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Control of *in vivo* blood clearance time of polymeric micelle by stereochemistry of amphiphilic polydepsipeptides

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ABSTRACT

Polymeric micelle, "Lactosome," is composed of amphiphilic polydepsipeptide with a hydrophobic block of helical poly(L-lactic acid) (PLLA) and a hydrophilic block of poly(sarcosine). Lactosome was labeled by incorporation of poly(lactic acid) having a near-infrared fluorescence (NIRF) chromophore, and studied on blood clearance and tumor imaging. *In vivo* blood clearance time of Lactosome was prolonged with incorporation of poly(D-lactic acid) (PDLA), but decreased with poly(D,L-lactic acid) (PDLLA). NIRF imaging with applying these Lactosomes to tumor-bearing mice revealed that the tumor/background intensity ratio increased with incorporation of PDLLA. Stereochemistry in the hydrophobic core of self-assemblies is thus an important factor for determining physical stability in the blood stream and consequently contrast in imaging.

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1. Introduction

Core-shell type polymeric micelle, "Lactosome," is composed of amphiphilic polydepsipeptide with a hydrophobic block of helical poly(L-lactic acid) (PLLA) and a hydrophilic block of poly(sarcosine) [1]. Sarcosine, *N*-methylated glycine, is a kind of natural amino acids found in muscles and other body tissues, and it is metabolized endogenously by sarcosine dehydrogenase [2]. PLLA is a well-known biocompatible and biodegradable polymer [3,4]. Lactosome is therefore considered to be biocompatible materials, which is confirmed by no acute toxicity of Lactosome by a single-dose toxicity test with using mice.

Lactosome has been examined on application for imaging probe of solid tumors. A characteristic point of Lactosome about *in vivo* disposition is a significantly low level of accumulation in liver resulting in a long *in vivo* blood clearance time [5,6]. Lactosome therefore accumulates selectively in solid tumors due to the enhanced permeability and retention (EPR) effect [7,8]. In the view point of imaging probe, however, nanoparticles with the long *in vivo* blood clearance time have a drawback of remaining signal at background for a long period, leading to insufficient contrast in images of tissues with large blood volume [9–11]. In the present study, we try to improve the signal ratio at tumor against background by controlling the *in vivo* blood clearance time of Lactosome.

Near-infrared fluorescence (NIRF) image is taken by using Lactosome containing NIRF-labeled poly(L-lactic acid) (PLLA) of 1.5 mol% [5]. It is known that stability of polymeric micelles *in vivo* is influenced by compounds entrapped in the hydrophobic core of the micelle [12–14]. The hydrophobic core of Lactosome polymeric micelles is constructed by dense molecular packing of helical PLLA blocks, where NIRF-labeled PLLA at the low content can be easily incorporated without changing the diameter of polymeric micelles. PLLA takes a left-handed helical structure and is known to form stereocomplex with right-handed helical poly(D-lactic acid) (PDLA) providing materials with high mechanical strength [15–17]. Physical stability of Lactosome in the blood stream is therefore considered to increase with the addition of PDLA through stereocomplex formation. On the contrary, Lactosome may be destabilized by the incorporation of poly(D,L-lactic acid) (PDLLA) into the hydrophobic core through disturbing the helix molecular packing.

We recently revealed that enantiopure amphiphilic blockpeptides having a hydrophobic block with a helix structure self-assembled into nanotubes [18]. On the other hand, a mixture of amphiphilic blockpeptides having right-handed and left-handed helices as the hydrophobic block yielded vesicles due to stereocomplex formation [19–21]. Stereochemistry in the hydrophobic core of self-assemblies is therefore an important factor for determining properties of self-assemblies. Lactosome with mixing PLLA, PDLA, and PDLLA is examined

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here on stability in the blood stream and consequently on tumor imaging.

2. Material and methods

2.1. Lactosome preparation

Synthetic method for ICG-labeled poly(lactic acid) is summarized in Supplementary data. Lactosome dispersion in saline was prepared as described in the previous paper [1,5].

2.2. Measurements

Circular dichroism (CD) spectroscopic data were taken using a JASCO J600 spectropolarimeter at room temperature with an optical cell of 0.1 cm optical path length. Malvern Zetasizer-Nano was used for dynamic light scattering (DLS) measurements. All measurements were performed at 25 °C.

2.3. In vivo imaging using tumor-bearing mice

Tumor-bearing BALB/c nu/nu nude mouse was prepared by transplantation of human pancreatic cell line of SUIT-2/pEF-Luc at right femoral region as previously reported.

To the tumor-bearing mice, a saline solution of ICG-labeled Lactosome (Lactosome containing PLLA, PDLA, or PDLLA with indocyanine green (ICG)) (1.0 mg/mL, 50 μL) was intravenously administrated from the tail vein. Concentration of ICG-labeled Lactosome was normalized by UV absorption intensity at 768 nm. *In vivo* NIRF image was taken by Clairvivo OPT (Shimadzu Corp.) with using a filter set of excitation of 785 nm and emission of 845 nm. During the imaging process, the mouse was held on the stage under anesthetized condition with 2.5% of isoflurane gas in air flow (1.5 L/min).

3. Results and discussion

3.1. Polymer synthesis

ICG-labeled poly(lactic acid) was chemically synthesized as illustrated in Scheme S1. Initiating from mono-*N*-Cbz-ethylenediamine, poly(lactic acid) was synthesized by the melt polymerization method with using three types of the lactide monomers, L,L-lactide, D,D-lactide, and D,L-lactide, to yield PLLA, PDLA, and PDLLA, respectively. After the polymerization, PLLA and PDLA were purified by precipitation in methanol. PDLLA was purified by size exclusion chromatography (SEC) of Sephadex LH-20 column on methanol. The Cbz group was deprotected by 25% HBr-AcOH, and to the amino group, ICG was covalently attached via amide linkage.

The degree of polymerization of the poly(lactic acid)s was set at ca. 30, which is a critical value for enantiopure poly(lactic acid)s to take a helical structure. PLLA and PDLA used here are noted with the determined residue numbers as PLLA-28 and PDLA-31, respectively, which were confirmed to take a left-handed and a right-handed helical structure, respectively, by CD measurements (Fig. S1). On the other hand, PDLLA (PDLLA-30) took a disordered structure based on no signal at CD spectrum.

3.2. Molecular assembly containing PLLA, PDLA, and PDLLA

The amphiphilic polydepsipeptide composed of a hydrophobic 30mer block of helical PLLA and a hydrophilic 70mer block of poly(sarcosine) self-assembled into core-shell type polymeric micelles with 35 nm diameter. The effect of PLLA-28 addition to the amphiphilic polydepsipeptide on the self-assembly morphology was examined by DLS measurements. With increase of the mixing PLLA-28 amount, the hydrodynamic diameters of Lactosome remained within 20% increase in the range of less than 25 mol% addition, and became significantly larger beyond 50 mol% addition (Fig. 1). PDLLA-30 also showed the same effect on the hydrodynamic diameters as PLLA-28 (Fig. 1). On the other hand, PDLA-31 induced drastic increase of the hydrodynamic diameters, which became over 100 nm with the addition of 25 mol% (Fig. 1). Further, white precipitation was observed with 75 mol% addition.

PLLA is known to form hexagonal crystalline structure. In the hydrophobic core of Lactosome, PLLA blocks are considered to be regularly aligned and molecularly packed to form locally the crystalline structure. The crystalline domains should be confined into the small space of the polymeric micelle core however with many structural defects. When PLLA-28 or PDLLA-30 was added to the Lactosome, these molecules may fill the defects in the polymeric micelle core, and thus the assembly sizes remained to be similar values within 25 mol% addition. On the other hand, PDLA-31 forms stereocomplex with the matrix polydepsipeptide of Lactosome. The addition of PDLA therefore directly increases the size of the polymeric micelle.

The T_g value of poly(lactic acid) is reported to be 50–60 °C. However, no peaks were observed by differential scanning calorimetry (DSC) measurements of PLLA and PDLA (5 mol%) (Fig. S2). On the other hand, the poly(lactic acid)s T_g peak was observed with the Lactosome dispersion when it was prepared at 0 °C. These data also support that PLLA-28 or PDLA-31 should be nicely packed in the hydrophobic core of the Lactosome constituents due to the heat treatment at 90 °C at the micelle preparation.

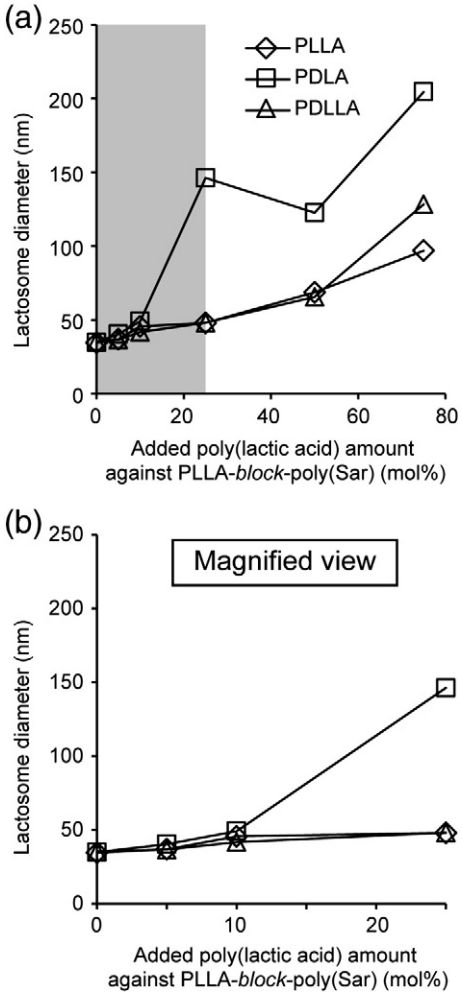

Fig. 1. Lactosome diameter containing 0–75 mol% three types of poly(lactic acid)s. Fig. 1b is a magnified view of the highlighted region in Fig. 1a.

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