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MODULATION OF ACTIVITIES OF STERYL GLUCOSIDE HYDROLASE AND UDPG:STEROL GLUCOSYLTRANSFERASE FROM *SINAPIS ALBA* BY DETERGENTS AND LIPIDS

MAŁGORZATA KALINOWSKA and ZDZISŁAW A. WOJCIECHOWSKI

Department of Biochemistry, Warsaw University, 02-089 Warszawa, Al. Zwirki i Wigury 93, Poland

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Key Word Index—*Sinapis alba*; Cruciferae; steryl β-D-glucoside hydrolase; UDPG:sterol glucosyltransferase; modulation by lipids and detergents.

Abstract—Interactions of detergents and lipid compounds on the activity of delipidated preparations of UDPG:sterol glucosyltransferase and steryl β-D-glucoside hydrolase (SG hydrolase) isolated from white mustard seedlings were studied. It has been found that various lipids exert diverse effects on the activity of SG hydrolase. This activity was distinctly stimulated by several neutral, relatively unpolar compounds such as phytol, tripalmitoylglycerol, methyl stearate or cholesteryl acetate and, to a lesser extent, by free fatty acids. On the other hand a number of phospho- and glycolipids were inhibitory. A particularly strong inhibition was observed with charged, zwitterionic phospholipids such as PC, PE or their 2-lyso derivatives. These results point to the possibility of *in vivo* regulation of the membrane-bound SG hydrolase by its lipid microenvironment. In contrast to SG hydrolase no evidence was found for a clear-cut effect of lipids on the activity of UDPG:sterol glucosyltransferase even after a pretreatment of the enzyme preparation with phospholipase C.

INTRODUCTION

Recent studies [1–6] indicate that steryl β-D-monoglucosides (SG) are components of plant membranes, along with free sterols, and it has been suggested [2, 7, 8] that reversible glucosylation–deglucosylation of membrane-bound sterols may have regulatory effects on membrane organization and functions due to induced alterations in lipid–lipid and/or lipid–protein interactions. Both *in vivo* [9] and *in vitro* [10] experiments have demonstrated that a dynamic equilibrium between free sterols and their glycoside derivatives exists in plant tissues. There are some indications that this equilibrium may be under control of phytohormones [11] or some environmental factors such as the light conditions [12].

The glucosylation of plant sterols is catalysed by a specific enzyme, UDPG:sterol glucosyltransferase, which occurs predominantly in dictiosomes [13–15] and/or in plasma membrane [16, 17]. Recently we have reported [18] that white mustard (*Sinapis alba*) seedlings contain steryl β-D-glucoside hydrolase activity which could be partially separated from other enzymes with β-D-glucosidase activity. This SG hydrolase is also present in membranous fractions and its subcellular distribution is similar to that of UDPG:sterol glucosyltransferase [18]. Although both UDPG:sterol glucosyltransferase and SG hydrolase were partly purified and some of their properties studied [1–3, 7, 13–22] very little is known on regulation of sterol glucosylation–deglucosylation at the enzyme level. It has been reported [7, 20–22] that preparations of UDPG:sterol glucosyltransferase from cotton fibres [7], pea seedlings [20] or maize seedlings [21, 22] are stimulated by some phospholipids suggesting possible regulatory role of these lipids in sterol glucosylation.

In this paper we report results of our comparative studies on the effects of detergents and lipids on activities of partly purified, delipidated preparations of UDPG:sterol glucosyltransferase and SG hydrolase from white mustard seedlings.

RESULTS

Our previous studies [18, 19] have shown that white mustard seedlings contain UDPG:sterol glucosyltransferase and steryl β-D-glucoside hydrolase (SG hydrolase) which both are membrane-bound enzymes occuring mainly in cellular structures sedimenting at 3000–15 000 *g*. Both enzymes can be solubilized, the former by delipidation of crude membrane fraction with acetone and subsequent extraction with 0.3% Triton X-100, the latter by delipidation with acetone followed by extraction with succinate buffer (see Experimental).

Figures 1 and 2 show the effect of some detergents, both ionic and non-ionic on the activities of delipidated UDPG:sterol glucosyltransferase and SG hydrolase, respectively. A wide range (0.01–1.0%) of detergent concentrations were used. The activity of UDPG:glucosyltransferase measured with [4-^{14}C]cholesterol and unlabelled UDPG as the substrates (Fig. 1) was distinctly stimulated by a number of non-ionic detergents. The highest activation was obtained with Triton X-100 which stimulated enzymatic glucosylation of [4-^{14}C]cholesterol over the whole concentration range. At 0.1% concentration of Triton X-100 about 6.5-fold stimulation was observed. All other non-ionic detergents tested also stimulated this reaction at low concentrations, however, to a much lower extent. The highest stimulation

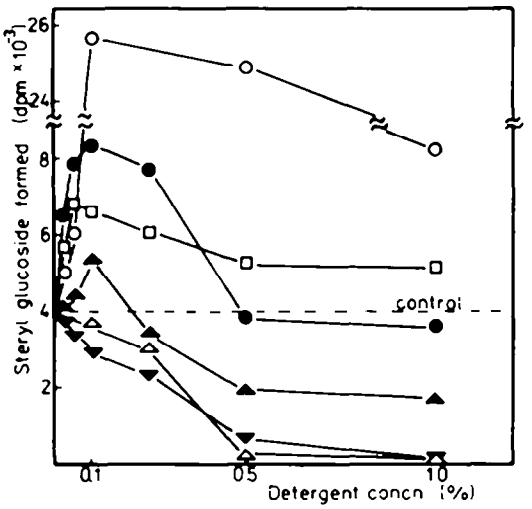

Fig. 1. Effect of some detergents on the activity of delipidated UDPG : sterol glucosyltransferase from *S. alba*. Enzyme preparation (Me_2CO precipitated enzyme, 0.210 mg protein) was incubated at 30° for 20 min with [4-^{14}C]cholesterol and unlabelled UDPG in the presence of Triton X-100 (○), Tween-40 (□), Tween-20 (●), Tween-60 (▲), sodium taurocholate (▼) or sodium deoxycholate (△). For other details see the Experimental.

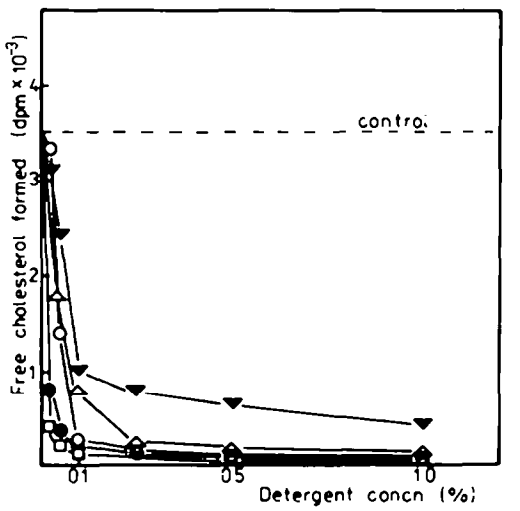

Fig. 2. Effect of some detergents on the activity of delipidated steryl glucoside hydrolase from *S. alba*. Enzyme preparation (Me_2CO precipitated enzyme, 0.056 mg protein) was incubated at 30° for 20 min with [4-^{14}C]cholesteryl β-D-glucoside in the presence of Triton X-100 (○), Tween-40 (□), Tween-20 (●), sodium taurocholate (▼) or sodium deoxycholate (△).

by Tween-20 was about 2-fold (at 0.1 % concentration). For Tween-40 and Tween-60 the enhancement of the glucosylation rate was respectively 70% (at 0.05% concentration) and 35% (at 0.1 % concentration). In contrast to the non-ionic detergents sodium taurocholate and sodium deoxycholate were strongly inhibitory over the whole concentration range. In the case of SG hydrolase from white mustard seedlings (Fig. 2) all tested detergents, both ionic and non-ionic, had very strong inhibitory effects on the rate of hydrolysis measured with [4-^{14}C] cholesteryl β-D-glucoside as the substrate. With Triton X-100, Tween-20 or Tween-40 the reaction was almost completely inhibited at 0.1 % concentration of detergent.

Our further experiments have shown that the activity of solubilized SG hydrolase can be modulated *in vivo* by the presence of various lipid compounds in the incubation medium. As it can be concluded from data shown in Fig. 3 a number of phospho- or glycolipids, typical for higher plant tissues, had a distinct inhibitory influence on the activity of SG hydrolase. Among compounds tested inhibitory properties increased in the following order: 2-lyso-phosphatidylethanolamine (2-lyso-PE) > 2-lyso-phosphatidylcholine (2-lyso-PC) > phosphatidylcholine (PC) > phosphatidylethanolamine (PE) > monogalactosyldiacylglycerol (MGDG). Phosphatidylinositol (PI) at low concentrations (up to 0.1 mM) increased the rate of SG hydrolysis (by about 10%) but at higher concentrations it had an inhibitory effect similar to that of all other phospholipids tested. On the other hand we have found (Fig. 4) that the activity of SG hydrolase is distinctly increased in the presence of some neutral lipids of low polarity such as phytol (200% at 0.02 mM concentration), methyl stearate (155% at 0.1 mM concentration), cholesteryl acetate (150% at 0.1 mM concentration) or tripalmitoylglycerol (145% at 0.1 mM concentration). Similar, but less pronounced stimulatory effects on the SG hydrolase activity were observed with free fatty acids such as palmitate or laurate. It is noteworthy that the stimulation by phytol could be completely abolished by 0.05% Triton X-100.

It has been mentioned in the Introduction that some phospholipids (particularly PE) were found to exert a stimulatory effect on the activity of UDPG : sterol gluco-

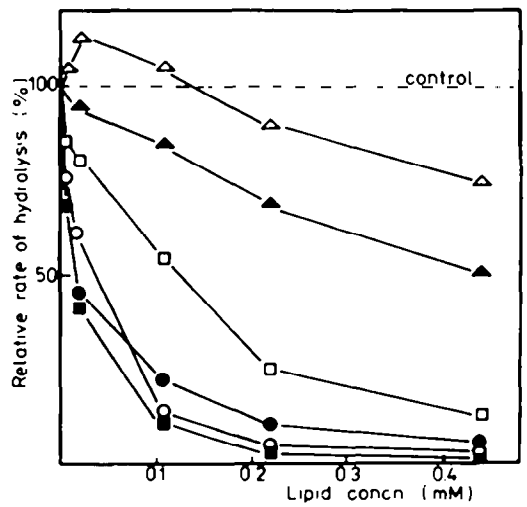

Fig. 3. Influence of some phospho- and glycolipids on the rate of steryl glucoside hydrolysis by solubilized, delipidated enzyme preparation from *S. alba*. The solubilized enzyme (0.030 mg protein) was used. Incubations were carried out as described in the legend to Fig. 2 in the presence of PI (△), MGDG (▲), PE (□), PC (●), 2-lyso-PC (○) or 2-lyso-PE (■). Sources of the above lipids are given in the Experimental.

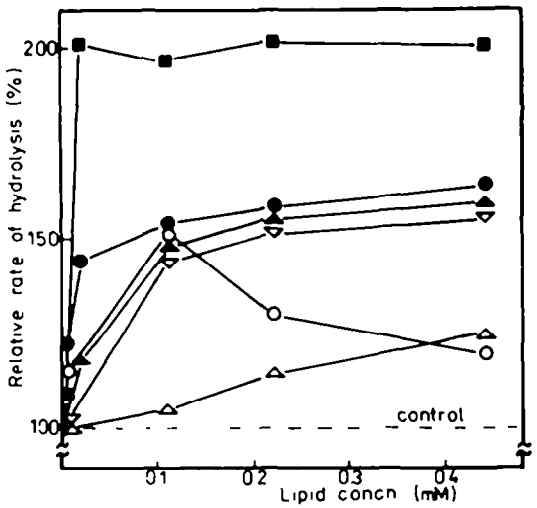

Fig. 4. Influence of some neutral lipids and free fatty acids on the activity of steryl glucoside hydrolase. Incubations were carried out as described in the legends to Figs 2 and 3 in the presence of phytol (■), methyl stearate (●), cholesteryl acetate (▲) tripalmitoylglycerol (▽), palmitic acid (○) or lauric acid (△).

syltransferase from some plants. Such a stimulation was observed with crude membrane preparations from pea seedlings [20] or partly delipidated enzyme preparations from cotton fibres [7] and maize seedlings [21, 22]. Our experiments with solubilized UDPG : sterol glucosyltransferase from white mustard could not demonstrate any enhancement of this activity by an addition of lipids to the incubation medium. Both in the presence and in the absence of Triton X-100 none of the tested lipids (see above) had a stimulatory effect on the rate of [4-^{14}C]cholesterol glucosylation. In fact, at the higher concentrations used (above 1.0 mM) we observed a slight inhibition (by about 10%) of this reaction by PC. As we have shown previously [19] solubilized UDPG : sterol glucosyltransferase from white mustard seedlings gives upon gel filtration on Sephadex G-200 a single peak of activity corresponding to an M_r of *ca* 140 000. However, it can not be excluded that this preparation still contains enough endogenous lipids necessary for optimum activity of glucosyltransferase. To test this possibility we carried out experiments in which a UDPG : sterol glucosyltransferase preparation from white mustard was preincubated for 60 min with phospholipase C prior to the incubation with [4-^{14}C]cholesterol and UDPG (see Experimental). It has been shown in control experiments that under preincubation conditions PE added to the incubation mixture (1 mg/ml) was completely hydrolysed to diacylglycerol. After such a pretreatment of the enzyme preparation with phospholipase C we were unable to find any stimulation of SG synthesis by the addition of lipids, among them PE and PC which were reported [7, 20–22] to stimulate the activity of UDPG : sterol glucosyltransferase from pea, maize or cotton plants.

DISCUSSION

It is well established [23] that membrane-bound enzymes are well adapted to act in a specific lipid environment present in membranes. Many of these enzymes, after their delipidation and solubilization, require the presence of amphipathic compounds such as various lipids or detergents for the expression of their full activity [23–26]. Studies on the interactions of small amphipatic molecules with enzymes isolated from membranous structures are of importance not only for establishing of optimum assay conditions *in vitro* but may also supply useful information on possible ways of regulating enzyme activities *in vivo* [23].

Both plant UDPG : sterol glucosyltransferase [13–17, 19] and SG hydrolase [18] are found only in membrane fractions. UDPG : sterol glucosyltransferase from various plant tissues can be solubilized exclusively with the use of detergents [7, 19, 21, 22]. A stimulatory effect of the non-ionic detergent Triton X-100 on the membranous preparations of this enzyme was observed in several cases [4, 13–17, 20–22]. This effect was explained by the increased permeability of sealed membrane vesicles to UDPG at low concentrations of Triton X-100 and by the solubilization of the enzyme at higher concentrations of detergent [21]. The present results indicate that low concentrations of Triton X-100, as well as of some other non-ionic detergents, have also a distinct stimulatory effect on the delipidated preparation of UDPG : sterol glucosyltransferase. It seems that this effect can be explained on the assumption that these detergents facilitate the interactions of the hydrophobic substrate (i.e. sterol) with the active site of the enzyme. It has been reported that partly delipidated preparations of UDPG : sterol glucosyltransferase from developing cotton fibres [7] or etiolated maize coleoptiles [21, 22] are stimulated by the addition of some phospholipids such as PE or PC. Likewise, experiments with crude membranous fractions from etiolated pea seedlings have shown [20] that a pretreatment of these preparations with phospholipases led to a partial loss (by 20–30%) of the activity of UDPG : sterol glucosyltransferase which could be restored by an addition of PE, PC or PS. Our studies with the solubilized glucosyltransferase could not confirm that it is a lipid-dependent enzyme. All tested lipids, among them PC and PE had little effect on the activity of solubilized UDPG : sterol glucosyltransferase from white mustard. Moreover, we were unable to find any inhibitory effect of the pretreatment with phospholipase C on the solubilized glucosyltransferase. It can not be excluded that UDPG : sterol glucosyltransferase from white mustard differs in its lipid dependence from similar enzymes present in cotton, pea or maize. However, another explanation is still possible. In all the cited studies [7, 20–22] glucosyltransferase activity was assayed using labelled UDPG and unlabelled sterol acceptor.

It is possible that in these cases the stimulatory effect of some phospholipids can be explained by the facilitated dispersion of relatively large amounts of sterol acceptor added to the incubation medium and by the facilitated utilization of endogenous sterols present in the enzyme preparation. It is well known that phospholipids readily form mixed micelles with sterols, especially in the presence of detergents and that these micelles are good substrates for some enzymes involved in sterol metabolism [27, 28]. In the present study we used a glucosyltransferase assay with labelled cholesterol of high specific activity which was added to the incubation mixtures in a very small amount ensuring a good dispersion.

In contrast to the effects upon UDPG : sterol glucosyl-

transferase we have found that various lipids exert distinct and diverse effects on the activity of solubilized SG hydrolase from white mustard seedlings. A particularly high stimulation of SG hydrolysis was observed with neutral lipids of low polarity such as phytol, tripalmitoylglycerol, methyl stearate or cholesteryl acetate. This demonstrates a requirement of SG hydrolase from white mustard for a highly hydrophobic environment. However, the structural diversity of the above mentioned compounds suggest rather a low specificity of interactions between these lipids and the enzyme. On the other hand, a number of phospholipids exerted a pronounced inhibitory effect on the activity of SG hydrolase. This is particularly evident in the case of zwitterionic phospholipids such as PC, PE and their 2-lyso derivatives. It is possible that the above described effects may have some importance for regulation of SG hydrolase *in vivo*.

In our previous work [18] we unexpectedly found that during solubilization and partial purification of SG hydrolase from white mustard, especially during subsequent precipitations of the enzyme with acetone, a *ca* 30-fold increase of the total enzyme activity took place. This result indicated clearly an activation of the enzyme during the purification procedure. Taking into account the present results it seems likely that the activation of SG hydrolase observed by us earlier [18] can be explained by a more or less selective removal of some inhibitory lipids at some stages of enzyme purification.

EXPERIMENTAL

Steryl glucoside hydrolase preparations. Cotyledons and hypocotyls of 7-day-old *S. alba* seedlings were homogenized with a 3-fold amount of 0.1 M Tris–maleate buffer, pH 5.2. The homogenate was filtered through a cheese-cloth and successively centrifuged at 1000 *g* (10 min) and 15 000 *g* (20 min). Organelles from the 15 000 *g* pellet were suspended in a small amount of 0.1 M Tris–maleate, pH 5.2 and added dropwise, with stirring, to a 20-fold amount of cold (−15°) Me_2CO. The ppt was collected by centrifugation, washed × 3 with cold Me_2CO and dried in a vacuum. This preparation ('Me_2CO precipitated enzyme') was stable at −20° for several months. For solubilization of SG hydrolase activity Me_2CO precipitated enzyme was suspended in 0.1 M succinate buffer, pH 5.2 (1 mg/1 ml) and centrifuged at 105 000 *g* for 1 hr. The supernatant ('the solubilized enzyme') was used immediately for incubations.

UDPG : sterol glucosyltransferase preparations. Me_2CO precipitated enzyme preparation was obtained as described above for SG hydrolase using 0.1 M Tris–HCl buffer, pH 7.2 (5 ml/g fresh plant material) for homogenization. This preparation was stable at −20° for at least 2 weeks. For solubilization of UDPG : sterol glucosyltransferase activity Me_2CO precipitated enzyme preparation was incubated, with stirring, in 0.1 M Tris–HCl, pH 7.2 containing 0.3% Triton X-100 (1.4 mg/ml) at 4° for 30 min and subsequently centrifuged at 105 000 *g* for 1 hr. The supernatant is referred in the text as 'the solubilized enzyme'.

Enzyme assays. For assay of SG hydrolase activity the standard reaction mixture contained in a total volume of 0.570 ml: Me_2CO precipitated enzyme (56 μg protein) or solubilized enzyme (30 μg protein); 50 nmol succinate, pH 5.2; 0.06 ml EtOH and 0.12 nmol [4-^{14}C]cholesteryl β-D-glucoside [18] (1.2 × 10^4 dpm). The labelled substrate, detergents and lipids were added as solns in EtOH. The reaction was run at 30° for 10–40 min. The isolation of labelled cholesterol was described earlier [18]. For assay of UDPG : sterol glucosyltransferase activity incubations were carried out in a total volume of 0.570 ml which included: Me_2CO precipitated enzyme (210 μg protein) or solubilized enzyme (32 μg protein); 50 nmol Tris–HCl, pH 7.2; 30 nmol UDPG, disodium salt; 0.6 nmol [4-^{14}C]cholesterol (6.0 × 10^4 dpm) and 0.06 ml EtOH. Incubations were run at 30° for 10–60 min. Labelled cholesteryl glucoside was isolated chromatographically as described previously [19, 29].

Phospho- and glycolipids used as enzyme effectors were of the following origin: phosphatidylethanolamine (PE) from *E. coli*, phosphatidylinositol (PI) from baker's yeast and phosphatidylcholine (PC) from egg yolk. 2-lyso-PC and 2-lyso-PE were obtained by enzymatic hydrolysis of PC and PE with phospholipase A. Monogalactosyldiacylglycerol (MGDG) was isolated from white mustard seedlings.

Other methods. Protein was estimated according to Lowry *et al.* [30]. Samples containing Triton X-100 were dialysed against H_2O for 48 hr before protein determination. Radioactivity was measured as described earlier [18]. Preincubations of glucosyltransferase preparations (Me_2CO precipitated enzyme or solubilized enzyme) from *S. alba* with phospholipase C (ex *Bacillus cereus*) in the presence of 13.3 mM Ca^{2+} were carried out as described by Fang and Baisted [20].

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