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Short Communication

Preparative Regioselective Acylation of Glycols by Enzymatic Transesterification in Organic Solvents

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ABSTRACT

Porcine pancreatic lipase can catalyze transesterification reactions in organic solvents in a highly regioselective manner. Lipase powder was suspended in solutions of various diols in ethyl carboxylates, and the mixtures were shaken at 30°C; as a result, primary monoesters of glycols were produced on a preparative scale.

Index Entries: Preparative acylation of glycols, by enzymatic transesterification; acylation of glycols, by enzymatic transesterification; glycols, acylation by enzymatic transesterification of; enzymatic transesterification, in the preparative acylation of glycols; regioselective acylation, of glycols by enzymatic transesterification; transesterification, of glycols, enzyme-catalyzed.

INTRODUCTION

Glycols are widely used as versatile building blocks for the synthesis of a variety of practically important compounds (*1–3*). In such applications, regioselective modifications, in particular acylation of the primary

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hydroxyl group of a primary–secondary glycol, are often required (*1–4*). Acylation of glycols by conventional means, e.g., with carboxylic acids, their anhydrides, or their chlorides, usually results in a complex mixture of mono-, di-, and oligoesters (*5–10*). For example, direct stoichiometric esterification (in the presence of acid or alkaline catalysts) of 1,2-propanediol or 1,3-butanediol affords mixtures containing 20–25% of the free glycol, 35–40% of the primary monoester, 15–20% of the secondary monoester, and 25% of the diester (*11*). Therefore, alternative, rather sophisticated, and expensive methods have been required to achieve reasonable regioselectivity (*12–18*).

Enzyme-catalyzed conversions are usually very selective (*19–21*), but are plagued by the need to carry them out in water, which is a poor reaction medium for most organic reactions. We have recently discovered (*22*) that porcine pancreatic lipase can catalyze transesterification reactions in virtually anhydrous organic solvents. In the present work, we have employed this ability of the enzyme for facile regioselective acylation of glycols.

MATERIALS AND METHODS

Porcine pancreatic lipase (EC 3.1.1.3) was purchased from Sigma Chemical Co. (St. Louis, MO) as a powder with a specific activity of 54 olive oil units/mg of solid; this preparation contains 3.6% (w/w) water (*22*). All chemicals used in this work were obtained from various commercial suppliers and were of the highest purity available.

The time course of all lipase-catalyzed transesterifications was followed by gas chromatography (measuring both disappearance of the alcohols and accumulation of the esters) using a 530 μm fused silica capillary column (Hewlett-Packard).

The sites of acylation in glycols was determined by nuclear magnetic resonance (NMR) spectroscopy using a 60 MHz Perkin-Elmer NMR spectrometer.

RESULTS AND DISCUSSION

Porcine pancreatic lipase (*23*) is an excellent candidate for a practical catalyst because it is readily commercially available, inexpensive ($24/500 g from Sigma), and requires no cofactors for its action. This enzyme has been used for asymmetric hydrolysis of racemic esters (*24,25*). In addition to hydrolysis, porcine pancreatic lipase, like other lipases (*26,27*), also should be able to catalyze the reaction of transesterification. To suppress the side reaction of hydrolysis, the water content in a transesterification mixture must be kept to a minimum. We have recently found that porcine pancreatic (*22*) and other (*28*) lipases can catalyze transesterifica-

tions in nearly anhydrous organic solvents. In this study we have found that if a glycol is used as a nucleophile, porcine pancreatic lipase is extremely regioselective in the transesterification process.

The target reaction was:

$$\text{Ethyl carboxylate} + \text{Glycol} \rightarrow \text{Monoacyl glycol}$$

An ethyl carboxylate (e.g., ethyl acetate) was employed both as an acylating agent and as the reaction medium; no water was added. The general procedure developed is illustrated by the enzymatic monoacetylation of 1,2-butanediol. Eighteen grams of 1,2-butanediol were dissolved in 344 g of ethyl acetate, and the mixture was placed in a 1 L screw-cap bottle. The transesterification reaction was initiated by the addition of 20 g of crude porcine pancreatic lipase. The suspension formed (the enzyme is not soluble in organic solvents) was shaken on an orbit shaker at 250 rpm and 30°C; periodically, aliquots were withdrawn and analyzed by gas chromatography. After 23 h, 97% of the glycol was acetylated and the reaction stopped. Analysis of the products by GC showed that almost all the 1,2-butanediol converted resulted in a monoester (the diester content was 1% by GC). The enzyme was removed by filtration; it retained nearly full initial catalytic activity and was successfully used for repeated transformations. Ethyl acetate was evaporated from the liquid phase, and the remainder was subjected to silica gel column chromatogaphy; as a result, 21.2 g (83% yield) of pure (as determined by GC) monoacetyl ester of 1,2-butanediol (bp 95–97°C at 15 mm Hg) were obtained. We examined the monoester by NMR and found that only the primary hydroxyl group in the diol was acetylated: Acylation of the secondary hydroxyl group (in contrast to the primary one) results in a significant downfield shift of the hydrogen adjacent to the same carbon as the hydroxyl group (*13*) [e.g., in the case of 1,2-butanediol, acetylation with acetic anhydride results in a shift (in $CDCl_3$) from 3.82 (sextet) to 4.75 (sextet)]. No such shift was observed in enzymatically acylated glycols which suggests that no secondary hydroxyl groups were acylated, indicating that lipase displays a rather remarkable regioselectivity. This finding is in accord with the results obtained in acylation of monohydric alcohols. If 1- and 2-butanols (mimicking the primary and secondary hydroxyl moieties in 1,2-butanediol) are used instead of the diol in the experiment described above, then after 26 h more than 90% of 1-butanol, but only about 5% of 2-butanol are acylated.

Similar experimental procedures were used to acylate (with different acyl groups) a variety of other glycols, and the results obtained are presented in Table 1. One can see that in all instances the enzyme exhibited the overwhelming preference for the primary over the secondary hydroxyl group. Only one hydroxyl group was acylated in a symmetrical diol (entry 3 in Table 1). In the case of 2-mercaptoethanol, only the OH group was acylated. This was determined by NMR, which showed that

Table 1
Porcine Pancreatic Lipase-Catalyzed Regioselective Acylation of Glycols[a]

Glycol		Ester			Composition of the glycol product (by GC), %			Yield of the primary monoester[c] after purification,[d] g	
Name	Amount, g	Name	Amount, g	Reaction time[b], h	Monoester	Diol	Diester		
1,2-Butanediol	18.0	Ethyl acetate	344	23	96	3	1	21.2	(83%)
1,2-Hexanediol	23.6	Ethyl acetate	338	40	96	2	2	30.0	(93%)
2-(2-Pyridyl)-1,3-propanediol	16.0	Ethyl acetate	540	16	96	2	2	16.0	(78%)
1-Morpholino-2,3-propanediol	16.2	Ethyl acetate	175	40	97	3	0	16.8	(82%)
1,3-Heptanediol	13.2	Ethyl butyrate	168	28	95	5	0	18.3	(91%)
1,3-Butanediol	9.0	Ethyl propionate	170	60	91	7	2	12.4	(85%)
1,3-Butanediol	3.4	Ethyl caprylate	59	44	91	9	0	7.2	(87%)
2-Ethyl-1,3-hexanediol	14.6	Ethyl acetate	167	40	88	12	0	14.6	(82%)
2-Mercaptoethanol	3.9	Ethyl acetate	86	40	85	15	0	4.9	(82%)

[a]Conditions: 50 mg of crude porcine pancreatic lipase/mL of the reaction mixture. The suspension was shaken (250 rpm) at 30°C.
[b]After which the acylation stops.
[c]It was shown by NMR that in all instances only the primary hydroxyl group of the glycol was enzymatically acylated.
[d]In all cases, following completion of the reaction the enzyme was removed by filtration, the acylating ester was evaporated under vacuum, and the remainder was applied to a silica gel column using hexane/ether mixtures as a solvent. The monoester obtained as a result of this procedure was 100% pure by GC.

the triplet corresponding to the hydrogen bonded to sulfur did not shift upon enzymatic acylation, whereas that bonded to oxygen disappeared. This conclusion was also confirmed by spectrophotometric titration of sulfhydryl groups in the enzymatically prepared monoester using Ellman's reagent (*29*).

Inspection of the data in Table 1 reveals that the lipase-catalyzed acylation is effective regardless of the nature of the glycol or the ester, the main variations being the fraction of the free glycol in the product and the reaction time. The former reflects the position of the equilibrium in the transesterification mixture (for a general discussion of such equilibria, *see* ref. *30*). The time of the reaction can be easily controlled by using more activated esters: e.g., when 1,2-hexanediol (the second entry in Table 1) was acylated with trichloroethyl butyrate or glyceryl tributyrate instead of ethyl acetate, the rates of the enzymatic process increased by 3.3- and 4.5-fold, respectively. Transition from ethyl to trichloroethyl esters also reduces the fraction of the free glycol in the system because of the equilibrium shift (*30*) (because ethanol is a much better nucleophile than trichloroethanol).

In conclusion, the strategy described herein affords a simple, practical method for preparative regioselective acylation of the primary hydroxyl moiety in glycols. In addition to transesterification, lipases can also catalyze many other reactions in organic solvents such as esterification, interesterification, aminolysis, thiotransesterification, and oximolysis (*28*). This paves the way to enzyme-catalyzed, preparative, and selective (regio-, stereo-, or geometrically) transformations of such major classes of organic compounds as alcohols, carboxylic acids and esters, amines, thiols, and oximes. Advantages of conducting enzymatic conversions in organic solvents as opposed to water include: (i) no need to immobilize enzymes because they are insoluble in organic solvents and hence can be easily recovered and reused after the process is completed; (ii) enhanced stability of enzymes (*22*); (iii) high solubility of most organic compounds in non-aqueous media; (iv) ability to carry out new reactions impossible in water due to kinetic or thermodynamic reasons; and (v) relative ease of product recovery from organic solvents as compared to water.

Recently, Inada et al. (*31,32*) reported that upon modification with polyethylene glycol lipoprotein lipase from *Pseudomonas fluorescens* becomes soluble in benzene and displays catalytic activity in that solvent. We have found that this modification is unnecessary either for lipoprotein lipase (*28*) or porcine pancreatic lipase (this work): although the unmodified lipases are insoluble in organic solvents, they exhibit full catalytic power in such media [indicating that, in contrast to the conclusion made in refs. *31–33*, solubility is not a prerequisite for enzymatic activity—a fact hardly surprising to those working with immobilized enzymes (*20*)].

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