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

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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

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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

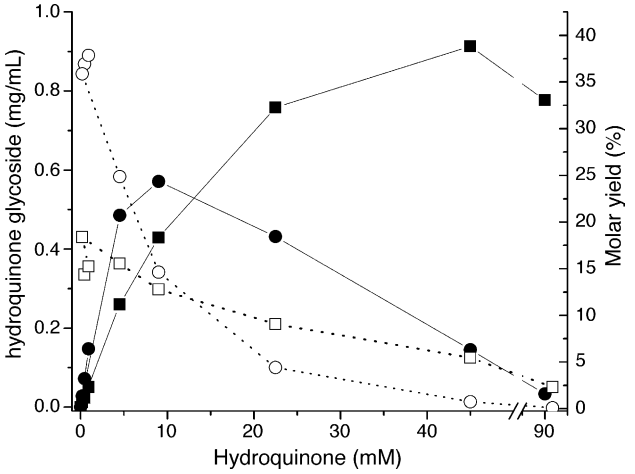

Fig. 1. Influence of hydroquinone concentration on molar yield and concentration of hydroquinone glycosides. The reaction mixture containing 0.1 M sodium citrate/phosphate buffer at pH 5.0, 1.5 M maltose and 10 U/mL of glucosidase was incubated for 20 h at 30 °C. (■) Hydroquinone α-glucoside concentration, (●) hydroquinone α-isomaltoside concentration, (□) hydroquinone α-glucoside yield, (○) hydroquinone α-isomaltoside yield.

taken at equilibrium. In order to increase further the reaction yield of hydroquinone α-glucoside we varied the concentration of hydroquinone and observed that with decreasing hydroquinone concentration a novel reaction product later characterized as hydroquinone α-isomaltoside was synthesized, see Fig. 1.

It can be seen in Fig. 1 that for concentrations of hydroquinone above 50 mM almost only one product, hydroquinone α-glucoside is formed. With 90 mM of hydroquinone, the obtained glucoside concentration was 0.75 mg/mL with a molar conversion yield of 2.5% with respect to hydroquinone. With a decreasing concentration of hydroquinone in the reaction mixture a novel reaction product isomaltoside was formed. Moreover, with hydroquinone under 15 mM, more isomaltoside was formed than glucoside. The total concentration of glycosides did not change but the molar conversion yield increased. Thus, hydroquinone at 9 mM gave 0.4 mg/mL of hydroquinone α-glucoside, with a molar conversion yield of 13% and 0.6 mg/mL of hydroquinone α-isomaltoside with a molar conversion yield of 15%. This yield of isomaltoside was much higher than in the previously reported synthesis of 1-*O*-benzyl α-maltoside by transglycosylation with α-amylase [15]. Also, the obtained yield of glycosides was about six times higher than in our previous report [14]. A further decrease in hydroquinone concentration led to an increased molar conversion yield and much more isomaltoside formed than glucoside but the total concentration of glycosides decreased. Glycosides of hydroquinone with more than two glucose units attached were not observed. This is probably because of the low specifity of α-glucosidase from baker's yeast for higher oligosaccharides [13].

The ability of α-glucosidase to synthesize isomaltosides was not observed in previous reports with menthyl and *n*-alkyl alcohols [11,12], but α-glucosidase was able to form phenyl maltoside from phenyl glucoside in 50% (v/v) acetonitrile [16]. That report contained no structural evidence for the phenyl maltoside described. Our results could be explained by the following mechanism of the transglucosylation reaction. The first step in the reaction is the splitting of maltose and formation of a glucosylated enzyme intermediate. This glucosylated enzyme could transfer glucose to a molecule of water (hydrolytic reaction) or alcohol acceptors present in the reaction mixture (transglucosylation reaction), see Fig. 2.

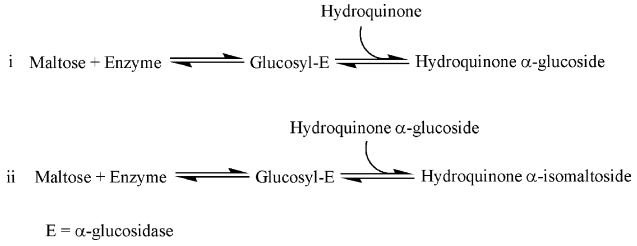

Fig. 2. Schematic representation of a possible mechanism of the transglucosylation reaction catalysed by α-glucosidase from baker's yeast.

When a high concentration of hydroquinone is present, the transglucosylation reaction i is favoured because the enzyme is saturated with hydroquinone. When the hydroquinone concentration is lower than 50 mM, transglucosylation reaction ii is favored due to the high affinity of α-glucosidase for glucosides of hydrophobic phenols (K_m for 4-nitrophenyl α-glucoside is 0.2 mM [13]) and synthesis of hydroquinone α-isomaltoside occurs. Maltose, hydroquinone α-glucoside and hydroquinone α-isomaltoside would be subject to simultaneous hydrolysis. This can be confirmed by the time course of the reaction. During the first 15 min of the transglucosylation reaction only hydroquinone α-glucoside could be detected, see Fig. 3.

After that, because of the hydroquinone α-glucoside formed, transglucosylation reaction ii takes place resulting in hydroquinone α-isomaltoside. Two hours after the beginning of the reaction, the steady state concentration of hydroquinone α-glucoside was reached and only the hydroquinone α-isomaltoside concentration increased. The maximum yield of total glycosides was obtained after 20 h of the transglucosylation reaction.

3.2. Optimization of reaction conditions

The transglucosylation reaction of hydroquinone catalysed by α-glucosidase from baker's yeast was further optimized with respect to pH, temperature and maltose concentration. The pH optimum for the synthesis of hydroquinone α-isomaltoside was in the range of 5.0–5.5 while for the glucoside it was in the range of 4.5–5.0, see Fig. 4.

Differences in the optimum pH values used for hydrolysis (7.0) and the transglucosylation (5.0) reaction catalysed by α-glucosidase from baker's yeast were also reported by Nakagawa et al [11].

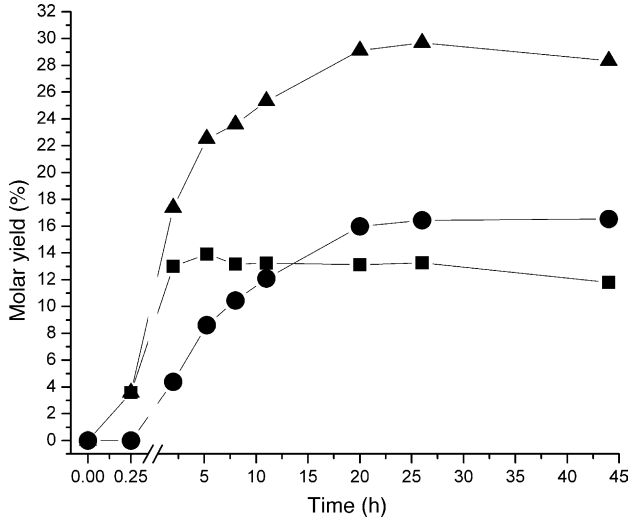

Fig. 3. Time course of the transglucosylation reaction catalysed by α-glucosidase from baker's yeast. The reaction mixture containing 0.1 M sodium citrate/phosphate buffer at pH 5.0, 9 mM hydroquinone, 1.5 M maltose and 10 U/mL of glucosidase was incubated for the indicated times at 30 °C. (■) Hydroquinone α-glucoside yield, (●) hydroquinone α-isomaltoside yield, (▲) total glycoside yield.

The temperature optimum for glycoside synthesis was determined to be between 30 and 40 °C, Table 1.

For 9 mM hydroquinone we obtained higher yields of glycosides at 40 °C than in our previous work with higher hydroquinone concentrations [14]. This is probably the result of instability of the enzyme in higher hydroquinone concentrations due to chemical modifications of the enzyme with benzoquinone, which is the oxidation product of hydroquinone. Its concentration in the reaction mixture is increased

Table 1
The effect of temperature on the transglucosylation of hydroquinone

Temperature (°C)	Glucoside (mol%)	Isomaltoside (mol%)
20	8.52	2.38
25	12.0	8.42
30	13.2	15.2
35	13.3	15.1
40	13.6	15.0

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 the indicated temperatures.

at higher concentrations of hydroquinone and at higher temperatures.

Maximum yields of isomaltoside and total glycosides of hydroquinone were obtained at 1.5 M maltose, see Fig. 5.

At higher maltose concentrations the reaction rate was slower due to the high viscosity of the reaction solution and larger amounts of glucoside than isomaltoside were obtained. At lower maltose concentrations low yields of both glycosides were obtained due to increased water concentration and higher rates of the hydrolysis reaction (Fig. 2). This was in accordance with previous results for menthyl glucoside synthesis [11].

3.3. Purification and structural analysis of products

In order to determine the structures of the products of the transglucosylation reaction catalysed by α-glucosidase from baker's yeast the reaction was performed under optimum conditions for hydroquinone α-isomaltoside synthesis as was previously determined. Under the conditions described in the Experimental section, specific products were detected in the

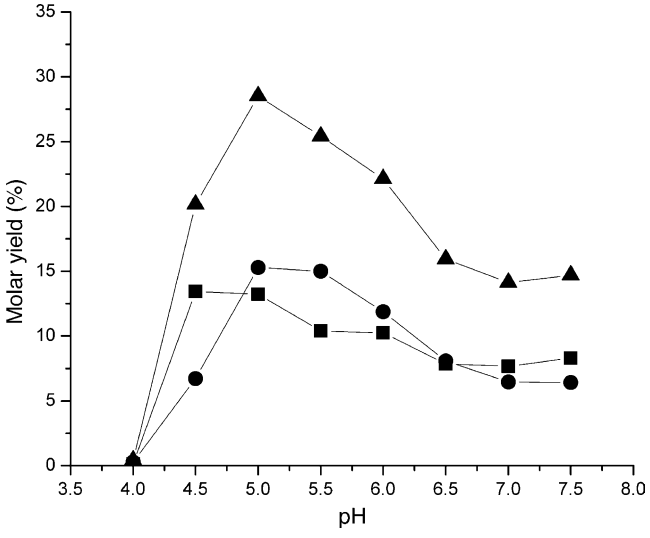

Fig. 4. Effects of pH on the transglucosylation of hydroquinone. The reaction mixture containing 0.1 M sodium citrate/phosphate buffer at different pHs, 9 mM hydroquinone, 1.5 M maltose and 10 U/mL of glucosidase was incubated for 20 h at 30 °C. (■) hydroquinone α-glucoside yield, (●) hydroquinone α-isomaltoside yield, (▲) total glycoside yield.

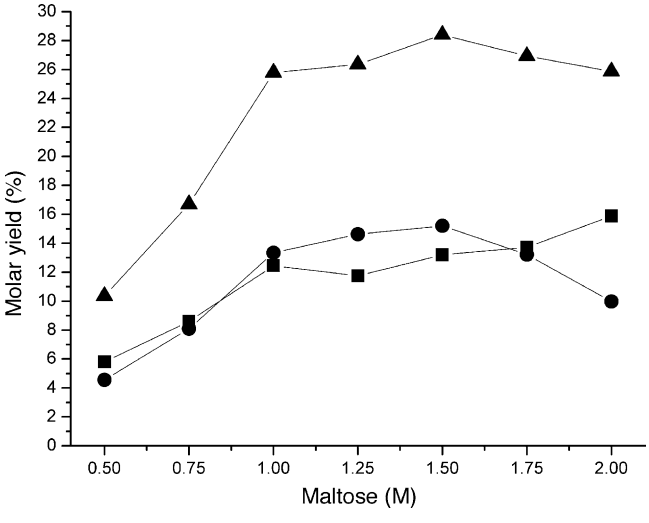

Fig. 5. Effects of maltose concentration on the transglucosylation of hydroquinone. The reaction mixture containing 0.1 M sodium citrate/phosphate buffer at pH 5.0, 9 mM hydroquinone, indicated maltose concentrations and 10 U/mL of glucosidase was incubated for 20 h at 30 °C. (■) hydroquinone α-glucoside yield, (●) hydroquinone α-isomaltoside yield, (▲) total glycoside yield.

reaction mixture by TLC analysis. The products show R_f values of 0.50 (hydroquinone α-glucoside), identical to that of β-arbutine and R_f of 0.15 (hydroquinone α-isomaltoside), very similar to the glucose R_f value of 0.10. After purification about 30 mg of hydroquinone α-glucoside and 40 mg of hydroquinone α-isomaltoside were obtained.

The ^{1}H NMR spectrum of the product with a higher R_f value (0.50) indicated the α-configuration of the glycosidic linkage, based on the value of the coupling constant (J = 3.6 Hz), of the signal of the anomeric proton at 5.14 [17]. NMR data provide evidence that this product is hydroquinone α-glucoside [17].

In the product with a lower R_f value (0.15) the glucose units are α (1–6) linked. In ^{13}C NMR spectrum the chemical shifts of two –CH_2– signals, unambiguously assigned using DEPT experiment, are not similar, which shows that one –CH_2OH group is free (chemical shift 61.3 ppm) and the other participates in formation of the glycosidic bond (chemical shift 66.7 ppm). The values of the chemical shifts are close to those of isomaltose in the SDBS NMR database [18]. Both glycosidic linkages have an α-configuration, as evidenced by coupling constants of anomeric protons (both J = 3.6 Hz) in the ^{1}H NMR spectrum. Therefore, the product can be identified as hydroquinone α-isomaltoside. To the best of our knowledge this compound has not been either isolated or synthesized so far.

These conclusions are further confirmed by enzymatic hydrolysis with glycosidases. Both products were rapidly hydrolyzed by α-glucosidase from baker's yeast in 0.1 M sodium phosphate buffer at pH 7.0 to hydroquinone and glucose which was confirmed by TLC. Hydroquinone α-isomaltoside was rapidly hydrolyzed by amyloglucosidase in 0.1 M sodium acetate buffer at pH 5.0 to hydroquinone α-glucoside and glucose which was also confirmed by TLC.

4. Conclusions

The ability of α-glucosidase from baker's yeast to synthesize hydroquinone α-isomaltoside from hydroquinone with maltose as the glucosyl donor is reported for the first time. Reaction conditions for selective synthesis of either glucoside or isomaltoside were determined. This could be very useful for synthesis of isomaltosides of other hydrophobic, physiologically active phenolic compounds containing the hydroquinone moiety and having very low solubility in water. The obtained isomaltosides of phenolic compounds such as hydroquinone could also have different pharmacological properties compared to glucosides (arbutin).

Acknowledgment

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