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The *in vitro* synthesis of diosgenin mono- and diglucoside by enzyme preparations from *Solanum melongena* leaves*

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Steroid glycosides of the spirostane type (steroid saponins) are rather common secondary plant metabolites. Their importance for chemical protection of plants against pathogenic microorganisms has been suggested [1]. There are, however, only sparse data on the biosynthesis of these compounds; in particular little is known about the biosynthesis of their oligosaccharide moieties [2, 3].

This study was aimed at elucidation of the mechanism of sugar chain formation in steroid saponins, as examplified by the so-called melongosides, i.e. steroid saponins which occur in *Solanum melongena* (egg plant) [4]. Melongosides are oligoside derivatives of diosgenin containing, at C-3 of the aglycone, sugar chains composed of two to five monosaccharides; the simplest of them, melongoside F, is a diosgenin 3-(β-2-D-glucopyranosyl)-β-D-glucopyranoside (see Fig. 1).

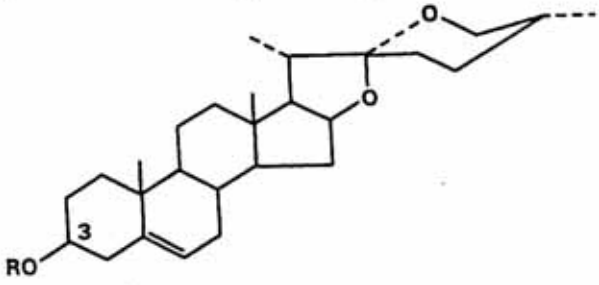
We found that crude, partially delipidated enzyme preparations ("acetone powders"), obtained by acetone treatment of the cytosolic fraction (the 105 000 *g* supernatant) from leaves of 2- to 3-week-old plants, can effectively catalyze the following two reactions:

–1) diosgenin + UDP-[^{14}C]glucose → diosgenin 3-β-D-[^{14}C]glucopyranoside + UDP

–2) diosgenin 3-β-D-glucopyranoside + UDP-[^{14}C]glucose → diosgenin 3-[^{14}C]diglucoside + UDP

The yield of the labelled product, after incubation of crude enzyme in Tris/HCl buffer, pH 8.7 (1 mg/ml) with UDP-[^{14}C]glucose (0.64 nmol = 4×10^5 d.p.m./ml) and an appropriate steroid acceptor (50 nmol/ml) for 30 min at 30°C, attained 17% in the first reaction and 5% in the second one (as calculated against labelled UDP-Glc).

The main radioactive product of the first reaction (see Fig. 2, lane b) was identified by co-chromatography and co-crystallization with original diosgenin 3-β-D-glucopyranoside obtained by chemical synthesis [5]. This labelled compound purified by t.l.c. (approx. 1.1×10^6 d.p.m.) was diluted with a large excess (25.3 mg) of the synthetic glucoside, whereupon the mixture was acetylated and the product was recrystallized five times from ethanol-water (99:1, v/v). During the whole procedure the specific radioactivity of the crystalline fractions



I R = — H

II R = — β-D-Glcp

III R = — β-D-Glcp (2←1)-β-D-Glcp

Fig. 1. Structures of diosgenin (24R-spirost-5-e n-3β-ol, **I**), *its 3-β-D-glucopyranoside* (**II**) *and melongoside F* (**III**)

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[1] Abbreviations: GT-ase, glucosyltransferase; t.l.c., thin-layer chromatography

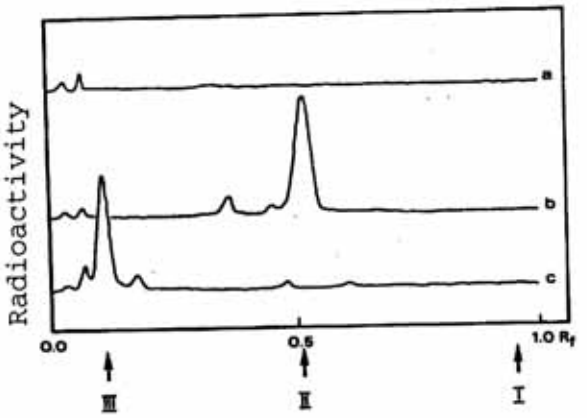

Fig. 2. T.l.c.[1] separation of the labelled products formed upon incubation of crude delipidated enzyme preparation from S. melongena leaves with: UDP-[^{14}C]Glc only (lane a); UDP-[^{14}C]Glc and diosgenin (lane b); UDP-[^{14}C]Glc and diosgenin 3-β-D-glucopyranoside (lane c)

Localization of the reference compounds is indicated by arrows: I - diosgenin, II - diosgenin 3-β-D-glucopyranoside, III - melongoside F. T.l.c. was performed on silica gel plates developed with chloroform-methanol (85:15 v/v). The radioactive spots were located by autoradiography and subsequent evaluation of autoradiograms using a UltroScan Laser densitometer (Pharmacia LKB)

remained constant (4.3×10^4 d.p.m./mg for the crude acetylation product and 4.8×10^4 d.p.m./mg after the fifth crystallization).

Glucosyltransferase (GT-ase)[1] catalyzing the conversion of diosgenin into its 3-β-D-glucopyranoside was partially purified by fractionation with ammonium sulphate (precipitation at 60 - 90% saturation) and gel filtration on Sephadex G-100 (a single activity peak). Some properties of this enzyme are listed in Table 1. Studies on its specificity with respect to a number of 3-OH steroids (some selected data are given in Fig. 3) showed that GT-ase is most active towards diosgenin and some closely related spirostanol sapogenins such as yamogenin (25*S*-epimer of diosgenin) or tigogenin (5,6-dihydro derivative of diosgenin). Natural spirostanols differing from diosgenin by the presence of an additional oxygen functions (i.e. β-chlorogenin or ruscogenin) or by spatial arrangement of the steroid ring system (i.e. smilagenin which is a 5-β-isomer of tigogenin) were glucosylated at evidently lower rates. Also steroidal alkaloids were much less effective substrates for this enzyme and only

Table 1

Some properties of UDP-Glc:diosgenin glucosyltransferase from S. melongena leaves

Molecular mass	approx. 55 kDa
Optimum pH	8.4 - 9.0
Optimal temperature	30°C
Activator	2-mercaptoethanol
Inhibitors	UDP, UMP, heavy metals (Hg^{2+}, Zn^{2+}, Cu^{2+}), *N*-ethylmaleimide, Triton X-100

traces of activity were observed in the case of typical sterols. These results clearly indicate that diosgenin is the natural substrate for this enzyme which, therefore, can be regarded as UDP-Glc:diosgenin GT-ase specifically involved also *in vivo*, in the initiation of sugar chain formation in the course of melongoside biosynthesis. The very low activity of the above-described enzyme towards typical sterols indicates that it has nothing in common with the well-known UDP-Glc:sterol GT-ase widely distributed in vascular plants [6]. It should be mentioned that crude homogenates of *S. melongena* leaves can glucosylate sterols at

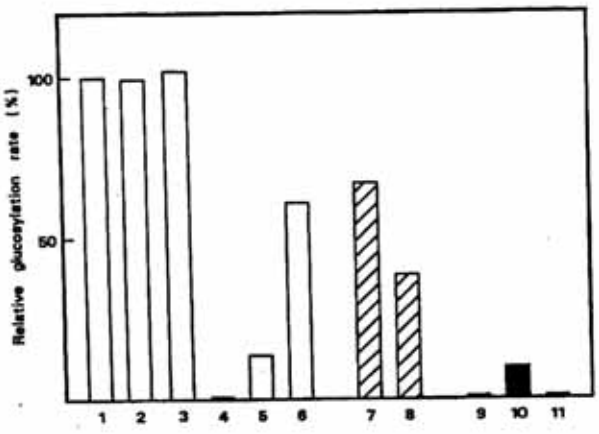

Fig. 3. Specificity of UDP-Glc:diosgenin glucosyltransferase towards various 3β-OH steroids.

Steroid sapogenins of the spirostane type - outlined bars (1, diosgenin; 2, yamogenin; 3, tigogenin; 4, smilagenin; 5, β-chlorogenin; 6, ruscogenin); steroidal alkaloids - hatched bars (7, tomatidine; 8, solasodine); phytosterols - black bars (9, cholesterol; 10, sitosterol; 11, stigmasterol)

a fairly high rate in the presence of UDP-Glc; in contrast to diosgenin glucosylation, this enzymic activity is, however, almost exclusively present in the membrane fractions sedimenting at 105 000 *g*, and is greatly stimulated by Triton X-100, similarly as it has previously been demonstrated for UDP-Glc:sterol GT-ases from other plants [6].

In addition to the synthesis of diosgenin 3-β-D-glucopyranoside, crude "acetone powders" obtained from the cytosolic fraction of *S. melongena* leaves can catalyze the UDP-Glc-dependent conversion of the latter glucoside into a compound with polarity expected for diosgenin diglucoside. This compound was tentatively identified as melongoside F on the ground of the identity of its chromatographic properties with those of original melongoside F obtained from the natural saponin fraction of *S. melongena* seeds (see Fig. 2, lane c).

The ability of the crude enzyme preparations from *Asparagus officinalis* and *A. plumosus* to synthesize monoglucosides of steroid sapogenins characteristic of these plant species, i.e. sarsasapogenin or yamogenin, respectively, has been reported by us [7, 8]. The present work demonstrates that a similar GT-ase, but with a clearly different specificity pattern, is present also in *S. melongena*. Moreover, we report here, to our knowledge for the first time, synthesis *in vitro* of natural spirostanol oligoside (melongoside F) by way of sequential, two-step addition, to the aglycone, of two monosaccharide residues from a nucleotide sugar precursor, i.e. from UDP-Glc.

Furthermore, the present work throws light on another important aspect of steroid saponin biosynthesis. So far it has generally been assumed [3, 9, 10], exclusively on the ground of indirect evidence, that formation of oligosaccharide chains in steroid saponins occurs at one of the early steps of the conversion leading from cholesterol to a spirostanol sapogenin of the spirostane type, i.e. before final closure of the spiroketal ring system (heterocyclic rings E-F). The occurrence in *S. melongena* of a quite specific UDP-Glc:diosgenin GT-ase as well as of an enzyme catalyzing further glucosylation of diosgenin 3-monoglucoside clearly testifies that the initiation and further elongation of the oligosaccharide chains can occur at the level of the completely formed spirostanol aglycone.

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