ENZYMATIC REGIOSELECTIVE ACYLATION OF 3-*O*-β-D-GALACTOPYRANOSYL-*SN*-GLYCEROL BY *ACHROMOBACTER* SP. LIPASE

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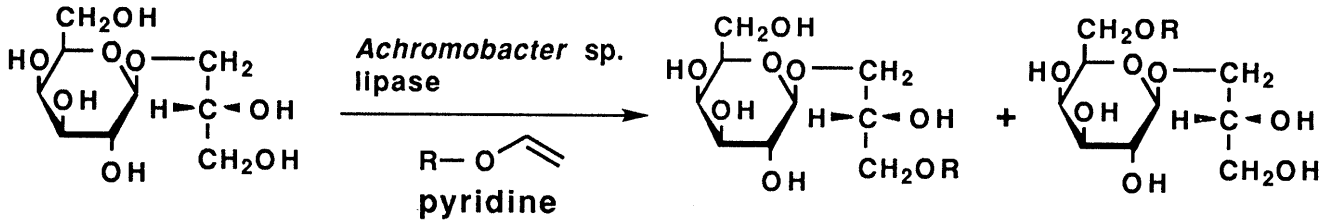
Achromobacter sp. lipase regioselectively acylated the hydroxyl group on *sn*-1 carbon among the two primary hydroxyl groups of 3-*O*-β-D-galactopyranosyl-*sn*-glycerol .

KEYWORDS regioselective acylation; lipase; *Achromobacter* sp.; lysoglyceroglycolipid; 3-*O*-β-D-galactopyranosyl-*sn*-glycerol

Lysoglycerophospholipids have been studied from various points of view in detail, because they were readily prepared by use of phospholipase A_1 and A_2. Thus lysophospholipids have been shown to have several biological activities and to play an important role in elucidating the metabolism and biosynthesis of glycerophospholipids.[2] In contrast, lysoglyceroglycolipids have been found in plants and bacteria,[3] but lack of practical methods of preparation[4] and low content have made it difficult to investigate their biological activities[5] and the metabolism and biosynthesis of glyceroglycolipids. Recently, it has been reported that lipase catalyzes regioselective acylations of hydroxyl groups in polyhydroxyl molecule.[6] Therefore we have investigated the application of crude lipases for preparation of glyceroglycolipids in the course of our studies on glycolipids. Here we describe a regioselective acylation of two primary hydroxyl groups in 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**1**) using *Achromobacter* sp. lipase.



1 **2** (92:8~95:5) **3**

R=acyl residue in fatty acid **2a: R=palmitoyl** **3a: R=palmitoyl**

First, we screened several lipases for acylation of **1**[7] with vinyl palmitate[8] as an acylating reagent. When DMF or DMSO was used as a solvent, acylation of **1** proceeded only slightly. In *t*-amyl alcohol or THF, either lipase gave a trace amount of a diester.[9] On the other hand, use of pyridine as a reaction medium gave a mixture of monoester in which the acyl residue was linked only to primary hydroxyl groups for all the lipases tested, *Achromobacter* sp. lipase (lipase AL), *Alcaligenes* sp. lipase (lipase PL),

Table I. Lipase Catalyzed Acylation of 3-*O*-β-D-Galactopyranosyl-*sn*-glycerol (**1**) with Vinyl Palmitate ($C_{16:0}$)

Lipase	Time (d)	Conversion (%)	**2a** : **3a**
AL	4	53	95 : 5
PL	4	61	84 : 16
PS	6	49	55 : 45
M	7	11	46 : 54
RDL	7	23	65 : 35

All reaction were carried out using **1** (0.2 mM) and acylating agent (0.6 mM) in the presence of lipase (150 mg) in pyridine (1.0 ml) at 40 °C.

Psudomonas sp. lipase (lipase PS), *Mucor javanics* lipase (lipase M), and *Rhizopus delemer* lipase (RDL)[10]. Among the lipases, lipase M, PS and RDL showed little selectivity, while lipase AL and PL regioselectively acylated at *sn*-1 position in 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**1**), lipase AL being the best (95 : 5).[11] Furthermore, we surveyed acylations with trichloroethyl ester, trifluoroethyl ester, and isopropenyl ester in order to enhance the yield and selectivity of **2a**, but found only decreases in reactivities and selectivities.

Glyceroglycolipids are known to alter biological activities and chemical properties with composition and distribution of fatty acid residues.[12] Thus, we applied lipase AL for preparation of various *sn*-2 lyso monogalactosyl diacylglycerols (MGDG) using several vinyl esters as acyl donors. Table II shows that acylations proceed as expected for all vinyl esters of six fatty acids to furnish *sn*-2 lyso MGDGs selectively. It is noteworthy that *sn*-2 lyso MGDG containing linoleic acid, which is liable to oxidation, was obtained in nearly the same yield as the others.

Table II. Lipase AL Catalyzed Acylation of **1** with Various Vinyl Esters of Fatty Acids

Acylating reagent	Time (d)	Conversion (%)	**2** : **3**
Vinyl laurate ($C_{12:0}$)	4	36	94 : 6
Vinyl myristate ($C_{14:0}$)	4	42	92 : 8
Vinyl palmitoleate ($C_{16:1}$)	3	47	93 : 7
Vinyl stearate ($C_{18:0}$)	4	53	94 : 6
Vinyl oleate ($C_{18:1}$)	3	53	94 : 6
Vinyl linoleate ($C_{18:2}$)	4	51	93 : 7

Consequently, we found the regioselective acylation of 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**1**) using *Achromobacter* sp. lipase and vinyl esters of fatty acid in pyridine. Although some regioselective acylations with lipases have been demonstrated so far, to our best knowledge this is the first example of differentiation of two primary hydroxyl groups in a polyhydroxyl molecule, which seems difficult with the usual organic reaction. It is worthy of note that this method is expected to be practical for facile synthesis of 2-lyso MGDG, because the substrate (**1**) is readily prepared.

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