Plant Physiol. (1987) 85, 51–55
0032-0889/87/85/0051/05/$01.00/0

Regulation by Phospholipids and Kinetic Studies of Plant Membrane-Bound UDP-Glucose Sterol β-D-Glucosyl Transferase[1]

Received for publication February 11, 1987 and in revised form May 13, 1987

PASCALINE ULLMANN, PIERRETTE BOUVIER-NAVÉ*, AND P. BENVENISTE
Laboratoire de Biochimie Végétale, Institut de Botanique, 28, rue Goethe, 67083 Strasbourg, Cédex, France

ABSTRACT

Solubilization and partial purification of the microsomal UDP-glucose sterol glucosyl transferase activity from maize coleoptiles by chromatography on DEAE-cellulose resulted in a highly delipidated (>95%) and inactive enzymic preparation. Addition of sterols revealed part of the activity and subsequent addition of phospholipids further increased the activity. Negatively charged phospholipids were shown to be by far the best activators. The purification step also produced the elimination of two interfering microsomal enzymic activities: UDPase and steryl glucoside acyl transferase. The removal of these two enzymic activities was a prerequisite for kinetic studies including product-inhibition studies, since the substrates of these two latter enzymes are the products of UDPG-SGTase activity. The results of the kinetic studies strongly suggest an ordered bi-bi mechanism for the glucosylation of sterols. Finally the effect of different phospholipids on the kinetic parameters of the reaction was studied. Both phosphatidylcholine and phosphatidylglycerol significantly decrease $K_{m\text{-sterol}}$ (and not $K_{m\text{-UDPglucose}}$) and increase the reaction V_{max}. The decrease of $K_{m\text{-sterol}}$ is similar with both phospholipids whereas the increase of V_{max} is much greater with phosphatidylglycerol than with phosphatidylcholine.

Steryl glucosides (SG)[2] and acylated steryl glucosides (ASG) are present with free and esterified sterols in all plant tissues investigated so far, most probably as components of membrane structures (30). The glucosylation of sterols is catalyzed by UDPG-SGTase, a plasma membrane bound enzyme of plant cells (11). The reaction consists of a glucose transfer from UDP-glucose to a phytosterol with formation of a β-glucosidic linkage between the anomeric carbon of glucose and the 3-hydroxyl group of the sterol (30):

$$\text{Sterol} + \text{UDP-glucose} \rightarrow \text{steryl glucoside} + \text{UDP}$$

Sterols are major constituents of the plasma membrane and their role in the regulation of membrane fluidity is now well established (9). By controlling the amount of free sterols in the membrane, UDPG-SGTase might participate in the regulation of membrane fluidity.

In a recent work we showed the phospholipidic dependence of maize coleoptile UDPG-SGTase: partial delipidation of the enzymatic preparation by acetone precipitation (29), or selective solubilization (5), results in a strong inhibition of the enzymic activity; the addition of PL to the delipidated enzymic preparation fully restores the activity (5, 29).

The regulation of membrane-bound enzymes by PL is well established (for a review, see Refs. 23 and 24), but papers dealing with the lipid-dependence of plant membrane-bound enzymes are just a few (8, 12, 14–16, 20, 23, 28). Some enzymes exhibit a certain specificity in the lipid requirement for optimal activity: most of them belong to bacterial or animal systems (23, 24); among plant lipid-dependent enzymes, only one case was described (20).

Investigation of the lipid dependence of UDPG-SGTase on a molecular basis required purification and total delipidation of the enzyme as well as knowledge of the kinetic parameters of the enzymic reaction. In the present paper we report the partial purification of the solubilized UDPG-SGTase activity from etiolated maize coleoptiles microsomes. The purification-associated delipidation allowed a study of the dependence towards different PL. In addition, the kinetic mechanism of the purified enzyme could be analyzed and we studied the effect of various PL on the kinetic parameters of the reaction.

Preliminary data from some aspects of this work have been reported (6).

MATERIALS AND METHODS

Chemicals. Technical sitosterol from Fluka, Switzerland consisted in 93% β-sitosterol, the remainder being campesterol, according to GLC analysis (3). Sitosteryl glucoside was a generous gift of Professor Pegel, Durban, South Africa. UDP, UDP-glucose, 98 to 99% pure phospholipids, and free and esterified fatty acids were from Sigma. UDP-[U-^{14}C]glucose (240 mCi/mmol) was purchased from Amersham (U.K.) and Emulphogene BC 720, from Gaf Corp. (New York).

Plant Material Preparation of Membrane Fraction and Solubilization of UDPG-SGTase Activity. The cultivation of maize seedlings (5) and preparation of microsomes from coleoptiles (29) have been described. The microsomal membranes were resuspended in 50 mM Tris-HCl (pH 8) containing 1 mM mercaptoethanol (buffer 1). To this suspension (protein concentration of approximately 8 mg/ml) Emulphogene BC 720 was added, so as to obtain a detergent to protein ratio of 2 (w/w). This emulsion was mixed for 15 min at 30°C, then centrifuged at 100,000*g* for 1 h.

[1] Part III in the series "Phospholipid-dependence of plant UDP-glucose sterol β-D-glucosyl transferase" (5, 29).

[2] Abbreviations: SG, steryl glucosides; ASG, acyl steryl glucosides; UDPG, UDP-glucose; UDPG-SGTase, UDP-glucose sterol β-D-glucosyl transferase; PL, phospholipids; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, Phosphatidyl-glycerol; PS, phosphatidylserine.

Ion Exchange Chromatography. A DEAE-cellulose DE-52 (Whatman) column (1 cm i.d.) was prepared and equilibrated in buffer 2 (= buffer 1 containing in addition 20% glycerol [v/v] and 0.2% Emulphogene [w/v]). The supernatant was loaded onto the column (3–4 mg protein/ml gel, flow rate: 0.25 ml/min), washed with buffer 2 (flow rate: 0.5 ml/min), and then eluted with a discontinuous gradient of KCl (0.050, 0.075, 0.100, 0.150, and 0.400 M KCl in buffer 2; for the profile of this gradient, see Fig. 1). The purification was carried out at 4°C.

UDPG-SGTase Assay. The enzymic preparations (70 μl of microsomal suspension or Emulphogene supernatant, both diluted 5 times, or 70 μl of the column fractions) were preincubated for 15 min at 30°C with 20 μl of (a) an Emulphogene solution (1.8% for microsomes, 1.1% for the supernatant and the DEAE fractions, so as to get the same final detergent concentration of 0.36% w/v); or (b) a sitosterol emulsion in Emulphogene (1.8 or 1.1%) so as to obtain 290 μM sterol in the final assay mixture except in the kinetic studies where this concentration was varied; or (c) an emulsion in Emulphogene of both sitosterol and different PL at various concentrations, usually 25 μg/100 μl final assay (*i.e.* about 300 μM); we checked that even at the highest sterol concentration used (290 μm), this substrate is totally solubilized by 0.36% Emulphogene, PL being present or not. Then UDP-[^{14}C]glucose (60,000 cpm in 10 μl of 50 mM Tris-HCl [pH 8] final concentration in the test usually 0.17 mM except in the kinetic studies) was added and the reaction was run as previously described (5).

The [^{14}C]glucosylated sterols were then extracted by addition of chloroform:methanol:water so as to get a final ratio of 4:1:1 (v/v/v). The water phase was discarded and the organic phase was washed with methanol:water (1:2, v/v), dried on Na_2SO_4, and transferred into a scintillation vial. The solvent was evaporated and the scintillation cocktail was added (5). It must be recalled that during incubation of the unpurified enzymic preparations, the sterols are not only glucosylated, but part of the SG is acylated into ASG. For this reason we considered the sum of the radioactivities associated with SG and ASG (both extracted by chloroform-methanol) as a measure of UDPG-SGTase. When separation of [^{14}C]SG and [^{14}C]ASG was needed for a measure of steryl glucoside acyltransferase, TLC was performed as described previously (5).

UDPase Assay. This activity was assayed by phosphate determination as described (21).

Phospholipid and Protein Determination. Total lipids were extracted according to Bligh and Dyer (4). All lipids, commercial or extracted, were stored in benzene:ethanol (4:1, v/v) under Ar at −20°C. Mineralization and phosphorus determination were performed as described by Rouser *et al.* (22). Proteins were assayed according to Schacterle and Pollack (25).

Kinetic Studies. UDPG-SGTase activity was measured with varying concentrations of UDPG in the presence of different fixed concentrations of sterol and vice versa. In both cases the unlabeled UDPG was varied whereas the UDP-[^{14}C]glucose amount was fixed (60,000 cpm/assay). The UDPG conversion yield was kept below 10 to 20% and the reaction rate was checked to be constant during the incubation (15 min). The added PL was usually soybean PC (50 nmol).

For the product-inhibition studies the concentration of one substrate was varied in the presence of the second substrate at different fixed (nonsaturating) concentrations and in the absence or in the presence of one product at different fixed concentrations. The product concentrations were of the order of magnitude of the concentrations inducing a 50% inhibition of the enzymatic activity ($I_{50\text{-UDP}}$ approximately 70 μM and $I_{50\text{-SG}}$ approximately 600 μM). Data were fitted using the iterative nonlinear regression methods of Cleland (7). The programs were written in Fortran and run on a DEC PDP-11 computer.

RESULTS

Solubilization of UDPG-SGTase. Triton X-100 was known to solubilize and activate efficiently plant microsomal UDPG-SGTase (5, 30). Here we used a structurally close detergent which does not absorb at 280 nm: Emulphogene BC 720. At a detergent to protein ratio of 2, 95% of the microsomal UDPG-SGTase was

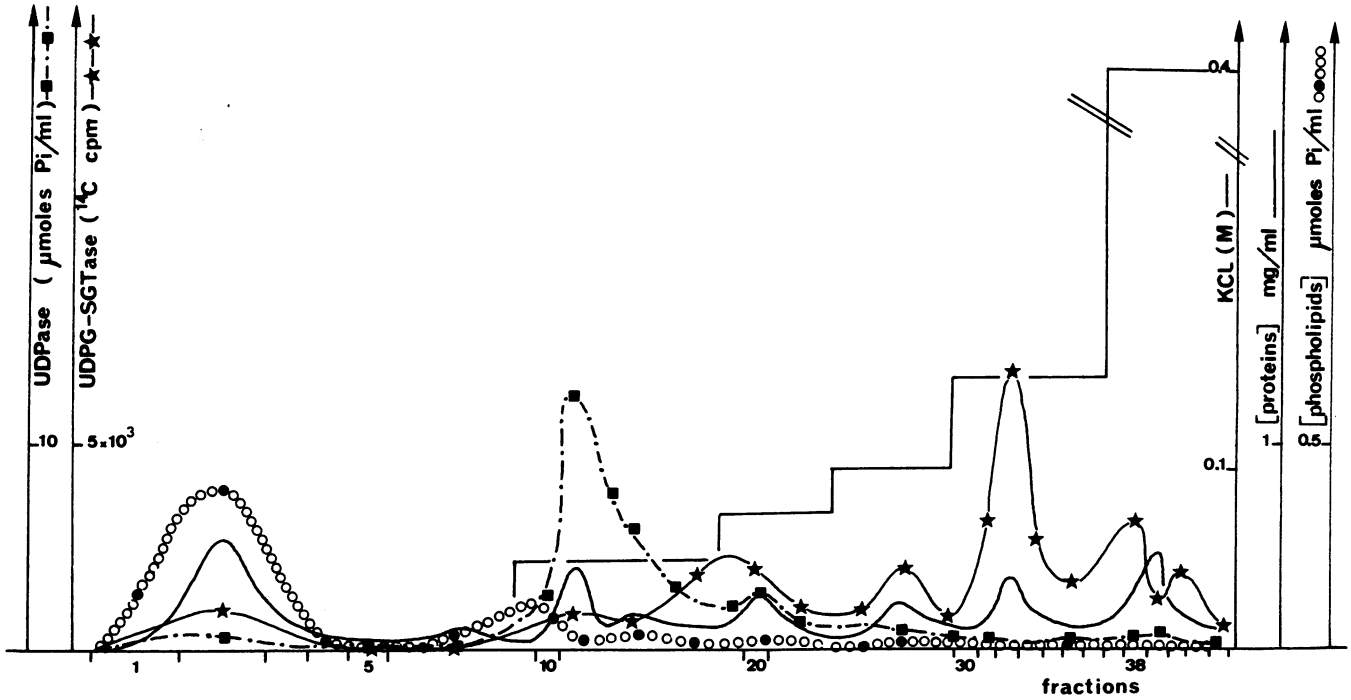

FIG. 1. Partial purification of UDPG-SGTase and separation from UDPase on a DEAE cellulose DE 52 column. Typical experiment where a 14 ml sample containing 66 mg protein was chromatographed on a 1 × 14 cm column as described under "Materials and Methods." Fraction size, 5 ml except for the very first ones. Volumes of the KCl steps: 0.05 M, 45 ml; 0.075 ml, 25 ml; 0.1 M, 25 ml; 0.15 M, 35 ml; 0.4 M, 30 ml.

recovered in a 100,000*g* supernatant (i.e. solubilized) with 65% of the total proteins.

Ion Exchange Chromatography. The 100,000*g* supernatant was fractionated by anion exchange chromatography on a DEAE-cellulose column. The bulk quantity of protein and phospholipid passed straight through the column (Fig. 1). Sterols were similarly eluted (data not shown). A discontinuous KCl gradient was then applied and UDPG-SGTase activity was mainly eluted by 0.15 M KCl whereas UDPase activity was chiefly eluted with 0.05 M KCl and virtually absent from the main UDPG-SGTase peak (fractions 30–38 in Fig. 1). Nor was SG acyl transferase detected in these active fractions (data not shown). The traces of sterol present in these fractions (measured according to Ref. 3), *i.e.* 0.7 μg/ml, represent about 1.5 μM endogenous sterol in the assay mixture (whereas $K_{m\text{-sterol}}$ equals approximately 200 μM).

Purification-Associated UDPG-SGTase Inhibition, and Restoration of Activity by Coarse Lipidic Extract. Table I shows the delipidation-associated inhibition of UDPG-SGTase activity, and the stimulation obtained by the simultaneous addition of sterols and a lipidic extract of microsomes. In fact sterols by themselves, as substrates, partially revealed the activity; the sterol amount used was shown to produce the maximal effect; the subsequent addition of the lipidic extract brought about a further stimulation by a factor 2.

Table I. *Partial Purification and Delipidation of UDPG-SGTase, after Chromatography on a DEAE-Cellulose DE 52 Column*

Results of a typical experiment (see also Fig. 1). The experimental error is ±10%.

Enzymatic Preparation	Phospholipids	Proteins	Specific UDPG-SGTase Activity	
			Without lipids[a]	With lipids[a]
	% of microsomes		*pmol/mg protein·s*	
Microsomes	100	100	8	14
Emulphogene supernatant	87	79	10	13
Fractions 1–7	>52	24	0	0
Fractions 30–38	<6	3	2	98[b,c]

[a] UDPG-SGTase activity was measured in the absence or in the presence of lipids:sterols (12 μg sitosterol, *i.e.* 290 μM, final concentration in the assay) + a lipidic extract of maize coleoptiles microsomes (extraction according to Bligh and Dyer (4); the added extract represents approximately 10 μg sterols and 50 μg PL). [b] In this experiment, the purification factor was 7.5. According to the experiments, it varied between 6 and 10. The total activity in fractions 30–38 represented 45% of the total activity loaded onto the column (in cpm). This yield varied between 40 and 70. [c] In fact sterols by themselves partially revealed the enzymic activity and the subsequent addition of the lipidic extract brought about a further stimulation by a factor 2.

Table II. *Reactivation Factor of Purified UDPG-SGTase, by Different Phospholipids*

The control activity (= 1) was measured in the absence of PL. All the assays (100 μl) contained: active fraction from DEAE-cellulose (approximately 10 μg prot), sitosterol (290 μM), and Emulphogene (0.36%).

Experiment	Fatty Acids Composition of PL	Added Phospholipid[a]					
		PC[b]	PE[b]	PI[b]	PS[b]	PG[b]	PA[b]
1	[b]	2.4	1.4	5.5	8.3	10.8	11.4
2	[b]	1.9	1.0	6.8	4.5	5.0	6.0
3	di $C_{18:1}$	2.1				5.8	
	di $C_{18:0}$	2.3				3.0	
	di $C_{16:0}$	0.9				4.5	

[a] 25 μg of these PL were present in the assay (approximately 300 μM). [b] In experiments 1 and 2, PC was PC di $C_{18:2}$; PE and PI were from soybean; PS from bovine brain and PG and PA, from egg yolk PC (via phospholipase D-catalyzed hydrolysis or transesterification).

Reactivation by Different Pure Phospholipids. We compared the reactivating power of different PL. Table II shows the results of two typical experiments (1 and 2). Whereas the general reactivation amplitude varies from one experiment to the other (more than six were performed), the reproducible feature is the much stronger activation obtained with the negatively charged PL (PA, PG, PI, and PS): they are 2 to 4 times more efficient than the zwitterionic PL (PE and PC).

The fatty acid composition of the natural PL described in experiments 1 and 2, Table II, is not known. Therefore, we compared the effects of synthetic zwitterionic and negatively charged PL with the same fatty acid composition (Table II, experiment 3). Whatever is this composition, PG is more efficient than PC in stimulating the enzyme.

The importance of the charge of the polar head of PL in the activation of UDPG-SGTase was further confirmed by a comparison of 5 different fatty acids ($C_{16:0}$ to $C_{18:3}$) with the corresponding methyl esters, for their ability to stimulate purified UDPG-SGTase. The free fatty acids were as efficient as PA in activating the enzyme, whereas the methylesters were one-half as efficient (A Ury, and P Bouvier-Navé, unpublished results).

Kinetic Studies. In order to determine the mechanism for UDPG-SGTase-catalyzed sterol glucosylation and the kinetic parameters of this reaction, we performed kinetic studies with the purified enzyme preparation in the presence of Emulphogene and soybean PC. When UDPG concentration was varied in the presence of different fixed concentrations of sterol (Fig. 2) and vice versa, intersecting patterns were obtained in Lineweaver-Burk plots.

These results clearly indicate that a sequential rather than ping-pong mechanism applies to UDPG-SGTase-catalyzed reaction (27). Thus, they confirm our preliminary data obtained with the acetone powder of maize coleoptiles microsomes (29). The double reciprocal plots and the secondary plots ($1/V_{max}$ apparent = $f(1/[S])$ allowed the determination of K_ms and V_{max} (mean of two experiments, with PC as the activator): $K_{m\text{-UDPG}}$ = 40 μM ± 10%, $K_{m\text{-sterol}}$ = 200 μM ± 30%, and V_{max} = 160 pmol/mg prot/s ± 25%.

Additional insight into the reaction mechanism of UDPG-SGTase was obtained by a study of the inhibition by the reaction products: UDP and SG. Because these products are the substrates of two microsomal enzymes, UDPase (21) and SG acyl transferase (30), these two interfering enzymic activities had to be removed to allow such a study. This was achieved by purification on DEAE cellulose (see Fig. 1 and the corresponding text). The type of inhibition produced by SG or UDP towards UDPG or sterol was examined both by Lineweaver-Burk plots (*e.g.* Fig. 3) and by a computer-based statistical analysis of the data, using Cleland's program (7). Whereas both methods failed to determine whether the inhibition by UDP towards UDPG is competitive or noncompetitive, although at least six different experiments were carried out, both methods were in accordance with the exclusion of a competitive type of inhibition for the couples SG/sterol, SG/UDPG, and UDP/sterol.

Thus, the product-inhibition pattern excludes the possibility of a random bi-bi mechanism for the reaction of sterol glucosylation (27). If UDP is a competitive inhibitor towards UDPG, the sterol glucosylation mechanism is simply ordered, UDPG being the first bound substrate and UDP the last released product. If, however, UDP is a noncompetitive inhibitor towards UDPG, then the product inhibition pattern is in agreement with a mono-iso-ordered mechanism in which a conformational change of the

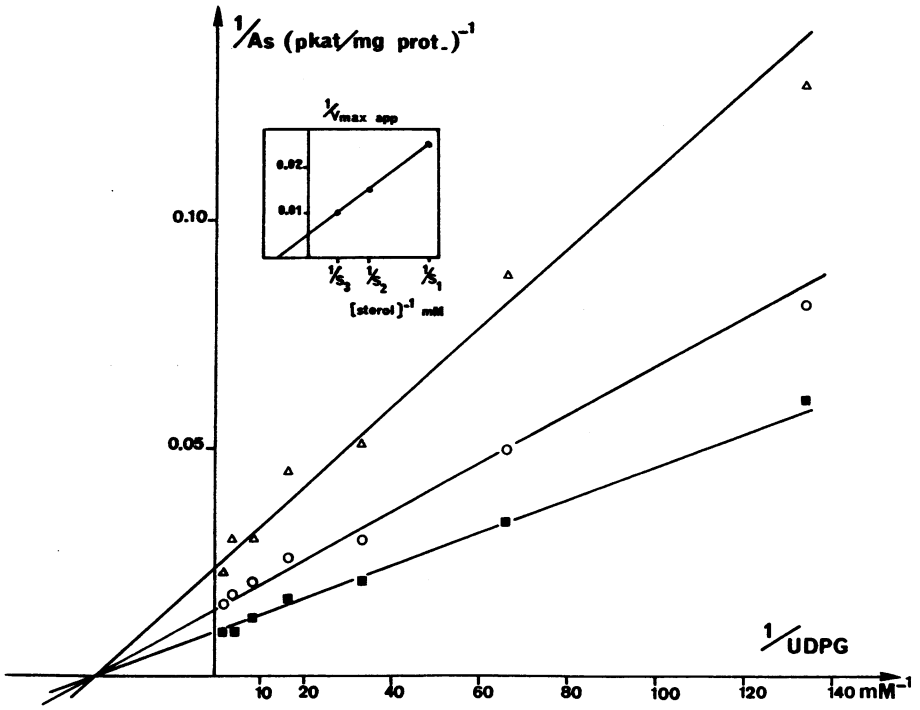

FIG. 2. Double reciprocal plots of initial velocities with UDPG as the variable substrate and sterol as the changing fixed substrate. ■ [sterol] = 72 μM (S_1), ○ [sterol] = 145 μM (S_2), △ [sterol] = 290 μM (S_3). *Inset*: intercept replot.

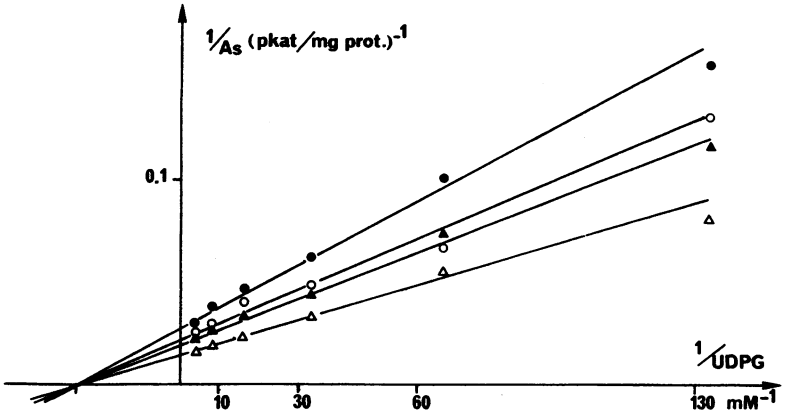

FIG. 3. Double reciprocal plots of initial velocities with UDPG as the variable substrate, sterol at two fixed concentrations in the absence or in the presence of SG as inhibitor. Noncompetitive pattern. ○ [sterol] = 97 μM, ● [sterol] = 97 μM and [SG] = 500 μM, △ [sterol] = 193 μM, ▲ [sterol] = 193 μM and [SG] = 500 μM.

enzyme is assumed during the course of the reaction (27). In this case the binding order of the substrates (and the release order for the products) cannot be predicted.

Effects of Phospholipids on the Kinetic Parameters of the Reaction. All the kinetic studies described above were carried out in the presence of Emulphogene and soybean PC. In order to evaluate the influence of PL on the K_ms and V_{max} of the reaction, we measured the $K_{m\text{-sterol}}$ (in the presence of 60 μM UDPG) and $K_{m\text{-UDPG}}$ (in the presence of 290 μM sterol) of the DEAE-cellulose-purified enzyme (a) in the absence of any PL, or (b) in the presence of PC, or PG (25 μg). The results show that $K_{m\text{-sterol}}$ is strongly lowered (from 800 to 160 or 140 μM, respectively) by either PC or PG whereas $K_{m\text{-UDPG}}$ is only slightly lowered (1.3 times). The apparent V_{max} is also varied by addition of PL, and the observed increase depends on the added PL: when PC increased V_{max} by a factor 1.9, PG increased it by a factor 3.8.

DISCUSSION

Anion exchange chromatography had already been described for partial purification of UDPG-SGTase solubilized from other plant sources (30). Thus, Forsee *et al.* (10) purified UDPG-SGTase from developing cotton fibers and seeds on a DEAE-cellulose and observed a 3-fold increase of the partially purified enzyme activity, following the addition of purified cotton lecithins. Our results are in agreement with their findings.

We took advantage of the purification-associated delipidation (Table I) of the maize coleoptiles microsomal UDPG-SGTase to confirm its phospholipid dependence (5, 29) and to look for a possible selectivity in this dependence; indeed, UDPG-SGTase exhibited a clear preference for negatively charged PL (Table II). Its behavior is thereby similar to the one found for potato microsomal NADH-Cyt *c* reductase (20).

The chromatography on DEAE-cellulose allowed a good removal of UDPase and SG acyl transferase from the UDPG-SGTase active fractions. Hence, a complete kinetic study was rendered possible. The data obtained in the absence of products confirm that UDPG-SGTase-catalyzed reaction follows a sequential mechanism (29).

The product inhibition study indicates an ordered addition of the substrates (and removal of the products) with or without an isomerisation of the enzyme. These results are in agreement with the kinetic mechanisms established for two soluble glucosyl transferases (18, 26).

Because UDPG-SGTase is a membrane-bound enzyme, its purification had to be carried out in the presence of detergent. Moreover, one of the substrates (sterol) and one of the products

(SG) are insoluble in water. Subsequently the kinetic studies were also carried out in the presence of detergent, *i.e.* with an isotropic system. However, the kinetic parameters of the reaction as well as the enzymic selectivity towards PL might be modified in this micellar system, as compared with a membrane system. We intend to perform these measurements in a reconstituted system consisting of the enzyme embedded into liposomes of controlled sterol and PL composition. In spite, or because, of these difficulties inherent to the hydrophobic character of the protein, the knowledge of the kinetic mechanism of the reaction—and of its overall molecular mechanism, including the PL role—is important. Up to now just a few plant membrane-bound enzymes have been studied as for their kinetic mechanism (1, 2). No PL dependence was described in these cases.

In order to approach the molecular basis of the activation by PL, we studied their effect on the kinetic parameters of the reaction. We showed that PL affect both the K_ms and the V_{max} of the UDPG-SGTase-catalyzed reaction. $K_{m\text{-sterol}}$ is much more strongly modified than $K_{m\text{-UDPG}}$ and is 5 times lower with either PC or PG than without. In addition, these PL affect the V_{max} with PG increasing it much more than PC.

We cannot exclude the possibility that PL might be interacting with unidentified inhibitory molecules in the preparations rather than directly with the enzyme. Such inhibitors would be present in the native membrane but kept aside from the enzyme by PL (even after solubilization). They should then co-migrate with the enzyme during the purification step, and be brought into contact with the enzyme, via the purification-associated delipidation. Exogenous PL added to the purified enzyme would then again discard the inhibitory molecules. Although we have never heard of such a situation for any membrane-bound enzyme described in the literature, this possibility remains.

On the other hand, the possibility of a PL role restricted to the 'solubilization' of hydrophobic enzymatic substrates has been reviewed, but in this case usually there is no selectivity towards any PL and even detergent can substitute for PL (23). It must be pointed out here that detergent is present in our assay but is unable in the presence of sterols, to reveal the full activity of the delipidated enzyme (Table II; also Refs. 5, 29). However, this possibility of a mere solubilizing role for PL was evoked for UDPG-SGTase (17). In order to test this hypothesis we incubated our DEAE-cellulose-purified enzymic preparation in the presence of labeled UDPG and different sterol concentrations (from 290 μM down to 2.9 μM) in the absence or the presence of PG at different concentrations. The results (data not shown) indicate that whatever the sterol concentration is, UDPG-SGTase activity is stimulated by PG. Moreover, the stimulation factor increases when the sterol concentration decreases. Therefore, we believe that negatively charged PL play much more than a simple role of solubilizing the hydrophobic substrate of UDPG-SGTase. In this respect the possibility of a lowering of the activation energy of the reaction should be considered; this has been clearly shown in the case of animal liver UDP-glucuronyl transferase (13, 19).

Acknowledgments—We wish to thank Dr L. Freysz, CNRS Neurochimistry Department in Strasbourg, for helpful discussions.

LITERATURE CITED

1. BACH TJ 1986 Hydroxymethylglutaryl-CoA reductase, a key enzyme in phytosterol synthesis. Lipids 21: 82–88
2. BENVENISTE I, B GABRIAC, F DURST 1986 Purification and characterization of the NADPH-cytochrome P-450 (cytochrome c) reductase from higher plant microsomal fraction. Biochem J 235: 365–373
3. BLADOCHA M, P BENVENISTE 1983 Manipulation by tridemorph, a systemic fungicide of the sterol composition of maize leaves and roots. Plant Physiol 71: 756–762
4. BLIGH EG, WJ DYER 1959 A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917
5. BOUVIER-NAVE P, P ULLMANN, D RIMMELE, P BENVENISTE 1984 Phospholipid-dependence of plant UDP-glucose sterol-β-D-glucosyltransferase. I. Detergent-mediated delipidation by selective solubilization Plant Sci Lett 36: 19–27
6. BOUVIER-NAVÉ P, P ULLMANN, P BENVENISTE 1987 UDP-glucose sterol β-glucosyl transferase, a plant sterol conjugating enzyme *In* L Freysz, S Gatt, eds, Enzymes of Lipid Metabolism. Plenum Press, New York
7. CLELAND WW 1979 Statistical analysis of enzyme kinetic data. Methods Enzymol 63: 103–138
8. COCUCCI M, A BALLARIN-DENTI 1981 Effect of polar lipids on ATPase activity of membrane preparations from germinating radish seeds. Plant Physiol 68: 377–381
9. DEMEL RA, B DE KRUYFF 1976 The function of sterols in membranes. Biochim Biophys Acta 457: 109–132
10. FORSEE WT, RA LAINE, AD ELBEIN 1974 Solubilization of a particulate UDP-glucose: sterol β-glucosyl-transferase in developing cotton fibers and seeds and characterization of steryl 6-acyl-D-glucosides. Arch Biochem Biophys 161: 248–259
11. HARTMANN-BOUILLON MA, P BENVENISTE 1978 Sterol biosynthetic capability of purified membrane fractions from maize coleoptiles. Phytochemistry 17: 1037–1042
12. HEINIGER U 1983 UDP-glucose-1,3-β-glucan synthetase in potato tubers solubilization and activation by lipids. Plant Sci Lett 32: 35–41
13. HOCHMAN Y, D ZAKIM, DA VESSEY 1981 A kinetic mechanism for modulation of the activity of microsomal UDP-glucuronyltransferase by phospholipids. Effects of lysophosphatidylcholines. J Biol Chem 256: 4783–4788
14. JOLLIOT A, P MAZLIAK 1973 Rôle des lipides dans diverses activités enzymatiques de la chaîne de transport des électrons d'une fraction mitochondriale isolée d'inflorescence de choufleur. Plant Sci Lett 1:21–29
15. JOLLIOT A, C DEMANDRE, P MAZLIAK 1981 Regulation by lipids of plant microsomal enzymes. II. Lipid dependence of the NADH-cytochrome c reductase of potato tubers. Plant Physiol 67: 9–11
16. JOLLIOT A, AM JUSTIN, E BIMONT, P MAZLIAK 1982 Regulation by lipids of plant microsomal enzymes. III. Phospholipid dependence of the cytidine-diphospho-choline phosphotransferase of potato microsomes. Plant Physiol 70: 206–210
17. KALINOWSKA M, ZA WOJCIECHOWSKI 1986 Modulation of activities of steryl glucoside hydrolase and UDPG:sterol glucosyltransferase from *Sinapis alba* by detergents and lipids. Phytochemistry 25: 45–49
18. KHOURI H, RK IBRAHIM 1984 Kinetic mechanism of a flavonol-ring-β-O-glucosyltransferase from *Chrysoplenium americanum*. Eur J Biochem 142: 559–564
19. MAGDALOU J, Y HOCHMAN, D VESSEY 1982 Factors modulating the catalytic specificity of a pure form of UDP-glucuronyltransferase. J Biol Chem 257: 13624–13629
20. MAZLIAK P, A JOLLIOT, AM JUSTIN, JC KADER 1985 Stimulation of potato microsomal NADH-cytochrome c reductase by acidic phospholipids. Phytochemistry 24: 1163–1168
21. M'VOULA-TSIERI M, MA HARTMANN-BOUILLON, P BENVENISTE 1981 Properties of nucleosides diphosphatases in purified membrane fractions from maize coleoptiles. I. Study of latency. Plant Sci Lett 20: 379–386
22. ROUSER G, S FLEISCHER, A YAMAMOTO 1970 Two dimensional thin layer chromatographic separation of plant lipids and determination of phospholipids by phosphorus analysis of spots. Lipids 5: 494–495
23. SANDERMANN H 1978 Regulation of membrane enzymes by lipids. Biochim Biophys Acta 515: 209–237
24. SANDERMANN H 1983 Lipid solvation and kinetic cooperativity of functional membrane proteins. Trends Biochem Sci 8: 408–411
25. SCHACTERLE GR, RL POLLACK 1973 A simplified method for the quantitative assay of small amounts of protein in biological material. Anal Biochem 51: 654–655
26. SCHMID G, H GRISEBACH 1982 Enzymatic synthesis of lignin precursors. Purification and properties of UDPglucose:coniferyl-alcohol glucosyltransferase from cambial sap of spruce (*Picea abies* L.). Eur J Biochem 123: 363–370
27. SEGEL IH 1975 Enzyme Kinetics. Wiley-Interscience, New York
28. TERPSTRA W, JWJ LAMBERS 1983 Interactions between chlorophyllase chlorophyll a, plant lipids and Mg^{2+}. Biochim Biophys Acta 746: 23–31
29. ULLMANN P, D RIMMELE, P BENVENISTE, P BOUVIER-NAVE 1984 Phospholipid-dependence of plant UDP-glucose-sterol-β-D-glucosyl transferase. II. Acetone-mediated delipidation and kinetic studies. Plant Sci Lett 36: 29–36
30. WOJCIECHOWSKI ZA 1983 The biosynthesis of plant steryl glycosides and saponins. Biochem Soc Trans 11: 565–568