doi:10.1246/cl.2010.250

Published on the web February 6, 2010

Preparation of Nanoaggregates through Self-assembly of Amphiphilic Polyrotaxane Composed of PLLA–PEG–PLLA Triblock Copolymer and α-Cyclodextrins

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An amphiphilic polyrotaxane (PRX) composed of biodegradable triblock copolymer of poly(ethylene glycol) (PEG) and poly(L-lactide) (PLLA), PLLA–PEG–PLLA, and many α-cyclodextrins (α-CD) threaded on the triblock copolymer was successfully synthesized. The PRX was found to be self-assembled into vesicle-like water-soluble nanoaggregates in an aqueous solution.

Molecules consisting of amphiphilic building blocks can provide diverse self-assembled structures in aqueous solution, because of their delicate balance of intermolecular hydrophobic interaction and solvation with water.[1] Such amphiphilic assemblies can be used in various applications from biology to materials.[2] Amphiphilic block copolymers have been a popular choice for such types of assemblies.[3]

On the other hand, supramolecular structure of physically interlocked molecules such as polyrotaxanes (PRXs) is also one of the key concepts to design novel functional materials such as nanomaterials and molecular machines. For example, many α-cyclodextrins (α-CDs) were found to be threaded by some polymer such as poly(ethylene glycol) (PEG) to form PRX structure,[4] which has inspired the development of interesting nanostructures such as molecular tubes,[5] as well as various supramolecular materials for electronics and biomaterials applications.[6] We previously reported the synthesis of polypseudorotaxane (**tri-pPRX**) consisting of many α-CDs threaded by biodegradable triblock copolymers of PLLA and PEG (PLLA–PEG–PLLA).[7] The **tri-pPRX** has amphiphilic supramolecular structure composed of hydrophobic PLLA and hydrophilic PEG with α-CDs. Therefore, it is expected that **tri-pPRX** having appropriate hydrophobic–hydrophilic balance can be self-assembled into nanoaggregates based on hydrophobicity of PLLA and hydrophilicity of PEG and α-CDs in aqueous media. Since the α-CDs of the **tri-PRX** were not covalently bound to the main PLLA-PEG-PLLA chain, they can slide and rotate along the main chain consisting outer shell of the nanoaggregate. Thus, such nanoaggregates should be novel functional nanomaterials having dynamic surfaces based on the PRX structure.

In this study, we designed amphiphilic PRX (**tri-PRX**) consisting of PLLA–PEG–PLLA and α-CDs (Figure 1). Moreover, we successfully prepared nanoaggregates of **tri-PRX** by self-assembly in aqueous solution and investigated their self-assembly behavior, size and the distribution, morphology, and the ability to encapsulate water-soluble molecules.

Bis(hydroxy-terminated) PLLA–PEG–PLLA with a M_n of 21.5 kDa and a narrow M_w/M_n (1.05) was obtained by ring-opening polymerization of L–LA in the presence of PEG with M_n of 20.0 kDa. Bis(amino-terminated) PLLA–PEG–PLLA was synthesized by coupling reaction of bis(hydroxy-terminated) PLLA–PEG–PLLA with *t*-butoxycarbonyl (Boc)–glycine, and removal of Boc groups (Scheme S1).[8,9] The **tri-pPRX** was synthesized according to a method reported previously.[7] The **tri-PRX** was then synthesized by coupling reaction of amino-termini of PLLA–PEG–PLLA in **tri-pPRX** with benzyloxycarbonyl-L-phenylalanine succinimide ester in methanol. The average number of threaded α-CD per PLLA–PEG–PLLA molecule was estimated to be 42 from the ^{1}H NMR integral ratio of the C(1)H signal assigned to α-CD and the CH_3 signal assigned to lactic acid of PLLA–PEG–PLLA in 10 wt % NaOD/D_2O (Table 1). Considering the stretched length of the lactide unit and the depth of the α-CD cavity, one α-CD molecule should be equivalently fit to two lactic acid units, as reported for the case of PEG.[10] Estimating from these theoretical parameters, the coverage for PLLA–PEG–PLLA by α-CD was 17%. The M_n of **tri-PRX** was estimated to be 61.8 kDa from the equation described in Table 1.

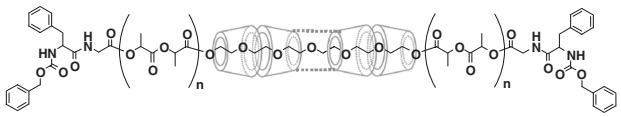

Figure 1. Structure of the PRX composed of PLLA–PEG–PLLA triblock copolymer and α-CDs.

Table 1. Characterization of the PRX and the triblock copolymer

Sample	M_n/kDa[a]	DP[b]	PEG content /wt %	α-CD[c]
Triblock	21.5	5	93	—
PRX	61.8	5	32	42

[a]M_n of the triblock copolymer was estimated by ^{1}H NMR in DMSO-d_6. M_n of the PRX was estimated from the following equation: M_n of the PRX = (M_n of the triblock copolymer) + (total molecular weight of threaded α-CD onto the triblock copolymer). [b]Degree of polymerization of L-lactide unit estimated by ^{1}H NMR in DMSO-d_6. [c]The average number of α-CD threaded was estimated by ^{1}H NMR in 10 wt % NaOD/D_2O after hydrolysis of PLLA at rt for 24 h in 10 wt % NaOD/D_2O.

Self-aggregates of **tri-PRX** were prepared by dialysis of **tri-PRX** in DMSO solution against water. After dialysis, slightly opaque aqueous solution of **tri-PRX** was observed, indicating that **tri-PRX** form water-soluble aggregates spontaneously (Figure S1).[9] In order to investigate self-assembly behavior of **tri-PRX** in water, the critical aggregation concentration (CAC)

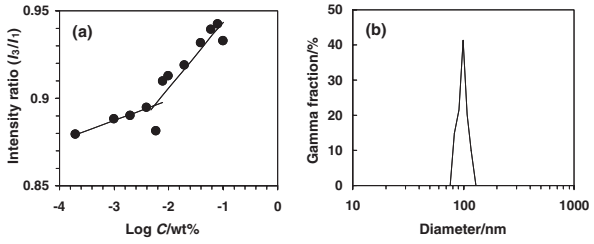

Figure 2. (a) Plots of the fluorescence intensity ration I_{394}/I_{374} from the pyrene emission spectra vs. log C for the PRX in water. (b) Size distribution of the PRX aggregates in water determined by DLS measurement. The PRX concentration was at 0.5 wt %.

of **tri-PRX** and the corresponding PLLA–PEG–PLLA was analyzed using pyrene as a fluorescence probe (Figure 2a). The CAC of **tri-PRX** and the PLLA–PEG–PLLA were 5.2×10^{-3} and 7.8×10^{-4} wt %, respectively. In general, PLLA–PEG–PLLA copolymers with a certain balance of hydrophilicity and hydrophobicity have been known to form nanosized micelles with a core–shell structure.[11] Although the higher CAC value of **tri-PRX** compared with that of the PLLA–PEG–PLLA means relatively weak tendency to form aggregates, this fluorescence study confirm that **tri-PRX** form aggregates in dilute aqueous solution. The hydrodynamic diameter (D_h) and the distribution of **tri-PRX** aggregates and the PLLA–PEG–PLLA micelles in water were investigated by DLS measurement (Figure 2b). The **tri-PRX** aggregates and the PLLA–PEG–PLLA micelles showed unimodal distribution. **Tri-PRX** aggregates showed two times larger D_h value (101 nm) than the PLLA–PEG–PLLA micelles (47 nm), indicating that they form different types of nanoaggregates.

In order to investigate the morphology of **tri-PRX** nanoaggregates, TEM measurement was carried out. Although we tried several ways by varying experimental conditions, a fine image was not obtained because of low stability of the nanoaggregates in dry state. On the other hand, ^{1}H NMR measurements of **tri-PRX** in DMSO-d_6 or D_2O, were carried out. All of the components, PLLA, PEG, and α-CD, are soluble in DMSO-d_6 (Figure 3a). Characteristic peaks of CH_3 and CH protons of PLLA were observed at 1.6 and 5.1 ppm, respectively. However, when **tri-PRX** nanoaggregates were redispersed in D_2O, these peaks drastically decreased (Figure 3b), indicating the formation of the core of PLLA segments. In contrast, characteristic peaks of PEG (3.6 ppm) and α-CD are still observed obviously in D_2O. These results indicate that the surface of the nanoaggregate is covered by PRX, where multiple α-CDs threaded on PEG chain, and PLLA cores formed by hydrophobic interaction are located inside of the nanoaggregate.

We then tried to prepare **tri-PRX** nanoaggregates loading calcein, a water-soluble fluorescence dye, by dialysis of DMSO solution containing **tri-PRX** and calcein against water. After isolation of the calcein-loaded **tri-PRX** nanoaggregates by dialysis, confocal laser scanning microscope (CLSM) observation was carried out. On the CLSM image, calcein-derived bright fluorescence spots were observed (Figure S2).[9] This result indicates that **tri-PRX** nanoaggregates have vesicle-like structure with interior aqueous phase which is capable of loading water-soluble molecules.

In summary, amphiphilic PRX consisting of multiple α-CDs and biodegradable PLLA–PEG–PLLA copolymer was successfully synthesized. The PRX form vesicle-like nanoaggregates in dilute aqueous solution. The nanoaggregate can be expected to be applied as a novel biodegradable drug carrier having dynamic surface based on PRX structure. The amphiphilic supramolecular assemblies could be an important concept to create advanced functional nanomaterials.

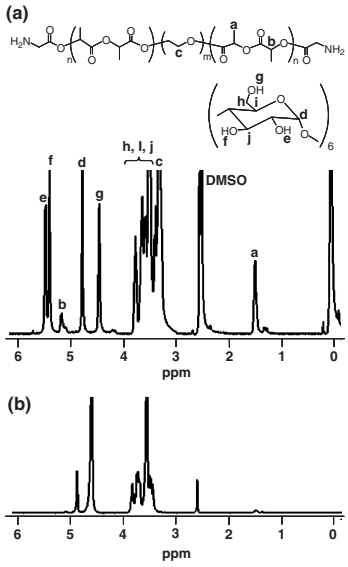

Figure 3. ^{1}H NMR spectra of (a) the PRX in DMSO-d_6 and (b) the PRX aggregate in D_2O.

A part of this work was financially supported by Grant-in-Aid for Scientific Research on Priority Areas (No. 20034055) and Scientific Research B (No. 19300170) from MEXT, Japan.

References and Notes

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