Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular Catalysis B: Enzymatic 35 (2005) 142–146
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Transglucosylation of hydroquinone catalysed by α-glucosidase from baker's yeast

Radivoje Prodanović [a,*], Nenad Milosavić [b], Dušan Sladić [a], Mario Zlatović [b], Branislav Božić [c], Tanja Ćirković Veličković [a], Zoran Vujčić [a]

[a] *Faculty of Chemistry, University of Belgrade, Studentski trg 12, 11000 Belgrade, Serbia and Montenegro*
[b] *Department of Chemistry, IHTM, Njegoševa 12, 11001 Belgrade, Serbia and Montenegro*
[c] *DugaNova,Viline Vode 6, 11000 Belgrade, Serbia and Montenegro*

Received 7 March 2005; received in revised form 17 June 2005; accepted 29 June 2005
Available online 28 July 2005

Abstract

Hydroquinone α-isomaltoside and hydroquinone α-glucoside were synthesized by transglucosylation in an aqueous system with baker's yeast α-glucosidase from hydroquinone and maltose as a glucosyl donor. Only one phenolic group was glucosylated, with α-selectivity, and the nature of the reaction products was governed by the concentration of hydroquinone. The optimal conditions for synthesis of glycosides were 9 mM hydroquinone and 1.5 M maltose in a 100 mM sodium citrate/phosphate buffer at pH 5.0 and 30 °C for 20 h. Under these conditions both hydroquinone α-glycosides were obtained in nearly equimolar amounts with a total molar yield of 28% with respect to hydroquinone and a total glycoside concentration of 1 mg/mL in the reaction mixture.

Keywords: α-Arbutine; Hydroquinone; Maltase; Isomaltoside; Transglycosylation

1. Introduction

Phenolic compounds are widely distributed in nature and they may have many physiological functions, exemplified by the antioxidative activity of catechins [1] and the antileukemic activity of avarol [2]. Glycosylation of phenolic compounds increases their solubility in water and could improve pharmacological properties. Hydroquinone is toxic while its glucoside arbutin has antibacterial and skin whitening effects [3]. The physiological activity and bioavailability of glycosides may also depend on the type or positions of sugars attached. For example, the skin whitening activity of α-arbutin is eight times higher than that of β-arbutin [3]. Therefore, new glycosides of phenolic compounds could have novel pharmacological properties.

Chemical methods for glycoside synthesis often require protection and deprotection steps for the substrates and products, as well as the use of toxic catalysts and solvents. Using enzymes, glycosides could be obtained in one step under mild conditions and without byproducts [4–7]. For this reason biocatalysts that could enable glycosylation of phenolic compounds are desirable. Only a few enzymes are reported to catalyse this reaction, such as sucrose phosphorylase from *Leuconostoc mesenteroides* [8], glucosyl transferase from *Xanthomonas campestris* [1] and α-amylase from *Bacillus subtilis* [9]. From the synthetic viewpoint the more readily available glycosyl hydrolases offer the advantage of a simple catalytic system and accept a broader structural range of alcohol acceptors than glycosyl transferases [10]. α-Glucosidase (maltase) is one of the most abundant glycosyl hydrolases present in baker's yeast. It has been previously used for the synthesis of menthyl- [11] and *n*-alkyl-glucosides [12].

In this paper we described for the first time synthesis of hydroquinone α-isomaltoside by α-glucosidase from baker's

* Corresponding author. Fax: +381 11 638785.
E-mail address: rprodano@chem.bg.ac.yu (R. Prodanović).

1381-1177/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2005.06.011

yeast (*Saccharomyces cerevisiae*). The reaction conditions were optimized and the products were characterized by TLC, HPLC, NMR, MS and by hydrolysis with α-glucosidase and amyloglucosidase.

2. Experimental

2.1. Enzymes and chemicals

Amyloglucosidase (3.2.1.3) was purchased from Mapol Warszawa. α-Glucosidase (3.2.1.20) was isolated from baker's yeast by a slightly modified previously published procedure [13]. It showed a single band on SDS-gel electrophoresis with an approximate molecular weight of 63 kDa. The specific activity of the purified enzyme was 80 U/mg protein and the K_m for 4-nitrophenyl α-D-glucopyranoside was 0.2 mM. Proteins were determined by the method of Bradford. 1 U of enzyme activity was defined as the amount of enzyme that liberates 1 μmol of glucose at 25 °C for 1 min from 4-nitrophenyl α-D-glucopyranoside. Hydroquinone, β-arbutin, silica gel 60 for column chromatography and all other chemicals were obtained from Merck Co.

2.2. Transglucosylation reaction

Unless otherwise indicated, glycosylation was carried out under the following conditions. The reaction mixture containing 0.1 M sodium citrate/phosphate buffer at pH 5.0, 9 mM hydroquinone, 1.5 M maltose and 10 U/mL of glucosidase was incubated for 20 h at 30 °C. The obtained glycosides were examined by TLC and HPLC.

2.3. Identification and quantification of products

The reaction was stopped by adding 0.1 M HCl to pH 3.0 (products were stable at that pH during HPLC time analysis, i.e. for at least one day) and acetonitrile to 10% (w/v). After that the reaction mixture was centrifuged, and analyzed by HPLC (column, Bondesil C18; mobile phase 10% (v/v) acetonitrile with 1 mM HCl; flow rate, 0.6 mL/min; spectrophotometric detection at 280 nm). TLC was carried out on silica gel 60 plates (E. Merck, Darmstadt, Germany) using the ascending method with ethyl acetate–methanol–water (10:1.7:1.4, v/v/v) as the solvent. Spots were made visible by spraying with 50% (w/v) H_2SO_4 followed by heating at 160 °C.

2.4. Purification and structural analysis of products

The reaction mixture containing 9 mM hydroquinone, 1.5 M maltose and 10 U/mL of α-glucosidase in 100 mL of 0.1 M sodium citrate/phosphate buffer at pH 5.0 was incubated for 20 h at 30 °C and then applied to a column packed with Purolite MN102, a synthetic macroporous polystyrene resin, purchased from Purolite Wales UK. The column was washed with water and hydroquinone compounds were eluted with 96% (v/v) ethanol. The effluent was concentrated and the residue applied to silica gel dry flash chromatography with ethyl acetate–methanol (9:1, v/v) as the eluent. Two glycosides were obtained and their NMR spectra were obtained using a Varian Gemini apparatus and 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Mass spectra were recorded on a LCQ Advantage Thermo Finningan ion trap mass spectrometer. Optical rotation was measured using a Autopol IV, Rudolph Research Analytical automatic polarimeter. Hydrolysis of glycosides was carried out in a 0.1 M sodium phosphate buffer at pH 7.0 with α-glucosidase and in a 0.1 M sodium acetate buffer at pH 5.0 with amyloglucosidase.

2.5. NMR spectra, mass data and optical rotations of products

2.5.1. Hydroquinone α-D-glucopyranoside

^{13}C NMR (50 MHz, DMSO-d_6): δ 152,7 (C-1), δ 150,4 (C-4), δ 119,2 (C-3, C-5), δ 116,0 (C2–C6), δ 99,6 (C-1′), δ 73,8 (C-3′), δ 73,5 (C-5′), δ 72,1 (C-2′), δ 70,4 (C-4′), δ 61,1 (C-6′). ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.91 (d, 2 H, J = 9.0 Hz, H-2, H-6), δ 6.67 (d, 2 H, J = 9.0 Hz, H-3, H-5), δ 5.14 (d, 1 H, J = 3.6 Hz, H-1′), δ 3.25-3.65 (m, 5 H, H-2′, H-3′, H-5′, H-6′A, H-6′B), δ 3.16 (t, 1 H, J = 9.0 Hz, H-4′). MS (ESI): m/z = 271.11 $[M-H]^-$. $C_{12}H_{16}O_7$: 272.25. $[\alpha]_D{}^{20}$ = +114° (c = 1.23, methanol).

2.5.2. Hydroquinone α-D-isomaltoside

^{13}C NMR (50 MHz, DMSO-d_6): δ 153,1 (C-1), δ 150,8 (C-4), δ 119,8 (C-3, C-5), δ 116,3 (C2–C6), δ 100,2 (C-1″), δ 98,8(C-1′), δ 73,7 (C-3′), δ 73,6 (C-3″), δ 72,9 (C-5″), δ 72, 4 (C-2′), δ 72,1 (C-2″), δ 72,0 (C-5′), δ 70,8 (C-4′), δ 70,5 (C-4″), δ 66,7 (C-6′), δ 61,3 (C-6″). ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.95 (d, 2 H, J = 9.0 Hz, H-2, H-6), δ 6.70 (d, 2 H, J = 9.0 Hz, H-3, H-5), δ 5.05 (d, 1 H, J = 3.6 Hz, H-1′), δ 4.64 (d, 1 H, J = 3.6 Hz, H-1″), δ 3.05–3.80 (m, 12 H, H-2′, H-2″, H-3′, H-3″, H-4′, H-4″, H-5′, H-5″, H-6′A, H-6′B, H-6″A, H-6″B). MS (ESI): m/z = 433.26 $[M-H]^-$. $C_{18}H_{26}O_{12}$: 434.40. $[\alpha]_D{}^{20}$ = + 128° (c = 1.35, methanol).

3. Results and discussion

3.1. Influence of acceptor hydroquinone concentration on the type of reaction products

In order to select an enzyme with the ability to catalyse glycosylation of hydroquinone we screened amyloglucosidase from *A. niger* and invertase and α-glucosidase from baker's yeast and succeeded in the synthesis of hydroquinone α-glucoside using α-glucosidase [14]. We determined that equilibrium was reached after 20 h from the beginning of the reaction with any hydroquinone concentration so that all other experiments were based on single point determinations