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Functional polylactide via ring-opening copolymerisation with allyl, benzyl and propargyl glycidyl ethers

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ABSTRACT

A versatile and simple strategy is presented to synthesize reactive polylactide derivatives and their block copolymers with polyethylene glycol. Commercially available glycidyl ethers with an allyl, benzyl or propargyl functional group were copolymerised with D,L-lactide. Tin(II)-2-ethylhexanoate-catalysis produced polymers with up to 4.6, 5.9 and 2.3 allyl, benzyl or propargyl groups per chain, respectively. In contrast, less than one reactive group per chain was obtained with the organocatalyst 1,5,7-triazabicyclo[4.4.0]dec-5-ene. By increasing the polymerisation feed ratio in glycidyl ether polymers with a higher number of reactive groups per chain were obtained, however a decrease in molar mass was observed. An azidocoumarin was conjugated to the propargylated polymers *via* copper-catalysed azide-alkyne cycloaddition. These dye-labelled polymers produced nanospheres with fluorescent properties and diameters in the 100-nm size-range, as characterised by asymmetric flow field flow fractionation hyphenated with fluorescence, static and dynamic light scattering detection. The functionalised polymers were obtained at gram-scale in one step from commercially available reagents; therefore providing a robust and easy to implement approach for the production of multifunctional nanomaterials.

1. Introduction

Polylactide is widely used in the field of biomaterials and drug delivery due to its exceptional biocompatibility and bioresorbability. The hydrophobic character of this polymer makes it suitable for the preparation of polymeric nanocarriers capable of encapsulating poorly soluble lipophilic drugs in aqueous suspensions. In such applications, where molar masses above 10,000 g mol^{-1} favour *in vitro* [1] and *in vivo* stability of the nanoparticles, PLA is prepared via ring-opening polymerisation (ROP). In order to allow the conjugation of drugs, biological ligands or medical imaging probes great efforts have been made to introduce functional groups along the polymer chain. The most successful approach consists in the copolymerisation of lactide with functional derivatives of lactide or glycolide, as recently reviewed in [2]. The similar reactivities of the co-monomers allow high levels of incorporation of the functional co-monomers. However, the applicability of this method is limited by the difficulty in synthesizing the functional cyclic esters, since the alpha-hydroxy acid precursors require tedious multi-step synthesis and purification. In addition, a “copolymerisation” approach is particularly appealing because it allows the preparation of block copolymers of functional PLA with polyethylene glycol (PEG), the amphiphilic block copolymer of choice for the preparation of long-

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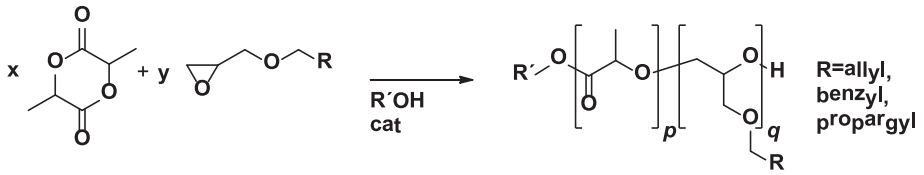

Scheme 1. Introduction of allyl, benzyl or propargyl functional group along a polylactide chain via copolymerisation of D,L-lactide with glycidyl ethers.

circulating nanocarriers [3]. Consequently, more readily-available functional co-monomers have been sought after for copolymerisation with lactide.

Within the library of compounds susceptible to undergo ring-opening (co)polymerisation, glycidyl ethers may be obtained from commercial sources with a variety of functional groups. In addition, conjugation chemistries with these monomers are already described for their homo- and copolymers [4]. In particular, allyl glycidyl ether (AGE) and propargyl glycidyl ether (PGE) incorporated in a polymer allow its direct modification under "click" chemistry conditions, such as thiol-ene for AGE [5] and thiol-yne and the 1,3-dipolar cycloaddition with organic azides for PGE [6]. The benzyl ether group in benzyl glycidyl ether (BGE) is a precursor to the hydroxyl group, readily obtained via catalytic hydrogenation, and for which derivatisation and coupling strategies are available.

Copolymerisation of lactide with glycidyl ethers is an attractive strategy towards functionalised polylactide derivatives for the ease of implementation. Nonetheless, limitations have already been identified, which are a low degree of incorporation of the epoxide in the copolymer, even at high epoxide feed ratio, and a detrimental impact of the initial epoxide feed ratio on the resulting polymer molar mass and molar mass distribution. In the present study we investigate the potential and limits of this approach in terms of co-monomer incorporation for three reactive glycidyl ethers, namely AGE, BGE and PGE (Scheme 1), and the impact on the copolymer molar mass and molar mass distribution. The influence of the ROP catalyst was studied by comparing the copolymer composition, monomer conversion and polymerisation times under $SnOct_2$ or TBD catalysis.

The present synthetic strategy was applied to the preparation of a macromolecular fluorescent probe, whereby an azide-functional coumarin was conjugated to alkyne-functional PLA or PEG-*b*-PLA. The conjugates self-assembled into fluorescent nanospheres, which narrow size distribution was assessed upon fractionation under asymmetric flow field flow fractionation (AF4) with fluorescence, multi-angle light scattering (MALS) and dynamic light scattering (DLS) characterisation.

2. Experimental

2.1. Materials

The monomer 3,6-dimethyl-1,4-dioxane-2,5-dione (D,L-lactide, Sigma, 98%) was purified by three recrystallisations from dry toluene. Benzyl alcohol, used as a polymerisation initiator, was distilled under reduced pressure (160 °C, 70 kPa). The polymerisation catalyst tin (II) 2-ethylhexanoate ($SnOct_2$, Sigma, 95%) was dried under reduced pressure (7×10^{-2} mbar) and stored under argon and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, Sigma-Aldrich, 98%) was dried by three azeotropic distillations with toluene and stored under argon. 3-(α-Azidoacetyl)coumarin was prepared from 3-(bromoacetyl)coumarin (Aldrich, 97%) according to a literature procedure [7]. Dry dichloromethane (Aldrich, 99%) was stored over 3 Å molecular sieves and degassed under a flow of argon for 30 min prior to use in polymerisations. Allyl glycidyl ether (AGE, Aldrich, > 99%), benzyl glycidyl ether (BGE, Aldrich, 99%), glycidyl propargyl ether (PGE, Aldrich, 95%), poly(ethylene glycol) monomethyl ether ($mPEG_{5k}$, M_n 5000 g mol^{-1} and $mPEG_{2k}$, M_n 2000 g mol^{-1}), poly(ethylene glycol) (PEG_{2k}, M_n 2050 g mol^{-1}), sodium azide (Sigma, 99.5%), dry toluene (Aldrich, 99%), anhydrous Na_2SO_4, $CuSO_4$, L-ascorbic acid, sodium bicarbonate, and PA grade dichloromethane, hexane, propan-2-ol and acetone from VETEC (Brazil) were used as received.

2.2. Characterisation

^1H and ^{13}C NMR experiments were recorded at 25 °C on a Bruker AVANCE DRX400 MHz spectrometer. Copolymers with BGE were analysed in DMSO-d_6 and all other compounds in $CDCl_3$, with tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) are given in parts per million (ppm). The molar mass distribution of the polymers was characterised by gel permeation chromatography (GPC) on an Agilent Technologies 1260 Infinity unit comprising a solvent degasser, isocratic pump, an Agilent 1260 Infinity UV detector (G1314F) and a differential refractive index detector (G1362A RID at 35.0 °C) in series, a Varian PLgel 5 μm MiniMix-D 50 × 4.6 mm pre-column and two Agilent PLgel 5 μm MiniMix-D 250 × 4.6 mm columns in series at 30.0 °C with HPLC grade THF with 250 ppm BHT stabilizer as the eluent at a flow of 0.25 mL/min. The samples were prepared at a concentration of 3 mg/mL in the eluent, filtered (0.22 μm PVDF Millipore filter) and the injection volume was 20 μL. The system was calibrated using Agilent Technologies EasiVial narrow dispersion polystyrene standards (162–371,100 g mol^{-1}). Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrometer.

The polymeric nanosphere suspensions were diluted at 1:500 in 1 mM NaCl and characterised in terms of Z-average hydrodynamic diameter (D_h) by dynamic light scattering (DLS) and zeta potential by electrophoretic light scattering on a Zetasizer Nano ZS (Malvern Instrument, UK) equipped with a He-Ne Laser at 633 nm. D_h were calculated with cumulant analysis