방향족 폴리에스터의 효소적 합성과 효소화학적 합성

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Enzymatic and Chemoenzymatic Approaches to Aromatic Polyesters Synthesis

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INTRODUCTION

Recently it has been shown that hydrolytic enzymes are highly stable in organic solvents and thus can be used for transformations that are difficult to perform in water [1]. The most common reactions are enzyme-catalyzed stereoselective esterification and transesterifications, which have been extensively used for the resolution of chiral acids and alcohols. Lipases[2] and proteases[3] have been used to catalyze aliphatic polyester synthesis in organic solvents. However, with the exception of alkyds[4], there appears to have been little effort to prepare aromatic polyesters with enzymes.

The major drawback with enzymatic polyester synthesis is nearly universally slow rate of catalysis and the fact that the molecular weights of the polymers are not yet as high as is desirable. A far more efficient approach would be to use enzymes only for the highly selective step(s) in polymer synthesis (such as monomer preparation) and to employ conventional chemical catalysts for the bulk polymer synthesis. This study outlines our success[5-6] in developing enzymatic synthesis of aromatic polyester and describes chemoenzymatic synthesis of aromatic polyester containing sucrose.

MATERIALS AND METHODS

Materials: Protease from *Bacillus licheniformis* (Optimase M-440) was obtained from Solvay Enzyme Co and other enzymes were obtained from commercial suppliers. Sigma Sil-A was obtained from Sigma Chemical Co to prepare TMS derivatives of sugars and all other chemicals and solvents in this work were of analytical grade and solvents were dried over 4A° molecular sieves for 24h prior to use.

Analytical Methods: The concentrations of trifluoroethyl esters and benzenedimethanol were determined by gas chromatography (GC; Varian model 3300) equipped with a flame ionization detector using a column packed with chromosorb W 80-100 mesh as solid phase and carbowax 20 M 10% as stationary phase. To determine the concentrations of all sugar derivatives, they were subjected to precolumn derivatization with 1,1,1,3,3,3-hexamethyldisilazane according to a general methodology[7] and 10-m Alltech AT-1 capillary column packed with polydimethylsiloxane was used. Helium was used as a carrier gas. Proton NMR spectra were carried out in $CDCl_3$ solution and the position of acylation in all enzymatically prepared compounds were established by ^{13}C NMR on a Bruker AMX 500. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel coated plastic or glass sheets (Merck). Spots were visualized with a UV lamp at 254 nm and were also developed by spraying with 10% H_2SO_4 in ethanol followed by heating.

Gel permeation chromatography (GPC) was carried out using a Waters Model CV 150 instrument, equipped with four connected μ-styragel columns (500, 10^3, 10^4, and 10^5A°)

and a waters RI detector using a 1 mL/min flow rate and THF as a mobile phase and the system was calibrated with polystyrene standards.

Synthesis of Trichloroethyl and Trifluoroethyl esters: Trichloroethyl and trifluoroethyl esters were synthesized from the corresponding acyl chloride and 2,2,2-trichloroethanol or 2,2,2-trifluoroethanol according to a general methodology [8].

Bis(2,2,2-trifluoroethyl) glutarate: mp = -9.33°C ; ^{1}H-NMR($CDCl_3$) : δ 2.01(multiplet, 2H, $-CH_2-\underline{CH_2}-CH_2-$) ; 2.52(triplet, 4H, $-\underline{CH_2}-CH_2-\underline{CH_2}-$) ; 4.49(quartet, 4H, $-CH_2-CF_3-$).

Bis(2,2,2-trichloroethyl) glutarate: mp = 44.40°C ; ^{1}H-NMR($CDCl_3$) : δ 2.00(multiplet, 2H, $-CH_2-\underline{CH_2}-CH_2-$) ; 2.49(triplet, 4H, $-\underline{CH_2}-CH_2-\underline{CH_2}-$) ; 4.44(quartet, 4H, $-CH_2-CCl_3-$).

2,2,2-trifluoroethylbenzoate: mp = -6.53°C ; ^{1}H-NMR($CDCl_3$) : δ 4.71(quartet, 2H, $-CH_2-CF_3$) ; 7.48(multiplet, 2H, ArH) ; 7.62(multiplet, 1H, ArH) ; 8.09(multiplet, 2H, ArH).

2,2,2-trichloroethylbenzoate: mp = -6.08°C ; ^{1}H-NMR($CDCl_3$) : δ 4.98(singlet, 2H, $-CH_2-CCl_3$) ; 7.49(multiplet, 2H, ArH) ; 7.63(multiplet, 1H, ArH) ; 8.13(multiplet, 2H, ArH).

Bis(2,2,2-trifluoroethyl)phthalate: mp= 42.7°C ; ^{1}H-NMR($CDCl_3$) : δ 4.64(quartet, 4H, $-CH_2-CF_3$) ; 7.63(multiplet, 2H, ArH) ; 7.79(multiplet, 2H, ArH).

Bis(2,2,2-trifluoroethyl)isophthalate: mp = 99.3°C ; ^{1}H-NMR($CDCl_3$) : δ 4.73(quartet, 4H, $-CH_2-CF_3$) ; 7.61(triplet, 1H, ArH) ; 8.30(doublet, 2H, ArH) ; 8.72(singlet, 1H, ArH).

Bis(2,2,2-trifluoroethyl)terephthalate: mp = 115.2°C ; ^{1}H-NMR($CDCl_3$) : δ 4.71(quartet, 4H, $-CH_2-CF_3$) ; 8.16(singlet, 4H, ArH).

Bis(2,2,2-trichloroethyl)terephthalate: mp = 128.7°C ; ^{1}H-NMR($CDCl_3$) : δ 4.97(singlet, 4H, $-CH_2-CF_3$) ; 8.25(singlet, 4H, ArH).

RESULTS AND DISCUSSION

Specificity of lipases and proteases toward aromatic diesters

To identify enzymes capable of catalyzing the synthesis of aromatic polyesters, the biocatalytic activity of commercially available hydrolytic enzymes from different sources was studied in the transesterification reactions of an activated ester donor, trifluoroethyl (TFE) terephthalate and 1,4-butanediol (Table 1). Nine commercially available enzymes were tested for aromatic polyester synthesis in anhydrous THF. After 2 days, five enzymes showed appreciable activity (>10% conversion), with Optimase M-440, an alkaline protease from *Bacillus licheniformis* showing the highest activity (Table 1). No significant reaction was detected in the absence of the enzyme.

Table 1 Effect of the type of lipase and protease on the transesterification between TFE-terephthalate and 1,4-butanediol in THF

Enzyme	Supplier	Conversion of TFE-terephthalate after 48h (%)
PPL	Sigma	67.4
CCL	Sigma	16.3
Lipase G	Amano	12.8
Lipase CES	Amano	94.7
Lipase N	Amano	0
Proleather	Amano	2.3
Protease N	Amano	0
Biozyme S	Amano	0
Optimase M-440	Solvay	100

Aromatic polyester synthesis

A number of aromatic oligomers were obtained by protease catalyzed transesterification of aromatic diesters with various diols. Table 2 summarizes experiments performed in the course of a survey of polytransesterification of aromatic diesters. For the diols with different chain lengths (C_2-C_6), the fastest initial reaction was obtained with 1,4-butanediol, although the differences were very small. GPC analysis against polystyrene standards provided a Mw of 400-1000 daltons, which indicated that the aromatic polyesters produced were mainly from "trimers" to "pentamers" (Table 2). In the reaction with 1,4-butanediol, oligomers obtained using TFE-isophthalate had lower molecular weight than those obtained using TFE-terephthalate although the initial reaction rate of the former was faster than that of the latter. It can be explained by steric effect. As TFE-isophthalate reacts into oligomers, it gives rise to steric hindrance and it prevents TFE-isophthalate from growing into longer oligomers. Also, higher molecular weights may be limited by poor enzyme-polymer interactions. TFE-phthalate underwent only a mono- and ditransesterification reaction.

Table 2 Enzymatic synthesis of aromatic polyester by transesterification of aromatic diesters and diols in THF

Diester	Diol	Initial reaction rate of ester (μmol/ml·h)	Molecular weight (M_w)
TFE-terephthalate	$HO(CH_2)_2OH$	16.7	554
TFE-terephthalate	$HO(CH_2)_3OH$	19.3	619
TFE-terephthalate	$HO(CH_2)_4OH$	22.3	848
TFE-terephthalate	$HO(CH_2)_5OH$	22.2	836
TFE-terephthalate	$HO(CH_2)_6OH$	18.5	745
TFE-terephthalate	$HOCH_2C_6H_{10}CH_2OH$	18.2	828
TCE-terephthalate	$HO(CH_2)_4OH$	13.7	485
TFE-phthalate	$HO(CH_2)_4OH$	11.0	377
TFE-isophthalate	$HO(CH_2)_4OH$	40.3	671

A number of aromatic oligomers were also obtained by protease-catalyzed polycondensation of diesters of glutarate and 1,4-benzenedimethanol or isomers of the latter (Table 3). In pyridine, steric effects appear to be critical in the polycondensation reaction.

Table 3 Enzymatic synthesis of aromatic polyester by polytransesterification of diesters and aromatic diols in organic solvents

Solvents	Diester	Diol	Initial reaction rate (μmol/ml·h)	Molecular weight (M_w)
pyridine	TFE-glutarate	1,2-benzenedimethanol	96.1	373
pyridine	TFE-glutarate	1,3-benzenedimethanol	92.4	680
pyridine	TFE-glutarate	1,4-benzenedimethanol	92.8	1214
pyridine	TCE-glutarate	1,4-benzenedimethanol	68.8	1128
acetone	TFE-glutarate	1,2-benzenedimethanol	72.8	999
acetone	TFE-glutarate	1,3-benzenedimethanol	66.5	1350
acetone	TFE-glutarate	1,4-benzenedimethanol	67.3	1243
acetone	TCE-glutarate	1,4-benzenedimethanol	40.0	1205

The molecular weights of the polyesters formed decreased as the steric hindrance around the hydroxyl moieties increase. Oligomers produced in acetone were larger than those

prepared in pyridine although the initial reaction rate in the former was slower than that in the latter.

Chemoenzymatic synthesis of sucrose containing aromatic polyester

The key step in the chemoenzymatic method for the synthesis of sucrose containing aromatic polymer, is the highly selective enzymatic esterification of a sucrose with aromatic diesters. This is difficult to perform chemically due to the lack of control in the acylation step and would result in multiple modified sugars. However, we found the enzyme that can efficiently catalyze the transesterification of aromatic compounds. Using the enzyme, sucrose was acylated with TFE-terephthalate in pyridine and sucrose monoester and sucrose diester were preparatively synthesized.

To determine the site of sucrose acylation, ^{13}C NMR analysis of the sucrose ester products was carried out. The monoester product showed a distinct downfield shift in the C1' carbon with a concomitant upfield shift in carbon C2' and the diester product shows clear downfield shifts in carbons C1' and C6 with upfield shifts in carbon C2' and C5 (data not shown). These chemical shifts enabled us to assign structures to the mono- and diester products with the sucrose monoester identified as sucrose 1'-terephthalate and the diester product identified as sucrose 6,1'-diterephthalate. Hence, sucrose was acylated at the 1' position followed by acylation at the 6 position (Fig. 1). The sucrose diester can be polymerized with diols such as ethylene glycol in the next chemical step(s) using conventional catalysts.

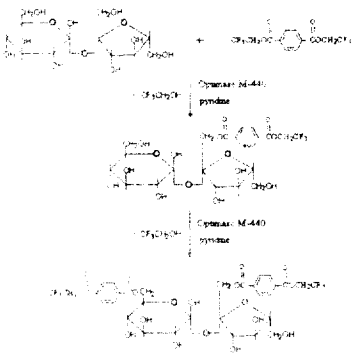

Figure 1. Scheme of sucrose acylation.

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