Purification and Characterization of Solanidine Glucosyltransferase from the Potato (*Solanum tuberosum*)

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The glycosylations of the spirostanol alkaloid solanidine in potatoes are considered to be the terminal steps in the synthesis of the potentially toxic glycoalkaloids α-solanine and α-chaconine. To better define the biosynthetic pathways to *Solanum* alkaloids, we describe an approximately 600-fold purification of the enzyme solanidine UDP-glucose glucosyltransferase (SGT) to near homogeneity from potato sprouts of the cultivar Lemhi. Purification was achieved by a combination of ammonium sulfate precipitation and FPLC anion-exchange, gel filtration, and chromatofocusing chromatography. The isolation of this enzyme was complicated by its copurification with patatin, the primary tuber storage protein. Separation of the two proteins was achieved only by binding the glycosylated patatin to a concanavalin A affinity resin under conditions where the SGT activity did not bind. Analysis of the purified enzyme by SDS/denaturing-PAGE revealed a major protein band of M_r 38 000. The native molecular weight of SGT determined by gel filtration chromatography was approximately 36 000, indicating that the native enzyme exists as a monomer. The purified enzyme preparation had a pH optimum of pH 6.4–6.8. Kinetic analysis showed K_m values of 37 μM for UDP-glucose and 20 μM for solanidine. The closely related spirosolane alkaloids tomatidine and solasodine were glucosylated at rates 2 and 3 times that of solanidine, respectively, whereas 3-β-hydroxy steroids lacking a ring nitrogen such as cholesterol, diosgenin, digoxigenin, and β-sitosterol did not serve as glucose acceptors. No enzymic activity was detected when UDP-galactose was used as a substrate, suggesting either that the pathway to α-solanine requires a separate galactosyltransferase activity or that γ-chaconine (glucosylsolanidine) is first converted to γ-solanine (galactosylsolanidine) by a specific epimerase. The significance of these findings for the control of the biosynthesis of glycoalkaloids at the molecular level in Solanaceous plants is discussed.

INTRODUCTION

Sterol glycosyltransferases play an important role in the biosynthesis of certain plant secondary metabolites. The sterol glycoside products of these enzymes and their acylated derivatives have multiple functions in the plant. For example, sterols such as sitosterol and campesterol are reportedly involved in the regulation of membrane fluidity and integrity (Demel and DeKruyff, 1976; Catz et al., 1985; Ullman et al., 1987; Mudd and McManus, 1980). Other plant steroids such as sapogenins, cardenolides, and bufadienolides exist as glucosides in higher plants, where they probably function in plant defense against insects and other pests (Kalinowska and Wojciechowski, 1986, 1987). Solanaceous plants, including potato and tomato, synthesize a variety of such natural defense compounds. Among the most commonly encountered are the glycoalkaloids, which are nitrogen-containing steroidal glycosides. In commercial potato cultivars, the primary glycoalkaloids are α-solanine and α-chaconine, which are triglycosylated derivatives of the aglycon solanidine (Morris and Lee, 1984).

Physiologically, glycoalkaloids act as cholinesterase inhibitors (Orgell, 1963; Patil et al., 1972; Bushway et al., 1987) and are reported to exhibit a range of toxic effects in animals and man [for reviews, see Jadhav et al. (1981) and Morris and Lee (1984)]. Well-documented episodes of human toxicity (McMillan and Thompson, 1979; Harvey et al., 1985) have led to the implementation of a guideline limiting glycoalkaloid content to 20 mg/100 g in a given potato cultivar. Potato glycoalkaloids are reported to have caused up to 30 deaths and over 2000 documented cases of poisoning (Morris and Lee, 1984). Considerable attention has been paid in recent years to the possible teratogenic effects of glycoalkaloids following a paper by Renwick (1972) linking anencephaly and spina bifida to the consumption of blighted potatoes during pregnancy (Chaube et al., 1973; Keeler et al., 1975; Allen et al., 1977; Brown and Keeler, 1978). While this relationship may or may not exist, further studies have since linked glycoalkaloids to other reproductive and birth abnormalities (Poswillo et al., 1972, 1973a,b; Swinyard and Chaube, 1973; Keeler et al., 1976; Allen et al., 1977). Such studies have clearly demonstrated that high levels of glycoalkaloids in potatoes, whether caused by genetic, environmental, or storage and processing factors, present a significant risk to human health.

The aglycon portion of the glycoalkaloid is believed to be considerably less toxic than the glycoside (Nishie et al., 1971; Osman, 1983). As a consequence, decreasing the activity of the enzyme(s) responsible for glycosylation of the aglycone should effectively lower the potential toxicity. A biosynthetic pathway to aglycons from acetyl-CoA via cholesterol has been proposed (Kaneko et al., 1976; Heftmann, 1983), but relatively little is known about the enzymes and the mechanisms involved in subsequent glycosylation steps. In theory, the sugar moieties could be added sequentially or as disaccharide or trisaccharide units. However, there is general agreement that glycosylations are the terminal steps in glycoalkaloid biosynthesis and that the sugars are added sequentially (Osman et al., 1980). Liljegren (1971) demonstrated the activity of a solasodine UDP-glucose glucosyltransferase from *Solanum laciniatum*, and Jadhav and Salunkhe (1973) and Lavintman

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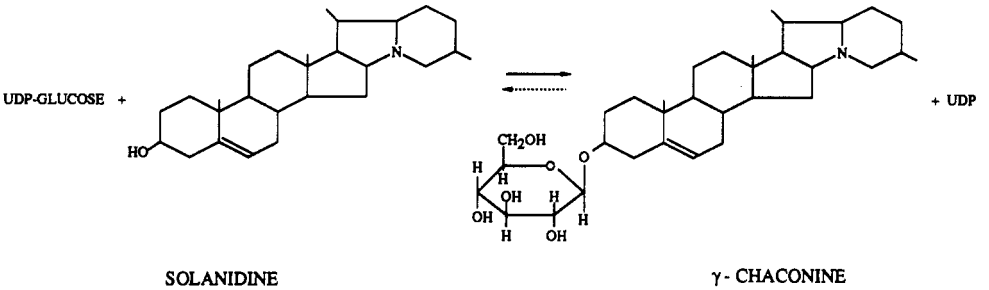

Figure 1. Glucosylation of solanidine to produce 3-β-*O*-glucosylsolanidine (γ-chaconine).

et al. (1977) provided evidence for the glucosylation of solanidine by an extract of potato sprouts and a crude extract of potato tuber, respectively. More conclusive evidence for a sequential addition mechanism was provided by Osman et al. (1980), who used gas–liquid chromatography, mass spectroscopy, and nuclear magnetic resonance to identify 3-β-*O*-glucosylsolanidine (γ-chaconine) and 3-β-*O*-glucosyl (glucosyl) solanidine as products of the enzyme-catalyzed reaction in potato tuber disks and cell suspensions.

As part of a program of potato improvement using molecular genetics in parallel with food safety evaluations, we are interested in isolating and characterizing the genes and translation products associated with the biosynthesis of the glycoalkaloids. As an initial step, we report here the isolation and characterization of an enzyme catalyzing the glucosylation of solanidine (Figure 1) from potato sprouts of the cultivar Lemhi.

MATERIALS AND METHODS

Lemhi tubers were obtained from the Potato Breeding Program, University of Idaho, Aberdeen, ID. The tubers were stored at 4 °C until brought to room temperature (in the dark) to encourage sprouting. Chromatography columns for FPLC were obtained from Pharmacia LKB Biotechnology (Piscataway, NJ). Precast gradient acrylamide gels, marker proteins, and anion-exchange resin AG1X8 acetate form, were obtained from Bio-Rad Laboratories (Richmond, CA). Gold stain for protein determination was obtained from Enprotech (Hyde Park, MA). Uridine diphospho-D-[6-^{3}H]glucose (14.7 Ci/mmol) and uridine diphospho-D-[6-^{3}H]galactose (19 Ci/mmol) were obtained from Amersham International (Arlington Heights, IL). All other reagents came from Sigma Chemical Co. (St. Louis, MO).

Enzyme Assay. Solanidine UDP-glucose glucosyltransferase was assayed by a newly developed technique that depends upon the capture of the negatively charged, radiolabeled substrate UDP-glucose by the strong anion-exchange resin AG1x8 acetate form. This occurs at a pH below the p*K* of the glucosylated product. Details of the procedure are as follows: The anion-exchange resin was washed several times with deionized water and resuspended to give a 1:1 slurry in 100 mM MES buffer, pH 5.0. This slurry was constantly stirred. For monitoring column fractions for enzyme activity, a typical assay mixture contained 10 μL of 1 mM solanidine (in DMSO), 50–200 μL of enzyme solution, 10 μL of [^{3}H]UDP-glucose (10 nmol/0.5 μCi), and 20 mM Bis-Tris propane, pH 6.6, containing 1 mM DTT and 5 mM $MgCl_2$, to give a final volume of 300 μL. Following incubation at 37 °C for 15 min, the reaction was terminated by boiling for 5 min and the mixture cooled to room temperature. Next, 100 μL of 100 mM MES buffer and 700 μL of the resin slurry were added, and the mixture was vortexed vigorously for 4 min. The assay mixture was centrifuged for 5 min at full speed (14000*g*) in a microfuge and 200 μL of the supernatant taken for scintillation counting in 4 mL of ICN Aquamix using a Beckman LS 3801 counter. Addition of approximately 10^6 dpm of [^{3}H]-UDP-glucose per assay resulted in typical values for assay blanks (boiled enzyme) of 4000 dpm.

Product Recovery and Identification. The assay was optimized in terms of recovery of the glucosylated product by using a pool of the supernatants from several assays. The ion-exchange resin was adjusted to pH values between 5.0 and 6.5 with 100 mM MES buffer and to between pH 7.0 and 9.0 with 100 mM Bis-Tris propane buffer. Following vortexing, centrifugation, and scintillation counting, the recovery of the labeled product was determined and plotted as a function of resin pH. The identity of the radioactive product was established by pooling and lyophilizing the supernatants from several assays, redissolving the product in methanol, and analyzing by TLC according to the method of Filadelfi and Zitnak (1983).

Polyacrylamide Gel Electrophoresis. SDS/denaturing-PAGE with silver staining was performed with a Bio-Rad Mini-Protean II cell with 4–20% polyacrylamide gradient gels using the manufacturer's recommended protocols. The protein standards (Bio-Rad low molecular weight prestained markers) were phosphorylase B (M_r 110 000), bovine serum albumin (M_r 84 000), ovalbumin (M_r 47 000), carbonic anhydrase (M_r 33 000), trypsin inhibitor (M_r 24 000), and lysozyme (M_r 16 000).

Estimation of Native Molecular Weight by Gel Filtration. The native molecular weight of the enzyme was determined by using a Superose 12 FPLC column calibrated with Bio-Rad gel filtration standards consisting of thyroglobulin (M_r 670 000), γ-globulin (M_r 158 000), ovalbumin (M_r 44 000), myoglobin (M_r 17 000), and vitamin B_{12} (M_r 1350). In addition, bovine serum albumin (M_r 66 000) was included. The eluent was 50 mM Bis-Tris propane, pH 6.6, containing 1 mM DTT, 5 mM $MgCl_2$, and 1 mM KCl at a flow rate of 0.5 mL/min.

Enzyme Extraction and Purification. Lemhi tubers were allowed to sprout for 4–8 weeks in the dark at room temperature. The sprouts were harvested when they measured between 5 and 10 cm in length. Typically, 100 g of sprouts was extracted with 400 mL of ice-cold 20 mM Bis-Tris propane, pH 6.6, containing 1 mM DTT, 5 mM $MgCl_2$, and 10 g of poly(vinylpyrrolidone) (PVP), by using an Omnimixer set at full speed for 2 min. The brei was filtered through eight layers of cheesecloth and centrifuged at 10000*g* (11 000 rpm, Beckman Ti 70 rotor) for 30 min. The supernatant from this step was filtered through one layer of Miracloth and recentrifuged at 100000*g* (40 000 rpm, Beckman Ti 70 rotor) for 60 min. Next, the cell-free extract was brought to 35% saturation by addition of solid ammonium sulfate over 30 min, stirred for 30 min, and then centrifuged at 17000*g* (12 000 rpm, Beckman JA20 rotor) for 30 min. The resulting supernatant was brought to 65% saturation with solid ammonium sulfate, and the above stirring and centrifugation step was repeated. The resulting pellet, containing the glucosyltransferase activity, was dissolved in a minimum amount of ice-cold homogenization buffer (minus PVP) and dialyzed overnight against 4 L of the same buffer. This enzyme preparation was then centrifuged at 10000*g* for 30 min to remove insoluble material. Finally, the preparation was filtered twice through Millipore 25-μm syringe filters prior to the first chromatographic step.

FPLC Protocol. All steps were performed at room temperature and the fractions placed on ice immediately after elution.

(*i*) A Mono Q HR 10/10 anion-exchange column was equilibrated overnight with 20 mM Bis-Tris propane, pH 6.6, containing 1 mM DTT and 5 mM $MgCl_2$ at a flow rate of 0.4 mL/min. The desalted and filtered enzyme preparation was applied via a Superloop. Nonbinding material was washed from the column with 40 mL of equilibration buffer at a flow rate of 4 mL/min. The column was developed by running a gradient to 0.25M KCl (in equilibration buffer) over 44 min at the same flow rate. The

fraction size was 4 mL, and 200-μL samples were assayed for the glucosyltransferase activity.

(*ii*) The pooled fractions containing the enzyme activity were passed through a 10-mL column of concanavalin A–Sepharose 4B (previously washed extensively with the equilibration buffer used in the first step) at a flow rate of 1 mL/min. The column was then washed with 10 mL of equilibration buffer. Under these conditions, between 80 and 90% of the applied SGT activity was recovered. The enzyme activity was concentrated to 0.5 mL by using an Amicon stirred cell (YM30 membrane) and Amicon Centricon 30 centrifugation concentration cells. When required, patatin was recovered from the affinity column by eluting with 15 mL of equilibration buffer containing 25 mM methyl α-D-glucopyranoside.

(*iii*) A Superose 12 gel filtration column was equilibrated with 25 mM piperazine, pH 5.7, containing 1 mM DTT and 5 mM $MgCl_2$ overnight at a flow rate of 0.5 mL/min. The concentrated enzyme from the negative Con A–Sepharose affinity step was applied and eluted at the same flow rate. Fractions (0.5 mL) were collected, and 50-μL samples were assayed.

(*iv*) A Mono P chromatofocusing column was equilibrated overnight with 25 mM piperazine, pH 5.7, containing 1 mM DTT and 5 mM $MgCl_2$ at a flow rate of 0.1 mL/min. The pooled fractions containing enzyme activity from the preceding step were applied to the column which was washed with 5 mL of equilibration buffer. The column was developed with 40 mL of Polybuffer 74 containing 1 mM DTT and 5 mM $MgCl_2$ at pH 4.0. The flow rate was 0.75 mL/min, and 0.75-mL fractions were collected. Samples of 100 μL per fraction were taken for enzyme assay.

(*v*) The active fractions from the Mono P step were concentrated to a small volumn with concomitant buffer exchange into 20 mM Bis-Tris propane, pH 6.6, by using Centricon 30 cells and chromatographed on a Mono Q HR 5/5 anion-exchange column under the same conditions described in step i except that the flow rate was 0.75 mL/min and a gradient to 0.25 M KCl was run over 33 min. Fractions (0.75 mL) were collected, and 100 μL was taken for enzyme assay.

Protein Sequence Analysis of Patatin. Samples of purified patatin eluted from the concanavalin A–Sepharose affinity column with 25 mM methyl α-D-glucopyranoside were precipitated with TCA and acetone, lyophilized, and sequenced by using an Applied Biosystems Model 470A protein sequencer, linked to a 120A PTH-analyzer (Eggum and Sorensen, 1989).

Protein Assay. Protein concentrations were determined by using a Coomassie Blue binding assay (Bio-Rad) and by a more sensitive gold stain (Enprotech), with bovine serum albumin as a standard. Experimental details are given in the manufacturer's literature.

Enzyme Kinetics. K_m values were calculated by using the nonlinear regression curve fitting program Enzfitter (Elsevier-BioSoft).

RESULTS

Assay Development. Previous assays for solanidine glucosyltransferase have relied on precipitation of the radiolabeled product with ammonium hydroxide (Jadhav and Salunke, 1973) or extraction with organic solvents (Liljegren, 1971; Osman et al., 1980) followed by analytical TLC. These techniques were unsuitable for establishing a purification protocol which required an assay capable of dealing with large numbers of column fractions. The assay reported here was based on the selective capture of the radiolabeled UDP-sugar substrate by an anion-exchange resin. At pH values below the pK of the glucosylated aglycon product, the positive charge of the alkaloid nitrogen evidently prevents its binding to the resin, and it is then quantitatively recovered from the assay mixture. Figure 2 shows the recovery of the labeled product as a function of resin pH. At pH 5.0, approximately 90% of the product was recovered. Due to the strong ionic characteristics of the Dowex resin, more than 99% of the UDP-sugar substrate was captured over the same pH range (data not shown), allowing consistently low blank counts. The specificity of the assay was established by experiments in which no counts were recovered from the assay without exogenously added solanidine but only after the enzyme had been purified by the first ion-exchange step. Prior to this step, appreciable solanidine-independent counts were obtained from the crude homogenate, cell-free extract, and salt-precipitated enzyme. This nonspecific enzyme activity was attributed to the presence of endogenous acceptors for the labeled substrate UDP-glucose. In addition, potatoes are reported to contain UDP-glucose pyrophosphorylate activity which could liberate labeled glucose 1-phosphate (Nakano et al., 1989).

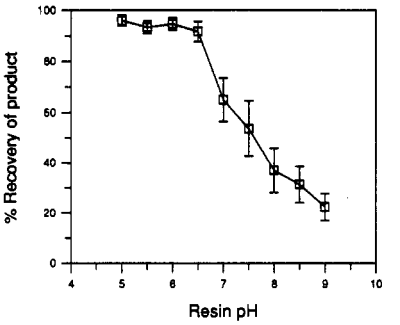

Figure 2. Recovery of the radiolabeled, glucosylated solanidine product from the assay mixture as a function of the anion-exchange resin pH. Results are the average of three experiments ± SEM. Details are given under Materials and Methods.

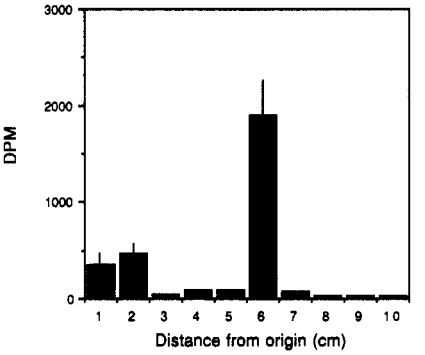

Figure 3. Identification of the glucosylated solanidine product as γ-chaconine by TLC. The radioactive product was concentrated by lyophilization, redissolved in methanol, and analyzed by TLC (for details, see Materials and Methods). Sections (1 cm) of the TLC plate were scraped and analyzed by scintillation counting. The large peak of radioactivity corresponded to the position of authentic γ-chaconine (R_f 0.53). Results are the average of three experiments ± SEM.

The identification of the major radiolabeled product as 3-β-*O*-glucosylsolanidine (γ-chaconine) from assays of such crude extracts with addition of exogenous solanidine was confirmed by TLC analysis using standards derived from the chemical hydrolysis of α-solanine (Figure 3). Some radioactivity was detected just above the baseline. This radioactivity did not correspond to α-solanine hydrolysis products and most likely represented products of the endogenous substrates. This assay was found to give linear correlation curves with respect to both time (up to 30 min) and protein concentration at all stages of enzyme purification (data not shown).

Enzyme Purification. Table I shows a summary of a typical purification protocol for solanidine glucosyltransferase. SGT was readily solubilized by homogenization with aqueous buffer. The ammonium sulfate precipitation step gave a partial purification but more importantly was found to remove a significant amount of insoluble material that otherwise interfered with subsequent chromatogra-

Table I. Summary of a Typical Purification of Solanidine Glucosyltransferase from Sprouts of the Cultivar Lemhi

purifn step	vol, mL	protein concn, mg/mL	total protein, mg	sp act., units[a]/mg	total act., units	purifn, x-fold	yield, %
crude homogenate	400	0.74	296	0.46	136	(1)	(100)
supernatant	380	0.55	209	0.50	105	1	77
ammonium sulfate ppt	23	4.1	94	0.75	71	1.6	52
ion-exchange Mono Q	45	0.21	9.5	2.5	24	5	18
Con A–Sepharose	55	0.1	5.5	3.9	22	8	16
gel filtration Superose 12	2.0	0.93	1.86	9.0	17	20	13
chromatofocusing Mono P	2.25	0.066	0.148	75.5	11	164	8
ion-exchange Mono Q	1.5	0.0026	0.004	270	1.1	587	0.8

[a] 1 unit = 1 nmol of solanidine glucosylated/min at 37 °C.

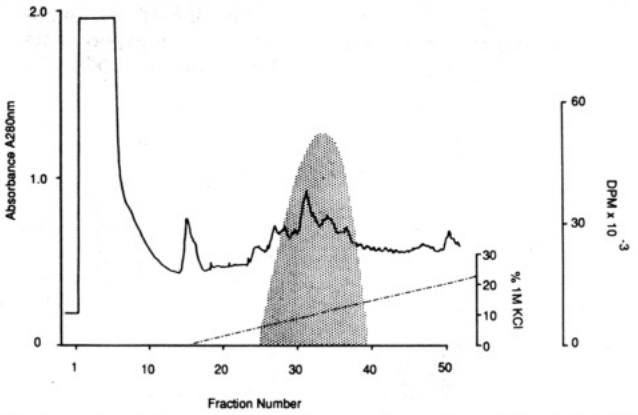

Figure 4. Anion-exchange chromatography. The 35–65% ammonium sulfate cut of the Lemhi supernatant was initially fractionated by using a Mono Q HR 10/10 column developed by a linear KCl gradient in 20 mM Bis-Tris propane, pH 6.6, at a flow rate of 4.0 mL/min. Enzyme activity (hatched area) was eluted between 80 and 150 mM KCl.

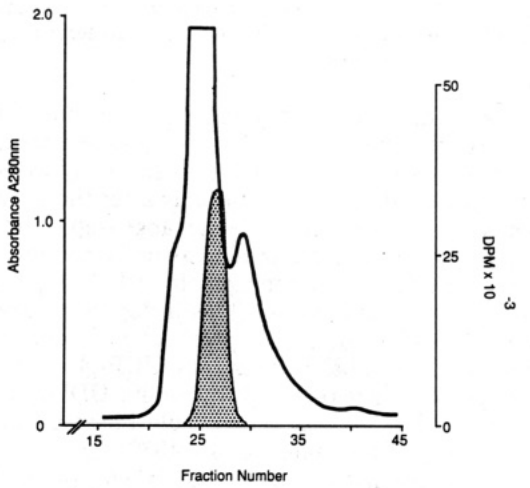

Figure 5. Gel filtration chromatography. SGT activity was fractionated on a Superose 12 column equilibrated in 50 mM Bis-Tris propane buffer, pH 6.6, containing 1 mM DTT and 5 mM $MgCl_2$. The flow rate was 0.5 mL/min. A single peak of glucosyltransferase activity was observed (hatched area).

phy steps. The first ion-exchange step resolved a broad peak of enzyme activity between 100 and 150 mM KCl (Figure 4). The negative affinity step using concanavalin A–Sepharose typically bound between 30 and 40% of the applied protein and gave a recovery of SGT activity of 80–90%. This nonbinding pool chromatographed as a single peak of enzyme activity by gel filtration (Figure 5), eluting just after the peak absorbance corresponding to the marker protein ovalbumin (Figure 6). The chromatofocusing step resolved one peak of enzyme activity at a Polybuffer pH of approximately 4.7 (Figure 7). The final ion-exchange step resolved a sharp peak of enzyme activity

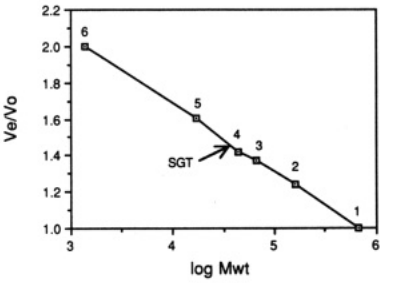

Figure 6. Estimation of native molecular weight by gel filtration chromatography. The native molecular weight of the enzyme was determined by using a Superose 12 column and cochromatographing with protein standards: (1) thyroglobulin (M_r 670 000); (2) γ-globulin (M_r 158 000); (3) BSA (M_r 66 000); (4) ovalbumin (M_r 44 000); (5) myoglobin (M_r 17 000); (6) vitamin B_{12} (M_r 1350). SGT eluted at an elution volume (V_e) corresponding to M_r 36 000.

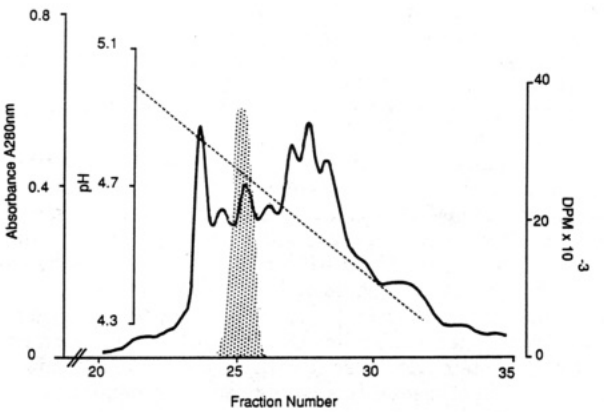

Figure 7. Chromatofocusing chromatography. SGT activity from the gel filtration step was fractionated on a Mono P HR 5/20 column. The column was initially equilibrated to pH 5.7 with 25 mM piperazine. Proteins were eluted by a pH gradient to pH 4.0 (see Materials and Methods). A single peak of glucosyltransferase activity (hatched area) was observed eluting at an approximate pH of 4.7.

at a salt gradient concentration of 130 mM KCl (Figure 8).

SDS/Denaturing–PAGE. The near homogeneity of the enzyme preparation after the final ion-exchange step was confirmed by SDS/PAGE. A major band corresponding to a molecular weight of 38 000 ± 2000 (n = 6) is ascribed to SGT (Figure 9). In addition, very faint bands corresponding to higher molecular weights (typically 57 000, 64 000, and 97 000) were also observed in some gels.

pH Profile and Ionic Requirements. Figure 10 shows the pH–activity profile of the purified enzyme (after the gel filtration step). SGT had a distinct optimum between pH 6.4 and 6.8. Mg^{2+}, Mn^{2+}, and K^+ at concentrations up to 10 mM had no significant effects on the activity of the enzyme.

Kinetic Studies and Substrate Specificity. In the presence of 200 μM solanidine, the partially purified

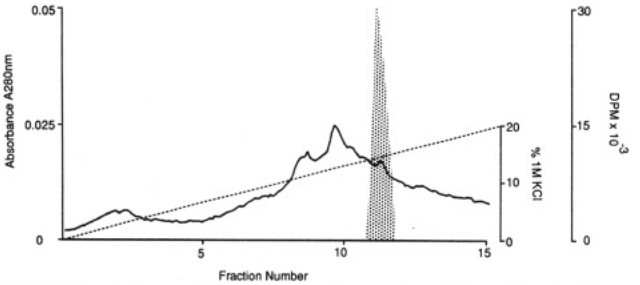

Figure 8. Anion-exchange chromatography. The active fractions from the chromatofocusing column were fractionated by using a Mono Q HR 5/5 column and a shallow gradient to 250 mM KCl in 20 mM Bis-Tris propane, pH 6.6. The flow rate was 0.75 mL/min. A single peak of enzyme activity eluted at 150 mM KCl.

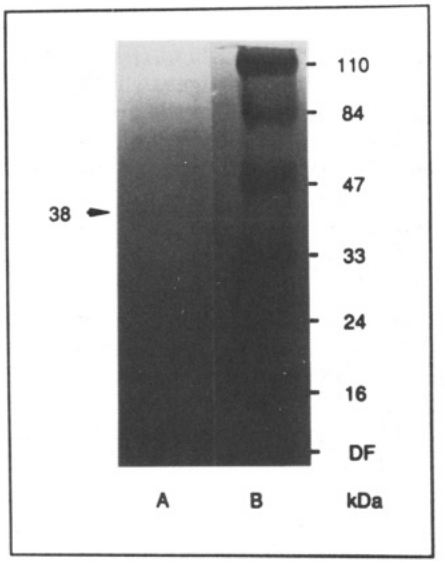

Figure 9. SDS/denaturing-PAGE. The peak fractions from the final Mono Q column were concentrated by lyophilization and denatured by boiling in the presence of SDS and mercaptoethanol. Electrophoresis (2.0 μg of protein) with silver staining was performed on a 4–20% precast gradient gel as described under Materials and Methods. The major protein band corresponded to a molecular weight of 38 000.

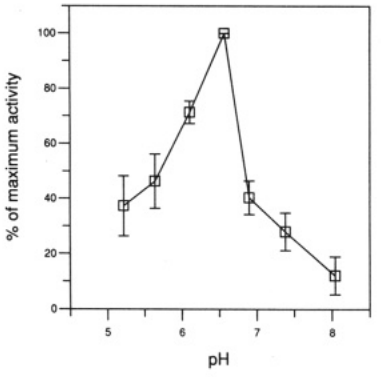

Figure 10. pH profile of partially purified SGT. Solanidine glucosyltransferase activity from the gel filtration step was assayed at pH 5.0–6.5 with 100 mM MES buffer and between pH 7.0 and 8.0 with 100 mM Bis-Tris propane. Results are the average of three experiments ± SEM.

enzyme (after Superose 12 chromatography) had a K_m for UDP-glucose of 37 ± 10 μM (n = 6). Under saturating UDP-glucose concentration (100 μM), the K_m for solanidine was 20 μM (n = 2). No enzymic activity was found with radiolabeled UDP-galactose as substrate. UDP-galactose also did not act as an inhibitor of the enzymic reaction when UDP-glucose was the radiolabeled substrate.

Table II. Relative Rates of Aglycon Glucosylation[a]

aglycon	rel act.	aglycon	rel act.
solanidine	100	sitosterol	0
solasodine	326 (±20)	diosgenin	0
tomatidine	204 (±7)	digoxigenin	0
cholesterol	0		

[a] SGT activity from the Superose 12 column was measured in the presence of 100 mM [^{3}H]UDP-glucose and 100 mM aglycon (dissolved in DMSO) for 15 min at 37 °C and 50 mM Bis-Tris propane buffer, pH 6.6. Results are the average (±SEM) of at least four determinations performed in triplicate.

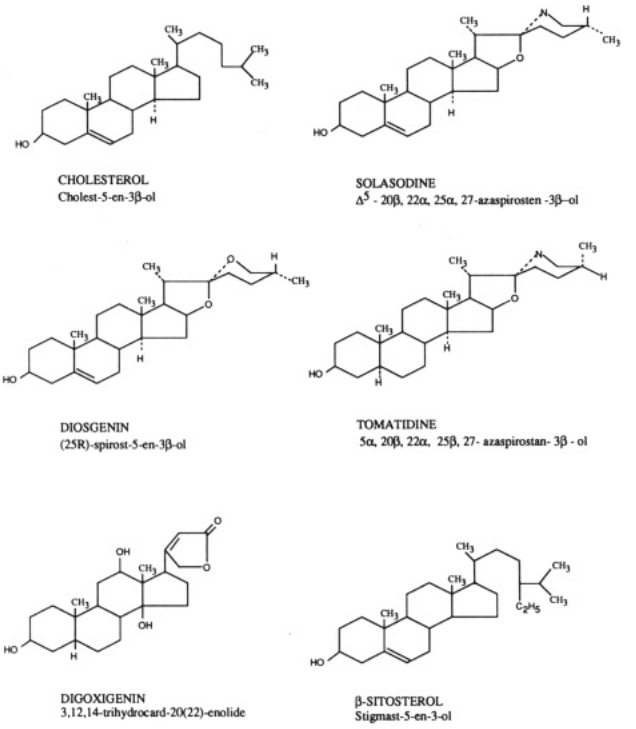

Figure 11. Structures of steroidal aglycons tested as glucosylation substrates (see Table II).

In the crude homogenate and salt-precipitated enzyme preparations, solanidine galactosyltransferase activity was very low compared to the glucosyltransferase activity (7:1 glucosyl:galactosyl transferase activity).

Table II shows the relative susceptibilities of a series of structurally different aglycons (Figure 11) to SGT-catalyzed glucosylation. The spirosolane alkaloids tomatidine and solasodine were glucosylated at rates 2 and 3 times that of solanidine, respectively. In contrast, 3-β-OH steroids without a ring nitrogen were not glucosylated.

DISCUSSION

To our knowledge, this is the first report of an extensive purification and characterization of an enzyme responsible for the glucosylation of solanidine from potatoes. The development of the new ion-exchange resin assay was instrumental in this respect due to its speed, specificity, and sensitivity. The assay may have application in the study of other UDP-sugar transferases.

The purification summary (Table I) shows that the overall yield of SGT activity was relatively low despite a purification factor of almost 600-fold. This loss of total activity is probably not due to proteolysis as the inclusion of protease inhibitors such as phenylmethanesulfonyl fluoride (PMSF), leupeptin, pepstatin, and EDTA to the chromatography buffers had no significant effect on the recovery of enzyme activity. The addition of substrates

and glycerol to the buffers used in the later chromatographic steps was also ineffective in maintaining enzyme activity. Following the final ion-exchange step, approximately 50% of enzymic activity was lost after the enzyme was frozen overnight. This instability may be an inherent characteristic of steroidal alkaloid glycosyltransferases in highly purified form. Keil and Schreier (1989) reported that treatment of crude homogenates with protamine sulfate prior to salt precipitation was critical for maintaining UDP-sugar transferase activities from plants. This treatment had no significant effect on the activity of solanidine glucosyltransferase in our study.

The isolation of SGT was complicated by its copurification with patatin. Patatin is a M_r 40 000 glycoprotein consisting of several isoforms and is considered to represent the major tuber storage protein (Racusen and Foote, 1980; Park et al., 1983). Preliminary calculations show that the ratio of patatin to SGT in sprouts is more than 1000:1. This large ratio may explain why these two proteins are difficult to resolve. The identification of patatin was verified by N-terminal amino acid sequencing following its desorption from the concanavalin A–Sepharose affinity column. We were unable to separate SGT from patatin by other purification methods including the high-resolution technique of chromatofocusing. Patatin has been reported to possess both lipid acyl hydrolase and acyl transferase activities (Racusen, 1984; Andrews et al., 1988). We confirmed these findings and also assayed patatin for steroidal alkaloid glucosyltransferase activity with negative results.

Solanidine glucosyltransferase was found to be very specific for steroidal alkaloids, as 3-OH steroids lacking a nitrogen-containing ring were not glucosylated (Table II). An unexpected observation was that the spirosolane aglycons, solasodine and tomatidine, were glucosylated at higher rates than solanidine. This activity may reflect the experimental conditions employed in this study for the in vitro assay of SGT or may reflect a greater conformational flexibility of the steroidal indolizidine F-ring skeleton of the spirosolanes (Brown and Keeler, 1978; Gaffield and Keeler, 1984) to fit the active site of the enzyme. Potato tissue has been reported to contain both spirostanol and spirosolane glycoalkaloids and also glycosylated 3-aminospirostanes, solanocapsines, and 22,26-epiminocholestanes (Van Gelder, 1990). It is not known how the glycosylation of each class of steroidal alkaloid is controlled in vivo and whether more than one glycosyltransferase is involved. We were surprised to find that crude extracts of potato sprouts had a very low activity of solanidine galactosyltransferase activity compared to glucosyltransferase activity and that UDP-galactose was neither a substrate nor a competitive inhibitor of the purified glucosyltransferase. Maga (1980) reported that the two major potato glycoalkaloids α-solanine and α-chaconine were generally found in a 40:60 ratio. From the results of this study, it seems reasonable to speculate that glucosylation of solanidine to produce γ-chaconine (glucosylsolanidine) may be the primary reaction and that γ-solanine (galactosylsolanidine) is then formed by the action of a highly specific epimerase that transforms a glucose to a galactose side chain. Further elucidation of these metabolic pathways would require the isolation of the galactosyltransferase or the epimerase.

Additional attempts were made to purify SGT by using affinity chromatography. The enzyme did not bind to UDP-glucuronic acid–Sepharose under various ionic conditions. Attempts to couple solanidine and solasodine via their 3-β-OH groups to epoxy-activated Sepharose were also unsuccessful due to aglycon insolubility at the high pH (pH >9) required for this reaction. The use of an affinity purification step would facilitate the preparation of a sufficient quantity of SGT for proteolytic digestion and amino acid sequencing, as preliminary results have indicated that the N terminus of SGT is blocked.

Further characterization of SGT, especially with regard to its catalytic mechanism, would benefit from a more stable enzyme preparation. Subsequent studies may reveal additional information concerning, for example, ion and/or cofactor requirements that would permit the isolation of an enzyme of higher specific activity. Several reports of plant membrane-bound UDP-glucose-sterol glucosyltransferase utilizing β-sitosterol as substrate have shown a requirement for phospholipids for activity following detergent solubilization (Bouvier-Nave et al., 1984; Ullmann et al., 1984, 1987). Although the enzyme reported here was easily solubilized without the use of detergents, it may be appropriate to investigate whether similar phospholipid factors could stabilize the enzyme activity.

In conclusion, the isolation of solanidine glucosyltransferase reported here is a first step toward our goal of understanding and controlling the biosynthesis of potato glycoalkaloids at the molecular level.

ABBREVIATIONS USED

FPLC, fast protein liquid chromatography; SGT, solanidine UDP-glucose glucosyltransferase; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; MES, 2-(*N*-morpholino)ethanesulfonic acid; TCA, trichloroacetic acid; DMSO, dimethyl sulfoxide; V_e, elution volume; V_0, void volume.

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