Process Biochemistry 47 (2012) 1894–1902

Contents lists available at SciVerse ScienceDirect

Process Biochemistry

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

Biocatalytic acylation of sugar alcohols by 3-(4-hydroxyphenyl)propionic acid

R. Croitoru [a], F. Fiţigău [a], L.A.M. van den Broek [b], A.E. Frissen [b], C.M. Davidescu [a], C.G. Boeriu [b,c,*], F. Peter [a,**]

[a] University "Politehnica" of Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, C. Telbisz 6, 300001 Timişoara, Romania
[b] Wageningen University and Research Centre, Food & Biobased Research, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands
[c] University "Aurel Vlaicu", Department of Chemical & Biological Sciences, Arad, Romania

ARTICLE INFO

Article history:
Received 3 May 2012
Received in revised form 8 June 2012
Accepted 15 June 2012
Available online 23 June 2012

Keywords:
Lipase
Sugar alcohol
Aromatic ester
Biocatalysis
Candida antarctica

ABSTRACT

Enzymatic synthesis of aromatic esters of four different sugar alcohols (xylitol, arabitol, mannitol, and sorbitol) with 3-(4-hydroxyphenyl)propionic acid was performed in organic solvent medium, using immobilized *Candida antarctica* lipase (Novozyme 435), and molecular sieves for control of the water content. The influence of reaction parameters on the conversion has been investigated, including reaction time, temperature, alcohol/acid molar ratio, and enzyme amount. The highest conversions (94% for xylitol, 98% for arabitol, 80% for mannitol, and 93% for sorbitol) were obtained in pure *tert*-butanol at 60 °C and 72 h reaction time, 0.3 alcohol/acid molar ratio, and 0.5 g/mol enzyme/substrate ratio. The isolated new sugar alcohols esters were identified by different spectral analyses. MALDI-TOF MS analysis showed the formation of monoesters, diesters, and small quantities of triesters for all investigated sugar alcohols. The catalytic efficiency of the enzyme was higher for the pentitol substrates, decreasing in the following order: arabitol > xylitol > sorbitol > mannitol. These new compounds could have interesting applications in food, pharmaceutical and cosmetic formulations.

1. Introduction

In recent years, there has been an increasing interest in the development of naturally occurring antioxidants, which are presumed safe compared with synthetic ones. In addition to many of their vital properties in biologic systems, such as anti-inflammatory and anticarcinogenic activities, most phenolic acids, as well as their derivatives, are known to be powerful antioxidants [1–3]. The use of sugar esters of aromatic carboxylic acids in tumor treatment has been described [4], while phenolic glucosides are known for their antimicrobial, antiviral, and anti-inflammatory activities [5,6]. Combining both aromatic glucose esters and phenolic glucosides resulted in novel aromatic arylglucoside esters, exhibiting putative pharmaceutical effects [7]. Phenolic compounds in the extracts of plants were shown to have a strong capacity to scavenge oxygen radical species and to inhibit oxidation and growth of pathogenic bacteria [8]. Phenethyl esters of caffeic acid and ferulic acid exist in honeybee propolis. These lipophilic hydroxycinnamic acid esters were found to possess significant biological activities [9].

Aromatic acids, which play an important role in metabolism of plants, are frequently found in nature in the form of esters with polyhydric alcohols and carbohydrates. However, their use is restricted by the difficulty of isolating them from plants. Synthesis of sugar alcohol esters by conventional chemical procedure requires high temperatures, high pressures, corrosive acid catalysts, and toxic solvents which could leave traces in the products [10].

In such circumstances, the biocatalytic way could be a viable solution for the synthesis of this kind of compounds. From all the biocatalysts considered, the synthetic utility of lipases has shown to be outstanding, because their mode of action can be reversed in organic media, such that they catalyze, with enantiomeric selectivity, ester synthesis or transesterification reactions [11,12]. Lipases were demonstrated to be the most important biocatalysts for synthetic organic chemistry in nonaqueous systems [13,14]. Among lipases, *Candida antarctica* B lipase emerged as the most robust and efficient catalyst for a diversity of biotransformations, including regioselective syntheses [15]. Lipase-catalyzed synthesis of fatty acid esters of sugars and sugar derivatives has been studied extensively, owing to numerous applications in food, pharmaceutical, and cosmetic industries. Although the major attention was paid to sugar esters, synthesis of sugar alcohol esters was subject of numerous investigations, as well. The most important difficulty

* Corresponding author at: Wageningen University and Research Centre, Food & Biobased Research, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands. Tel.: +31 317 480168; fax: +31 317 483011.
** Corresponding author. Tel.: +40 256 404216; fax: +40 256 403060.
E-mail addresses: Carmen.Boeriu@wur.nl (C.G. Boeriu), francisc.peter@chim.upt.ro, Francisc52@gmail.com (F. Peter).

1359-5113/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.procbio.2012.06.015

in these reactions was the solubilization of sugars in the organic medium [16]. Several strategies have been developed to overcome this problem, as utilization of a solid-phase reaction system with a small amount of organic solvent serving as adjuvant [17], or a mixture of tertiary alcohol and polar solvent as a compromise between enzyme activity and sugar solubility [18]. Not only sugars, but also sugar acids have been successfully esterified with aliphatic and aromatic alcohols [19].

The synthetic strategies for esters of sugar alcohols were essentially the same as for sugar esters. However, the solubility problem was not so critical, allowing utilization of tertiary alcohols as reaction media. Sorbitol monooleate and other esters of sugar alcohols with surfactant properties were obtained by enzymatic esterification catalyzed by Novozyme 435, in *tert*-amyl alcohol, at reduced pressure. Excess of the hydroxyl donor yielded more than 90% monoester [20]. Monoesters and diesters of sugar alcohols with saturated fatty acids were continuously produced in acetone at 60 °C, with Novozyme 435. Fatty acid excess and other reaction conditions were set to obtain larger amounts of monoesters, which were considered more interesting for several applications [21]. Adnani et al. synthesized xylitol esters of capric and caproic acids in a solvent-free reaction system, with 74% and 60.5% conversions, respectively [22]. A comprehensive study about acylation reactions of polyols (including sorbitol) revealed that selective esterification of one or two of the primary hydroxyl groups can be achieved through the appropriate selection of reaction conditions: temperature, solvent, and excess of polyol substrate [23].

Enzymatic esterification of functionalized phenols with fatty acids, as well as of phenolic acids with various alcohols, has been successfully accomplished [24–26]. Among various phenolic acids, 3-(4-hydroxyphenyl)propionic acid (HPPA) was employed for acylation of flavonoid glycosides, yielding 62% and 49% naringin and isoquercetin esters, respectively [27]. However, at our best knowledge, enzymatic synthesis of sugar alcohol esters with HPPA as acyl donor was not yet reported. HPPA is a natural non-toxic aromatic hydroxy acid, also interesting as possible source of biodegradable copolymers [28,29]. In the present study, the effect of different reaction parameters on the synthesis of such esters was investigated, using lipase B from *C. antarctica* (Novozyme 435) as biocatalyst. The reactions were analyzed with respect to conversion, initial reaction rate, and monoester and diester distribution. The selection of Novozyme 435 was based on our previous screening of several native and immobilized lipases, whereby Novozyme 435 exhibited the highest yield of products [30].

2. Materials and methods

2.1. Materials

Dimethylsulfoxide (DMSO, 99.5%) and acetonitrile (99.5%) were products of Fluka and Merck, respectively. Novozyme 435 was a generous gift of Novozyme. Xylitol, arabitol, 3-(4-hydroxyphenyl)propionic acid (HPPA, 97%), *tert*-butanol (≥99%), molecular sieves (4 Å, 4–8 mesh), and 2,5-dihydroxybenzoic acid (≥98%) were from Sigma–Aldrich. Mannitol and sorbitol were purchased from Acros Organics.

2.2. Enzymatic esterification of sugar alcohols

HPPA and different sugar alcohols (xylitol, arabitol, mannitol, and sorbitol) were used as substrates for optimization of the reaction conditions, with regard to sugar alcohol esters synthesis. A sugar alcohol, at different initial concentrations (0.05–0.15 mol/L), and 3-(4-hydroxyphenyl)propionic acid, at different alcohol/acid molar ratios (between 5 and 0.3), were added to 1 mL binary mixtures of *tert*-butanol and dimethylsulfoxide (at 100:0, 95:5, 90:10, or 80:20% volume ratio, respectively), in 4 mL glass reaction flasks. Immobilized lipase from *C. antarctica* B (Novozyme 435) has been used as catalyst. The activity of Novozyme 435 for this type of aromatic substrate was previously assayed as 25 U/g, in the transesterification reaction of methyl-3-(4-hydroxyphenyl)-propionate with 1-octanol, in *tert*-butanol reaction medium, at 40 °C [30]. Molecular sieves (4 Å, 0–0.1 g), and Novozyme 435 (0.01–0.05 g) were subsequently added. The reactions were performed in an incubator (ILW 115 STD; Pol-Eko-Aparatura, Poland) equipped with an orbital shaker (MIR-S100; Sanyo, Japan) at 250 rpm, at different temperatures (40, 50, 60, 70 °C) and for different time periods (12, 24, 48, 72 h). The progress of the esterification reaction was monitored by HPLC. All experiments were performed in duplicate, and the sampling was also done in duplicate. Results are given as the mean value of the four measurements. The conversions were calculated based on the consumption of the phenolic acid, while monoester and diester yields were calculated based on calibration curves of the pure compounds, isolated and purified in our laboratory. Identification of the products has been accomplished by MALDI-TOF MS (matrix assisted laser desorption-ionization time of flight mass spectrometry), based on the similarity between the *m/z* values identified in the spectra and the calculated values of the appropriate mono-, di-, and triesters (Fig. 1).

2.3. Kinetic study

Kinetic study was accomplished for acylation of sugar alcohols (xylitol, arabitol, mannitol, and sorbitol) by 3-(4-hydroxyphenyl)propionic acid in pure *tert*-butanol at 50 °C, at different initial sugar alcohol concentrations (from 10 to 150 mM), at the same biocatalyst concentration, 1 g/mol alcohol. The initial reaction rates were calculated for the first hours of every reaction (typically the first 3 h), when only monoester was produced and the ester formation rate was linear. Based on the initial reaction rates, the Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) values were obtained from Lineweaver–Burk plots and used for calculation of the kinetic constant k_{cat} and catalytic efficiency (k_{cat}/K_m).

2.4. Reutilization study

Arabitol (0.1 mmol) and 3-(4-hydroxyphenyl)propionic acid (0.3 mmol) were added to 2 mL *tert*-butanol in a 4 mL glass reaction flasks. Molecular sieves (4 Å, 0.04 g), and Novozyme 435 (0.05 g) were subsequently added. The reactions were performed at 60 °C for 72 h, with orbital shaking, as previously described. Samples were taken at fixed intervals and analyzed by HPLC. After each cycle, the reaction product was separated, the enzyme was washed with *tert*-butanol, the molecular sieves were replaced, and the reaction was repeated in the same conditions.

2.5. Separation and purification of sugar alcohol esters

After completing the reaction, the enzyme and molecular sieve were separated by filtration, *tert*-butanol was evaporated and the resulted syrup was eluted on a silica gel column with ethyl acetate:dichloromethane (1:1 ratio) for isolation of the diester, followed by elution with dichloromethane:methanol (8:1 ratio), for isolation of the monoester. The purified mono- and diesters were used as standards for the HPLC analysis.

2.6. Reaction monitoring by HPLC

The reaction mixture was analyzed by HPLC (Jasco HPLC system, equipped with PU-2089 Plus quaternary pump and UV-2070 Plus detector), on a reverse phase Synergy 4u Hydro-RP 80A, 250 mm × 4.6 mm column (Phenomenex). The eluting components were detected at 276 nm. The compounds were eluted using an acetonitrile/water mixture gradient from 2% to 98% (17.5 min), followed by isocratic elution with 98:2 acetonitrile/water (4.5 min), at a flow rate of 0.6 mL/min.

2.7. Structural analysis

MALDI-TOF MS, ^{1}H NMR, ^{13}C NMR, and FT/IR were used for the identification and characterization of aromatic sugar alcohol esters. The mass spectra were recorded on an Ultraflex Workstation, using FlexControl and FlexAnalysis software packages for acquisition and processing of the data (Bruker Daltonics, Germany). The instrument was calibrated using a mixture of maltodextrins with known molecular masses (Avebe, The Netherlands). One μL of the reaction mixture was mixed with 9 μL of matrix solution (10 mg 2,5-dihydroxy benzoic acid in 1 mL acetonitrile: water, 1:1). Two μL of this mixture was subsequently transferred to a target plate and dried under a stream of dry air. Measurements were performed in the positive mode. Ions were accelerated with a 25 kV voltage after a delayed extraction time of 200 ns. Detection was performed in the reflector mode. The lowest laser intensity needed to obtain a good quality spectrum was applied.

NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400.17 MHz (^{1}H) and 100.62 MHz (^{13}C). DMSO-d_6 (99.9 at.% D, Aldrich) was used as the solvent and it was used as received. For ^{1}H NMR the signal of the solvent peak was set at δ 2.5000 ppm and for ^{13}C NMR the signal of the solvent peak was set at δ 39.5000 ppm. Attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectra were recorded on a Jasco FT/IR-430 spectrophotometer.

Eight new mono- and diesters of sugar alcohols with HPPA acid were isolated and characterized (Fig. 2):

2.7.1. 1-Mannityl-3-(4-hydroxyphenyl)propionate (**1**)

FTIR (cm^{-1}): 3270 (ν(O—H)), 2921 (ν(C—H)), 1705 (ν(C=O) ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.22 (br s, 1H, H-1′), 7.01 (d, 2H, J = 8.5 Hz, H-3′), 6.66 (d, 2H, J = 8.5 Hz, H-2′), 4.6–3.3 (multiple couplings, 13H, H-1 + H-2 + H-3 + HJ-4 + H-5 + H-6 + OH of carbohydrate moiety), 2.75 (t, 2H, J = 7.6 Hz, H-5′), 2.55 (t, 2H, J = 7.6 Hz, H-6′); ^{13}C NMR (100 MHz, DMSO-d_6, δ) and proton-decoupled DEPT-135 (negative