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QUARTERLY

UDP-glucose:solasodine glucosyltransferase from eggplant (*Solanum melongena* L.) leaves: Partial purification and characterization

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Uridine 5′-diphosphoglucose-dependent glucosyltransferase which catalyzes the glucosylation of solasodine, i.e. UDP-glucose:solasodine glucosyltransferase, is present in leaves, roots, unripe fruits and unripe seeds of eggplant (*Solanum melongena* L.). The glucosylation product is chromatographically identical with authentic solasodine 3β-D-monoglucoside, a putative intermediate in the biosynthesis of solasodine-based glycoalkaloids characteristic of the eggplant. The enzyme was purified about 50-fold from crude cytosol fraction of eggplant leaves by ammonium sulphate precipitation and column chromatography on Q-Sepharose and Sephadex G-100. The native enzyme has a molecular mass of approx. 55 kDa and pH optimum of 8.5. Divalent metal ions are not required for its activity but the presence of free –SH groups is essential. Besides solasodine (K_m = 0.04 μM), the enzyme effectively glucosylates tomatidine, another steroidal alkaloid of the spirosolane type, but it is virtually inactive towards the solanidane-type steroidal alkaloids such as solanidine or demissidine. The enzyme is specific for UDP-glucose (K_m = 2.1 μM) since unlabelled ADP-, GDP-, CDP- or TDP-glucose could not effectively compete with UDP-[^{14}C]glucose used as the sugar donor for solasodine glucosylation. Moreover, no synthesis of labelled solasodine galactoside was observed when UDP-[^{14}C]glucose was replaced with UDP-[^{14}C]galactose.

Steroidal glycoalkaloids are common constituents of numerous plants belonging to the Solanaceae family including such economically important crop plants as potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) or garden eggplant (*Solanum melongena*) [1]. The reported toxicity of steroidal glycoalkaloids for animals and humans [2–4] as well as their postulated physiological function in the plant chemical protection against pathogenic fungi and insects [5–7] have attracted for years the attention of scientists. Although numerous studies concerning the occurrence, chemical structure and biological activities of steroidal glycoalkaloids have been published, still very little

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Abbreviations: GlcUA, glucuronate; NEM, *N*-ethylmaleimide; pCMB, *p*-chloromercuribenzoate.

is known about their biosynthesis, especially at the enzyme level. The major part of information concerning the biosynthesis of glycoalkaloids was derived from experiments in which more or less distant labelled precursors, e.g. acetate, mevalonate or cholesterol, were fed to the intact plants, plant organs or cell cultures [8]. These experiments have elucidated in outline the biosynthetic pathways leading to the formation of steroidal alkaloids, i.e. aglycones of glycoalkaloids, but until recently almost nothing was known about the synthesis of oligosaccharide chains present in glycoalkaloids.

A few years ago three independently working teams [9–11] reported on the occurrence in potato leaves, tubers and sprouts of a glucosyltransferase catalyzing the glucosylation of solanidine in the presence of UDP-glucose (UDPGlc). The product of this reaction (γ-chaconine) may be regarded as an intermediate in the biosynthesis of α-chaconine, one of the two main glycoalkaloids typical of potato. Subsequently, this glucosyltransferase was purified and thoroughly characterized [12, 13]. More recently a short report has been published [14] on the presence, in tomato leaves, of an UDP-galactose-dependent galactosyltransferase catalyzing the formation of tomatidine monogalactoside and it has been suggested that this compound is the first intermediate on the way from tomatidine to α-tomatine — the main glycoalkaloid of tomato.

In the course of our studies on the biosynthesis of another class of plant steroidal glycosides, i.e. steroidal saponins of the spirostane type, we have found [15,16] that the crude cytosol fraction obtained from eggplant leaves can effectively glucosylate, in the presence of UDPGlc, diosgenin and tigogenin — aglycones of the so-called melongosides, spirostane-type saponins which are known to accumulate in eggplant seeds [17]. The reaction products have been identified as diosgenin or tigogenin 3-monoglucosides, possible intermediates in the synthesis of melongosides. We have shown further [16] that the ability of the crude cytosol fraction from eggplant leaves to catalyze glucosylation of diosgenin or tigogenin is accompanied by a high capacity for solasodine glucosylation. The product of the latter reaction may be regarded as an intermediate in the biosynthesis of solasodine-based glycoalkaloids which are known to accumulate in the pulp of unripe eggplant fruits [18, 19].

The present study was aimed at purification and more precise characterization of UDP-glucose:solasodine glucosyltransferase activity present in the eggplant. We expected to find some clues to the question whether the parallel glucosylation of spirostane sapogenins, i.e. diosgenin or tigogenin, and spirosolane alkaloid, solasodine, is catalyzed by a single enzyme or by two different glucosyltransferases specific for sapogenins and solasodine, respectively.

MATERIALS AND METHODS

Q-Sepharose and Sephadex G-100 were acquired from Pharmacia. Commercial preparations of solasodine, tomatidine, diosgenin and tigogenin (from Sigma) were additionally purified by recrystallization from ethanol immediately before use. Solasodine and diosgenin 3β-D-glucopyranosides were synthesized chemically as described earlier [16].

Plant material. Eggplant (cv. Black Beauty) seeds were sown on trays containing moist garden soil-sand, 1:1, and germinated in darkness at 26°C. After 5–6 days the trays were transferred into light (16 h per day). Leaves of 6 to 9-week-old plants were used in most experiments.

Crude enzyme preparations. Crude lipid-depleted enzyme preparations ("acetone powders") from the cytosol fraction (105 000 ***g*** supernatant) or from the membranous fraction (3 000–105 000 ***g*** pellet) were obtained from the crude homogenate exactly as described previously [20, 21]. These preparations could be stored for several months at –20°C without any perceptible loss of the glucosyltransferase activity. The synthesis of labelled glucosidic products upon incubation of acetone powders obtained from the cytosol fraction with labelled UDP-glucose, in the absence of exogenously added solasodine, was negligible. When a delipidated enzyme preparation from the membranous fraction was used, a small amount of labelled product

with chromatographic mobility of sitosterol glucoside [22] was synthesized in the absence of an exogenous steroid acceptor.

Purification of UDPGlc:solasodine glucosyltransferase. In a typical purification procedure the acetone powder preparation obtained from the cytosolic fraction of leaves of 9-week-old plants (250 mg, 26 mg protein) was suspended in 50 ml of 0.05 M Tris/HCl buffer, pH 7.3, containing 10 mM 2-mercaptoethanol and then centrifuged at 20000 × ***g*** for 20 min. The insoluble material, containing about 30% of the protein present in the initial preparation, was discarded as it contained only traces of the glucosyltransferase activity. The clear supernatant was then fractionated with solid ammonium sulphate. Protein precipitated between 45% and 80% $(NH_4)_2SO_4$ saturation was collected by centrifugation, resuspended in 1 ml Tris/HCl buffer, as above, and dialyzed overnight against the same buffer. The dialysate was applied to a Q-Sepharose column (15 mm × 60 mm) equilibrated with the buffer. Elution was carried out using in succession the following Tris/HCl buffers, pH 7.3, containing 5 mM 2-mercaptoethanol: 0.05 M (15 ml), 0.20 M (20 ml), 0.35 M (13 ml) and 0.50 M (13 ml). Fractions emerging between 25 and 35 ml were pooled and proteins precipitated with solid ammonium sulphate added up to 75% saturation. The precipitate was dissolved in 1 ml of 0.05 M Tris/HCl, pH 8.5, and applied to a Sephadex G-100 column (10 mm × 400 mm). Elution was carried out with the same buffer and fractions emerging between 17 and 22 ml were pooled and used as the the purified enzyme preparation.

Standard glucosyltransferase assays. The enzyme activity was measured essentially as described earlier [16]. Unless otherwise stated, incubations were carried out at 30°C for 30 min. A typical incubation mixture contained in a total volume of 0.52 ml: 0.25 ml of the enzyme (30 μg or 0.6 μg protein for crude or purified enzyme, respectively) in 0.1 M Tris/HCl, pH 8.5; UDP-[^{3}H]Glc (1.5 nmol, 3.33 kBq) or UDP-[^{14}C]Glc (6.8 pmol, 3.33 kBq) in 0.01 ml of 50% ethanol, solasodine (0.4 nmol) or another steroid acceptor added in 0.01 ml of ethanol, and 0.25 ml of H_2O or an equivalent amount of an enzyme effector solution in H_2O. The reaction was stopped and the labelled glucosidic product separated from the labelled nucleotide sugar precursor by extraction with butan-1-ol, exactly as described earlier [22]. Aliquots of the butanolic extract were counted in a liquid scintillation counter or analysed by thin-layer chromatography on silica gel plates (DC-Plastikfolien Kieselgel 60, Merck) using chloroform/methanol (17:3) as the solvent. The R_f (0.20) value of the labelled glucoside formed upon incubation of the purified enzyme preparation with solasodine and UDP-[^{14}C]Glc was identical with that of authentic solasodine 3β-D-glucopyranoside. When solasodine was replaced with tomatidine, diosgenin or tigogenin the labelled products had different R_f values (0.36, 0.51 and 0.51, respectively).

Other methods. Protein was assayed by the method of Bradford [23]. The molecular mass of native glucosyltransferase was determined by gel filtration on a Sephacryl S-200 HR column (10 mm × 400 mm) calibrated with the following protein standards: horse cytochrome *c*, myoglobin, chymotrypsinogen A, ovalbumin, bovine serum albumin and yeast alcohol dehydrogenase. The column was equilibrated and eluted with 0.05 M Tris/HCl, pH 7.3. Fractions of 1 ml were collected. In order to determine the kinetic parameters of the UDPGlc:solasodine glucosyltransferase, the enzyme activity was measured with varying concentrations of UDP-Glc (0.03–0.40 μM) at a fixed concentration of solasodine (0.23 μM) or with varying concentrations of solasodine (0.02–0.15 μM) at a fixed concentration of UDPGlc (3.0 μM). The K_m values were deduced from double reciprocal plots of the initial reaction velocities.

RESULTS AND DISCUSSION

Data summarized in Table 1 confirm our earlier report [16] on the presence of UDPGlc:solasodine glucosyltransferase activity in young eggplant seedlings. This activity was also detected in various organs of older plants: leaves, stems, roots and unripe fruits. In pericarp parenchyma of mature fruits the activity was negligible but it was rather high in ripening seeds.

In all cases the major part of the total enzyme activity was present in the fraction of soluble proteins, i.e. in the 105000 *g* supernatant obtained from crude homogenate, however, in most cases a rather high activity was found also in the membranous fraction (3000–105000 *g* pellet). Smilarly, specific activities of UDPGlc:solasodine glucosyltransferase present in the membranous fraction were usually several times lower than those found in the fraction of soluble proteins but they were still quite high. Therefore it is very difficult to ascertain whether this enzyme is a true cytosolic protein or is a peripheric membrane protein which can be partially solubilized during the homogenization and centrifugation procedure. The latter possibility is quite likely — the literature data indicate that various glycosyltransferases acting on highly hydrophobic sugar acceptors are usually more or less tightly bound to some membranous structures; as examples may serve UDPGlc:sterol glucosyltransferase [22, 24] or UDPGal:diacylglycerol galactosyltransferase [25] from plant tissues.

Purification of the eggplant UDPGlc:solasodine glucosyltransferase is presented in Table 2. Most of the enzyme activity was precipitated from the buffer extract of the crude delipidated cytosol fraction over the 45–80% $(NH_4)_2SO_4$ saturation range. This step resulted in a 7-fold increase of the specific activity, accompanied by an about 45% increase of the total activity. Ion-exchange chromatography on a Q-Sepharose column using a discontinuous Tris/HCl concentration gradient gave only one activity peak. Subsequent gel filtration on a Sephadex G-100 column demonstrated also a single activity peak. The combined purification steps resulted in a 48.6-fold increase in the specific activity as compared with the activity of crude delipidated cytosol.

Table 1. Expression of UDPGlc : solasodine glucosyltransferase in various organs of developing eggplants

Plant age (weeks)	Organ	Specific activity (pkat per g protein)	
		3000–105000 *g* pellet	105000 *g* supernatant
3	Whole shoots	240	820
6	Leaves	210	800
	Roots	120	630
9	Leaves	300	820
	Stems	70	380
	Roots	190	810
11	Leaves	260	830
22	Unripe fruits (4 cm)		
	Whole berries	150	240
	Calices	<10	<10
23	Ripe fruits (30 cm)		
	Pericarp parenchyma	<10	<10
	Unripe seeds	<10	680

It appears noteworthy that a substantial, about 2-fold increase of the total activity was noticeable at the early steps of enzyme purification, i.e. $(NH_4)_2SO_4$ precipitation and Q-Sepharose chromatography. This evident activation could be caused by separation of the investigated glucosyltransferase from some unidentified endogenous inhibitors or/and some enzymes degrading the labelled substrate, i.e. UDP-[^{3}H]Glc. A similar activation of several other plant glycosyltransferases has been described: e.g. for UDPGlc:betanidin glucosyltransferase from *Dorotheanthus bellidiformis* [26] or UDPGlc:*p*-hydroxybenzoate glucosyltransferase from *Lithospermum erythrorhison* [27].

Table 2. Typical protocol for the purification of UDPGlc:solasodine glucosyltransferase from the cytosol fraction of eggplant leaves

Purification step	Protein (mg)	Specific activity (pkat × mg^{-1})	Purification (fold)	Yield (%)
Crude delipidated cytosol	25.7	0.81	1.0	100
Extract with 50 mM Tris/HCl, pH 7.3	17.6	0.91	1.1	79
$(NH_4)_2SO_4$ precipitate (45–80% saturation)	5.2	5.65	7.0	145
Q-Sepharose	2.3	16.82	20.8	190
Sephadex G-100	0.5	39.38	48.6	97

Some selected data on the properties of UDPGlc:solasodine glucosyltransferase are listed in Table 3.

Using 0.05 M Tris/HCl or Hepes buffers, high rates of solasodine glucosylation were observed within a fairly wide pH range of 7.0–9.5, with a maximum at pH = 8.5. The optimal temperature was about 35°C, however, the enzyme was highly thermolabile; at 45°C less than 10% of the activity could be detected, as compared to that found at 30°C.

enzyme activity, confirmed that the eggplant glucosyltransferase had no requirement for metal cofactors. On the other hand, heavy metal ions (Cu^{2+}, Zn^{2+}, Hg^{2+}) exerted very strong inhibitory effects.

The eggplant enzyme was strongly inhibited by pCMB (*p*-chloromercuribenzoate) and, to a lesser extent, by NEM (*N*-ethylmaleimide), however, this inhibition could be almost completely reversed by addition of 10 mM 2-mercaptoethanol. The above observations clearly indicate a requirement for reduced cysteine residues in the enzyme protein for its full activity.

Table 3. Some properties of UDPGlc:solasodine glucosyltransferase

Molecular mass*	about 55 kDa
Optimal pH	8.5
Optimal temperature	about 30°
Apparent K_m:	
for UDPGlc	2.10 μM
for solasodine	0.04 μM
Strong inhibitors:	UDP (I_{50} = 68 μM); oxidized UDP (I_{50} = 2.5 μM);
	pCMB (I_{50} = 10 μM);
	High ionic strength (I_{50} at 80 mM NaCl);
	Cu^{2+}, Hg^{2+} and Zn^{2+} (>90% inhibition at 1 mM);
No effect:	Mn^{2+}, Mg^{2+}, Ca^{2+} (0.1–10 mM); EGTA or EDTA (1 mM)

*As determined by gel filtration on Sephacryl S-200 HR

In contrast to numerous other glycosyltransferases of plant origin [28], the eggplant enzyme was not stimulated by divalent metal ions, such as Mg^{2+}, Ca^{2+} or Mn^{2+}, within a wide concentration range of 0.1–10 mM. The fact that some divalent metal chelators, such as EDTA or EGTA, were without effect on the

The glucosylation of solasodine was distinctly reduced in the presence of UDP, i.e. one of the reaction products. Feedback inhibition of a number of UDP-sugar-dependent

plant glycosyltransferases acting on various acceptors, such as terpenoids, phenols or flavonoids, has been frequently reported [24, 27–29]. It is noteworthy that oxidized UDP (periodate oxidized UDP, i.e. UDP-2′,3′-dialdehyde) was a much more potent inhibitor than unmodified UDP (I_{50} at 2 μM or 68 μM concentration, respectively). A similar potent effect of oxidized UDP has been observed with some other glycosyltransferases [30, 31] and was explained by covalent linking of oxidized UDP to the catalytic site of the enzyme [31]. Thus, labelled oxidized UDP could act as a suitable reagent for affinity labelling of UDPGlc:solasodine glucosyltransferase isolated from eggplant.

55 kDa. The K_m for UDPGlc was rather similar to K_m values reported for a number of other UDPGlc-dependent glucosyltransferases of plant origin [24, 26–28], however the K_m for solasodine was much lower than K_m values reported for various aglycones utilized by other known plant glucosyltransferases [24, 26–28] pointing to an unusually high affinity of the enzyme for its substrate, i.e. solasodine.

Specificity of the eggplant UDPGlc:solasodine glucosyltransferase with respect to the glucosyl moiety acceptor was studied using various 3β-OH steroids and UDP-[^{14}C]Glc for incubations. Chemical structures of steroidal alkaloids and sapogenins used in these experiments are given in Fig. 1. In all cases when an appreciable incorporation of [^{14}C]Glc into the glycoside fraction could be detected, thin-layer chromatography and subsequent autoradiography showed the presence of a single labelled product with chromatographic properties of monoglucoside of a given steroid (see Materials and Methods). Under standard conditions (see Table 4, the first column) the partially purified enzyme from eggplant leaves displayed high activities with both spirosolane-type steroidal alkaloids (solasodine, tomatidine) and spirostane-type steroidal sapogenins

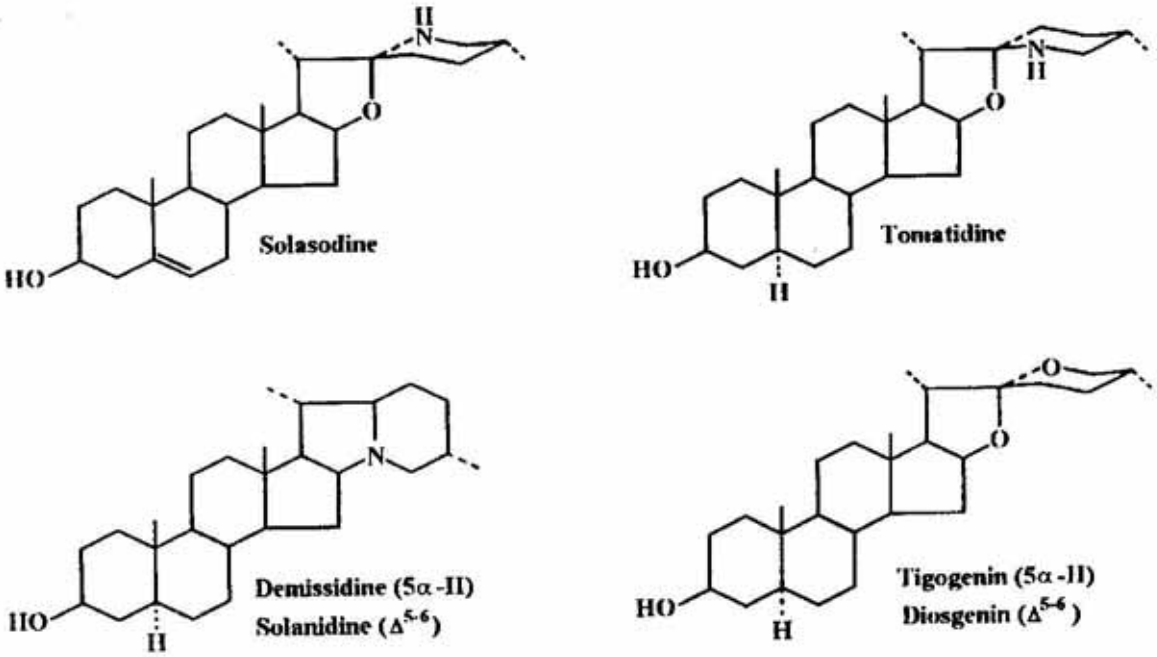

Figure 1. Structures of some steroidal alkaloids and sapogenins.

The eggplant glucosyltransferase was very sensitive to increased ionic strength of the incubation medium. An almost complete inhibition of solasodine glucosylation was observed at 0.3–0.4 M concentration of various salts tested, e.g. NaCl, KCl, KF, CH_3COONH_4. This property created a serious problem in our attempts to purify the enzyme by elution from various beds, e.g. hydroxylapatite, with eluent of increasing ionic strength.

Gel filtration of the partially purified enzyme on a calibrated Sephacryl S-200 HR column gave a single, symmetric activity peak with an apparent molecular mass of

(diosgenin, tigogenin). Typical sterols (cholesterol, sitosterol) and solanidane-type steroidal alkaloids (solanidine, demissidine) were glucosylated at very low rates (if any). At first sight these results suggested that UDPGlc-dependent glucosylation of diosgenin observed by us earlier in crude cytosol of eggplant leaves [15, 16] and the glucosylation of solasodine by partially purified enzyme described in the present paper might be catalyzed by the same glucosyltransferase. We found, however, on studying the effect of non-ionic detergent, Triton X-100, a clear-cut difference between glucosylation of spirosolane-type alkaloids and of spirostane-type sapogenins (see Table 4, the second column). Triton X-100 at very low concentrations exerted a very potent inhibitory effect on the glucosylation of diosgenin or tigogenin but had little effect on the glucosylation of solasodine or tomatidine (in fact, 0.05–0.30% Triton X-100 slightly stimulated glucosylation of solasodine). The differentiating effect of Triton X-100 is illustrated (see Fig. 2) by an additional experiment in which diosgenin and solasodine were added together to the incubation medium and glucosyltransferase assays were carried out in the presence of increasing amounts of Triton X-100. It should be mentioned that similar effects were observed with several other non-ionic detergents having quite different chemical structures, e.g. Tyloxapol, Tween 20 or octyl glucoside. It would be very difficult to explain the above described effects of Triton X-100 and some other detergents on the assumption that spirostane-type sapogenins and spirosolane-type alkaloids are glucosylated by a single enzyme. Therefore, we suggest that eggplant leaves contain two similar though separate enzymes with glucosyltransferase activity specific for spirosolane alkaloids and spirostane sapogenins, respectively. Most likely, these two glucosyltransferases co-purify under purification conditions described in the present paper.

Table 4. Specificity of eggplant glucosyltransferase towards some typical plant steroids

Steroidal substrate	Relative glucosylation rate (%)*	
	–Triton X-100	+ 0.1% Triton X-100
Solasodine	100	110
Tomatidine	90	60
Solanidine	<3	<3
Demissidine	<3	<3
Cholesterol	<3	<3
Sitosterol	<3	<3
Diosgenin	99	8
Tigogenin	97	10

*Glucosylation of solasodine under conditions described in the Material and Methods (without addition of the detergent) was taken as 100%

In order to gain some information on the specificity of the enzyme with respect to the sugar donor, we tested the effects of addition of excess amounts of various unlabelled (cold) nucleotide sugars on the synthesis of labelled solasodine glucoside from UDP-[^{3}H]Glc (see Table 5). Under experimental conditions in which an excess of cold UDPGlc caused an about 90% reduction in the formation of solasodine [^{3}H]glucoside, the same molar amounts of cold ADPGlc, GDPGlc or CDPGlc had only slight inhibitory effects. A significant inhibition (about 50%) was found, however, in the case of cold TDPGlc. These results indicate that among glucose-containing nucleotide sugars only TDPGlc can effectively compete with UDPGlc for the active site of the investigated glucosyltransferase. This is not surprising since TDPGlc is the closest structural analogue of UDPGlc. The ability to utilize limited amounts of TDPGlc has been reported for some other UDPGlc-dependent glucosyltransferases of plant origin, e.g. UDPGlc:coniferyl alcohol glucosyltransferase from *Picea abies* [32] or UDPGlc:sterol glucosyltransferase from *Gossypium* sp. [33].

UDP-glucuronate (UDPGlcUA), and UDP-xylose (UDPXyl) had little effect on the incorporation of [^{3}H]Glc into solasodine glucoside but the addition of cold UDP-galactose (UDPGal) and, particularly, UDP-mannose (UDPMan) strongly reduced the formation of labelled solasodine glucoside. In our previous paper [16] we have shown, using a crude cytosol fraction from eggplant leaves, that UDP-[^{14}C]Gal can be used for galactosyla-

tion of solasodine, however, at a much lower rate as compared with the formation of solasodine glucoside from UDP-[^{14}C]Glc. In the present study, using partially purified enzyme, we were unable to detect any synthesis of labelled solasodine galactoside in the presence of UDP-[^{14}C]Gal. These results clearly indicate that the formation of labelled solasodine galactoside in the presence of UDP-[^{14}C]Gal (as observed with the crude enzyme preparations) was catalyzed by a separate enzyme with galactosyltransferase activity. This enzyme either becomes separated from UDPGlc:solasodine glucosyltransferase during the purification procedures or is much less stable and loses its activity in the course of purification. Whether UDPMan can serve as a sugar donor or it can only compete with UDPGlc for binding to the active site of UDPGlc:solasodine glucosyltransferase remains to be elucidated. It should be mentioned, however, that a similar very potent inhibitory effect of UDPMan on the UDP-Glc:sterol glucosyltransferase from oat leaves has been recently described [24].

To sum up, the eggplant UDPGlc:solasodine glucosyltransferase described in the present paper shares several properties with other glycosyltransferases acting on steroidal alkaloids which have been characterized hitherto, i.e. UDPGlc:solanidine glucosyltransferase from potato [9–13] and UDP-Gal:tomatidine galactosyltransferase from tomato leaves [14]. The major part of the activities of each of these enzymes is present in the fraction of soluble proteins obtained from crude homogenates by high-speed centrifugation. This indicates that all these glycosyltransferases are either true cytosolic proteins (what seems to be rather surprising in view of highly hydrophobic nature of their substrates) or they are peripheric proteins loosely bound to some unidentified membranous structures. This problem requires further studies. All these glycosyltransferases exhibit the highest activities within an alkaline pH range (7.0–9.0) and do not require divalent metal cofactors. The apparent molecular mass of the eggplant glucosyltransferase (55 kDa) and those of other alkaloid-specific glycosyltransferases are also similar. The values of 38, 40 and 50 kDa have been reported for UDPGlc:solanidine glucosyltransferase isolated from potato sprouts [10,

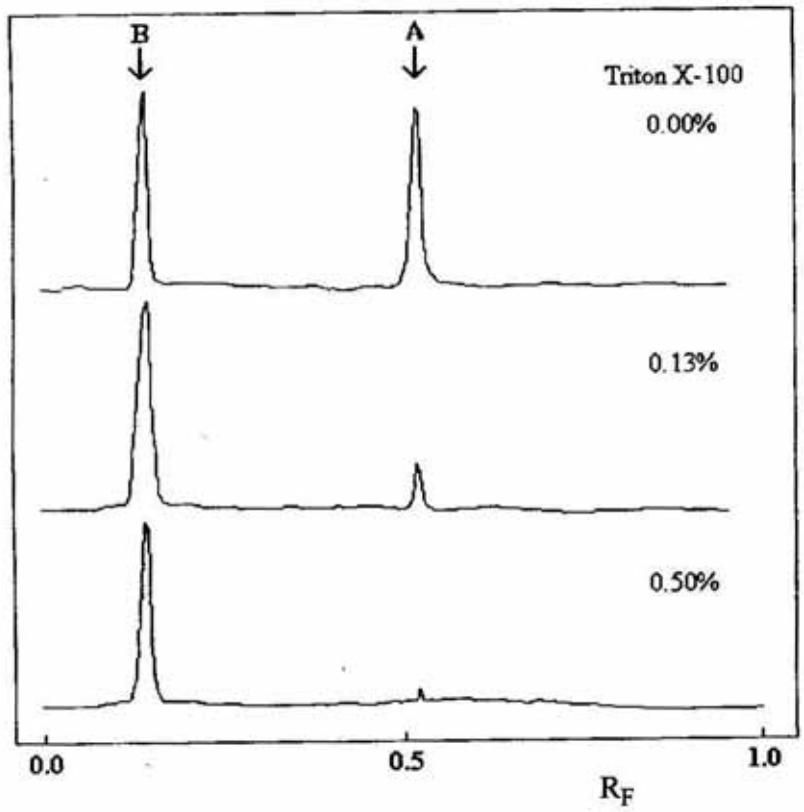

Figure 2. Effect of increasing concentrations of Triton X-100 (0.00–0.50%) on glucosylation rate of solasodine and diosgenin.

Enzyme preparation was incubated simultaneously with solasodine, diosgenin and UDP-[^{14}C]Glc. The labelled reaction products were separated by thin-layer chromatography and their radioactivities evaluated by densitometric scanning of autoradiograms. Localization of synthetic reference glucosides: diosgenin glucopyranoside (A) and solasodine glucopyranoside (B) is indicated by arrows.

13], tubers [11] or leaves [9, 12]. The value of about 50 kDa has been estimated for UDP-Gal:tomatidine galactosyltransferase from tomato leaves [14].

Table 5. Effect of unlabelled nucleotide sugars on the synthesis of labelled solasodine glucoside from UDP-[^{3}H]Glc*

Unlabelled nucleotide sugar added (13.6 nmol)	Relative rate of synthesis of labelled solasodine glucoside (%)
None	100
UDPGlc	12
ADPGlc	85
GDPGlc	82
CDPGlc	85
TDPGlc	54
UDPGal	68
UDPGlcUA	92
UDPXyl	81
UDPMan	<2

*In this experiment the labelled UDP-[^{3}H]Glc (6.8 pmol; 3.33 kBq) was applied at non-saturating concentration.

The substrate specificity of UDPGlc:solasodine glucosyltransferase present in eggplant leaves greatly differs, however, from substrate specificities reported for potato or tomato enzymes. In contrast to the eggplant glucosyltransferase, which efficiently glucosylates only spirosolane-type alkaloids, the enzyme from potato has been reported to be highly active with both the solanidane- and spirosolane-type steroidal alkaloids [9–13]. It has been claimed [10, 13] that highly purified enzyme from potato sprouts shows even somewhat higher activity towards solasodine or tomatidine than with its natural substrate, i.e. solanidine. The sugar-acceptor specificity of eggplant UDPGlc:solasodine glucosyltransferase is rather similar to that reported for UDPGal:tomatidine galactosyltransferase from tomato leaves [14] as both these enzymes are specific for the spirosolane-type steroidal alkaloids. However, the partially purified enzyme from eggplant leaves is completely unable to catalyze galactosylation of solasodine or tomatidine — in contrast to the enzyme from tomato leaves which preferentially utilizes UDPGal for glycosylation of the spirosolane-type steroidal alkaloids [14].

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