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Metabolism of conjugated sterols in eggplant. Part 1. UDP-glucose : sterol glucosyltransferase

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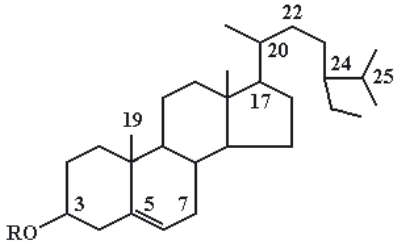
A membrane-bound UDP-glucose : sterol glucosyltransferase from *Solanum melongena* (eggplant) leaves was partially purified and its specificity as well as molecular and kinetic properties were defined. Among a wide spectrum of 3-OH steroids (i.e. typical plant sterols, androstane, pregnane and cholestane derivatives, steroidal alkaloids and sapogenins) and triterpenic alcohols, the highest activity was found with 22-oxycholesterol. UDP-glucose appeared to be the best sugar donor. The enzyme preparation was also able to utilize UDP-galactose, TDP-glucose and CDP-glucose as a sugar source for sterol glucosylation, however, at distinctly lower rates. The investigated glucosyltrasferase was stimulated by 2-mercaptoethanol, Triton X-100 and negatively charged phospholipids, and inhibited in the presence of UDP, mono-, di- and triacylglycerols, divalent cations such as Zn^{2+}, Co^{2+}, high ionic strength, cholesteryl glucoside, galactoside and xyloside and some amino acid-modifying reagents (SITS, DIDS, PLP, DEPC, pCMBS, NEM, WRK and HNB). Our results suggest that unmodified residues of lysine, tryptophan, cysteine, histidine and dicarboxylic amino acids are essential for full enzymatic activity and indicate that a glutamic (or aspartic) acid residue is necessary for the binding of sugar donor, i.e. UDP-glucose in the active site of the GT-ase while histidine and cysteine residues are both important for the binding of the nucleotide-sugar as well as of the steroidal aglycone.

Keywords: plant sterols, *Solanum melongena,* steryl glucoside, UDP-glucose : sterol glucosyltransferase

INTRODUCTION

Apart from free sterols, their conjugated forms, i.e. steryl esters, steryl 3-*O*-β-D-monoglycosides (SG) and steryl 6′-*O*-acyl-β-D-glycosides are widely distributed common constituents in higher plant cells (see Scheme). They were also found in ferns, mosses and fungi, while their presence in algae and prokaryotes has been reported in a few cases only (Wojciechowski, 1991; Tannes *et al*., 2000). Plant SG usually consist of a mixture of individual "molecular species" differing in sterol and/or saccharide moiety. The predominating sugar moiety found



R = H	sitosterol
R = acyl residue	sitosteryl ester
R = glucopyranose	sitosteryl glucoside
R = 6'-*O*-acylglucopyranose	sitosteryl 6'-*O*-acylglucopyranose

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Abbreviations: DEPC, diethylpyrocarbonate; DGDG, digalactosyldiacylglycerols; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; DTE, dithioerythritol; DTT, dithiothreitol; GTase(s), glucosyltransferase(s); HNB, 2-hydroxy-5-nitrobenzyl bromide (Koshland I Reagent); MGDG, monogalactosyldiacylglycerols; NAI, *N*-acetylimidazole; NEM, *N*-ethylmaleimide; PA, phosphatidic acid; PC, phosphatidylcholine; pCMBS, *p*-chloromercuribenzenesulfonic acid; PE, phosphatidylethanolamine; PG, phenylglyoxal; PI, phosphatidylinositol; PLP, pyridoxal 5′-phosphate; PMSF, phenylmethylsulfonyl fluoride; SG, steryl glucosides; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid; WRK, Woodward's Reagent K.

in SG is glucose, but galactose, xylose and others also occur (Jares *et al*., 1990; Wojciechowski, 1991; Jahan *et al*., 1995; Casabuono & Pomilo, 1997). Besides the monoglycoside steryl derivatives, glycosides with an oligosaccharide chain (up to five sugar residues) have been found (Fujino & Ohnishi, 1979; Kojima *et al*., 1989). SG and their 6′-*O*-acyl derivatives in general occur in small amounts, but plants from the *Solanum* genus, e.g. *S. tuberosum*, *S. lycopersicon*, *S. dulcamara*, demonstrate a unique abundance of SG and ASG (acetylated steryl glycoside), as the glycosylated forms of sterols make up to 30–50% of total sterols in these plants (Duperon *et al*., 1984; Duperon & Duperon, 1989; Zimowski & Wojciechowski, 1996). In some papers one can find suggestions that SG present in the above plants are a mixture of steryl β-D-monoglucosides and β-D-monogalactosides (Duperon *et al*., 1984; Duperon & Duperon, 1989).

Enzymatic glucosylation of sterols in the presence of UDP-glucose has been demonstrated in cell-free preparations and in various crude membraneous fractions obtained from vascular plants (Wojciechowski & Nguyen Van Uon, 1975; Fang & Baisted, 1976; Baisted, 1978; Wojciechowski *et al*., 1979; Pączkowski *et al*., 1990; Wojciechowski, 1991; Zimowski 1992), green algae (Forsee *et al*., 1974; Hopp *et al*., 1978) and non-photosynthesizing microorganisms (Esders & Light 1972; Wojciechowski *et al*., 1979; Warnecke *et al*., 1999). Only in a few cases have the respective enzymes been partially purified and characterized (Wojciechowski & Nguyen Van Uon, 1975; Wojciechowski *et al*., 1979; Yoshikawa & Furuya 1979; Ullmann *et al*., 1993; Warnecke & Heinz, 1994). Recently, sterol GTases from oat, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*, *Candida albicans*, *Pichia pastoris* and *Dictyostelium discoideum* were cloned and expressed in *Escherichia coli* (Warnecke *et al*., 1997, 1999).

Whether SG are active metabolic compounds or not remains an unsolved problem. Some hypotheses consider these conjugates as final products of sterol metabolism or reserve forms of sterols in plant cell. Others describe them as metabolically active substances, which may participate in intracellular transport of glucose (Murakami-Murofushi *et al*., 1997) or in specific transglucosylation processes, e.g. in cellulose (Peng *et al*., 2002; Peters, 2002) or cerebroside biosynthesis (Cantatore *et al*., 2000). On the other hand, the processes of sterol glucosylation-deglucosylation or sterol glucoside acylation-deacylation, which take place in membranes, can play a significant role in the properties of these membranes.

It is expected that UDP-glucose : sterol GTase, as an enzyme involved in the above-mentioned transformations, may play a significant physiological role. Glucosylation of free sterols represents a metabolic process most probably ubiquitous in plants and may participate in the regulating of free sterol concentration in the cell. Except the GTase from oat, data about basic molecular and catalytic properties of the enzyme involved in sterol glucosylation is still lacking. In the presented paper we determined biochemical properties of UDP-glucose : sterol GTase isolated from eggplant leaves.

MATERIAL AND METHODS

Plant material. Eggplant (*Solanum melongena* L., cv Black Beauty) plants were grown on artificial support (Perlite) in a greenhouse at a 16/8 h photoperiod, at 25/20°C day/night. Leaves were cut off from 9-week old plants.

Enzyme preparation. Fresh leaves of eggplant (100 g fresh mass) were homogenized with 200 ml of ice-cold 0.1 M Tris/HCl buffer, pH 7.3, containing 10 mM 2-mercaptoethanol (buffer A). The homogenate was filtered through cheesecloth and centrifuged at 3 000 × *g* (10 min). Supernatant was centrifuged at 20 000 × *g* (20 min) and the 20 000 × *g* pellet (the membrane fraction) was resuspended in buffer A and added dropwise to a 10-fold volume of cold (minus 20°C) acetone so "acetone powder I" was obtained as previously described (Wojciechowski *et al*., 1979). "Acetone powder I" was resuspended in buffer A containing 0.1% Triton X-100 (buffer B) and centrifuged at 20 000 × *g* (20 min). Supernatant (partly delipidated enzyme preparation) was added dropwise to a 10-fold volume of acetone (4°C) and "acetone powder II" was obtained as previously described (Wojciechowski *et al*., 1979).

Glucosyltransferase purification. Gel filtration was carried out on a Sephadex G-150 column (2 × 40 cm) equilibrated with buffer B. The enzyme preparation (acetone powder II, 10 mg/ml) was applied onto the column and eluted with buffer B at a flow rate of 0.4 ml/min. Fractions exhibiting the highest GTase activity were combined (12–20 ml) and then applied onto a Q-Sepharose (Sigma) column (1 × 3 cm), previously equilibrated with buffer B and eluted at a flow rate of 0.4 ml/min. Unbound proteins were washed out from the column with 30 ml of buffer B and then the column was developed with 20 ml of 0.5 M Tris/HCl buffer, pH 7.3, containing 10 mM 2-mercaptoethanol and 0.1% Triton X-100 (flow rate 0.4 ml/min). This fraction was dialyzed overnight against buffer B and GTase activity was assayed.

Glucosyltransferase assay. The standard reaction mixture contained in a total volume of 0.52 ml: enzyme preparation (50 μg protein in 0.5 ml of buffer B); 25 nmol of sitosterol (or another sugar acceptor) or [4-^{14}C]cholesterol (Reachim, 3.0 × 10^5 dpm, 2.5

nmol) in 0.01 ml ethanol, and UDP-[^{14}C]glucose (Amersham, 2.0×10^5 dpm, 6 nmol) or unlabelled UDP-glucose (Sigma, or another sugar donor, 15 nmol) in 0.01 ml 50% ethanol. Once UDP-glucose was added, the reaction was run at 30°C for 30 min and stopped by adding of 1 ml of methanol and heating for 3 min in a boiling water bath. Subsequently samples were extracted with 4 ml of 1-butanol saturated with water (3 times) and the butanolic extracts were washed several times with water saturated with 1-butanol. The samples were air-dried and applied on silica gel plates (Merck) and developed with chloroform/methanol (9 : 1, v/v), as a solvent. Labelled products were localized by autoradiography and their chromatographic mobility was compared with those of authentic reference compounds: 3-*O*-β-D-monoglucopyranoside of sitosterol (R_F = 0.32) and 3-*O*-β-D-monogalactopyranoside of sitosterol (R_F = 0.27). The radioactivity measurements of butanolic extracts or purified radioactive products were carried out as previously described (Zimowski, 1991).

Other methods. Unlabelled steroidal monoglycosides, i.e. cholesteryl, sitosteryl, stigmasteryl 3-*O*-β-D-monoglucopyranosides or 3-*O*-β-D-monogalactopyranosides were obtained as previously described (Janiszowska *et al*., 1980).

RESULTS

Preliminary incubations performed in the presence of [^{14}C]cholesterol and non-radioactive UDP-glucose with homogenate obtained from 9-week old *S. melongena* leaves indicated that this preparation was able to catalyze the formation of labelled cholesteryl glucoside. Similar incubations with subcellular fractions separated by differential centrifugation indicated that the investigated GTase activity copurified with membrane fractions. The fraction sedimenting between 3000–20000 × *g* was the most active, exhibiting a 3–4-times higher total activity than the remaining membrane fractions.

The investigated enzyme was partially purified by gel filtration on Sephadex G-150 and by filtration on Q-Sepharose. The purified enzyme preparation showed a 21-fold increase of specific activity and a 2-fold decrease of total activity comparing with the crude homogenate.

Molecular and kinetic properties of UDP-glucose : sterol glucosyltransferase from eggplant leaves

The partially purified enzyme exhibits apparent K_m values, 14.29 μM for UDP-glucose, 1.43 μM for sitosterol. Divalent metal ions, such as Mg^{2+}, Ca^{2+}, Mn^{2+} and some metal chelators (EDTA, EGTA) show no effect on the GTase activity. On the other hand, some heavy metal ions had a strong inhibitory effect (I_{50} for Zn^{2+} and Co^{2+} was 0.09 and 0.53 mM, respectively). The GTase activity was strongly inhibited in the presence of UTP, UDP (I_{50} = 0.035, 0.065 mM, respectively) and some amino acid-modifying reagents i.e. SITS, DIDS, PLP, DEPC, pCMBS, NEM, WRK and HNB (I_{50} value was 0.28, 0.021, 1.43, 1.0, 0.003, 0.043, 0.8, and 1.2 mM, respectively). The enzyme catalyzing the biosynthesis of SG was also sensitive to higher ionic strength of the incubation medium (I_{50} for NaF and NaCl was 0.52 M and 0.29 M, respectively). Unlabelled (cold) steryl glycosides, e.g. cholesteryl glucoside, galactoside and xyloside added to the incubation mixtures in the presence of cholesterol and UDP-[^{14}C]glucose caused a considerable decrease of the formation of labelled cholesteryl glucoside (I_{50} = 4.6, 10.8, 61.8 μM, respectively). Detergents commonly used to solubilize and/or to activate membrane-bound enzymes, e.g. Tween 20, Tween 40, Tween 60, Tween 80, Tyloxapol, sodium deoxycholate showed practically no effect on the investigated GT-ase. On the other hand, *n*-octyl thioglucoside irreversibly inhibited (I_{50} = 4.8 mM) while Triton X-100 strongly activated (approx. 130-fold at 0.1% conc.) the studied enzyme.

Specificity of UDP-glucose : sterol glucosyltransferase from eggplant leaves towards sugar moiety acceptor and donor

The partially purified enzyme preparation was used to study the substrate specificity of the investigated GTase towards the sugar moiety acceptor in the presence of UDP-[^{14}C]glucose. Typical sterols and their oxidized derivatives, some C_{19} and C_{21} steroids, steroidal alkaloids, sapogenins and triterpenic alcohols were tested as potential sugar acceptors (Table 1). In all cases when incorporation of radioactivity into the butanol extract was found, TLC analysis with subsequent autoradiography showed the presence of a single labelled product with chromatographic mobility expected for the monoglucoside of a given steroid. Among the natural plant sterols tested sitosterol was the best sugar acceptor. A comparison of the configuration of the OH- group at C-3 and/or the coupling of A and B rings (*cis* or *trans*) in saturated isomers of C_{27}-stanols (cholestanol, epicholestanol, epicoprostanol and coprostanol) showed that only cholestanol served as sugar acceptor. Thiocholesterol and triterpenic alcohols were not active as sugar acceptors. The presence and/or the position of double bonds in the steroid molecule seem to play an important role in glucosylation rate. This effect is distinct when comparing the data for glucosylation of sterols differing in the number and/or position of double bonds. The glucosylation rates

Table 1. Specificity of UDP-glucose : sterol GTase from *S. melongena* leaves towards glucosyl moiety acceptors

Acceptor	Steryl [^{14}C]glucoside formation (%)*
Steroid alkaloids	
a) Spirosolane type	
Tomatidine (25*S*-5α,22β*N*-spirosolanin-3β-ol)	2.8
Solasodine (25*R*-22α*N*-spirosol-5-enin-3β-ol)	0.0
b) Solanidane type	
Solanidine (22*S*,25*S*-solanid-5-enin-3β-ol)	0.0
Demissidine (22*S*,25*S*-5α-solanidanin-3β-ol)	1.4
Steroid sapogenin	
Nuatigenin (22*S*,25*S*-epoxy-furost-5-en-3β,26-diol)	16.1
Isonuatigenin (25*R*-spirost-5-en-3β,25β-diol)	13.4
Hecogenin (25*R*-5α-spirostan-3β-ol-12-on)	6.3
Diosgenin (25*R*-spirost-5-en-3β-ol)	3.5
Tigogenin (25*R*-5α-spirostan-3β-ol)	2.1
Sarsasapogenin (25*S*-5β-spirostan-3β-ol)	0.0
Yamogenin (25*S*-spirost-5-en-3β-ol)	0.0
Sterols and their derivatives	
Sitosterol (stigmast-5-en-3β-ol)	100.0
Stigmasterol (stigmasta-5,22-dien-3β-ol)	49.2
Stigmasta-5,24(28)-dien-3β-ol	82.4
Stigmastan-3β-ol	38.7
Cholesterol (cholest-5-en-3β-ol)	98.5
25-Hydroxycholesterol (cholest-5-en-3β,25-diol)	89.4
Cholest-5-en-3β,20α-diol	90.8
Cholest-5-en-3β,19-diol	9.2
Cholest-5-en-3β-ol-7-on	5.6
22-Oxycholesterol (cholest-5-en-22-on-3β-ol)	133.1
5α-Cholest-7-en-3β-ol	14.1
Thiocholesterol (cholest-5-en-3β-tiol)	0.0
5α-Cholestan-3β-ol (cholestanol)	12.0
5β-Cholestan-3β-ol (coprostanol)	0.0
5α-Cholestan-3α-ol (epicholestanol)	0.0
5β-Cholestan-3α-ol (epicoprostanol)	0.0
Androstane or pregnane derivatives	
Androstenolon (androst-5-en-3β-ol-17-on)	16.0
Pregnenolon (pregn-5-en-3β-ol-20-on)	33.1
Triterpenic alcohols	
Lanosterol (4,4′,14-trimethyl-5α-cholest-8-en-3β-ol)	0.0
β-Amyrin (olean-12-en-3β-ol)	0.0

*The glucosylation rate for sitosteryl glucosylation (355 fkat) was taken as 100%. Incubations were carried out in the presence of unlabelled sugar moiety acceptor (25 nmol) and UDP-[^{14}C]glucose as described in Material and Methods.

of these sterols show the following sequence: $C_{29}\ \Delta^5 > C_{29}\ \Delta^{5,24(28)} > C_{29}\ \Delta^{5,22} > C_{29}\ \Delta^0$ and $C_{27}\ \Delta^5 > C_{27}\ \Delta^7 \geq C_{27}\ \Delta^0$. The modification of sterol side chain structure also has an important effect on glucosylation rate. C_{29}-sterols (i.e. sterols with an additional alkyl group in the side chain at C-24) were better glucose acceptors than C_{27}-sterols (sitosterol *versus* cholesterol, stigmastanol *versus* cholestanol). Pregnane and androstane derivatives (C_{21} and C_{19} steroids, respectively) as well as some sapogenins and steroidal alkaloids (C_{27} sterol derivatives) tested were glucosylated at a much lower rate (nuatigenin, isonuatigenin, hecogenin, diosgenin, androstenolon, pregnenolon) or were not glucosylated (sarsasapogenin, yamogenin, solasodine, solanidine). The incorporation of oxygen atom into the sterol molecule side chain had practically no effect (25-hydroxycholesterol, cholest-5-en-3β,20α-diol) or stimulated the appropriate glucoside biosynthesis (22-oxycholesterol). On the other hand, incorporation of oxygen atom into the sterol planar ring system strongly inhibited the formation of the appropriate glucoside (cholest-5-en-3β-ol-7-on, cholest-5-en-3β,19-diol).

To determine the substrate specificity of the enzyme with respect to the sugar donor, the formation of [^{14}C]cholesteryl monoglycosides in the presence of [^{14}C]cholesterol and various unlabelled nucleotide-sugars (e.g. UDP-glucose, UDP-galactose, UDP-mannose, UDP-xylose, CDP-glucose, TDP-glucose, ADP-glucose and GDP-glucose) was tested. Our results indicate that only UDP-glucose, UDP-galactose, TDP-glucose and CDP-glucose were sugar donors for glycosylation of [^{14}C]cholesterol, however at different rates (48.3, 12.8, 13.0 and 7.5 fkat, respectively). An autoradiographic analysis of labelled product(s) formed in the presence of the above-mentioned nucleotide-sugars showed that in all cases only one radioactive compound with chromatographic mobility of cholesteryl monoglucoside was synthesized.

Effect of chemical compounds selectively modifying amino-acid residues on steryl glucoside formation

Significant inhibition was obtained in the presence of reagents modifying lysine ε-NH_2 group (DIDS, SITS and PLP), histidine (DEPC), dicarboxylic amino acids (WRK), tryptophan (HNB) and free thiol group in cysteine (pCMBS, NEM) (see above). On the other hand, reagents acting on serine, arginine and tyrosine, i.e. PMSF, PG and NAI, respectively, had no effect on steryl glucoside formation. In order to identify the amino acids involved in substrate binding, the effect of various amino acid-modifying reagents on steryl glucoside biosynthesis was investigated after preincubation of the enzyme preparation with the substrate, i.e. UDP-glucose or

Table 2. Eggplant GT-ase activity protection in the presence of amino acid-modifying reagents by UDP-glucose or sitosterol.

Enzyme preparation was preincubated for 5 min with or without sitosterol (46 μM) or with or without UDP-[^{14}C]glucose (33.5 kBq, final conc. 0.29 μM). Then modifying reagent was added and preincubation was continued for 10 min. The enzymatic reaction for samples preincubated with sitosterol or UDP-[^{14}C]glucose was initiated by an addition of labelled UDP-glucose or sitosterol, respectively. For samples preincubated without substrate the reaction was started by the use of equivalent amount of unlabelled sitosterol and UDP-[^{14}C]glucose.

Amino acid modified	Amino acid-modifying reagent	Concentration of reagent (mM)	Relative activity of GTase from eggplant leaves (%)*		
			Preincubation		
			without substrates	with sitosterol	with UDP-glucose
	DIDS	0.05	20 ± 7	20 ± 7	27 ± 4
Lysine	SITS	0.5	17 ± 7	18 ± 8	24 ± 1
	PLP	2.0	19 ± 4	24 ± 3	32 ± 3
Cysteine	NEM	0.1	**19 ± 5**	**37 ± 1**	**42 ± 5**
Histidine	DEPC	2.0	**29 ± 6**	**57 ± 7**	**54 ± 7**
Tryptophan	HNB	2.3	24 ± 8	23 ± 5	24 ± 9
Aspartic and glutamic acid	WRK	2.0	26 ± 7	20 ± 6	**94 ± 1**

*Enzyme activity without amino acid-modifying reagent was taken as 100%.

sitosterol (Table 2). Noticeable protection of the enzyme against inactivation with NEM or DEPC was found when GTase was preincubated with sitosterol or with UDP-glucose. Preincubation of the enzyme with UDP-glucose, but not with sitosterol, practically completely eliminated the inhibitory effect of WRK.

The influence of some lipids on sterol glucosylation

Preliminary investigations showed that various lipids (phospholipids, glycolipids and acylglycerols) had no effect on sitosteryl glucoside biosynthesis catalyzed by native enzyme preparation (i.e. homogenate or subcellular fractions). In contrast, sitosterol glucosylation was distinctly modified in the presence of lipids when a completely delipidated enzyme preparation was used. Among the lipid tested, an inhibitory effect on the formation of steryl glucoside was observed with acylglycerols, e.g. 1-monopalmitoyl-, 1,2-dipalmitoyl-, 1,3-dipalmitoyl- and tripalmitoylglycerol (27–35% inhibition), whereas typical chloroplast glycolipids (MGDG and DGDG from wheat) and zwitterionic phospholipids (e.g. synthetic 1,2-dipalmitoyl-PC and 1,2-dipalmitoyl PE) had no and a slightly stimulatory effect, respectively. However, negatively charged phospholipids, i.e. phosphatidylglycerol (synthetic 1,2-dipalmitoyl-), PA (synthetic 1,2-dipalmitoyl-) and PI (from soya bean) significantly stimulated steryl glucoside biosynthesis in comparison with the control without lipid added (175, 146 and 123% stimulation, respectively). In order to establish whether the above-described effects depended on acyl composition of the phospholipid molecule, several "molecular species" of phosphatidic acids and phosphatidylglycerols differing in fatty acids' chain length or saturation were tested. The obtained results indicated that the chain length of fatty acid residue (from C_{12} to C_{16} in the case of synthetic PA) had no effect on steryl glucoside formation by GT-ase from eggplant leaves. The presence of an unsaturated fatty acid residue in the phosphatidylglycerol molecule evidently stimulated (about 1.6-fold) sterol glucosylation (in the presence of 1,2-di$C_{18:0}$ — or di$C_{18:1}$ phosphatidylglycerol the rate of formation of sitosteryl glucoside was 522.8 or 849.4 fkat, respectively).

DISCUSSION

Glucosylation of sterols in the presence of UDP-glucose was demonstrated for the first time 40 years ago with the use of cell-free preparations from immature soybean seeds (Hou *et al.*, 1967). Our results indicate that an enzyme preparation obtained from *S. melongena* leaves by differentil centrifugation is also able to catalyze the formation of steryl glucosides in the presence of exogenously added sugar donor and sugar acceptor (i.e. UDP-glucose and [^{14}C]cholesterol or UDP-[^{14}C]glucose and sitosterol). The investigated membraneous enzyme, i.e. UDP-glucose : sterol GTase from eggplant, shares some properties with other GTases acting on sterols. All of them are bound with membranes and, except for the enzyme from *Physarum polycephalum* (Wojciechowski

& Nguyen Van Uon, 1975), are strongly activated by the non-ionic detergent Triton X-100 (Wojciechowski, 1991). Solubilization of membrane-bound UDP-glucose : sterol GTase with Triton X-100 (Wojciechowski & Nguyen Van Uon, 1975; Hopp *et al*., 1978; Wojciechowski, 1991) or *n*-octyl-β-D-thioglucoside (Warnecke & Heinz, 1994) followed by typical purification methods results in the 30–100-fold or 12 500-fold increase of enzyme activity, respectively. The eggplant GTase was solubilized and strongly activated by Triton X-100 only. The purification procedure with the use of this detergent leads to a 21-fold increase of the enzyme activity. The *n*-Octyl derivative cannot be use for eggplant GTase purification as it causes irreversible inhibition of the enzyme activity.

In contrast to a partially purified GTase from pea seedlings (Fang & Baisted, 1976), the partially purified GTase from eggplant (like the enzyme preparations from *Calendula officinalis* (Wojciechowski & Nguyen Van Uon, 1975), potato plant (Zimowski, 1992) or *Sinapis alba* seedlings (Wojciechowski *et al*., 1979)) failed to be stimulated by Mg^{2+} or Ca^{2+} or by chelating factors. It was shown that most GTases are strongly inhibited by one of the reaction products, i.e. UDP (Wojciechowski, 1991). The GTase from eggplant, apart from UDP, is also inhibited by the second reaction product, i.e. steryl glucoside. The apparent molecular masses of UDP-glucose : sterol GTases range from 56 kDa for oat GTase (Warnecke & Heinz, 1994) to 140 kDa for the enzyme from white mustard seedlings (Wojciechowski *et al*., 1979). The molecular mass for the eggplant enzyme was established as 120 kDa. Apparent K_m values for sitosterol range from 5.0 μM (white mustard seedlings GTase (Wojciechowski *et al*., 1979)) to 200 μM (etiolated maize coleoptyle GTase (Ullmann *et al*., 1993)), while for UDP-glucose they range from 6.3 μM (potato plant GTase (Zimowski 1992)) to 130 μM (*Physarum polycephalum* GT-ase (Wojciechowski *et al*., 1979)). The eggplant enzyme shows the highest affinity for the sugar acceptor among all GTases studied. The K_m value for sitosterol, 1.43 μM, is lower than that for the white mustard seedling enzyme (3-fold).

The influence of lipids on the activity of membrane-bound glycosyltransferases is a well-known phenomenon (Curruthers & Melchior, 1986). Stimulatory or inhibitory effects are not surprising since lipids make a natural environment for these membrane-bound enzymes. A lipid dependence has also been shown for UDP-glucose : sterol GTases isolated from several plants. It was observed that treatment of membraneous preparations of UDP-glucose : sterol GTase from etiolated pea seedlings with phospholipases caused a partial loss of the enzymatic activity (Fang & Baisted, 1976), which could be restored by some phospholipids (especially phosphatidylethanolamine). Phospholipid influence on UDP-glucose : sterol GTase activity was also observed for partly and completely delipidated enzyme preparation from etiolated maize coleoptiles (Bouvier-Navé *et al*., 1984; Ullmann *et al*., 1984). Negatively charged phospholipids, i.e. phosphatidic acid, phosphatidylglycerol, phosphatidylinositol were much more effective than zwitterionic phospholipids, e.g. phosphatydylcholine and phosphatidyletanolamine (Ullmann *et al*., 1987). We observed a similar influence of negatively charged and zwitterionic phospholipids on eggplant GTase. In addition, eggplant GTase activity was modified (inactivated) in the presence of acylglycerols. It is interesting that some of them, i.e. 1,2-diacylglycerols, also play a role in membraneous secondary messenger signalling pathways. The results of the above experiments strongly suggest the possibility of *in vivo* regulation of plant UDP-glucose : sterol GTase activity by natural lipid microenvironment.

The substrate specificity of eggplant GTase towards sugar acceptors and sugar donors is similar to those observed for sterol glucosyltransferases from *Calendula officinalis*, *Sinapis alba*, *Asparagus plumosus*, *Physarum polycephalum* or *Solanum tuberosum* (Wojciechowski & Nguyen Van Uon, 1975; Wojciechowski *et al*., 1979; Pączkowski *et al*., 1990; Zimowski, 1992). The presence of a double bond at C-5 or the *trans* coupling of rings A and B, which are prerequisites for the planeness of the sterol ring system, as well as β configuration of the 3-OH group are necessary for the activity of all of the investigated enzyme. On the other hand, we have shown that incorporation of the SH- group at position C-3β makes glucosylation of such a thiol steryl derivative impossible. All sterol GTases seem to exhibit a rather broad specificity pattern if a planar ring system and the β configuration of the 3-OH group is fulfilled. Glucosylation rate can be influenced by several structural factors in the sterol molecule such as the presence and/or the position of a double bond, substitution at the side chain or at the ring system and modification of side chain (Yoshikawa & Furuya, 1979; Zimowski, 1992). It is very interesting that the cytosolic eggplant UDP-glucose : solasodine GTase which also catalyzes the glucosylation of steroids, i.e. steroidal alkaloids and sapogenins, practically does not galactosylate sterols (Pączkowski & Wojciechowski, 1994).

The substrate specificity of plant UDP-glucose : sterol GTase towards the sugar moiety donor was studied by several researchers (Forsee *et al*., 1974; Wojciechowski & Nguyen Van Uon, 1975; Hopp *et al*., 1978; Wojciechowski *et al*., 1979; Warnecke & Heinz, 1994; Warnecke *et al*., 1997). In

all cases UDP-glucose was definitely the best sugar donor. Other nucleosidediphosphate glycosides were much poorer substrates for the enzyme. For the eggplant enzyme UDP-glucose is also the best glucose donor, but relatively high glycosylation rates were observed with UDP-galactose, TDP-glucose and CDP-glucose. However, the fact that when UDP-galactose was used as a substrate the cholesterol glycosylation product could not be separated from synthetic cholesteryl glucoside by TLC in several solvent systems or by HPLC (unpublished) suggests that UDP-galactose utilization is preceeded by epimerization to UDP-glucose by 4'-epimerase present in the enzyme preparation. Besides, we could not confirm *in vivo* sterol galactosylation or the occurrence of sterol galactosides (Zimowski & Wojciechowski, 1996) in eggplant.

Several genes of plant GT-ases catalyzing nucleotide-sugar-dependent glycosylation of various secondary metabolites have been cloned recently. All these genes contain a consensus sequence, so called PSPG-box (putative secondary product glycosyltransferase-box) (Vogt & Jones, 2000). This PSPG-box is considered to represent the nucleotide-sugar binding site and contains highly conserved histidine and glutamic acid residues which are proposed to be involved in the transfer of the sugar moiety from the nucleotide-sugar donor to the sugar acceptor. Our results confirm that lysine, tryptophan, cysteine, histidine and dicarboxylic amino-acid residues are essential for steryl glucoside formation catalyzed by UDP-glucose : sterol GTase from *S. melongena*. They also indicate that glutamic (or aspartic) acid residue(s) is necessary for binding of sugar donor, i.e. UDP-glucose, in the active site of the enzyme and that two other amino acids, i.e. histidine and cysteine are important for the binding of nucleotide-sugar and of the steroidal aglycone. Similar data concerning essential amino-acid residues in the active site of cytosolic UDP-glucose : solasodine GTase from *S. melongena* have already been obtained. In that case lysine, tryptophan, tyrosine, cysteine, histidine and dicarboxylic amino acid residues of the enzyme are indispensable for glucosylation of steroidal alkaloids. However, only the presence of a histidine residue(s) (for binding sugar acceptor) and a dicarboxylic amino-acid residue(s) (for binding both sugar donor and its acceptor) were required in active site of the enzyme (Nawłoka *et al*., 2003).

The classical biochemical investigations presented in these paper supply data necessary to study regulation of the activity of plant membraneous enzymes as well as their role in changing the properties of plant membranes stress.

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