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Uridine Diphosphate Glucose–Sterol Glucosyltransferase and Nucleoside Diphosphatase Activities in Etiolated Pea Seedlings

By MICHAEL J. STAVER, KEVIN GLICK and DEREK J. BAISTED
Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

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1. UDP-glucose–sterol glucosyltransferase and nucleoside diphosphatases were isolated in a particulate fraction from 7-day-old etiolated pea seedlings. The glucosyltransferase and UDPase (uridine diphosphatase) are stimulated by Ca^{2+} cation, less so by Mg^{2+} cation, and inhibited by Zn^{2+}. 2. Each activity has a pH optimum near 8. 3. The glucosyltransferase is specific for UDP-glucose as the glucosyl donor and is inhibited by UDP. Partial recovery from UDP inhibition is effected by preincubation of the enzyme. 4. Freeze–thaw treatment and subsequent sucrose-density-gradient centrifugation of the particulate fraction shows the glucosyltransferase to be widely distributed among cell fractions but to be most active in particles with a density of 1.15g/ml. UDPase is most active in particulate material with a density of over 1.18g/ml but an activity peak also appears at 1.15g/ml. Of several nucleoside diphosphatase activities, UDPase activity is most enhanced by the freeze–thaw and sucrose-density-gradient-fractionation procedures. 5. Detergent treatment with 0.1% sodium deoxycholate allows the partial solubilization of the glucosyltransferase and UDPase. The two activities are similarly distributed between pellet and supernatant after high-speed centrifugation for two different time intervals. 6. A role for UDPase in the functioning of glucosylation reactions is discussed.

The enzymic synthesis of sterol 3β-D-glucosides has been demonstrated with preparations from a variety of plants (Hou *et al.*, 1968; Kauss, 1968; Ongun & Mudd, 1970; Laine & Elbein, 1971; Bush & Grunwald, 1974; Forsee *et al.*, 1974; Wojciechowski *et al.*, 1976; Lezica *et al.*, 1976). The intracellular location of the activity is not known with any certainty. In some cases it appears to be associated with a microsomal fraction (Péaud-Lenoël & Axelos, 1971), but it has also been located in a high-speed supernatant from carrot root (Eichenberger & Grob, 1970; Eichenberger & Siegrist, 1975), a mitochondrial fraction from pea-seedling roots (Ongun & Mudd, 1970) and a number of cellular fractions that may be Golgi, plasma and tonoplast membrane fragments as well as mitochondria from pea-seedling-axis tissue (Fang & Baisted, 1976).

A histochemical study of onion root tip (Goff, 1973) has shown nucleoside diphosphatase activity to be associated with a number of cellular membranes, including endoplasmic reticulum, Golgi, plasma membrane and tonoplast. These membranes also showed high labelling after treatment of the plant tissue with [^{3}H]glucose, from which it was inferred that glycosyltransferase and nucleoside diphosphatase activities are related. It has been suggested that the role of nucleoside diphosphatase in polysaccharide synthesis is to remove UDP or GDP formed by glycosyl- or other transferases, thus driving the reaction towards synthesis (Ernster & Jones, 1962; Novikoff *et al.*, 1971).

In the present paper we examine the properties of a UDP-glucose–sterol glucosyltransferase and of several nucleoside diphosphatases, especially UDPase. In several respects the two enzymes have biochemical properties consistent with UDPase providing the means of enhancing the glucosylation reaction.

Abbreviations used: UDPase, uridine diphosphatase; CDPase, cytidine diphosphatase; IDPase, inosine diphosphatase; ADPase, adenosine diphosphatase; GDPase, guanosine diphosphatase.

Experimental

Materials

Pisum sativum L. cultivar Alaska (W. Atlee Burpee Co., Riverside, CA, U.S.A.) was used. UDP-[U-^{14}C]glucose (155μCi/μmol) was from International Chemical and Nuclear, Irvine, CA, U.S.A. Unlabelled nucleoside diphosphoglucoses were from Calbiochem, La Jolla, CA, U.S.A., and Sigma Chemical Co., St. Louis, MO, U.S.A. Cholesteryl glucoside was a generous gift from Dr. J. J. Schneider, Department of Medicine, Jefferson Medical College, Philadelphia, PA, U.S.A. Precoated t.l.c. sheets were obtained from Sargent and Co., Los Angeles, CA, U.S.A. Crystalline bovine serum albumin was obtained from Calbiochem. Miracloth is a product of Chicopee Mills, New York, NY, U.S.A.

Methods

Seed germination. Seeds were germinated as described by Fang & Baisted (1976).

Protein concentration. This was determined by a modification of the Lowry procedure (Potty, 1969) with bovine serum albumin as standard.

Preparation of the UDP-glucose–sterol glucosyltransferase. The preparation and assay of this enzyme was conducted essentially as described previously by Fang & Baisted (1976). The axis tissue of 7-day-old pea seedlings was homogenized for 10s in a Waring blender in ice-cold 50mM-Tris/HCl, buffer, pH8, containing 0.4M-sucrose. The buffer contained 2mM-EDTA and 10mM-2-mercaptoethanol for the experiments involving detergent solubilization of the enzyme activities. The volume of buffer was 1.5 times the seedling tissue weight. After filtration through one layer of Miracloth, the crude homogenate was successively centrifuged at 1000*g* ($r_{av.}$ 9.0cm) for 5min, 10000*g* ($r_{av.}$ 9.0cm) for 15min and 19000*g* ($r_{av.}$ 9.0cm) for 20min. The 10000*g*/19000*g* pellet, designated the 19000*g* pellet, was washed once by suspension in the homogenizing buffer and centrifugation again at 19000*g* ($r_{av.}$ 9.0cm) for 20min. The washed pellet was resuspended in 50mM-Tris/HCl buffer, pH8, and adjusted to a protein concentration of approx. 1–2mg/ml. All operations were conducted at 2–4°C. The enzyme was frozen in liquid N_2 in volumes of 3–5ml and stored at −20°C. For the detergent solubilization studies and those examining the influence of nucleoside mono- and di-phosphates, the pellet was resuspended in the buffer containing EDTA and 2-mercaptoethanol.

UDP-glucose–sterol glucosyltransferase assay. Reaction mixtures contained 10μmol of $CaCl_2$, 0.5μmol of UDP-[U-^{14}C]glucose (0.05μCi), 0.5mg of protein and 35μmol of Tris/HCl, pH8, in a total volume of 0.75ml. For the effect of adducts on the assay, the appropriate adduct replaced Ca^{2+} cation in the mixture. For the determination of pH optima, equal volumes of the 19000*g* preparation were centrifuged at 78500*g* ($r_{av.}$ 7.8cm) for 15min and the pellets resuspended in 50mM-Tris/HCl of the appropriate pH. The pH optima and the influence of nucleoside diphosphates on the transferase activity were determined in the presence of 13mM-Ca^{2+} cation.

For the time course of recovery of transferase activity after UDP inhibition, the enzyme was preincubated for times ranging from 5 to 30min with 6μmol of UDP and the remaining components of the reaction mixture except UDP-glucose. Control experiments were run in which UDP was omitted from the reaction mixture. The preincubated mixture was then assayed by the addition of substrate. The reactions were run at 28°C and stopped after 15min by agitation with 1.5ml of chloroform/methanol (2:1, v/v). The phases were separated by low-speed centrifugation, the organic layer removed and the aqueous phase extracted twice more with a a total volume of 3.0ml of chloroform. The ^{14}C content was measured as described previously by Fang & Baisted (1976).

Nucleoside diphosphatase assays. Reaction mixtures contained 3mM-nucleoside diphosphate, 4mM-Mg^{2+} cation, 20μg of protein and 24μmol of Tris/HCl buffer, pH8, in a total volume of 0.5ml. Reactions were run at 28°C for 3–5min and were stopped by the addition of 0.5ml of 20% (v/v) trichloroacetic acid. Then 1ml of a solution containing 1mg of bovine serum albumin was added and the precipitated protein was sedimented by low-speed centrifugation. Supernatant (1ml) was used for phosphorus determination by a modification of the method of Fiske & SubbaRow (Leloir & Cardini, 1957). Attempts to measure nucleoside 5′-monophosphatase were made in the same way, substituting 3mM-nucleoside monophosphate for the diphosphate.

Influence of unlabelled ADP-glucose, GDP-glucose, CDP-glucose and UDP-glucose on ^{14}C incorporation from UDP-[U-^{14}C]glucose with the 19000g pellet. The sterol glycosyltransferase assay was carried out as described above, but reaction mixtures contained in addition 0.5, 1.0, 2.0 or 3.0μmol each of the appropriate nucleoside diphosphoglucose. Sterol glucoside was isolated as described previously (Fang & Baisted, 1976).

Freeze–thawing and sucrose-density-gradient centrifugation of the 19000g pellet. The frozen 19000*g* pellet was slowly thawed at room temperature (21°C). Several ice-cold samples were centrifuged at 78500*g* ($r_{av.}$ 7.8cm) for 20min and the pellets combined and resuspended in 50mM-Tris/HCl buffer, pH8, to give protein concentrations of 4mg/ml. A portion (2ml) of this freeze–thawed suspension was layered on a 35ml discontinuous gradient of sucrose. The gradient consisted of a 7ml cushion of 1.4M-sucrose and 7ml each of 1.2M-, 1.0M-, 0.8M- and 0.5M-sucrose in 50mM-Tris/HCl, pH8. Two such tubes were centrifuged in a Sorval OTD-2 ultracentrifuge with a Spinco SW27 rotor at 76000*g* ($r_{av.}$ 11.8cm) for 2h at 2°C. Protein bands from the interfaces were removed with a Pasteur pipette, diluted with sucrose-free 50mM-Tris/HCl, pH8, and centrifuged at 76000*g* ($r_{av.}$ 11.8cm) for 30min. The pellets were resuspended in the same buffer and assayed for the glucosyltransferase and UDPase.

Effect of detergents on glucosyltransferase and UDPase activities. For the glucosyltransferase, solutions of deoxycholate, Triton X-100 and cetyltrimethylammonium bromide were added to separate tubes containing 0.44mg of protein of the 19000*g*-pellet fraction suspended in 50mM-Tris/HCl buffer, pH8, containing 2mM-EDTA and 10mM-2-mercapto-

ethanol. The final concentrations of detergent ranged from 0.1 to 1.0% in a volume of 0.71 ml of 50mM-Tris/HCl, pH8. The mixtures were cooled in ice for 5min and then the transferase activity was assayed as described above by the addition of Mg^{2+} cation (10μmol) and UDP-[U-^{14}C]glucose (0.5μmol, 0.05μCi) to give a final reaction volume of 0.75ml.

For the UDPase assay, the detergents were added in the same concentration range to separate tubes containing 18μg of protein in a volume of 480μl of 50mM-Tris/HCl, pH8. After 5min on ice, the UDPase was assayed as described above by the addition of Mg^{2+} cation (2.0μmol) and UDP (1.5μmol) to give a final volume of 0.5ml.

Distribution of soluble and particulate glucosyltransferase and UDPase in the deoxycholate-treated 19000g fraction. To the 19000*g* fraction in the Tris buffer containing EDTA and 2-mercaptoethanol was added deoxycholate to give a final concentration of 0.1% (w/v) in detergent. The mixture was cooled on ice for 5min at 0°C and then divided into two portions. One portion was centrifuged at 78500*g* ($r_{av.}$ 7.8cm) for 5min and the other at the same speed for 60min. The pellets in each case were resuspended in the suspending buffer containing 0.1% deoxycholate. The pellets and supernatant from each centrifugation were assayed for glucosyltransferase and UDPase activities in the presence of 13mM-Mg^{2+}.

Results and Discussion

Glucosyltransferase and UDPase activities are increased by Ca^{2+} and Mg^{2+} cations. The magnitude of the activation varies among preparations, but in all preparations the stimulation of both activities by Ca^{2+} is greater than by Mg^{2+}. An interesting difference between the two activities is in their response to the chelating agents EDTA and EGTA. The glucosyltransferase retains 30% of its original activity in the presence of 13mM-EDTA but the UDPase is completely inhibited. The residual transferase activity may reflect the presence of an enzyme that is not dependent on Mg^{2+} cation or is located in an environment not accessible to EDTA. Evidently there is an absolute requirement for Mg^{2+} cation for UDPase activity. In the presence of the powerful Ca^{2+}-chelating agent EGTA at a concentration of 13mM, both activities suffer approx. 50% inhibition. For UDPase, the residual activity presumably results from the presence of residual Mg^{2+} cation.

The response of each activity to a range of concentrations of Ca^{2+} and Mg^{2+} is shown in Fig. 1. The glucosyltransferase (Fig. 1*a*) is stimulated over a broad range of cation concentrations. A 1:1 mixture of Ca^{2+} and Mg^{2+}, each at a concentration of 15mM, gives a transferase activity midway between that for each cation, showing that there are not two separate

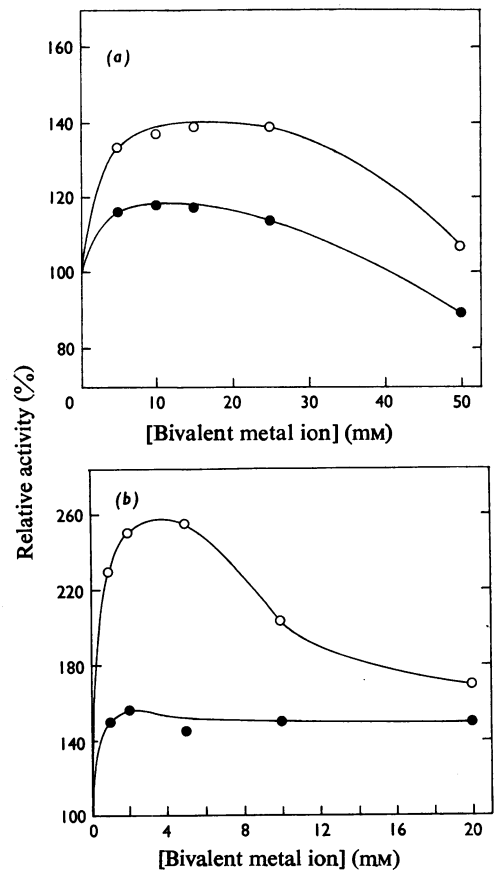

Fig. 1. *Influence of Ca^{2+} and Mg^{2+} on UDP-glucose–sterol glucosyltransferase and UDPase activities*
Glucosyltransferase (*a*) and UDPase (*b*) activities were assayed on the 19000*g* particulate preparation in 50mM-Tris/HCl, pH8, in the presence of either Ca^{2+} (○) or Mg^{2+} (●) as described in the Experimental section. The activity of each enzyme in the absence of metal ion is arbitrarily set at 100%.

activities, one stimulated by Ca^{2+} and the other by Mg^{2+}. The response of UDPase is shown in Fig. 1(*b*).

Both activities have broad pH optima near 8. The stimulation by the same cations and similar pH–activity profiles might suggest that the two enzymes function in a similar environment or compartment of the cell.

A possible role for UDPase is evident on examination of Fig. 2. In the presence of a range of concentrations of ADP and GDP the transferase suffers no inhibition. However, with UDP there is a loss of activity of approx. 60% of the control value. The product of UDP hydrolysis, UMP, is also inhibitory, but to a much smaller extent. P_i has no

effect on the activity (results not shown). The residual transferase activity in the presence of UDP approximates to that found in the presence of EDTA and may represent membrane-bound transferase that is similarly as inaccessible to UDP as it is to EDTA. As the transferase and UDPase are present in the same particulate preparation, it would be expected that the transferase should recover from UDP inhibition as the UDPase hydrolyses the inhibitor. Fig. 3 shows partial recovery of activity to a value representing inhibition of the enzyme by UMP, the product of UDP hydrolysis. There is no 5'-nucleotidase activity in this preparation, so that further degradation of UMP cannot occur. Surprisingly, the recovery is not as rapid as would have been expected on the basis of activity of UDPase in this 19000*g* fraction being sufficient to hydrolyse μmol amounts of UDP per min (Table 1).

Further fractionation of the glucosyltransferase was attempted by subjecting the 19000*g* pellet to rapid freezing in liquid N_2, followed by thawing and centrifugation again at 19000*g* ($r_{av.}$ 9.0cm) for 20min. The resuspended thawed pellet was placed on a discontinuous sucrose-density gradient and centrifuged at 78500*g* for 2h. The activity was distributed throughout the more-dense layers with

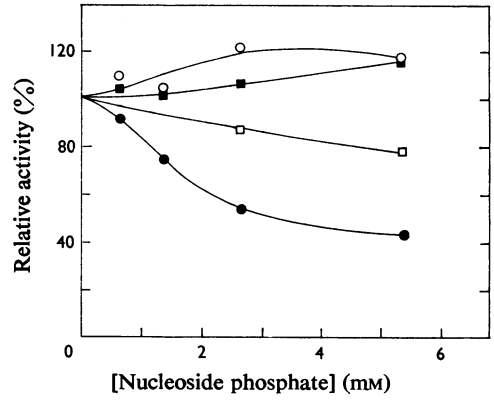

Fig. 2. *Influence of nucleoside phosphates on UDP-glucose–sterol glucosyltransferase activity*
UDP-glucose–sterol glucosyltransferase activity was measured in the 19000*g* particulate fraction in the presence of 13mM-Ca^{2+}. The assay mixtures contained, in addition to the UDP-[U-^{14}C]glucose, increasing concentrations of either unlabelled ADP (○), GDP (■), UDP (●) or UMP (□). The details of the assay are described in the Experimental section. In the absence of nucleoside diphosphate the activity of the transferase is arbitrarily set at 100%.

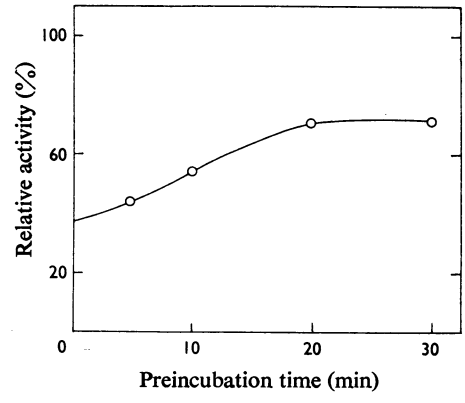

Fig. 3. *Time course of recovery of glucosyltransferase activity from UDP inhibition*
The 19000*g* particulate fraction was prepared in the presence of EDTA and 2-mercaptoethanol. It was preincubated with 13mM-Ca^{2+} and 8mM-UDP at 28°C. Assay of glucosyltransferase activity after preincubation was initiated by the addition of UDP-[U-^{14}C]glucose. Details of the procedure are given in the Experimental section. The activity values are relative to a control in which UDP is absent.

Table 1. *Distribution of UDP-glucose–sterol glucosyltransferase activity after freeze–thawing and density-gradient centrifugation*
Glucosyltransferase activity was measured in the presence of 13mM-Ca^{2+} cation, and UDPase activity in the presence of 4mM-Mg^{2+} cation. Each enzyme was assayed in a freshly prepared 19000*g* pellet and in a thawed sample that had been frozen in liquid N_2 and stored at −20°C for 24h. The activities were again measured in the protein accumulating at the sucrose-density interfaces after subjecting a thawed 19000*g* pellet to the gradient centrifugation. Details of the centrifugation procedure are described in the Experimental section.

	19000*g* pellet	Freeze–thaw pellet	Sucrose-density interfaces (g/ml) 1.00/1.06	1.06/1.11	1.11/1.14	1.14/1.16	1.16/1.18	Sucrose-gradient pellet
Specific activity of glucosyltransferase (nmol/h per mg of protein)	35	58	—	—	61	94	77	30
UDPase activity (μmol/min per mg of protein)	2.2	2.5	—	—	1.3	3.6	3.3	4.4

Table 2. *Relative nucleoside diphosphatase activities of samples in Table* 1

Nucleoside diphosphatase activities were measured in the presence of 4mM-Mg^{2+} cation and 50mM-Tris/HCl, pH8, in the fresh 19000*g* pellet, the freeze–thawed 19000*g* pellet, and the protein accumulating at the 1.14/1.16 (g/ml) interface of the sucrose-gradient centrifugation shown in Table 1. The five nucleoside diphosphate substrates were present at concentrations of 3mM in the reaction volume. Details of the assay are described in the Experimental section.

	19000*g* pellet	Freeze–thawed pellet	Fraction of density 1.15g/ml
UDPase	1.00	1.00	1.00
CDPase	1.52	0.94	0.79
ADPase	1.64	1.43	1.38
GDPase	1.63	1.51	1.08
IDPase	1.24	1.10	0.95

some concentration in particles with a density of 1.15g/ml (Table 1). Particles with a density of 1.15g/ml have been reported to be a denser class of Golgi-apparatus membrane (Ray *et al.*, 1969; Shore & Maclachlan, 1975; Leonard & VanDerWoude, 1976).

Nucleoside diphosphatase activity, represented by UDPase, was also measured on the 19000*g* pellet, the pellet obtained from freeze–thaw treatment and the density-gradient fractions (Table 1). The distribution of UDPase activity does not parallel the glucosyltransferase activity distribution. UDPase is most active in the pellet (density over 1.18g/ml) from the gradient, but some concentration of activity occurs in a fraction with density 1.15g/ml. Several other nucleoside diphosphatases were also measured in this same fraction. The results are shown in Table 2. It is evident that UDPase is the only activity that increases in specific activity. All the other nucleoside diphosphatases suffer losses in activity ranging from approx. 50% for CDPase to 16% for ADPase. Interestingly, ADPase and GDPase are more active than the UDPase both in the crude 19000*g* pellet and also in the gradient-centrifugation fraction.

As the ADPase and GDPase were so active and and their location in the same membrane fraction may be related to glycosylation reactions involving ADP-glycose and GDP-glycose, we investigated the possibility that sterol glucosyltransferase might utilize ADP-glucose and/or GDP-glucose in addition to UDP-glucose as a sugar donor. The experiments were conducted with the 19000*g*-pellet fraction by using ^{14}C-labelled UDP-glucose in the presence of increasing amounts of unlabelled ADP-glucose, GDP-glucose or CDP-glucose. The results are shown in Fig. 4. The addition of unlabelled UDP-glucose served as a control and showed the expected decline in incorporation of ^{14}C from labelled UDP-glucose

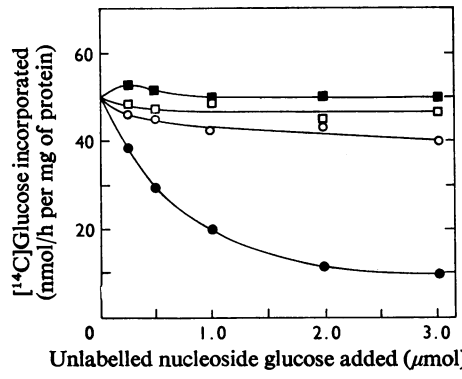

Fig. 4. *Effect of unlabelled nucleoside diphosphate glucoses on the incorporation of UDP-[U-^{14}C]glucose into sterol glucoside*

UDP-glucose–sterol glucosyltransferase was assayed in the 19000*g* particulate fraction in the presence of 13mM-Ca^{2+}. The assay mixtures contained, in addition to UDP-[U-^{14}C]glucose, increasing concentrations of unlabelled UDP-glucose (●), GDP-glucose (○), CDP-glucose (□) or ADP-glucose (■). The details of the assay are described in the Experimental section.

into sterol glucoside as a consequence of dilution of the radioactive substrate. Clearly, of the four glucose donors only UDP-glucose serves as an effective glucose donor in sterol glucoside synthesis in this system. GDP-glucose shows slight inhibition (20%) at a concentration six times that of the added UDP-[^{14}C]glucose. The slight stimulation of incorporation of label from UDP-[^{14}C]glucose in the presence of low concentrations of ADP-glucose always occurred, but varied in magnitude among different enzyme preparations. It is possible that low concentrations of ADP-glucose afford a sparing effect on UDP-glucose, thereby making more UDP-glucose available for sterol glucosylation than in the absence of ADP-glucose.

An examination of the ease of removal of the transferase and UDPase from the membrane was made. The action of a range of concentrations of a cationic, a neutral and an anionic detergent on the activities of the two enzymes is shown in Fig. 5. The cationic detergent severely inhibits the activity of both enzymes. Triton X-100 does not affect UDPase, but has an overall moderate inhibitory effect on glucosyltransferase, especially at a low concentration of the detergent. Only with deoxycholate at a concentration of 0.1% were the two activities not inhibited. UDPase at this detergent concentration was actually stimulated, but the magnitude of this effect varied from preparation to preparation. Based on these studies a partial solubilization of a

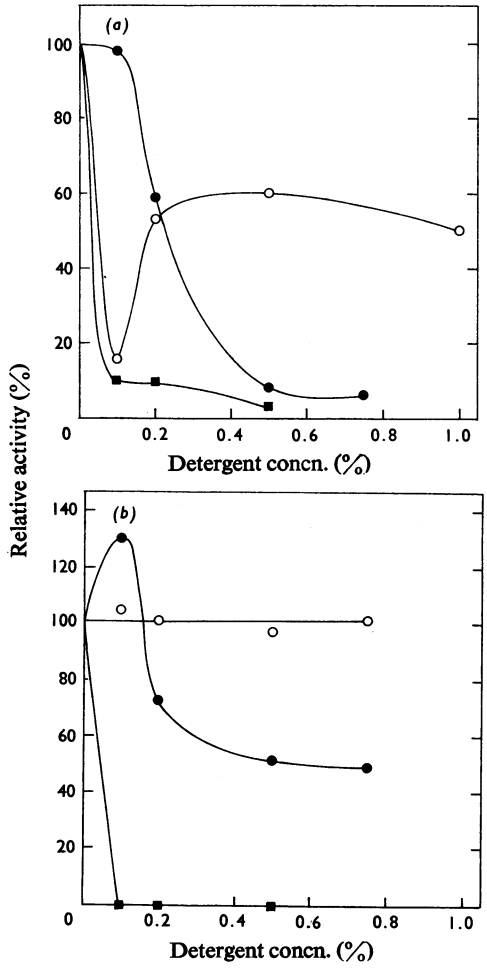

Fig. 5. *Effect of detergents on UDP-glucose–sterol glucosyltransferase and the UDPase activities*

The glucosyltransferase (*a*) and UDPase (*b*) activities were assayed on a 19000*g* particulate fraction prepared in the presence of EDTA and 2-mercaptoethanol. The assays were carried out in the presence of 13mM-Mg^{2+} and in the presence of a range of concentrations of sodium deoxycholate (●), Triton X-100 (○) or cetyltrimethylammonium bromide (■). Details of the experimental procedures are described in the Experimental section. The activities are relative to a control (set arbitrarily at 100%) in which detergents are omitted.

19000*g* preparation was effected with 0.1% deoxycholate. Glucosyltransferase and UDPase activities were measured in the supernatant and pellet fractions resulting from centrifugations for 5min and for 60min of this partially solubilized preparation. The results are shown in Table 3. The stabilities

Table 3. *Distribution of glucosyltransferase and UDPase between supernatant and residual pellet from deoxycholate solubilization of* 19000*g fraction*

Glucosyltransferase and UDPase activities were assayed in the presence of 13mM-Mg^{2+} cation in the 19000*g* pellet in 50mM-Tris/HCl, pH8, containing 10mM-2-mercaptoethanol, 2mM-EDTA and 0.1% deoxycholate. The activities were also measured on the pellet and supernatant resulting from centrifugation of the pellet after treatment with 0.1% deoxycholate. Two samples of the detergent-treated pellet were centrifuged at 78500*g* ($r_{av.}$ 7.8cm), one for 5min and the other for 60min. Details of the procedure are given in the Experimental section. The pellet was assayed in the presence of 0.1% deoxycholate.

	Relative total activities (%)			
Time of centrifugation (min)	... 5		60	
	Glucosyltransferase	UDPase	Glucosyltransferase	UDPase
19000*g* fraction	100	100	100	100
Pellet	23	28	28	53
Supernatant	60	87	25	57
Pellet+supernatant	83	115	53	110
Pellet / Supernatant	0.38	0.32	1.1	0.93

of the two enzymes in the presence of the detergent are quite different. After short exposure time, 83% of the glucosyltransferase activity is recovered, but long exposure decreases this value to 53%. UDPase, on the other hand, shows slight stimulation even on long exposure time. The distribution of the transferase between particles and supernatant shows increased recovery in particles with increased centrifugation time, from a ratio of 0.38 to 1.1. UDPase behaves similarly, with an increased ratio from 0.32 to 0.93. This similarity in the sedimentation of the two activities points to a similar degree of association of each activity with membrane lipid.

There have been several studies in which nucleoside diphosphatase activities have been shown to be membrane-bound (Ernster & Jones, 1962; Kuriyamo, 1972; Klohs & Goff, 1973). The activities appear to be distributed predominantly in Golgi, endoplasmic reticulum and plasmalemma membranes. Klohs & Goff (1973) have reported that a fraction from onion root tip, which is enriched in Golgi membranes but also contains other membranes, including microsomal ones, is very active in the hydrolysis of IDP, UDP and GDP and less active in the hydrolysis of ADP and CDP. Poux (1967) has localized ADPase and IDPase in the plasmalemma and in the adjacent cell wall of cucumber root.

The metabolic role of nucleoside diphosphatase remains largely speculative. A relationship between high activity and secretion of glycose-containing products is evident, and the localization of activity in the endoplasmic reticulum would indicate a possible additional involvement in such activities as phospholipid synthesis.

In the present study, ADPase, GDPase and UDPase appear extremely active under conditions that are optimal for the sterol glucosyltransferase. As the transferase is inhibited by UDP, a product of the transferase reaction, a metabolic role for UDPase is evident. Preincubation of the transferase in the presence of UDP produces a relief from such inhibition. Both enzymes appear in particles with a density of 1.15g/ml and are equally easily removed from the membrane by 0.1% deoxycholate. The enzymes are stimulated by Ca^{2+} and Mg^{2+} cations and have similar pH profiles. An examination of Table 1 reveals that UDPase is three orders of magnitude more active than the transferase. The conditions producing this substantial difference in activity are unlikely to prevail in the cell, but, as UDPase has an absolute requirement for Mg^{2+} cation, its activity may be governed by this ion. The activity of UDPase is potentially so great that it may serve as a means for disposing of not only UDP resulting from sterol glucosylation but also from other UDP-glycose-requiring glycosylation reactions that may be proximally located in the same membrane.

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References

Bush, P. B. & Grunwald, C. (1974) *Plant Physiol.* **53**, 131–135
Eichenberger, W. & Grob, E. C. (1970) *Chimia* **24**, 394–396
Eichenberger, W. & Siegrist, H. P. (1975) *FEBS Lett.* **52**, 153–156
Ernster, E. L. & Jones, C. L. (1962) *J. Cell Biol.* **15**, 563–578
Fang, T.-Y. & Baisted, D. J. (1976) *Phytochemistry* **15**, 273–278
Forsee, W. T., Laine, R. A. & Elbein, A. D. (1974) *Arch. Biochem. Biophys.* **161**, 248–259
Goff, C. W. (1973) *Protoplasma* **78**, 397–416
Hou, C. T., Umemura, U., Nakamura, M. & Funahashi, S. (1968) *J. Biochem. (Tokyo)* **63**, 351–360
Kauss, H. (1968) *Z. Naturforsch. Teil B* **23**, 1522–1526
Klohs, W. D. & Goff, C. W. (1973) *J. Histochem. Cytochem.* **21**, 417–425
Kuriyamo, Y. (1972) *J. Biol. Chem.* **247**, 2979–2988
Laine, R. H. & Elbein, A. D. (1971) *Biochemistry* **10**, 2547–2553
Leloir, L. F. & Cardini, C. E. (1957) *Methods Enzymol.* **3**, 840–850
Leonard, R. T. & VanDerWoude, W. J. (1976) *Plant Physiol.* **57**, 105–114
Lezica, R. P., Romero, P. A. & Dankert, M. A. (1976) *Plant Physiol.* **58**, 675–680
Novikoff, P. M., Novikoff, A. B., Quintana, N. & Houw, J. J. (1971) *J. Cell Biol.* **50**, 859–886
Ongun, A. & Mudd, J. B. (1970) *Plant Physiol.* **45**, 255–262
Péaud-Lenoël, C. & Axelos, M. (1971) *C. R. Hebd. Séances Acad. Sci. Ser. D* **273**, 1057–1060
Potty, V. H. (1969) *Anal. Biochem.* **29**, 535–539
Poux, N. (1967) *J. Microsc. (Paris)* **6**, 1043–1058
Ray, P. M., Shininger, T. L. & Ray, M. M. (1969) *Proc. Natl. Acad. Sci.* U.S.A. **60**, 605–612
Shore, G. & Maclachlan, G. A. (1975) *J. Cell Biol.* **64**, 557–571
Wojciechowski, Z. A., Zimowski, J. & Zieleńska, M. (1976) *Phytochemistry* **15**, 1681–1683