Preparation of Novel Polymer Assemblies, "Lactosome", Composed of Poly(L-lactic acid) and Poly(sarcosine)

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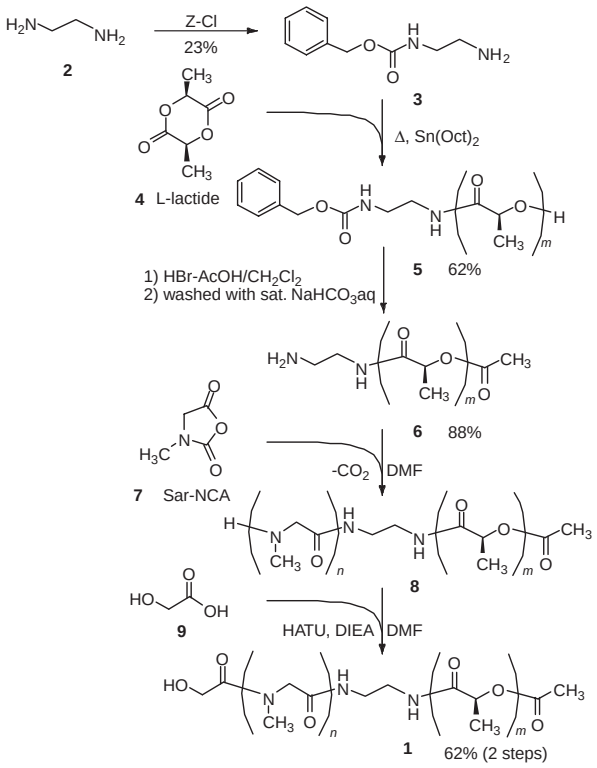
Novel amphiphilic block polymers composed of poly(L-lactic acid) (PLLA) and poly(sarcosine) (PS) were synthesized by successive polymerizations of lactide and sarcosine *N*-carboxy anhydride (NCA). The degree of polymerization of each block was controlled by the monomer/initiator ratio in feed. The block polymers were successfully dispersed in buffer with taking various morphologies of micelle, lamellar, and vesicle depending on the hydrophobic/hydrophilic balance of the copolymers. The size of the molecular assemblies was in the range from 20 to 200 nm, which may be available for drug delivery system (DDS) as nanocarrier.

Recently, DDS using small particles has attracted much attention in terms of controlled release and targetting.[1] Amphiphilic block polymers are known to form various types of molecular assemblies such as spherical micelles, rod micelles, and vesicles.[2] The shape and size of the molecular assemblies are generally controllable by choosing a proper hydrophobic/hydrophilic balance of the block polymer. The advantages of using polymers for the molecular assemblies are the physical stability compared with liposome made of lipids,[3] and their facile size adjustment in the range from a few ten nm to a hundred nm. In the present study, biodegradable polymers of poly(L-lactic acid) (PLLA) and polypeptide were chosen for the components of copolymers, which may be applicable to DDS as nano-scaled molecular particles.

A vesicular molecular self-assembly, named peptosome, has been reported with using amphiphilic polypeptides.[4,5] The key design of the peptosome is a helical peptide segment as the hydrophobic part. The regular packing of the helices should contribute to the physical stability of the thin membrane. On the basis of the concept of peptosome, another helical and biodegradable polymer of PLLA was used here for preparation of molecular assemblies. PLLA is well known to form a 3_{10} helical structure.[6] Core–shell-type micelle was already prepared from di- and tri-block polymers composed of PLLA and poly(ethylene glycol) (PEG).[7] Instead of using PEG, which is pointed out to cause allergic reactions,[8] poly(sarcosine) (PS) was used here for the hydrophilic segment. Sarcosine is a naturally occurring amino acid and highly water-soluble. Copolymers composed of PLLA and PS are thus considered to be biodegradable and biocompatible.

The synthetic route for the amphiphilic block polymers is illustrated in Scheme 1. One amino group of 1,2-ethylenediamine (**2**) was protected by benzyloxycarbonyl (Cbz) group and the other amino group was utilized as initiator for preparation of the PLLA block. A mixture of the initiator **3**, L-lactide (**4**), and 0.2 wt % tin(IV) octanoate as catalyst was heated up to 120 °C for 12 h. The reaction mixture was washed with ice-cooled alcohol to remove low molecular weight products. The polydispersity index (PDI) M_w/M_n values of PLLA were 1.05–1.14. After the deprotection of the Cbz group, the amino terminal was used as initiator for polymerization of sarcosine *N*-carboxy anhydride (NCA) (**7**). The degree of polymerization of PS was controlled by choosing a proper ratio of NCA and initiator in the feed. Finally, the N terminal of PS was endocapped with glycolic acid (**9**). All synthetic products were identified by ^{1}H NMR.[9]



Scheme 1. Route for the synthesis of PLLA–PS block polymer **1**. The yield is shown in the case of Entry 4.

Aqueous dispersion of **1** was prepared by the film rehydration technique. A solution of polymer **1** (6.0 mg) in chloroform was evaporated to dryness till a thin film was formed on the internal surface of a test tube. To the test tube, 10 mM Tris-HCl buffer (2.0 mL) was added and treated with a bath-type sonicator. The dispersion was subjected to dynamic light-scattering (DLS) analysis,[10,11] transmission electron microscope (TEM) observation, and circular dichroism (CD) measurement.[12]

The composition of the synthetic amphiphilic block polymers **1** and the hydrodynamic diameter of corresponding molecular assemblies by DLS measurements are summarized in Table 1.

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Table 1. Composition of PLLA–PS block polymers **1** and the hydrodynamic diameter of their molecular assemblies

Entry	Composition of **1**[a]		Yield from	Diameter	Polydispersity
	PLLA	PS	**3**/%	/nm[b]	index
1	30	50	35	precipitation	—
2	30	91	41	24	0.28
3	30	143	38	30	0.14
4	25	150	34	52, 204*	0.05
5	16	45	6	160, 785*	0.34
6	16	22	28	217, 1092*	0.59

[a]The composition of **1** was determined by ^{1}H NMR spectrometry. [b]Asterisks on diameter represent the predominant molecular assembly.

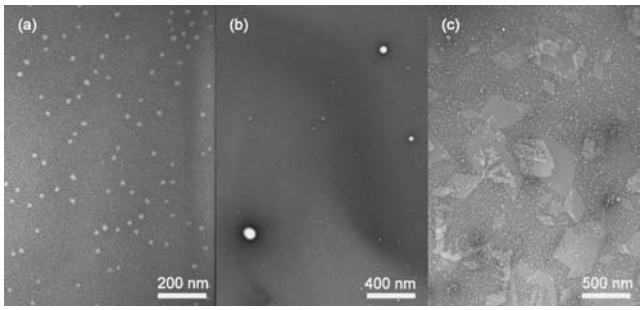

Figure 1. Transmission electron micrographs of PLLA–PS molecular assemblies negatively stained with uranyl acetate: (a) PLLA30–PS143 (Entry 3), scale bar: 200 nm.; (b) PLLA25–PS150 (Entry 4), scale bar: 400 nm.; (c) PLLA16–PS45 (Entry 5), scale bar: 500 nm.[13]

The block polymer PLLA30–PS50 (Entry 1) could not be dispersed in buffer to generate white precipitates because of the shortage of the hydrophilic property. With the elongation of the PS block, PLLA30–PS91 polymer (Entry 2), a stable dispersion was obtained but the size distribution was broad (diversity index of 0.28). When the length of the hydrophilic block was long enough, the size distribution of the molecular assemblies became uniform to be ca. 30 nm (Entry 3). Figure 1a shows the TEM image of the dispersion prepared from PLLA30–PS143. The average diameter of the molecular assembly analyzed from the TEM image was consistent with that evaluated from the DLS measurement. On the basis of its size and the spherical shape, the molecular assembly should be core-shell-type micelle.

PLLA25–PS150 block polymer (Entry 4) yielded two types of molecular assemblies with diameters of 52 and 204 nm. The latter assembly was predominant and considered to be vesicles from its size, TEM image (Figure 1b) and the encapsulation experiment described in the following section. It is surprising that a small decrease of the PLLA block in the length caused a large difference in morphology of the molecular assembly. Hydrophobicity, helical content, and rigidity of the PLLA block should influence the hydration process of the block polymers, which may be different between these two block polymers to yield different morphologies. However, the factor determining the morphology is not straightforward. For example, the molar residue ellipticity per the lactate unit of PLLA25–PS150 was stronger by ca. 10% than that of PLLA30–PS143. The helical structure of the latter may be distorted owing to the large curvature of the micelles. Interestingly, block polymers with a short PLLA (Entries 5 and 6) formed large lamellar assemblies as shown by the large diameter from DLS measurements (Table 1) and the TEM image (Figure 1c). The CD measurements showed positive cotton effects at 210 nm, indicating helical conformation of the PLLA block.[12] However, the molar residue ellipticity per the lactate unit was decreased to half with decreasing the length of the PLLA block from 25 to 16 (degree of polymerization), suggesting the PLLA block taking disordered structure in PLLA16–PS45 but helical structure in PLLA30–PS143. The cross section area of the PLLA block in PLLA16–PS45 may be therefore larger than that of the diameter of the cylindrical shape in PLLA30–PS143, which is considered as the reason for the change in morphology between the two copolymers.

To get information on morphology of PLLA25–PS150 in buffer, the encapsulation experiment with using a water soluble pseudo-drug was carried out. FITC-dextran 4000 was used as a pseudo-drug.[12] In the elution profile of the molecular assembly of PLLA25–PS150 through a size exclusion chromatography (SEC) showed the coelution of the molecular assembly and FITC-dextran. Further, the fraction of the molecular assembly did not loose the encapsulated FITC-dextran upon rechromatography on the SEC, showing no leakage or release of FITC dextran from the molecular assembly. On the contrary, FITC-dextran was scarcely detected in the fraction of the molecular assembly of PLLA30–PS143 (micelle) on the SEC. Taken together, the molecular assembly of PLLA25–PS150 was mainly vesicle, which is therefore named "lactosome".

In conclusion, a small change in the PLLA length of the block polymers influence the morphology of the molecular assembly. As a result, various morphologies, micelle, lamellar, and vesicle, can be prepared from PLLA–PS block polymers.

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12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
13 Magnified images of Figure 1 are shown in the Supporting Information.

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