Transactions of the Materials Research Society of Japan 32[3] 769-771 (2007)

Synthesis and Biological Properties of Glycopolymer-Polylactide Conjugate

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The conjugates of polyester and glycopolymer were synthesized using poly(lactide) macromer. The macromer of poly(lactide) was synthesized by the ring opening polymerization with hydroxyethyl methacrylate, and the macromer was co-polymerized with α-glucosyloxyethyl methacrylate (GEMA) with radical initiator. The physical properties of poly(GEMA)-poly(lactide) was dependent on the monomer ratio in the polymer. The conjugate showed the specific affinities to the α-glucose recognition lectin.

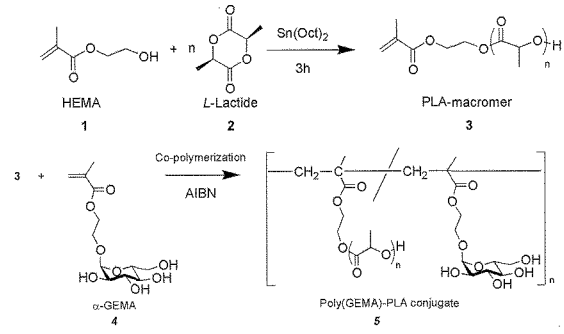
Key words: Glycopolymer, Polylactide, Lectin, Glyco-cluster effect

1. INTRODUCTION

Carbohydrate on cell surfaces serves as significant biological signals and play important roles in numerous intercellular recognition processes.[1] The interactions of isolated carbohydrates ligands with carbohydrate-binding proteins (lectins) are low in affinity and broad in specificity. The carbohydrate signals in biological system are often amplified by the multivalency of carbohydrates or the "glyco-cluster effect".[2] Synthetic glyco-clusters are also reported to amplify the carbohydrate signals similarly to the natural carbohydrate ligands. In particular, synthetic glycopolymers have attracted a great attention as synthetic glyco-clusters, because of the biomedical applications such as tissue engineering, drug delivery systems, and treatment of infection disease.[3]

Taking the biomedical applications into consideration, the biocompatibility and biodegradability are important factor for polymers.[4] For that reason, biodegradable polymers of poly(lactic acid) (PLA) or poly(glycolic acid) (PGA) has attractive features: the degradation products are natural metabolites.[5] Therefore, the conjugation of glycopolymer with biodegradable polyester is idealistic for biodemedical applications. However, the glycopolymer-polyester conjugate is difficult to be synthesized due to the degradable properties of polyester and the protective groups of hydroxyl group. Though some groups reported the glycopolymer-polyetser conjugate with elaborate synthesis, the general strategy for the conjugation has not been reported.[6,7]

On the other hand, the use of biodegradable macromers of PLA or PGA readily enables the conjugate materials, which are utilized for the synthesis of biodegradable gels, microspheres and biomaterials.[8] The conjugate materials of the macromers and the glycopolymer have the ideal properties for biomedical use due to the biocompatibility, biorecognition ability and bioabsorbability.



Scheme 1 Synthesis of glycopolymer-polyester conjugate with PLA macromer.

In this paper, we aimed to develop a biodegradable glycopolymer for biomedical applications. We synthesized PLA macromer with methacrylate backbone, which can be copolymerized with glucosyloxyethyl methacrylate (GEMA).[9] The PLA macromer gave the brush-like structure. The physical properties of poly(GEMA)-PLA conjugate was varied with the monomer ratio. The subsequent polymer was composed of the hydrophobic PLA segment and the hydrophilic sugar segment, and the polymer had the self-assembling properties to form hydrogel, porous structure and spheres.

2. EXPERIMENTAL

Materials

The following reagents were used as received. Dioxane, dimethylsulfoxide (DMSO), ethanol, tetrahydrofuran (THF) (Kishida, Osaka), concanavalin A (Con A), *Ricinus communis* agglutinin120 (RCA_{120}) (Seikagaku Co. Ltd., Tokyo) and Stannous octoate ($Sn(Oct)_2$, 95 %) (Aldrich, St. Luois). *L*-lactic acid (LA) (Kishida) was recrystalized three times from ethyl acetate under nitrogen. 2-Hydroxyethylmethacrylate (HEMA) (Kishida) was dried over 3Å molecular sieve and

Table 1 Co-polymerization of GEMA and PLA macromer with varying feeding ratio and solvent.[a]

Run No.	Feed ratio (mol %)		Polymer component ratio (mol %)[c]		Solvent	Yield (%)	$M_W(10^{-5})$	M_w/M_n
	GEMA	PLA	GEMA	PLA				
1	95	5	92	8	Dioxane/DMSO	63	1.3	2.0
2	95	5	90	10	Dioxane/DMSO	61	1.5	3.3
3	95	5	93	7	Dioxane/Ethanol	76	1.6	2.2
4[b]	95	5	n.d.	n.d.	Dioxane	74	n.d.	n.d.
5	89	11	88	12	Dioxane/DMSO	48	1.3	2.4
6[b]	89	11	n.d.	n.d.	Dioxane	58	n.d.	n.d.
7	89	11	92	8	Dioxane	59	1.7	2.5
8[b]	68	32	n.d.	n.d.	Dioxane/Ethanol	58	n.d.	n.d.
9[b]	68	32	n.d.	n.d.	THF/Etanol	49	n.d.	n.d.
10	100	0	100	0	H_2O	88	3.2	3.9

[a] The polymerization was initiated with 1 mol% 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPD), [b]The sample was insoluble, [c]The component ratio was determined by ^{1}H-NMR.

distilled under vacuum before use. α-Glucosyloxyethyl methacrylate (GEMA) (Nippon Fine Chemicals, Osaka) was purified by recrystalization from 2-propanol.

Characterizations

^{1}H (500 MHz) and ^{13}C(125 MHz) NMR spectra were recorded on a Varian Inova 500 equipped with a Sun workstation. FTIR spectra were recorded in the form of a KBr disk using a JASCO FTIR-230. Size exclusion chromatography (SEC) measurements were conducted with a JASCO 800 high-performance liquid chromatography on Shodex B804+B805 columns with PBS or Shodex KF602 column with THF. Molecular weight was estimated with pullulan standards. Scanning electron microscopy (SEM) was performed with a JSM-6330F (JEOL Ltd., Japan). Fluorescence spectroscopy was carried out on a JASCO FP-6500 (JASCO, Tokyo, Japan) and on a KingFisher 96 deep well (Thermo Fisher Sci., Walthman, MA, U. S. A.). Dynamic Light Scattering (DLS) measurements were performed on a DLS-6600HK (Otsuka Electronics, Osaka Japan) equipped with a 5 mW He-Ne laser. The diameters of the polymer micelles were calculated by the cumulant analysis.

Syntheses of glycopolymer-PLA conjugate.

Methacrylated PLA macromer was synthesized according to a literature by the ring opening polymerization of lactide.[8] *L*-Lactide (1.0g, 6.9 mmol) was dried under the vacuum for 3 h in shlenk tube, and $Sn(Oct)_2$ (6 μmol) and HEMA (9.0 mg, 6.9 μmol) were added. The reaction mixture was heated up to 130 °C for 3h, and the polymer was precipitated in methanol. The obtained macromer was analyzed by ^{1}H-NMR and SEC (Mw=990, Mw/Mn=1.2) (Scheme 1). The conjugates were obtained by radical copolymerization of PLA macromer and α-GEMA with changing feed ratio (Table 2). The obtained copolymer was precipitated in acetone. The conjugates with higher PLA content were difficult to dissolve in solvents (THF, $CHCl_3$, DMSO, DMF, methanol, ethanol and toluene). The polymers of run No. 4, 6, 8 and 9 were totally insoluble due to the self-assembling properties of PLA segment.

Table 2 The diameter of the polymers estimated by DLS

Run No. in Table 1	Polymer component ratio (mol %)[a]		Diameter (nm)
	GEMA	PLA	
3	93	7	135.8
1	92	8	179.3
7	92	8	63.3
2	90	10	45.1
5	88	12	92.8
10	100	---	35.3

3. RESULTS and DISCUSSION

Physical Properties of glycopolymer

The physical properties of poly(GEMA)-PLA were dependant on the polymer component and the solvent in the polymerization. The polymers with lower PLA content were able to be dispersed in the aqueous solution (run No. 1, 2, 3 and 5 in Table 1). In addition, the solubility of the polymer was depending on the solvent used in the polymerization. The polymers of run No. 4, 6, 8 and 9 formed gel and didn't solve in the soluvents.

The polymer was dispersed in the aqueous solution as micelles, and the size of the polymer micelles were analyzed by dynamic light scattering (DLS) (Table 2). The diameters of poly(GEMA)- PLA were 40-180 nm,

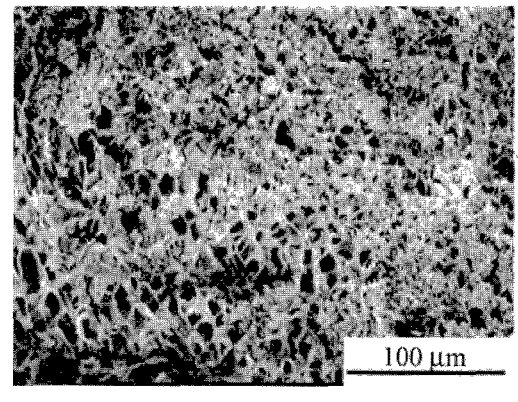


Figure 1 SEM micrograph of glycopolymer-PLA (run No. 2).

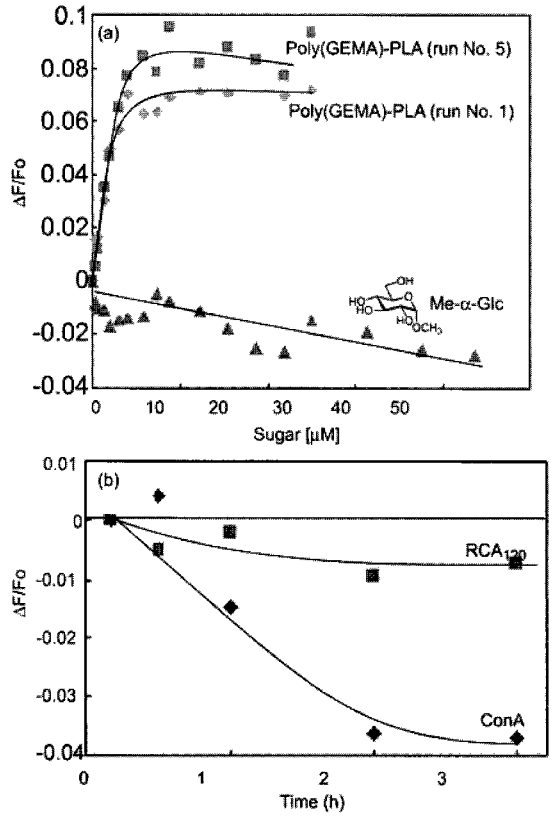


Figure 2 Protein recognition abilities of glycopolymer-PLA conjugates. (a) Fluorescence intensity changes of FITC-labeled ConA with varying sugar concentration of methyl α-glucose and glycopolymer-PLA conjugates (run No. 1 and 5). (b) Fluorescence intensity change of FITC-labeled Con A and RCA_{120} by addition of glycopolymer-PLA conjugate (run No. 9) with changing the incubation time.

which was much larger than that of poly(GEMA). The results suggests the self-assembling properties of poly(GEMA)- PLA due to the amphiphilic properties. Interestingly, the polymer with high sugar ratio showed the larger diameter.

The polymer with higher poly(lactide) had a tendency to form gel due to the self-assembling property and the hydrophobicity. The gel of the polymer swelled in the aqueous solution and the weights were increased to 1200-1400 %. The polymer gels were observed by SEM micrographs, and were revealed to form porous structure with about 10 μm diameter (Figure 1).

Lectin recognition abilities

Poly(GEMA)-PLA should have the biorecognition abilities due to α-Glc unit. The biological abilities were evaluated by the lectin recognition with Con A. The polymer with lower PLA content was evaluated by the fluorescence spectroscopy with FITC-labeled Con A (α-Glc recognition lectin) (Figure 2).[10] The fluorescence was changed with the addition of the polymers. The relative change in the fluorescent intensity (ΔF/Fo) at 518 nm was plotted against the carbohydrate concentration. The association constants were estimated by the Scatchard plot. Poly(GEMA)-PLA (Run No. 1 in Table 1) induced the strong fluorescence decrease and the affinity constants of the polymer (8.6×10^5 (M^{-1})) were much higher than those of monomeric methyl-α-glucose (2.4×10^4 (M^{-1})) due to the glyco-cluster effect.

The polymer with higher PLA (run No.9) content was evaluated in terms of lectin recognition ability (Figure 3). The FITC-labeled lectin was added to the gel of polymer and the fluorescence change was measured to compare the lectin affinities between Con A and RCA_{120}. In the first 30 min, the fluorescence changes were not noticeable and almost the same between Con A and RCA_{120}. However, after 1 h, the fluorescence of Con A was gradually decreased, while that of RCA_{120} seldom changed. The results suggests that the gel of poly(GEMA)-PLA had the specific affinities to α-Glc recognition protein. Poly(GEMA)-PLA showed the strong and specific affinities to lectin, regardless of polymer morphology and structures, which can be utilized for medicinal applications.

4. CONCLUSION

The conjugate of glycopolymer and polyester was synthesized using macromer. Poly(GEMA)-PLA with various sugar and poly(lactide) content was prepared, and the physical properties were changed with the monomer ratio. The polymer with low PLA content was dispersed in aqueous solution to form polymer micelle, while that with high PLA content formed hydrogel. The polymers showed strong and specific lectin recognition abilities.

5. Acknowledgements

We acknowledge Dr. Nobuyoshi Nomura (Nagoya University) for poly(lacide) synthesis. This work was supported by the Industrial Technology Research Grant Program in 2003 from New Energy and Industrial Technology Development Organization (NEDO), JST seeds research, JAIST Foundation, Inamori Foundation, Iketani Foundation and Shisedai Foundation.

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(Received January 17, 2007;Accepted May 22, 2007)