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QUARTERLY

Synthesis of γ-chaconine and γ-solanine are catalyzed in potato by two separate glycosyltransferases: UDP-glucose:solanidine glucosyltransferase and UDP-galactose:solanidine galactosyltransferase

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UDP-glucose:solanidine glucosyltransferase and UDP-galactose:solanidine galactosyltransferase from cytosol of potato sprouts were partially separated by Sephadex G-100 and Q-Sepharose chromatographies, proving the existence of different glycosylation systems in biosynthesis of α-chaconine and α-solanine.

Steroid glycoalkaloids are nitrogen-containing secondary plant metabolites found in many plants. These compounds have been reported to be potentially toxic for animals and human beings [1, 2]. On the other hand, they are involved in a plant self-defence against pathogenic organisms and insects [3–7].

The potato plants contain two steroid glycoalkaloid series (Scheme 1) α-chaconine and α-solanine, which have the same aglycone–solanidine, but differ in the structure of sugar chain.

Until now, very little has been known about the mechanism and regulation of biosynthesis of the glycoalkaloid sugar chain. There are only few reports on the enzymes catalyzing glucosylation of steroid alkaloids in potato [8–14], eggplant [15] and *Solanum lacciniatum* [16]. Most of them refer to the formation of solanidine glucoside, a suggested precursor of α-chaconine. This glucosylation is catalyzed by a specific, cytosolic UDPGlc:solanidine glucosyltransferase, found in potato leaves, tubers and sprouts. The ability of crude enzyme preparations from potato [10–12], eggplant [15] and tomato [17] to synthesize solanidine, solasodine and tomatidine galactoside, respectively, in the presence of UDP-galactose has also been demonstrated. However, until now UDP-galactose:solanidine galactosyltransferase activity has not been separated from UDP-glucose:solanidine glucosyltransferase activity in potato.

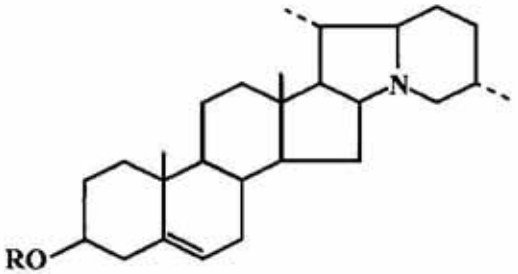
EXPERIMENTAL

Enzyme preparations. Sprouts of 2–3-week-old potato (*Solanum tuberosum* L., cv Irys) plants (100 g of fresh wt.) were homogenized with 200 ml of ice-cold 0.02 M Tris/HCl buffer, pH 7.3, containing 10 mM 2-mercaptoethanol (buffer A). The homogenate was filtered through cheesecloth and centrifuged at 105000 × ***g*** for 1.5 h. The 105000 × ***g*** supernatant (crude cytosol fraction) was

added dropwise to cold (–20°C) acetone (1:10, v/v) and the "acetone powder" was obtained as previously described [11].

Chromatography of glycosyltransferase. The cytosolic enzyme preparation ("acetone powder" from the 105000 × *g* supernatant) was suspended in buffer A (10 mg/ml) and centrifuged at 20000 × *g* for 10 min. The supernatant was applied onto the Sephadex G-100 (1 cm × 40 cm) column and eluted with the same buffer at a flow rate of 0.4 ml/min. Fractions of 2.7 ml were collected and glycosyltransferase activities were assayed in each fraction. Fractions exhibiting enzymatic activity (12–20 ml) were combined and then applied onto a Q-Sepharose ion-exchange column (1 cm × 3 cm), previously equilibrated with buffer A. The unbound material was washed out from the column with 22 ml of buffer A at a flow rate of 0.4 ml/min and active fractions were eluted by running a gradient up to 0.3 M NaF in buffer A for 100 min at the same flow rate. Fractions of 3.0 ml were collected, dialyzed overnight against buffer A and the glycosyltransferase activities were assayed.

Glycosyltransferase assays. The standard reaction mixture contained in a total volume of 0.52 ml: 0.5 ml of the fraction eluted from the Q-Sepharose column; 10 µmol Tris/HCl (pH 7.3), 5 µmol 2-mercaptoethanol, 25 nmol solanidine in 0.01 ml of ethanol and UDP-[^{14}C]glucose (2.2×10^5 d.p.m., 0.35 nmol) or UDP-[^{14}C]galactose (2.2×10^5 d.p.m., 0.37 nmol) in 0.01 ml of 50% ethanol. Once the labelled UDP-glucose or UDP-galactose was added, the reaction was run at 30°C for 2 h and then stopped by adding 1 ml of methanol and heating for 3 min on a boiling water bath. Subsequently, samples were extracted as previously described [13]. The samples were air-dried and applied on silica gel plates and developed with chloroform/methanol/28% NH_3 aq. (65:35:2, by vol.) as a solvent system. The chromatographic mobilities of radioactive products were compared with those of: solanidine 3-*O*-β-D-monoglucopyranoside (R_F 0.43) and solanidine 3-*O*-β-D-monogalactopyranoside (R_F 0.38) as reference compounds. Labelled compounds were localized by autoradiography and eluted from silica gel with methanol containing 0.1% NH_3 aq. Radioactivity was measured as previously described [11].



I R = -H
II R = -β-D-Gal*p*
III R = -β-D-Glc*p*
IV R = -β-D-Gal*p* → (2→1)-α-L-Rha*p*, → (3→1)-β-D-Glc*p*
V R = -β-D-Glc*p* → (2→1)-α-L-Rha*p*, → (4→1)-α-L-Rha*p*

Scheme 1. Structures of solanidine (25*S*,25*S*-solanid-5-enin-3β-ol; I), its 3β-D-galactopyranoside (γ-solanine; II) and 3β-D-glucopyranoside (γ-chaconine; III), α-solanine (IV) and α-chaconine (V).

RESULTS AND DISCUSSION

A partly lipid-depleted cytosolic enzyme preparation ("acetone powder" from 105000 × *g* supernatant) from potato sprouts was found to be able to synthesize solanidine monogalactoside (1.37×10^3 d.p.m.) as well as its monoglucoside (6.26×10^3 d.p.m.) in the presence of solanidine and UDP-[^{14}C]galactose in the incubation mixture. On the other hand, when the same enzyme preparation was incubated with solanidine and UDP-[^{14}C]glucose as a sugar donor, only formation of solanidine monoglucoside (20.92×10^3 d.p.m.) was observed.

The synthesis of solanidine monoglucoside on incubation with UDP-galactose as a sugar donor may suggest that the enzyme preparation contained UDP-glucose 4'-epimerase which transforms UDP-galactose into UDP-glucose, and this second nucleotide-sugar is then utilized for solanidine glucosylation.

Much lower incorporation of the radioactivity into solanidine glycosides when UDP-galactose was a donor of sugar moiety in comparison with radioactivity incorporated

into solanidine glucoside in the presence of UDP-glucose was also observed with the potato tuber enzyme [10, 12]. However, α-solanine and α-chaconine occur in potato sprouts in comparable amounts [18], so it could be expected that their precursors (i.e solanidine galactoside and solanidine glucoside) are synthesized with a similar yield. The evident differences in the apparent efficiency of solanidine glucoside and solanidine galactoside formation could be explained by a higher lability of solanidine galactoside, or by a possible partial inactivation of galactosyltransferase during the separation procedure. In fact it has been shown that solanidine galactoside is hydrolyzed at a much higher rate than solanidine glucoside by hydrolases present in cell-free preparations from potato [19, 20]. However, this can not account for the observed differences since during incubation of unlabelled solanidine galactoside (54 nmol per sample) with UDP-[^{14}C]galactose radioactive solanidine glycosides were formed at a very low rate (0.73 $\times$ 10^3 d.p.m.). This implies the presence of a single enzyme with different affinity towards UDP-glucose and UDP-galactose, or the presence of two separate enzymes.

The loss of solanidine galactosylation activity during the purification of the UDP-glucose:solanidine glucosyltransferase [14] implies the latter possibility.

In an attempt to separate these two glycosyltransferase activities, a two step procedure, involving gel filtration on Sephadex G-100 and ion-exchange chromatography on Q-Sepharose, was undertaken.

Figure 1 presents the result of chromatographic fractionation of crude cytosolic enzyme on Sephadex G-100 column. In each fraction the glycosyltransferase activities were assayed in the presence of solanidine and labelled UDP-galactose or UDP-glucose. A single activity peak (with apparent molecular mass of about 50 kDa) of galactosyl- and glucosyltransferase activities was found. A single glycosyltransferase activity peak was also reported by Bergenstråhle *et al.* [12] for the enzyme from potato tubers. However, these authors reported on the formation of only solanidine galactoside in the presence of UDP-galactose. It seems that those discrepancies between the results of Bergenstråhle *et al.* [12] and our data may be due to different conditions of separation of the reaction products by TLC chromatography. The R_F values for solanidine glucoside and its galactoside in our experiments were 0.43 and 0.38, respectively, which allowed to separate these two glycosyltransferases activities while the

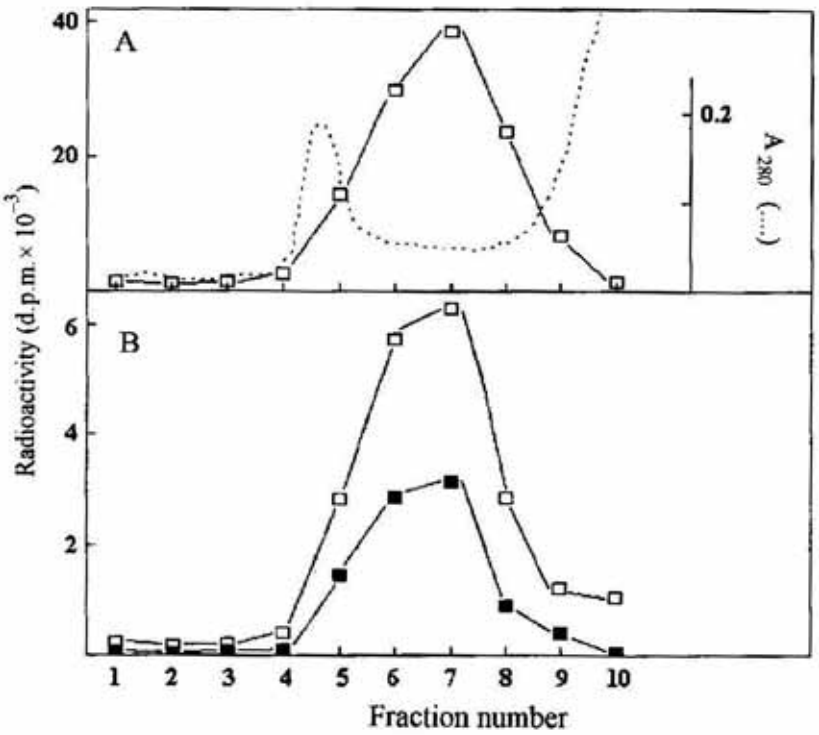


Figure 1. Chromatography of crude cytosolic enzyme preparation from potato sprouts on Sephadex G-100 column.

The glycosyltransferase activities were measured in the presence of solanidine and UDP-[^{14}C]glucose (A), and UDP-[^{14}C]galactose (B). The formation of labelled solanidine glucoside (□) and solanidine galactoside (■) was determined.

difference between the corresponding values reported by Bergenstråhle *et al.* (0.76 and 0.75, respectively) was too small to distinguish between those activities.

Further purification of the fraction collected from Sephadex G-100 column by ion-exchange chromatography on Q-Sepharose resulted in a partial separation of the two glycosyltransferase activities in the presence of UDP-galactose as a sugar donor (Fig. 2B). The first peak is ascribed to the formation of solanidine glucoside and the second to solanidine galactoside. In the presence of UDP-glucose as a sugar donor only the single activity peak, corresponding to the formation of solanidine glucoside, was observed (Fig. 2A).

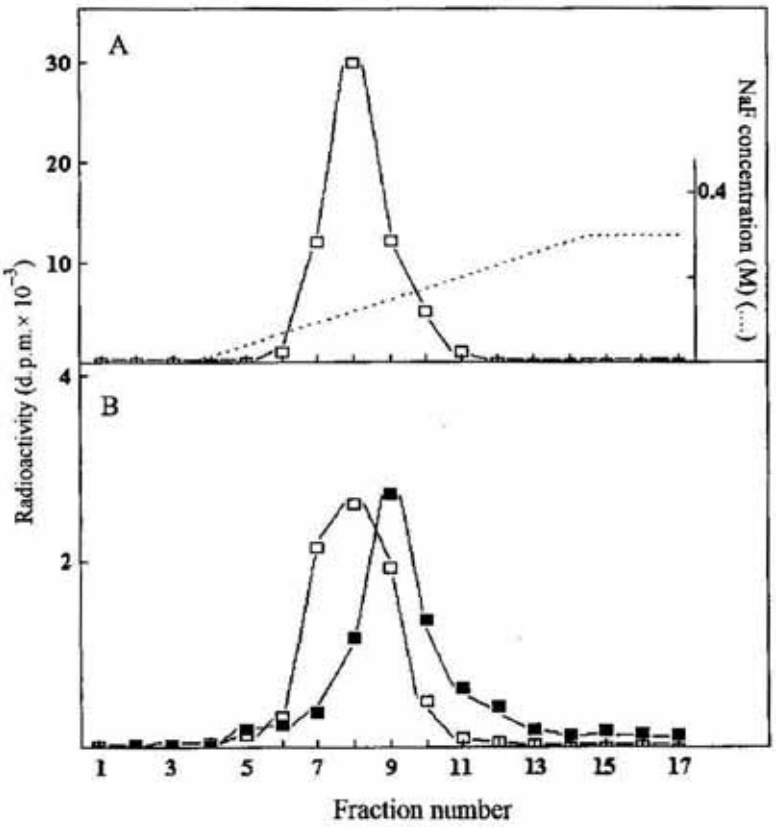


Figure 2. Chromatography of the enzymatically-active fractions from Sephadex G-100 on Q-Sepharose column.

Legend as in Fig. 1.

Table 1. Effect of some factors on glycosylation of solanidine by cytosolic enzyme preparation from potato sprouts.

For details see Methods.

Effector	2-Mercapto-ethanol (10 mM)	Formation of solanidine galactoside		Formation of solanidine glucoside	
		(d.p.m. × 10^{-3})	(%)	(d.p.m. × 10^{-3})	(%)
Control	+	2.7	100	41.3	100
None	–	0.5	21	34.4	83
$MgCl_2$ (1 mM)	+	2.6	96	40.9	99
$CaCl_2$ (1 mM)	+	2.8	104	42.1	102
EDTA (1 mM)	+	2.7	100	41.7	101
NaF (0.5 M)	+	1.2	44	14.5	35
Triton X-100 (0.05%)	+	1.5	58	33.1	80

In contrast to numerous other glycosyltransferases of plant origin [21], solanidine glycosylating enzymes failed to be stimulated by divalent metal ions, Mg^{2+} and Ca^{2+}. The fact that some divalent metal chelators, e.g. EDTA, were without effect on either enzyme activity, confirmed the lack of requirement for metal cofactors. Potato glycosyltransferases (i.e. galactosyltransferase and glucosyltransferase) were inhibited by 0.5 M NaF and 0.05% Triton X-100 but stimulated by 10 mM 2-mercaptoethanol (Table 1).

The results presented testify to the occurrence of two different glycosyltransferases in the cytosolic fraction from potato sprouts, i.e. UDP-glucose:solanidine glucosyltransferase catalyzing the formation of γ-chaconine (solanidine glucoside) and galactosyltransferase synthesizing γ-solanine (solanidine galactoside) in the presence of solanidine and UDP-galactose. This means that at least the first reactions in the synthesis of sugar chains in α-chaconine and α-solanine can be independently regulated.

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