Enzymatic Synthesis of α-Tocopherol Derivative Glycoside, Daidzein Glycoside, Daidzein Oligosaccharide, Resveratrol Oligosaccharide, and Curcumin Oligosaccharides and Their Anti-Allergic Activity and Neuroprotective Activity

Hiroki Hamada, Hatsuyuki Hamada, Kohji Ishihara, Atsuhito Kuboki, Takafumi Iwaki, and Yuya Kiriake

Abstract
Enzymatic glycosylations of an α-tocopherol derivative, daidzein, resveratrol, and curcumin were investigated. The plant polyphenol, resveratrol, was incubated with glucosyltransferase from Phytolacca americana. The resveratrol glycoside obtained was then incubated with cyclodextrin glucanotransferase to obtain resveratrol oligosaccharide. Daidzein and curcumin were also converted into daidzein glycoside, daidzein oligosaccharide, and curcumin oligosaccharides. Also, α-tocopherol derivative, that is, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-ol, was glycosylated. The glycosides and oligosaccharides had strong anti-allergic activity such as suppression of IgE formation, inhibition of histamine release, and inhibition of \( \text{O}_2^- \) generation. In addition, the glycosides and oligosaccharides showed efficient neuroprotective activity by inhibition of phosphodiesterase.

Keywords
glycoside, oligosaccharide, resveratrol, daidzein, α-Tocopherol derivative, curcumin, anti-allergic activity, neuroprotective activity

Received: February 19th, 2021; Accepted: May 19th, 2021.

Daidzein is one of the most important soybean isoflavones, α-tocopherol is a constituent of wheat malt, and resveratrol is a component of grape extracts. Turmeric, a spice that has long been recognized for its medicinal properties, contains curcumin. These plant phenolic compounds have versatile biological effects, including reduction of inflammation, antioxidant, antiaging, and anticancer.1-5 On the other hand, these compounds have shortcomings, such as water-insolubility and poor absorption after oral administration, limiting further pharmacological exploitation of these plant phenols. Synthesis of water-soluble derivatives of these lipophilic compounds is carried out by chemical methods, including tedious protection-deprotection procedures. Several attempts have been made to synthesize water-soluble glycosides of resveratrol by chemical methods, but these resulted in low yields.6

Cultured plant cells have been studied as useful agents for biotransformation reactions because of their potential to produce specific secondary metabolites, such as flavors, pigments, and agrochemicals. The reactions involved in the biotransformation of organic compounds by cultured plant cells include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, hydrolysis, and glycosylation. Glycosylation is a characteristic biotransformation reaction in cultured plant cells because glycosyltransferases are widespread in plants. Plant cell cultures and enzymes would be useful for practical preparation of glycosides, due to the high potential of plant glycosyltransferases to synthesize glucosides through a one step enzymatic glycosylation reaction.

1Faculty of Science, Department of Life Science, Okayama University of Science, Japan
2National Institute of Fitness and Sports in Kanoya, Kagoshima, Japan
3Faculty of Science, Department of Biochemistry, Okayama University of Science, Japan
4Faculty of Medicine, Department of Biophysics, Oita University, Japan
5Faculty of Medicine and Health Sciences, Yamaguchi University, Japan

Corresponding Author:
Hiroki Hamada, Faculty of Science, Department of Life Science, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan.
Email: hamada@dls.ous.ac.jp
In this study, we investigated the syntheses of daidzein glycoside, daidzein oligosaccharide, α-tocopherol-derivative glycoside, resveratrol oligosaccharide, and curcumin oligosaccharides and their pharmacological properties, such as anti-allergic and neuroprotective activities.

The glucosyltransferase from P. americana was used as a biocatalyst. This enzyme transformed the substrate, resveratrol, into a product, the chemical structure of which was determined on the basis of spectroscopic analyses as resveratrol 4'-β-glucoside. The glycosylation position of the glucoside was confirmed by comparison of its spectrum with authentic glucoside. Further glycosylation of resveratrol 4'-β-glucoside by cyclodextrin glucanotransferase gave resveratrol 4'-β-oligosaccharide (please see Figure 1).

Glycosylation of the α-tocopherol derivative, i.e., 2, 5, 7, 8-tetramethyl-2-(4, 8-dimethylxynonyl)chroman-6-ol, produced a β-glucoside (please see Supplemental data). When daidzein was the substrate, the glycosylated product, isolated by preparative HPLC, was determined to be daidzein 4'-β-glucoside, based on spectroscopic analyses. The glycosylation position of the glucoside was confirmed by comparison of its spectrum with an authentic sample. Authentic daidzein 7-β-glucoside was glycosylated by cyclodextrin glucanotransferase to daidzein 7-oligosaccharide. Curcumin was converted into curcumin β-oligosaccharides, such as curcumin β-maltoside, and curcumin β-maltotrioside, via glycosylation of curcumin β-glucoside by cyclodextrin glucanotransferase.

The inhibitory activities of the α-tocopherol derivative β-glucoside, daidzein 4'-β-glucoside, and daidzein 7-β-oligosaccharide for O2− generation from rat neutrophils were as high as that of the positive control, mequitazine. The percentage inhibitions of the α-tocopherol derivative β-glucoside, daidzein 4'-β-glucoside, and daidzein 7-β-oligosaccharide were 60, 52, and 55, respectively. The α-tocopherol derivative β-glucoside showed higher anti-allergic activity (IgE level 74) against glutenin than the positive control, hydrocortisone (IgE level 320), and α-tocopherol derivative (IgE level 384). The anti-allergic activity of daidzein 4'-β-glucoside (IgE level 128) and daidzein 7-oligosaccharide (IgE level 96) against 7S-globulin was stronger than that of hydrocortisone and daidzein (IgE level 427). The effects of glycosides on compound 48/80-induced histamine release from rat peritoneal mast cells were examined. Rat peritoneal mast cells released a high level of histamine when stimulated with compound 48/80. Resveratrol 4'-β-oligosaccharide, α-tocopherol derivative β-glucoside, daidzein β-glucoside, and daidzein β-oligosaccharide effectively inhibited compound 48/80-induced histamine release from rat peritoneal mast cells (The percentage inhibitions of resveratrol 4'-β-oligosaccharide, α-tocopherol derivative β-glucoside, daidzein β-glucoside, and daidzein β-oligosaccharide were 34, 34, 27, and 36, respectively). These results indicate that resveratrol oligosaccharide, α-tocopherol derivative glycoside, daidzein glycoside, and daidzein oligosaccharide would be useful anti-allergic agents.

The neuroprotective activity of the glycosides was examined by determining their inhibitory effects on phosphodiesterase (PDE) activity. Resveratrol 4'-β-oligosaccharide, curcumin β-maltoside, and curcumin β-maltotrioside showed high PDE inhibitory activity with IC50 values of 57, 51, and 41 µM, respectively. The IC50 values of resveratrol and curcumin were 216 and 180 µM. The oligosaccharide modification enhanced the neuroprotective activity of resveratrol and curcumin: the neuroprotective activity of resveratrol oligosaccharide and curcumin oligosaccharides was much higher than that of resveratrol and curcumin. These results indicate that resveratrol oligosaccharide and curcumin oligosaccharides could be useful anti-Alzheimer’s disease agents.

Further studies on the pharmaceutical properties of the glycosides are now in progress in our laboratory.

**Experimental**

**General**

HPLC was carried out with a YMC-Pack R&D ODS column (150 × 30 mm) (detection: UV (280 nm); flow rate: 1.0 mL/min).
Enzymatic Glosylolation

Plasmids containing cDNA of glucosyltransferase from *P. americana* (PgGT) were transformed into *Bacillus subtilis* cells. The purified enzyme solution was dialyzed with 50 mM Tris-HCl (pH 7.2) containing 5 mM dithiothreitol, and stored at −80 °C. Glosylolation reactions were performed at 35 °C for 24 hours in 5 ml 50 mM potassium phosphate buffer (pH 7.2) supplemented with 50 mM substrate, 100 µM UDP-glucose, and 5 µM enzyme. The incubation was stopped by adding 1.5% trifluoroacetic acid, and the reaction mixture was analyzed by HPLC. The reaction mixture was extracted with *n*-BuOH, the *n*-BuOH fraction was concentrated by evaporation, and the residue dissolved in water. The water fraction was applied to the Diaion HP20 column, washed with water, and eluted with methanol. The methanol solution was subjected to preparative HPLC.

Glosylation by Bioconversion

Cultured plant cells were subcultured for 2 months on solid Murashige and Skoog medium containing 3% sucrose, 10 mM 2,4-dichlorophenoxyacetic acid, and 1% agar (adjusted to pH 5.7) at 25 °C. A suspension culture was started by transferring the cultured cells to liquid medium in a 300 ml conical flask, and incubated on a rotary shaker (120 rpm) at 25 °C. Prior to use in this work, some of the callus tissue was transferred to freshly prepared MS medium and grown with continuous shaking for 2 days on a rotary shaker (120 rpm). The substrates were individually biotransformed by using plant cultured cells as biocatalysts. To a 300 ml flask containing culture medium and suspension cultured cells (25 g) 15 mg of substrate was added. The culture was incubated at 25 °C for 2 days on a rotary shaker (120 rpm) in the dark. After the incubation period, the cells and medium were separated by filtration with suction. The filtered medium was extracted with ethyl acetate (EtOAc). The cells were extracted by homogenization with 50 mM citrate buffer solution (pH 5.6) was added cyclo-dextrin glucanotransferase (CGTase). The reaction mixture was stirred at 40 °C for 24 hours, and then extracted with *n*-butanol. The *n*-butanol extracts were purified by preparative HPLC.

Anti-Allergic Activity

Effects of compounds on *O₂*₂ generation from rat neutrophils were examined as follows. Male Wistar rats, each weighing 250 g, were used. Under ether anesthesia, whole blood was collected from the carotid artery and diluted twice with Hanks’ balanced salt solution (HBSS) (pH 7.4). Neutrophils were purified by Percoll density gradient centrifugation. *O₂*₂ generation from rat neutrophils was measured by cypridina luciferin analog-dependent chemiluminescence. Neutrophil suspensions (10⁶ cells/mL) were incubated for 3 minutes in HBSS containing 0.4 mM of cypridina luciferin analog and sample at 37 °C in the dark. Five s later, fMLP (2.5 µM) was added to the assay mixture. Cypridina luciferin analog-dependent chemiluminescence was monitored. The results are expressed in terms of the percentage reduction of *O₂*₂ generation by the test compounds from rat neutrophils at 5 minutes after the administration of fMLP.

The effects of resveratrol and its glycoside on compound 48/80-induced histamine release from rat peritoneal mast cells were examined as follows. Peritoneal mast cells were collected from the abdominal cavities of rats (Male Wistar rats, Nippon SLC) and purified to a level higher than 95%. The purified mast cells were suspended in a physiological buffered solution (PBS) containing 145 mM NaCl, 2.7 mM KCl, 1.0 mM CaCl₂, 5.6 mM glucose, and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4) to give approximately 10⁶ mast cells/mL. Cell viability was always greater than 90%, as judged by the trypan blue exclusion test. Mast cells were preincubated with the test compound (1 µM) for 15 minutes at 37 °C, and subsequently exposed to compound 48/80 at 0.35 µg/mL. Histamine release was determined by a fluorometric assay.

Inhibition of Phosphodiesterase

BIOMOL Green, which is used for calculation of the amount of phosphate released, was purchased from Funakoshi Co. Phosphodiesterase (PDE) activity was measured by using a cyclic nucleotide phosphodiesterase assay kit from Enzo Life Sciences (BML-AK800), according to the manufacturer's instructions, except that PDE 100 mU per well was used. Stock solutions of inhibitors were prepared as 100 mM in DMSO and diluted to the appropriate concentrations. In the assay, 1 µL of each inhibitor solution was added before the addition of PDE enzyme solution. The PDE reaction was conducted at 37 °C for 60 minutes and terminated by the addition of BIOMOL Green and further incubation at room temperature for 30 minutes. The color reaction was measured by reading OD at 620 nm.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.
Supplemental Material

Supplemental material for this article is available online.

References

1. Marotta F, Mao GS, Liu T, et al. Anti-inflammatory and neuroprotective effect of a phytoestrogen compound on rat microglia. *Ann N Y Acad Sci*. 2006;1089:276-281. doi:10.1196/annals.1386.033

2. Marquardt D, Williams JA, Kučerka N, et al. Tocopherol activity correlates with its location in a membrane: a new perspective on the antioxidant vitamin E. *J Am Chem Soc*. 2013;135(20):7523-7533. doi:10.1021/ja312665r

3. Stewart JR, Artine MC, O’Brian CA, Jubilee RS, Marlene CA, Catherine AO. Resveratrol: a candidate nutritional substance for prostate cancer prevention. *J Nutr*. 2003;133(7 Suppl):2440S-2443S. doi:10.1093/jn/133.7.2440S

4. Sugimoto K, Hanai H, Tozawa K, et al. Curcumin prevents and ameliorates trinitrobenzene sulfonic acid-induced colitis in mice. *Gastroenterology*. 2002;123(6):1912-1922. doi:10.1053/gast.2002.37050

5. Salh B, Assi K, Templeman V, et al. Curcumin attenuates DNB-induced murine colitis. *Am J Physiol Gastrointest Liver Physiol*. 2003;285(1):G235-G243. doi:10.1152/ajpgi.00449.2002

6. Orsini F, Pelizzoni F, Bellini B, Miglierini G. Synthesis of biologically active polyphenolic glycosides (combretastatin and resveratrol series). *Carbohydr Res*. 1997;301(3-4):95-109. doi:10.1016/S0008-6215(97)00087-6