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Synthesis of poly[(5-benzyloxy-trimethylene carbonate)-*co*-(5,5-dimethyl-trimethylene carbonate)] catalyzed by immobilized lipase on silica particles with different size

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Abstract

Porcine pancreas lipase (PPL) immobilized on silica particles with different size were prepared and employed successfully for ring-opening co-polymerization of 5-benzyloxy-trimethylene carbonate (BTMC) with 5,5-dimethyl-trimethylene carbonate (DTC) for the first time. Three kinds of silica particles with different sizes (150–250, 75–150 and 1 μm) were selected as carriers for enzyme immobilization. The structure of copolymers were confirmed by ^{1}H and ^{13}C NMR which showed no decarboxylation occurrence during the polymerization. The (M_n) of poly(BTMC-*co*-DTC) decreased rapidly with the increasing of immobilized PPL concentration. The carrier size of immobilized PPL affected both the catalytic activity and the polymer yield. The highest molecular weight ($M_n = 26,400$) of poly(BTMC-*co*-DTC) was obtained at around 0.1% concentration of immobilized PPL on silica particles with size of 75–150 μm.

Keywords: Enzymatic ring-opening co-polymerization; Polycarbonates; Immobilized enzyme

1. Introduction

Ring-opening polymerization has been regarded as an efficient route for the synthesis of biodegradable polymers to gain high molecular weights, such as polyester, polycarbonates and polyphosphates. In addition, it is also a convenient method to synthesis copolymers, which can provide polymer materials with different properties to fill various applications [1].

Aliphatic polycarbonate, one kind of surface erosion biodegradable polymer materials, is generally prepared by ring-opening polymerization of 6-membered carbonates (i.e. TMC and DTC). Aliphatic polycarbonate has been used in drug controlled release system and other biomedical applications due to its good biocompatibility, favorable mechanical properties and some elasticity. However, the rather lower hydrophilicity and hydrodegradability introduced by the presence of the carbonate groups in the polymer chain decreases their compatibility with soft tissues and lowers their biodegradability [2]. Recently, a number of aliphatic polycarbonates with pendent functional groups were reported [3,4], which can be used to adjust the properties of polycarbonates and can facilitate covalent prodrug attachment as well as other modifications [5].

In general, chemical methods for the ring-opening (co)polymerization of biodegradable polymers always need extremely pure monomers and anhydrous conditions as well as organometallic initiators, which must be completely removed, especially for medical application. To avoid these restrictions, enzymatic polymerizations have become known as new methodology of polymer syntheses [6,7], which can easily ensure the good biocompatibility and reliability of resulting polymer materials and can provide an environmentally friend process. Up to now, the method of enzymatic ring-opening polymerizations has been applied successfully to synthesize several kinds of biodegradable and biocompatible polymers, such as polyester [8], polycarbonate [9,10], polyphosphate [11,12], etc. At the same time, the enzymatic ring-opening co-polymerization has thus far received little attention. Till now, only few kinds of copolymers have been prepared in this way, such as polyester [13] and poly(ester-co-carbonate)s [14]. Lately,

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enzymatic ring-opening co-polymerizations of different carbonates were reported firstly by Bisht and co-workers [4,15]. But the number-average molecular weight (M_n) of the resulting copolymers was no higher than 9000.

In our previous studies, we have found that porous silica bead is one good kind of carrier for enzyme immobilization [12]. Immobilization of porcine pancreas lipase (PPL) on porous silica beads and narrow distributed micron glass beads significantly enhanced its catalytic activity for the polymerization of cyclic phosphates [12] and cyclic carbonates [9]. In addition, the size of carrier particles seemed to affect the catalytic activity of immobilized enzymes directly.

We herein firstly report enzymatic ring-opening co-polymerization of 5-benzyloxy-trimethylene carbonate (BTMC) [3] with 5,5-dimethyl-trimethylene carbonate (DTC) catalyzed by immobilized PPL (Scheme 1). The protecting benzyl groups of resulting poly(BTMC-*co*-DTC) could be removed subsequently by catalytic hydrogenation to give a polycarbonate containing pendant hydroxyl groups offering a wide range of opportunities for further modification and functionalization. More importantly, three kinds of silica particles with different sizes (150–250, 75–150 and 1 μm) were selected to study the relationships between the carrier size of immobilized enzyme, the catalytic activity for ring-opening co-polymerization and the polymer yield. The structure of the copolymers were confirmed by ^{1}H and ^{13}C NMR.

2. Experimental section

2.1. Materials

PPL was purchased from Sigma with an activity of 198.90 U/mg protein (at pH 6.0, using olive oil as substrate) and used without further purification. Silica particles (150–250 and 75–150 μm) were purchased from Shanghai Wusi Chemical Plant of China. Silica particles (narrow distribution, 1 μm) were obtained from Dupont Company. Glutaraldehyde (25% (w/v)) was purchased from Merk. 3-Aminopropyl-triethoxysilane was obtained from the Chemical Plant of Wuhan University of China and redistilled before use (bp 213–216 °C). Olive oil was of chemical grade and without any treatment before use. BTMC and DTC were synthesized according to Refs. [3,16], and were re-crystallized for several times before use. The melting points of BTMC and DTC were 144–145 and 102–103 °C, respectively. All other reagents used in this study were analytic grade.

2.2. Enzyme immobilization

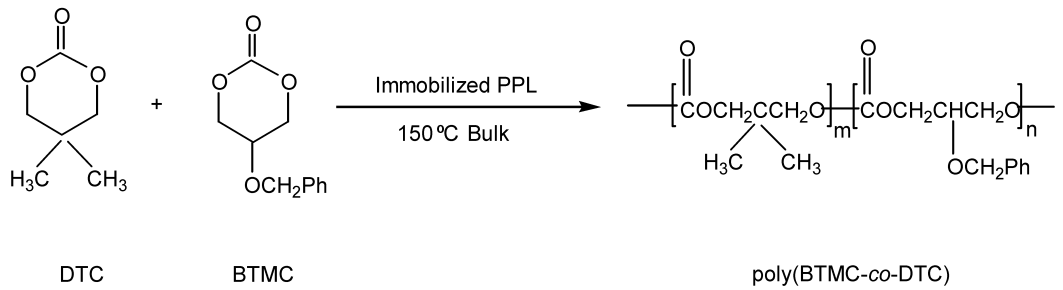
Silica particles with different size were treated as the similar method described previously [17]. The concentrations of bound amino group of 150–250, 75–150 and 1 μm silica particles are 1.48, 1.61 and 1.59 mmol/g (due to the losing in the period of separation), respectively. According to He [12] and Feng [9], PPL was covalently immobilized on these functional silica particles using glutaraldehyde as cross-linking agent. The coupling yields were equivalent to 26.55 mg of native PPL/1 g of 150–250 μm silica particles (IE-1), 37.34 mg of native PPL/1 g of 75–150 μm silica particles (IE-2), and 53.2 mg of native PPL/1 g of 1 μm silica particles (IE-3).

2.3. Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-VX 300 apparatus using tetramethylsilane (TMS) as an internal standard in chloroform ($CDCl_3$). M_n was measured by Gel-permeation chromatography (GPC). GPC was carried out on a Waters HPLC system equipped with a model 2690D separation module, a Module 2410 refractive index (RI) detection system and a Shodex K803 column. Chloroform was used as eluent at a flow-rate of 1.0 ml/min. Waters millennium^{32} module software was used to calculate molecular weights based on a universal calibration curve generated by narrow molecular weight distribution polystyrene standards. Enzymatic activity assay, using olive oil as substrate, was carried out according to the method described previously [12].

2.4. Enzymatic ring-opening co-polymerization of BTMC with DTC

All enzymatic ring-opening co-polymerization reactions were carried out in bulk at 150 °C for 24 h. A typical



Scheme 1. Ring-opening co-polymerization of BTMC with DTC by immobilized PPL on silica particles with different size.

synthesis of poly(BTMC-*co*-DTC) with an M_n of 16,500 (entry 6 in Table 1) was carried out as follows. A mixture of monomers (equal mole ratio) and IE-2 (0.2 wt%) were introduced to a thoroughly dried and sealed glass tube placed in a thermostated air bath with a magnetic stirring bar at 150 °C for 24 h. After the reaction, the reaction mixture was dissolved in dichloromethane and the insoluble immobilized enzyme was removed by filtration (IE-1 and IE-2) or centrifuge (IE-3). Then the solvent was concentrated under reduced pressure to obtain the crude polymer, which was further purified by re-precipitation (dichloromethane as a good solvent and methanol as a poor solvent). The molecular weight relative to polystyrene and the corresponding molecular weight dispersion as measured by GPC were 16,500 and 1.84, respectively.

3. Results and discussion

BTMC and DTC could be co-polymerized at the presence of immobilized PPL on silica particles with different size. The resulting copolymers (poly(BTMC-*co*-DTC)) had a M_n of up to 26,400 when the carrier size was 75–150 μm.

The structure of poly(BTMC-*co*-DTC) were confirmed by ^{1}H and ^{13}C NMR. ^{1}H NMR spectrum contained distinct resonances for the BTMC and DTC component of the copolymer (Fig. 1). Both BTMC and DTC segments have indistinguishable signals at 4.2 ppm due to O–C**H**$_2$–C– protons. The spectrum shows that 0.95 ppm is due to DTC repeat unit –C**H**$_3$ protons, and signals at 7.3, 4.6, and 3.8 ppm are assigned to –C$_6$**H**$_5$, –C**H**$_2$–C$_6$H$_5$, and O–C**H**–CH$_2$ protons of the BTMC repeated units, respectively.

Table 1
Ring-opening co-polymerization of BTMC with DTC catalyzed by immobilized PPL on silica particles with different size

Entry	Immobilized lipase[a]	Conc. (wt%)	Yield (%)	M_n[b]	M_w/M_n[b]
1	IE-1	0.1	82	13,600	2.05
2	IE-1	0.2	80	11,400	2.88
3	IE-1	0.5	82	6500	2.36
4	IE-1	1.0	86	5900	2.75
5	IE-2	0.1	83	26,400	1.74
6	IE-2	0.2	77	16,500	1.84
7	IE-2	0.5	82	10,000	2.22
8	IE-2	1.0	83	6900	2.66
9	IE-3	0.1	94	19,600	1.80
10	IE-3	0.2	92	15,300	3.16
11	IE-3	0.5	73	5500	2.75
12	IE-3	2.0	39	4700	2.28
13[c]	–	–	–	~4000	2.09

Reactions were carried out at 150 °C for 24 h with equal feed mole ratio of BTMC/TMC.

[a] The carrier sizes of immobilized PPL are 150–250 μm (IE-1), 75–150 μm (IE-2) and 1 μm (IE-3), respectively.

[b] Determined using GPC.

[c] Control sample.

There is no evidence for decarboxylation occurrence during the polymerization because no methylene protons of ether-linked repeated units could be detected ($\delta = 3.4$ ppm). In addition, Fig. 2 shows the ^{13}C NMR spectrum of poly(BTMC-*co*-DTC). Assignments of BTMC and DTC carbonate signals in copolymer were based on comparison with the ^{13}C NMR spectra of homopolymer of BTMC [3] and DTC, respectively. The signals at 155.5, 155.3, and 155.0 ppm are assigned to the **C**=O of DTC–DTC, BTMC–DTC or DTC–BTMC, and BTMC–BTMC diad sequence, respectively.

Table 1 lists the results of ring-opening co-polymerization of poly(BTMC-*co*-DTC) catalyzed by silica particles with different size while the monomer feed mole ratio was unvaried (1:1). The polymerization was carried out in bulk at 150 °C for 24 h as well as immobilized PPL concentration ranging from 0.1 to 2.0 wt%.

It was reported that the pure TMC monomer could be spontaneously polymerized at 100 °C [14]. In fact, we also found that BTMC and DTC can be thermally oligomerized at 150 °C in this study. But both the M_n and yield of the oligomers were significantly lower than the polymers obtained by lipase-catalyzed polymerization. These results indicate that the lipase enzymes actually promoted the ring-opening co-polymerization.

The enzyme concentration made much influence on the (M_n) of poly(BTMC-*co*-DTC). The (M_n) of copolymers decreased rapidly with the increasing of immobilized PPL concentration from 0.1 to 2.0 wt%, regardless of the kind of immobilized PPL. The highest (M_n) of poly(BTMC-*co*-DTC) was obtained at a immobilized PPL concentration of around 0.1%. This might be ascribed to the number variation of the initiating species by increasing the enzyme concentration.

The catalytic activity of immobilized PPL was affected directly by the particle size of the carrier used. In the range of selected experimental conditions, the immobilized PPL on silica particles with medium size (75–150 μm, IE-2) exhibited the highest activity, which led to the resulting poly(BTMC-*co*-DTC) with the highest (M_n) 26,400. In addition, the activity of immobilized PPL on silica particles with smaller size (1 μm, IE-3) was higher than that of immobilized PPL on silica particles with larger size (150–250 μm, IE-1). These results were not identical with the results of enzymatic activity assay. The reasons may be include three aspects: (I) the different substrate used in the enzymatic activity assay (olive oil) and in the enzymatic ring-opening co-polymerization (beginning with BTMC/DTC and subsequently with the chain end of copolymers); (II) the different reaction media used in the enzymatic activity assay (phosphate buffer solution) and in the enzymatic ring-opening co-polymerization (in bulk); (III) two different monomers used in the co-polymerization reaction. It is well know that enzyme has many characteristic features, such as regio-, stereo-, and chemo-selectivities. In our previous studies of enzymatic synthesis

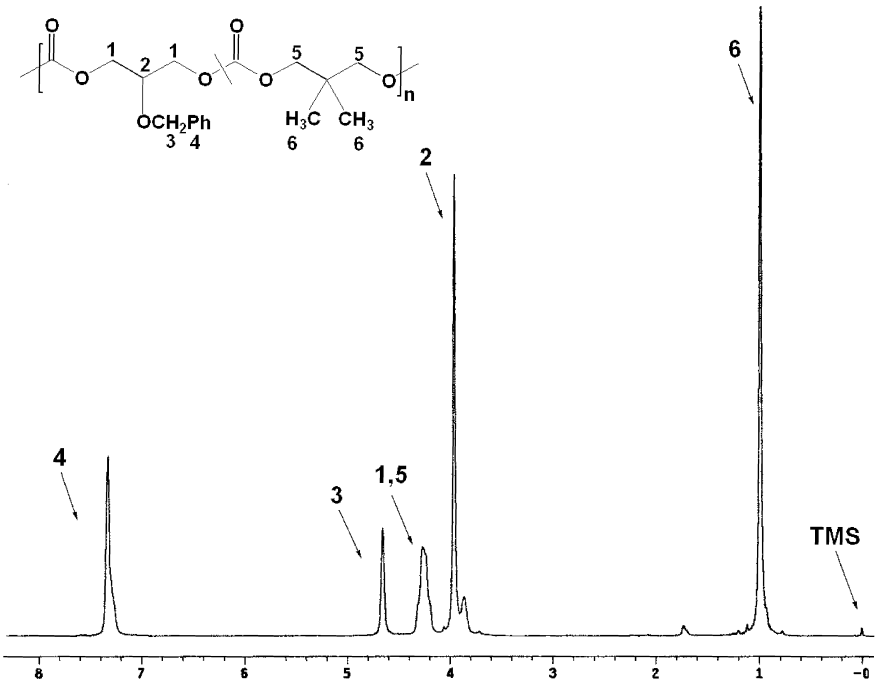


Fig. 1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of poly(BTMC-*co*-DTC) (entry 5, Table 1) obtained by immobilized PPL catalyzed ring-opening co-polymerization.

of homopolyphosphate, we found the result of enzymatic activity assay was agreed with that of enzymatic polymerization [12]. Enzyme should exhibit different catalytic activity to different monomer, such as BTMC and TMC, which would lead to unusual results in the co-polymerization reaction.

The polymer yield was also related to the kind of immobilized PPL. The yield of copolymers catalyzed by IE-1 and IE-2 was almost invariable with the increasing of immobilized PPL concentration. On the other hand, the yield of copolymers catalyzed by IE-3 decreased with the increasing of immobilized PPL concentration.

Although few studies on enzymatic ring-opening co-polymerization have been reported, it would be likely to attract more and more attentions in the future, due to its combined advantages of co-polymerization and enzymatic polymerization. In this paper, enzymatic ring-opening co-polymerization of BTMC with DTC was achieved in bulk catalyzed by immobilized PPL on three kinds of silica particles with different size. To the best of our knowledge, a number-average molecular weight (M_n) of 26,400 is the highest (M_n) of copolymers prepared from two kinds of cyclic carbonates so far. The size of immobilization carrier affected both the catalytic activity and the polymer yield. It is important that the results of enzymatic activity assay could not reflect the real catalytic capability in the co-polymerization reaction. The copolymers, after debenzylation, will provide pendent hydroxyl groups for further modification and functionalization to fit the various

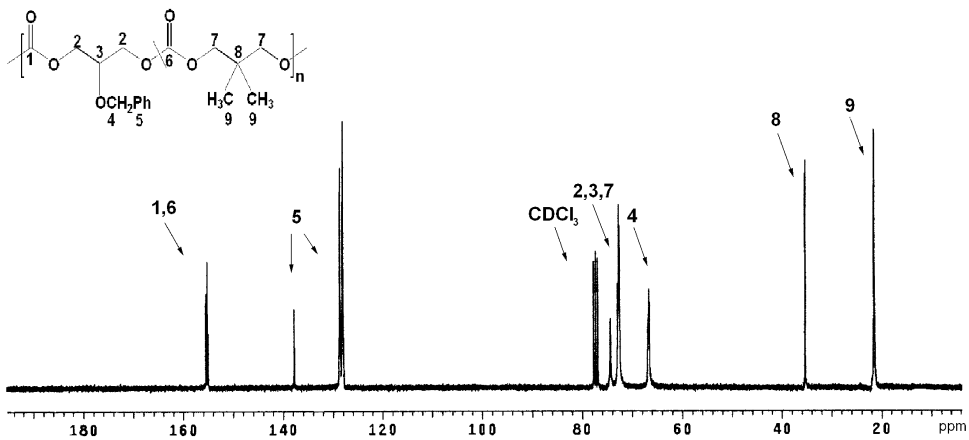


Fig. 2. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of poly(BTMC-*co*-DTC) (entry 5, Table 1) obtained by immobilized PPL catalyzed ring-opening co-polymerization.

biomedical applications. Physical, chemical, and biodegradation evaluation of poly(BTMC-*co*-DTC) with different monomer contents are currently underway in our laboratory.

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