Enzymatic Alcoholysis Reaction of Soy Phospholipids

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ABSTRACT: Lipase-catalyzed alcoholysis of soy phospholipids was investigated to simultaneously make lysophospholipids and fatty acid esters of individual alcohols. Alcoholysis was carried out by stirring a mixture of soy phospholipids and individual alcohols in equimolar proportions with 10% (by weight of reactants) *Mucor miehei* lipase at 55°C for 24 h. The products were isolated by column chromatography after removal of the lipase. Lysophospholipids (in 69–78% molar yield) were obtained from soy phospholipids, and the yield of esters of various alcohols also conformed nearly with theoretical yields.
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KEY WORDS: Alcoholysis, column chromatography, long-chain alcohol esters, lysophospholipid, *Mucor miehei* lipase, short- and long-chain alcohols, soy phospholipid.

In recent years, lipase-catalyzed transformation reactions of lipids have become important for making ester derivatives from fats and oils for various specific applications (1,2). Phospholipids, which constitute a class of industrially important surfactants, are also receiving attention for various lipase-catalyzed transformations. The conversion of phospholipids to lysophospholipids and fatty acids is usually performed by the action of phospholipases A_1 and A_2 (3–5). There are also some triacylglycerol lipases that can cleave fatty acids from the *sn*-1 and/or *sn*-2 positions of diacylglycerophospholipids to yield lysophospholipids with useful functional properties (6–9).

Conversion of phospholipid to lysophospholipid by reptilian phospholipases suffers from two distinct disadvantages. Considerable difficulties are faced in deactivation of the enzymes after completion of the reaction. Also, the phospholipases show poor activity in primary alcohols or in organic solvents (10,11). On the contrary, fungal lipases function perfectly well in nearly anhydrous alcohols, and some of them have been shown to accept phospholipid as substrate (12–15).

We are aware of only two literature reports concerning synthesis of lysophospholipids from soy phospholipids by lipase-catalyzed alcoholysis. One communication, published by Samey *et al.* (16), shows the possibilities of modifying phospholipids by alcoholysis reaction with alcohols such as ethanol, isopropanol and *n*-butanol, catalyzed by lipase from *Rhizomucor miehei,* to produce lysophospholipids. In the other publication (17), lysophospholipids were produced by glycerolysis of soy phospholipids with an immobilized form of this same lipase as a catalyst.

Production of long-chain alcohol esters of C_{16}–C_{18} fatty acids is industrially important because these quite hydrophobic compounds are widely used as lubricants, plasticizers, and in cosmetics. Depending on the chainlength of the fatty acids and alcohols, products vary in consistency from liquid to solid waxes (18). The production of long-chain alcohol esters of fatty acids by alcoholysis involving chemical catalysts is well established. However, there are certain disadvantages of the chemical catalyst route. It is nonspecific and leads to total conversion of fatty acid moieties to long-chain esters. With phospholipids, the long-chain esters of fatty acids will be formed but will not produce lysophospholipids (19). The chemical catalysts (acid, alkali) react on the phosphate bond on continuation of the reaction, which does not occur when lipozyme or phospholipases A_1 and A_2 are used as catalysts. Often, products with an undesirable dark color are obtained by reaction with chemical catalysts, whereas this kind of discoloration of products is not associated with lipases as catalysts. With lipase catalysis, it may be possible to make esters that are difficult to obtain by chemical methods. Mild reaction conditions and the high enzymatic regiospecificity are other advantages of lipase technology, especially if the lipids are to be used in human food or for medical purposes.

The present study deals with the production of lysophospholipids, along with fatty acid esters of short- and long-chain alcohols, from soy phospholipids by lipase-catalyzed alcoholysis.

MATERIALS AND METHODS

Substrate and enzyme. Crude soy phospholipid was supplied by M/s. S.M. Dyechem (Indore, India) and was deoiled by extraction of neutral lipids with acetone by standard method (20). The immobilized lipase of *Mucor miehei* (Lipozyme IM 20) was a gift of Novo A/S (Copenhagen, Denmark). Butanol and other fatty alcohols were purchased from E. Merck (India) Limited, Worli (Bombay, India). Unless otherwise specified, all other chemicals were reagent-grade.

Enzymatic alcoholysis reaction. Deoiled phospholipid (about 1.0 g, accurately weighed) and an alcohol in approxi-

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TABLE 1
Fatty Acid Composition of Various Alcohol Esters and Lysophospholipids[a]

Samples	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Original soy phospholipids	16.8	3.1	20.2	54.3	5.4
Alcohol esters (C_4–C_{18} alcohol) produced by enzymatic alcoholysis of soy phospholipids	43.0–45.2	4.9–5.4	13.2–14.5	32.5–34.5	0.8–1.2
Lysophospholipids produced by enzymatic alcoholysis of soy phospholipids	10.3–11.8	0.7–1.2	10.5–11.2	75.5–76.7	2.0–2.5

[a]Mean of three determinations.

mately 1:1 molar ratio were placed in a 50-mL round-bottom flask and stirred by a magnetic stirrer at 55 ± 2°C for 24 h in the presence of 10% (by weight of reactants) of *M. miehei* lipase. Hexane (5 mL) was used to make a homogeneous reaction mixture. The lipase contained 10% (w/w) water to maintain its activity. A typical example of the proportion of reactants used in the preparation of esters was 1 g (1290 µmole) of phospholipids (average molecular weight of phospholipid as 775) and 0.095 g (1248 µmole) *n*-butanol (C_4-alcohol). Samples were taken periodically and assayed for the presence of products. After the reaction, the product mixture was filtered to remove the enzyme, and hexane was evaporated under vacuum. The product mixture was analyzed by standard adsorption column chromatographic methods with silica gel as the adsorbent (21) for separation and estimation of esters, alcohols, phospholipids, and lysophospholipids.

Estimation of percentage yield of esters and lysophospholipids. The yield of esters and lysophospholipids by weight from each alcoholysis reaction was estimated by standard column chromatographic method. Silica gel (60–120 mesh size) was packed into a glass column (30 × 300 mm), and about 100 mg of an accurately weighed product mixture was applied. The flow rate of eluting solvent was maintained at 2.5 mL/min. The eluants were, in order of addition: 150 mL hexane/diethyl ether (98:2, vol/vol) to elute the ester fraction; 90 mL hexane/diethyl ether (92:8, vol/vol) to elute the unreacted alcohol fraction; 100 mL chloroform/methanol (75:25, vol/vol) to elute unreacted phospholipids; and 60 mL methanol to elute lysophospholipids. After each elution, the solvents were removed by evaporation under 8–10 mm Hg pressure and ambient temperature (28–30°C).

Analysis of fatty acids of alcohol esters and lysophospholipids. The fatty acid compositions of the various alcohol esters and the corresponding lysophospholipids were determined by gas chromatography (GC) of the methyl esters of the total fatty acids of the individual esters and lysophospholipids. The methyl esters of the fatty acids of the alcohol esters were prepared with methanol–boron trifluoride as methylating agent, according to the standard procedure of Metcalfe and Schmitz (22), after hydrolyzing the ester with methanolic KOH. The methyl esters of lysophospholipids were prepared by the standard methanolic KOH method of Litchfield (23). The fatty acid composition was then analyzed by standard GC procedure (24).

The molecular weights of esters and lysophospholipids were calculated from their fatty acid compositions.

RESULTS AND DISCUSSION

The fatty acid compositions of the original soy phospholipids (deoiled) and of the lysophospholipids and alcohol esters obtained by enzymatic alcoholysis are given in Table 1. Because phosphoglycerides most commonly contain a saturated acyl chain at C_1 and an unsaturated at C_2 (25), the result shown in Table 1 proves that the Lipozyme is *sn* 1-specific when it acts on phospholipids.

Results presented in Tables 2 and 3 show that percentage molar yield of ester and lysophospholipid and percentage

TABLE 2
Yields of Esters[a] and Lysophospholipids[b] on the Basis of Phospholipid (after 24 h)

Alcohols	Yield of ester (mole %)	Yield of lysophospholipid (mole %)
C_4	82.4	75.5
C_8	83.5	77.6
C_{10}	82.7	77.1
C_{12}	81.8	76.3
C_{18}	78.7	68.9

[a]Ester yield (mole %) = moles of ester obtained/moles of phospholipid originally present × 100.
[b]Lysophospholipid yield (mole %) = moles of lysophospholipid obtained/moles of phospholipid originally present × 100.

TABLE 3
Conversion of Phospholipid to Lysophospholipid[a] and of Alcohol to Alcohol Esters[b] (in 24 h)

Alcohol	Conversion of phospholipid (%)	Conversion of alcohol (%)
C_4	79.5	77.3
C_8	81.8	81.2
C_{10}	81.2	78.8
C_{12}	80.4	77.7
C_{18}	72.6	70.6

[a]Percentage conversion of phospholipid to lysophospholipid: (moles of phospholipid originally present – moles of unreacted phospholipid)/moles of phospholipid originally present × 100.
[b]Percentage conversion of alcohol to alcohol esters: (moles of alcohol originally present – moles of unreacted alcohol)/moles of alcohol originally present × 100.

molar conversion of phospholipid and alcohol are comparable due to the 1:1 molar ratio of substrate used. The conversions were in the range of 71–83% in 24 h, and the reaction rates were constant at 3–24 h.

The most significant result of this work is the documentation of the lack of selectivity of the lipase with respect to fatty alcohol chainlength, which is also evident from the work of Ghosh Ray and Bhattacharyya (26) in which the substrate of the alcoholysis reaction was castor oil.

These data establish the utility of lipase-catalyzed alcoholysis for the synthesis of lysophospholipids and fatty acid esters. The advantages of the method are that the feedstock is readily available and that the process results in high yields of product, which should be of high quality due to the mild reaction conditions employed.

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