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Characterization of UDP-galactose:tomatidine galactosyltransferase from tomato (*Lycopersicon esculentum*) leaves*

Jan Zimowski

Institute of Biochemistry, Warsaw University, Al. Żwirki i Wigury 93, 02–089 Warsaw, Poland

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Tomatine (3-*O*-[β-D-xylopyranosyl(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside of tomatidine; Fig. 1) is the main steroid glycoalkaloid occurring in tomato leaves and immature fruits. This compound, similarly as other plant glycoalkaloids, is considered to play a role in plant self-protection against pathogenic organisms [1]. Whereas the biosynthesis of the tomatine aglycone, i.e. tomatidine, has been the topic of many studies and was partly elucidated [2], the biosynthesis mechanism of the oligosaccharide fragment of this glycoalkaloid has not so far been investigated. Same sparse studies of the mechanism of oligosaccharide chain formation in steroid glycoalkaloids deal almost exclusively with potato plants [3–7]. However, the aglycone of potato glycoalkaloids, i.e. solanidine, greatly differs from tomatidine and belongs to another structural type [5].

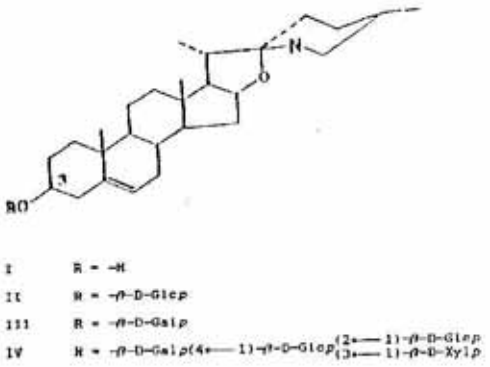

Fig. 1. Structures of tomatidine (25S-5α,22βN-spirosolanin-3β-ol, I), its 3β-D-monoglucopyranoside (II), 3β-D-monogalactopyranoside (III), tomatine (IV).

The present study concerns the process underlying the beginning of the biosynthesis of the sugar chain of tomatine, i.e. galactosylation of tomatidine.

The presented results, indicate that crude lipid-depleted enzyme preparations ("acetone powders"), obtained by acetone treatment of the cytosolic fraction (the 105000 × *g* supernatant) from leaves of 6-week old tomato plants, suspended in 0.1 M Tris/HCl buffer, pH 7.3 (1 mg/ml), in the presence of labelled UDP-galactose and tomatidine (50 nmol/ml) catalyse formation of two radioactive metabolites in about 9% yield (Fig. 2A). Co-chromatography of these labelled metabolites with tomatidine 3β-D-monoglycopyranosides (obtained by chemical synthesis [8]), showed that the main one (88% of radioactivity) was tomatidine 3β-D-monogalactopyranoside, whereas tomatidine 3β-D-monoglucopyranoside was found to be the second product. Formation of the tomatidine glucoside during incubation with UDP-galactose, a sugar residue donor, could indicate that the enzyme preparation contained UDP-glucose 4'-epimerase that transforms UDP-galactose into UDP-glucose. This possibility was additionally confirmed by the result of the experiment in which both these tomatidine monoglycosides were formed in the presence of UDP-[^{14}C]glucose as sugar residue donor.

The enzyme that catalyses formation of tomatidine monogalactoside from tomatidine and

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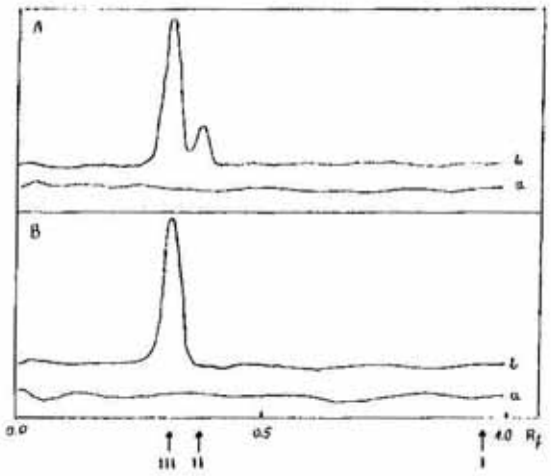

Fig. 2. Thin-layer chromatography of labelled products formed upon incubation of a crude, delipidated enzyme preparation from L. esculentum leaves (A), or upon incubation of an enzyme preparation partly purified by gel filtration on Sephadex G-100 and DEAE-Sephacel (B), with: UDP-[^{14}C]galactose only (lane a) or UDP-[^{14}C]galactose and tomatidine (lane b).

Location of the reference compounds is indicated by arrows: I, tomatidine; II, tomatidine 3β-D-monoglucopyranoside; III, tomatidine 3β-D-monogalactopyranoside. Thin-layer chromatography 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 the autoradiograms with the use of an UlroScan Laser densitometer (Pharmacia LKB).

UDP-galactose was partly purified by gel filtration on Sephadex G-100 in 0.1 M Tris/HCl buffer, pH 7.3, in the presence of 0.01 M 2-mercaptoethanol (a single activity peak), and by filtration on DEAE-Sephacel gel (elution with 0.5 M NaF in the above-mentioned buffer, followed by 24-h dialysis). The purified enzyme preparation showed a 28-fold increase in specific activity, a 6-fold rise in total activity as compared with the crude extract, and a virtually complete loss of the ability to synthesise tomatidine 3β-D-glucopyranoside when UDP-galactose served as the sugar residue donor (Fig. 2B). The increase in total activity of the enzyme isolated from the crude preparation seems to suggest that its purification resulted in elimination of the compound(s) directly inhibiting the activity of the enzyme galactosylating tomatidine. Alternatively, purification could result in removal of the compounds acting indirectly as factors activating the enzymes that catalyse degradation of UDP-galactose or of those competing for this nucleotide-sugar in other reactions. On the other hand, it is also possible that the purification eliminated UDP-glucose 4'-epimerase that maintains the UDP-galactose ↔ UDP-glucose equilibrium, by decreasing the UDP-glucose concentration in the incubated samples. The latter possibility is supported by the fact that the partly purified enzyme preparation was completely unable to synthesise tomatidine monogalactoside with UDP-glucose as the sugar residue donor. Studies on the effect of an excess (relative to radioactive UDP-galactose) of various unlabelled nucleotide-sugars (UDP-glucose, UDP-galactose, UDP-glucuronic acid) showed that during incubation an evident drop in radioactivity incorporation into tomatidine monogalactoside occurred only in the presence of unlabelled UDP-galactose (at a 1000-fold molar excess — to 5.6% of control value), whereas in the case of UDP-glucose and UDP-glucuronic acid there was a slight decrease to only 78.4% and 98%, respectively. These results suggest that the investigated enzyme is a galactosyltransferase which specifically utilizes UDP-galactose and is unable to utilise other UDP--sugars.

Some properties of the investigated galactosyltransferase are presented in Table 1. Studies of its specificity for several 3β-OH steroids (some selected data are given in Fig. 3) indicate that this enzyme utilizes at the highest rate tomatidine (a steroid alkaloid of spirostane type) as sugar residue acceptor. Another alkaloid of this type, solasodine, is a somewhat less effective substrate. In contrast, steroid alkaloids of solanidane type (solanidine, demissidine) and phytosterols (sitosterol, cholesterol) virtually fail to be utilized as sugar residue acceptors in the galactosylation process. These results clearly indicate that tomatidine is the natural substrate for this enzyme which, therefore, may be regarded as UDP-galactose:tomatidine galactosyltransferase. Also *in vivo* this enzyme is involved in the initiation of sugar chain formation in tomatine biosynthesis. The lack of activity of this enzyme towards typical plant sterols indicates that it is not engaged in the biosynthesis of sterol galactosides found to be present alongside with sterol glucosides in tomato leaves [9].

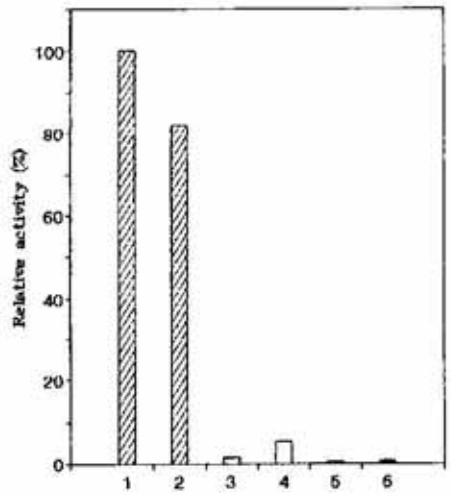

Fig. 3. Specificity of UDP-galactose:tomatidine galactosyltransferase towards various 3β-OH steroids.

Galactosylation of: 1, tomatidine; 2, solasodine (steroid alkaloids of spirosolane type); 3, solanidine; 4, demissidine (steroid alkaloids of solanidane type); 5, sitosterol; 6, cholesterol (phytosterols).

Earlier reports [3–6] demonstrated that the enzyme preparations from *Solanum tuberosum* were able to synthesize solanidine monoglucoside and monogalactoside, i.e. the precursors of α-chaconine and α-solanine, respectively, which are steroid glycoalkaloids characteristic of this plant. The loss of the ability of solanidine galactosylation, observed during purification of the enzyme preparation from potato sprouts [3], could indicate that there were two different enzymes: one of which glucosylates and the other galactosylates solanidine. The results presented [3–5] indicated that the enzyme preparations from potato tubers and sprouts unlike those from tomato leaves, display a markedly higher activity in the glucosylation of solanidine than in its galactosylation. Moreover, there are marked differences in specificity between the enzymes responsible for galactosylation of steroid alkaloids, i.e. solanidine (alkaloid of solanidane type in potato) and tomatidine (alkaloid of spirosolane type in tomato). The enzyme preparation from potato galactosylates both — steroid alkaloids of solanidane type and those of spirosolane type [5], whereas the enzyme preparation from tomato utilizes virtually in the galactosylation process only the alkaloid of spirosolane type.

Table 1

Some properties of UDP-galactose:tomatidine galactosyltransferase from Lycopersicon esculentum leaves

Molecular mass	about 50000 Da
Activator	2-mercaptoethanol
Inhibitors	UMP, UDP, UTP, heavy metals (Zn^{2+}, Hg^{2+}, Cu^{2+}), *N*-ethylmaleimide, Triton X-100, increased concentration of salts (NaCl, NaF)
No effect	Mg^{2+}, Ca^{2+}, Mn^{2+}, EDTA, EGTA

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