Thermally stable and photoreactive polylactides by the terminal conjugation of bio-based caffeic acid†

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Received (in Cambridge, UK) 4th March 2008, Accepted 23rd May 2008
First published as an Advance Article on the web 2nd July 2008
DOI: 10.1039/b803615b

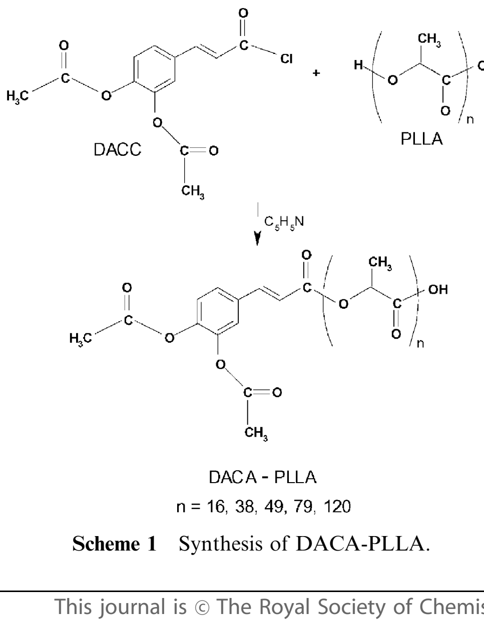
Caffeic acid terminally conjugated with polylactide showed high thermal stability and photoreactivity, and may be useful as a functional polylactide in the environmental and medical fields.

Poly-L-lactide (PLLA) has received much interest in recent years because it is synthesized from renewable resources, is resorbable in the human body and is nontoxic after biodegradation.[1] Therefore, it is expected to have various applications not only as a biomedical material, but also as a biodegradable plastic material. Many researchers have reported synthesis of polylactides by ring-opening polymerization of lactides with various initiators or catalysts.[2] Recently, the copolymerization of PLLAs with other monomers or polymers such as photoreactive cinnamic acid, 4-hydroxycinnamic acid (4HCA) or PEG has been reported to improve the thermal and mechanical properties, but less the functionalities of PLLA.[3] In previous studies, we reported that coumaric acid derivative homopolymers and copolymers showed liquid crystal phases, photoreactivities, degradabilities and cell compatibilities.[4] The bio-based coumaric acid derivative 3,4-dihydroxycinnamic acid: caffeic acid (DHCA) has photoreactive, biometabolizable and nontoxic properties. We reported that the copolymerization of 4HCA with DHCA enhanced its mechanical and thermal properties.[4c] Therefore, the modification of PLLA with DHCA can produce high performance PLLAs applicable to the environmental and biomedical fields.

In this paper, we report for the first time a novel thermally stable and photoreactive 3,4-diacetoxycinnamic acid (DACA)-terminally conjugated PLLA (DACA-PLLA).

The DACA-PLLAs were synthesized according to Scheme 1, and Table 1 shows their synthetic conditions and properties. 3,4-Diacetoxycinnamoyl chloride (DACC) was conjugated to the terminal hydroxyl group of the PLLAs, and DACA-PLLAs were obtained. The FT-IR and ^{1}H-NMR spectra suggested a successful conjugation of DACC to the PLLAs (see ESI†), and DACA-PLLAs of various molecular weights were obtained in high yield (Table 1). The composition ratio of L-lactic acid (LLA) and DACA in the modified PLLAs was calculated by the integral of the peak area ratio of the vinylene proton in DACA, and the C-H proton in PLLA (Table 1). The calculated LLA units from the ^{1}H-NMR spectra agreed well with the results from gel permeation chromatography (GPC) measurements. DACA-PLLAs dissolved in tetrahydrofuran (THF), chloroform, dichloromethane (DCM) and aprotic amidic solvents, and their solubilities were identical to that of a PLLA of the same molecular weight (see ESI†).

The thermal properties of the DACA-PLLAs were analyzed by DSC and TGA measurements. The melting points (T_m) of the DACA-PLLAs ranged from 137 °C to 169 °C depending on the molecular weight, and these values were about 10 °C higher than for PLLA of the same molecular weight (Table 1 and see ESI†). The crystallization temperatures (T_c) of the samples were 15 °C higher than for PLLA of the same molecular weight. Surprisingly, the 10% weight-loss temperatures (T_{10}) of the DACA-PLLAs ranged from 330 °C to 340 °C, which was over 100 °C higher than for PLLA of the same molecular weight (Fig. 1a). Although this increase in the T_m and T_{10} of PLLA *via* copolymerization with hydrophobic monomers has been reported,[3f,5] to the best of our knowledge, an extreme increase of over 100 °C to the thermal stability of PLLA by only terminal conjugation has never been reported. The detailed mechanism and reason for this phenomenon have not been clarified yet, but we speculate that one of the reasons might be the intermolecular stacking interaction of the terminal DACA units in DACA-PLLAs.[6]



Scheme 1 Synthesis of DACA-PLLA.

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† Electronic supplementary information (ESI) available: Detailed synthesis and evaluation, by DSC measurements, XRD analyses, and fluorescent dye conjugation, of PLLAs and DACA-PLLAs. See DOI: 10.1039/b803615b

Table 1 Synthetic conditions and properties of DACA-PLLAs[a]

Sample[b]	M_n[c] Before UV	M_n[c] After UV	M_w/M_n[c] Before UV	M_w/M_n[c] After UV	LLA/DACA[d] (unit)	Yield[e] (wt%)	T_m[f]/°C	T_c[f]/°C	T_{10}[g]/°C
DACA-PLLA16	1440	3070	2.1	1.6	16/1	96	137	117	333
DACA-PLLA38	2090	3950	2.1	2.1	38/1	92	157	103	329
DACA-PLLA49	3690	5450	2.8	2.5	49/1	93	147, 158	107	333
DACA-PLLA79	6010	11 310	2.8	1.9	79/1	92	168	106	336
DACA-PLLA120	8250	13 200	1.4	1.5	120/1	93	169	106	343

[a] The conjugation of DACA into the PLLA end chains was carried out at 0 °C for 2 h, and at room temperature for 24 h. [b] The numbers in the sample names are the unit numbers of LLA. [c] The molecular weights were estimated by GPC in THF with polystyrene standards (UV irradiation time: 90 min, λ >280 nm, intensity: 56 mW cm^{-2}). [d] The composition ratio of LLA and DACA (LLA/DACA) was estimated by ^{1}H-NMR spectroscopy. [e] The yields present results after the purification. [f] The T_m and T_c were measured by DSC upon a second heating (10 °C min^{-1}), and the "T_c" means the crystallization temperature. [g] The 10% weight-loss temperatures, T_{10}, were measured by TGA under nitrogen (20 °C min^{-1}).

The crystallinity of the DACA-PLLAs was analyzed by wide-angle X-ray diffraction (WAXD) (see ESI†). The results from DACA-PLLAs without annealing showed amorphous phases. When they were annealed at 100 °C for 1 h, some peaks were observed at 2θ = 15, 16.7, 19.1 and 22.5° (θ = diffraction angle) corresponding to spacings of 5.9, 5.3, 4.6 and 3.9 Å, respectively. This is consistent with the peaks at 15, 16, 18.5 and 22.5° reported by Ikada *et al.*[7] Furthermore, DSC results indicated that DACA-PLLA49 formed α' + α-phases and the others formed α-phase with 10_3 helical chain conformation (see ESI†).[8] In addition, their crystallization degrees were above 80%, the same as for PLLAs (see ESI†). Crossed-polarizing microscopic observations showed that the crystal domains of the DACA-PLLAs were similar to those of the PLLAs. These results suggested that the crystallinity of the PLLAs was maintained after the conjugation of DACA to the terminal PLLAs.

DACA-PLLAs are expected to be photoreactive because of the photoreactive terminally-conjugated DACA. The time dependent UV absorption changes of DACA-PLLA38 dissolved in DMF are shown in Fig. 1b. The maximal absorption peak at λ_{max} = 286 nm decreased with increasing UV irradiation time, and the other DACA-PLLAs and DACA monomer also showed the same behavior (Fig. 1c). All DACA-PLLAs showed an approximately 80% conversion after UV irradiation at λ >280 nm for 100 min, the same as the photoreactivities of DACA, indicating that the terminally conjugated DACA maintained their photoreactivity. The photoreaction of DACA at λ >280 nm is known to be a [2+2] cycloaddition,[4c] and thus we confirmed the photoreaction of

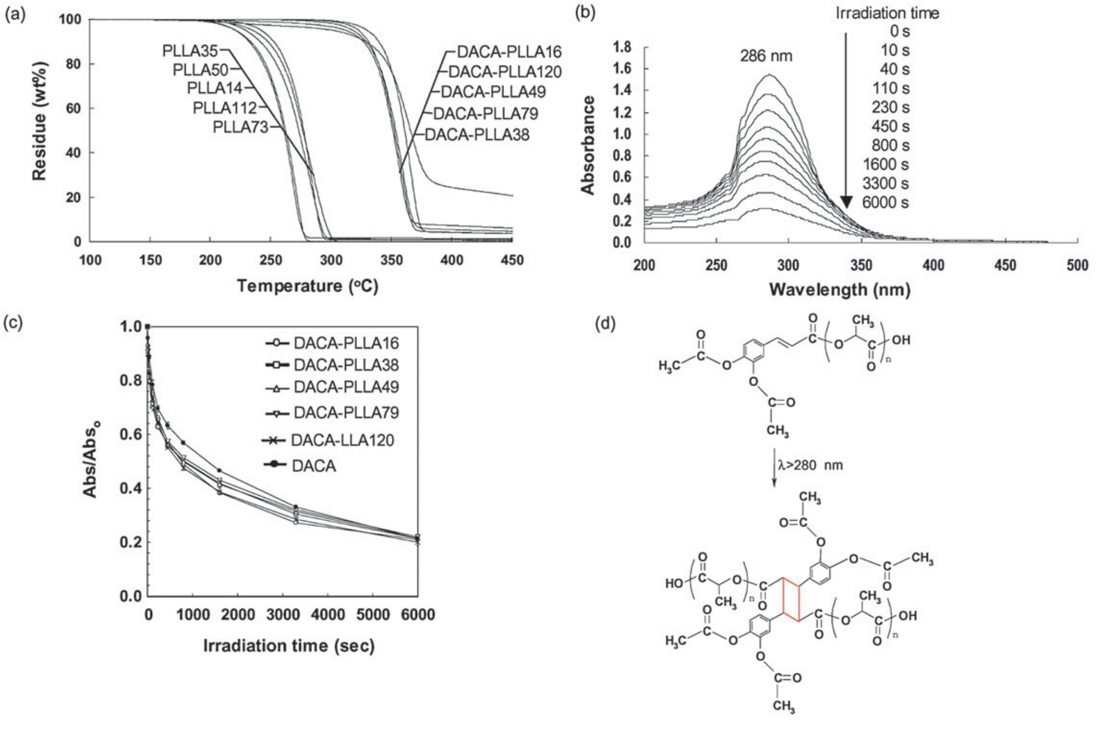

Fig. 1 (a) TG curves of all PLLAs and DACA-PLLAs at 20 °C min^{-1}; (b) UV absorption change of DACA-PLLA38 in DMF at 0.25 mg ml^{-1} during UV irradiation at λ >280 nm; (c) maximal absorption (λ_{max} = 286 nm) change of DACA-PLLAs and DACA during UV irradiation at λ >280 nm; and (d) photoreaction scheme of DACA-PLLA by UV irradiation at λ >280 nm.

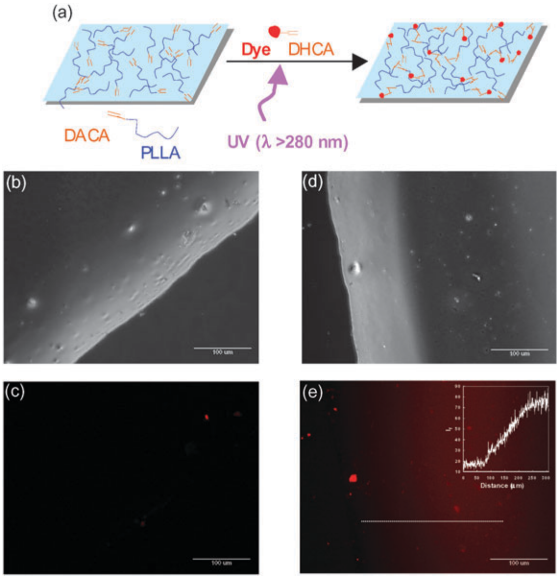

Fig. 2 (a) Schematic illustration of the UV conjugation of a fluorescent dye onto a DACA-PLLA38 film; photograph of PLLA36 and DACA-PLLA38 films in (b, d) phase contrast and (c, e) fluorescence images after UV irradiation at $\lambda > 280$ nm for 100 min, respectively. The UV irradiation was performed with the fluorescent dye and the films were washed with pure water. The insert in (e) is fluorescence intensity of the conjugated dye on DACA-PLLA38 film by line scanning. All scale bars are 100 μm.

DACA-PLLAs by ^{1}H-NMR measurements before and after UV irradiation (see ESI†). After the UV irradiation, a new peak appeared at 4.0–4.2 ppm, suggestive of cyclobutane formation by intermolecular [2 + 2] cycloaddition, as shown in Fig. 1d. The conversion of cyclobutane formation was calculated at about 77% from the ^{1}H-NMR. The solubility of all the DACA-PLLAs was not changed after *versus* before UV irradiation. When the molecular weight of the DACA-PLLAs after UV irradiation at $\lambda > 280$ nm was measured by GPC, the elution times were earlier (see ESI†) and the calculated molecular weights were about two-fold higher than those before UV irradiation (Table 1). In other words, a dimerization of the PLLA molecules had occurred *via* the photoreaction of terminally conjugated DACA.

These photoreactive DACA-PLLAs are expected to have biomedical applications as photoreactive PLLAs. We performed the photo-conjugation of a fluorescent dye as a model molecule onto a DACA-PLLA38 film. DHCA-conjugated Alexa fluor555 (see ESI†) was dissolved in PBS and dropped onto a DACA-PLLA38 film, and the subsequent UV irradiation was performed at $\lambda > 280$ nm for 100 min. After the photoreaction, the film was washed with pure water and observed by confocal fluorescence scanning microscopy (Fig. 2). The DACA-PLLA38 film showed a clear fluorescence image assigned to Alexa fluor555, although the PLLA36 film itself did not show any fluorescence. The DHCA conjugated dye could be reacted with DACA-PLLA38 to form cyclobutane by UV irradiation. These results demonstrated that DACA-PLLAs are useful as photo-conjugative PLLAs which can conjugate target molecules containing photoreactive coumarin groups like DHCA-conjugated Alexa fluor555.

In conclusion, DACA-terminally conjugated PLLAs were successfully synthesized, and their thermal properties were significantly improved, especially the T_{10}. However, the crystallinities of the PLLAs were well-maintained after the conjugation of DACA. All DACA-PLLAs showed photoreactivities related to the terminal DACA, and DHCA-conjugated fluorescent dyes were clearly attached onto a DACA-PLLA film by UV irradiation. DACA-PLLA may be useful as a photoreactive PLLA with high thermal stability for biomedical and environmental applications. These DACA-PLLAs easily formed branched copolymers by melt-polycondensation, and may be interesting as functional biodegradable polymers. The synthesis and evaluation of branched DACA-PLLA copolymers are now in progress.

This research was supported by an Osaka University 21st Century COE Program "Center for Integrated Cell and Tissue Regulation". We acknowledge Prof. H. Uyama of the Department of Applied Chemistry, Graduate School of Engineering, Osaka University for the TGA measurements.

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