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Helical structure of sugar-carrying polystyrene in aqueous solution by circular dichroism

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Abstract

Radical polymerization of *N*-*p*-vinylbenzyl-D-lactonamide (VLA) gave an optically active helical polymer. The stereoregularity of poly(*N*-*p*-vinylbenzyl-D-lactonamide) (PVLA) measured by ^{13}C NMR spectroscopy showed a well-resolved sharp-line width, which was assigned to the phenyl C-1 carbon of the isotactic polystyrene (PS). The helical structure of PVLA shown by circular dichroism (CD) indicated that the aromatic groups were chirally supramolecular-packed giving optically active disaccharide units in the side chain covalently linked via an amide linkage with PS, the original PS not being optically active. The intensity of CD for PVLA (a) decreased with increasing temperature due to the change in the conformation of the phenyl group or to the breakdown of intermolecular hydrogen bonding of amide groups and (b) increased in a mixture of water and alcohol due to the increased hydrophobicity. The CD intensity for maltose-carrying PS (PVMA) was slightly higher than that of PVLA CD due to the more hydrophobic property of PVMA than PVLA.
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Keywords: Helical structures; Sugar; poly(*N*-p-vinylbenzyl-D-lactonamide); Isotactic polystyrene; Circular dichroism

1. Introduction

Recently, much attention has been given to the design of sugar-carrying polymers for studies of carbohydrates in cell functions such as embryo genesis, tissue formation, infection, pathology, and cell recognition.[1] Specific cells are known to have carbohydrate-recognizing receptors; for example, hepatocytes, alveolar macrophages, and L 1210, mouse leukemia cell, recognize galactose/*N*-acetyl-β-galactosamine,[2] α-mannose[3] and α-fucose,[4] respectively. Sugar-carrying polymers exhibit functions different from those of low molecular weight carbohydrates, for example carbohydrate moieties introduced to the vinyl polymer, polypeptide, and polysaccharides.[5]

Most naturally occurring polymers such as proteins, nucleic acids, and polysaccharides are optically active, and helical structures are often observed in these biopolymers. In addition, they show characteristic functionalities including molecular recognition and catalytic activity in relation to the helices.[6] Polymers would be optically active due to their helical structure such as polyisocyanides,[7] polychloral,[8] polyisocyanates,[9] poly(2,3-quinoxaline)s,[10] poly(triaryl methyl methacrylate,[11] and polyacetylenes,[12] which were prepared by the polymerization of monomers having a chiral substituent.[13] It has been reported that the nature of glycosidic linkages and the substituents in the sugar rings determine the optical characteristics[7] and interaction properties of polysaccharides.[8] Thus, optically active synthetic polymers having sugar moieties in the side chain, similar to those of living systems, could contribute to studies of the biological functions of the carbohydrate chains.

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In this study, we report on the helical structures of poly(*N*-p-vinylbenzyl-D-lactonamide) (PVLA) and poly(*N*-p-vinylbenzyl-D-maltonamide) (PVMA) in aqueous solution using circular dichroism (CD). PVLA, particularly, has been found to be a superior surface-coating material for culturing hepatocytes,[9,10] which adhere to PVLA through highly specific interaction of the cell surface asialoglycoprotein receptors and the galactose moiety in PVLA.[11] In addition, PVLA was found to be represented by a molecular bottle brush, composed of a large helix of polystyrene (PS) backbone, and lactose brushes through small-angle X-ray scattering and molecular modelling.[12] Furthermore, PVLA formed a polymeric micelle in water due to the presence of hydrophobic PS backbone and hydrophilic sugar moieties.[13] CD is a powerful tool for determining the absolute configuration of the sugar residues and is sensitive to the chirality of a carbohydrate because chromophores of the molecule sense the asymmetry of their surroundings.[14] The relationship between CD and the secondary structure of the polypeptide is also well recognized.

Thus, the difference in the cellular recognition ability of PVLA from that of PVMA on the plastic plate[10] is expected to be closely related to the conformation of this glycoconjugate polymer.

2. Experimental

2.1. Materials

N-p-Vinylbenzyl-D-lactonamide (VLA) was synthesized and polymerized using methods reported previously.[15]. Briefly, 1 g (2.1 mmol) of VLA monomer and 20 mg (2 wt.%) of azobisisobutyronitrile (AIBN) as an initiator were dissolved in 3 mL of DMSO in a glass tube. The solution was degassed for 20 min under reduced pressure, and the glass was then sealed. Polymerization was carried out for 24 h at 60 °C. After the reaction, the solution was poured into ethanol to obtain a precipitate, which was then dissolved in a small amount of water, the solution was dialysed in distilled water (yield 83 wt.%). The average molecular weight of PVLA was measured by gel permeation chromatography. The polymerization of VMA was performed using a method similar to that of PVLA. The chemical structures of PVLA (a) and PVMA (b) are shown in Fig. 1. Atactic and isotactic polystyrenes were supplied by Aldrich Chem. Co. (Milwaukee, WI).

2.2. Measurement of CD spectra

CD spectra were measured with a JASCO Model J-700 recording CD spectropolarimeter (Japan Spectroscopic Co. Ltd., Tokyo, Japan).

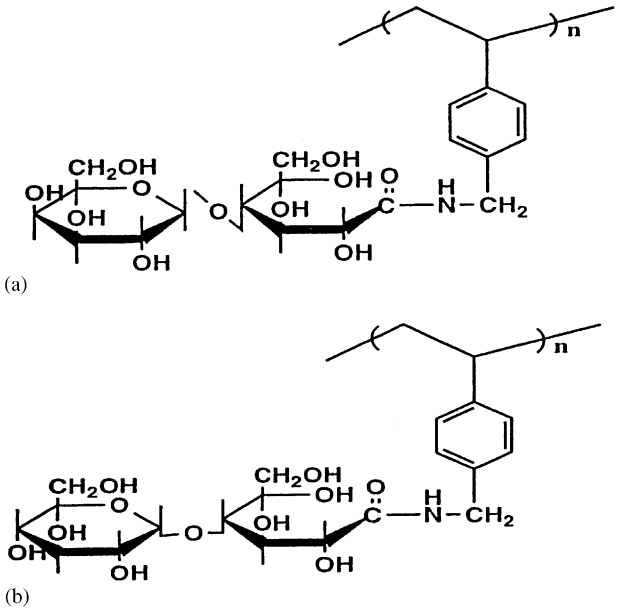

Fig. 1. Chemical structures of PVLA (a) and PVMA (b).

3. Results and discussion

Fig. 2 shows the CD spectra of VLA monomer and PVLA in aqueous solution. PVLA gave an optically active polymer of higher ordered structure with patterns similar to those of synthetic polypeptides such as poly(D-glutamic acid) and poly(D-lysine). The intensity of the CD for PVLA increased with an increase in the degree of polymerization of PVLA. The stereoregularity of PVLA was investigated by ^{13}C NMR spectroscopy to deduce whether the optical activity arose from the

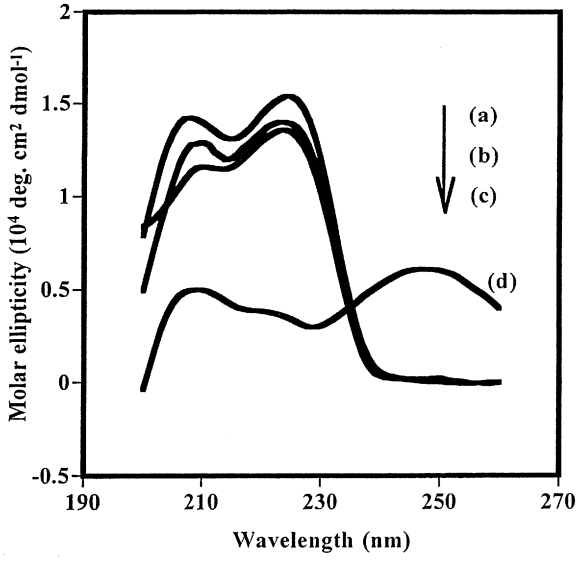

Fig. 2. CD spectra of VLA monomer and PVLA in aqueous solution at 25 °C against degree of polymerization: PVLA (DP:106) (a); PVLA (DP:76) (b); PVLA (DP:29) (c); and VLA (d).

aromatic groups being chirally supramolecular-packed due to the chiral side groups of the sugars. The resonance centered at around 147 ppm with a well-resolved sharp-line width was assigned to the phenyl C-1 carbon of the isotactic PS, although PVLA had a chemical shift of phenyl C-1 carbon different from that of isotactic PS, due to the incorporation of the sugar group to PS backbone, and had a low resolution due to the micelle formation even at 80 °C (Fig. 3). On the other hand, the commercial amorphous PS showed broad signals at around 145 ppm, indicative of the atactic PS. The chiral VLA monomer did not show any CD bands at 208 and 222 nm except for a small band at 250 nm. PVLA exhibited bands similar to those of the synthetic polypeptide at 208 and 222 nm assigned to π–π^* and n–π^*,[16] respectively, from the amide linkage between PS backbone and sugar unit in the side chain. The energy difference of twist sense per monomer unit may be small; however, due to the rare occurrence of helix reversals, it is amplified by the high cooperativity of long helical segments as similarly observed in the optically active polyisocyanate.[17] Therefore, the optical activeness of PVLA could be due to an excess of monomer units in the preferred helical sense.[12] Another possibility is that the helical structure of PVLA can be induced by the polymerization of a styrene derivative bearing a bulky and chiral substituent such as a disaccharide unit. Yashima and coworkers have reported that the helical conformation of polyacetylene derivatives is closely related to the steric effects of bulky side groups.[18]

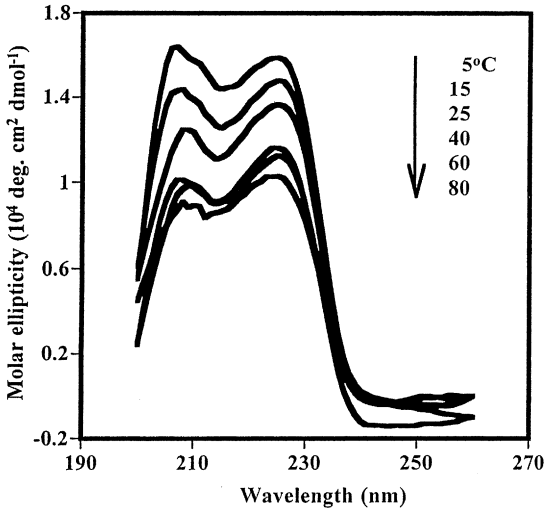

Fig. 4. CD spectra of PVLA in aqueous solution against temperature.

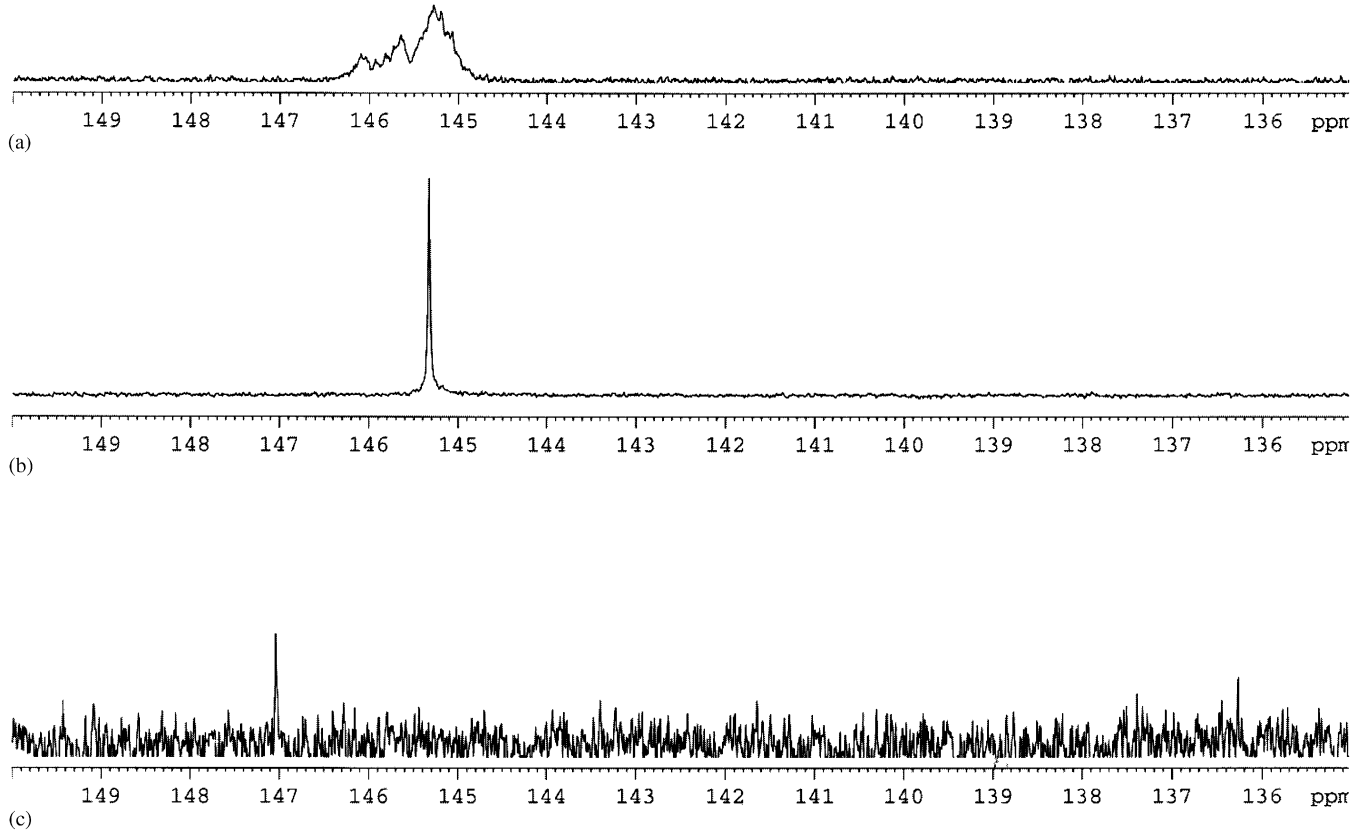

Fig. 3. ^{13}C NMR spectra of atactic PS (a); isotactic PS (b); and PVLA (c). a and b were measured using benzene at 60 °C, whereas c was measured using DMSO at 80 °C.

Fig. 4 shows the CD spectra of PVLA in aqueous solution varying into temperature. The results reveal that intensity of CD for PVLA decreased with an increase of temperature. The molar ellipticity at 208 nm against temperature is plotted in Fig. 5. The molar ellipticity linearly decreased with increasing temperature. About 38% of the helicity of PVLA at 5 °C decreased at 80 °C. This decrease in the stability of the PVLA helical structure was probably due to a change in the conformation of the phenyl group or to the breakdown of intermolecular hydrogen bonding of amide groups with increasing temperature. The molar ellipticity of PVLA with temperature change was thus reversible without showing hysteresis.

Fig. 6 shows CD spectra of PVLA in mixtures of water and various alcohols (7/3 in v/v). The intensity of CD in PVLA was higher in the mixture of water and alcohol than in water only. In addition, the intensity of molar ellipticity at 208 nm in PVLA increased with an increase of alkyl chain length in the alcohol. Therefore, the increased CD in PVLA could be attributed to the increased hydrophobicity with an increase of alkyl chain length in the alcohol.

Fig. 7 shows CD spectra of PVLA and PVMA in aqueous solution at 25 °C. CD bands of PVMA and PVLA were similar in shape and positions; however, PVMA showed slightly increased positive ellipticity near 208 nm than PVLA, a suggestion of more induced CD of PVMA, which may be due to more hydrophobic property than PVLA as revealed by the contact angles of water on PVMA and PVLA surfaces of 38.3 and 36.2°, respectively. Therefore, it can be said that the hepatocyte specificity to PVLA is closely related to the hydroxyl group position of galactose[19] instead of the helical structure of the PVLA.

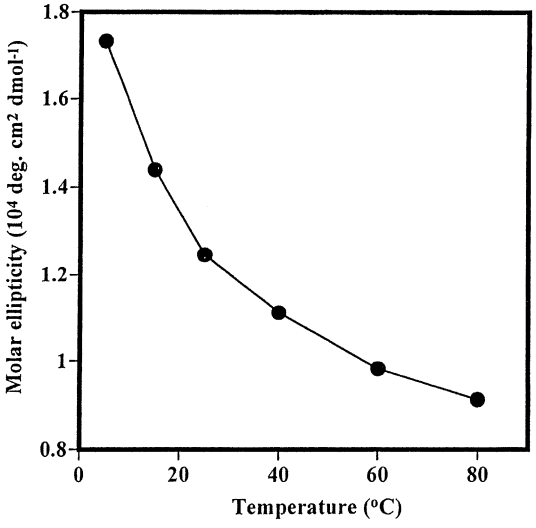

Fig. 5. Plots of molar ellipticity of PVLA at 208 nm against temperature.

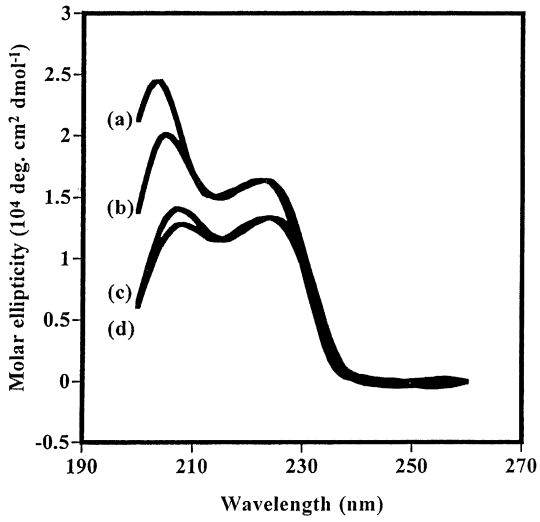

Fig. 6. CD spectra of PVLA in mixtures of water and various alcohols: H_2O+n-propanol (a); H_2O+ethanol (b); H_2O+methanol (c); and H_2O (d).

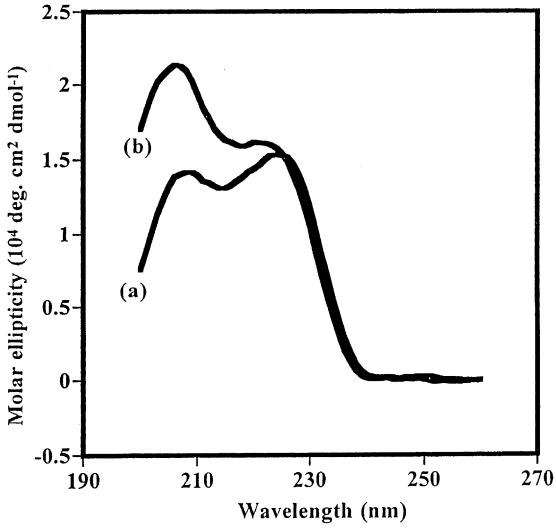

Fig. 7. CD spectra of PVLA (a) and PVMA (b) in aqueous solution at 25°C.

Acknowledgements

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