60° at concentrations lower than 0.1% to avoid aggregation. The results are shown in Table I, where the values of the radius of gyration are also reported.

The dimensions of the molecules in DMF are in agreement with an α-helical conformation, as can be inferred from the comparison between the length $(\bar{L}^2)^{1/2}$ deduced from the radius of gyration assuming a rod-like shape, and the length L_w calculated from the molecular weight of an α helix with 1.5 Å per residue (Table I). Furthermore, the asymptotic behavior at high angles of the reciprocal of the intensity of scattered light *vs.* sin $\theta/2$ gives, for samples PPLG-7, -9, and -11, linear density values of 172, 196, and 200 Å^{-1}, not far from the theoretical value 164 for an α helix.

Clear solutions were difficult to obtain in dichloroacetic acid (DCA) and only one light-scattering measurement was done on sample PPLG-11 (dn/dc = 0.112 ml/g at 5460 Å). A molecular weight of 134,000 was found, a value somewhat smaller than in DMF (Table I), which may indicate that some aggregation still occurs in this solvent. For the other samples, the molecular weights were calculated from the values of the limiting viscosity number $[\eta]$ measured for PPLG in DCA and assuming that the relationship between $[\eta]$ and $\bar{M}_w$ for PBLG[11] in this solvent also holds for PPLG. The agreement with the values obtained in DMF is rather good (Table I). Using these values for $\bar{M}_w$, a plot of log $[\eta]_{\mathrm{DMF},60^\circ}$ *vs.* log $\bar{M}_w$ has a slope of about 1.64, which indicates an elongated and rather rigid conformation in DMF. The same conclusion is reached when considering the value

Table II
Limiting Viscosity Numbers for Different Samples of PPLG in DMF at 60° and in DCA at 25°[a]

	Sample										
	1	2	5	6	7	8	9	10	11	12	14
$[\eta]_{DMF}$		36	215.5	152	340.5	6.90	240	191	161.5	118	116
$[\eta]_{DCA}$	13.4	43.6			143	20.6	118	105	96.5	82	73

[a] The k' coefficient of the Mark-Houwink relation is 0.5 in DMF and 0.3 in DCA.

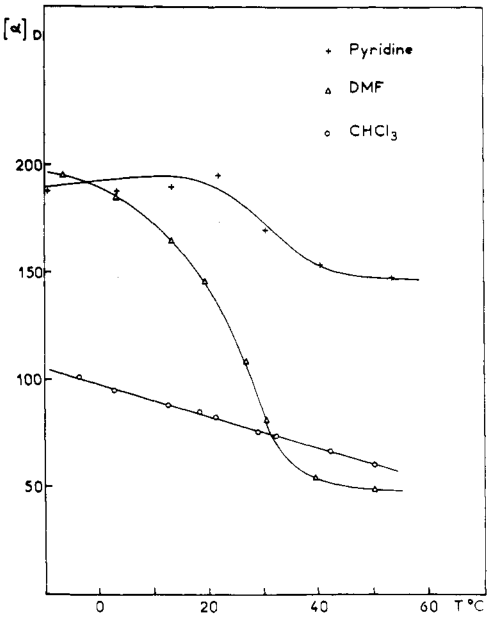


Figure 1. Temperature effect on the behavior of $[\alpha]_D$ of sample PPLG-2 in various solvents; $10 \leq c \leq 2$ g/l. in DMF and pyridine; $c = 2$ g/l. in $CHCl_3$.

of 2.0 found for the slope of the straight line representing log $[\eta]_{DMF}$ *vs.* log $[\eta]_{DCA}$ (Table II). Again, the values of $[\eta]_{DMF}$ are in agreement with the expected values for an α helix of a given molecular weight.

We conclude that the behavior of PPLG is very similar to that of PBLG in DMF (α helix) or DCA (random coil) when the results of hydrodynamic or light-scattering measurements are analyzed. Optical rotatory dispersion (ORD) experiments, on the other hand, confirm this behavior in DCA only (Moffitt coefficient $b_0 = -40°$); in DMF, even at 60°, the Moffitt plot is not a straight line.[6] This discrepancy will be examined in the next section.

Optical Rotation. From the point of view of ORD measurements, we have already shown[6] that three types of helicogenic solvents can be distinguished: (i) HFIP; (ii) chloroform, dichloromethane, TMP; (iii) DMF, pyridine.

In HFIP the value of b_0 lies between −500 and −700 depending on the sample, indicating a right-handed α-helical conformation.

In solvents other than HFIP, PPLG exhibits properties different from those usually observed for right-handed α-helical polypeptides; the Moffitt plots are nonlinear. In the chlorinated solvents $[\alpha]_D$ varies linearly with temperature and no gel formation is observed, whereas in DMF or pyridine the variation of $[\alpha]_D$ shows a sharp transition at 25° (Figure 1) and for high molecular weight samples the decrease of temperature is accompanied by the formation of a nonflowing gel even at concentrations as low as 2×10^{-3} g ml^{-1}.

Examination of the ORD behavior of random co(PLG–BLG) and co(PLG–BLA) in DMF at high temperature has suggested that the screw sense of the helical conformation of PPLG is right handed and that the Moffitt plots are disturbed by side-chain effects.[6] This is also probably true in chlorinated solvents, in pyridine, and in the gel-forming solutions. These points are now further examined.

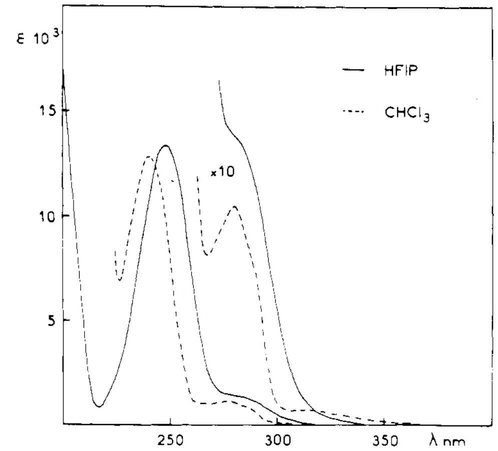


Figure 2. Ultraviolet absorption spectra of acetophenone in HFIP and $CHCl_3$ at room temperature and obtained on 1.20×10^{-3} molar solutions.

Hexafluoro-2-propanol. The observed CD spectra of HFIP solutions between 200 and 230 nm are characteristic of a right-handed α-helical conformation by the positions of the bands and their intensities.[2] Above 230 nm weak bands are observed, indicating side chain–side chain interactions, but their intensities are much smaller than in chloroform. Ultraviolet examination led us to conclude that the weakness of these interactions has its origin in the existence of a hydrogen bond between the solvent and the carbonyl function of the phenacyl group. The difference between the spectra of acetophenone used as a model for the side chain of PPLG in HFIP and chloroform is shown in Figure 2. The influence of the higher polarity of HFIP compared to chloroform is characterized by a blue shift for the n–π* transition and a red shift for the π–π* transition. But a shift of 8 nm (from 240 to 248 nm) for the π–π* transition is larger than expected and suggests the existence of a hydrogen bond between the solvent and acetophenone. The same behavior is observed for the polymer. These observations are corroborated by nmr experiments in $CDCl_3$–HFIP mixtures. As expected, addition of HFIP to pure $CDCl_3$ generates a linear downfield shift of the hydroxyl proton with increasing proportion of HFIP. This behavior is characteristic of self-association of HFIP molecules through hydrogen bond formation. If the experiment is repeated in the presence of PPLG, the hydroxyl proton moves first upfield, which indicates that at low concentrations of HFIP this solvent is initially strongly bonded to the polymer. Further additions of HFIP lead to the behavior observed in the solvent mixture without polymer. If PBLG is used in-

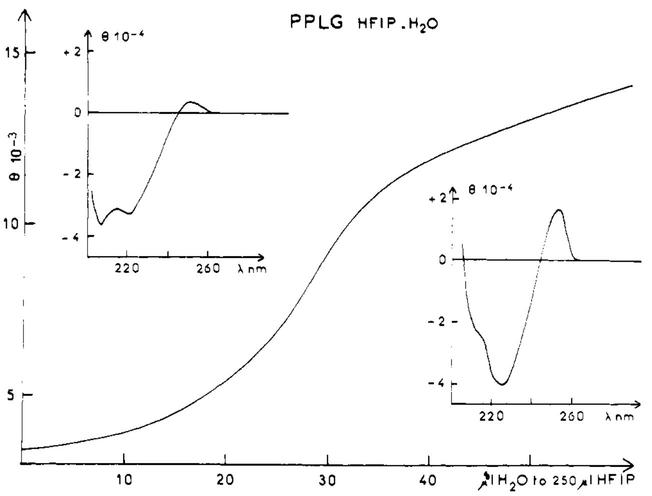


Figure 3. Effect of the addition of water on the CD spectrum of PPLG in HFIP. Spectra at the beginning and at the end of addition are shown in the inserts. Initial concentration in pure HFIP; $c = 0.15$ g/l. The ellipticity θ is given in (deg cm^2)/dmol.

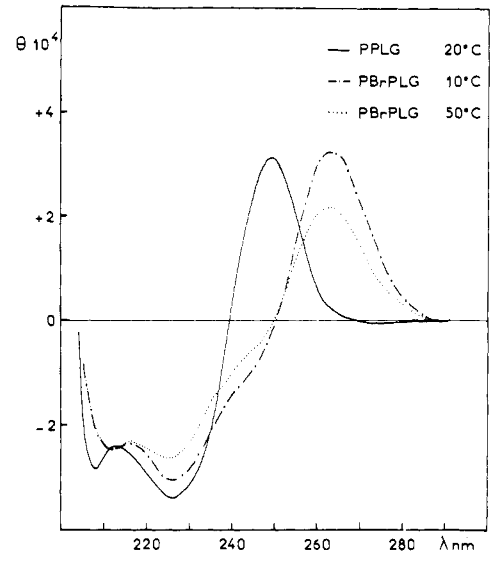


Figure 4. CD spectra of PPLG and PBrPLG in TMP; $c = 0.15$ g/l. The ellipticity θ is given in (deg cm^2)/dmol.

stead of PPLG the same observations are made. This means, as already shown by other techniques,[12] that HFIP forms hydrogen bonds with the polymers, either with the peptide carbonyl group or the ester, or more probably both. Examination of the position of the aromatic protons of PPLG reveals that addition of HFIP generates an upfield shift of the protons in the ortho position (7.80 to 7.70 ppm), whereas the meta and para protons remain unaffected (7.39 ppm). This clearly indicates that HFIP also forms a hydrogen bond with the phenacyl carbonyl group. This hydrogen bonding is also revealed by examination of a solution of PPLG in HFIP when water is added. Under these conditions, the CD spectrum is modified in the 200–300 nm region and, at the same time, one observes an increase of the band corresponding to the side chain–side chain interactions at 252 nm. Moreover, the variation of θ_{252} shows a titration point corresponding to the addition of 1 mol of water per mole of solvent (Figure 3). The same result is obtained at different concentrations of PPLG. This indicates the preference for HFIP to form a hydrogen bond with water rather than with the polypeptide.

TMP and Chloroform. The CD spectra in TMP and chloroform show certain similarities, although observations at lower wavelengths are only possible in TMP (Figure 4). One can see at 228 and 208 nm two negative bands which could be attributed to the peptide n–π^* and π–π^* transitions of a right-handed α helix. In chloroform and in TMP the spectra show the existence of optically active bands in the absorption region of the side chain (phenacyl chromophore), indicating that side chain–side chain interactions occur. This is confirmed by the CD study of co(PLG–BLG) and co(PLG–BLA) which show in both solvents at room temperature a nonlinear variation of the ellipticity at 250 nm with the composition of the copolymers while at the same time the variation of the ultraviolet absorption is linear.

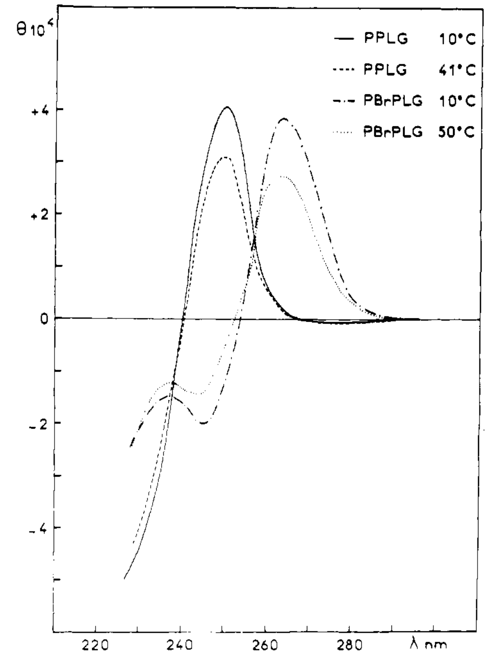


Figure 5. CD spectra of PPLG and PBrPLG in CHCl$_3$; $c = 0.15$ g/l. The ellipticity θ is given in (deg cm^2)/dmol.

The ellipticity at 228 nm for PPLG in chloroform solutions is much higher than the one usually observed for right-handed α-helical polypeptides. This suggests that the peptide chromophore region is also perturbed by side-chain dichroic bands. In order to test this hypothesis we have made observations on poly(γ-*p*-bromophenacyl L-glutamate) solutions. The introduction of a bromine atom on the phenacyl group produces a red shift of the absorption bands and we indeed observe a negative dichroic band at 246 nm, while its π–π^* transition is also shifted to a higher wavelength (Figure 5). In TMP similar behavior is observed (Figure 4).

It should be mentioned here, as previously observed by Loucheux and Duflot[4] with another system, that monomeric models, *i.e.*, *N*-acetyl-γ-phenacyl L-glutamate-*N*-ethylamide and *N*-acetyl-γ-phenacyl L-glutamate–γ-phenacyl L-glutamate-*N*-ethylamide, also exhibit optical activity in the absorption bands of the phenacyl group ($\theta = -15$ (deg cm^2)/dmol at 310 nm and -1000 (deg cm^2)/dmol at 250 nm for the monomeric model and -5000 (deg cm^2)/dmol at 250 nm for the dimeric model). As the sign of the optical activity of the models at 250 nm and above is opposite that of the polymer, we tend to conclude that the helical conformation and the resulting side-chain interactions contribute to the optical properties.

From this study we can now state that PPLG and its *p*-bromo derivative adopt a right-handed α-helical conformation in HFIP, TMP, and chloroform, but that strong interactions occur between the side chains except in anhydrous HFIP. In DMF or pyridine less information can be obtained from the CD measurements. However, the helical sense is probably the same, since the CD behavior appears to be similar to that observed in the above mentioned solvents in the transparent region.[6] Nevertheless, an important difference remains in the thermal behavior of the solutions. Cooling a solution of PPLG in chloroform or TMP gives a linear variation of θ_{250}, whereas in DMF or pyridine a sharp transition occurs for $[\alpha]$D and a gel is formed.

Aggregation

We have studied by dielectric measurements between 16 and 58° the aggregation process in pyridine, which, as mentioned above, occurs below 25° in dilute solutions of high molecular weight samples of PPLG.

For experiments run above 38° with sample PPLG-2 of molecular weight about 60,000 and PPLG-8 ($\bar{M}_w$ = 26,000) a single region of absorption was found whose critical frequency and dielectric increment are comparable to those observed for a PBLG sample having the same degree of polymerization. This result can be explained by the existence of isolated rod-like helices of PPLG having the same shape and dipole moment as PBLG. This confirms that PPLG has the same behavior in hot pyridine and DMF.

The general trend of the variation of the total dielectric increment as temperature is lowered is a sudden decrease at 38° for sample PPLG-2. Below 38°, two regions of dielectric absorption are observed, indicating that two kinds of particles of different sizes are simultaneously present. This is confirmed by ultracentrifugation experiments in which two peaks are found at 22°. The particles having the smaller sedimentation constant are somewhat less concentrated than the others. At 22°, the high frequency dielectric absorption is centered at an eight-fold higher critical frequency than that observed above 38°, thus indicating that the smaller of the two species has smaller overall dimensions than those of an α helix. Infrared spectroscopy has shown that the polymer remains in a helical conformation in pyridine at these temperatures, thus ruling out a coiled conformation for this species. Since the dielectric constant characterizing this high frequency absorption is low and since the relative concentration of these particles, as shown by sedimentation experiments, is of the order of ⅓ to ½, they have small dipole moments. The molecular model which can be proposed is that of a folded helix. As the temperature is raised, the reduced critical frequency ηf_c (η = solvent viscosity) decreases, which could correspond to an unfolding of the helix.

The critical frequency corresponding to the low-frequency absorption is about ten times smaller than that observed above 38°, *i.e.*, for a rod-like α helix. This is probably due to the presence of aggregates. Considering the small values of the dielectric constant relative to this low-frequency absorption, we suggest that they may be formed of associations of folded helices rather than of elongated α helices, since associations of the latter usually give high overall dipole moments.[13,14] Their size does not change in the 16–38° range of temperature and no concentration effect is observed whatever the temperature is for the concentration range (w in weight/weight) $3 \times 10^{-3} < w < 6 \times 10^{-3}$.

From these experiments, gel formation can be explained as follows. A decrease of temperature leads to the folding of helices favored by side chain–side chain interactions as shown by optical rotation measurements. The gel is then formed by intermolecular associations.

As the molecular weight is lowered, the folding of the α helix is apparently no longer possible. For PPLG-8 the dielectric absorption curves are less precise than in the preceding case; however, the general trend of the results is very different. Only one region of absorption is detected. The reduced critical frequency ηf_c does not change appreciably in the region 20–50°, whereas the dielectric increment $\Delta\epsilon/w$ suddenly decreases around 30–35° as the temperature increases (a sudden increase was observed for a PPLG $\bar{M}_w$ = 60,000). These results suggest side by side mainly parallel associations of rod-like helical molecules[13] at the lower temperatures.

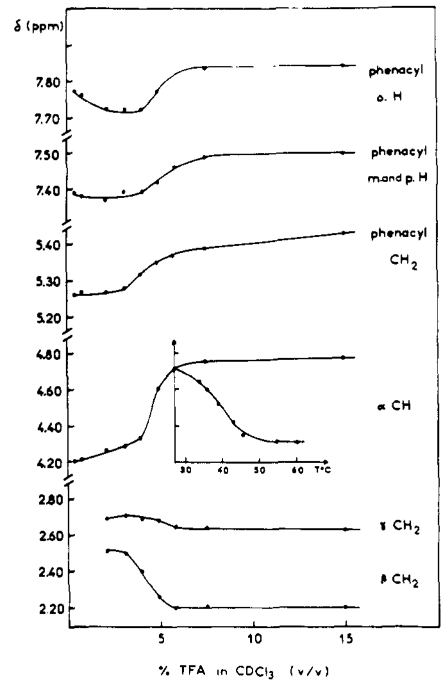


Figure 6. Nmr behavior of the different protons of sample PPLG-8 during the helix–coil transition in $CDCl_3$–TFA; c = 20 g/l.

The molecular weight and temperature-dependent solubility of PPLG in some solvents is thus accounted for by side chain–side chain interactions leading to specific ternary structures which, in turn, depend on the molecular weight of the polypeptide.

Helix–Coil Transition in Chloroform–TFA Mixtures

Addition of trifluoroacetic acid (TFA) to $CDCl_3$ solutions of PPLG leads first to the destruction of aggregates and then to the helix–coil transition, characterized like that of PBLG[15] by a downfield shift of the α-CH protons (Figure 6) and an inverse temperature effect. The transition can also be followed by examination of the behavior of the phenacyl CH_2 and aromatic protons (Figure 6). That this variation does not arise from the binding of TFA to the side chain is shown by the linear decrease of θ_{252} down to zero by addition of TFA up to 7%.

As already described in DMF-d_7,[6] in $CDCl_3$ an important difference of 0.15 ppm downfield is observed for the α-CH proton chemical shifts of α-helical conformations of PPLG and PBLG. The same difference is observed between the chemical shifts of the two polymers in the random coil conformation. In order to shed light on this behavior we have prepared and studied the corresponding monomeric models, *N*-acetyl-γ-phenacyl- and *N*-acetyl-γ-benzyl L-glutamate-*N*-ethylamide. Under the same solvent conditions ($CDCl_3$–TFA), a difference of 0.12 ppm is observed for the monomeric models. However, in DMF-d_7 a difference of only 0.02 ppm is observed between the models whereas it is of 0.20 ppm for the polymers. This result is not in contradiction with the conclusion of Bradbury, *et al.*,[16] who attributed such a difference in chemical shifts of

Table III

Wave number, cm^{-1}	θ, deg	Assignment
1740	57	C═O stretching (ester)
1705	60	C═O stretching (ketone)
1450	57	C—C stretching (phenyl B_1)
1165	53–54	C—O stretching (ester)

α-CH protons for different polypeptides in the same conformation to different magnetic anisotropies arising from different interactions between side chain and backbone.

Side-Chain Conformation in the Solid State

X-Ray Diffraction. The X-ray diffraction pattern given by an oriented fiber reveals a hexagonal lattice with 15.6 Å for parameter a. Because of the poor crystallinity of the polypeptide it was impossible to obtain a meridional reflection. However, from the density of the polymer (1.293 g cm^{-3}) measured on a compressed pellet, we calculated the molar weight per unit length along the c axis to be 164 Å^{-1}, close to the value of 165 Å^{-1} obtained by assuming that the polypeptide has an α-helical conformation with 1.50 Å per residue length.

Infrared. The α-helical conformation is corroborated by ir measurements on oriented films: amide A (3290 cm^{-1}) and I (1657 cm^{-1}) bands show a parallel dichroism whereas the amide II (1553 cm^{-1}) band has a perpendicular dichroism. By deuteration, an amide V band is found at 615 cm^{-1}, also in agreement with a right-handed α-helical conformation.[17]

As the vibration bands corresponding to the side chains are dichroic it was possible to make suggestions about the side-chain conformation. Using the peptide group geometry given by Tsuboi[17] and others[18] we calculated the angles θ between the transition moments of several groups and the fiber axis (Table III).

We note that the orientation of the ester group is close to that of PBLG. Considering the planarity of the group -C(O)-Ph and assuming that the directions of the C═O bond and of its transition moment are the same, the phenacyl group is found to be in a plane defined by the angles of the two vibrations phenyl B_1 and C═O ketone with the fiber axis and by the angle of 34° between these two vibrations.

In order to propose a model for the side-chain conformation we made the following assumptions: (1) the conformation from the α carbon atom up to the ester group is the same as that of PBLG; (2) the five atoms C-O-C(O)-C of the ester group are coplanar.

Numerous conformations of the side chain can be built. They were checked with molecular models, and by elimination of models mainly on the basis of steric hindrance between the phenacyl group and the C═O and C-O-C groups of the ester function, two possibilities remained. They are shown in Figure 7.

The first model (Figure 7, top) can be characterized by antiparallelism of the two carbonyl groups and near coplanarity of the ester and phenacyl groups. In the second possibility (Figure 7, bottom) these two groups are nearly perpendicular.

Assuming that all side chains on a right-handed α-helical backbone have the same conformation it appears that the first model favors interactions between the phenacyl groups of the nth and the (n + 4)th residues and the second model between the nth and the (n + 3)th residues. In the first model the distance between the aromatic rings is about 10 Å and is smaller than in the second model.

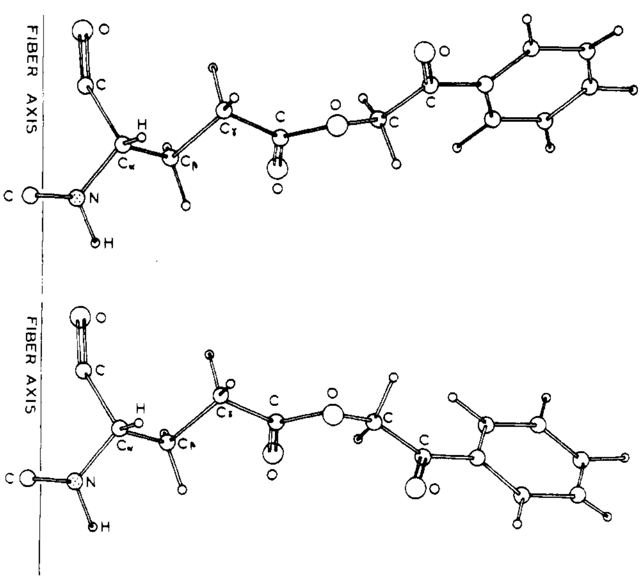


Figure 7. Proposed models for the conformation of the side chain of PPLG.

Experimental Section

All the solvents were distilled before use except deuterated solvents which were used without further purification. After distillation, DMF, TMP, and HFIP were stored over molecular sieves. Benzene for polycondensation was distilled over sodium.

Light-scattering measurements were made on a Sofica photogoniodiffusiometer. Solutions were made dust free by filtration on α-6 Metricelles: pore size 0.45 μ. Concentrations were verified after filtration by uv absorption at 280 nm. Refractive index increments were determined on a Brice Phoenix differential refractometer. Viscosities were measured using Ubbelohde viscosimeter (Cannon Cusmu).

Optical rotation measurements were carried out on a Perkin-Elmer polarimeter Model 141 M equipped with a 10-cm long thermostated cell. CD spectra were recorded on a Roussel–Jouan dichrograph equipped with a thermostated cell holder using 1 mm thick cells. Uv spectra were recorded on a Cary 14 spectrophotometer.

Nmr measurements were carried out with a Bruker HFX-90 spectrometer. Temperature was determined using ethylene glycol.

Ir spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. Ir dichroic spectra were made on films cast from m-cresol solutions and recorded on a Beckman IR 11 equipped with a gold grid polarizer. N-Deuterated polymer was prepared by dissolution of the polymer in deuterated trifluoroacetic acid and precipitation with hexane.

Dielectric absorption measurements were made in the 200 Hz–2 MHz range using water-jacketed stainless steel capacitors of 11, 33, and 89 pF when empty. The impedance bridges used were described previously.[19]

Conclusion

In this study we have shown that PPLG exhibits some peculiar optical rotatory and aggregation properties due to solvent dependent interactions of the side chains.

In helix breaking solvents (TFA and DCA) this polymer has the usual hydrodynamic and optical properties of a polypeptide in a random coil conformation. However, helicogenic solvents can be divided into three groups. The CD behavior of PPLG in HFIP can easily be explained by solvatation of the side chains. In chlorinated solvents and TMP on one hand, and basic solvents such as DMF and pyridine on the other, side chain–side chain interactions occur, leading to the existence of optically active bands in the absorption regions of the phenacyl group. The difference between these two latter classes of solvents lies essentially in the formation of gel-like solutions at low temperature in DMF and pyridine. This discrepancy is difficult to explain but seems to be related to the basicity and bulkiness of the solvent molecules. Although aggregation occurs in chloroform as revealed by nmr spectroscopy in pure $CDCl_3$, the aggregates in this solvent are not of the same type as observed in pyridine by dielectric measurements.

Side-chain conformation may play an important role in these interactions. Two possible models are proposed on the basis of infrared measurements in the solid state, but there is no evidence at this stage that they are also found in solution.

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Free Radicals from Crotonic Compounds Initiated with Redox Systems

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ABSTRACT: The reactions of crotyl alcohol (CAL), croton aldehyde (CAD), crotonic acid (CAC), and crotonitrile (CNI) with free radicals (R′) from redox reactions ($HO\cdot$, $H_2N\cdot$, and $H_3C\cdot$) have been studied in aqueous solution using esr measurements with a flow system. As a rule two types of radicals were formed from the croton compounds ($C_1{-}C_2{=}C_3{-}R$): (A) radicals by addition of R′ to C_3, and (B) radicals by addition of R′ to C_2. The concentration ratio of A to B is a function of R and decreases in the order $CH_2OH > COOH > CN > CHO$. This is interpreted as related to increasing resonance effects of the substituents in the radicals formed which would favor radical B. The concentration ratio of A to B is also affected by the nature of R′ and is found to decrease in the order $H_3C > HO > H_2N$; *i.e.*, the ratio decreases with increasing electron affinity of the attacking radical R′. Some exceptions are found. CAL with $H_3C\cdot$ gives predominantly an allylic type radical by hydrogen abstraction ($CH_3{-}CH \mathrel{\overset{\cdots}{-}} \dot{C}H \mathrel{\overset{\cdots}{-}} CHOH$). For CAD with $HO\cdot$ only B radicals were found. CAD with $H_2N\cdot$ and $H_3C\cdot$ gave only weak spectra with overlapping lines which could not be assigned to specific radicals. The α-proton coupling constants (a_H^{α}) are 20.0–22.1 G for the various radicals (19.5 G for B radicals of CNI and 16.4 G for B radicals of CAD). The a_H^{β} constants vary more with the nature of the substituents and decrease in the order $HO > H_3C > H_2N$ and $CN > CHO > CH_2OH > COOH$; *i.e.*, they decrease with increased bulkiness of the groups. The $a_{CH_3}{}^{\gamma}$ for the protons decrease in the order $COOH > CHO > CH_2OH > CN$ and $HO \simeq H_3C > H_2N$ which is interpreted as related to the spin density on the γ-CH_3 groups.

1. Introduction

In previous papers from this laboratory,[1-5] the radical polymerization of vinyl esters, butadiene, trimethylolpropane monoallyl ether, and related copolymerization reactions have been studied, using the flow technique developed by Dixon and Norman.[6,7] More recently, we have investigated initiation reactions of allyl[8] and methallyl compounds,[9] and copolymerization reactions of acrylonitrile with several water-soluble monomers[10] using the same method. Similar investigations by Fischer, *et al.*,[11-15] have dealt with various acrylic and methacrylic monomers initiated with several systems. Smith, *et al.*,[16] studied allyl alcohol and Griffiths, et al.,[17] studied vinyl chloride and vinyl fluoride using the flow technique. Two reviews of this field have been published.[18,19]

1,2-Substituted unsaturated compounds have not been homopolymerized but can be copolymerized with other monomers. Not much is known about the free radical mechanisms of the reactions of 1,2-substituted unsaturated compounds, which are complex reactions. Esr measurements using the flow technique have proved very useful for studies of initiation reactions of these monomers. Previously, however, only maleic acid,[7] fumaric acid,[7,12] crotonic acid,[7,12] and crotyl alcohol[17] have been investigated in separate studies. No systematic investigations have been reported.

In this paper, we describe reactions of three types of free radical initiators ($HO\cdot$, $H_2N\cdot$, and $H_3C\cdot$) with four types of crotonic compounds. The reactivity of these monomers to various radicals, the polymerizability of the monomers, the structure and the steric conformation of the transient monomer radicals formed, and the influence of the substituents on coupling constants have been derived from the esr measurements.