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Journal of Molecular Catalysis B: Enzymatic 36 (2005) 40–42
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
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Optimization of lipase-catalyzed glucose fatty acid ester synthesis in a two-phase system containing ionic liquids and *t*-BuOH

Franka Ganske, Uwe T. Bornscheuer *

Department of Technical Chemistry and Biotechnology, Institute of Chemistry and Biochemistry, Greifswald University, Soldmannstr. 16, D-17487 Greifswald, Germany

Received 12 July 2005; received in revised form 15 August 2005; accepted 15 August 2005
Available online 12 September 2005

Abstract

Selective lipase-catalyzed synthesis of glucose fatty acid esters in two-phase systems consisting of an ionic liquid (1-butyl-3-methyl imidazolium tetrafluoroborate [BMIM][BF_4] or 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIM][PF_6]) and *t*-butanol as organic solvent was investigated. The best enzyme was commercially available lipase B from *Candida antarctica* (CAL-B), but also lipase from *Thermomyces lanuginosa* (TLL) gave good conversion. After thorough optimization of several reaction conditions (chain-length and type of acyl donor, temperature, reaction time, percentage of co-solvent) conversions up to 60% could be achieved using fatty acid vinyl ester as acyl donors in [BMIM][PF_6] in the presence of 40% *t*-BuOH with CAL-B at 60 °C.

Keywords: Sugar ester; Ionic liquid; CAL-B; Two-phase system; Enzymatic synthesis

1. Introduction

Sugar fatty acid esters are nonionic detergents. Because of their amphiphilic properties they are often used in the pharmaceutical, cosmetic and food industry. Synthesis of sugar esters in organic solvents is more difficult due to the low solubility of sugars. Only a few solvents (e.g., pyridine or DMF) are able to dissolve the highly polar sugar and the nonpolar fatty acid [1]. However, these solvents often inactivate the enzyme and are incompatible with food applications. Using sugar derivatives or alkyl glycosides [2] on the one hand solubility of the sugar increases; on the other hand this method needs extra protecting and deprotecting steps, because the products show different properties compared to the non-derivatized sugar fatty acid esters. A further method developed by our group used a solid-phase system [3], which allows quantitative sugar ester yield, but shows problems during up-scaling and reactions cannot be run continuously.

Ionic liquids (IL) are considered as environmentally friendly alternatives to organic solvents because of their very low vapor pressure and good chemical and thermal stability. Biocatalysis in ionic liquids is currently strongly investigated [4], for instance, Park and Kazlauskas reported that lipase-catalyzed acetylation of glucose proceeds with considerably higher regioselectivity compared to reactions in conventional solvents [5]. Kim et al. described the selective enzymatic acylation of alkyl glycosides in ionic liquids and noticed enhanced reactivity and regioselectivity [6]. Recently, we described sugar fatty acid ester synthesis in pure ionic liquids by using a polyethylene glycol modified lipase B from *Candida antarctica* [7].

In this work we investigated several enzymes for the sugar fatty acid ester synthesis in a two-phase system consisting of an ionic liquid and an organic solvent. Selected systems were optimized with respect to chain-length and type of acyl donor, temperature, type of co-solvent and IL, concentration of enzyme and reaction time.

2. Materials and methods

2.1. Enzymes and chemicals

Lipase PS-C (*Pseudomonas cepacia*, immobilized) and Acylase (*Aspergillus* sp.) were a gift from Amano Pharmaceuticals Co., Nagoya, Japan. Free and immobilized lipase A and B from

* Corresponding author. Tel.: +49 3834 86 4367; fax: +49 3834 86 80066.
E-mail address: uwe.bornscheuer@uni-greifswald.de (U.T. Bornscheuer).
URL: http://www.chemie.uni-greifswald.de/~biotech.

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doi:10.1016/j.molcatb.2005.08.004

C. antarctica (CAL-A, Chirazyme L5; CAL-B, Chirazyme L2) and *Candida rugosa* Lipase (Chirazyme L3) were obtained from Roche Diagnostics, Penzberg, Germany. Immobilized lipases from *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosa* (Lipozyme TL IM) were received from Novozymes, Bagsvaerd, Denmark. Fatty acid vinyl esters were from TCI, Tokyo, Japan. Ionic liquids 1-butyl-3-methyl imidazolium tetrafluoroborate [BMIM][BF_4], 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIM][PF_6], 1-butyl-3-methyl imidazolium octylsulfate [BMIM][OSO_4], 1,3-dimethyl imidazolium methylsulfate [DMIM] [$MeSO_4$] and 1,3-dimethyl imidazolium dimethylphosphate [DMIM][DMP] were a gift from Solvent Innovation, Köln, Germany. All other chemicals were obtained from Merck, Darmstadt, Germany or Sigma–Aldrich, Munich, Germany at the highest purity available.

2.2. General procedure for enzymatic reactions

Typically 0.25 mmol β-D-glucose and 0.5 mmol fatty acid (vinyl ester) were placed in a capped glass vial. Five percent (w/v) enzyme, 10% (w/v) activated molecular sieves (4 Å), 300 μl ionic liquid and 200 μl *t*-butanol were added. The reaction took place at 60 °C in an Eppendorf Thermoshaker at 900 rpm to achieve proper mixing of substrates, ionic liquid and *t*-BuOH. After 72 h the samples were extracted two times with 400 μl tetrahydrofuran. The major part of the product was present in the *t*-butanol-phase, some was also found in the ionic liquid phase. From the combined extracts excess solvent was removed by evaporation under nitrogen and the solid was dissolved in a defined amount of methanol/acetonitrile (70/25). From this solution, the sugar ester content was determined qualitatively by thin layer chromatography and quantitatively by HPLC.

2.3. Thin layer chromatography

TLC analysis was performed on non-activated silica gel plates (Alugram® Sil G/UV, Roth, Düren, Germany) using chloroform:methanol:acetic acid:water (70:20:8:2, v/v/v/v) as developing system. The plates were treated with cerium-reagent (25 g molybdato phosphoric acid, 1 g cerium-(IV)-sulfate, 80 ml conc. sulfuric acid and 1000 ml distilled water) and visualized by heating.

2.4. HPLC

All reaction mixtures were analyzed by a HPLC system (Merck Hitachi, Darmstadt, Germany) equipped with a Hypersil ODS 120-5C_{18} column. Analysis was performed using a mobile phase consisting of methanol:acetonitrile:water (75:20:5, v/v/v) and a flow rate of 0.25 ml/min. The sugar esters were detected with a light scattering detector (PL-ELS 1000, Polymer Laboratories, Darmstadt, Germany) at 40 °C.

2.5. Structure determination

Position of the fatty acid in the glucose ester and monoacylation were confirmed by NMR spectroscopy. ^1H and ^{13}C NMR-specra were recorded on a Bruker Avance 600 spectrometer (^1H at 600 MHz, ^{13}C at 150 MHz) and matched literature data [8].

3. Results and discussion

3.1. Enzyme screening

Lipase B from *C. antarctica* (CAL-B) is the most frequently used enzyme for sugar ester synthesis in organic solvents [9]. It was also found to be active in solvent systems containing ionic liquids [10]. Thus, it was surprising to us, that commercially available CAL-B showed no activity in the synthesis of sugar esters in pure ionic liquids (data not shown). To enable enzyme activity, *t*-butanol was added to the solvent system as it was already known that sugar ester synthesis can take place in this solvent [3].

However, this created a two-phase system as *t*-BuOH is immiscible with [BMIM][BF_4] or [BMIM][PF_6] (Fig. 1). Without proper mixing, *t*-BuOH was in the top phase and the enzyme particles were observed to be at the interface, rather than at the bottom of the two-phase system.

Highest conversion was achieved with immobilized CAL-B (59%). NMR analysis confirmed that the 6-*O*-lauric acid monoester was formed exclusively. Satisfying activity was also observed using the lipases from *T. lanuginosa* (TL IM, 33%) and *R. miehei* (RM IM, 8%). All other enzymes showed conversions below 5%. Thus, immobilized CAL-B seemed to be the most suitable enzyme for this two-phase reaction system.

3.2. Influence of chain-length of the acyldonor and temperature

To investigate the influence of the acyldonor chain-length, vinyl esters of fatty acids with chain lengths between C_6 and C_{16} were used as substrates. In addition, these reactions were performed at different temperatures (Table 1).

At 60 °C the best acyl donor was lauric acid vinyl ester. All other vinyl esters lead to considerably lower conversions not exceeding 30%. At 50 °C myristic acid vinyl ester was converted best (60%). CAL-B is known to not accept long-chain fatty acids, which explains the low conversion found for palmitic acid vinyl ester. At 40 °C only low conversion was observed, which is in accordance to the temperature optimum of CAL-B reported to

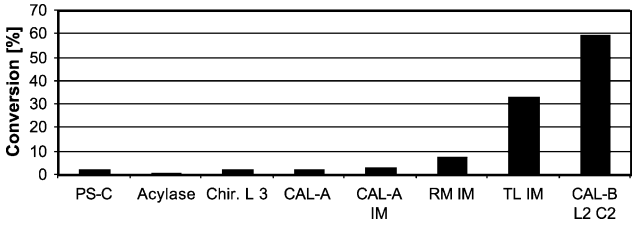

Fig. 1. Conversion obtained in the screening of several enzymes. For enzyme abbreviations, see Section 2. Reaction conditions: 0.25 mmol glucose, 0.5 mmol lauric acid vinyl ester, 2–5% (w/v) enzyme, 10% (w/v) activated molecular sieves (4 Å), 400 μl [BMIM] [BF_4], 100 μl *t*-butanol, 60 °C, 900 rpm, 72 h.

Table 1
Conversion of fatty acid vinyl esters at different temperatures using CAL-B

Temperature (°C)	C_6-VE	C_8-VE	C_{10}-VE	C_{12}-VE	C_{14}-VE	C_{16}-VE
40	18	0	13	4	18	10
50	12	17	17	52	60	16
60	24	18	27	59	11	8

C_6: caproic acid; C_8: octanoic acid; C_{10}: decanoic acid; C_{12}: lauric acid; C_{14}: myristic acid; C_{16}: palmitic acid; VE: vinyl ester; for reaction conditions see Fig. 1.

Table 2
Conversions in a two-phase system consisting of [BMIM][PF_6] and *t*-butanol with lauric acid vinyl ester (LAVE) and free palmitic acid as acyl donors

	IL/*t*-BuOH						
	100/0	90/10	80/20	60/40	40/60	20/80	0/100
LAVE	0	3	10	62	53	6	77
Palmitic acid	0	4	9	45	20	33	15

Reaction conditions: 0.25 mmol glucose, 0.5 mmol fatty acid (vinyl ester), 5% (w/v) CAL-B L2 C2, 10% (w/v) activated molecular sieves (4 Å), 60 °C, 900 rpm, 72 h.

be between 60 and 80 °C [11]. Higher temperatures could not be investigated, as the sugar started to decompose.

3.3. Optimal *t*-butanol content

As shown above, the addition of *t*-butanol allowed for sugar ester synthesis in ionic liquids resulting in a two-phase system. Next, experiments were performed to identify best ratios between *t*-BuOH and IL. The results for [BMIM][PF_6] with lauric acid vinyl ester and palmitic acid as acyl donors are given in Table 2.

For lauric acid vinyl ester, highest conversion (77%) was achieved in pure *t*-butanol. However, at 60% [BMIM][PF_6], almost as good conversion was feasible. Similar results were found using 60% [BMIM][BF_4] (65% conversion). Interestingly, conversions were substantially higher in biphasic mixtures compared to pure *t*-butanol for reactions using free palmitic acid as acyl donor. Maximum (45%) conversion was found at 60% [BMIM][PF_6] and only 15% in pure *t*-BuOH. Even under optimized conditions, no reactions took place with all other ionic liquids listed in Section 2 (data not shown).

3.4. Reaction time course

In literature, reaction times for enzymatic glucose ester synthesis varied between 6 h [12] and 72 h [3]. The time courses for the solvent system composed of 60% IL and 40% *t*-BuOH are presented in Fig. 2.

After 72 h more than 60% glucose fatty acid ester is synthesized using lauric acid vinyl ester in both solvent systems with no further increase after prolonged reaction times. Free fatty acids as acyl donors resulted in lower conversions (45% using palmitic acid) and the synthesis is also much slower compared to reactions using vinyl esters. No substantial differences between the two ILs were observed.

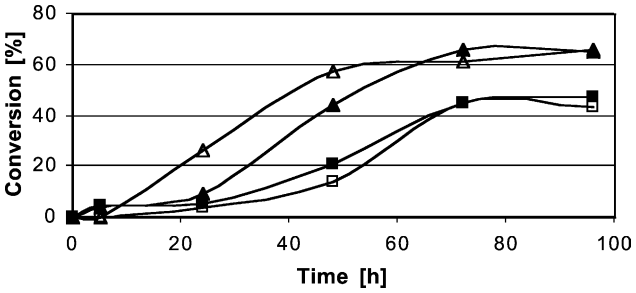

Fig. 2. Time course for the synthesis of 6-*O*-glucose laurate or palmitate using lipase B from *Candida antarctica*; (▲), 60% [BMIM][BF_4] with 40% *t*-butanol using lauric acid vinyl ester as substrate; (△), [BMIM][PF_6] with 40% *t*-butanol using lauric acid vinyl ester as substrate; (■), [BMIM][BF_4] with 40% *t*-butanol using palmitic acid as substrate; (□), [BMIM][PF_6] with 40% *t*-butanol using palmitic acid as substrate.

4. Conclusions

After optimization, similar or even higher conversions compared to reactions in pure *t*-butanol were possible using a biphasic mixture containing at best 60% ionic liquid ([BMIM][BF_4] or [BMIM][PF_6]). Enzymatic sugar ester synthesis was possible using activated fatty acid vinyl esters but also with free fatty acids and commercial CAL-B was identified as the best lipase.

Acknowledgements

We thank Solvent Innovation GmbH (Köln, Germany) for the ionic liquids, Roche Diagnostics (Penzberg, Germany) for samples of immobilized CAL-B. Financial support was provided by the BMBF (Bonn, Germany, Grant PTJ-BIO 0312737B).

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