Enzyme-catalysed condensation polymerization of 11-hydroxyundecanoic acid with lipase from *Candida cylindracea*

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The enzyme-catalysed condensation polymerization of 11-hydroxyundecanoic acid in hexane with the lipase from *Candida cylindracea* is reported. Molecular weights up to 35 000 are attained. A time course study of the polymerization process reveals that oligomers are formed relatively rapidly and that these oligomers then condense to generate higher molecular weight polyesters.

(Keywords: condensation polymerization; lipase; 11-hydroxyundecanoic acid)

Introduction and results

A number of papers[1–4] have reported the enzyme-catalysed condensation polymerization (ECCP) of diols and diacids/diesters to generate polyesters. In these systems, polyesters with $M_w \leqslant 14\,900$ have been reported[2]. Similarly, the ECCP of hydroxy acids and esters has been demonstrated[5] with porcine pancreatic lipase. Recently we reported[6] the ECCP of 10-hydroxydecanoic acid with the lipase from *Candida cylindracea*. The polymerization was carried out under optimized conditions in hexane at 55°C with added molecular sieves. After 48 h a material with $M_n = 9346$, $M_w = 12\,065$ and a polydispersity of 1.29 was isolated.

One problem in our initial study[6] was that the 10-hydroxydecanoic acid monomer used was contaminated with about 1% of the C_{10} diacid, sebacic acid. This led to end-group capping and resulted in approximately half of the polymer molecules containing carboxylic acid residues at each end. Analysis by 400 MHz ^1H n.m.r. demonstrated that the ratio of the $HOCH_2$– to –CH_2CO_2H end groups was 1:3. It was conjectured that the resultant diacid molecules are unable to participate further in enzyme-mediated elongation and therefore we were concerned that the sebacic acid was suppressing the average molecular weight of the polyester. We have been unable to obtain pure 10-hydroxydecanoic acid and turned our attention to 11-hydroxyundecanoic acid which we could prepare in a straightforward manner. Under optimized conditions (hexane, 55°C, 3 Å molecular sieves, 15 g lipase)[6] (*Scheme 1*), a material with $M_w = 22\,430$ was isolated after 7 days. It was clear from 400 MHz ^1H n.m.r. analysis that the ratio of the $HOCH_2$– to –CH_2CO_2H end groups was 1:1, confirming our suspicion that, in the C_{10} hydroxy acid experiment, sebacic acid was involved in end-capping the polymer chains.

A time course study of the polymerization of the C_{11} monomer was conducted, and gel permeation chromatography (g.p.c.) profiles at various times over an 8 day period are shown in *Figure 1*. In the early stages, low molecular weight oligomers (traces b–d) are rapidly assembled, and from these data and n.m.r. analysis it is clear that all of the monomer is consumed within 5 h. The oligomers continue to increase in molecular weight with time and the g.p.c. traces clearly show that there is an upper limit at $M_w = 35\,000$. This limit is well defined and is almost certainly set by the insolubility of the high molecular weight polyester chains in hexane. Towards the later stages of polymerization (>5 days), there is a gradual increase in low molecular weight oligomers which compromises the polydispersity of the polyester. This is most obvious in traces g and h, and it would appear that the polyester is slowly disproportionating to thermodynamically more stable oligomeric species. The polymerization has been studied up to 20 days (data not shown), and these low molecular weight species become increasingly significant over this time. Our data suggest, therefore, a rapid (~5 h) lipase-catalysed assembly of low molecular weight oligomers by sequential condensation of the monomer onto the developing chain. This is followed by a second phase of oligomer condensation to generate the higher molecular weight polyester. The length specificity displayed by the enzyme for the C_{10} and C_{11} monomers over the C_8 and C_{12} monomers, which do not polymerize, is presumably exercised in this early phase of oligomer assembly. However, the enzyme appears to have an additional capacity to mediate the condensation of oligomers, albeit at a reduced rate. Our efforts are now focused on studying ECCP with *C. cylindracea* using pure C_9 and C_{10} hydroxyacid monomers to test whether similar molecular weights can be achieved, and to probe in further detail the mechanism of this remarkable polymerization process.

Experimental

N.m.r. spectra were recorded on a Varian VXR-400S spectrometer (^1H n.m.r. at 400 MHz and ^{13}C n.m.r. 100.5 MHz). G.p.c. was performed on Waters 590 Series chromatographic equipment. Three columns (300 × 7.7 mm, Polymer Laboratories Ltd) packed with polystyrene PL gel, with particle size 5 μm and pore sizes 100,

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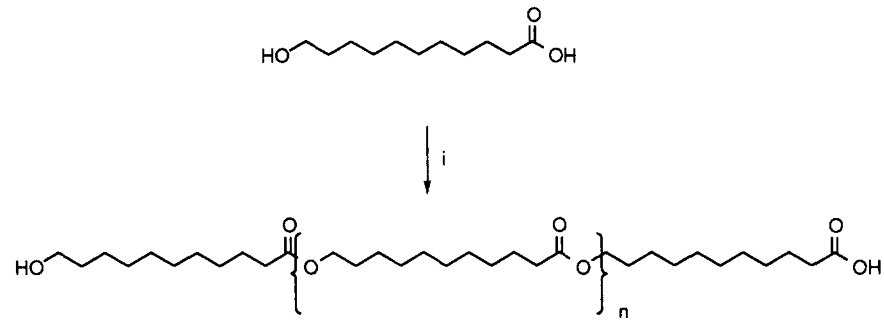

Reagents and conditions: i, hexane, 55°C, 3Å mol sieves, *C. cylindracea* lipase

Scheme 1

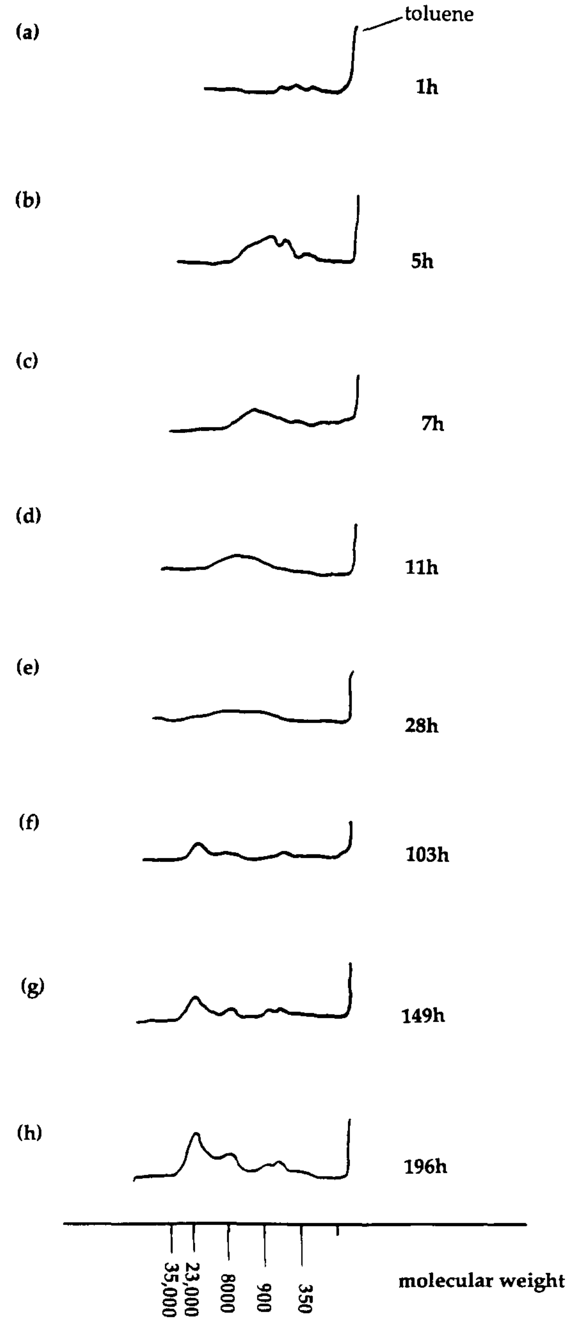

Figure 1 The traces a–h are g.p.c. chromatogram profiles of poly(11-hydroxyundecanoic acid) taken at various time intervals from the *C. cylindracea* lipase-mediated polymerization of 11-hydroxyundecanoic acid. The molecular weight scale is referenced relative to commercial polystyrene standards

10^3 and 10^5 Å, were used in series. A Waters differential refractometer (model 401) was used as a detector. Polyester samples were dissolved in chloroform. The molecular mass averages were computed using Polymer Lab software, and calibrated against commercial polystyrene standards.

Preparation of 11-hydroxyundecanoic acid. A solution of 11-bromoundecanoic acid (25 g, 0.1 mol) and potassium hydroxide (15 g, 0.27 mol) in water (150 ml) was heated under reflux for 7 days. The reaction mixture was cooled, filtered and the filtrate evaporated under reduced pressure. The resultant residue was recrystallized twice from methanol and was then dissolved in water (200 ml) and acidified to pH 1 with 6N HCl. The aqueous solution was extracted into diethyl ether (2 × 150 ml) and the ether extracts were washed with water and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded a crude product, which was recrystallized from ethyl acetate, to give the title compound (8.6 g, 45%) as a white amorphous solid, m.p. 67–70°C (lit.[7] 65–67°C). ν_{max} (cm^{-1}) (KBr), 3351 (OH), 2850–2935 (CH), 1714 (C=O); δ_H ($CDCl_3$) 6.4–6.7 (bs, 2H, OH), 3.63 (t, 2H, $\underline{CH_2}OH$), 2.33 (t, 2H, CH_2CO), 1.7–1.2 (m, 16H, CH_2s); δ_C ($CDCl_3$) 24.65 (CH_2), 25.63 (CH_2), 28.97 (CH_2), 29.13 (CH_2), 29.25 (CH_2), 29.28 (CH_2), 29.41 (CH_2), 32.53 (CH_2), 34.03 (CH_2), 62.91 (CH_2O), 179.45 (C=O). Found: C, 65.51, H, 11.29; $C_{11}H_{22}O_3$ requires C, 65.35, H, 10.89%.

Preparation of poly(11-hydroxyundecanoic acid). The *C. cylindracea* lipase (15 g, 860–2425 units mg^{-1} protein, Sigma Chem. Co. type VII) was added to a 100 cm^3 conical flask containing a suspension of 11-hydroxyundecanoic acid (1.5 g, 7.4 mmol) and pellets (4.2 mm) of activated 3 Å molecular sieves (20 g, Sigma Chem. Co.) in hexane (70 ml^3). The flask was stoppered with a septum seal and placed on a shaker (200 rev min^{-1}) at 55°C for 103 h. Aliquots (4 cm^3) were extracted periodically for g.p.c. analysis. The mixture was then filtered under suction and the insoluble material washed with dichloromethane (3 × 10 cm^3). Removal of the solvent under reduced pressure afforded the title polyester as a white solid, m.p. 78.5°C. ν_{max} (cm^{-1}) (KBr), 3446 (OH), 2925–2850 (CH), 1728 (C=O) and 1186 (C–O); δ_H ($CDCl_3$) 4.05 (t, OCH_2), 3.62 (t, OCH_2, end group), 2.35 (t, CH_2CO_2, end group), 2.29 (t, CH_2CO_2), 1.6 (m, CH_2), 1.55 (bs, CH_2) and 1.29 (bs, CH_2); δ_C ($CDCl_3$) 174.48 (C=O), 64.88 (OCH_2), 34.87 (CH_2CO_2), 29.95 (CH_2), 29.87 (CH_2), 29.73 (2 × CH_2), 29.64 (CH_2), 29.13 (CH_2), 26.41 (CH_2), 25.49 (CH_2). $M_n = 19967$, $M_w = 22430$, $M_w/M_n = 1.12$ (g.p.c. after 7 days).

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