Molecular Catalysis 460 (2018) 63–68

Contents lists available at ScienceDirect

Molecular Catalysis

journal homepage: www.elsevier.com/locate/mcat

6-*O*-glucose palmitate synthesis with lipase: Investigation of some key parameters

Dounia Arcens[a,b], Etienne Grau[a,b], Stéphane Grelier[a,b], Henri Cramail[a,b,*], Frédéric Peruch[a,b,*]

[a] Univ. Bordeaux, CNRS, Bordeaux INP/ENSCBP, Laboratoire de Chimie des Polymères Organiques, UMR 5629, 16 avenue Pey-Berland, F-33607, Pessac Cedex, France
[b] Centre National de la Recherche Scientifique, Laboratoire de Chimie des Polymères Organiques, UMR 5629, 16 avenue Pey-Berland, F-33607, Pessac Cedex, France

ARTICLE INFO

Keywords:
Glycolipids
Enzymatic synthesis
Lipase

ABSTRACT

Fatty acid sugar esters represent an important class of non-ionic bio-based surfactants. They can be synthesized from vinyl fatty acids and sugars with enzyme as a catalyst. Herein, the influence of the solvent, the lipase and the temperature on a model reaction between vinyl palmitate and glucose *via* enzymatic catalysis has been investigated and the reaction conditions optimized. Full conversion into 6-*O*-glucose palmitate was reached in 40 h in acetonitrile starting from a reactant ratio 1:1, at only 5%-wt loading of lipase from *Candida antarctica* B (CALB) without the presence of molecular sieves.

1. Introduction

Fatty acid sugar esters are non-ionic surfactants that can be synthesized from inexpensive natural resources. Because of their amphiphilic nature, non-toxicity and biodegradability [1,2], they find a wide range of applications in many fields such as food [3], pharmaceutical [4], detergents and cosmetics [5]. Depending on the chosen carbohydrate and acyl moieties, fatty acid sugar esters have been shown to exhibit anti-oxidant [6], antimicrobial [7–9], insecticidal [10], and antitumoral [11] properties. They can be obtained by a chemical route using alkaline catalysts [12], but this strategy requires high temperatures and the use of hazardous solvents such as DMF or pyridine, which are not compatible with food applications. Besides, as all the carbohydrate hydroxyl groups exhibit similar reactivity, it usually results in mixtures of esters, without any control of the composition [13]. Enzymes such as lipases, proteases and esterases are also able to catalyze fatty acid sugar ester synthesis with high selectivity, directly yielding mono-esters without need of additional protection/deprotection steps. Among them, lipases are the most used enzymes to catalyze fatty acid sugar esters synthesis. These enzymes are active in many organic solvents and at lower temperatures. Enzymatic route has therefore been widely studied as a milder and greener alternative to synthesize fatty acid sugar esters, but it presents some drawbacks such as longer reaction times, lower yields and an important cost, as large quantities of lipase are usually required (around 20 wt.%). Another major issue is also to find an appropriate solvent that can both solubilize the carbohydrate and the fatty acid moieties, without deactivating the lipases. Hydrophobic solvents enhance lipases activity [14], but poorly solubilize carbohydrates. Tertiary alcohols such as *tert*-butanol [15], and 2-methyl-butan-2-ol [16] are generally used as their relative polarity enables a good solubility of carbohydrates. Mixtures of two solvents such as *tert*-butanol/pyridine [17] or 2-methyl-butan-2-ol/DMSO [6,18] have also been tested in order to increase the carbohydrate solubility. More recently, ionic liquids [19–22] have also been explored. Another challenge is to increase the final conversions into fatty acid sugar esters. Esterification leads to the formation of water, which must be removed to shift the equilibrium toward fatty acid sugar ester formation, for instance by adding molecular sieves to the reaction media [23–26]. Ducret et al. developed a process of fatty acid sugar esters synthesis under reduced pressure to remove water [27]. Another strategy is to start from fatty acid vinyl esters. In that case, the transesterification sub-product is acetaldehyde, which is easily removed, leading to fast and high conversions [18,22,28–33]. In the present work, 6-O-glucose palmitate was synthesized in classical organic solvents from a 1:1 ratio of glucose and vinyl palmitate mainly with Lipase B from *Candida antarctica* (CALB) as the catalyst. Only 5%-wt of the supported lipase were used and the influence of the solvent, reaction time and presence of molecular sieves investigated. Several commercially available lipases were compared and the influence of the reaction temperature was also examined.

* Corresponding authors at: Univ. Bordeaux, CNRS, Bordeaux INP/ENSCBP, Laboratoire de Chimie des Polymères Organiques, UMR 5629, 16 avenue Pey-Berland, F-33607, Pessac Cedex, France.
E-mail addresses: cramail@enscbp.f (H. Cramail), peruch@enscbp.fr (F. Peruch).

https://doi.org/10.1016/j.mcat.2018.09.013
Received 7 May 2018; Received in revised form 18 July 2018; Accepted 12 September 2018

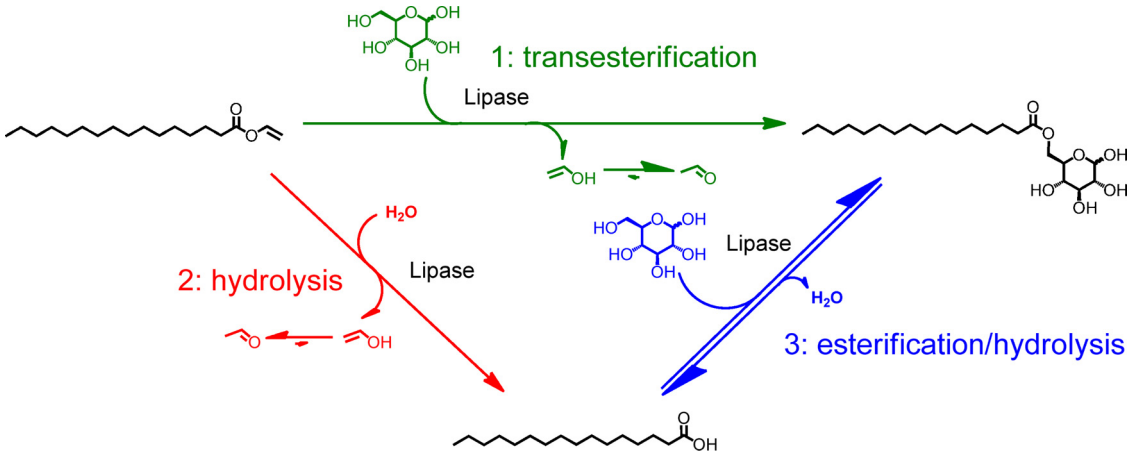

Fig. 1. Reaction scheme between vinyl palmitate (VP) or palmitic acid (PA) with glucose to produce 6-*O*-glucose palmitate (GP) in the presence of CALB.

2. Materials and methods

2.1. Materials

Vinyl palmitate was purchased from TCI Europe and was dried under dynamic vacuum overnight prior to use. Anhydrous glucose was purchased from Fluka and lipase B from *Candida antarctica* supported on acrylic beads (activity > 5000 U/g) was purchased from Sigma Aldrich. Supported lipases IMMCALB-T2-150 from *Candida antarctica* B (2500 U/g), IMMCALA-T2-150 from *Candida antarctica* A (3000 U/g), IMMRML-T2-150 from *Rhizomucor miehei* (1500 U/g), IMMTLL-T2-150 from *Thermomyces lanuginosa* (10,000 U/g), IMMABC-T2-150 from *Pseudomonas cepacia* (1500 U/g) and IMML51-T2-150 from *Fusarium solani pisi* (5000 U/g) were purchased from Chiral Vision. All lipases were used as received. Acetonitrile, THF, DMF, DMSO, and cyclohexane were purchased from Fluka, HPLC grade. Dichloromethane, HPLC grade and acetone, technical grade, were purchased from Sigma Aldrich. *tert*-Butanol, extra pure, was purchased from Acros Organics. Pyridine and dioxane were purchased from TCI. Solvent drying procedures are described in Supporting Information. Molecular sieves, 3 Å, were purchased from Acros Organics and activated by heating at 400 °C in a muffle furnace for 6 h then flamed several times under dynamic vacuum. Once activated, the latter were stored in a glovebox. Deuterated DMSO was purchased from Euriso-top.

2.2. General synthesis of 6-O-glucose palmitate catalyzed by CALB

In a typical synthesis of 6-*O*-glucose palmitate, 0.9 mmoL (249 mg) of vinyl palmitate and 0.9 mmoL (162 mg) of glucose were poured into an oven-dried Schlenk with 10 mL of solvent under an argon flux. 20 mg of supported CALB were then added. When needed, 100 mg of activated 3 Å molecular sieves beads were added. The reaction was carried out during 72 h, under magnetic stirring at 250 rpm and heated at 45 °C by means of a thermoset oil bath. For kinetic studies, 0.2 mL samples were sampled out and analyzed by ^{1}H NMR spectroscopy. Four different compounds were identified: glucose, vinyl palmitate, 6-*O* glucose palmitate and palmitic acid. In each case, the primary alcohol of glucose was only esterified; all the secondary alcohols remaining untouched. At the end of the reaction, the solvent was evaporated. THF was poured in the crude mixture and the obtained mixture was filtered under vacuum in order to remove the lipase and most of the glucose. The soluble part was evaporated. The obtained solid was dispersed into water then filtrated on a Büchner in order to remove traces of glucose. 5–10 mL of acetone was then added to dissolve the unreacted fatty chains and the suspension was filtrated again. The remaining insoluble white powder was characterized by ^{1}H NMR spectroscopy and was found to be pure 6-*O*-glucose palmitate. No significant loss was observed during the purification and 6-*O*-glucose palmitate was obtained with a yield of 90%. ^{1}H NMR (*d6*-DMSO, 400 MHz, δ (ppm)): 0.8 (3H, t, CH_3), 1.1–1.2 (H, m, alkyl chain CH_2), 1.4 (2H, q, CH_2CH_2CO), 2.3 (2H, t, CH_2CO), 3.1 (1H, m, H4), 3.2 (1H, m, H2), 3.4 (1H, m, H3), 3.7 (1H, m, H5), 4.0 (1H, m, H6a), 4.25 (1H, m, H6b), 4.6 (1H, d, OH3), 4.7 (1H, d, OH2), 4.9 (1H, t, H1), 5.0 (d, OH4β), 5.1 (d, OH4α), 6.2 (d, OH1β), 6.55 (d, OH1α)

2.3. Analysis

2.3.1. NMR spectroscopy

NMR experiments were performed at 298 K on a Bruker Avance 400 spectrometer operating at 400 MHz. Deuterated DMSO was used as solvent.

2.3.2. HPLC

HPLC analysis were performed on a HPLC apparatus with an evaporating light scattering detector (ELSD, Varian 380-LC) and a Prevail carbohydrate ES 5µ column. The evaporator and nebulizer temperatures were set at 90 °C and 40 °C, respectively. 50 µL of the samples were injected. The eluent was a solution of 75/25/5 v/v/v methanol/acetonitrile/water with a flow rate of 0.5 mL min^{-1}.

3. Results and discussion

Enzymatic fatty acid sugar ester synthesis is a complex process where several reactions can take place. CALB can catalyze vinyl palmitate transesterification into 6-*O*-glucose palmitate (reaction 1, Fig. 1). Nevertheless, because of the presence of residual water in the reaction medium, the enzyme is also able to catalyze vinyl palmitate hydrolysis (reaction 2, Fig. 1). Those two reactions are irreversible. Besides, an equilibrium takes place between the so-formed palmitic acid and 6-O-glucose palmitate (reaction 3, Fig. 1).

3.1. Effect of the solvent

Reaction between vinyl palmitate and glucose was carried out in ten organic solvents in the presence of CALB. Each solvent was tested as received (Conditions A) and also after drying (Conditions B). In the cases of acetone, acetonitrile, THF, *tert*-butanol and dioxane, the influence of the presence of 3 Å molecular sieves beads in the reaction medium was investigated and kinetic studies of the corresponding reactions were performed (Conditions C). Conversions into 6-*O*-glucose palmitate after 72 h of reaction are given in Table 1. In Fig. 2, simultaneous variations of vinyl palmitate, palmitic acid and 6-*O*-glucose palmitate contents with time are shown for each solvent, without and in the presence of molecular sieves. From those plots, the initial reaction