Side-Chain Interactions in α-Helical Poly(γ-phenacyl L-glutamate)†

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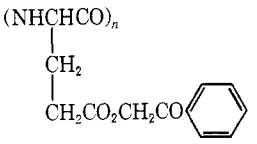
ABSTRACT: A right-handed α-helical conformation is proposed for poly(γ-phenacyl L-glutamate) in different solvents on the basis of hydrodynamic and spectroscopic measurements. In some solvents, especially dimethylformamide, strong interactions between side chains occur at low temperatures, leading to peculiar optical properties, gel formation, and energy transfer. In hexafluoroisopropyl alcohol, these interactions do not take place due to the formation of hydrogen bonds between the side chains and the solvent.

Preliminary results[1] have shown that poly(γ-phenacyl L-glutamate) (1) displays peculiar optical rotatory dispersion properties in some helicogenic solvents like dimethylformamide, pyridine, and chloroform. These are characterized by nonlinear Moffitt plots, leading to approximate positive or near zero values for the slope, depending on the temperature and the solvent. For instance, in the temperature range 0–60°, the optical rotation changes almost linearly in chloroform, but shows a sharp transition near 25° in dimethylformamide or pyridine, below which temperature gel formation is observed even at concentration as low as 2×10^{-3} g ml^{-1} for high molecular weight samples. Study of random copolymers with benzyl glutamate shows the existence of extrinsic Cotton effects. From these results it was not possible to determine the conformation of the polymer, presumably helical, in these solvents.



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In hexafluoroisopropyl alcohol (HFIP) the optical rotatory dispersion at room temperature is in agreement with a right-handed α-helical conformation, and in dichloroacetic acid the usual random-coil behavior is observed.

As this polymer showed properties which appeared related to side-chain–side-chain or side-chain–solvent interactions, we have studied its solutions in dimethylformamide, chloroform, HFIP, and trimethyl phosphate by circular dichroism, nuclear magnetic resonance, and phosphorescence. Light scattering and viscosity measurements demonstrate that in dimethylformamide at temperatures around 60° the molecules behave as rod-like rigid particles having the dimensions of the α helix. We have found the following relationship between intrinsic viscosity (ml g^{-1}) and molecular weight: $\eta = 4.4 \times 10^{-7} M^{1.64}$.

Circular Dichroism Experiments. The circular dichroism spectra obtained in different solvents are presented in Figure 1a,b. They are characterized by the existence of several positive and negative bands between 240 and 350 nm usually not seen for other esters of poly(glutamic acid), and which explain the nonlinear Moffitt plots.

At wavelengths between 200 and 270 nm (Figure 1a), we see that the behavior of 1 is not the same when dissolved in dry HFIP, chloroform, or trimethyl phosphate. In trimethyl phosphate and HFIP where it is possible to record the spectra down to 200 nm, we find the two characteristic negative bands (n–π* and π–π* parallel) corresponding to the peptide bond in a right-handed α helix. In HFIP the n–π* transition lies at 222 nm whereas in trimethyl phosphate the position of the band cannot be well specified because of the presence of a side-chain band, as explained below.

The positive band near 250 nm is much weaker in HFIP than it is in trimethyl phosphate or in chloroform, which may indicate that interactions between side chains take place to a lesser extent in the former solvent. However it seems that they are not completely inhibited as shown by the residual band at 250 nm and the shoulder at 225 nm. We ascribe the disappearance of the interactions to the existence of an hydrogen bond between the hydroxyl proton of the solvent HFIP and the carbonyl oxygen of the phenacyl group. Indeed, addition of water to a solution of 1 in HFIP increases the interactions between the side chains as shown by an increase of the CD intensities of the shoulder at 225 nm and of the band at 250 nm. This is very likely due to hydrogen bonding of HFIP to water rather than to the polypeptide.

In dimethylformamide, the circular dichroism spectrum shows positive bands centered around 328 nm and corresponding to the n–π* transition of the carbonyl group of the phenacyl residue. The intensity of these bands decreases when the temperature is raised as shown in Figure 1b. We conclude that, in dimethylformamide, the transition observed when $[\alpha]_D$ is plotted against temperature has its origin in the decrease of the interactions between the side chains when temperature is raised.[1] When dissolved in HFIP or in chloroform at room temperature, the spectra of 1 over 300 nm is similar to that obtained in dimethylformamide at high temperatures. This suggests that 1 α-helical molecules in dimethylformamide are also in the right-handed form.

Nmr. When dissolved in $CDCl_3$, the behavior of 1 observed by nuclear magnetic spectroscopy is analogous to that of poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$) when trifluoroacetic acid is added to the solution; *i.e.*, first destruction of aggregates, then helix–coil transition. However it should be noticed that the chemical shifts of the α-CH protons of both polymers are not the same even after the helix–coil transition has taken place (δ = 4.8 ppm for 1 and 4.65 for (BzlGlu)$_n$).

In dimethylformamide-d_7, at temperature below 25°, 1 forms aggregates and no nmr spectrum of the polymer can be observed. When the temperature is raised, the spectrum of the polymer appears (Figure 2a) and the chemical shifts do not change with temperature, especially that of the α-CH proton. For (BzlGlu)$_n$, no aggregation occurs in this solvent and the spectrum can be observed whatever the temperature[2] (Figure 2b). However, in dimethylformamide-d_7 as in mixtures of $CDCl_3$ and CF_3COOH the

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(1) F. Heitz and G. Spach, *C. R. Acad. Sci., Ser. C*, **269**, 679 (1969).

(2) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. W. E. Rattle, *Nature (London)*, **220**, 69 (1968).

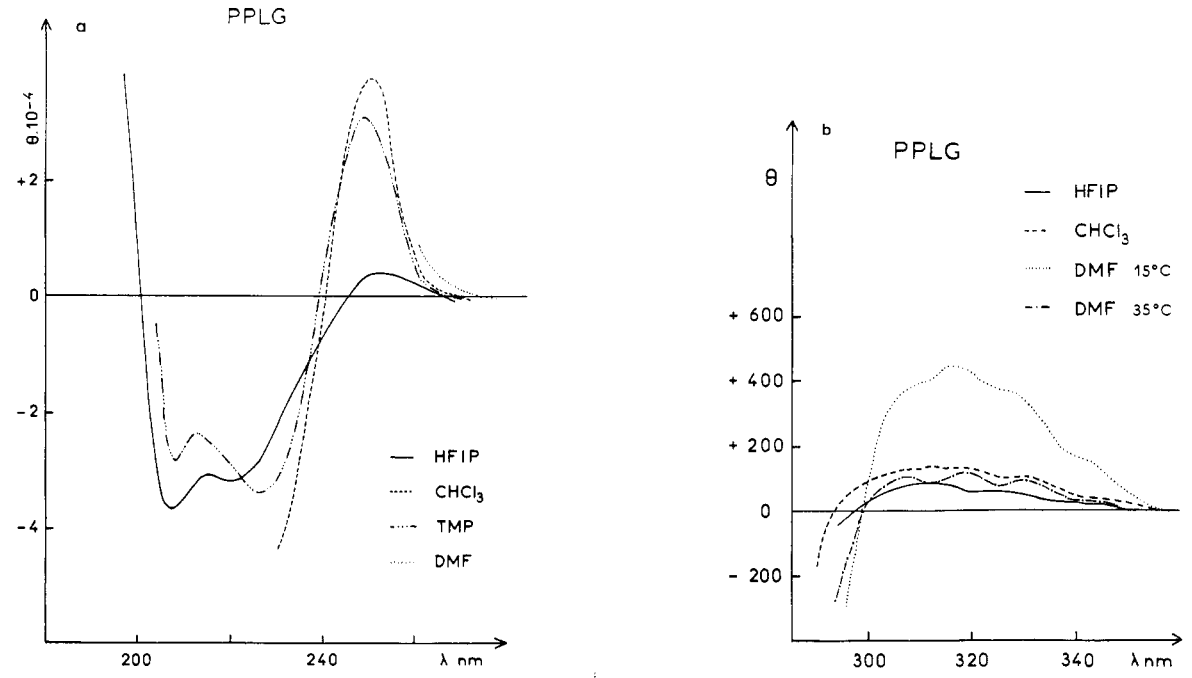

Figure 1. Circular dichroism spectra of PPLG (Compound 1) in different solvents. The ellipticity θ is given in (deg cm^2)/dmol. In HFIP, TMP (trimethylphosphate), and chloroform the spectra were recorded at room temperature, and in DMF (dimethylformamide) as indicated.

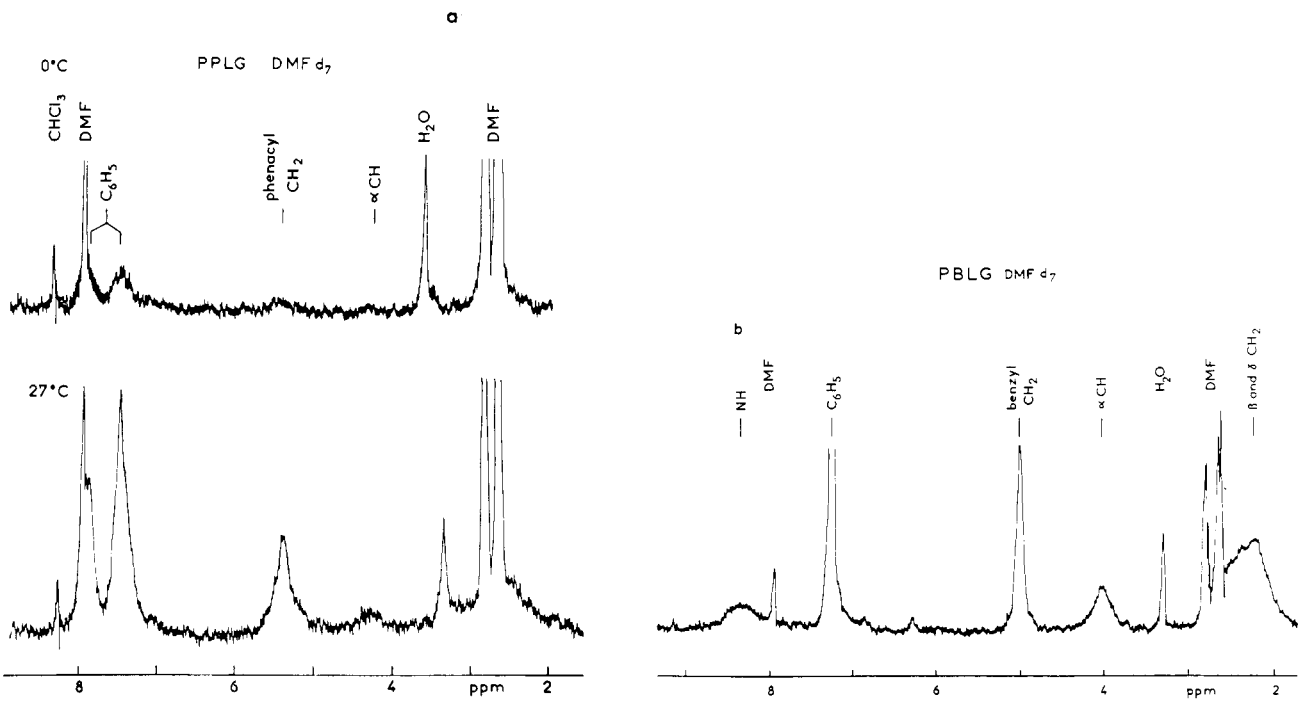

Figure 2. Observed nmr spectra of PPLG at 27 and 0° and PBLG ((BzlGlu)$_n$) in DMF-d_7 (2% w/v). Hexamethyldisilane was used as external reference, and $CHCl_3$ as an internal calibration. The α-CH peaks lie at 4.2 ppm for PPLG and at 4.0 ppm for PBLG.

α-CH protons of both polymers do not have the same chemical shift, the resonance of 1 being shifted about 0.2 ppm downfield as compared to that of (BzlGlu)$_n$. This may reflect the influence of the side chains as both polymers have the same conformation.

When increasing amounts of HFIP are added to a $CDCl_3$ solution of 1 the nmr observation of the hydroxyl proton of HFIP indicates that this solvent is gradually bonded to the polymer, probably by hydrogen bonding to the carboxyl group of the phenacyl residues as was expected from circular dichroism experiments.

Phosphorescence. Side-chain interactions have also been investigated by luminescence measurements. Phenacyl residues are not fluorescent, so that 1 and random copolymers with benzyl L-glutamate or *p*-bromophenacyl L-glutamate dissolved in dimethylformamide have only been studied by phosphorescence at 77°K under excitation at 300 nm.

In the copolymers with benzyl L-glutamate when the average number of neighboring phenacyl L-glutamate residues increases, the phosphorescence intensity decreases and the phosphorescence spectrum is shifted toward longer wavelengths indicating strong interactions between the phenacyl residues (Figure 3). These strong interactions lead to an efficient transfer of the excitation energy at the triplet level. This is demonstrated by a phosphorescence

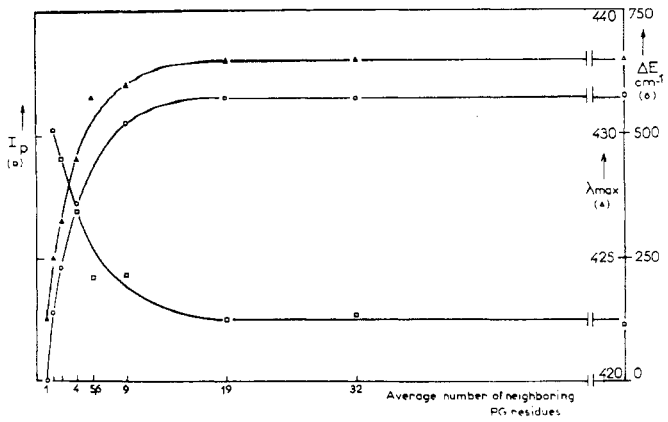

Figure 3. Phosphorescence intensity (I_p), phosphorescence maximum (λ_{max}) and change in energy of the phosphorescent triplet state relative to the monomer (ΔE) for random copolymers of benzyl and phenacyl L-glutamate. The average number of neighboring phenacyl residues was calculated from the overall composition of the polypeptide.

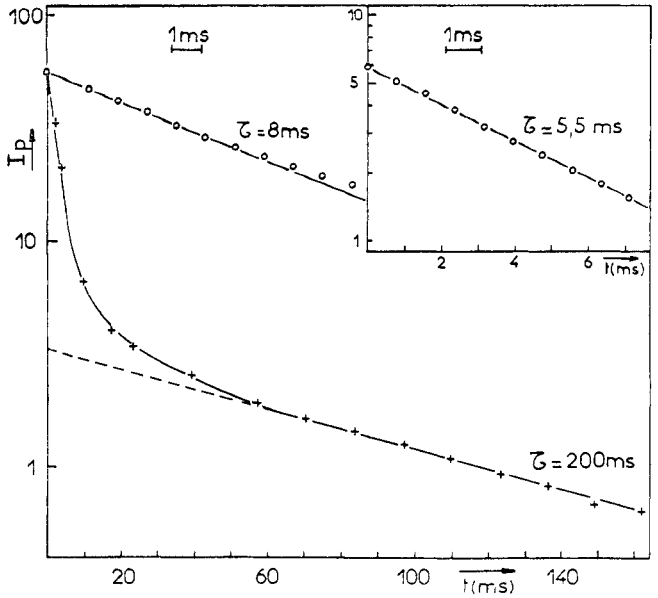

Figure 4. Phosphorescence decay of a random copolymer of *p*-bromophenacyl L-glutamate (0.5%) and phenacyl L-glutamate (99.5%). The short component is shown in the upper part on an expended scale. (The lifetime of phenacyl L-glutamate residue is about 200 msec.) Inset: phosphorescence decay of *p*-bromophenacyl bromide.

study of copolymers containing small amounts of *p*-bromophenacyl residues whose triplet state has an energy lower than that of the phenacyl residue and can thus act as an energy trap. Practically, all the excitation energy of the phenacyl residues is transferred at the triplet level to the *p*-bromophenacyl residues, even when only 0.5% of this residue is included in the polymer chain. This is more clearly seen in the decay curve where more than 90% of the decay originates from the short-lived triplet state of the *p*-bromophenacyl residue (Figure 4).

Using the same method it is possible to confirm that almost no interactions occur when 1 is dissolved in HFIP. The lifetime of the triplet state and the phosphorescence spectrum are the same for the polymer and for phenacyl acetate which was used as a monomer model.

Experimental Section

Synthesis and Characterization of the Polypeptides. Samples of 1 were prepared by polymerization in chloroform of the corresponding *N*-carboxyanhydride. The *N*-carboxyanhydride of the *p*-bromo derivative was used to prepare the random copolymers. They were purified by precipitation in methanol.

The molecular weight of the samples used for the experiments described in this paper were over 60,000 as measured by light scattering in dimethylformamide at 60° and in dichloroacetic acid. The spectroscopic behavior of different samples are identical.

Solvents. All the solvents were distilled and kept on molecular sieves except deuterated solvents which were used without further purification. Nmr measurements on solutions of 1 and $(BzlGlu)_n$ have been made at the same time using solvents coming from the same container.

Instruments. CD spectra were recorded with a Roussel-Jouan dichrograph equipped with a thermostated cell holder using 1-mm thick cells.

Nmr measurements were carried out with a Brüker HFX 90 spectrometer.

Phosphorescence spectra were recorded with a Jobin-Yvon spectrofluorimeter. The sample was contained in a quartz tube 2 mm in diameter and cooled to 77°K in liquid nitrogen contained in a quartz dewar. The position of the tube was mechanically adjusted so as to obtain maximum luminescence intensity. Phosphorescence decays were recorded with a Tektronic oscilloscope equipped with a polaroid camera.

Conclusion

From all the results reported above we can conclude that in helicogenic solvents 1 adopts a right-handed α-helical conformation. In dimethylformamide at low temperatures strong interactions occur between side chains leading to the appearance of circular dichroic bands above 300 nm, gel formation, and energy transfer. Heating above 35° leads to molecularly dispersed solutions, without however destroying all interactions. In HFIP, these interactions do not exist to a large extent. Rather the side chains are hydrogen bonded to the solvent.

It is not yet possible to give a picture of the conformation of the side chains. Only from the phosphorescence experiments can we say that the interacting phenacyl groups are distant of about 5–7 Å.