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Synthesis of curcumin β-maltooligosaccharides through biocatalytic glycosylation with *Strophanthus gratus* cell culture and cyclodextrin glucanotransferase

Kei Shimoda,[a] Takafumi Hara,[b] Hatsuyuki Hamada[c] and Hiroki Hamada[d,*]

[a]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Oita University, 1-1 Hasama-machi, Oita 879-5593, Japan*
[b]*Department of Applied Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[c]*National Institute of Fitness and Sports in Kanoya, 1 Shiromizu-cho, Kagoshima 891-2390, Japan*
[d]*Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*

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Abstract—A two step synthesis of a series of curcumin β-maltooligosaccharides through sequential biocatalytic glycosylation using *Strophanthus gratus* cell culture and cyclodextrin glucanotransferase (CGTase) is reported. Cultured plant cells of *S. gratus* converted exogenously added curcumin into unnatural curcumin β-D-glucoside. Furthermore, four unnatural β-maltooligosaccharides, that is, α-Glc-1→(4-α-Glc-1→)$_{n-1}$4-β-D-glucosides ($n = 1$–4), of curcumin were produced from curcumin β-D-glucoside in the presence of starch by CGTase-catalyzed glycosylation.

Curcuma longa Linn. has been used as a spice for centuries worldwide. Also it has been used in folk medicines for the treatment of a variety of inflammatory conditions.[1] Its intake reduces the risk of certain kinds of cancers and renders other protective pharmacological effects in human.[1] These medicinal properties have been attributed mainly to the curcuminoids, and the main component present in *C. longa* L. is curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] that has been widely studied for its anticancer, anti-inflammatory, antiaging, antiangiogenic, wound healing, and antioxidant effects.[2–6] Recently, it has been reported that curcumin prevented alcohol-induced liver disease.[7] Irrespective of such pharmacological activities, its use as a medicine has been limited, because of its water insolubility and poor absorption after oral administration. Glycosylation allows the conversion of water-insoluble compounds into corresponding water-soluble and stable ones to improve their bioavailability and pharmacological properties, for example, oligosaccharide conjugates of medicines have been used as prodrugs and have shown enhanced ability to be targeted to a particular organ within the living body, that is, liver.[8–10] From the physiological point of view, the oligosaccharide conjugates of curcumin can be of pharmacological interest and importance.

We report here the synthesis of a series of curcumin β-maltooligosaccharides. Our synthetic method involves stereoselective β-glucosylation by cultured plant cells of *Strophanthus gratus* followed by cyclodextrin glucanotransferase (CGTase)-catalyzed α-(1→4)-glucooligosaccharide formation.

Recently, chemical glucosylation of curcumin with acetobromoglucose in the presence of Et_3BnNBr has been reported to give corresponding mono-glucoside and symmetrical di-glucoside in yields of 8% and 3%, respectively.[11] Mohri et al. reported the condensation of arylaldehyde and glucosylarylaldehyde with acetylacetone–B_2O_3 complex to afford mono-glucoside (34%) and di-glucoside (4%) of curcumin.[12] In this study, curcumin β-D-glucoside (**2**) was prepared by biocatalytic glucosylation of curcumin (**1**) with cultured plant cells to avoid the time-consuming protection–deprotection steps necessary in chemical synthesis. Cultured suspension cells of three different plant species, that is, *S. gratus*,

* Corresponding author. Tel.: +81 86 256 9473; fax: +81 86 256 8468; e-mail: hamada@das.ous.ac.jp

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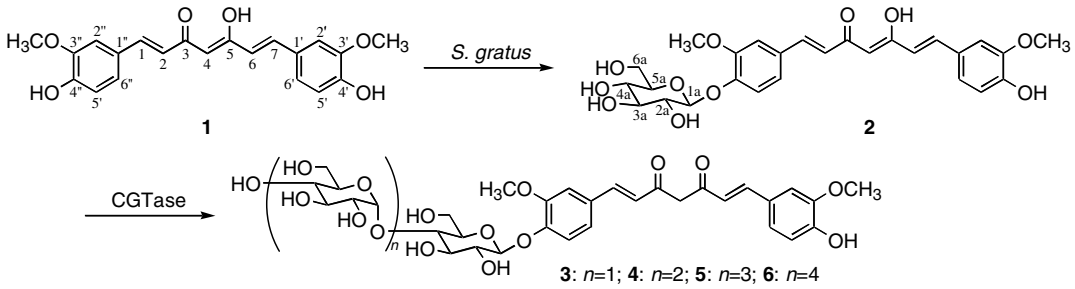

Figure 1. Synthesis of curcumin β-maltooligosaccharides by *S. gratus* cell culture and CGTase.[17,18]

Phytolacca americana, and *Nicotiana tabacum*, were screened for their ability to glucosylate curcumin (**1**). These cell cultures individually supplied with **1** were harvested after three days incubation and extracted with MeOH according to the previously reported method.[13] Glycoside products were not detected in the medium but in the MeOH extract of the cells by HPLC analyses. The structures of products were determined by comparison of their ^{1}H and ^{13}C NMR, and HRFABMS spectra with previously reported data.[12] Incubation with *S. gratus* suspension cells gave **2** as the sole product in 68% yield. Cultured cells of *P. americana* converted **1** into **2** (27%) and doubly glucosylated product (5%). On the other hand, no glycosylation products were isolated from the cultured *N. tabacum* cells which had been treated with **1**. The difference in the ability of glucosylation of curcumin between the three plant species is probably due to the substrate specificity of glucosyltransferases in these plant cells. Glucosylation of curcumin with *S. gratus* described here is considerably efficient method to give curcumin β-D-glucoside rather than chemical glucosylation.

Chemical synthesis of β-maltooligosaccharides of α-tocopherol using BF_3-etherate has been reported so far.[14] In this study, biocatalytic glycosylation of curcumin β-D-glucoside with CGTase was attempted to synthesize curcumin β-maltooligosaccharides.[15–18] As a result, products **3** (28%), **4** (20%), **5** (14%), and **6** (10%) were obtained after 24 h incubation of curcumin β-D-glucoside (**2**), which had been prepared by glucosylation of curcumin (**1**) with *S. gratus*, with CGTase in the presence of soluble starch. Incubation for a shorter period (12 h) resulted in the production of only three products **3**–**5**. A typical procedure for product identification is as follows. The HRFABMS spectrum of product **6** showed a pseudo-molecular ion peak $[M+Na]^{+}$ at *m*/*z* 1201.3785 (calculated for $C_{51}H_{70}O_{31}Na$, 1201.3799), indicating the presence of five hexoses in **6**. From the chemical shifts of the sugar carbon resonances in the ^{13}C NMR spectrum of **6**, the sugar component in **6** was determined to be glucose. The assignment of each signal in the NMR spectra of product **6** was performed by H–H COSY, C–H COSY, NOE, and HMBC analyses. The stereochemistry at 1-position of hexoses in the sugar moiety of **6** was determined by the coupling pattern of signals of the protons attached to the anomeric carbons in the ^{1}H NMR spectrum of **6**: an β-anomer (5.01 (1H, d, $J = 8.0$ Hz, H-1a)) and four α-anomers (5.15 (2H, d, $J = 3.6$ Hz, H-1b, 1c), 5.16 (1H, d, $J = 4.0$ Hz, H-1d), and 5.23 (1H, d, $J = 4.0$ Hz, H-1e)). In the ^{13}C NMR spectrum of **6**, C-4 sugar carbon resonances of inner glucoses shifted downfield: δ 80.6 (C-4a) and 81.3 (C-4b, C-4c, and C-4d). The HMBC spectrum of **6** showed correlations between the proton signal at δ 5.01 (H-1a) and the carbon signal at δ 150.9 (C-4″), between the proton signal at δ 5.15 (H-1b) and the carbon signal at δ 80.6 (C-4a), between the proton signal at δ 5.15 (H-1c) and the carbon signal at δ 81.3 (C-4b), between the proton signal at δ 5.16 (H-1d) and the carbon signal at δ 81.3 (C-4c), and between the proton signal at δ 5.23 (H-1e) and the carbon signal at δ 81.3 (C-4d). These findings established that the sugar moiety of **6** was a straight-chain α-(1→4)-glucooligosaccharose. Therefore, the structure of **6** was determined to be curcumin β-maltopentaoside. Similarly, products **3**–**5** were identified as curcumin β-maltoside (**3**), curcumin β-maltotrioside (**4**), and curcumin β-maltotetraoside (**5**). These curcumin β-maltooligosaccharides **3**–**6** were new compounds.[17] In comparison with chemical glycosylation which requires tedious protection–deprotection procedures, one-step biocatalytic glycosylation such as *S. gratus*-catalyzed glucosylation and CGTase-catalyzed oligosaccharide formation is more convenient. Furthermore, the combination of *S. gratus*-catalyzed glucosylation and CGTase-catalyzed glycosylation can provide a series of β-maltooligosaccharides, the polymerization degrees of which are different by a glucose unit, at the same time.

The antioxidant activities of curcumin β-glycosides **2**–**6** were determined by in vitro bioassay for their α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity.[19,20] The antioxidant activities for **2**–**6** were 67%, 49%, 55%, 70%, and 73%, respectively. This result suggests that curcumin β-glycosides having a phenolic hydroxyl group would be useful free-radical scavenging antioxidants which are soluble in aqueous solution.

Thus, the synthesis of curcumin β-maltooligosaccharides has been achieved, for the first time, by combination of *S. gratus* cell culture-catalyzed glucosylation and CGTase-catalyzed oligosaccharide formation (Fig. 1). The present system using these two biocatalysts would be useful for the preparation of drug-maltooligosaccharose conjugates as highly water-soluble prodrugs bearing an oligosaccharide transporter. Studies on the physio-

logical properties of curcumin β-maltooligosaccharides are now in progress.

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15. The synthesis of curcumin β-maltooligosaccharides was performed by incubating the reaction mixture (10 mL) containing 0.2 mmol of curcumin β-D-glucoside (**2**), 5 g of soluble starch, and 200 units of CGTase from *Bacillus macerans*, which acts as an useful biocatalyst to prepare oligosaccharides in the presence of starch,[16] in 25 mM sodium phosphate buffer (pH 7.0) at 40 °C for 24 h. The mixture was centrifuged at 3000*g* for 10 min. The supernatant was subjected on to a Sephadex G-25 column equilibrated with water. The fractions containing glycosides were lyophilized, resolubilized, and purified by HPLC to give products.[17,18]
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17. Spectral data for new compounds; product **3**: HRFABMS: *m/z* 715.2202 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD, δ in ppm): δ 3.27–3.89 (14H, m, H-4, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, 6a, 6b), 3.90 (6H, s, OCH_3), 4.99 (1H, d, $J = 7.6$ Hz, H-1a), 5.21 (1H, d, $J = 3.6$ Hz, H-1b), 6.62 (1H, d, $J = 15.6$ Hz, H-6), 6.66 (1H, d, $J = 15.6$ Hz, H-2), 6.80 (1H, d, $J = 8.2$ Hz, H-5′), 7.09 (1H, d, $J = 8.2$ Hz, H-5″), 7.15 (1H, s, H-2′), 7.16 (2H, d, $J = 8.2$ Hz, H-6′, 6″), 7.24 (1H, s, 2″), 7.54 (1H, d, $J = 15.6$ Hz, H-7), 7.56 (1H, d, $J = 15.6$ Hz, H-1); ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): δ 56.0 (C-4), 56.4 (3′-OCH_3), 56.7 (3″-OCH_3), 61.8 (C-6b), 62.7 (C-6a), 71.4 (C-4b), 74.1 (C-2b), 74.3 (C-5b), 74.7 (C-2a), 75.0 (C-3b), 76.8 (C-5a), 77.4 (C-3a), 80.7 (C-4a), 101.9 (C-1a), 102.8 (C-1b), 111.6 (C-2′), 112.3 (C-2″), 116.5 (C-5′), 117.3 (C-5″), 122.1 (C-6), 123.2 (C-2), 123.7 (C-6′), 124.1 (C-6″), 128.2 (C-1′), 131.3 (C-1″), 140.8 (C-7), 142.4 (C-1), 149.3 (C-3′), 149.5 (C-3″), 150.6 (C-4′), 150.8 (C-4″), 183.3 (C-5), 185.4 (C-3). Product **4**: HRFABMS: *m/z* 877.2731 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 3.26–3.89 (20H, m, H-4, 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b, 4c, 5a, 5b, 5c, 6a, 6b, 6c), 3.90 (6H, s, OCH_3), 5.01 (1H, d, $J = 7.6$ Hz, H-1a), 5.16 (1H, d, $J = 4.0$ Hz, H-1b), 5.21 (1H, d, $J = 4.0$ Hz, H-1c), 6.62 (1H, d, $J = 15.6$ Hz, H-6), 6.68 (1H, d, $J = 15.6$ Hz, H-2), 6.81 (1H, d, $J = 8.0$ Hz, H-5′), 7.10 (1H, d, $J = 8.0$ Hz, H-5″), 7.17 (1H, s, H-2′), 7.18 (2H, d, $J = 8.0$ Hz, H-6′, 6″), 7.26 (1H, s, 2″), 7.55 (1H, d, $J = 15.6$ Hz, H-7), 7.57 (1H, d, $J = 15.6$ Hz, H-1); ^{13}C NMR (CD_3OD): δ 56.0 (C-4), 56.4 (3′-OCH_3), 56.7 (3″-OCH_3), 61.9 (C-6b), 62.1 (C-6c), 62.7 (C-6a), 71.4 (C-4c), 73.3 (C-5b), 73.7 (C-2b), 74.2 (C-2c), 74.3 (C-3c, C-5c), 74.9 (C-2a, C-3b), 76.8 (C-5a), 77.5 (C-3a), 80.7 (C-4a), 81.3 (C-4b), 101.9 (C-1a), 102.6 (C-1b), 102.8 (C-1c), 111.7 (C-2′), 112.3 (C-2″), 116.5 (C-5′), 117.3 (C-5″), 122.2 (C-6), 123.3 (C-2), 123.8 (C-6′), 124.1 (C-6″), 128.3 (C-1′), 131.3 (C-1″), 140.9 (C-7), 142.4 (C-1), 149.2 (C-3′), 149.6 (C-3″), 150.4 (C-4′), 150.9 (C-4″), 183.4 (C-5), 185.4 (C-3). Product **5**: HRFABMS: *m/z* 1039.3259 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 3.25–3.89 (26H, m, H-4, 2a, 2b, 2c, 2d, 3a, 3b, 3c, 3d, 4a, 4b, 4c, 4d, 5a, 5b, 5c, 5d, 6a, 6b, 6c, 6d), 3.91 (6H, s, OCH_3), 5.01 (1H, d, $J = 8.0$ Hz, H-1a), 5.15 (1H, d, $J = 3.2$ Hz, H-1b), 5.16 (1H, d, $J = 3.6$ Hz, H-1c), 5.23 (1H, d, $J = 4.0$ Hz, H-1d), 6.62 (1H, d, $J = 15.6$ Hz, H-6), 6.69 (1H, d, $J = 15.6$ Hz, H-2), 6.82 (1H, d, $J = 8.0$ Hz, H-5′), 7.10 (1H, d, $J = 8.0$ Hz, H-5″), 7.17 (1H, s, H-2′), 7.18 (2H, d, $J = 8.0$ Hz, H-6′, 6″), 7.26 (1H, s, 2″), 7.56 (1H, d, $J = 15.6$ Hz, H-7), 7.57 (1H, d, $J = 15.6$ Hz, H-1); ^{13}C NMR (CD_3OD): δ 56.0 (C-4), 56.4 (3′-OCH_3), 56.7 (3″-OCH_3), 62.1 (C-6b, C-6c, C-6d), 62.7 (C-6a), 71.5 (C-4d), 73.3 (C-5b, C-5c), 73.7 (C-2b, C-2c), 74.2 (C-2d), 74.4 (C-3d, C-5d), 75.0 (C-2a, C-3b, C-3c), 76.8 (C-5a), 77.5 (C-3a), 80.6 (C-4a), 81.3 (C-4b, C-4c), 102.0 (C-1a), 102.5 (C-1b), 102.6 (C-1c), 102.8 (C-1d), 111.7 (C-2′), 112.3 (C-2″), 116.5 (C-5′), 117.4 (C-5″), 122.2 (C-6), 123.3 (C-2), 123.8 (C-6′), 124.1 (C-6″), 128.3 (C-1′), 131.3 (C-1″), 140.9 (C-7), 142.4 (C-1), 149.3 (C-3′), 149.6 (C-3″), 150.4 (C-4′), 150.9 (C-4″), 183.4 (C-5), 185.5 (C-3). Product **6**: HRFABMS: *m/z* 1201.3785 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 3.20–3.91 (32H, m, H-4, 2a, 2b, 2c, 2d, 2e, 3a, 3b, 3c, 3d, 3e, 4a, 4b, 4c, 4d, 4e, 5a, 5b, 5c, 5d, 5e, 6a, 6b, 6c, 6d, 6e), 3.92 (6H, s, OCH_3), 5.01 (1H, d, $J = 8.0$ Hz, H-1a), 5.15 (2H, d, $J = 3.6$ Hz, H-1b, 1c), 5.16 (1H, d, $J = 4.0$ Hz, H-1d), 5.23 (1H, d, $J = 4.0$ Hz, H-1e), 6.63 (1H, d, $J = 15.6$ Hz, H-6), 6.69 (1H, d, $J = 15.6$ Hz, H-2), 6.82 (1H, d, $J = 8.0$ Hz, H-5′), 7.11 (1H, d, $J = 8.0$ Hz, H-5″), 7.17 (1H, s, H-2′), 7.18 (2H, d, $J = 8.0$ Hz, H-6′, 6″), 7.27 (1H, s, 2″), 7.57 (1H, d, $J = 15.6$ Hz, H-7), 7.58 (1H, d, $J = 15.6$ Hz, H-1); ^{13}C NMR (CD_3OD): δ 56.0 (C-4), 56.4 (3′-OCH_3), 56.7 (3″-OCH_3), 62.0 (C-6b, C-6c, C-6d, C-6e), 62.7 (C-6a), 71.5 (C-4e), 73.3 (C-5b, C-5c, C-5d), 73.8 (C-2b, C-2c, C-2d), 74.2 (C-2e), 74.4 (C-3e, C-5e), 74.9 (C-2a, C-3b, C-3c, C-3d), 76.8 (C-5a), 77.5 (C-3a), 80.6 (C-4a), 81.3 (C-4b, C-4c, C-4d), 102.0 (C-1a), 102.6 (C-1b, C-1c, C-1d, C-1e), 111.7 (C-2′), 112.4 (C-2″), 116.5 (C-5′), 117.4 (C-5″), 122.2 (C-6), 123.3 (C-2), 123.8 (C-6′), 124.1 (C-6″), 128.4 (C-1′), 131.3 (C-1″), 140.9 (C-7), 142.4 (C-1), 149.3 (C-3′), 149.6 (C-3″), 150.4 (C-4′), 150.9 (C-4″), 183.5 (C-5), 185.5 (C-3).
18. The ^1H NMR spectra of **1** and **2** included a signal of enolic olefin proton responsible for H-4 at δ 5.96 (1H, s). On the other hand, those of **3**–**6** showed no signals of enolic olefin proton.[17] The ^{13}C NMR spectra of **1** and **2** exhibited

carbon signal for C-4 at δ 101.8, whereas the carbon resonance for C-4 shifted upfield (δ 56.0) in the ^{13}C NMR spectra of **3**–**6**.[17] These findings indicate that **3**–**6** prefer to have the β-diketone structure rather than enol structure.

19. The DPPH radical scavenging activities of curcumin β-glycosides were measured according to the reported method.[20] The results are expressed in terms of the percentage reduction (E) of the initial DPPH adsorption by the test compound: $E(\%) = [(A_c - A_t)/A_c] \times 100$, where A_t and A_c are the respective absorbance at 515 nm of 0.2 mM DPPH solution with and without the test compound.[20]
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