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Research paper

Lipase-catalyzed synthesis of sucrose monoester: Increased productivity by combining enzyme pretreatment and non-aqueous biphasic medium

Pranee Inprakhon[a,b,*], Natcha Wongthongdee[a,d,e], Taweechai Amornsakchai[c], Thunyarat Pongtharankul[a], Panya Sunintaboon[c], Lars O. Wiemann[b], Alain Durand[d,e], Volker Sieber[b]

[a] Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand
[b] Bio, Electro and Chemocatalysis BioCat, Straubing Branch, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Schulgasse 11a, 94315 Straubing, Germany
[c] Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand
[d] CNRS, LCPM, UMR 7375, Nancy F-54001, France
[e] Université de Lorraine, LCPM, UMR 7375, Nancy F-54001, France

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ABSTRACT

Sucrose monocaprate was synthesized by carrying out a lipase-catalyzed transesterification in a non-aqueous biphasic medium. Vinyl caprate was mechanically dispersed into a solution of sucrose in DMSO. The use of DMSO allowed increasing sucrose concentration up to 0.7 M (in DMSO). The denaturing effect of DMSO on lipase was avoided by pretreatment of lipase by pH adjustment in the presence of crown ether. This pretreatment maintained a significant catalytic activity which led to 0.2 M sucrose monoester within 1 h at 50 °C, which represented higher productivity than already reported. Detailed structural characterization revealed that only monoester was recovered and the 2-*O*-acylated sucrose monocaprate was the major isomer in the final product.

1. Introduction

Sucrose esters have appeared as an important class of carbohydrate-based surfactants (Behler et al., 2001; Daudé et al., 2012). They were found very convenient ingredients for food and cosmetic formulations due to very mild dermatological properties, non-toxicity, bio-degradability and the possibility to be synthesized from bio-based reactants (Khan and Rathod, 2015; Queneau et al., 2008; Shi et al., 2011). Sucrose esters have been synthesized by esterification or transesterification of sucrose with either fatty acids or methyl or vinyl esters with the aim to obtain preferably higher content of mono- and diesters. Indeed, these compounds found more potential applications as compared to highly substituted derivatives which exhibited low solubility in water. In this respect, one important characteristic of the esterification was its selectivity which was quite difficult to achieve considering the high functionality of sucrose (eight hydroxyl groups). Current manufacturing processes rely on base-catalyzed transesterification in polar organic solvents (dimethyl sulfoxide, DMSO, and dimethyl formamide, DMF) or in bulk at about 130 °C and exhibit low selectivity (Zhao et al., 2014). The use of enzymes as alternative catalysts has been actively explored over years at the laboratory scale (Shi et al., 2011). Although, the selectivity of the reaction was greatly improved, the productivity remained quite low. The main difficulties encountered for improving productivity arose from the low solubility of carbohydrates in organic solvents except in the very polar ones. Unfortunately, most of biocatalysts had a low activity in these polar solvents (like DMSO and DMF). Only the proteases of the subtilisin-family were shown to be active in such solvents (Ritthitham et al., 2009). Adding water to the organic solvent or using organic solvent with high polarity increased the solubility of sucrose, but decreased enzyme activity and favored unwanted side reactions like hydrolysis (Chamouleau et al., 2001; Humeau et al., 1998). Similarly, increasing the temperature increased sucrose solubility, but also affected the catalytic activity of the enzyme (Arcos et al., 1998; Coulon et al., 1996). Thus there was a great challenge to find treatments for enzymes that could enhance their stability and their activity in polar solvents like DMSO or DMF. This would allow increasing the concentration of sucrose in the reaction medium and consequently increasing the productivity while maintaining selectivity of esterification. According to the state of the art, the highest reported sucrose loaded concentration was 0.15 mol/L in reaction media containing either DMF or DMSO (Ashrafuzzaman et al., 2014; Pedersen et al., 2003; Shi et al., 2011; Wang et al., 2012). Nevertheless, the

* Corresponding author at: Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand.
E-mail address: pranee.inp@gmail.com (P. Inprakhon).

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maximal solubility of sucrose in DMSO has been reported to exceed 0.8 mol/L (Gaylord Chemical Company, 2014). Thus the limiting parameter has been enzyme catalytic activity which prevented the use of good solvents for sucrose substrate. In addition, the times needed for reaction ranged between 6 h and several days, which could be serious drawback for industrial developments.

This work examined the synthesis of sucrose monoesters by lipase-catalyzed transesterification in a non-aqueous biphasic reaction medium in which vinyl caprate was dispersed into a concentrated solution of sucrose in DMSO. We selected vinyl ester as the substrate in order to suppress possible limitation by thermodynamic equilibrium. In addition, the choice of vinyl caprate was made by reference to substrate preference of *Candida rugosa* regarding the number of carbon atoms in the alkyl chain of the acyl donor (Janssen et al., 1996; Kaewprapan et al., 2011). The main goal of this work was to increase sucrose concentration as compared to previously reported conditions. The effect of several operational parameters (pre-treatment of lipase by pH-adjustment, sucrose concentration in reaction medium) on monoester production was examined. The chemical structure of sucrose ester was determined using different techniques (LC–MS, ^{1}H NMR, ^{13}C NMR). The surface activity of synthesized sucrose ester was characterized and compared to that of a commercial product.

2. Materials and methods

2.1. Materials

The lipase from *Candida rugosa* (Lipase AY) with 32,800 U g^{-1} was purchased from Amano Enzyme Co. (Nagoya, Japan). Vinyl caprate, sucrose and dimethyl sulfoxide (DMSO, CROMASOV®) were purchased from Sigma-Aldrich (Buchs, Switzerland) and used without further purification. All other chemicals were of highest commercial purity and used without further purification.

2.2. Methods

2.2.1. Preparation of pH-adjusted lipase AY co-lyophilized with 18-Crown-6 ether

Firstly, the mixed solution of 18-Crown-6 ether (43 mM) in phosphate buffer solution was prepared according to literature (Kaewprapan et al., 2011). Twenty milliliters of 20 mM phosphate buffer pH 7.5 were mixed thoroughly with 0.23 g (0.86 mmol) of 18-Crown-6 ether. Subsequently, 1 g of *Candida rugosa* lipase AY was added to the solution of 18-Crown-6 ether in 20 mM phosphate buffer at pH 7.5 and the mixture was stirred at room temperature, with 250 rpm of agitation speed for 1 h. Lastly, the mixed solution was flash-frozen in liquid nitrogen and lyophilized with a freeze drier (Labcongo Corp., USA). The pretreated *Candida rugosa* lipase AY was recovered as a white powder and yielded 1.15 g (approximately 94%).

When required, the protein content of native and pretreated lipase AY was increased from 2.3 wt% to 22 wt% by ultrafiltration The crude enzyme was dissolved in 50 mM KPi (pH 6) and concentrated using an Amicon ultrafiltration unit (MWCO 10 KDa, Millipore, Massachusetts, USA) according to the manufacturer's recommendation.

2.2.2. Determination of lipase activity by pH-stat

The lipase activity in catalyzed-hydrolysis of standard tributyrin (substrate) was measured by automatically titrating the amount of released acid with a 0.01 M NaOH solution (Kaewprapan et al., 2007). The equivalence of mole of NaOH and released acid by pH-stat titration (C30 Compact Karl Fischer Coulometer, Mettler Toledo, Germany) was used to calculate the catalytic activity with the reaction mixture sample. The initial rate was calculated from the slope of the linear variation of the amount of released acid as a function of time (0–10 min). The experiments were repeated in triplicate at fixed concentration of *Candida rugosa* lipase AY (1 mL, concentration of 1 mg mL^{-1}), stabilizer (4 mL, 10% w/w gum arabic solution in Tris–HCl buffer) and standard tributyrin (146.5 μL, concentration 25 mM) in the final reaction mixture of Tris–HCl buffer (20 mL, 0.28 mM Tris–HCl, 150 mM NaCl, 1.4 mM $CaCl_2$). The results were reported as mean values ± standard deviation (SD).

2.2.3. Deactivation of pretreated lipase AY co-lyophilized with 18-Crown-6 ether

Thermal deactivation of pretreated lipase AY was carried out by suspending 0.125 g of pretreated lipase AY in 5 mL of distilled water. The solution of pretreated enzyme was refluxed at 100 °C for 5 h and then allowed to cool down at room temperature.

Afterward, the samples were flash-frozen in liquid nitrogen and lyophilized with a freeze drier (Labcongo Corp., USA) for 48 h (Kaewprapan et al., 2007). Total deactivation of enzyme activity could be verified by using the methodology for determination of lipase activity described in Section 2.2.2.

2.2.4. Lipase-catalyzed transesterification in a non-aqueous biphasic system

The experiment was designed to study catalytic ability of different forms of *Candida rugosa* lipase including native lipase AY (non-pretreated lipase), pretreated lipase AY (pH-adjusted and co-lyophilized with 18-Crown-6 ether), deactivated pretreated lipase AY, and without adding any lipase (using salts as control-catalyst instead of enzyme) for catalyzed-transesterification of sucrose with vinyl caprate in an organic biphasic system. Typically, a 0.3 M sucrose solution in DMSO was first prepared by dissolving 0.103 g of sucrose in 1 mL of DMSO under stirring with magnetic barrel at 50 °C. A 0.84 M of vinyl caprate (188 μL) was mixed thoroughly with the sucrose solution and kept under stirring at 50 °C for 5 min before adding 8.1 mg of enzyme (6.8 mg mL^{-1}). The enzyme-catalyzed synthesis reaction was allowed to proceed for 1 h. The progress of the reaction was detected by TLC. For comparison, salts (0.07 mg of potassium phosphate monobasic (KH_2PO_4) and 0.49 mg of potassium phosphate dibasic (K_2HPO_4) were added separately in the reaction medium of DMSO (1 mL) instead of *Candida rugosa* lipase AY as a control experiment. Afterwards, 0.10 g of sucrose was dissolved in the salt solution and kept under stirring with magnetic barrel at 50 °C until complete dissolution. Subsequently, 0.84 M of vinyl caprate (188 μL) was mixed thoroughly with the sucrose solution and kept under stirring with magnetic barrel at 50 °C, 500 rpm for 1 h. The progress of reaction was monitored by TLC. Additionally, vinyl caprate droplet size and density were visualized by inverted microscope BX51 & DP70 Digital Camera System (Olympus Corporation, Tokyo, Japan) at high power objective (x40 Phase contrast Microscopy) coupled with a microscope imaging software (Olympus DP Controller software) for imaging and size measurement.

2.2.5. Scale up of sucrose monoester synthesis and purification procedure

Sucrose (3.08 g, 9 mmol) was dissolved in DMSO (30 mL) at 50 °C with magnetic stirrer agitation speed of 500 rpm. Then vinyl caprate (5.64 mL, 25.31 mmol) was added continuously and kept under magnetic stirring for 30 min until vinyl caprate was well dispersed in the reaction mixture (a cloudy white emulsion reaction mixture formed). The enzymatic reaction was started by adding 0.24 g pretreated lipase AY as the biocatalyst and the reaction was carried out during 3 h of incubation time. At the end of reaction time, the turbid reaction mixture turned into a clear solution. The reaction was stopped and the presence of sucrose ester was checked by ^{1}H NMR analysis. Afterwards, the separation of products was carried out by liquid–liquid extraction to eliminate residual vinyl caprate. Hundred milliliters of *n*-hexane were added to the reaction mixture (50 mL) and mixed vigorously at room temperature. The *n*-hexane phase (clear upper phase) was separated to eliminate the residual vinyl deaconate. The remaining turbid liquid phase that contained sucrose, sucrose ester and DMSO was mixed with 50 mL of water and extracted with 200 mL of cyclohexane: 1-butanol (95:5, v/v) three times. Subsequently, the organic phases (upper phase)