Biocatalytic Synthesis and in Vitro Release of Biodegradable Linear Polyesters with Pendant Ketoprofen

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Enzyme-catalyzed polycondensation for the synthesis of polyester prodrugs of ketoprofen was reported. Lipase acrylic resin from *Candida antarctica* (CAL-B) was used to synthesize the linear polyesters with pendent ketoprofen groups based on ketoprofen glycerol ester, poly(ethylene glycol), and divinyl sebacate. The products were characterized by GPC and ^{1}H NMR. The results indicated that the molecular weight and yields of the polyesters depend on experimental conditions such as temperature and feed ratio. The in vitro study showed that the drug release from the polyester was slow under physiological conditions, which indicated that the polyester could be a promising prodrug with extended pharmacological effects by delayed release of ketoprofen.

1. Introduction

Ketoprofen is an important 2-Arylpropionic acid class of nonsteroidal anti-inflammatory drugs (NSAIDs), which is widely used in the treatment of pain and inflammation under many conditions.[1] However, the long-term use of NSAIDs may cause some side effects such as gastrointestinal irritation (GI), bleeding, and ulceration. The prodrug of NSAIDs for temporarily masking the carboxylic group of NSAIDs is promising to reduce or abolish the GI toxicity due to the localized effect.[2]

In recent years, polymeric prodrugs have gained prominence in the pharmaceutical field. Conjugating of drugs to polymers and their modification through the formation of covalent bonds such as ester or amide should be relatively stable to prevent drug release during its transport before the cellular localization of the drug.[3] Polymeric prodrugs of NSAIDs have been developed to reduce gastrointestinal side effects and improve delivery problems such as solubility and release rate.[4] Among synthetic polymer, poly(ethylene glycol) (PEG) has been the lead polymer for drug conjugation because of its well-known low toxicity and good biocompatibility. Choi et al. reported that ketoprofen−PEG conjugates could be a promising prodrug with extended pharmacological effects by delayed release.[5] However, the poor drug-loading capacity accompanying the available methoxy or diol forms of PEG presents a crucial limitation in the case of low-molecular-weight drug PEG conjugates.[6] Moreover, most of polymeric prodrugs have been prepared by chemical polymerization, which often used toxic catalyst and harsh reaction conditions.

An enzyme can act as a powerful catalyst for the production and chemical recycling of green and sustainable polymers. Enzyme-catalyzed synthesis of biodegradable polyesters has been developed as a novel methodology that has received much attention owing to its environmentally benignness, energy savings, atom economy, and so on.[7] Recently, Kobayashi and Makino reviewed the enzymatic polymer synthesis, which may provide an opportunity for conducting green polymer chemistry.[8] Herein we report the enzyme-catalyzed polycondensation of divinyl sebacate, PEG, and ketoprofen glycerol ester for the preparation of the polyester prodrug of ketoprofen. The in vitro study showed that the polyester can release ketoprofen effectively under physiological conditions, which indicated that the polyester could be a promising prodrug.

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2. Experimental Section

2.1. Materials. Lipase from acrylic resin from *Candida antarctica* (CAL-B), lipase from porcine pancreas (PPL), glycerol, and PEG were purchased from Sigma-Aldrich. Ketoprofen was obtained from Wuhan Gang Zheng Biology Technology (China). Ketoprofen vinyl ester was synthesized and purified as described by Wang et al.[9] Divinyl sebacate was synthesized according to the literature.[10] All other chemicals used in this work were of analytical grade and were first dried over 3 Å molecular sieves for 24 h prior to use.

2.2. Instrumental Methods. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DMX 400. Spectra were run in $CDCl_3$ and referenced to an internal TMS standard. Electrospray ionization (ESI) mass spectrometry experiments were performed on Bruker Daltonics Bio TOF mass spectrometer. The quantitative analysis of samples was made by HPLC on a reverse phase column (Welchrom-C18, 5 μm, 4.6 × 150 mm, Welchrom) using a Shimadzu LC-2010A$_{HT}$ apparatus equipped with a UV detector at 254 nm. For the analysis of ketoprofen, methanol/water 70:30 (v/v) was used as eluent (flow rate, 0.8 mL/min). Gel permeation chromatography (GPC) analyses of the copolymer molecular weight and its distribution were performed on an Agilent-1100 system consisting of 5 μm × 10 000 Å and 5 μm × 100 Å PLgel columns and a refractive index detector. The eluting solvent was tetrahydrofuran at a flow rate of 1.0 mL/min at 35 °C. The retention times were calibrated against polystyrene standards.

2.3. Synthesis of 1-*O*-Ketoprofen Glycerol Ester. The typical reaction mixture consisted of 15 mmol ketoprofen vinyl ester, 15 mmol of glycerol, 100 mg of CAL-B, and 25 mg/mL of 3 Å molecular sieves in 50 mL of acetone. The mixtures were refluxed at 70 °C for 12 h. Then, the reaction mixture was filtered and the organic solvent was removed in vacuum. All reactions were detected by thin layer chromatography (TLC) plates using petroleum ether/ethyl acetate (3:1, v/v) as eluent. 1-*O*-Ketoprofen glycerol ester: Yellow liquid (yield: 48.5%). ^{1}H NMR ($CDCl_3$, ppm): 7.82−7.43 (m, 9H, ph-H), 4.23−4.13 (m, 2H, $-CH_2$), 3.91−3.85 (m, 2H, −CH), 3.59−3.47 (m, 2H, $-CH_2$), 1.55 (d, J = 7.2 Hz, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, ppm): 196.7 (CO-ph), 174.2 (CHCO), 140.9, 137.7, 137.1, 132.7, 131.7, 129.9, 128.5 (−CH), 70.0 (−CH), 65.5 ($-CH_2$), 63.2 ($-CH_2$), 45.0 (−CH), 18.3 ($-CH_3$). ESI-MS (m/z) calcd. for [M+Na], 351.1208; found, 351.1201.

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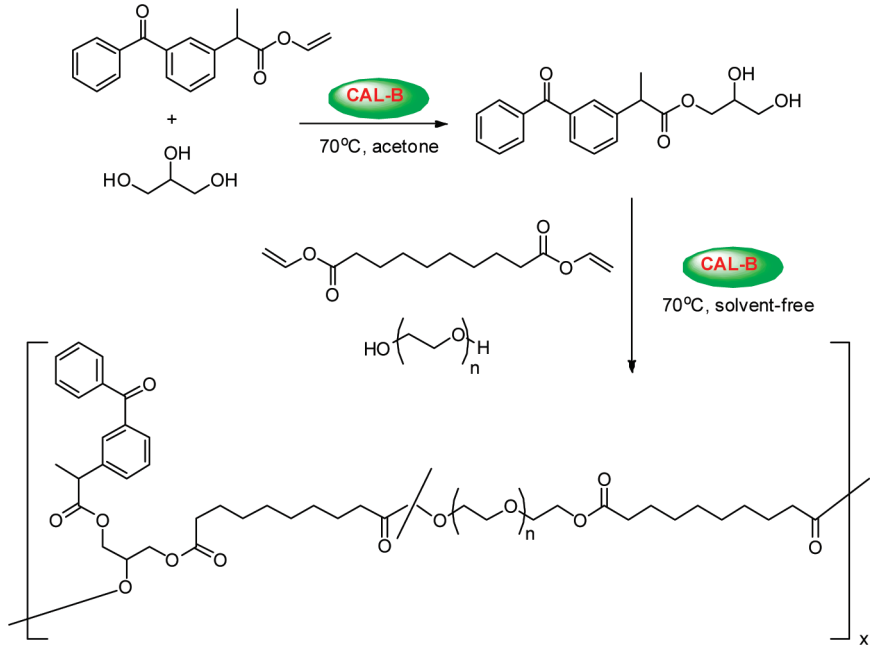
Scheme 1. Enzyme-Catalyzed Polycondensation of Ketoprofen Glycerol Ester, PEG, and Divinyl Sebacate



2.4. General Procedure for Enzyme-Catalyzed Synthesis of Polyester Prodrug. A mixture of divinyl sebacate, ketoprofen glycerol ester, PEG, and CAL-B was placed in a dried test tube. The mixture was gently stirred at 70 °C for 24 h. A small amount of chloroform was added to the mixture, and the organic solution was separated by filtration. The filtrate was poured in a large amount of a mixture of water and methanol (50:50, v/v). The resulting oily precipitates were collected by centrifugation, followed by drying in vacuum.

2.5. In Vitro Release Studies. The chemical and enzymatic hydrolysis of the polyester prodrugs was studied using pH 7.4 phosphate buffer, artificial small intestinal fluid (pH 6.8 phosphate buffer with trypsin), and artificial gastric juice (pH 1.2 hydrochloric acid aqueous solution with pepsin). The polyester prodrug for hydrolysis was suspended in 70 mL of buffer solution, which was shaken at 37 °C. At appropriate time intervals, samples were withdrawn and diluted with mobile phase for analysis by HPLC. We determined hydrolysis rates of polyester prodrug by monitoring the production of parent drug with HPLC.

3. Results and Discussion

3.1. Enzyme-Catalyzed Transesterification between Glycerol and Ketoprofen Vinyl Ester. Enzymatic selective synthesis of 1-*O*-ketoprofen glycerol ester is shown in Scheme 1. The ketoprofen vinyl ester was used in the transesterification with glycerol catalyzed by CAL-B in acetone because the vinyl alcohol freed from the reaction rapidly tautomerized to acetaldehyde, making the process irreversible and simple for product isolation. The products were purified by silica gel chromatography and characterized by MS and NMR spectrometries.

The acylation position in the products was characterized by NMR. According to the general strategy described by Yoshimoto et al., acylation of a hydroxyl group of glycerol would lead to the *O*-acylated carbon downfield, whereas it would lead to the adjacent carbon upfield in the ^{13}C NMR.[11] Analysis of ^{13}C NMR spectra of the products revealed that the reactions occurred on the 1-OH position of the glycerol. In the ^{13}C NMR spectrum of products, the peak of C1 results in a downfield shift from 63.5 to 65.6 ppm, whereas the peak of C2 results in an upfield shift from 72.3 to 69.9 ppm.

3.2. Enzyme-Catalyzed Polycondensation for the Preparation of Polyester Prodrug. Similarly, divinyl dicarboxylates were used as effective monomers for enzymatic polymerization,[12] and some fatty acid such as sebacic acid have been shown to be biocompatible both in vitro and in vivo.[13] PEG can be safely administrated in vivo, and covalent conjugation of PEG to drug can increase the plasma residence time.[14] The biodegradable polyester of PEG and diacid monomers may be useful for tissue engineering applications and drug delivery systems;[15] then, the enzyme catalyst copolymerization of glycerol ester and PEG and divinyl sebacate was investigated.

Polycondensation of PEG, ketoprofen glycerol ester, and divinyl sebacate with CAL-B as the catalyst are shown in Scheme 1. The reactions were carried out at 70 °C at a molar ratio of PEG, ketoprofen glycerol ester, and divinyl sebacate of 1:1:2 without solvent. The yields and molecular weight of the polymers are shown in Table 1.

The temperature effect on the polymerization has been examined (Table 1, polyesters A−C). Three independent experiments have been done in triplicate for each temperature condition, and we found that the reproducibility was good. The temperature was found to influence not only the polymer molecular weight but also the yield of the resulting polyester. The polymer molecular weight increased as a function of temperature, whereas the polymer composition was almost the same. The highest yield (73.7%) resulted at 70 °C. Therefore, we chose 70 °C for the further investigation of the polycondensation.

The polymerization in the different feed ratio of ketoprofen glycerol ester and PEG was examined (polyesters C−E). The use of 1:2:3 ratio led to higher M_w of the final polymer than the use of either 1:1:2 or 2:1:3 ratio, but the 1:1:2 ratio obtained better yield than the others. These data suggest that the polymer structure can be controlled by changing the feed ratio.

Similar experiments were performed while the PEG or divinyl dicarboxylates were changed (polyester F−H). The unit ratio between the PEG and glycerol ester and divinyl units was shown in Table 1, which was relatively close to the feed ratio. The molecular weight and yield of polyester F (PEG400 and divinyl

Table 1. Synthesis of Ketoprofen Polyester with PEG and Divinyl Dicarboxylates[a]

code	PEG	temp (°C)	K/P/D feed ratio	obsd K/P/D (mol %)[b]	yield (%)[c]	M_w (g/mol)[d]	M_w/M_n
A	800	50	10:10:20	10:11:21	55.4	2700	2.1
B	800	90	10:10:20	10:12:22	61.9	7300	4.4
C	800	70	10:10:20	10:10:20	73.7	4500	3.1
D	800	70	10:20:30	10:19:29	27.7	5400	3.9
E	800	70	20:10:30	20:12:32	63.4	3700	3.9
F	400	70	10:10:20	10:12:22	74.5	4900	2.7
G	200	70	10:10:20	10:12:22	66.4	3500	3.3
H	800	70	10:10:20	10:8:18	36.1	3600	3.6

[a] Reaction conditions: bulk, divinyl sebacate (polyesters A−G), divinyl adipate (polyester H), CAL-B (10% w/w of monomers), reaction time was 24 h. [b] K is ketoprofen glycerol ester, P is PEG, and D is divinyl sebacate or divinyl adipate; the mol % was determined by ^{1}H NMR. [c] Methanol/water (50/50 vol %) insoluble part. [d] From GPC.

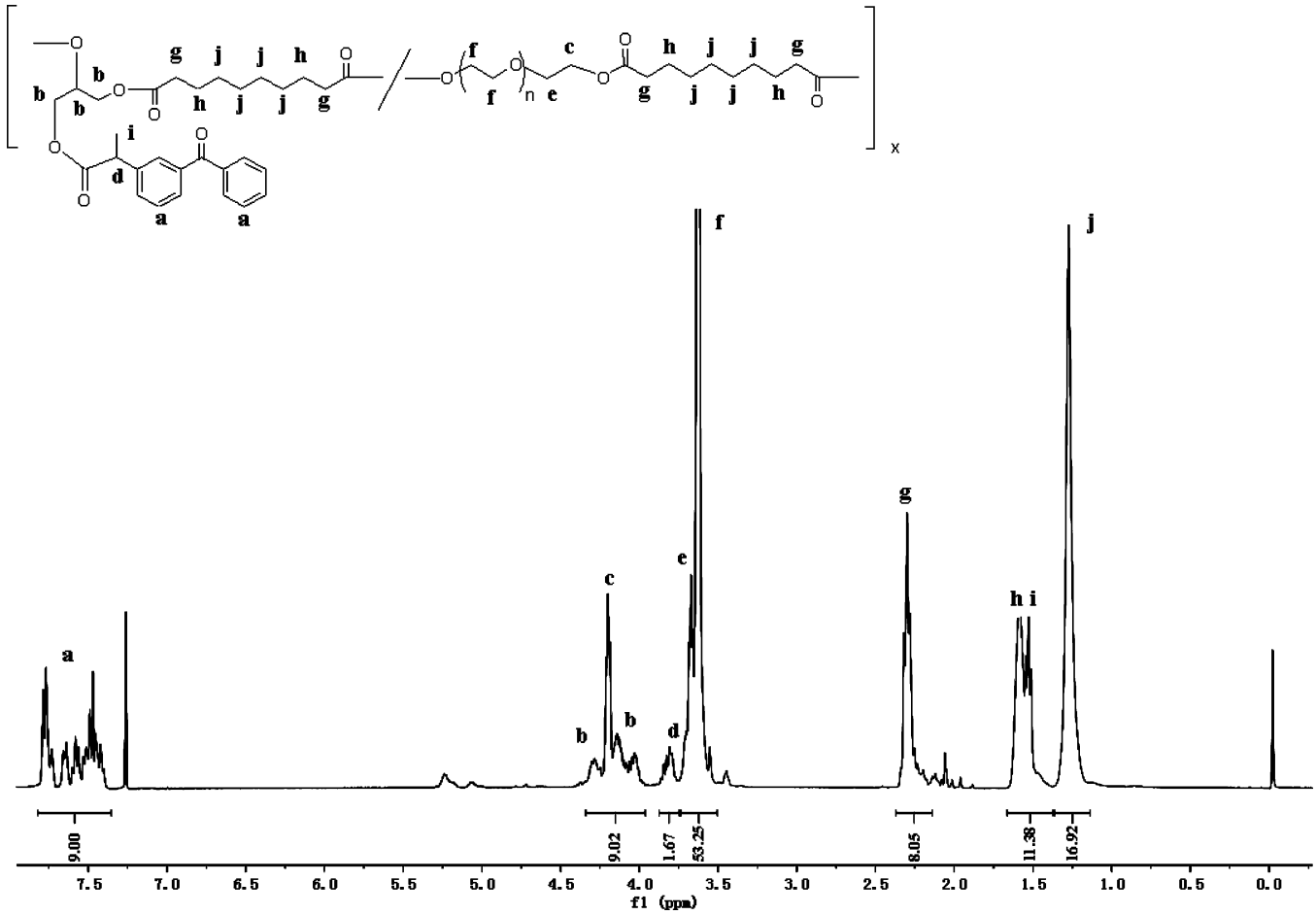


Figure 1. ^{1}H NMR spectrum of polyester C in Table 1.

sebacate) was 4900 g/mol and 74.5% respectively, which is higher than that of polyesters C (PEG800 and divinyl sebacate), G (PEG200 and divinyl sebacate), and H (PEG800 and divinyl adipate). This may be a result of the difference in enzymatic recognition for the PEG and divinyl dicarboxylates.

The structures of the copolymers formed by enzymatic condensation polymerization were analyzed by their ^{1}H NMR, as shown in Figure 1. Signals at δ 1.27, 1.6, and 2.3 were assigned to the protons from the backbone of the junction unit, whereas signals of the protons of the vinyl ester end group were hardly visible. This indicates that divinyl sebacate was reacted and the backbone was incorporated into the polymers. The broad signal at δ 3.5−3.7 was assigned to the main chain protons of the PEG. The characteristic signal at δ 4.2−4.3 was assigned to the glyceride units and repeating methylene protons of ester linkage between sebacic acid and PEG.

The molar ratio of PEG units to ketoprofen units in the polyester was determined by the ratio of the integrated ^{1}H NMR signals attributed to the methylene protons of PEG at 3.7 ppm and the phenyl proton of ketoprofen at 7.5 ppm.

3.3. In Vitro Hydrolysis of Polyester Prodrug. The in vitro hydrolysis behavior of polyester prodrug was studied under physiological conditions (aqueous phosphate and artificial small intestinal fluid and artificial gastric juice at 37 °C).

The drug release of polyester C in different buffer solutions was investigated. As shown in Figure 2, the release amount of ketoprofen was 17.6% within 14 days in the pH 7.4 phosphate-buffered saline (PBS), which may have been due to the slow rate of polymer degradation. To study the enzymatic hydrolysis of the polyester, we introduced PPL in the pH 7.4 PBS, and we observed the two-phase-release profile was. There was an initial

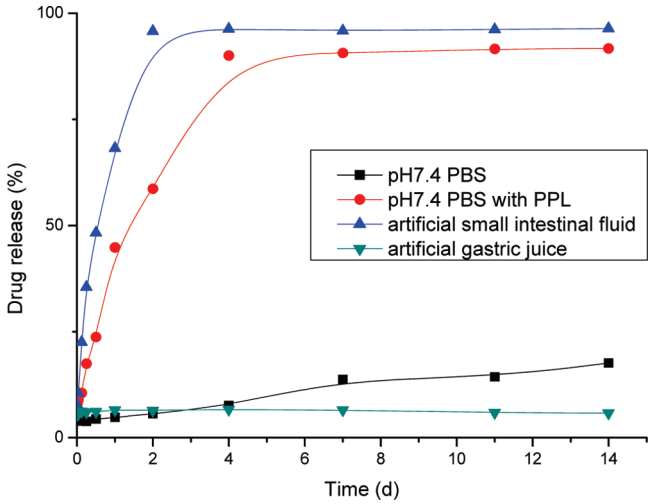


Figure 2. Drug release from polyester C incubated under physiological conditions.

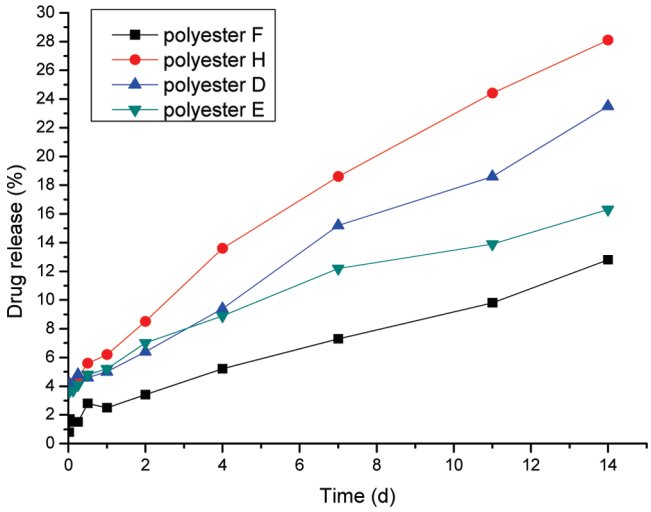


Figure 3. Drug release from polyester incubated at pH 7.4 PBS at 37 °C.

burst release of 44.8% for the initial 24 h, and the release of the ketoprofen was prolonged over 4 days.

In the in vitro release study, the polyester C showed an initial burst release of 68.2% for the initial 24 h and controlled the release for 2 days in artificial small intestinal fluid, but no parent drug was found in the artificial gastric juice over 14 days. This confirmed that polyester C can be stable in the gastric environment with the potential to release ketoprofen during body circulation.

As shown in Figure 3, the drug release from the polyester was investigated while the PEG or divinyl dicarboxylates were changed. A slow release of 23.5 and 16.3% was observed for polyesters D and E, respectively, in the pH 7.4 PBS over 14 days. The hydrolysis of polyester F was slower compared with the others, whereas polyester H was released faster with the release of 28.1%.

The CAL-B catalyst polymerization between ketoprofen glycerol ester and divinyl sebacate was studied in our previous work, but the product was so hydrophobic because all of the available −OH groups were occupied by ketoprofen and could not release the parent drug in the buffer solution. These results suggest that the introduction of PEG to the polyester may increase the release of the parent drug.

4. Conclusions

In this study, we have successfully synthesized the biodegradable polyester via enzyme-catalyzed polymerization of PEG, divinyl sebacate, and ketoprofen glycerol ester. Under the selected conditions, polyesters with molecular weights of several thousands were obtained in good yields. The hydrolysis under physiological conditions was investigated. The drug release from the polyester was slow, and the polyester was stable in the gastric environment, which may reduce the drug toxic effects. These results indicate that this polyester could be a promising NSAIDs prodrug with extended pharmacological effects by delayed release of parent drug.

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