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Purification of a Membrane-Bound UDP-Glucose:Sterol β-D-Glucosyltransferase Based on Its Solubility in Diethyl Ether[1]

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Membrane-bound UDP-glucose:sterol β-D-glucosyltransferase (UDPG-SGTase) catalyzes the formation of steryl glucosides from UDP-glucose and free sterols. This enzyme was purified from etiolated oat shoots (*Avena sativa* L. cv Alfred) in five steps. UDPG-SGTase was solubilized from a microsomal fraction with the detergent *n*-octyl-β-D-thioglucopyranoside and then extracted into diethyl ether. Subsequent removal of the organic solvent, resolubilization with an aqueous buffer, and two column chromatographic steps on Q-Sepharose and Blue Sepharose resulted in a 12,500-fold overall purification. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the final preparation revealed a 56-kD protein band, the intensity of which correlated with enzyme activity in the respective fractions. Polyclonal antibodies raised against this 56-kD protein did not inhibit enzyme activity but specifically bound to the native UDPG-SGTase. These results suggest that the 56-kD protein represents the UDPG-SGTase. The purified enzyme was specific for UDP-glucose (K_m = 34 μM), for which UDP was a competitive inhibitor (inhibitor constant = 47 μM). In contrast to the specificity with regard to the glycosyl donor, UDPG-SGTase utilized all tested sterol acceptors, such as β-sitosterol, cholesterol, stigmasterol, and ergosterol.

Plant cells contain sterols in free and derivatized forms, among which steryl esters, steryl glycosides, and acylated steryl glycosides predominate. These membrane lipids are ubiquitously present in higher plants and widely distributed among ferns, mosses, and fungi, but their presence in algae has been reported in only a few cases (Eichenberger, 1977; Wojciechowski, 1991). Steryl glycosides have not been detected in prokaryotes except for *Mycoplasma* (Smith, 1971) and *Borrelia* (Livermore et al., 1978).

Apart from the predominant steryl glucosides, mannosides, galactosides, and several other glycosides also may occur in plants (Wojciechowski, 1991). In addition to the monoglycosides, higher homologs with two, three, or even four glycosyl residues in different linkages have been found (Kojima et al., 1989). Of the various enzymes required for synthesis and interconversion of these compounds, UDPG-SGTase has been studied in most detail.

Investigations of the subcellular distribution of free sterols, SG, and ASG indicated that plasma membrane, tonoplast, and Golgi membranes contain the major part of these lipids (Yoshida and Uemura, 1986; Hartmann and Benveniste, 1987; Haschke et al., 1990). This agrees with the intracellular distribution of UDPG-SGTase activity, which was found to be associated with plasma membrane and Golgi vesicles (Dupéron and Dupéron, 1987; Ullmann et al., 1993). Occasionally it was also detected in tonoplasts (Verhoek et al., 1983).

In contrast to the detailed knowledge of the structure, occurrence, intracellular location, and biosynthesis of SG and ASG (recently reviewed by Wojciechowski, 1991), very little is known about the functions of these lipids. The role of free sterols in the modulation of membrane fluidity and permeability is well established (Demel and De Kruyff, 1976; McKersie and Thompson, 1979; Schuler et al., 1991). The thermal phase transition of phospholipids was eliminated by free sterols as well as by SG and ASG (Mudd and McManus, 1980), but the ability to reduce membrane leakage was lost upon glucosylation of free sterols (Grunwald, 1971). Further studies on the behavior of SG and ASG in membranes have not been carried out.

On the other hand, experiments with *Arabidopsis* (Hugly et al., 1990) and rye indicated that the proportion of the various forms of sterols may be correlated with low temperature resistance of plants. In particular, cold acclimation of winter rye was paralleled by an increase of free sterols at the expense of SG and ASG in plasma membranes (Lynch and Steponkus, 1987). Thus, the ability to modulate free sterol proportions either by de novo synthesis or by interconversion of sterol derivatives may be one aspect of how plants respond to low temperature stress to preserve membrane function. For further studies in this field we isolated the UDPG-SGTase that glucosylates free sterols to SG.

MATERIALS AND METHODS

Plant Material

Oat seeds (*Avena sativa* L. cv Alfred) were germinated on moist vermiculite in the dark for 7 d at 22°C.

Enzyme Assay

The standard assay mixture contained, in a total volume of 50 μL: 60 mM Tris-HCl, pH 8.5, 0.2% Triton X-100, 1 mM

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Abbreviations: ASG, acylated steryl glucosides; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate; K_i, inhibitor constant; OTG, *n*-octyl-β-D-thioglucopyranoside; SG, steryl glucosides; UDPG-SGTase, UDP-Glc:sterol β-D-glucosyltransferase.

β-sitosterol, 2.5 mM DTT, 1 ng to 10 μg of protein, and 72 μM UDP-[U-^{14}C]Glc (specific activity 257 MBq mmol^{-1}, 50,000 dpm). After incubating for 10 min at 30°C, the reaction was terminated by the addition of 0.4 mL of 0.45% NaCl solution and 1.6 mL of ethyl acetate. The mixture was vortexed and phases were separated by centrifugation (3 min at 1000*g*). The radioactivity in the upper organic phase was counted in a liquid scintillation spectrometer. The incorporation of ^{14}C into the organic phase was linear for 10 to 15 min.

Identification of Reaction Product

The lipids extracted from an enzyme assay were separated by TLC with chloroform:methanol (85:15, v/v). The radioactivity on the silica gel plate was measured by a Berthold TLC scanner. SG was identified by co-chromatography with an authentic sample. The identification was verified by submitting the reaction product to alkaline saponification and acidic hydrolysis. It resisted the former treatment, but radioactivity was found in the aqueous phase only after the latter. When the enzyme assay was performed with labeled cholesterol and unlabeled UDP-Glc (see Table II), acidic cleavage of the reaction product resulted in the release of labeled cholesterol.

Preparation of Microsomes

All operations took place in a cold room at 4°C. Two kilograms of etiolated oat shoots were cut 2 cm above the caryopses and then homogenized with 4 L of buffer (0.1 M Tris-HCl, pH 8.0, 150 mM sorbitol, 150 mM NaCl, 10 mM DTT) in a Waring Blendor for 20 s. The homogenate was filtered through two layers of nylon gauze (150-μm mesh size) and centrifuged for 5 min at 1,800*g* in 1-L beakers. The supernatant was centrifuged at 33,000*g* for 30 min and the resulting pellets were resuspended in 100 mL of buffer (50 mM Tris-HCl, pH 8.0, 15% glycerol, 5 mM DTT). After adjusting the crude microsomal fraction to a protein concentration of 10 mg mL^{-1}, it was stored at −20°C. Typically, 2 kg of fresh oat seedlings yielded about 165 mL of microsomal suspension.

Enzyme Purification

All purification steps were carried out at 4°C if not otherwise stated. Buffers: A, 50 mM Tris-HCl (pH 8.0), 40 mM *n*-octyl-β-D-thioglucopyranoside, 15% glycerol, 1 mM DTT; B, 50 mM Mops-NaOH (pH 7.0), 10 mM CHAPS, 15% glycerol, 2.5 mM DTT; C, buffer B supplemented with 50 mM NaCl; D, buffer B supplemented with 125 mM NaCl.

Step I: Solubilization

Microsomes from 4 kg of oat seedlings (333 mL) were mixed with buffer A to give a total volume of 1 L, containing 50 mM Tris-HCl, pH 8.0, 27 mM OTG, 15% glycerol, 2.5 mM DTT, and 3.3 mg protein mL^{-1}. After gentle stirring for 30 min the mixture was centrifuged at 100,000*g* for 1 h and the supernatant was used for further purification.

Step II: Extraction with Diethyl Ether

The supernatant was thoroughly mixed with an equal volume of ice-cold diethyl ether in stoppered glass centrifuge tubes. After centrifugation at 5000*g* for 30 min at 12°C, the major part of the protein was precipitated, forming a layer at the aqueous:organic interface. This protein layer was moved aside with a Pasteur pipette before drawing off the upper organic phases, which were only slightly contaminated by precipitated protein. The combined organic phases were centrifuged two times at 5000*g* for 15 min at 12°C to sediment precipitated protein. Subsequently, diethyl ether was removed from the supernatant by rotary evaporation. The resulting residue was resuspended in buffer B to resolubilize the UDPG-SGTase.

Step III: Ion-Exchange Chromatography

The resolubilized diethyl ether extract was adjusted to 50 mM NaCl and applied to a Q-Sepharose Fast Flow column (Pharmacia, Freiburg, Germany; 2.5 × 6 cm) that had been equilibrated with buffer C. After washing with 100 mL of the same buffer, proteins were eluted with a linear gradient of NaCl (100 mL, 50–600 mM, 1.5 mL min^{-1} flow rate, 5-mL fraction size).

Step IV: Affinity Chromatography

The fractions from ion-exchange chromatography containing enzyme activity were pooled, adjusted to 125 mM NaCl with buffer B, and loaded onto a HiTrap Blue column (a prepacked Cibacron Blue Sepharose column, Pharmacia; 1.7 × 2.4 cm, equilibrated with buffer D). Most proteins and all carotenoids passed through the column. After the column was washed with 15 mL of buffer D, the UDPG-SGTase was eluted with a linear gradient of NaCl (20 mL, 0.125–1.0 M, 1.5 mL min^{-1} flow rate, 2-mL fraction size).

SDS-PAGE, Electroblotting of Proteins, and Western Blot Analysis

SDS-PAGE was performed in polyacrylamide gels (10 or 12.5%, 50 × 80 × 0.75 mm^3) according to Laemmli (1970). Gels were stained with Coomassie brilliant blue G-250. Alternatively, proteins were electroblotted onto Immobilon-P polyvinylidene difluoride membranes (Millipore, Eschborn, Germany) for N-terminal sequencing and onto nitrocellulose membranes for western blot analysis. Anti-56-kD-protein antibodies in a rabbit antiserum were detected by western immunoblot assay using peroxidase-conjugated goat anti-rabbit IgG with 4-chloro-1-naphthol as substrate.

Production of Antibodies and IgG Purification

Immunization was performed by Eurogentec (Seraing, Belgium). Polyclonal antibodies were raised in one rabbit by multiple injections of the 56-kD protein (total 100 μg) obtained by SDS-PAGE from purified UDPG-SGTase fractions. Immunoglobulins were purified from preimmune and immune serum by chromatography on HiTrap protein A Sepharose columns (Pharmacia).

Binding of Native UDPG-SGTase by Purified Antiserum-IgG

Solubilized microsomal protein or fractions with purified enzyme were incubated for 30 min at 23°C with different amounts of purified immune and preimmune serum IgG. After addition of Protein A-Sepharose, the mixture was incubated for another 30 min. The Protein A-Sepharose-IgG-antigen complexes were sedimented by short centrifugation at 500*g* and UDPG-SGTase activity was determined in the supernatant.

Studies with Purified UDPG-SGTase

For inhibition studies the UDPG-SGTase assay was supplemented with various nucleotides or sugar nucleotides in concentrations between 50 μM and 1.0 mM. For substrate specificity studies various sugar nucleotides were substituted for UDP-Glc and different sterol acceptors were substituted for β-sitosterol.

Protein Determination

Protein in purified fractions was estimated by comparison with known amounts of serum albumin on Coomassie-stained gels or determined according to Bramhall et al. (1969).

RESULTS AND DISCUSSION

Purification of UDPG-SGTase

The purification of integral membrane proteins requires their solubilization by detergents (Helenius and Simons, 1975; Hjelmeland and Chrambach, 1984) or organic solvents (Boyan and Clement-Cormier, 1984; Van Renswoude and Kempf, 1984; Findlay, 1987). We made use of both techniques by combining detergent solubilization with subsequent extraction of enzyme activity into diethyl ether.

The purification of UDPG-SGTase started with the preparation of microsomal membranes from etiolated oat shoots. These membranes were solubilized with the nonionic detergent OTG. Subsequent centrifugation resulted in a yellow-colored supernatant fraction containing 96% of UDPG-SGTase activity and the major part of carotenoids. This supernatant was thoroughly mixed with diethyl ether and subjected to phase separation by centrifugation (Fig. 1). This harsh treatment resulted in an almost colorless subphase that was capped by a layer of denatured and precipitated protein. The ethereal phase above the protein layer was divided into a small green band below a yellow solution of larger volume. The whole ethereal phase was drawn off and found to contain about 20% of enzymic activity but only 0.7% of protein compared to the solubilized supernatant. This represents a 30-fold enrichment of UDPG-SGTase, and, therefore, the low yield of activity was accepted. Additional activity could not be detected in either the precipitated proteins or the aqueous subphase.

The diethyl ether was removed from the combined organic phases by rotary evaporation and the residue was redissolved in a detergent-containing buffer. Apart from the enrichment of enzymic activity, the extraction step reduced the volume

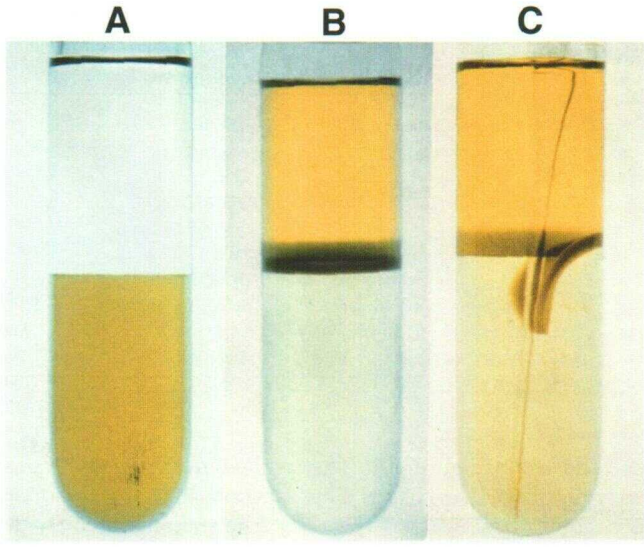

Figure 1. Extraction of the UDPG-SGTase with diethyl ether. A, The solubilized microsomal fraction (yellow due to carotenoids) and an equal volume of diethyl ether were placed in a glass centrifuge tube with stopper. B, Thorough mixing and centrifugation resulted in two ethereal phases (yellow and green). The major part of the protein was precipitated, forming a layer at the aqueous-organic interface. UDPG-SGTase activity was found exclusively in the ethereal phases and accounted for approximately 20% of the activity solubilized from microsomes. The aqueous phase and the precipitated proteins were inactive. C, The same as B but the precipitated protein was moved aside by a Pasteur pipette to ease the removal of the upper organic phases. Protein from the combined ethereal phases was used for further purification of the enzyme.

and the protein quantity from 1 L of solubilized fraction with 1700 mg of protein to 141 mL of resolubilized ether extract with 10.6 mg of protein. This fraction was suitable for subsequent column chromatography.

When the ether extraction was performed with unsolubilized microsomal membranes or with microsomes that had been solubilized with detergents other than OTG (such as Triton X-100, CHAPS, cholate, octyl glucoside), enzyme activity could not be recovered from the organic phase. In this context it may be of relevance that among these detergents only OTG is easily soluble in diethyl ether. Substitution of other organic solvents for diethyl ether resulted in lower yields of enzyme activity extracted into the organic phase. This unique combination of solubilization plus extraction may be useful for purification of other membrane-bound proteins.

The resolubilized ether extract was subjected to column chromatography using two different matrices in succession. Ion-exchange chromatography on Q-Sepharose FF resulted in a 4.7-fold enrichment of enzyme activity with a yield of 54%. The final HiTrap Blue column did not bind the carotenoids and the major part of the proteins, thus achieving a 19-fold increase of specific activity with a yield of 45%. Based on the homogenate, the overall purification was about 12,500-fold with a yield of 2.7%. The results of a representative purification are summarized in Table I.

The protein patterns at various steps of this purification

Table I. *Purification of UDPG-SGTase from etiolated oat seedlings*
Typical data for 4 kg fresh weight.

Purification Step	Volume	Total Protein	Specific Activity	Yield	Purification Factor
	mL	*mg*	*nmol SG min^{-1} mg^{-1}*	%	
Homogenate	10,000	13,000	1.1	100	1.0
Microsomal fraction	333	3,300	2.7	60	2.4
Solubilized microsomal fraction	950	1,615	5.0	58	4.5
Resolubilized diethyl ether extract	141	10.6	155	11	139
Q-Sepharose FF eluate	36	1.2	731	5.9	656
HiTrap Blue Sepharose eluate	14	0.028	14,927	2.7	12,500

are shown in Figure 2A. The protein dominating in the enzymically active HiTrap Blue fractions had an apparent molecular mass of 56 kD (Fig. 2C). The staining intensity of this band correlated with UDPG-SGTase activity in the respective fractions, and therefore we assume that this 56-kD protein represents the UDPG-SGTase. In fractions eluting later from the HiTrap Blue column, the 56-kD protein was contaminated by a protein of slightly lower molecular mass, but the elution pattern of this band did not parallel enzymic activity (data not shown).

The blotted 56-kD protein was submitted to N-terminal amino acid sequencing, which yielded a series of unequivocal signals at each position analyzed, including the first one. The resulting sequence of 14 amino acids (data not shown), which was reproduced in a second, completely independent preparation and analysis, will be useful in future experiments on the cloning of a corresponding cDNA. On the other hand, the protein of slightly lower molecular mass (see above) was resistant to N-terminal sequencing.

In addition, the 56-kD protein was used to raise polyclonal antibodies. Western blot analysis (Fig. 2B) gave a strong signal with the antiserum, whereas preimmune serum was inactive (data not shown). The faint staining above the 56-kD protein was attributed to unspecific binding, which was also observed when only buffer was subjected to SDS-PAGE, electroblotting, and immunostaining. The signal below the 56-kD protein may be due to the smaller protein mentioned above contaminating the bands excised from the gel for immunization. Since the western blot shown was not carried out under conditions of proportionality between color development and protein content, the intensity of the signal of the lower band may greatly exaggerate the actual content of this contamination.

Enzymic activity was not inhibited by purified IgG that was isolated from the antiserum. This is not unusual for antibodies directed against enzymes that were denatured by SDS prior to immunization (Werck-Reichhart et al., 1993). On the other hand, the purified IgG was able to bind to the native UDPG-SGTase both in purified fractions (Fig. 3) and in solubilized microsomes (data not shown). This was shown by low-speed sedimentation of the enzymic activity caused

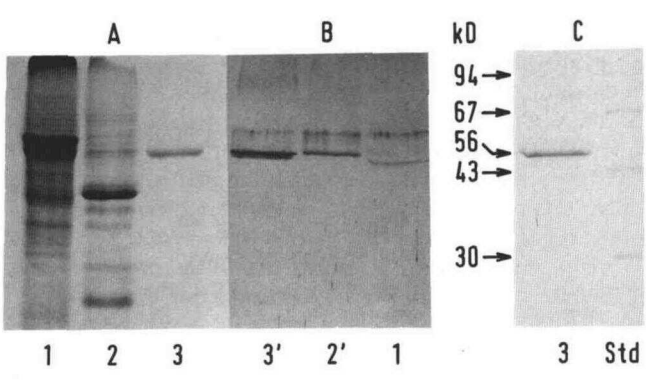

Figure 2. A, SDS-PAGE at various steps of UDPG-SGTase purification. B, Western immunoblot corresponding to A, using the anti-56-kD-protein antiserum. Lanes 1, Solubilized microsomal fraction (30 μg); lanes 2 and 2′, Q-Sepharose FF eluate (10 and 1 μg, respectively); lanes 3 and 3′, HiTrap Blue Sepharose eluate (1 and 0.1 μg, respectively). Relative enzymic activities applied to the various lanes were 1 (lanes 1), 40 (lane 2), 88 (lane 3), 4 (lane 2′), and 8.8 (lane 3′). C, SDS gel for determination of mol wt. The major protein in lanes 3 and 3′ had a mol wt of 56,000. The SDS gels were stained with Coomassie blue.

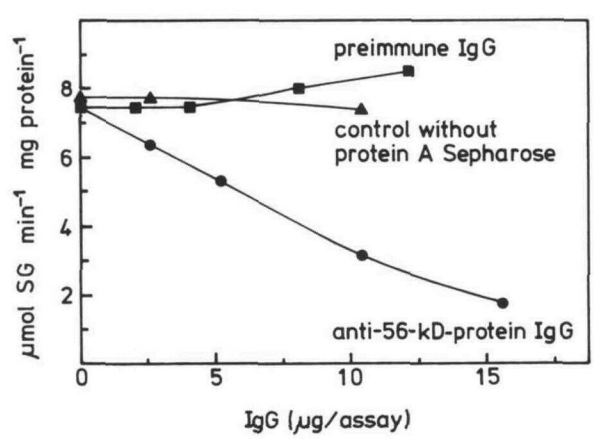

Figure 3. Binding of native UDPG-SGTase by purified IgG. Purified UDPG-SGTase (7.5 ng of protein) was incubated with various amounts of IgG isolated from preimmune serum and anti-56-kD-protein antiserum. After addition of Protein A-Sepharose (or buffer, as a control), the mixture was incubated for 30 min. Antibody-antigen complexes were sedimented by a short centrifugation, and UDPG-SGTase activity in the supernatant was determined by the standard assay procedure.

Table II. *Substrate specificity of purified UDPG-SGTase*

Reaction mixtures contained 5 ng of protein of purified UDPG-SGTase preparations, 0.2% Triton X-100, and 60 mM Tris-HCl, pH 8.5. Incorporation with UDP-Glc and cholesterol was taken as 100%.

Donor	Concentration	Acceptor	Concentration	Incorporation into Radioactive Product
				%
UDP-[U-^{14}C]Glc	72 μM	β-Sitosterol	250 μM	100
UDP-[U-^{14}C]Glc	72 μM	Cholesterol	250 μM	100
UDP-[U-^{14}C]Glc	72 μM	Ergosterol	250 μM	69
UDP-[U-^{14}C]Glc	72 μM	Stigmasterol	250 μM	63
UDP-[U-^{14}C]Glc	72 μM	Ceramide	250 μM	0
UDP-Glc	1 mM	[4-^{14}C]cholesterol	65 μM	100
UMP-Glc	1 mM	[4-^{14}C]cholesterol	65 μM	0
UDP-Man	1 mM	[4-^{14}C]cholesterol	65 μM	0
UDP-Gal	1 mM	[4-^{14}C]cholesterol	65 μM	0

by binding of the antibody-enzyme complexes to Sepharose-coupled protein A.

Properties of Purified UDPG-SGTase

In the standard assay purified UDPG-SGTase exhibited a broad pH optimum between 8.0 and 9.0, confirming previous results with a partially purified enzyme (Forsee et al., 1974). UDPG-SGTase glucosylated common plant sterols such as β-sitosterol, stigmasterol, and cholesterol as well as ergosterol, which is abundant in yeasts (Table II). Ceramide was not accepted, which excludes the possibility of co-purifying UDP-Glc:ceramide glucosyltransferase. These results are in agreement with those of previous investigations of substrate specificity of UDPG-SGTase (Wojciechowski and Van Uon, 1975; Yoshikawa and Furuya, 1979; Ullmann et al., 1984; Zimowski, 1992). The K_m for β-sitosterol determined under standard assay conditions varied between 240 and 540 μM. We attribute this variation to difficulties in the reproduction of identical substrate dispersions. Enzyme preparations from maize seedlings had K_m values in the range of 140 to 800 μM for the same acceptor under different conditions (Ullmann et al., 1987). The glucosylation of ergosterol may be of importance for future experiments with yeasts. These organisms, synthesizing sterols but not steryl glucosides, could be suitable for transformation with plant cDNAs. Expression of UDPG-SGTase would provide the possibility of studying the function of SG and its correlation with stress resistance in these organisms.

UDPG-SGTase could utilize UDP-Glc, but not UMP-Glc, UDP-Gal, or UDP-Man as glycosyl donors. In these experiments unlabeled sugar nucleotides and labeled cholesterol were used as substrates (Table II). Sugar nucleotides with a base other than uracil (e.g. TDP-, CDP-, or ADP-Glc) were not tested in these experiments, but we expect that they will hardly function as glycosyl donors, since they displayed very low affinity to UDPG-SGTase activity in inhibition experiments (see below, Table III). The apparent K_m for UDP-Glc (34 μM, Fig. 4) was similar to values between 23 and 40 μM determined previously in different plants (Péaud-Lenoël and Axelos, 1971; Forsee et al., 1974; Ullmann et al., 1987).

Table III. *Inhibition of purified UDPG-SGTase activity by nucleotides, sugar nucleotides, and some derivatives*

Standard enzyme assays with 5 ng of protein of purified UDPG-SGTase preparations were supplemented with various possible inhibitors in concentrations between 50 μM and 1.0 mM.

Compound	Inhibitory Effect
AMP, ADP, ATP CMP, CDP, CTP, CDP-Glc GDP, GDP-Glc TDP, TDP-Glc, UMP, UDP-2′,3′-dialdehyde, UDP-Gal, UDP-GlcNAc	Very weak inhibition, less than 25% at concentrations of 1.0 mM
UTP, desoxyUDP, UDP-xylopyranose, UMP-Glc	Weak inhibition, between 25 and 50% at concentrations of 1.0 mM
UDP, UDP-Man, 4-S-UDP	Strong inhibition, more than 80% at concentrations of 1.0 mM. Inhibitor concentration inducing 50% inhibition: 40 μM UDP-Man, 70 μM UDP, 160 μM 4-S-UDP

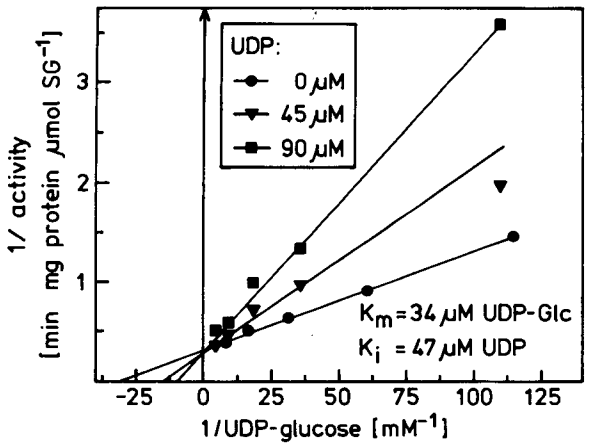

Figure 4. Inhibition of purified UDPG-SGTase by UDP. Standard assays were performed with 25 ng of protein of purified UDPG-SGTase preparations and various concentrations of UDP and UDP-Glc at a constant concentration of 1 mM β-sitosterol.

We also determined the inhibitory effects of various nucleotides and sugar nucleotides on SG synthesis, since the results could be interesting for two reasons. First, they could help to characterize the binding site for UDP-Glc. Second, they could be useful in the selection of reagents for photoaffinity labeling and media for affinity chromatography, which was particularly relevant at the beginning of our studies. Among the compounds tested (Table III) only UDP, UDP-Man, and 4-thio-UDP showed a strong inhibitory effect. The K_i for UDP was found to be 47 μM, which agrees with the value of 50 μM determined previously (Ullmann et al., 1993). UDP proved to be a competitive inhibitor with regard to UDP-Glc (Fig. 4). 4-Thio-UDP is easily photooxidized to reactive intermediates that can add to protein-bound nucleophiles (Hanna, 1989). Thus, labeled derivatives of this compound, such as 4-thio-UDP-[^{14}C]Glc, could represent suitable reagents for photoaffinity labeling.

Compounds differing only slightly from UDP or UDP-Glc did not inhibit UDPG-SGTase (Table III), except for 4-thio-UDP and UDP-Man. With reference to these results, it is not surprising that the enzyme did not bind to UDP-hexanolamine- and UDP-glucuronic acid-agarose. UDPG-SGTase was not inhibited by TDP and TDP-Glc, which differ from UDP and UDP-Glc only by a methyl group at the C5 position of the base.

For this reason, we did not carry out experiments with labeled 5-azido-UDP-Glc, which has been used for photoaffinity labeling of several other glycosyltransferases (Drake and Elbein, 1992). It also seemed unlikely that UDPG-SGTase would display a specific binding to the 5-Hg-UDP-thiopropyl group of an affinity medium in which this ligand is coupled via C5 of the base to a Sepharose matrix. In contrast to this expectation, the retention of UDPG-SGTase by this matrix (whether by specific affinity or unspecific binding) was used for an efficient purification step resulting in a 20-fold enrichment of activity (Ullmann et al., 1993).

In summary, we isolated the UDPG-SGTase, raised antibodies against the purified 56-kD protein, and obtained a partial N-terminal amino acid sequence. With these tools in hand, we have started experiments on the cloning of a cDNA coding for UDPG-SGTase, which will enable a new approach toward the elucidation of SG functions.

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