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Enzyme mediated polyester synthesis with the lipase from *Candida rugosa*

Preparation of an enantiomerically enriched polymer from an A-B monomer

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Summary

We have previously demonstrated an efficient polyester synthesis of ω-hydroxyacids catalysed by the *Candida rugosa* lipase in dry hexane (1,2). This method is now extended to the polymerisation of racemic 10-hydroxyundecanoic acid to explore the polymerisation of a secondary alcohol and the ability of the lipase to display stereoselectivity. The resultant polyester (~1000 Mwt) contained monomer units with an average enantiomeric excess of 60%ee favouring the (*S*) enantiomer. The residual monomer was recovered with a 33%ee favouring the (*R*) enantiomer. The origin of this stereoselectivity has been evaluated and it has been shown to arise due to the immediate chiral environment surrounding the esterification event between the acylated enzyme and the secondary alcohol nucleophile. It is not due to recognition of the remote secondary alcohol functional group by the enzyme during initial acylation.

Introduction

The ability of lipase enzymes to catalyse polyester formation has been recognised now for over a decade and there are many examples of polyesters of the A-A/B-B (eg. diols and diacids) (3-7) and A-B (eg. hydroxyacids (1,2), hydroxyestersand lactones (10, 11)) types which have been generated in this way. In general the molecular weights are low rising to maximums of ~20,000, however several patents (12, 13) have highlighted the potential of this technology in the commercial arena. One of the potential attractions of using enzymes in polymer synthesis lies in the ability of enzymes to mediate stereoselective processes. The possibilities here were demonstrated in an early report (14) and patent (15) by Wallace and Morrow on polyester preparation using porcine pancreatic lipase where an A-A/B-B polyester was generated by lipase catalysis between butane diol and (±)-bis(2,2,2-trichloroethyl)epoxyadipate. An enantiomerically enriched polyester was recovered which contained the epoxide monomer with an average enantiomeric excess of 95%ee. This example advertised the potential of lipase to generate optically enriched polyesters from racemic monomers, however it remains one of few examples (16) in the literature of such enantiomeric enrichments. A recent report (17) by Kobayashi has demonstrated that the enantiomerically pure monomers of the lactone 3)-methyl-4-oxa-6-hexanolide are polymerised at significantly different rates by a lipase, however the ability of the enzyme to *resolve* a racemate was not reported. There are no reports of lipase induced enantioselectivity for the generation of an A-B type polyester, from eg. an (ω-1)-hydroxy acid, ester or lactone.

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We have been exploring the polymerisation of ω-hydroxy acids with the lipase from the yeast *Candida rugosa* (1, 2). The lipase is a good catalyst for polyester synthesis and the initial rates of polymer assembly are particularly rapid. This therefore provided an attractive experimental system in which to explore the polymerisation of a secondary alcohol and to investigate the enantioselectivity of the process. The results of an investigation using racemic 10-hydroxyundecanoic acid as a monomer are discussed below.

Experimental

Preparation of 10-hydroxyundecanoic acid (19); Undecylenic acid (3.7g, 20.0 mmol) was added to a stirred solution of mercuric acetate (6.4g, 20.0 mmol) in water : THF (1 : 1, 40ml). After 30 min a 10% solution of NaOH (10ml) and a 1.3M solution of $NaBH_4$ in 10% NaOH (15ml) was added and after stirring for 1 h the layers were allowed to separate and the organic phase was removed, (1.9g, 48%); mp 32.5°C (lit. (18) 34°C); δ_H ($CDCl_3$, 400MHz), 3.79 (1H, m, $CH_3C\underline{H}OH$), 2.34, (2H, t 7.6Hz, $C\underline{H}_2COOH$), 1.61 (2H, m, $C\underline{H}_2CH_2COOH$), 1.34 (12H, m, CH_2's), 1.18 (3H, d 6.4Hz, CH_3); Anal: found C 65.21, H 11.12, $C_{11}H_{22}O_3$ requires C 65.35, H 10.89%.

Polymer preparation; A suspension of 10-hydroxyundecanoic acid (200mg) and the *Candida rugosa* lipase (2.0g, Sigma Chem Co. Type VII) in dry hexane (50ml) was placed in a 100ml flask and stoppered with a septum seal. The flask was then placed on an orbital shaker (200 rpm) and incubated at 55°C for 6h. removal of the solvent gave a product which was purified over silica gel (CH_2Cl_2: acetone, 7:1) to separate the polymer (Mw 1000, PDI = 1.3) from the residual monomer.

Chiral analysis; The polymer was treated with aqueous NaOH and the reaction acidified (aq. H_2SO_4) and extracted into ether to regenerate the monomer. Both the residual monomer and this recovered monomer were then derivatised using (*R*)-MTPA chloride following a standard protocol in pyridine with DMAP (20). The resultant (*R*)-MTPA esters were then purified by chromatography (CH_2Cl_2: acetone, 20:1) and isolated as colourless oils, and gave satisfactory analytical data. (*R*)-2-Octanol and racemic octanol were similarly derivatised to provide reference compounds for absolute configuration assignment by ^{1}H-NMR analysis.

Results

In previous studies the parameters for the *C. rugosa* mediated polymerisation of ω-hydroxyacids have been explored (1,2). When these reactions are carried out using molecular sieves as an agent to remove water from the system relatively high molecular weights (~20,000) can be achieved (2, 4, 6). However over time the resultant materials display progressively larger polydispersities, and this has been attributed to non enzymatic hydrolytic reactions occurring on the surfaces of the sieves. In an attempt to control the polydispersity of the resultant polyesters, albeit at the expense of compromising molecular weight, the progress of these reactions has been explored without molecular sieves. The resultant molecular weight/time profiles are shown in Figure 1 for C_8, C_{10} and C_{11} ω-hydroxycids. A rapid initial reaction is apparent over the first hour and then the rate plateaus as the monomer is consumed (as determined by GPC and ^{1}H-NMR analysis). The oligomers then condense in a slower assembly phase. In these reactions all of the monomer is consumed within a 1 hour period.

By contrast Kobayashi has prepared (10, 11) A-B polyesters using macrocyclic lactones in a lipase mediated ring opening polymerisation. In these reactions water is not released during each condensation and there is no requirement to add a drying agent. In Figure 2

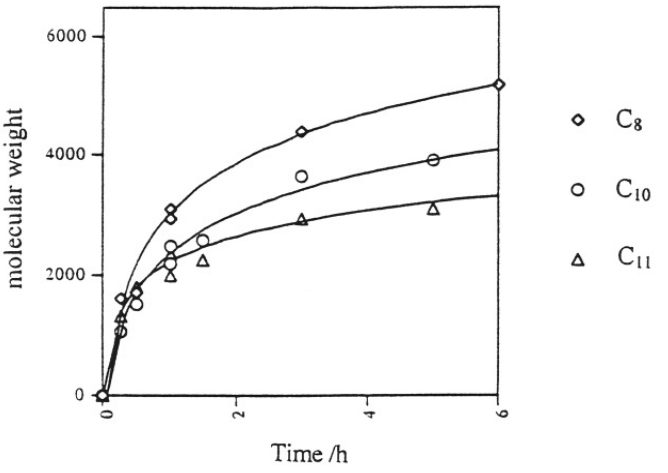


Figure 1 Comparative relationship of MWt (GPC determined) versus time (h) for the progress of *C. rugosa* lipase mediated polymerisations of C_8, C_{10} and C_{11} ω-hydroxyacids in hexane.

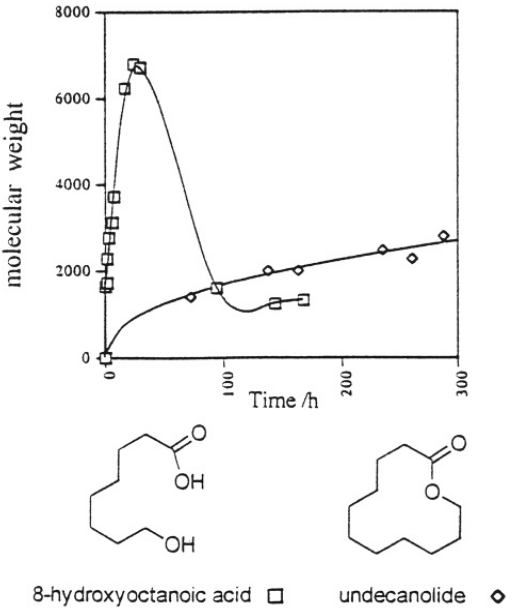
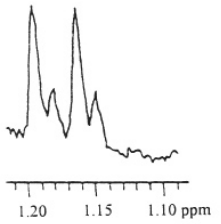


Figure 2 Comparative relationship of MWt (GPC determined) versus time (h) for the progress of *C. rugosa* lipase mediated polymerisations of 8-hydroxyoctanoic acid in hexane and neat undecanolide.

a comparison is made between the rates of polymer assembly of 8-hydroxyoctanoic acid and the lactone undecanolide. Initially lipase mediated polymerisation of 8-hydroxyoctanoic acid is much more rapid than that of the lactone, however after ~50 hours the ω-hydroxy acid derived polymer becomes substantially hydrolysed as the concentration of the water in the system increases, and the average molecular weight falls until an equilibrium is reached providing an oligomeric mixture. By contrast the undecanolide polymerisation shows a slow but steady increase in molecular weight over time.

Despite the limitations in achieving higher molecular weights under these conditions the rapid initial rates of these ω-hydroxy acid reactions offered a system where enantioselectivity could be investigated. Polymerisation of racemic 10-hydroxyundecanoic acid was explored in an attempt to generate an enantiomerically enriched polyester. The initial rate of this polymerisation was sluggish as anticipated for a secondary alcohol and the reaction progressed to approximately 50% completion after 6 hours. The polyester (~1000Mwt, 1.3 PDI) was separated from the residual monomer by chromatography and was hydrolysed back to 10-hydroxyundecanoic acid. The resultant hydroxy acid and the residual monomer were then both subjected to enantiomeric purity analysis by preparation of their respective (*R*)-Mosher's ester (MTPA) derivatives (20). The resultant diasteromeric excess was determined after ^{1}H-NMR analysis to be 60% de as shown by the region of the methyl group signals in Figure 3. The residual monomer had a 33% de. In order to assign the absolute stereochemistry to the major diastereoisomer (*R*)-Mosher's esters of (*R*)-(-)-2-octanol and racemic 2-octanol were prepared. 2-Octanol was judged to be sufficiently similar to the hydroxyl end of the monomer to act as a reliable reference compound for ^{1}H-NMR and this comparison revealed that the (*S*)-monomer was the favoured enantiomer for polymerisation.



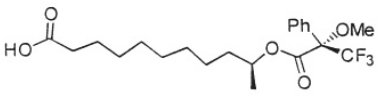


Figure 3 Selected region of the ^{1}H-NMR spectra of (*R*)-Mosher's esters of 10-hydroxyundecanoic acid recovered after hydrolysis of the polyester, showing the terminal methyl group region (1.0 – 1.2ppm). A set of diastereomers is evident in a ratio of 4:1 (60%ee) favouring the (*R, S*)-diastereoisomer.

Discussion

The enzyme has displayed a preference for incorporating the (*S*)-monomer into the resultant polymer. The discrimination by the lipase in favour of (*S*)-10-hydroxyundecanoic acid can have one of two origins as illustrated in Figure 4. The (*S*)-monomer could be acylated preferentially over the (*R*)-monomer by the enzyme, to form a predominantly (*S*)-acyl enzyme complex. If this is the case a binding interaction, between the enzyme and the stereogenic centre, remote from the site of acylation, must

control the stereoselectivity. Alternatively the enzyme does not discriminate between the (*R*)- and (*S*)- monomers during the acylation event, but the (*S*)-monomer is the preferred nucleophile for deacylation of the acylated enzyme.

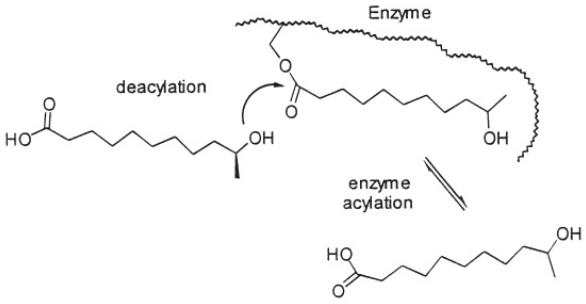


Figure 4 Catalysis involves acylation of the enzyme by monomer followed by deacylation by the hydroxyl group of another monomer. High enantioselectivity in the resultant polymer demands that both of these events are stereoselective and that the stereoselectivity is reinforcing.

In order to delineate these two possibilities the *C. rugosa* lipase mediated hydrolysis of racemic methyl 10-hydroxyundecanoate was explored to determine if the enzyme could distinguish each enantiomer during the enzyme acylation event. The reaction, which was carried out in buffer, was stopped at 50% conversion and the product hydroxy acid and the residual ester were each subjected to chiral analysis. In the event both the hydroxy acid and the residual methyl ester were shown to be racemic, after derivatisation and ^{1}H-NMR analysis of their Mosher's esters in the manner described above. In the light of this study it is concluded that the *C. rugosa* lipase *cannot discriminate* the stereogenic centre of 10-hydroxyundecanoate during acylation of the enzyme. Clearly therefore the observed enantiomeric bias in the polymer must arise at *esterification* where the secondary alcohol acts as a nucleophile for deacylation of the acyl-enzyme complex. Therefore the following sequence of events is envisaged. During the early stages of the polymerisation both the (*R*) and (*S*) monomers are acylated by the enzyme at a similar rate, however there is a preference for attack of the acyl-enzyme complex by the (*S*)-monomer, leading to a set of dimers enriched with the (*S*)-enantiomer. Those dimers with (*S*)-ends will then react faster in further deacylation events to generate trimers and tetramers of increasing enantiomeric enrichment and so on. In the event the resultant enantioselectivity suggests that the enantioselectivity of the deacylation step was moderate (> 60%ee).

Conclusion

A low molecular weight polymer was prepared from racemic 10-hydroxyundecanoic acid. The material was enriched in the (*S*)-enantiomer to a level of 60% ee and the enantiomeric enrichment arises due to a preference for the (*S*)- over the (*R*)- alcohol acting as a nucleophile during deacylation of the enzyme. It follows that to generate a polymer of high enantiomeric excess from a racemic A-B monomer at least two criteria must be met. Both the generation of the acyl-enzyme complex and the transacylation

event require to be stereoselective, and the stereoselectivity of these two independent events must reinforce each other. This problem can be overcome in an A-A/B-B polymerisation if only one of the monomers is chiral as previously demonstrated by Wallace and Morrow (15). The generation of a polyester of high enantiomeric purity from a racemic A-B monomer remains a challenge for this reason.

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