NOTES

Poly(*O*-acyl-hydroxy-L-proline) III. Conformation of Poly(*O*-acyl-hydroxy-L-proline)

Tadayoshi KAWASAKI, Takashi KOMAI, and Toshifumi HIRAOKI

*Department of Polymer Science, Faculty of Science,
Hokkaido University, Nishi 8-chome, Kita 10-jo,
Kita-ku, Sapporo 060, Japan*

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Poly(α -amino acid)s are promising as biomedical materials in view of the fact that modified protein components of natural tissues or organs are finding wide use in reconstructive surgery and in general exhibit good biocompatibility.^{1,2} The surface of poly(α -amino acid) containing benzyl-L-glutamate or leucine has been found to have a comparatively good blood compatibility and the film of the polymer containing acylated L-lysine appears very promising as a substitute material for bladder tissue.^{3,4}

When considering materials for blood contacting surfaces, especially in the case of glutaraldehyde treated umbilical cord vessels (GA-UCV) implanted in human beings as artificial blood vessels in reconstructive surgery,^{5,6} it should be noted that the surface of GA-UCV is comprised of collagen, lipid and polysaccharide, and is relatively hydrophobic.⁷⁻¹¹

In spite of successful utilization of GA-UCV in reconstructive surgery, the characteristics and functional properties of these conjugated materials still remain to be clarified, mainly because of their complicated chemical structures. An examination of model compound simulating the GA-UCV surface chemically should provide some understanding of this. Accordingly, we synthesized and polymerized *O*-acyl-hydroxy-L-proline (*O*-acyl-Hyp) with

various lengths of acyl groups in side chain hydroxy groups¹² and investigated its practical use as a material for a blood contacting surface.¹³ Its wetting characteristics, solubility properties and blood clotting profiles were found to depend strongly on the lengths of the side chain acyl groups. It was also found that the surface of the polymer having a longer aliphatic acyl group had comparatively good antithrombogenic properties.¹³ The surface characteristics of this material may be due not only to its wettability but other factors such as inter or intramolecular interactions, a secondary or tertiary structure, and side chain conformation. For determination of these factors, which may possibly lead to simulation of the luminal surfaces of GA-UCV, a structural study of poly(*O*-acyl-Hyp) was carried out.

This paper presents the results of conformational studies using IR, ¹H NMR and ¹³C NMR and discuss them on the basis of surface characteristics previously established.¹³

EXPERIMENTAL

Poly(*O*-acyl-Hyp)s were prepared by the method reported previously.¹² The molecular weight of poly(*O*-acetyl(Ac)-Hyp), poly(*O*-butyryl(But)-Hyp), poly(*O*-hexanoyl(Cap)-Hyp), poly(*O*-dodecanoyl(Lau)-Hyp), and poly(*O*-benzoyl(Bz)-Hyp) were 22000, 2000,

6300, 4500, and 7000, respectively.¹⁴ The IR absorption spectra were taken by the KBr pellet method using a JASCO-A-302 IR spectrophotometer. ¹³C and ¹H NMR spectra were taken with a JEOL FX-100 (25 MHz) and FX-400 (400 MHz) spectrometers in CDCl₃ at room temperature, respectively.

RESULTS AND DISCUSSION

The IR spectra of poly(*O*-Ac-Hyp), poly(*O*-But-Hyp), poly(*O*-Cap-Hyp), poly(*O*-Lau-Hyp) and poly(*O*-Bz-Hyp) are shown in Figures 1a and 1b. The spectra in Figure 1a

were obtained with samples cast from acetic acid and those in Figure 1b with those from chloroform.

Several absorption bands were found to be sensitive to the main chain and side chain conformations of the polypeptides and are shown in Table I.

The spectra of poly(*O*-acyl-Hyp)s having shorter acyl groups such as poly(*O*-Ac-Hyp) and poly(*O*-But-Hyp) cast from acetic acid were similar to those of poly(Pro), (1650, 1440, 1340 and 620 cm⁻¹ etc.). Poly(Pro) is known to take the Form II conformation corresponding to all *trans* imide bonds in acetic acid.¹⁵⁻¹⁷

Figure 1a. IR spectra of poly(*O*-acyl-Hyp)s cast from acetic acid. (A), (B), (C), and (D) are corresponding to poly(*O*-Ac-Hyp), poly(*O*-But-Hyp), poly(*O*-Cap-Hyp), and poly(*O*-Lau-Hyp).

Figure 1b. IR spectra of poly(*O*-acyl-Hyp)s cast from chloroform. (A), (B), (C), (D), and (E) are corresponding poly(*O*-Ac-Hyp), poly(*O*-But-Hyp), poly(*O*-Cap-Hyp), poly(*O*-Lau-Hyp), and poly(*O*-Bz-Hyp).

Table I. IR specific absorptions of poly(*O*-acyl-Hyp)scm⁻¹

Polymer	Cast from acetic acid					Cast from chloroform				
	Pro	<i>O</i> -Ac-Hyp	<i>O</i> -But-Hyp	<i>O</i> -Cap-Hyp	<i>O</i> -Lau-Hyp	<i>O</i> -Ac-Hyp	<i>O</i> -But-Hyp	<i>O</i> -Cap-Hyp	<i>O</i> -Lau-Hyp	<i>O</i> -Bz-Hyp
(Mode)										
Amide A	3450	3450	3450	3450	3500	3450	3450	3450	3450	3450
Amide I	1640	1650	1650	1660	1660	1650	1650	1650	1650	1650
							1630 _s			1600
										1590
δC-H	1440	1440	1440	1440	1460		1460	1460	1470	1490
					1440	1440	1430	1440	1460	1450
							1420	1420	1440	1430 _s
Amide III		1380	1380	1380	1380	1375	1380 _s	1380 _s	1380 _s	
	1350		1350	1350	1350		1350	1355	1355	1340
	1340	1340					1340	1320	1310	1320
		1320	1310	1320	1320		1310			
Acyl C-O	1260	1260	1260	1250	1260			1270		1270
						1250	1250	1250	1250 _s	
								1230	1230	1230
CH ₂ and Ph			750	740	725	750	750	735		755
	710	710	710			710	710	710	720	710
Amide V	680	635				665				670
	620	610	580			630	630	640		620
						610		610		
Back bone	550	560	550	560	560	560	560	560	560	560
			520	530	530	520	520	525	525	525

Therefore, the secondary structure of poly(*O*-Ac-Hyp) and poly(*O*-But-Hyp) cast from acetic acid are considered to be ordered and similar to that of poly(Pro) Form II. The spectra of poly(*O*-Cap-Hyp) and poly(*O*-Lau-Hyp) exhibited some more bands in addition to those characteristic of the poly(Pro) Form II conformation (1460 and 1440 as amide III, 1338—1310, 1230—1220, and 700—550 cm⁻¹). The conformations of these polypeptides thus seem to be a mixture of poly(Pro) Form II and other conformations. Since poly(*O*-Bz-Hyp) did not dissolve in acetic acid, no film from this solvent could be obtained.

Figure 1b shows the spectra of poly(*O*-acyl-Hyp)s cast from chloroform to be complex and have absorption bands assignable to the Form II and the other conformations.

Thus the conformation of poly(*O*-Ac-Hyp) and poly(*O*-But-Hyp) apparently changes from Form II in acetic acid to another confor-

mation as the poly(Pro) Form I conformation in chloroform, depending on the casting solvent. The conformations of the polymers having longer acyl groups were not affected by the solvent. They were found to be complicated conformation which was comprised of Form I, Form II, and others and could not be defined their composition only by the data of IR.

The influence of side group length on the polymer conformation is consistent with the previously established relation between the length of acyl group and wetting characteristics on the polymer film surface.¹³

The ¹³C NMR spectra of poly(*O*-acyl-Hyp)s in chloroform are shown in Figure 2. Methyl and methylene signals can be seen in a high field region and carbonyl signals in a low field region. The pyrrolidine ring carbon signals of *O*-acyl-Hyp are indicated by C^α, C^β, C^γ, and C^δ, and the carbonyl signals of the side chain acyl group by C_x'.

Figure 2. ^{13}C NMR spectra of poly(*O*-acyl-Hyp)s in CDCl_3 at 25 MHz. Concentration 50 mg ml^{-1} . Chemical shifts in ppm from tetramethylsilane (TMS).

Figure 2 shows poly(*O*-Ac-Hyp), poly(*O*-But-Hyp), poly(*O*-Lau-Hyp) and poly(*O*-Bz-Hyp) to have broad pyrrolidine signals and relatively sharp methyl and methylene signals, while poly(*O*-Cap-Hyp), an unusually sharp spectrum, although all these spectra were taken under the same conditions. These broad spectra can be ascribed to an aggregation of

the polymers in chloroform. The mobility of these polymer may be lessened by aggregation in chloroform and the aggregates can not be dissociated by the addition of TFA.

As shown in Figure 3, broadening also occurred in the ^1H NMR spectra of poly(*O*-Ac-Hyp) having the shortest acyl chain and poly(*O*-Lau-Hyp) having the longest chain.

Figure 3. ^1H NMR spectra of poly(*O*-acyl-Hyp)s in CDCl_3 at 400 MHz. Concentration 50 mg ml^{-1} . Chemical shifts in ppm from TMS.

In regard to the aggregation of poly(*O*-acyl-Hyp)s, the following two mechanisms may be considered: hydrogen bonding between the carbonyl group of the side chain ester group and the hydrogen of the α -carbon in the peptide backbone, and a hydrophobic interaction in the side chain methylene region. We conclude that aggregation of poly(*O*-acyl-Hyp)s having shorter acyl groups may be ascribed to the intermolecular hydrogen bonding and that of poly(*O*-acyl-Hyp) having longer acyl groups to the hydrophobic interaction in the side chain region.

In a previous paper,¹³ it was shown that the dispersion and nondispersion components of surface tension on the surfaces of poly(*O*-acyl-Hyp) films varied with the length of acyl groups but their differences became very small for polymers with side chains longer than hexanoyl. A similar trend was found in the relationship between side chain length and the blood clotting time on these polymer films. The excellent thromboresistant properties and larger dispersion contribution of the surface tension on the film surfaces of poly(*O*-acyl-Hyp)s having longer acyl side chains may arise mainly from the aggregated structure of this polymer in the side chain region. The aggregated methylene group may cover up the peptide backbone and control the surface characteristics of the polymer. From the conformational characteristics of poly(*O*-acyl-Hyp)s mentioned above and the surface characteristics of their films, they may be concluded to be suitable model compounds for lipid conjugated collagen.

Research is now being carried out on the molecular motion of these polymers by the spin probe ESR technique to elucidate the relation between chain length and aggregation in solid state.¹⁸

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