Multi-enzymatic Glucosylation Using Eucalyptus UDP-Glucosyltransferase Coupled UDPglucose-Fermentation by Bakers’ Yeast

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The enzymatic synthesis of glucosides compounds using a membrane-associated UDP-glucosyltransferase fraction from Eucalyptus perriniana cultured cells as a water-insoluble catalyst (N. Nakajima, et. al., J. Ferment. Bioeng., 84 (5), pp. 455-460, 1997) has been effectively done by coupling UDP-glucose-fermentation by bakers’ yeast. For example, β-thujaplicin (hinokitol) and p-aminobenzoic acid were converted respectively to their corresponding β-D-monoglucoisides with the conversion rate of around 24-26% by the multi-enzymatic system with UDPglucose as a glucose donor, which is produced by yeast cells from glucose and 5’-UMP. Addition of either cellubiose, a substrate of β-glucosidase, or 1,2-anhydro-myo-inositol, an inhibitor for the enzyme in the reaction mixture, could increased the yield of these β-D-monoglucosides. This new enzymatic system could also be used for the synthesis of flavonoid glucosides such as isouqueritin (quercetin 3-O-β-D-glucoside).

Key words: UDP-glucosyltransferase; glucosylation; Eucalyptus perriniana; UDPglucose-fermentation; bakers’ yeast

Glycosylation is an important method for the structural modification of various exogenous compounds, resulting in the conversion of water-insoluble compounds to stable, water-soluble ones.1-4 For example, β-thujaplicin (hinokitol), a naturally occurring antimicrobial compound, and p-aminobenzoic acid, a sunscreen for ultraviolet light absorption, are used widely as medicines, food additives, preservatives, and cosmetics. Such useful biofunctional abilities of these compounds could be improved by glycosylation due to the elimination of disadvantageous properties such as water insolubility, heat instability, and degradability by light and oxygen. Furthermore, flavonoids, naturally occurring plant pigments such as isouqueritin, have been reported to be immunomodulators, antioxidants, and antiviral factors.3,5,6

Until now, various procedures for enzymatic glycosylation have been reported with the transglycosylation reaction by glycosidases.6-8 Enzymatic in situ regeneration of UDP-sugars such as UDPglucose from 5’-UDP and glucose-6-phosphate was demonstrated for the UDP-glucosyltransferase reaction,8,9 however, little attention has been paid to synthesis of glucosides by the direct use of UDP-glucosyltransferase due to the high cost and instability of UDPglucose as a glucose donor.

Recently, we found that Eucalyptus perriniana cultured cells have a rather stable UDP-glucosyltransferase in their membrane fraction, which act on the hydroxy groups of aromatic, phenolic, polyphenolic, and non-phenolic compounds to produce the corresponding β-D-glucosides.10

On the other hand, a useful method for UDPglucose production has been developed with UDPglucose-fermentation by yeast cells,11 and recently the synthesis of trehalose 6-phosphate from glucose and 5’-UMP as substrates was also reported with an energy coupling fermentation by the yeast.12

Herein, we report an efficient multi-enzymatic glycosylation system for biofunctional compounds such as β-thujaplicin,13 p-aminobenzoic acid,14 and quercetin3,10 using a membrane-associated UDP-glucosyltransferase from the cultured cells by coupling UDPglucose-fermentation by bakers’ yeast. In the enzymatic glycosylation system, the UDP-glucosyltransferase in the membrane fraction as a water-insoluble catalyst could use UDPglucose as a glucose donor, which was produced by the yeast from glucose and 5’-UMP in the presence of magnesium and phosphate ions (Fig. 1.).

The membrane fraction containing the membrane-associated UDP-glucosyltransferase from Eucalyptus perriniana cultured cells (cultivation for 2 weeks at 25°C) was prepared by the procedure described previously.10 The β-D-glucoside compounds formed in the coupled enzymatic system were identified by comparison of the retention time on HPLC with the corresponding authentic β-D-glucosides isolated as we reported previously.10,13,14 The conversion rate of the β-D-glucoside compounds produced was calculated by the molar ratio against substrate-aglycons added to the reaction mixture.

We found that β-thujaplicin,13 a substrate-aglycone was converted to β-thujaplicin β-D-monoglucoside (4-isopropyltropolone 2-O-β-D-glucopyranoside and 6-isopropyltropolone 2-O-β-D-glucopyranoside) most efficiently under the following conditions. The reaction

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mixture contained 10 μmol β-thujaplicin, 40 μmol of 5'-UMP, 400 μmol of glucose, 2.5 μmol of magnesium chloride, 50 mg of dried bakers’ yeast (Oriental Yeast Co., Japan), 200 mg of the membrane fraction (wet weight) containing the membrane-associated UDP-glucosyltransferases to which corresponded 0.8 g of the cells (wet weight) collected by filtration, and 0.2 M potassium phosphate buffer (pH 7.8) in a final volume of 1 ml. The optimum temperature for the reaction was 30°C, and the optimum pH was between 7.5 and 7.8. When β-thujaplicin was added to the reaction mixture, the amount of the β-D-monoglucoside increased with prolonged incubation time, followed the decrease of glucose consumed by yeast as shown in Fig. 2.

The reaction reached equilibrium after 23 h of incubation, when nearly 24% of β-thujaplicin was converted to the β-D-glucoside and the added glucose had almost disappeared. The maximum production of the β-D-glucoside was 0.79 mg in the reaction mixture (1 ml) containing 1.64 mg of β-thujaplicin added. Addition of larger amounts of the substrate, the yeast cells, and the enzyme fraction resulted in decreased yield of the β-D-glucoside. More than 25 h of incubation also decreased the yield of the product. The decreased yield of the β-D-glucoside was probably due to the action of β-glycosidases present in the membrane fraction as we described previously.

The amount of UDPglucose produced by the yeast in the reaction mixture with or without the addition of β-thujaplicin was kept at about 20 μmol after 10 h of incubation, when measured using UDPglucose dehydrogenase and NAD+. Although almost of the UDP glucose fermented was not used for the glucosylation, the ratio of the β-D-glucoside produced (nearly 24%) was larger than that in the our previous procedure by addition of the same amount of UDPglucose (20 μmol) into the reaction mixture (23–24 h of incubation). The β-D-glucoside was not synthesized at all in the absence of ether 5'-UMP and glucose or bakers’ yeast. From these results, we concluded that UDPglucose in this multi-enzymatic glucosylation system with energy coupling fermentation by the yeast was cheaper than the previous method.

Addition of either cellulose (10 μmol, a substrate of β-glucosidase) or DL-1,2-anhydro-myo-inositol (0.1 μmol, an inhibitor for the enzyme) to the reaction mixture also effective for the production of a larger amount of the β-glucosides (the increased yield, about 5% in each case). The increased yield seemed to be caused by the inhibition of β-glucosidase in the membrane fraction.

We also converted p-aminobenzoic acid to p-aminobenzoyl β-D-glucopyranoside in the same manner with the conversion rate of nearly 26%. This procedure for enzymatic glucosylation could be applicable to the regioselective synthesis of flavonoid monoglucosides, a naturally occurring plant pigment such as isorqueritrin (quercetin 3-O-β-D-glucoside) with the rate of about 6% from the water-insoluble polyphenol-aglycone (quercetin), when the polyphenol as an ethanol solution (100 μl) added into the reaction mixture (1 ml).

In the production of β-thujaplicin β-D-monoglucoside and p-aminobenzoyl β-D-glucopyranoside, TDPglucose also acted as a glucose donor as UDPglucose in the UDP-glucosyltransferase reaction, however, GDPglucose, ADPglucose, and CDPglucose did not
work at all. Furthermore, β-thujaplicin β-D-gentiobioside (6-isopropyltropolone 2-O-β-D-gentiobioside) was found to be synthesized slightly in the presence of UDPglucose or TDPglucose, only when the corresponding β-thujaplicin β-D-monoglucoside (6-isopropyltropolone 2-O-β-D-glucopyranoside) was added as a substrate in the reaction mixture. Although, in biotransformation with the suspension cells of E. perriniana, p-(N-β-D-glucopyranosylamino) benzoyl β-D-glucopyranoside was produced from p-aminobenzoic acid added to the medium, the synthesis of the β-D-diglucoside from p-aminobenzoic β-D-glucopyranoside could not be done with this system.

These β-D-glucoside compounds produced through this multi-enzymatic system could be separated easily by liquid chromatography from the reaction mixtures, and the results of structural analysis of the β-D-glucosides separated coincided to those from our previous reports.10,13,14 Therefore, we have developed a new enzymatic system for the selective synthesis of β-D-monoglucosides, which consisted of a combination of the glucosylation reaction by the UDP-glucosyltransferase and the UDPglucose-fermentation by the yeast cells.11

It was considered that further increases in yield of the β-D-glucosides by this enzymatic system must come both form the larger productivity of UDPglucose by the yeast cells in the reaction mixture and to the higher reactivity of the UDP-glucosyltransferase for the aglycones added. The application of the enzymatic method in more large scale production is now on progress and will be reported in the following paper.

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