Biodegradable Polymersomes from Poly(2-hydroxyethyl aspartamide) Grafted with Lactic Acid Oligomers in Aqueous Solution

Hyun Jin Lee, Seung Rim Yang, Eun Jung An, and Jong-Duk Kim*

Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

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Amphiphilic molecules self-assemble into various structures such as spherical and cylindrical micelles, lamellae, and vesicles in aqueous media, organic solvent, or a mixture of both of these items.[1−3] Those self-assemblies have been extensively studied for the past decade due to their unique characteristics and the variety of applications in many areas including the biomedical field. In particular, the vesicular aggregates made of block copolymers, known as polymersomes,[4] have been paid a great deal of recent attention with their high potential use as cell-mimicking systems,[5] biosensors,[6] containers or reactors,[7] and vehicles for delivery of bioactive agent.[5,8,9]

But until now, studies of polymer vesicles were focused on di- or multiblock copolymers.[5,10−13] Although graft copolymers could easily have diverse functionalities by conjugation of functional moieties or drugs onto the polymer backbone, in most cases, spherical micelles were formed as shown in our previous works.[14−16]

Here, we report the synthesis of novel amphiphilic graft copolymer, poly(2-hydroxyethyl aspartamide) grafted with lactic acid oligomers (PHEA-*g*-LA), and the structural transition of the self-aggregates as a function of DS (degree of substitution, mole percent of grafted LA segments) which is related to hydrophobic/hydrophilic block ratio. To our best knowledge, we first report the formation of the polymer vesicles from comblike traditional graft copolymers. Besides, the use of aqueous media and the biocompatibility and biodegradability of copolymer material of our system would be essential for later application as delivery carriers.

All synthetic routes and the molecular structure of PHEA-*g*-LA are shown in Scheme 1. The precursor polymer, poly(succinimide) (PSI), was presynthesized by the acid-catalyzed polycondensation of L-aspartic acid using phosphoric acid as the catalyst as previously reported.[16] PHEA containing amine group was prepared by aminolysis of PSI with 0.9 equiv ethanolamine and excess amount of ethylenediamine, respectively. Lactic acid oligomers (LAs) were synthesized by ring-opening polymerization of L-lactide using 1-octanol as initiator, and stannous octate as catalyst and purified with diethyl ether.[17] The number-average molecular weight and PDI of LA measured by MALDI−TOF mass spectroscopy were 2412 and 1.02, respectively. The hydroxyl group at the chain terminus of LAs was activated with coupling agent *N*,*N*-carbonyldiimidazole (CDI) (LA−CDI), for the grafting reaction. LAs were grafted to the backbone through the formation of carbamate linkage by reaction of amine group in the backbone polymer and purified LA−CDI. In this step, we can control the DS of copolymer just by varying the feed mole ratio LA−CDI. The reaction mixture was extensively dialyzed against DMF and distilled water, respectively. After freeze-drying, the final product PHEA-*g*-LA was obtained.

Scheme 1. Synthetic Route of PHEA Grafted with LA Oligomers

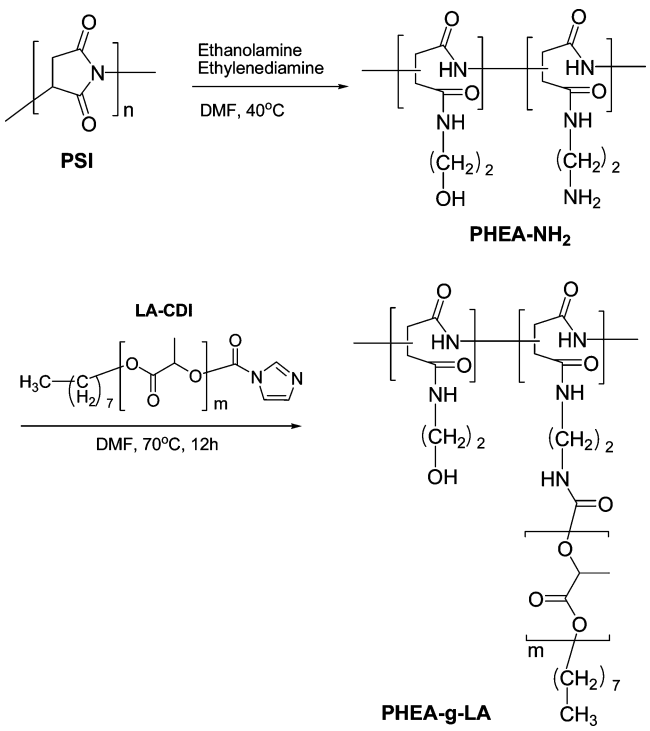

Table 1 shows the molecular characterization of synthesized copolymers. DS values were determined with ^{1}H NMR spectroscopy (Figure S1 and S2) and elemental analysis (EA), and based on that, the weight fraction of LA ($f_{LA}(w)$) was calculated. We prepared five copolymers having different hydrophilic/hydrophobic ratio within a broad range.

The self-aggregates in aqueous solution were prepared by precipitate-dialysis method using DMSO.[16] Self-aggregates are formed during the precipitation process and the formation is completed by removal of DMSO through extensive dialysis against water. The aggregate size of each copolymer was measured with DLS (Figure S3) and the results is largely different from those of our previous works about spherical micelles of various graft polymer systems.[14−16] We had found that size of micellar aggregates is continuously decreased with increase of DS since the aggregates become compacter and smaller due to stronger hydrophobic interaction. That experimental result goes well with the theoretical prediction about micelle size based on the change of degree of polymerization of corona and core blocks.[18]

DLS result of the present system shows that P-LA2.5 and P-LA5.0 formed small aggregates about 15−20 nm, but P-LA7.5, P-LA10.0, and P-LA12.5 formed larger aggregates about 40 nm and the diameter was slightly increased with the increase of DS. In addition, the size distribution of every each copolymer except P-LA7.5 is monodisperse. P-LA7.5 shows bimodal distribution, in which one part is around 12 nm and the other one is around 35 nm. Therefore, on the basis of the different aggregation behavior and $f_{LA}(w)$ values of copolymers, we can expect that two different structures of aggregates are

* Corresponding author. jdkim@kaist.ac.kr. Telephone: +82-42-869-3961. Fax: +82-42-869-3910. E-mail: jdkim@kaist.ac.kr

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Table 1. Molecular Characterizations of Copolymers

feed (mole %)[a]	polymers	DS (%)[b]	f_{LA}(w)[b]	DS (%)[c]	f_{LA}(w)[c]
2.5	P-LA2.5	1.38	0.17	1.43	0.18
5.0	P-LA5.0	2.98	0.31	3.17	0.32
7.5	P-LA7.5	4.37	0.39	4.30	0.39
10.0	P-LA10.0	6.34	0.49	6.59	0.49
12.5	P-LA12.5	9.16	0.58	9.11	0.57

[a] Feed mole ratio % of LA−CDI/PHEA−NH_2 in the grafting reaction. [b] Determined based on ^{1}H NMR of copolymers. [c] Determined based on elemental analysis.

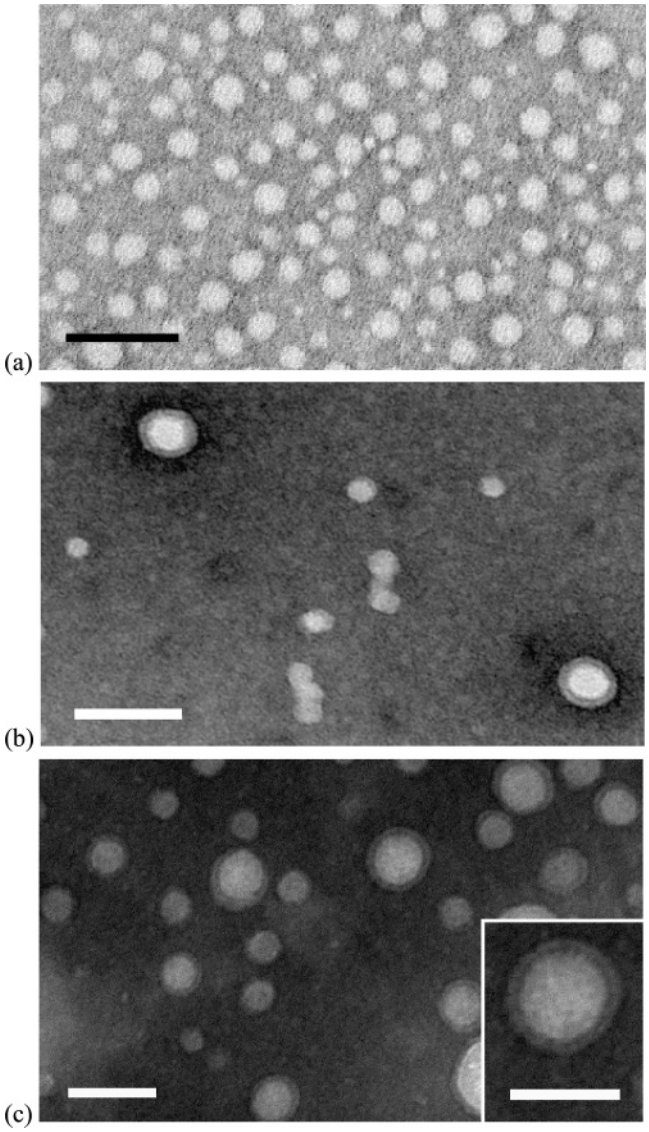

Figure 1. TEM images of aggregates of (a) P-LA5.0, (b) P-LA7.5, and (c) P-LA10.0 in aqueous solution negatively stained with phosphotungstic acid (2 wt %). The scale bars in large images represent 40 nm and the one in inset image of (c) represents 30 nm.

possible according to DS. DS about 4.3−4.7 at which $f_{LA}(w)$ becomes 0.39, would be the structural transition point. The formation of aggregates and their structures were confirmed by TEM. Figure 1 shows the TEM images of aggregates in aqueous solution negatively stained with phosphotungstic acid (2 wt %). The image of P-LA5.0 shows only micelle structure, and that of P-LA7.5 shows coexistence of micelles and vesicles, while in the image of P-LA10.0 all aggregates are vesicles. The two structures, micelles and vesicles, are definitely distinct in the images. The vesicle structure via formation of polymer bilayer is clearly visualized with the contrast difference as shown in the inset of Figure 1c, high-magnified image of one vesicle. TEM images are exactly consistent with DLS data in terms of size and size distribution. Therefore, our expectation is proved and the two different structures are identified as spherical micelle and vesicle.

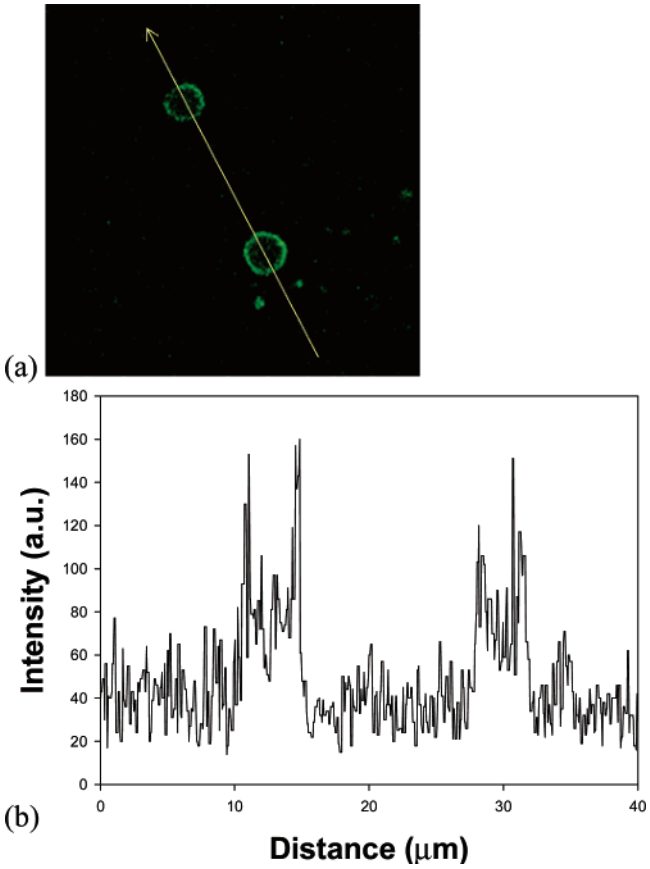

Figure 2. (a) CLSM image and (b) fluorescent intensity profile of microvesicles from P-LA12.5.

The DS would not be only related to the hydrophilic/hydrophobic block ratio itself but also affect the molecular shape and ultimately flexibility and the bending behavior of polymer backbone (Figure S4). In the graft copolymer system, that bending behavior of backbone might be considered as an additional factor induced from the amount of grafted counterparts, DS, and eventually affects the aggregates structure. As the number of grafted segments is increased, the polymer chain becomes bulky and rigid and the bending of backbone part and gathering of grafted parts in one place becomes difficult. Copolymer chains would rather form bilayer structure than hydrophobic core enclosed with hydrophilic backbones.

In the formation of vesicle, preparation method is another important factor which largely affects the size and stability of aggregates. Depending on the preparation method, even the same polymer material can form nanosized vesicle or microsized one.[10] We tried to form the microvesicles from P-LA12.5 by using the method of film-hydration with sonication, general method to form liposome and micron-sized vesicles, and the final concentration of solution was fitted to be 1 mg/mL. FM 1−43, a fluorescent probe that is frequently used for visualization of bilayer membrane,[19] was dissolved in water prior to the hydration step for the observation of microvesicle with confocal laser scanning microscope (CLSM). We could observe microvesicles that are 5 μm in diameter with clear hydrophobic membrane loaded with FM 1−43 as shown in the CLSM image and the fluorescent intensity profile (Figure 2).

In summary, a series of amphiphilic graft copolymer, PHEA-*g*-LA with various DS was successfully synthesized via the formation of carbamate bond. Self-aggregates in aqueous solution were prepared with precipitate-dialysis method and characterized with DLS and TEM. The copolymers self-assembled into two different aggregates, spherical micelle and vesicle, as a function of DS. The structural transitional point is DS 4.30 where f_{LA} becomes 0.39, and this assembly behavior was discussed in the view of composition and geometry of a copolymer molecule. Microvesicles were also obtained by a film-hydration method and confirmed with CLSM. It is expected that these new products have potential applications as delivery carriers in biotechnological, pharmaceutical, and cosmetic fields. More advanced characterizations and the investigation for possibility of use as delivery carrier will be covered in future work.

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Supporting Information Available: Figures showing the ^{1}H NMR spectra, DLS data, and a schematic diagram of the structural transition. This material is available free of charge via the Internet at http://pubs.acs.org.

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