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Spin-Probe Studies of Poly(γ-benzyl L-glutamate)

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ABSTRACT: Spin-probe techniques have been employed to study the molecular motion of poly(γ-benzyl L-glutamate) (PBLG) in the solid state. Electron spin resonance (ESR) spectra of an organic nitroxide radical, 2, 2, 6, 6-tetramethyl-4-hydroxy-piperidine-1-oxyl, dissolved in PBLG have been measured as a function of temperature in the range from −180 to 130°C. Rotational correlation times were estimated on the basis of recent theories of motional effects on ESR spectra. Two motional processes were observed, below and above room temperature. Activation energies, calculated by the Arrhenius plots of the correlation times, were 3.5 and 10.5 kcal/mol for the lower and higher temperature motional processes, respectively. These motional processes have a strong correlation with the side-chain relaxations observed by other measurements, such as dielectric ones. It was found that the tumbling rate of the nitroxide radical dissolved in PBLG in the solid state is as rapid as in the liquid above room temperature, indicating that the side-chain region surrounding the α-helical backbone is essential in the liquid-like state.

KEY WORDS Poly(γ-benzyl L-glutamate) / ESR / Spin-Probe / Nitroxide Radical / Correlation Time / Side-Chain /

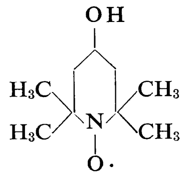
Molecular motions of α-helical poly(amino acid)s have been studied by means of nuclear magnetic resonance, dynamic mechanical, and dielectric measurements.[1-17] These studies showed that the side-chains are in a rapid motional state above an appropriate temperature, while the α-helical backbone is regarded to be essential in the solid state.

The methods of studying molecular motions of poly(amino acid)s mentioned above all involve making a direct measurement on the system. It is well established that the spin-probe technique can give us information about the motional freedom of the environment surrounding the spin-probe, by studying its motion with electron spin resonance (ESR) measurements.[18-21] The spin-probe which has been proved most suitable for such use is a nitroxide radical. The spin-probe technique can be considered to be another approach by which to study the molecular motion in poly(amino acid)s. Recent theories of motional effects on ESR spectra of the nitroxide radical show that we can obtain information about molecular motions with tumbling frequencies from 10^6 to 10^{11} Hz,[18,22-24] which are much higher than those studied by the other methods mentioned above.[1-17]

In this work, we measured ESR spectra of 2, 2, 6, 6-tetramethyl-4-hydroxypiperidine-1-oxyl dissolved in poly(γ-benzyl L-glutamate) (PBLG) in the solid state over a wide range of temperature. The rotational correlation time was estimated from ESR spectra. The results are discussed in terms of the motional freedom of the side-chain region surrounding the α-helical backbone.

EXPERIMENTAL

The PBLG used in this work was prepared by polymerizing the *N*-carboxyanhydride of γ-benzyl L-glutamate in dioxane with a trace of triethylamine as an initiator. The molecular weight determined from the intrinsic viscosity in *N*, *N*-dimethylformamide solution was 160,000. The nitroxide radical 2, 2, 6, 6-tetramethyl-4-hydroxy-piperidine-1-oxyl (nitroxide-I) was prepared using a procedure similar to that reported by Rozantzev and Krinitzkaya.[25]



Nitroxide-I

The samples used for the ESR measurements were films approximately 0.5 mm in thickness which were cast from a chloroform solution containing 1×10^{-3} mol of nitroxide-I to a polymer residue.

ESR measurements were made with a JEOL ESR spectrometer, which was operated at approximately 9.5 GHz. The temperature was changed from -180 to 130°C.

RESULTS AND DISCUSSION

The ESR spectra of nitroxide-I in PBLG are shown in Figure 1. At lower temperatures the spectra are broad, and asymmetric triplets. The spectrum slowly narrows as the temperature is raised. In the vicinity of room temperature a second, narrower component appears superimposed on the broad one. Ultimately, at temperatures above 80°C, the spectrum is composed of only the narrow component and becomes a sharp triplet.

The peak-to-peak separations of the two outermost extrema are plotted against temperature in Figure 2. The extrema separation-temperature curve shows two inflection points, at -100 and 35°C, the latter being more apparent. Figure 2 indicates that the motion of nitroxide-I in PBLG starts to occur from far below room temperature and suddenly becomes more rapid in the vicinity of room temperature.

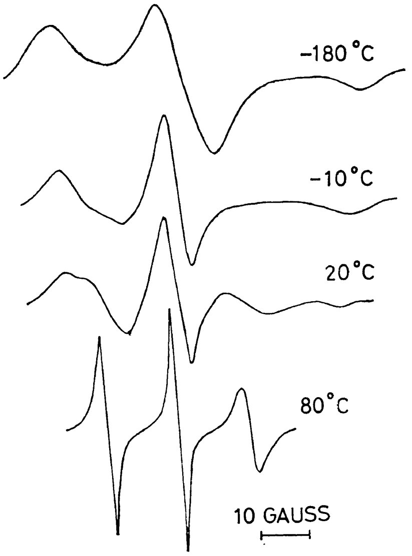


Figure 1. ESR spectra of nitroxide-I in poly(γ-benzyl L-glutamate) at the indicated temperatures.

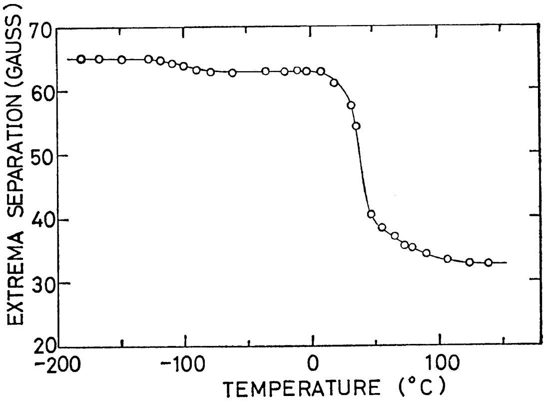


Figure 2. Plots of extrema separation *vs.* temperature in ESR spectra of nitroxide-I in poly(γ-benzyl L-glutamate). Each data point shows an averaged value from 3~5 spectra.

Freed, Bruno, and Polnaszek have simulated the spectra of the nitroxide radical for a range of slow rotational correlation times ($10^{-9}\,\text{sec} < \tau_c < 10^{-6}\,\text{sec}$) using the stochastic Liouville method.[23] Goldman, Bruno, and Freed[24] have shown that it is possible to describe the rigorous results of Freed, *et al.*,[23] by the analytical expression

$$\tau_c = a(1-S)^b \qquad (1)$$

where *a* and *b* are constants which depend upon the diffusion model adopted and the intrinsic line width of the spectrum. S is the ratio of the extrema separation to its rigid lattice value, the latter being 65.5 gauss for nitroxide-I in PBLG, as seen in Figure 2. For a Brownian rotational diffusion model, the following values of a and b were taken from Goldman, *et al.*,[24] with the intrinsic line width of 3 gauss, for use in our calculation:

$$a=5.4\times10^{-10}$$

$$b=-1.36$$

For the case of a rapidly tumbling nitroxide radical ($\tau_c<10^{-9}$ sec), the correlation time is expressed, according to Stone, Buckman, Nordio, and McConnell,[18] as

$$\tau_c=h_0[(I_0/I_+)^{1/2}+(I_0/I_-)^{1/2}-2]C \qquad (2)$$

where h_0 is the peak-to-peak line width of the central line, I_+, I_0, and I_- are the peak-to-peak amplitudes of the low-, middle-, and high-field peaks, respectively, and C is a constant which is characteristic of the hyperfine tensor anisotropy.

We attempted to estimate the correlation time by an application of these theories to our ESR results. For spectra below 20°C and above 50°C, the slow- and rapid-motional theories were applied, respectively.

The Arrhenius plot of the correlation time is shown in Figure 3, here a and b are for the slow- and rapid- motional regions, respectively, which give two straight lines. The slopes of such straight lines afford values for the rotational activation energies of the nitroxide radical. The calculated activation energies are 3.5 ± 0.5 and 10.5 ± 0.5 kcal/mol for the slow- and rapid-motional regions, respectively. These results indicate that nitroxide-I in PBLG undergoes molecular motion with two types of motional processes, the one at lower temperatures having a smaller activation energy and the other at higher temperatures having a larger activation energy.

It is known that PBLG shows two relaxations due to the motion of side-chains in the vicinity of room temperature and far below room temperature; these are denoted as β- and γ-relaxations.[12] β- and γ-relaxations have been interpreted in terms of a large scale motion involving the entire side-chain and a small scale motion such as an oscillation of the side-chain with a small amplitude, respectively; the activation energy and the strength of relaxation are considerably larger for the former than for the latter.[12,26]

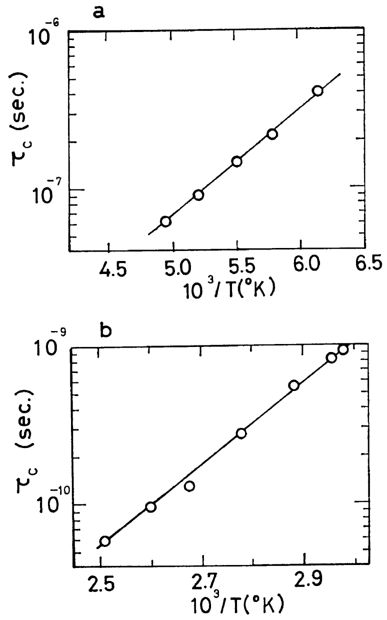


Figure 3. Arrhenius plot of the rotational correlation times τ_c as calculated by eq 1 and 2 in the text; a and b refer to the lower and higher temperature regions, respectively.

Comparing the two motional processes of nitroxide-I with their side-chain relaxations with respect to their temperature regions, we can recognize that there is a strong correlation between the motion of nitroxide-I and the side-chain: the slow-and rapid-motional processes of nitroxide-I correspond to the γ- and β-relaxations of the side-chain, respectively. It seems reasonable to consider that the environment which surrounds nitroxide-I is a side-chain region, if nitroxide-I has no specific interaction with the helical core. It can be concluded that nitroxide-I monitors the motion of the side-chain.

We have found that the relaxation time of the β-relaxation of PBLG obeys the Williams-Landel-Ferry equation (WLF-equation),[12,17] which usually describes the glass transition phenomena of amorphous polymers.[27] Recently, we have also found that PBLG shows a break point in the specific volume-temperature curve in the temperature range of β-relaxation;[28] the coefficients of thermal expansion below and above this break point are quite similar to those obtained at the glass transition region in amorphous polymers. On the basis of these facts, we have suggested that the side-chain regions are in a state quite similar to the amorphous regions in crystalline polymers.[12,17,28] From the viewpoint of molecular motion, we can state that the side-chain regions of PBLG are in a quite mobile state above room temperature, essentially in the liquid state, as in the case of amorphous polymers above their glass transition points. The ESR results strongly support such an idea, since the extrema separation of nitroxide-I in PBLG decreases rapidly to its motionally narrowed isotropic value above room temperature, indicating that the motional freedom of the environment surrounding nitroxide-I (side-chain region) is as high as in the low-molecular-weight liquid. This is confirmed by our additional observation that the correlation time of nitroxide-I in chloroform is $\sim 10^{-10}$ sec, which corresponds to the value in PBLG above 80°C.

CONCLUSION

ESR measurements on nitroxide-I dissolved in PBLG revealed that the environment surrounding this radical is in the mobile state far below room temperature. The estimation of correlation times showed that two motional processes which have different tumbling rates and activation energies appear, below and above room temperature. It was found that there is a strong correlation between the motions of nitroxide-I and the side-chains; nitroxide-I is thought to monitor the motion of the side-chains which form its environment. The value of the correlation time above room temperature shows that the side-chain regions are regarded essencially to be in the liquid-like state. This supports our earlier considerations about side-chain motions in PBLG. Thus, the spin-probe study as a function of temperature has proved to be a useful method in helping to understand the motional freedom of side-chain regions around α-helical poly(amino acid)s backbones.

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