Enzymatic Biosynthesis of Novel Resveratrol Glucoside and Glycoside Derivatives

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A UDP glucosyltransferase from Bacillus licheniformis was overexpressed, purified, and incubated with nucleotide diphosphates (NDP) D- and L-sugars to produce glucose, galactose, 2-deoxyglucose, viosamine, rhamnose, and fucose sugar-conjugated resveratrol glycosides. Significantly higher (90%) bioconversion of resveratrol was achieved with α-D-glucose as the sugar donor to produce four different glucosides of resveratrol: resveratrol 3-O-β-D-glucoside, resveratrol 4'-O-β-D-glucoside, resveratrol 3,5-O-β-D-diglucoside, and resveratrol 3,5,4'-O-β-D-triglucoside. The conversion rates and numbers of products formed were found to vary with the other NDP sugar donors. Resveratrol 3-O-β-D-2-deoxyglucoside and resveratrol 3,5-O-β-D-di-2-deoxyglucoside were found to be produced using TDP-2-deoxyglucose as a donor; however, the monoglycosides resveratrol 4'-O-β-D-galactoside, resveratrol 4'-O-β-D-viosaminoside, resveratrol 3-O-β-D-rhamnoside, and resveratrol 3-O-β-D-fucoside were produced from the respective sugar donors. Altogether, 10 diverse glycoside derivatives of the medically important resveratrol were generated, demonstrating the capacity of YjiC to produce structurally diverse resveratrol glycosides.

Resveratrol is a phytoalexin that is naturally produced by various plants and is present in grapes, peanuts, blueberries, and hops (1–4). Resveratrol is often produced as a defense against microbial infections by Botrytis cinerea (5), radical damage, UV irradiation (6), and other stressors. The trans and cis configurations of resveratrol exist in glucosides, dimers (pallidol) (7), trimers (grandiphenol C) (8), α-viniferin (9), and polymers (Fig. 1). Moreover, the biological activities of resveratrol are mostly associated with its trans configuration (10–12). Other modified resveratrol derivatives with potent biological activities, such as antioxidant, cardioprotective, neuroprotective, anticancer, anti-estrogenic, and antiaging properties, have also been identified in different plant sources, including pterostilbene, trans-polydatin (trans-piceid), piceatannol (synonym astringinin), and astringin (Fig. 1) (12–14).

Due to the popularity of the “French paradox,” a wide array of studies on resveratrol have been performed. As a result, the biological activities of resveratrol against various diseases, including type II diabetes (15–17), obesity, atherosclerosis (18, 19), Alzheimer’s disease (20), cardiovascular diseases (including hypertension (21, 22) and ischemic injury (23–25)), and various cancers (26), have been demonstrated. Similar to other antioxidants, resveratrol neutralizes self-generated free radical species, including reactive oxygen species and reactive nitrogen species, and prevents radical damage to organs. Thus, most of the biological activities of resveratrol are attributed to its intrinsic radical-scavenging activity (27). Resveratrol exhibits anti-inflammatory properties, as demonstrated by its ability to inhibit the production of nitric oxide (NO) and proinflammatory cytokines, including tumor necrosis factor alpha and interleukin-6 (IL-6), from macrophages (28–30). Resveratrol also has numerous functional targets inside cells, including receptors (either membrane bound or intracellular), signaling molecules (sirutins [31] and the 5’-AMP-activated kinase signaling pathway [32]), transcription and DNA repair factors, and oxidative enzymes (33, 34). Furthermore, based on the database available at the U.S. National Institutes of Health website (http://www.clinicaltrials.gov/), a series of clinical investigations of resveratrol are in progress, as well. However, additional studies are needed to address the safety, pharmacokinetics, pharmacodynamics, and clinical efficacy of resveratrol, as these parameters are essential for the advancement of resveratrol for clinical application (35).

Most biologically active natural products (NPs) and recently used therapeutics, including calicheamicin (36), daunomycin (37), streptomycin (38), vancomycin (39), digitotoxin (40), and amphotericin (41), are decorated with diverse types of sugar moieties to enhance their physical, chemical, and biological properties. These added sugar moieties can also be important for the recognition of different target molecules in a cell (42). Moreover, the adsorption, distribution, metabolism, and excretion properties of drugs are also influenced by the type of sugar moiety attached to them (43). Hence, glycosylation has been developed as an efficient tool for generating biologically potent and novel NP glycoside compounds with enhanced drug efficacy.

Various NPs have been successfully glycodiversified, either by enzymatic or by chemical synthesis approaches. Digitoxin (44), novobiocin (45), vancomycin (46), colchicine (47), and 4-methyl-
umbelliferone (48) have been glycorandomized, thereby producing a number of unusual sugar-bearing NPs. Among these, some have shown enhanced biological properties compared to their respective parent molecules (44). In addition, glycosyltransferases (GTs) (e.g., oleandomycin GT [OleD] and its variants [49, 50], kanamycin GT-KanE, and vancomycin GT-GtfE [51]) have been engineered to increase their flexibility in accepting various donor and acceptor substrates. For the present study, a GT, YjiC (GenBank accession no. AAU40842.1) from Bacillus licheniformis DSM 13, with a flexible donor and acceptor substrate profile was selected in order to synthesize glycodiversified resveratrol derivatives. YjiC has been shown to accept geldanamycin analogues (52), epothilone A (53), various flavonoids (54, 55), chalcone (56), and isoflavonoids (57) as substrates for glucosylation. In this study, resveratrol was used as an acceptor substrate and various rare nucleotide diphosphate sugars were used as sugar donor substrates to produce glycodiversified resveratrol derivatives that have not been previously described.

**MATERIALS AND METHODS**

**Chemicals and reagents.** Resveratrol, UDP-β-glucose, UDP-β-galactose, UDP-β-glucuronic acid, and UDP-β-N-acetylglucosamine were purchased from Sigma-Aldrich (St. Louis, MO, USA). TDP-β-rhamnose and GDP-β-fucose were purchased from GeneChem (Daejeon, South Korea). TDP-β-2-deoxyglucose and TDP-β-viosamine were gifts from Dae Hee Kim (GeneChem, Daejeon, South Korea). All other chemicals and reagents were of the highest chemical grade.

**Gene cloning and protein expression and purification.** The details of the methodology for the cloning, expression, and purification of YjiC are described in our previous reports (53–57).

**General laboratory-scale glycosyltransferase enzymatic reaction.** The glycosylation reactions using YjiC were carried out in a total reaction mixture volume of 100 μl containing 100 mmol Tris-Cl (pH 8.0), 4 mmol nucleotide diphosphate (NDP) sugar, 2 mmol resveratrol, 10 mmol MgCl₂ · 6H₂O, and 50 μg/ml (final concentration) enzyme. The reaction mixtures were incubated at 37°C for 3 h. Assay mixtures lacking enzyme served as controls. The reaction was quenched by adding 400 μl chilled methanol and mixed by vortexing for several minutes. Then, the aliquots were centrifuged at 12,000 rpm to remove denatured proteins, and the...
reaction product was directly monitored by high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) after proper dilution.

**Preparative-scale reaction of resveratrol with UDP-β-D-glucose.** The reaction mixture contained purified YjiC enzyme (500–μg/ml final concentration in the reaction mixture), 20 mmol (final concentration) of UDP-β-D-glucose (≈122 mg) as a donor substrate, 5 mmol resveratrol (11.4 mg dissolved in dimethyl sulfoxide [DMSO]) as an acceptor substrate, and 100 mmol Tris-Cl (pH 8.0) buffer containing 10 mmol MgCl2·6H2O. The reaction was carried out in a 10-ml final volume for 12 h at 37°C and stopped by adding a triple volume of chilled methanol. Then, the reaction mixture was mixed by vortexing and centrifuged at 12,000 rpm for 30 min at 4°C to remove denatured protein. The supernatant was concentrated by evaporation and lyophilization. Finally, the compounds were dissolved in 2 ml HPLC grade methanol and subjected to preparative HPLC purification (Shimadzu, Tokyo, Japan). The purified compounds were dried, lyophilized, and dissolved in methanol-D2 and further analyzed by nuclear magnetic resonance (NMR).

**Deglycosylation reaction assay.** The deglycosylation assay was carried out using purified resveratrol 3-O-β-D-glucoside as the substrate. The reaction mixture contained 100 mmol Tris-Cl (pH 8.0), 10 mmol UDP, 10 mmol MgCl2, and a 50-μg/ml final concentration of enzyme. The reaction mixture was incubated at 37°C for 24 h. Ten microliters of reaction sample was taken and analyzed by HPLC-PDA after proper dilution.

**Analytical methods.** For the thin-layer chromatography ( TLC) analysis of reaction mixtures, samples were loaded on a normal-phase silica TLC plate and developed in an ethyl acetate-methanol-water-toluene (10: 2:1.5:1.0:0.2 ml) mixture in a closed TLC chamber. After a complete run of the samples, the plate was air dried and visualized under UV light (UV) at 254 nm. The reverse-phase HPLC-PDA analysis was performed with a C18 column (YMC-Pack ODS-AQ; 4.6 mm internal diameter [i.d.], 150 mm long, 5 μm particle size) connected to a PDA (308 nm) using binary conditions of H2O (0.1% trifluoroacetic acid buffer) and 100% acetonitrile (ACN) at a flow rate of 1 ml/min for 25 min. The ACN concentrations were as follows: 20% (0 to 5 min), 50% (5 to 10 min), 70% (10 to 15 min), 90% (15 to 20 min), and 10% (20 to 25 min). The purification of compounds was carried out by preparative HPLC with a C18 column (YMC-Pack ODS-AQ; 150 mm long, 20 mm i.d., 10 μm particle size) connected to a UV detector (308 nm) using a 36-min binary program with ACN at 20% (0 to 5 min), 40% (5 to 10 min), 40% (10 to 15 min), 90% (15 to 25 min), 90% (25 to 30 min), and 10% (30 to 35 min) at a flow rate of 10 ml/min. The high-resolution quantitative time of flight electrospray ionization mass spectrometry (HRQTOF–ESI-MS) was used to identify the reaction product. The deglycosylation reaction product was directly monitored by high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) after proper dilution.

**RESULTS**

**Enzymatic synthesis of resveratrol glucosides.** The HPLC-PDA analysis of the reaction mixture containing resveratrol, UDP-β-D-glucose, and the YjiC enzyme showed four different glucoside peaks at 308 nm (Fig. 2). By analyzing the same reaction mixture with an HPLC-PDA combined with HRQTOF–ESI-MS, the exact mass for each peak was determined. Peaks P1 and P3 had retention times (tR) of 13.32 min and 12.9 min, respectively. Both peaks had an exact mass [(P1 or P3) + H+] of ~391.1384 and represented monoglucosides of resveratrol. The third peak (P2) had a tR of 11.4 min with an exact mass, [P2 + Na+], of ~757.1755 and represented a resveratrol with two glucose conjugated. Similarly, the fourth peak (P4) had a tR of 10.1 min and an exact mass, [P4 + Na+], of ~737.2266, representing a triglucoside of resveratrol (see Fig. S1 and Tables S1 and S2 in the supplemental material). To further verify the reaction products obtained, degradative scale reaction was carried out in a 10-ml reaction volume. The products were purified as described in Materials and Methods and were subjected to various NMR analyses, including 1H-NMR, 13C-NMR, and two-dimensional NMR analyses (COSY, ROESY, HSQC, and HMBC) (see Fig. S2 to S4 in the supplemental material). The P2 product was identified as (E)-1-(3,β-D-glucopyranosyl-5-hydroxyphenyl)-2-(4-hydroxyphenyl) ethene (common name, resveratrol 3-O-β-D-glucoside) (1.81 mg; 465 μmol; ~9.3%), which has previously been identified from different plant sources, as well as by enzymatic reactions (62, 63). The P4 product was identified as (E)-1-(3,5-dihydroxyphenyl)-2-(4-β-D-glucopyranosyl-oxyphenyl) ethene (common name, resveratrol 4′-O-β-D-glucoside) (3.63 mg; 931.5 μmol; ~18.6%), as described previously (62, 63). According to the mass analysis performed, P3 is hypothesized to represent a diglucoside of resveratrol identified as (E)-1-(3,5-bis-β-D-glucopyranosylxylophenyl)-2-(4-hydroxyphenyl) ethene (common name, resveratrol 3,5-di-O-β-D-glucoside) (14.58 mg; 2639 μmol;
The purity of each product was greater than 95%, and the available reactive hydroxyl groups of resveratrol were glucosylated by UDP-D-galactose, a C-6 position-modified sugar (UDP-D-glucuronic acid), and a C-4 and C-6 position-modified sugar (UDP-D-glucopyranosyloxyphenyl)-2-(4'-O-acetylglucosamine), a C-4 position-modified sugar (UDP-N-acetylglucosamine), and UDP-D-glucuronic acid were used as sugar donors. Specifically, C-2 position-modified sugars (TDP-D-2-deoxyglucose, NDP-L-fucose, and GDP-L-fucose), four different types of sugar donors were used. Two resveratrol monoglucosides (resveratrol 3-O-β-D-glucoside [P₁] and resveratrol 4'-O-β-D-glucoside [P₂]), a diglucoside (resveratrol 3,5-O-β-D-diglucoside [P₃]), and a triglucoside (resveratrol 3,5,4'-O-β-D-triglucoside [P₄]) were produced in the reaction mixture.

Because of the limited availability and difficulties in enzymatic synthesis of various NDP sugars, all products were characterized by HPLC-PDA followed by HRQTOF–ESI-MS analysis in positive mode finally confirmed the conjugation of various sugars with resveratrol (Fig. 3; see Fig. S6 and Tables S1 and S2 in the supplemental material). Both resveratrol 3-O-β-D-2-deoxyglucoside (tᵢR = 12.6 min; [M + Na]⁺ ~ 396.1412) and resveratrol 4'-O-β-D-galactoside (tᵢR = 13.8 min; [M + Na]⁺ ~ 413.1229) and resveratrol 4'-O-β-D-viosaminoside (tᵢR = 12.6 min; [M + Na]⁺ ~ 396.1412) exhibited tᵢR values similar to those of the corresponding resveratrol 4'-O-β-D-glucoside.

![Figure 2](http://aem.asm.org/)

**FIG 2** HPLC-PDA analysis. The chromatogram presents the reaction products of resveratrol and UDP-α-D-glucose catalyzed by the YjiC enzyme. The inset provides the full chromatogram, and the shaded region is magnified. Above each peak, the structure of the identified product is presented. Two resveratrol monoglucosides (resveratrol 3-O-β-D-glucoside [P₁] and resveratrol 4'-O-β-D-glucoside [P₂]), a diglucoside (resveratrol 3,5-O-β-D-diglucoside [P₃]), and a triglucoside (resveratrol 3,5,4'-O-β-D-triglucoside [P₄]) were produced in the reaction mixture.

~52.8%) (62–64). The triglucoside product, P₄, was identified as (E)-1-(3,5-bis-β-D-glucopyranosyloxyphenyl)-2-(4-β-D-glucopyranosyl-oxophenyl) ethene (common name, resveratrol 3,5,4'-tri-O-β-D-glucoside) (3.28 mg; 460 μmol; ~9.2%), and all available reactive hydroxyl groups of resveratrol were glucosylated (Fig. 2). The purity of each product was greater than 95%, and the overall conversion rate of resveratrol to its glucoside forms was 89.92%. In addition, YjiC exhibited nonregiospecific enzyme activity by conjugating glucose at all the available reactive hydroxyl positions of resveratrol. In our previous report, YjiC exhibited high flexibility when phloretin was used as an acceptor molecule, producing five different glucosylated derivatives, including two monoglucosides, two diglucosides, and a triglucoside. Recently, Zhou and colleagues reported the production of two monoglucosides and two diglucosides of resveratrol using OleD and its variants (ASP, AIP, TDP16, and 3–1-H12) (63). However, the authors did not report the detection of a triglucoside resveratrol.

**Biosynthesis of diverse resveratrol glycosides.** To characterize the donor substrate specificity of YjiC for different NDP sugars using resveratrol as an acceptor substrate, NDP-α-sugars and NDP-β-sugars were used in glycosyltransferase assays. For the NDP-α-sugars, four different types of sugar donors were used. Specifically, C-2 position-modified sugars (TDP-β-D-2-deoxyglucose and UDP-β-D-glucose), a C-4 position-modified sugar (UDP-β-D-galactose), a C-6 position-modified sugar (UDP-β-D-glucuronic acid), and a C-4 and C-6 position-modified sugar (TDP-β-D-viosamine) were used. Two different NDP-β-sugars, TDP-β-D-rhamnose and GDP-β-D-fucose, were also used to assay the activity of YjiC with resveratrol under identical reaction conditions. As a preliminary analysis, TLC on normal-phase silica plates (F₂₅₄; Merck, Darmstadt, Germany) was performed. Extra spots with a lower tᵢR than that of unmodified resveratrol were distinctly observed in most of the reaction mixtures (see Fig. S5 in the supplemental material). All the reaction mixtures were further analyzed by HPLC-PDA (Fig. 3), which detected the conjugation of various sugars with resveratrol at 308 nm. HPLC-PDA and HRQTOF–ESI-MS analysis in positive mode finally confirmed the conjugation of various sugars with resveratrol (Fig. 3; see Fig. S6 and Tables S1 and S2 in the supplemental material).
in the supplemental material). These products had $t_R$s similar to that of resveratrol 3-0β-d-glucoside (see Table S1 in the supplemental material).

The substrate conversion percentage, as well as the number of products formed, varied with the NDP sugars used in the reaction mixtures. The conversion percentages of resveratrol to resveratrol glycosides were determined at different time intervals in all reaction mixtures (Fig. 4). The highest conversion of resveratrol to resveratrol glucosides was obtained with UDP-d-glucose (~90%), followed by TDP-d-viosamine (~49%), UDP-d-galactose (~45%), and TDP-2 deoxy-d-glucose (~45%). In comparison, the reactions that used TDP-l-rhamnose and

FIG 3 HPLC-PDA analyses of reaction mixtures of resveratrol and YjiC with different NDP sugar donor substrates. (A) Resveratrol standard; (B) TDP-2-deoxy-d-glucose; (C) UDP-d-galactose; (D) TDP-d-viosamine; (E) TDP-l-rhamnose; (F) GDP-l-fucose; (G) UDP-d-glucuronic acid; (H) UDP-N-acetylglucosamine. The open circles represent resveratrol standard, the filled circles are products, and the four-pointed stars represent unidentified products. The structures of the resveratrol glycoside derivatives identified are presented, along with HPLC chromatograms. The structures were identified by HPLC-PDA coupled with HRQTOF–ESI-MS analyses and by comparing the retention times with those of resveratrol glucoside analogues identified by NMR.
Concomitantly, the concentration of resveratrol increased (Fig. 4) and it decreased to 20% of the initial concentration in 12 h at 37°C. Decrease by 20% (to 80% of the initial concentration) within 1 h, respectively (Fig. 4). However, an unexpected result was that the concentration of resveratrol and UDP-D-glucose in a reverse-glycosylation reaction decreased after 1 h, and this may be due to a lack of excess UDP-D-glucose in the reaction mixture. Taken together, these results indicate that YjiC can deglucosylate resveratrol glucoside to produce UDP-D-glucose and resveratrol, and these products can eventually be utilized by the same enzyme to produce other products (Fig. 5).

**Docking of resveratrol in the YjiC model.** OleD from *S. amybioticus* (PDB ID 2IYA) (65) was selected as a template to build the YjiC model using Accelrys Discovery Studio 3.1 software (Accelrys Inc., San Diego, CA) based on the high similarity and identity of the amino acid sequences (see Fig. S7 in the supplemental material). The three-dimensional (3D) structure of the YjiC model resembles the Rossmann-like domains belonging to the GT-B fold of GTI family proteins (Fig. 6A). Following active-site optimization, molecular dynamics were used to dock resveratrol to the YjiC model. The study of all docked conformations of resveratrol in the YjiC model showed that resveratrol has two binding modes (head-first versus tail-first) (Fig. 6). In head-first mode, 3-OH and 5-OH groups were in close proximity to UDP in a deep cleft formed between N-terminal (acceptor binding pocket) and C-terminal (donor binding pocket) domains. In the tail-first mode, the 4’-OH group was near the UDP (Fig. 6B and C). Thus, there was a possibility of production of multiple glycosylated products of resveratrol. This *in silico* docking result is consistent with our *in vitro* glucoylation reaction elucidating the production of four different resveratrol glucoside derivatives while using UDP-D-glucose as a glucose donor substrate (Fig. 2), as well as the conjugation of other sugars at different positions of resveratrol by YjiC (Fig. 3). Similar binding modes of resveratrol were also observed in docking studies of resveratrol in OleD and its variants, producing two monoglucosides and two diglucosides of resveratrol (63).

**DISCUSSION**

Besides a number of biological activities of resveratrol and its derivatives beneficial to health (12–14), recent evidence has also demonstrated an important role of resveratrol in the activation of SIRT1, a protein that has been shown to lengthen the life spans of con resveratrol. UDP-D-glucose and resveratrol were also detected after deglucosylation of resveratrol 3-O-β-D-glucoside occurred and were utilized by YjiC to produce the newly formed products. However, resveratrol 3,4’-O-β-D-triglucoside was not detected, and this may be due to a lack of excess UDP-D-glucose in the reaction mixture. Taken together, these results indicate that YjiC can deglucosylate resveratrol glucoside to produce UDP-D-glucose and resveratrol, and these products can eventually be utilized by the same enzyme to produce other products (Fig. 5).
of yeast, worms, flies, fish, and mice (31, 66, 67). Upregulation of SIRT1 has also been shown to reduce cellular oxidative stress in the diabetic milieu (68). In this study, we attempted to expand the number of resveratrol derivatives by means of an enzymatic glycosylation approach that could help to generate novel therapeutics. Since the YjiC glycosyltransferase has been identified as accepting diverse kinds of NDP sugars, as well as NDP sugars, as donor substrates, the use of the same enzyme has eased the production of diverse resveratrol glucosides and glycosides by in vitro glycosylation. We have recently reported the wide promiscuity of the enzyme, which raised the possibility of production of diverse flavonol glycosides. As resveratrol is one of the most studied plant-derived lead compounds and is under clinical trials for development as a drug for diverse diseases, generation of novel resveratrol glycosides could facilitate the development of drugs to treat oxidative-stress-induced diseases and to provide antiaging functions.

Resveratrol occurs in two forms, cis-resveratrol and trans-resveratrol. The latter form of resveratrol changes to the cis form upon exposure to UV light. Importantly, the trans form of resveratrol is biologically potent and more abundantly present in nature than the cis form (69–71). The glycosylation of resveratrol could make it more stable, since most studies have proven that glycosylation enhances the stability of the compound (72–74). Furthermore, glycosylation increases its solubility in water, which ultimately enhances the bioavailability of the compound for diverse medical and cosmetic applications. Importantly, the conjugation of resveratrol with diverse sugars could modify its biological potency, as well.

The sugar moieties of most NPs have been found to be involved in cell-cell recognition, stable binding of the compound at the target site, and other biochemical processes (42). Most of the plant-derived glycosides contain sugars, like D-glucose, D-galactose, D-xylose, D-arabinose, D-rhamnose, and D-fucose. However, most of the microbe-originated therapeutics were found to be conjugated with deoxy sugars and amino sugars. Thus, we used diverse NDP sugars, including deoxy sugars (TDP-D-2-deoxyglucose, TDP-1-glucose, and GDP-1-fucose) and amino sugars (TDP-D-viosamine and UDP-D-N-acetylglucosamine) for the production of nonnatural sugars bearing resveratrol glycosides, along with glycosides and galactosides. We successfully produced 10 diverse resveratrol glycosides—including resveratrol 3-O-β-D-glucoside, resveratrol 4′-O-β-D-glucoside, resveratrol 3,5-O-β-D-diglucoside, resveratrol 3,5,4′-O-β-D-triglucoside, resveratrol 3-O-β-D-2-deoxyglucoside, resveratrol 3,5-O-β-D-di-2-deoxyglucoside, resveratrol 4′-O-β-D-galactoside, resveratrol 4′-O-β-D-viosaminoside, resveratrol 3-O-β-1-rhamnoside, and resveratrol 3-O-β-1-fucoside—by in vitro glycosylation reactions. Most of the resveratrol derivatives produced are novel compounds, except for two monoglucosides and a diglucoside, which were isolated from different plant sources. The diverse resveratrol glycosides could exhibit different bioactivities in comparison to the parent lead compound.

In general, the secondary metabolites produced by plants and actinomycetes are unstable, toxic, hydrophobic, or volatile and are postmodified to glycosylated forms by GTs (75). Moreover, these glycosylated compounds have been found to exhibit increased stability and solubility in water while also being nontoxic. Currently, the relevance of metabolic stability following deglycosylation of NP-glycosides remains unknown. Thus, the formation of resveratrol 4′-O-β-D-glucoside and resveratrol 3,5-O-β-D-diglucoside followed by deglycosylation of resveratrol 3-O-β-D-glucoside is as yet unknown, but this could be due to the higher thermodynamic stability of resveratrol 4′-O-β-D-glucoside and resveratrol 3,5-O-β-D-diglucoside than of resveratrol 3-O-β-D-glucoside. The HPLC-PDA analysis of the resveratrol glucosylation reaction (Fig. 2) also detected a greater abundance of resveratrol 4′-O-β-D-glucoside and resveratrol 3,5-O-β-D-diglucoside than of resveratrol 3-O-β-D-glucoside. However, it is also possible that resveratrol 3-O-β-D-glucoside is directly converted to the more stable resveratrol 3,5-O-β-D-diglucoside following sequential glycosylation of resveratrol 3-O-β-D-glucoside at the C-5 hydroxyl position.

The molecular modeling and docking studies showed that resveratrol has two binding modes (head first versus tail first) with YjiC, similar to OleD. The dual binding modes of resveratrol have supported the possibility of production of multiple glycosylated products. Thus, glycosylation can occur at the 3-, 5-, and 4′-hydroxyl positions of resveratrol. Our in vitro results, which included the detection of diglucoside and triglucoside forms of resveratrol, are consistent with this model. Detailed studies of protein structure, as well as mutagenesis studies, are needed to further elucidate the mechanistic details of adopting various sugars in the donor substrate binding pocket, as well as harboring the substrate in dual conformations by YjiC, which could be a key follow-up study to explore the substrate promiscuity of the enzyme. Though the biological activities of the newly synthesized resveratrol glucosides and various glycoside analogues in this

FIG 6 Molecular modeling of YjiC and docking of resveratrol in the YjiC model. (A) Superimposed ribbon diagram of the YjiC model (light brown) with the crystal structure of OleD (yellow). The ribbon diagram of the 3D structure of YjiC resembles the Rossmann-like domains belonging to the GT-B fold of GT1 family proteins. The C-terminal domains of the enzyme, like those of other family 1 glycosyltransferases, lodge nucleotide sugar donors, whereas the N-terminal domain accommodates acceptors. (B) Docking of resveratrol in head-first mode. (C) Docking of resveratrol in tail-first mode.

Glycosylation of Flavonoids
study are still unknown, they could be beneficial for the generation of novel therapeutics.

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