Poly(O-acyl-hydroxy-L-proline) IV. Studies of Molecular Motions in Poly(O-acetyl, butyryl, hexanoyl, dodecanoyl, and benzoyl-hydroxy-L-proline) Films by the Spin Probe Technique

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ABSTRACT: The molecular motion in poly(*O*-acetyl (Ac), butyryl (But), hexanoyl (Cap), dodecanoyl (Lau), and benzoyl (Bz)-hydroxy-L-proline (Hyp)) films was investigated by ESR measurements with 2,2,6,6-tetramethyl-4-hydroxypiperidine-1-oxyl (TEMPO) as the spin probe. As the length of acyl side chains increased, the temperature at which the separation of the outer extrema of ESR spectra reached 50 gausses (T_{50}) decreased. The rotational correlation times (τ_c) obtained from the ESR spectra at -70° C for series of polymers were nearly the same. The values of τ_c at 130°C decreased with an increase in the acyl side chain length until the number of carbon atoms in the acyl chains reached 6. The activation energies of TEMPO in the polymers were less than 3 kcal mol⁻¹ at low temperatures. At high temperatures, the activation energies of poly(*O*-Ac-Hyp) and poly(*O*-But-Hyp), poly(*O*-Cap-Hyp), and poly(*O*-Lau-Hyp) were enhanced and particularly that of poly(*O*-Cap-Hyp), exceeding 10 kcal mol⁻¹.

KEY WORDS ESR / Poly(O-acyl-Hyp)s / Spin Probe / Side Chain Length / TEMPO / Activation Energy /

In previous papers, the preparation of poly-(*O*-acyl-hydroxy-L-proline) having acetyl (Ac), butyryl(But), hexanoyl(Cap), dodecanoyl(Lau) and benzoyl (Bz) groups in the side chains, its solubility, surface free energy and thromboresistance were reported.^{1,2}

The solubility of poly(*O*-acyl-Hyp)s in ordinary organic solvents was improved by increasing the chain length of acyl groups attached to the hydroxy group of Hyp. Poly(*O*-Lau-Hyp) having the longest side chain in the series of polymers showed the characteristics of both lipids and polypeptides.

The wettability of the polymer films depended on the type of acyl groups attached to Hyp. Values for the dispersion and nondispersion components of the surface free energy were closely related to the length of the side chain groups. The values of both components varied with an increase in the length of the acyl groups.

The clotting time of blood on the film surfaces of the poly(*O*-acyl-Hyp)s also depended on the type of acyl groups. The blood clotting time increased with an increase in the length of the side acyl chains. Variations in both wettability and blood clotting time were oberved in the derivatives with hexanoyl or shorter acyl groups, but no further variation was observed in the dodecanoyl derivative. The effects of acyl chain length on surface properties such as wettability and blood compatibility were most pronounced in the hexanoyl derivative. These phenomena appear to arise from differences in molecular motion according to the type of acyl side chain.

Furthermore, these polymers were shown to aggregate in chloroform solution by the measurement of carbon-13 NMR.

The spin probe technique, using mixed paramagnetic probes in ESR spectroscopy has been employed to obtain information on the degree of motional freedom.³⁻⁶ Recently, Tsutsumi *et al.*⁷ and Kaifu *et al.*,⁸ reported the use of this technique in investigating the motional freedom of side chain regions on polymer backbones.

In this study, the spin probe technique was used to study the motion of poly(*O*-acyl-Hyp)s. The results were correlated with the chain length of side chain acyl groups.

EXPERIMENTAL

Materials

Poly(*O*-acyl-Hyp)s were prepared by the polymerization of *N*-carboxy anhydrides (NCA) of *O*-acyl-Hyp.¹ In Table I, the structural formulas of the polymers are illustrated.

2,2,6,6-Tetramethyl-4-hydroxypiperidine was purchased from Aldrich Chemical Co. and oxidized to 2,2,6,6-tetramethyl-4-hydroxypiperidine-1-oxyl (TEMPO) with hydrogen peroxide.⁹

Sample Preparation for ESR Measurements

Poly(*O*-Ac, But, Cap, Lau, and Bz-Hyp) were dissolved in chloroform solutions of TEMPO, each containing 2×10^{-3} equiv. mol of TEMPO per amino acid residue of Hyp derivatives. These solutions were placed in 5 mm quartz sample tubes and dried under nitrogen for 1 h and then *in vacuo* at 50°C for 2 days. The atmosphere of the tubes were replaced with nitrogen and sealed.

A JEOL ESR spectrometer Model JES-FE1X equipped with a variable temperature controller was used for ESR measurements over a temperature range from -130 to 160° C.

RESULTS AND DISCUSSION

Figure 1 shows the typical ESR spectra of poly(O-Lau-Hyp) obtained, respectively at -121, -10, 21, and 132.5° C. At low temperatures, the spectra were broad and asymmetric triplets, and the separation of the outer extrema (W) was about 70 gausses. This W value indicates that the mobility of the spin probe was restricted due to the rigid polymer matrix. W became narrower as the temperature was raised, and a new narrow component appeared superimposed on the original broad component. At high temperatures, the broad component disappeared and the narrow one turned



Table I. Structural formulae of poly(O-acyl-Hyp)s and acyl groups



Figure 1. ESR spectra of spin probed poly(*O*-Lau-Hyp) film at various temperatures.

into a sharp triplet as in the spectrum in the liquid state. Relationship between the separation of the two outer extreme (W) and temperature for respective poly(O-acyl-Hyp) derivatives are shown in Figure 2. In each sample, the narrowing due to the increase in the mobility of the spin probe was observed. It was of interest that the temperature regions of the narrowing for the samples were different from one another and appeared to depend on the length of the aliphatic side chains.

To clarify the above results, the temperature at which the W value reached 50 gausses (T_{50}) was plotted against the length of the acyl side chains, as shown in Figure 3. The length of the side chain acyl groups are represented by the number of carbon atoms contained. The T_{50} value for poly(O-Ac-Hyp) having the shortest aliphatic side chains (number of carbon atoms = 2) was 63.5° C. When the number of carbon atoms increased to 6, the T_{50} values abruptly changed to 22.0°C. The T_{50} value for the poly(O-acyl-Hyp) derivative having 12 carbons in the side chains was 4.5°C. At room temperature, the film of poly(O-Ac-Hyp) was rigid, while that of poly(O-Lau-Hyp) was flexible. The flexibility of the poly(O-acyl-Hyp) films was remarkably influenced by the length of the acyl chains even though the polymers had the same backbones. Furthermore, these effects became pronounced when the number of carbon atoms was less than 6 and then became small with an increase in the number of carbon atoms up to 12. The T_{50} value for poly(O-Bz-Hyp) having aromatic side chains was higher than that of poly(O-Ac-Hyp). This suggests that the rigidity of films was due to the stacking of the aromatic side chains.

The rotational correlation time (τ_c) for TEMPO in the polymer films was estimated as follows. In the fast motional region at high temperatures, where the spectra were sharp triplets, τ_c was calculated using an equation based on the theory of Kivelson,¹⁰

$$\tau_{\rm c} = C \left[(h_0/h_1)^{1/2} + (h_1/h_{-1})^{1/2} - 2 \right] W_{\rm o}$$

where h_1 , h_0 , h_{-1} are the heights of three peaks of spectra from a low magnetic field. W_0 is the width of the central peak in gausses. A constant C was calculated from the anisotropy of highperfine constants for TEMPO. In the slow motional region at low temperatures where the spectra were broad and asymmetric triplets, τ_c was calculated using the method developed by Freed *et al.*,^{11,12}

$$\tau_{\rm c} = a(1-S)$$
$$S = A_{\rm z}'/A_{\rm z}$$

where A_z' is the measured separation of the outer extrema and A_z , the rigid limit value for frozen TEMPO. Selection for the *a* and *b* parameter values had to be made according to

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Figure 2. Relationship between the separation of the two outer extreme (W) and temperature for poly(O-acyl-Hyp) films.



Figure 3. Relationship between the side chain length of poly(O-acyl-Hyp)s and T_{50} .

the diffusion model. In this study, by using the Brownian diffusion model,¹² a and b have been estimated to be 5.4×10^{-10} and -1.36, respectively. The τ_c values calculated by the two methods mentioned above showed reason-

able variation over the measured temperature range.

The τ_c values at -70 and 130° C are plotted against the length of the acyl side chains in Figure 4. At -70° C, the τ_c values were nearly

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Figure 4. Relationship between the side chain length of poly(O-acyl-Hyp)s and T_c at -70 and 130°C.



Figure 5a and 5b. Arrhenius plots of the T_c for poly(O-acyl-Hyp)s in the lower (5a) and higher (5b) temperature regions: (a) poly(O-Ac-Hyp); (b) poly(O-But-Hyp); (c) poly(O-Cap-Hyp); (d) poly(O-Lau-Hyp); (e) poly(O-Bz-Hyp).

the same in spite of the differences in side chain lengths. At 130°C, the τ_c value decreased with an increase in the number of carbon atoms up to 6. No further decrease was observed when the number increased to 12. The τ_c value of poly(*O*-Bz-Hyp) was nearly the same as that of poly(*O*-Ac-Hyp) and both polymers were fairly rigid in the higher temperature region.

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Figure 6. Relationship between the side chain length of poly(*O*-acyl-Hyp)s and activation energy in the lower (\bigcirc) and higher (\bigcirc) temperature regions.

Figures 5a and b show the Arrhenius plots of τ_c in the series of polymers in the lower and higher temperature regions, respectively. The Arrhenius plots of all the polymers were linear in both temperature regions. With these plots, the activation energies of TEMPO in these temperature regions were estimated.

As can be seen from Figure 6, the activation energies in the lower temperature region of all polymers were all less than 3 kcal mol⁻¹. In the higher temperature region, the activation energies of poly(O-Ac-Hyp) and poly(O-Bz-Hyp) differed scarcely from those in the lower temperature region. The activation energies of poly(O-But-Hyp), poly(O-Cap-Hyp) and poly(O-Lau-Hyp) were, however, enhanced and the value for poly(O-Cap-Hyp) was well beyond 10 kcal mol⁻¹. The difference in the activation energy among the polymers in the higher temperature region was considered to arise from the differences in the lengths of the side chains.

It has been reported that the molecular motion of the spin probe is a function of various factors such as oscillation, rotation and translation.⁶ The activation energy of small scale motion consisting of oscillation and rotation was less than 10 kcal mol⁻¹ and that of large scale motion including translational diffusion greater than 10 kcal mol⁻¹.¹³ This explains the variation in the activation energy of the poly(*O*-acyl-Hyp)s. In the lower

temperature region, the motional process of all the polymers were oscillation and slower rotation since the spin probe was trapped in the polymer matrix. In poly(O-Ac-Hyp) and poly(O-Bz-Hyp) films, this motional process did not change in the higher temperature region and consequently these polymers had only one motional process consisting of oscillation and slower rotation in the polymer matrix in the measured temperature range. The degree of motional freedom in the side chain region was apparently not sufficiently enough to bring about translation of the TEMPO in the higher temperature region. In regard to the long side chain polymers, an increase in the temperature resulted in a phase transition of the side chain region, eventually inducing an increase in the activation energy. These phase transition temperatures, depending on the length of the side chain acyl groups, were ca. 50, 30 and 0° C for poly(O-But-Hyp), poly(O-Cap-Hyp) and poly(O-Lau-Hyp), respectively. The activation energy of the spin probe in poly(O-Cap-Hyp) was over 10 kcal mol⁻¹, indicating that TEMPO translated and jumped throughout the polymer matrix above the phase transition temperature. Poly-(O-But-Hyp) and poly(O-Lau-Hyp) showed activation energies smaller than poly(O-Cap-Hyp). The poly(O-But-Hyp) and poly(O-Lau-Hyp) values were about 8.5 kcal mol⁻¹ suggesting their motional process to have faster rotation and slow translation. In the case of poly(O-But-Hyp), this is considered due to relatively short side chain length. It is of interest that poly(O-Lau-Hyp) having the longest side chains in the series showed such a low activation energy. The interaction between the long aliphatic chains seemed to prevent the free translation of TEMPO in the side chain region.

Based on the above results, the molecular motions in the films of poly(O-acyl-Hyp)s were influenced by differences in the side chains, this influence being strongest when the number of carbon atoms was 6. This tendency showed

remarkably good correlation with their wettability and blood compatibility.² The effect of an increase in side chain length on wettability was greatest when the number of carbon atoms was 6. The blood clotting time of poly(*O*-acyl-Hyp) was improved with the increase in the side chain length and it reached maximum when the number of carbon atoms was 6. This is evidence that molecular motion affects such surface properties.

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