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UDP-GLUCURONIC ACID: TRITERPENE GLUCURONOSYLTRANSFERASE ACTIVITY IN CULTURED LICORICE CELLS

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Key Word Index—*Glycyrrhiza glabra*; Leguminosae; triterpene; glucuronosyltransferase; cell culture; soyasapogenol B; 24-hydroxyglycyrrhetinic acid.

Abstract—UDP-glucuronic acid:triterpene glucuronosyltransferase activities for soyasapogenol B, soyasapogenol C, 24-hydroxyglycyrrhetinic acid and 24-hydroxyglycyrrhetinic acid methyl ester were detected in cultured licorice cells. The relative ratio of the activities for different substrates varied in culture strains. The pH dependency of the activity for soyasapogenol B was different from that for 24-hydroxyglycyrrhetinic acid methyl ester.

INTRODUCTION

Stolons and roots of licorice, *Glycyrrhiza glabra* L., contain a large amount of glycyrrhizin (GL), an oleanane-type triterpene saponin, which is used as a natural sweetener. Cell suspension cultures of *G. glabra* produced no detectable amount of GL [1, 2], but they produced soyasaponins (SS) [3]. Both GL and SS are saponins which are glucuronylated at the C-3 hydroxyl group of oleanane-type triterpenes. There are some reports on plant glucuronosyltransferase (UGT) activities for triterpenoids [4, 5] and flavonoids [6–8], and the UGT was shown to participate in the metabolism of exogenous glycyrrhetinic acid by the licorice cell cultures [5]. In the present study, we examined the UGT activities for different triterpenes in low and high soyasaponin-producing culture strains of *G. glabra*.

RESULTS AND DISCUSSION

Figure 1 shows the substrate specificity of UGT activities in the microsomal fractions of a low SS-producing strain (RDS-2A strain, nine days old) and a high SS-producing strain (RNS-1B strain, 17 days old) of licorice cell cultures. In this experiment, a small amount of radioactivity was incorporated into a *n*-butanol extract, as a background, for the heat denatured microsome as well as in the absence of exogenous substrate. The incorporation of radioactivity into the *n*-butanol extract was greatly increased in the presence of soyasapogenol B (3β,22β,24-trihydroxyolean-12-ene, SB), soyasapogenol C (3β,24-dihydroxyolean-12,21-diene, SC) 24-hydroxyglycyrrhetinic acid (24G) and 24-hydroxyglycyrrhetinic acid methyl ester (24GM), which are 24-hydroxyoleanane-type triterpenes. On the other hand, no increase was observed for β-amyrin (3β-hydroxyolean-12-ene, AM), sophoradiol (3β,22β-dihydroxyolean-12-ene, SD) and glycyrrhetinic acid (GA), which have no 24-hydroxyl group. The relative ratio of the UGT activities for SB and 24GM varied in both culture strains, suggesting that at least two types of UGT existed in the cultured cells of *G. glabra*, and the activity for SB in the high SS-producing strain was higher than that in the low SS-producing strain. Figure 2 shows the pH dependency of the UGT activities for SB and 24GM in microsome of the high SS-producing strain. The pH optimum (pH 6.0) for SB was different from that (pH 5.5) for 24GM.

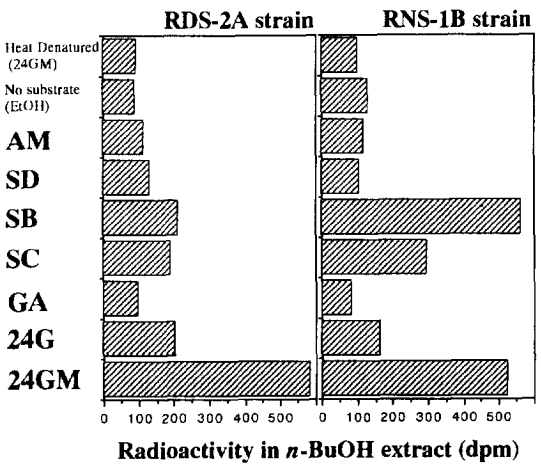

Fig. 1. Substrate specificity of UGT activity in microsome of a low SS-producing strain (RDS-2A strain, 9 days old) and a high SS-producing strain (RNS-1B strain, 17 days old) of licorice cell cultures. Incorporation of radioactivity from UDP-[U-^{14}C]-GlcUA into *n*-BuOH extract (glycoside fraction) was determined for different substrates. The pH of incubation mixture was 6.0. Mean of two replicates. 28 μg protein per assay (RDS-2A strain) and 16 μg protein per assay (RNS-1B strain).

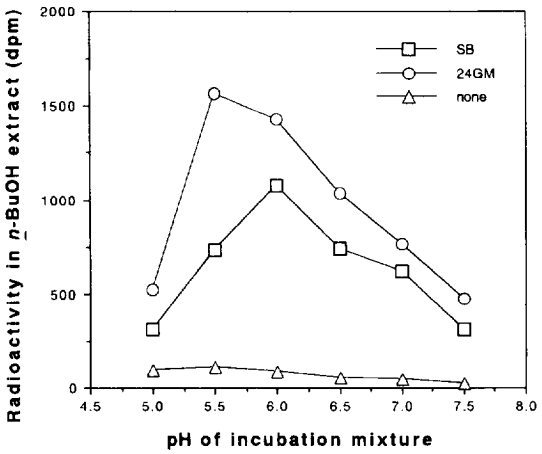

Fig. 2. pH Dependency of UGT activity in microsome of a high SS-producing strain (RNS-1B, 17 days old) of licorice cell cultures. Incorporation of radioactivity from UDP-[U-^{14}C]-GlcUA into *n*-BuOH extract (glycoside fraction) was determined for different substrates in different pH. Mean of two replicates. 16 μg protein per assay.

Since SB is the aglycone of SS, the UGT activity for SB seems to be responsible for the biosynthesis of SS. Although the glucuronylation of GA was not detected in the cultured cells, the UGT for GA seems to be involved in the biosynthesis of GL, which is localized in the thickening roots and stolons of *G. glabra* [9, 10].

EXPERIMENTAL

Chemicals. AM was obtained from Funakoshi Ltd, Japan. SB was obtained from Wako Pure Chemical Industries, Japan. GA and 24G were isolated from a commercial prepn of GA as reported previously [5]. 24GM was isolated from crude GA after treatment with 5% HCl in MeOH–$CHCl_3$ (1:1). SD and SC were obtained from Prof. I. Kitagawa (Kinki University, Japan).

Cell cultures. Cell suspension cultures were derived from seedlings of *G. glabra* and maintained as described elsewhere [3]. RNS-1B strain was transferred to LS medium containing 10^{-6} M NAA and 10^{-5} M BAP to induce SS production.

Preparation of microsomal fraction. All operations were carried out at 0–4°. Cells (70 g fr. wt) were homogenized in liquid N_2, and extracted with 0.1 M K-Pi buffer (pH 7.5, 630 ml) containing 2 mM EDTA, 20 mM mercaptoethanol, 0.25 M sucrose and 7 g insoluble PVPP. The homogenate was centrifuged successively at 10 000 *g* (30 min) and 100 000 *g* (60 min). The 100 000 *g* pellet was suspended using a Potter–Elvehjem homogenizer in 0.1 M K-Pi buffer (pH 7.5, 18 ml) containing 2 mM EDTA, 20 mM mercaptoethanol, and 20% glycerol (the microsomal fr.). Protein determination was performed according to ref. 11] with BSA as standard.

Assay of UGT activity. UGT activity was measured by a modified version of the previous method [4, 5]. The standard assay mixt. (total vol. 300 μl) contained: 25 μl microsomal fr.; 245 μl 0.1 M K-Pi buffer (standard pH 6.0) containing 2 mM EDTA, 20 mM mercaptoethanol and 20% glycerol; 2 μl 7.5 mM triterpene in ethanol (50 μM); 3 μl 1 M $MgCl_2$ (10 mM); 25 μl 12.5 nCi UDP-[^{14}C-U]-glucuronic acid (ARC, 300 mCi mmol^{-1}). The mixt. was incubated for 60 min at 15°. The reaction was terminated by addition of 30 μl M HCl and extracted with 400 μl *n*-BuOH. The radioactivity of a 100 μl aliquot of the *n*-BuOH extract was measured in a liquid scintillation counter (Aloka LSC-700). The ^{14}C-glucuronide products for SB, 24G and 24GM were identified by TLC as reported previously [5]. The enzyme reactions were linear with time up to 60 min at 15°. Protein concns up to 35 μg per assay resulted in a linear increase in the activity. The microsomal fr. could be stored at −80° for >6 months without significant loss of the activity.

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