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ORIGINAL ARTICLE

Candida antarctica lipase B catalyzed enantioselective acylation of pyrimidine acyclonucleoside

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Abstract
The influence of solvent and acyl group donor on selectivity of the transesterification reaction of 1-[1′,3′-dihydroxy-2′-propoxymethyl]-5-methyluracil, a structural analogue of ganciclovir was examined. Lipase (EC 3.1.1.3) B from *Candida antarctica* (CALB) enabled desymmetrization of prochiral hydroxyl groups when 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]) was used as a reaction medium. It was observed that CALB was up to 2.7–4 times more enantioselective in the ionic liquid [Bmim][PF_6] than in conventional organic solvents.

Keywords: *lipase B from* Candida antarctica*, transesterification, desymmetrization, ionic liquids*

Introduction

The catalytic potential of lipases has been the subject of intensive investigation for many years. The chiral structure of the enzyme facilitates enantioselective reactions giving kinetic resolution of racemic mixtures (Ha et al. 2008; Brem et al. 2009) or production of enantiopure products from achiral precursors (Mahapatra et al. 2008; Köhler & Wünsch 2006; Arai et al. 2004). This is particularly significant when the enantiomer is a biologically active compound, and its therapeutic properties depend on its optical purity. The catalytic properties of lipases have been used to obtain optically pure compounds by enantioselective hydrolysis, esterification and transesterification (Schöfer et al. 2001; Ducret et al. 2000; Utzig et al. 2001).

Lipases generally display a high tolerance towards structural changes in substrates as well as a wide spectrum of activity (they work not only in aqueous systems but also in organic solvents). When planning enzymatic synthesis, the choice of solvent used as a reaction medium is very significant. The basic criterion of this choice is its hydrophilic and hydrophobic properties, which influence the activity, stability and selectivity of the enzyme. Along with an increase in medium polarity in an enzymatic reaction, the amount of water needed to activate the enzyme decreases because its molecules enter into interaction with polar solvent. As a consequence, unfavourable conformational changes in the enzyme bring about a decrease in activity and selectivity (Rantwijk et al. 2006; Kaar et al. 2003). The removal of an essential monolayer of water from the protein in polar solvents is usually the reason for deactivation of enzymes. Lipases are most selective in hydrophobic organic solvents, but their activity is typically lower than in a suitable polar solvent (Lozano et al. 2001; Hernández-Fernández et al. 2007).

Increasingly, enzymatic reactions are being performed in ionic liquids (Ha et al. 2008; Noël et al. 2004; Eckstein et al. 2002). The polarity of the majority of imidazole based ionic liquids is comparable to low molecular weight alcohols (methanol, ethanol, 1-butanol). The length of the alkyl chain attached to the imidazolium ring as well as the accompanying anion influences the hydrophilicity and hydrophobicity of the liquid (Vidya & Chadha 2009). A properly

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selected ionic liquid not only increases the activity of enzymes but also improve stereoselectivity.

In this work, the enzymatic transesterification of the acyclonucleoside 1-[1′,3′-dihydroxy-2′-propoxymethyl]-5-methyluracil (**1**) has been investigated. Interest in acyclonucleosides arises from their biologically active properties; 1-[1′,3′-dihydroxy-2′-propoxymethyl]-5-methyluracil (**1**) is known as an *E. coli* uridine phosphorylase inhibitor in the micromolar range and compounds similar in structure inhibit the growth of cancer cells *in vitro* (Drabikowska et al. 1987).

The influence of the solvent and the acylating agent were examined for their effects on the enantioselectivity of lipase B from *Candida antarctica* (CALB), one of the few lipases that does not require interfacial activation. Since the lipase is stable and active in organic solvents, acylation reactions were carried out under anhydrous conditions.

Materials and methods

Lyophilized CALB was from Fluka (Switzerland), 10.9 U/mg.

Other chemicals of analytical grade were commercially available: 1-phenylethanol from Fluka (Switzerland), 1-[1′,3′-dihydroxy-2-propoxymethyl]-5-methyluracil was obtained from the Medical Academy (Poland) (Drabikowska et al. 1987), vinyl acetate from Fluka (Germany), vinyl butyrate and vinyl benzoate from Fluka (Japan), vinyl laurate from Fluka (Switzerland), 1-butyl-3-methylimidazolium hexafluorophosphate and 1-butyl-3-methylimidazolium tetrafluoroborate from Fluka (Switzerland), pyridine, cyclohexane, dioxane, n-hexane for High-performance liquid chromatography (HPLC), 2-propanol for HPLC from POCH (Poland).

General procedure

Typical enzymatic reactions were performed in a solution containing substrate (0.0001 mol), CALB (1 mg), acyl donor (0.0001 mol) in a solvent (1 mL) and were kept at constant temperature (37–50°C). After the reaction methanol was added, the enzyme removed by filtration and the resulting solution concentrated. In the case of ionic liquids, the mixture was first extracted with hexane: 2-propanol (80:20), and the organic phase concentrated.

The product in the residue was characterized by ^{1}H NMR. The enantiomeric ratios were determined on an HPLC system using a chiral column, using a mobile phase of hexane: 2-propanol.

Analysis

HPLC analyses were performed on a Shimadzu SCL–10A *VP*, analytical column Chiralcel OJ 250 × 4, 6 mm, 10 μm, Daicel (France).

^{1}H NMR spectra were recorded on Bruker 300 Hz apparatus using tetramethylsilane (TMS) as an internal standard.

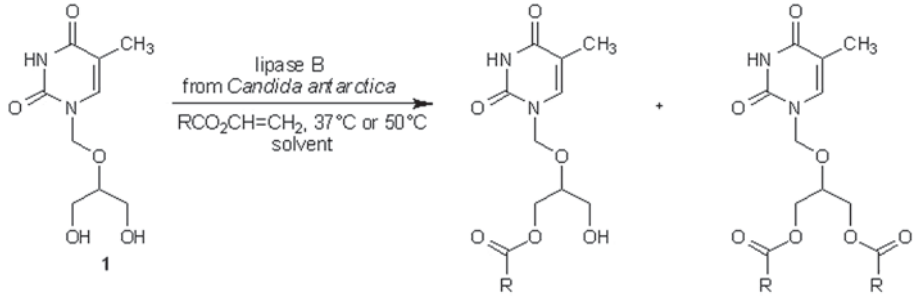
Results and discussion

Enzymatic transesterification of 1-[1′,3′-dihydroxy-2′-propoxymethyl]-5-methyluracil ***(1)***

On the basis of a model reaction, solvents were selected for transesterification of the prochiral diol (Scheme 1).

In the transesterification reaction of compound **1** three different organic solvents (cyclohexane, pyridine and dioxane) were tested and reactions were carried out at 37 and 50°C. The reaction in cyclohexane was carried out only at 50°C due to poor solubility of the substrate. Vinyl acetate was used as an acylating agent.

CALB allows regioselective acylation of primary hydroxyl groups in nucleosides (Kołodziejska & Dramiński 2004). Part of **1** imitates a sugar fragment of nucleosides, however, in this case, the hydroxyl groups are both primary. From the prochiral acyclonucleoside, an optically pure compound could be



Scheme 1. Desymmetrization of the prochiral diol 1-[1′,3′-dihydroxy-2′-propoxymethyl]-5-methyluracil by enzymatic transesterification.

obtained if CALB allowed desymmetrization of these OH groups.

To investigate the course of the transesterification of **1** and assess the optical purity of the resulting homoesters, analyses were conducted on post-reaction mixtures taken at specified intervals. The analyses were carried out by HPLC on a Chiralcel OJ column in hexane: 2-propanol (60:40 or 80:20) system, with a flow rate of 1 mL/min. The results are shown in Tables I—III. Transesterification of vinyl acetate in the presence of lipase CALB proceeded at a high rate. Apart from the monoacetylated derivative, completely acetylated acyclonucleoside was also obtained. The maximum enantiomeric excess (ee) (26%) was obtained in dioxane after 10 h at 37°C, and at 50°C in dioxane after 23 h (26%) (Table I), with a slightly lower excess (22%) in pyridine after 24 h of reaction (Table II).

The enzyme failed to give desymmetrization of the prochiral hydroxyl groups in pyridine, with both pro-(*S*) and pro-(*R*) groups being acetylated (racemic mixture after 1 h of reaction). Presumably, the 22% ee after 24 h was the result of different reaction rates of the enantiomers (Table II).

Partial selectivity for acylation of only one primary –OH group in cyclohexane and dioxane made it possible to obtain an excess of the enantiomer of the opposite configuration. In this case, the ee might have resulted from partial desymmetrization of prochiral –OH groups.

Lowering the temperature did not have a significant influence on enantioselectivity (comparable ee at 37 and 50°C). In the transesterification reaction of **1** conducted in pyridine at room temperature, after 2 months 17% ee was obtained (30% yield).

Table I. Transesterification of diol with vinyl acetate in dioxane.

T [°C]	Time [h]	Mono [%]	Di [%]	ee [%]
37	1	26.1	4.1	18
	4	43.6	23.5	18
	6	46.2	27.6	14
	8	43.1	32.2	15
	10	39.2	44.6	26
	12	34.8	56.1	22
50	1	31.8	13.8	10
	2	38.2	22.9	5
	4	43.6	23.5	18
	7	44.5	27.1	18
	9	30.1	54.3	16
	11	36.4	54.4	17
	13	34.9	55.8	13
	16	28.2	69.7	24
	21	20.8	76.6	23
	23	18.4	79.4	26
	26	18.2	79.6	18
	33	8.6	89.3	24

Table II. Transesterification of diol with vinyl acetate in pyridine.

T [°C]	Time [h]	Mono [%]	Di [%]	ee [%]
37	1	20.2	10.5	5
	4	28.3	20.1	8
	6	36.9	26.6	4
	8	42.7	27.3	16
	10	49.2	28.2	5
	12	44.9	32.4	10
50	1	26.8	0	1
	2	39.2	8.2	6
	3	41.3	14.1	8
	5	44.2	16.6	13
	9	44.9	21.9	4
	10	48.2	31.9	7
	12	46.7	40.8	10
	14	45.9	44.6	18
	18	42.8	49.4	19
	20	38.2	55.6	8
	24	32.1	61.8	22
	30	33.1	63.9	22
	36	34.8	64.0	19

Additional attempts at kinetic resolution of the racemic monoester failed to bring expected results. In pyridine, CALB introduced the acetyl group into the hydroxyl group of both enantiomers, 17% ee was obtained. The reaction in pyridine proceeded rapidly and after 24 h the diacetyl derivative was obtained with a high yield (77%).

In order to improve the stereoselectivity of acylation of **1**, the hydrophobic ionic liquid [Bmim][PF_6] was used and the results compared with those obtained in pyridine. The reaction was carried out for 24 h at a temperature of 50°C, with various acylating agents. Apart from vinyl acetate, vinyl esters of butyric, benzoic and lauric acids were used. The application of [Bmim][PF_6] increased the enantioselectivity of the reaction, giving a higher ee than with the less polar pyridine.

In pyridine, the best results were obtained in transesterification with vinyl acetate and contrary to expectations, elongation of the acyl donor chain did not influence the enantioselectivity. Ionic liquid [Bmim][PF_6] appeared to be a much better medium for the reaction. Acylation with vinyl acetate and benzoate proceeded with about 60% ee.

Table III. Transesterification of diol with vinyl acetate in cyclohexane.

T [°C]	Time [h]	Mono [%]	Di [%]	ee [%]
50	1	3.6	0	10
	4	7.1	2.4	16
	7	28.8	18.1	9
	11	38.0	31.3	10
	13	32.5	43.9	15

Table IV. Results of enzyme – catalyzed transesterification of diol.

Acyl agent	Solvent	Time [h]	Mono [%]	Di [%]	ee [%]
Vinyl acetate	Pyridine	30	33.1	60.9	22
	[Bmim][PF_6]	24	40.7	48.7	61
Vinyl butyrate	Pyridine	24	57.3	26.4	13
	[Bmim][PF_6]	24	18.1	28.2	6
Vinyl benzoate	Pyridine	24	13.9	63.2	14
	[Bmim][PF_6]	24	45.1		57
Vinyl laurate	Pyridine	24	38.9	0.6	5
	[Bmim][PF_6]	24	9.8		5

In [Bmim][PF_6] the reaction with vinyl benzoate yielded only monoesters after 24 h, whereas the same reaction carried out in pyridine proceeded much faster and mainly the diacetyl derivative was obtained (monoester 14% ee). The results of transesterification of diol in pyridine and [Bmim][PF_6] are demonstrated in Table IV and Figures 1 and 2.

Unexpectedly, transesterification reactions with vinyl butyrate and laurate, proceeded almost completely non-selectively. In both pyridine and ionic liquid, practically racemic mixture was obtained.

Ionic liquid [Bmim][PF_6] is an ideal environment for enzymatic transesterification reactions. [Bmim][PF_6] undoubtedly has useful properties, it is as polar as ethanol (identical value of E_T^N) and hydrophobic at the same time (slightly soluble in water – 0.13%), with the accompanying anion showing only weak nucleophilic character. With imidazole based ionic liquids of comparable polarity, the nucleophilic character of the accompanying anion mainly dictates the properties of the liquid (hydrophobicity, miscibility with organic solvents, solubility of organic and non-organic substances) and consequently its usefulness for a catalyzed reaction. Together with the increase in nucleophilicity, the accompanying ion coordinates more strongly in the active site of the enzyme contributing to damaging conformation changes of a catalyst, which influence enzyme activity and stability (Kaar et al. 2003; Vidya & Chadha 2009). Consistent with the argument, acetylation of **1** in another imidazolic ionic liquid derivative [Bmim][BF_4], whose accompanying anion is a stronger nucleophile than PF_6^- ion, showed that the reaction gave a lower ee (29%) and a low yield (28%).

[Bmim][PF_6] provides an excellent microenvironment for the enzyme, leading to a more compact conformation capable of giving both high activity and stability; CALB was 2.7–4 times more in [Bmim][PF_6] than in a conventional organic solvent.

Encouraged by these initial results, we are continuing our studies to look for a more efficient biocatalyst. Therefore, the work is being extended using a variety of commercially available lipases.

Conclusions

We have reported desymmetrization of identical hydroxyl groups of a prochiral diol in an enzymatic

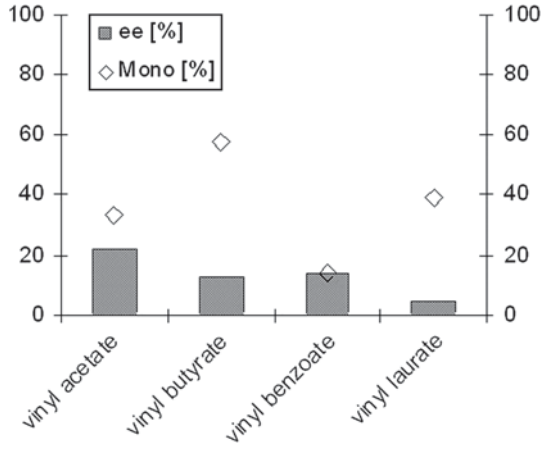


Figure 1. The transesterification of diol catalyzed by CALB in pyridine.

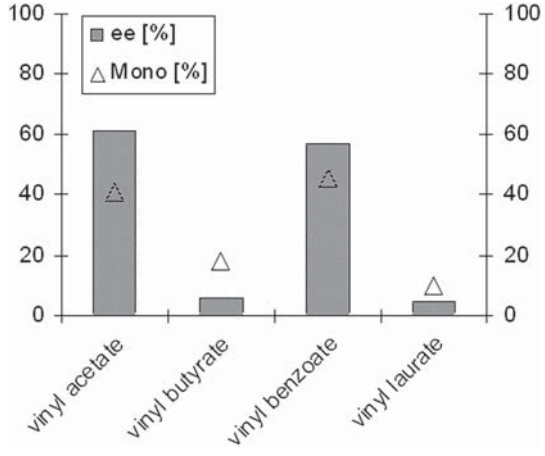


Figure 2. The transesterification of diol catalyzed by CALB in [Bmim][PF_6].

transesterification reaction. Both the solvent and the acyl group donor used influenced the enantioselectivity of the mono-acylated derivatives. Compared with organic solvents, ionic liquids provided the optimal environment for the enzyme. In [Bmim][PF_6], CALB selectively acylated enantiotopic hydroxyl groups. CALB was up to 2.7–4 times more enantioselective in ionic liquid ([Bmim][PF_6]) than in conventional organic solvents.

Supporting information

Detailed information on product characterization specifications (^{1}H NMR spectra and retention time in HPLC analysis) is available in the Supplementary Supporting Information, at http://informahealthcare.com/doi/abs/10.3109/10242422.2012.715637

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Supplementary material available online

Supplementary Supporting Information