Synthesis of poly (glycerol-*co*-dioate-*co*-butanedioate-*co*-xanthorrhizol) ester and a study of chain length effect on pendant group loading

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ABSTRACT

Natural *R*-(−)-xanthorrhizol possess a number of therapeutic activities including anti-cancer. The pharmacokinetic properties of that poorly aqueous soluble compound could be improved by incorporating it into polymeric materials. Glycerol can produce a functionalized polymer through a polycondensation process. Enzymatic polycondensation of glycerol and divinylesters was studied and xanthorrhizol was covalently loaded *via* a butanedioate linker to the polymer backbone. It was observed that xanthorrhizol loading to the polymer backbone increases with the increasing of the chain length of a dioate moiety. Enzyme-mediated xanthorrhizol release from a polymer backbone shows that the polymeric prodrug is able to release xanthorrhizol in a sustained manner. Therefore, the approach described here might be valuable for controlled loading and release of such phenolic sesquiterpenes from the polymeric prodrug.

1. Introduction

Lipase-catalyzed polycondensation of polyol and divinylesters is an efficient technique to produce highly functionalized polyesters. Polymerization can proceed by a chemical or enzyme catalysis. However, the enzymatic process has advantages over the chemical process from the viewpoints of protection–deprotection, regioselectivity [1], and green chemistry [2]. Polyol polymers were described as "sweet polyesters" [3]. Glycerol is one of the main by-products of the bio-fuel industry, which is getting priority as a monomer for polymerization [4]. The synthesis and

characterization of glycerol-based polyesters has been reported earlier [5–7]. There are a huge number of reports on the synthesis of sweet polyester, but reports on drug-incorporated glycerol-based polyester are rare. Recently ketoprofen glycerol esters and other monomers were used directly for enzymatic polymerization processes [8]. Due to its biodegradability and low toxicity this functional polymer became a promising target for use as a polymeric vehicle for drug loading and subsequent use in delivery systems. Glycerol-based polyester was used as a nano-vehicle for the encapsulation and delivery of a hydrophilic drug [9]. According to that report, drug encapsulation efficiency was improved but actual drug loading was low. Usually a physical drug encapsulation process allows random drug loading to the polymer matrix, thus making it difficult to achieve the required loading for improved therapeutic efficacy. Improved drug encapsulation has been observed when using cyanoacrylate polymers, but those systems exhibit toxicity [10,11]. So studies on the drug loading efficiency of polyol polyester have recently become interesting and need to be addressed.

Post modification of a polymer backbone is the convenient way of loading a pendant group. Functional groups of the functionalized polymer were used for covalent drug loading using a biodegradable linker. The main advantage is the absence of additional protection/deprotection steps. Moreover, a chemical drug loading process is able to load drugs to free primary and secondary alcohol positions of glycerol moiety. That is really important to increase drug loading efficiency. So a study on the drug loading efficiency of such polyesters would be interesting and could be approached by using a number of glycerol-based polyesters. Based on that assumption we used several well-defined polyesters and incorporated a

Table 1
Properties of glycerol-*co*-dioate polymer.

Polymer	M_w (KD) and PDI[a]	Regioselectivity (primary)[b] %	Regioselectivity (secondary) %	DB[b]	DS[b]
Poly (glycerol-*co*-hexanedioate) (PGH)	11, 2.18	69	31	61	2.19
Poly (glycerol-*co*-octanedioate) (PGO)	15, 1.93	74	26	55	2.00
Poly (glycerol-*co*-dodecanedioate) (PGDo)	12, 1.50	80	20	59	1.82
Poly (glycerol-*co*-tridecanedioate) (PGT)	12, 1.33	73	27	55	1.95

GPC analytical conditions: Younglin Instrument, refractive index (RI) detector at 35 °C with three Shodex LF-804 columns (300 mm × 8.0 mm I.D.) and THF as mobile phase with a flow rate 0.6 mL min^{-1}. Standard and sample concentration was 0.1% (w/w) in THF, the calibration curves were obtained using polystyrene standards with narrow r^2 value of 0.99999. M_w of calibration standards was 1.32×10^3, 4.38×10^3, 1.32×10^4, 3.27×10^4, 5.40×10^4 and 5.18×10^5 respectively. M_w of polymer sample was determined by Autochro-GPC 1.0 software automatically.

  [a] Determined by GPC under the conditions described below.
  [b] Determined from the height of glycerol carbon signal of ^{13}C-IG NMR as indicated in Fig. 2 as well as using the formula described in [7].

xanthorrhizol moiety as a pendant drug. We chose phenolic sesquiterpene as the pendant drug for their natural abundance and potential therapeutic value.

R-(−)-xanthorrhizol is the major component of the essential oils of *Curcuma xanthorrhiza* [12], a bisabolane-type natural sesquiterpene containing a stereogenic centre at the benzylic position. Natural sesquiterpenes, especially xanthorrhizol, have a variety of activities such as anti-cancer [13], anti-bacterial [14], anti-metastatic [15], anti-inflammatory [16], and estrogenic [17]. Similarly curcumin is a phenolic compound which has huge anti-cancer potential [18]. Usually compounds in this family are hydrophobic with low bioavailability [19]. Polymer drug-delivery systems can often compensate some shortcomings of small molecular drugs, such as side effects, limited water solubility, poor biocompatibility, biostability, and immunogenicity [20]. Thus developing a universal drug loading process for polyol polyesters might be crucial for the effective delivery of phenolic compounds. Stimuli-response is the predominant strategy that activates drug release from the polymeric materials [21]. To achieve a stimuli-responsive release, a linker between the drug and polymer should be enzymatically degradable, pH-sensitive, or reductively labile. That facilitates either bond-breaking between drug and carrier or destabilization of the carrier upon reaching the intended site of action. A number of enzymes work in our biological system including lipase, so we have chosen three different kinds of lipase to check the enzymatic release of xanthorrhizol. In this study, we optimized the method of synthesis and checked the enzymatic release profile and dependency of drug loading on a dioate chain length of a polymer.

2. Materials and methods

2.1. Materials

Lipase enzymes from *Candida antarctica* B (CAL-B), *Porcine pancreas* (PPL), and *Aspergillus oryzae* (AOL) were used with an activity of 120 U/mg, 20 U/mg, and 2 U/mg, respectively. Enzymes collected from Novozyme Co., Amano and Aldrich. CAL-B, commercially known as Novozyme-435, had been dried over P$_2$O$_5$ at RT for 2 days prior to use. Succinic anhydride, divinyl hexanedioate, and other reagents were purchased from Sigma–Aldrich and TCI with sufficient purity. Divinyl esters of other diacids were synthesized with little modification to the described transvinylation reaction with more than 90% yield [22]. Briefly, diacids were treated with an excess amount of vinyl propionate in presence of mercuric acetate and sulfuric acid as catalysts. Several backbone polymers were synthesized according to this method [9] with little modification. Plant extract of *C. xanthorrhiza* containing 20% (w/w) of *R*-(−)-xanthorrhizol was obtained from Genofocus Inc. Korea Ltd.

2.2. Characterization

SEC analysis was carried out using a Younglin Instrument equipped with a refractive index (RI) detector at 35 °C under the following conditions: three Shodex LF-804 GPC columns were connected in a series and the THF eluent was at a flow rate of 0.6 mL min^{-1}. The calibration curves were obtained using polystyrene standards with a narrow r^2 value of 0.99999. ^{1}H, ^{13}C, and ^{13}C-IG NMR were recorded by 400 and 100 MHz respectively, with a Bruker Avance 400 instrument using DMSO-d_6 or CDCl$_3$ as a solvent and TMS as an internal standard. The regioselectivity, the degree of substitution (DS), and the degree of branching (DB) of those polymers were calculated from ^{13}C IG NMR as reported [7]. Xanthorrhizol loading (%) in the polymer was determined by ^{1}H NMR as well as quantitatively (w/w) by chemical hydrolysis of the polymer network. Backbone and prodrug polymer characterization data are provided in Tables 1 and 2, respectively. The lipase-catalyzed release profile of xanthorrhizol was analyzed by a Younglin binary gradient HPLC system equipped with a UV detector at 254 nm, chiral column: Chiralpak AS-H (Daicel chemical Ind. Ltd.), 250 mm × 4.6 mm, 5 μm, mobile phase: hexanes: IPA = 97:03 (v/v) with a flow rate of 0.4 mL min^{-1} at RT. The retention time of xanthorrhizol was 13.5 min under the above condition. The solvents used for HPLC analysis were collected from JT Baker.

2.3. General process for synthesis of glycerol-co-dioate polymer

A series of backbone polymers were synthesized using glycerol and a variety of divinyl esters as shown in Scheme 1. Briefly for poly (glycerol-*co*-hexanedioate) (PGH), a 500 mL one-necked bottle equipped with a centered mechanical stirrer was charged with divinyl hexanedioate (49.55 g, 250 mM), glycerol (23.38 g, 250 mM), and 20 mL anhydrous THF. This mixture was stirred for 30 min to allow reactants to warm to the oil bath temperature of 50 °C. The enzyme Novozyme-435 (1.45 g, 2% of the monomer's weight) was then added to the mixture and stirred at 200 rpm using an overhead Labortechnik IKA RW11 basic stirrer fitted with an SS paddle for 24 h. During the reaction, a sample was drawn at 1, 4, 19, and 24 h and analyzed by ^{1}H NMR to check the reduction of the vinyl signal. Finally the reaction mixture was diluted with sufficient THF and Novozyme-435 was removed by suction filtration using Whatman filter paper followed by washing with 50 mL of THF. The solvents were removed by rotary evaporation followed by high vacuum for 3–4 days. 43 g of polyester (*i.e.* 59% yields for PGH, calculated on the basis of used monomer weight) was collected as a pale yellow viscous liquid, which was used for the next step without further purification. The polymer was characterized by GPC, ^{1}H, and ^{13}C-IG NMR. Polymer properties and analytical data are tabulated in Table 1. Other glycerol-*co*-dioate polymers were synthesized by a similar process using glycerol