Received: January 18, 2014 | Accepted: February 4, 2014 | Web Released: February 8, 2014 CL-140032

Studies on Synthesis, Characterization, and Functionalization of Poly(3,4-dihydroxy-L-phenylalanine)

Dongjian Shi,[1,2] Rongjin Liu,[1] Fengduo Ma,[1] Daoyong Chen,[2] Mingqing Chen,*[1] and Mitsuru Akashi[3]
[1]*The Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, P. R. China*
[2]*State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, P. R. China*
[3]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871*

(E-mail: mqchen@jiangnan.edu.cn)

In this paper, hyperbranched PDOPA polyester with precise structure was synthesized by thermal polycondensation. PDOPA showed good solubility, degradability, and biocompatibility. Functional polymers based on PDOPA were prepared by reactions of amine groups in PDOPA with pyrenecarboxylic acid and polylactide, respectively. Wide applications of the PDOPA polyester are expected after modified with functional groups.

Biobased polymers are a diverse class of materials that have potential applications to adhesives, absorbents, lubricants, cosmetics, drug delivery carriers, and food packaging products in various fields.[1] There are many biobased polymers such as cellulose, poly(hydroxybutyrate) (PHB), poly(lactic acid) (PLA), poly(amino acid)s, polysaccharides, and so on. One of the most attractive biobased polymers is poly(amino acid).[2] 3,4-Dihydroxyphenylalanine (DOPA) is a specialized amino acid, which is commonly found in many marine organisms and plant sources and can be used for the treatment of neural disorders such as Parkinson's syndrome.[3] DOPA has unique characteristics such as easy crosslinking and strong wet adhesion properties with metals, ceramics, organics, and polymers.[4] In an effort to duplicate these characteristics of DOPA in synthetic polymers, DOPA was incorporated into synthetic polymers on side chains or end groups to make them with good adhesive and mechanical properties for application to imaging agents, surface treatments, and coatings.[5] Although the obtained polymers showed good properties, some evidence suggest that catechols are oxidized to *o*-quinones or semiquinone and undergo nucleophilic addition reactions with primary amines such as lysine via Michael addition.[6] Thus, the exact structures of the resultant DOPA derivatives or polymers are difficult to confirm and control. Although Messersmith et al. synthesized polydopa with exact structure by amidation reaction, the polymer existed with only two or three repeat units, possibly due to the steric hindrance of benzene groups.[7] To widen applications of polydopa in engineering and biomedicine, it is necessary to prepare functional polydopa as a main chain with precise structure.

In our previous reports, we have prepared a series of linear and hyperbranched aromatic polyesters based on cinnamic acid derivatives by thermal polycondensation, which showed high performance, biocompatibility, and degradability.[8] In this paper, synthesis and characterization of hyperbranched poly(3,4-dihydroxy-L-phenylalanine) (PDOPA) polyester with precise structure is presented. In addition, pyrene and polylactide (PLA) polymer chain as functional groups were introduced into PDOPA side chains to prepare functional PDOPA, which might widen PDOPA engineering and biomedical applications.

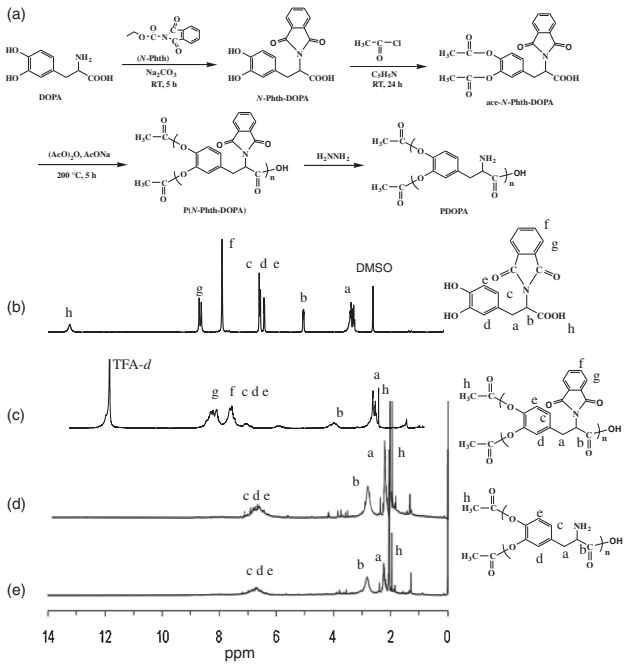

Figure 1. (a) Synthetic scheme for the PDOPA polymer and ^{1}H NMR spectra of *N*-Phth-DOPA in DMSO-d_6 (b), P(*N*-Phth-DOPA) in TFA-*d* (c), PDOPA in acetone-d_6 before degradation (d), and PDOPA in acetone-d_6 after degradation (e).

In order to obtain precise structure of PDOPA polyester, the amine group in DOPA should be protected to avoid reacting with carboxy groups during polymerization. Boc- or Fmoc-strategies are most prominent today.[9] However, the Boc- or Fmoc-protected groups will be deprotected at temperatures above 180 °C, resulting in formation of a polymer mixture. Bergmann and Zervas discovered an easily removable benzoxycarbonyl protecting group,[10] which is stable at high temperature. Thus, *N*-(ethoxycarbonyl)phthalimide (*N*-Phth, 1.2 mol) was used to react with DOPA (1 mol) in the presence of borax, as shown in Figure 1a. With disappearance of absorbances of amine groups at 3100–3400 cm^{-1} and appearance of ester absorbances at 1710 cm^{-1} in FT-IR spectra (Figures S1a and S1b),[11] amine groups were confirmed to be protected by *N*-Phth. Peaks at 7.9 and 8.5 ppm in the ^{1}H NMR spectrum (Figure 1b) assigned to benzene groups in *N*-Phth, suggested the successful synthesis of *N*-Phth-DOPA. Hydroxy groups in DOPA easily auto-oxidize to form quinones, which causes loss of activity and turns the product black. Thus, acetyl chloride was used to protect hydroxy

groups before polymerization, and the structure of Ac-*N*-Phth-DOPA was confirmed by the FT-IR spectrum (Figure S1c). Then, Ac-*N*-Phth-DOPA (50 mmol) was heated at 200 °C using acetic anhydride (10 mL) as a condensation reagent and sodium acetate as a catalyst for transesterification. After purification in methanol, a brown product P(*N*-Phth-DOPA) was obtained. The ^{1}H NMR spectrum showed multiple peaks in the region of the chemical shift of 6.9–7.2 ppm assigned to aromatic protons in DOPA and a single peak at 2.5 ppm assigned to the acetylate end group (Figure 1c). The *N*-Phth group can be cleaved in weak alkaline solution, and thus deprotection of the amino groups in P(*N*-Phth-DOPA) was performed in the present of hydrazine. The FT-IR spectrum of PDOPA (Figure S1d) confirmed the presence of amide at 3050 and 3250 cm^{-1} and disappearance of Phth at 1710–1780 cm^{-1}. The ^{1}H NMR spectrum (Figure 1d) also confirmed the disappeared of Phth groups at 7.9–8.5 ppm to obtain PDOPA polyester with active amine groups. The degree of branching of PDOPA was 0.356 (Figure S2), which was calculated from the ^{1}H NMR spectrum (Figures 1c and 1d) based on a reported method.[12]

The resultant PDOPA showed good solubility in acetone, THF, DMF, DMSO, and other common organic solvents. This result indicated that PDOPA could be easily processed and further modified. Molecular weight (M_n) and distribution were determined by GPC in THF to be 11990 g mol^{-1} and 1.89 (Figure S3), respectively. DSC showed T_g of PDOPA at about 106 °C and T_m higher than 200 °C (Figure S4), which were higher than the values of some biobased polymers (such as $T_{g\ PLA}$: 55 °C and $T_{m\ PLA}$: 160 °C).

Degradation of biobased polymers is a very important factor for materials in biomedical applications. Therefore, the hydrolytic degradation of PDOPA was investigated in alkaline buffer at pH 12 as an accelerated test. The weight of PDOPA decreased significantly to approximately 28% after 30 min of hydrolysis (Figure 2a), and to 83% after 150 min. These results suggested that PDOPA has high degradability. The molecular weight of remaining PDOPA after degradation was 11480 g mol^{-1}, which is similar to that of PDOPA before degradation, as shown in Figure S3. The ^{1}H NMR spectrum (Figure 1e) of the remaining PDOPA after degradation was the same as before degradation, suggesting no structure change. To evaluate the degraded structure, the product yielded by degradation in the supernatant solution was analyzed by high-performance liquid chromatography (HPLC). The results (Figure S5) showed monomer peak together with other peaks presumably assigned to oligomers. The mass of the degraded products in the supernant solution was about 326.1 (Figure S6), which was approximately two DOPA molecules. These results indicated that PDOPA degraded to monomer and oligomers, and the oligomer might be due to the melamine formation by DOPA auto-oxidation. We further confirmed the cell compatibility of PDOPA through a cell adhesion test using the MTT method. After L929 fibroblasts (5×10^4 cells) were incubated on the PDOPA film for 24 h at 37 °C in PBS, the cells adhered and grew to 14.2×10^4 cells (Figure 2b), and the cells on tissue culture polystyrene (TCP, as a control sample) numbered 11.6×10^4 cells. There was no significant difference in cell adhesion of PDOPA with TCP.

In order to prepare functional PDOPA, 1-pyrenebutyric acid (Py) was employed to react with PDOPA through amidation

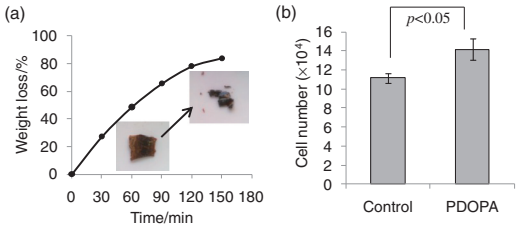

Figure 2. (a) Weight loss of PDOPA during hydrolytic degradation. (b) Cell adhesion after 24 h on PDOPA film ($n = 6$). Tissue culture polystyrene as a control sample. Statistically significant differences using a two-sample *t*-test for comparison.

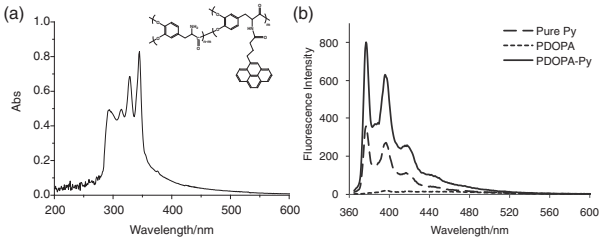

Figure 3. (a) UV–vis and (b) fluorescence spectra of PDOPA–Py polyester (1×10^{-5} mol L^{-1}).

reaction (Scheme S1a). ^{1}H NMR spectrum (Figure S7) confirmed the structure of PDOPA–Py and the grafting degree of Py was about 21.1%. The fluorescence of PDOPA–Py was confirmed by UV–vis and fluorescence spectra, as shown in Figure 3. Both UV–vis and fluorescence spectra showed the special adsorptions of Py, suggesting Py was introduced into the PDOPA polymer. Moreover, the fluorescence intensity of the PDOPA–Py polymer was higher than pure Py and PDOPA (Figure 3b). These results suggested the obtained PDOPA–Py polymer was fluorescent.

Moreover, carboxy polylactide (PLA) was also a functional group to react with PDOPA (Scheme S1b). The structure was confirmed by ^{1}H NMR spectra (Figure S8). The properties of PDOPA-*g*-PLA are now being determined.

In conclusion, hyperbranched PDOPA polyester with precise structure was prepared and confirmed to have good solubility, biodegradability, and biocompatibility. PDOPA could be modified by pyrene and PLA to obtain functional polymers based on PDOPA. Since PDOPA has active amine groups, PDOPA can be modified with many functional compounds or polymers, such as cyclodextrins, chitosan, and so on. Therefore, the PDOPA polyester can be a novel biopolymer to be used in many fields.

This study was supported by the National Natural Science Foundation of China (Nos. 21004029 and 51173072) and State Key Laboratory of Molecular Engineering of Polymers (Fudan University, K2011-08). The authors thank Dr. Koji Kadowaki for cell adhesion experiments in Osaka University.

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