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SOLANIDINE METABOLISM IN POTATO TUBER TISSUE SLICES AND CELL SUSPENSION CULTURES

S. F. OSMAN, R. M. ZACHARIUS and D. NAGLAK

Eastern Regional Research Center*, Philadelphia, PA 19118, U.S.A.

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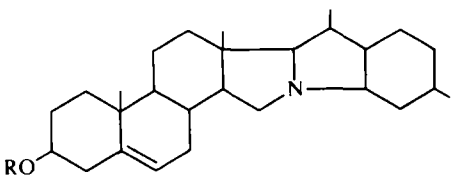
Key Word Index—*Solanum tuberosum*: Solanaceae; solanidine metabolism; glycosylation; glycoalkaloids.

Abstract—Two metabolites have been isolated from potato tuber tissue slices or cell suspension cultures that have been incubated with labeled solanidine. Initially glucosyl solanidine is formed which subsequently is glycosylated to a diglucosyl solanidine.

INTRODUCTION

The biosynthesis of solanidine (**1**), the aglycone of the two major potato (*Solanum tuberosum* L.) glycoalkaloids, α-solanine (**2**) and α-chaconine (**3**) has been, for the most part, elucidated [1]. The aglycone, however, is generally only found in *S. tuberosum* as the glycoalkaloids, α-solanine and α-chaconine. The pathway from aglycone to glycoalkaloid has not been determined. Recently, some evidence has been provided for the enzymatic glucosylation of solanidine by potato tuber tissue [2] and crude enzyme extracts of potato tuber [3]. However, in neither case were metabolites conclusively identified. Similar evidence has been obtained for the glucosylation of solasodine; in addition the conversion of solasodine to the glycoalkaloids solamargine and solasonine by *S. lactiniatum* was also demonstrated [4].

Our interest in potato glycoalkaloids has led us to re-investigate the metabolism of solanidine. As an initial step we wished to confirm that glycosylation of solanidine is a general metabolic pathway. In this report we provide conclusive evidence for the *in vivo* formation of 3-β-*O*-glucosylsolanidine (γ-chaconine) from solanidine by potato tissue. Evidence is also presented for the turnover of the initial glycosylated product to a disaccharide, 3-β-*O*-glucosyl(glucosyl)solanidine which has not previously been demonstrated in this system. While the diglucosyl solanidine compound has not been found in potato tissue, the stepwise glycosylation process reported herein may be indicative of how naturally occurring glycoalkaloids are formed in plant tissue, i.e. via a stepwise addition of sugars.



1 R = H
2 R = Galactose < Glucose, Rhanmose
3 R = Glucose < Rhamnose, Rhamnose

RESULTS AND DISCUSSION

Tritiated solanidine is rapidly consumed when added to aqueous solutions containing potato tuber discs (Fig. 1) or potato cell suspensions. The initial product that is formed under these conditions is, in turn, rapidly metabolized. Quantities of both metabolites were prepared in this manner using labeled solanidine diluted with unlabeled solanidine. Hydrolysis of the initial metabolite (M − 1) yielded solanidine, as determined by GLC, MS and NMR and glucose (GLC, MS). The EI–MS for M − 1 was almost identical to that of γ-chaconine. Differences in the MS are best explained by the difference in mass spectrometer operating conditions which produced an $[M-18]^+$ artefact in the metabolite sample. Since the metabolite

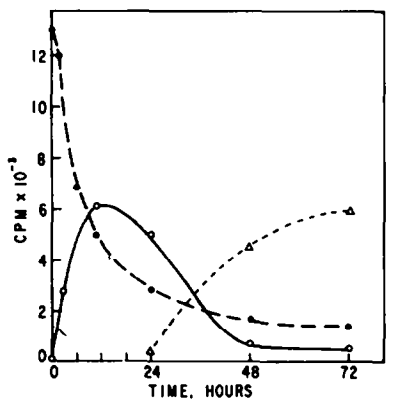


Fig. 1. Metabolism of solanidine by potato tuber slices. (●--●) = solanidine, (○—○) = 3-β-*O*-glucosylsolanidine = M − 1, (△---△) = 3-β-*O*-glucosyl(glucosyl)solanidine = M − 2.

* Agricultural Research, Science and Education Administration, U.S. Department of Agriculture.

M − 1 was also identical in chromatographic behavior and NMR to γ-chaconine, we concluded it was γ-chaconine. The second metabolite (M − 2) could either be produced directly from solanidine or by adding γ-chaconine to water, containing tuber discs. The molecular weight of M − 2, as determined by FD–MS, was 721. The only hydrolytic products of M − 2 were solanidine and glucose; thus M − 2 is a glucose disaccharide of solanidine. The glucose–glucose linkage was not determined.

Although γ-chaconine (M − 1) has been found in *Solanum* tissue, M − 2 has never been reported in the native tissue. Normal glycoalkaloid biosynthesis, which may be highly compartmentalized, would be expected to produce α-solanine and α-chaconine, neither of which contain the glucose–glucose structure.

Incubation of [^{3}H]-solanidine with autoclaved tissue resulted in almost quantitative recovery of solanidine establishing the enzymatic nature of the conversion.

After centrifugation of cell-free potato preparations at 100 000 *g*, enzymatic activity resided in the centrifugate, therefore the glycosylation enzymes are apparently membrane-bound.

The enzymatic glycosylation of solanidine by potato tuber discs [2] and crude enzyme preparations from potato tubers [3] has only been shown indirectly; the characterization of products relied exclusively on solubility and chromatographic behavior. The validity of the identification of products in some of the aforementioned experiments is questionable. Lavintman *et al.* [3], using a crude potato enzyme preparation and [^{14}C]-UDP-glucose, isolated a number of labeled products that were identified as glycosylated solanidines based on TLC analysis. However, solanidine was not added exogenously. The authors conclude that solanidine must have been present in the potato enzyme preparation but this is highly unlikely since free solanidine is rarely found in potato tubers and even if it were present it would have been removed in the partial purification of the enzyme preparation. Liljegren [4] has demonstrated the conversion of solasodine to its 3-β-glucoside by enzyme preparations from *S. lactiniatum* using both [^{14}C]-solasodine and [^{14}C]-UDP-glucose. He also demonstrated the conversion of solasodine to solasonine and solamargine by an excised stem of *S. lactiniatum*. Again, in these experiments, chromatographic behavior of the radioactive products were the main method of characterization. To our knowledge, our results are the first report of the chemical characterization of the solanidine metabolites formed under the conditions described. More importantly, the turnover of M − 1 to M − 2 suggest that 3-*O*-glycosylated products containing more than one sugar can be formed in a stepwise synthesis. In our systems there is a high concentration of glucose (and possibly glucosyl nucleotides) which may account for isolating only glucose-containing metabolites. This hypothesis is supported by preliminary evidence from experiments in this laboratory; the partial replacement of rhamnose for glucose in incubation experiments with suspension cultures results in the formation of a metabolite we have tentatively identified as 3-β-*O*-glucosyl-(rhamnosyl)solanidine.

The high conversion of solanidine to glycosylated products may explain why free solanidine is usually not found in healthy tissue. When it is observed there is good reason to believe that it may be the product of enzymatic hydrolysis of the indigenous glycoalkaloids, α-solanine and α-chaconine [5, 6]. We have not been able to isolate glycoalkaloids from cultured potato callus except for tissues that form roots or root initials [7], and this failure may result from lack of aglycone synthesis, since this study demonstrates that cultured tissues have the capacity to glycosylate solanidine.

EXPERIMENTAL

MS were obtained on a Varian-Mat 311A spectrometer operated in the electron impact or field desorption mode. [^{3}H]-Solanine was obtained from New England Nuclear Corp.; Spectrofluor from Amersham/Searle. TLC plates were supplied by Analtech or E. Merck. ECNSS-M coated Gas Chrom-Q was from Applied Science. 2,4-Dichlorophenoxyacetic acid was from Eastman Kodak. α-Solanine and α-chaconine were isolated from potatoes at this laboratory.

Tuber disc preparation. Katahdin potato tubers were scrubbed with light detergent, held in 1% NaOCl for 20 min and then rinsed with sterile water. Using aseptic conditions, 3 mm slices were cut from which 2.2 cm dia disks were removed with a cork borer. The disks were rinsed in sterile water and rerinsed with a stream of sterile water.

Tissue culture preparation. Callus was initiated from explants of *S. tuberosum* L. vars. Kennebec, Katahdin and Merrimack. Tissue borings were removed under sterile conditions with a No. 2 cork borer and slices of 0.2 and 0.4 mm thickness were selected. The explants were cultured on a modified 0.8% agar medium (RM-1964) of Linsmaier and Skoog [8] in which indoleacetic acid was replaced with α-naphthaleneacetic acid (1.0 mg/l.) and kinetin (0.2 mg/l.) was added. The tissues were incubated at 28° in darkness. The developing calli were transplanted to fresh agar media every 4 weeks. After three transfers a suspension cell culture was developed from callus tissue in 1B5C liquid media [9]. The suspension culture was maintained on a shaker at 150 RPM under illumination at 27°. The tissue was subcultured every 7 days.

Bacterial monitoring. After incubation of the disks or suspension cell cultures with solanidine was completed, the liquid medium was streaked on bacterial TGE plates and incubated at 37° for as long as 4 days.

Preparation of [^{3}H]-solanidine. Tritiated solanine (5×10^6 cpm) diluted with 2 mg of unlabeled solanine was hydrolysed in 1 N H_2SO_4–EtOH (1:1) for 2 hr at 95°. The soln was cooled to room temp., EtOH was removed under a stream of N_2 and the product was diluted with an equal vol. of H_2O. The soln was adjusted to pH 10 with conc NH_4OH and the resultant ppt. was extracted with C_6H_6. The solanidine in this soln was isolated by prep. TLC on Si gel G with $CHCl_3$–MeOH (1:1). The purity of the isolated solanidine was established by MS and mp (220°).

Preparation of γ-chaconine. α-Chaconine (50 mg) was dissolved in 5.0 ml 0.1 N H_2SO_4 and heated for 7 hr at 85°. Aliquots were removed every 2 hr and examined by TLC to determine the extent of hydrolysis. After 7 hr the soln was cooled and the glycoalkaloids were pptd with conc NH_4OH. Pure γ-chaconine was isolated by prep. TLC. Identity of the γ-chaconine was confirmed by MS and mp (250°).

Incubation of solanidine. Routinely 100 000 cpm of [^{3}H]-solanidine and 1–2 mg of unlabeled solanidine were incubated in 75 ml H_2O containing 7–8 g of tissue for the desired time at 28° with constant shaking. For control purposes [^{3}H]-solanidine was also incubated with tuber disks that had been autoclaved for 30 min at 15 psi and 105°. The solanidine was thoroughly dispersed in the aq. soln by sonication before the tissue was added. For kinetic studies individual incubations were made for each point on the curve.

Isolation of solanidine metabolites. The aq. medium, after the required incubation period, was decanted into a separatory funnel and extracted with 2 × 100 ml CH_2Cl_2 to recover unmetabolized solanidine. The tissue and media were extracted 2 × with 100 ml MeOH–$CHCl_3$ (1:1). The MeOH–$CHCl_3$ soln was concd to near dryness *in vacuo* and the residue was taken up in MeOH. Insoluble material was removed by centrifugation and the MeOH soln was analysed for labeled metabolites.

Metabolite analysis. The MeOH fractions were analysed by TLC on Si gel G with the mobile phase MeOH–$CHCl_3$ (1:1) saturated with 1% NH_4OH. After exposure to I_2 vapor the stained zones that were present in samples cultured with solanidine and not present in the controls were taken as fractions. Metabolites were purified by repeated NH_4OH precipitations and by prep. TLC. Fractions from the TLC plate were also measured for radio activity.

Characterization of metabolites. Following hydrolysis in 1 N H_2SO_4–EtOH (1:1) for 3 hr, the metabolite soln was neutralized, concd *in vacuo* and extracted with C_6H_6. The aglycone was identified by GC–MS, NMR and TLC and GLC behavior [10]. An aliquot of the aq. soln from the hydrolysis was concd and the residue was taken up in a pyridine soln of NH_2OH (100 μl) and heated for 0.5 hr at 75°; 100 μl Ac_2O was added and the soln was reheated at 75° for an additional 0.5 hr. The soln was concd and the residue was taken up in CH_2Cl_2. The aldononitriles of the sugars were characterized by GC retention values on ECNSS-M [11].

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