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# INTRACELLULAR DISTRIBUTION OF THE STEROIDAL GLYCOALKALOID α-TOMATINE IN *LYCOPERSICON ESCULENTUM* FRUIT

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**Key Word Index**—*Lycopersicon esculentum*; Solanaceae; tomato; intracellular distribution; steroidal glycoalkaloid; α-tomatine.

**Abstract**—Pericarp tissue from green tomato fruits was homogenized and separated into organelle fractions by differential centrifugation. Tomatine was found mainly in the final (105000*g*) supernatant, with small amounts in the microsomes. Expressed sap from intact tissue was also rich in the alkaloid. It is suggested that tomatine accumulates in the vacuoles and/or soluble phase of the cytoplasm and is possibly synthesized in microsomal organelles.

## INTRODUCTION

It is widely accepted that the sterols of animal cells are components of cell and organelle membranes [1–3], and there is accumulating evidence that plant sterols are similarly located in the plant cell [4, 5]. However, some plants also elaborate other steroids such as alkaloids, sapogenins and cardiac genins [6], and these may accumulate in relatively large quantities in certain organs. It has been calculated that the concentration of α-tomatine in tomato leaves may be as high as 1 mM [7], and such a value raises questions about the location of tomatine within the cell. Although a considerable amount of information exists relating to the distribution of tomatine in the whole plant [8], little is known of its distribution at the subcellular level. This communication describes attempts to provide information on this subject.

## RESULTS

Homogenous tomato tissue was obtained by using pericarp from small and large green fruits. Ripe fruits were not used as these are usually tomatine-free [9]. The organelle composition of the various fractions was determined by electron microscopy. The 500*g* fraction was composed mainly of cell debris, nuclei and nuclear material, and large chloroplasts; the 2500*g* fraction of smaller chloroplasts and dispersed nuclear material; the 16000*g* fraction of mitochondria and proplastids/small chloroplasts; and the 105000*g* fraction of microsomes and small membrane-bound vesicles.

Tomatine was not detected in any of the particulate fractions from large green fruit tissue but was present in the final (105000*g*) supernatant, although in relatively small amounts (Table 1). On the other hand, tissue from small green fruits was much richer in tomatine, most of which (78.6%) was again present in the final supernatant, although smaller amounts were detected in the tissue residue (16.9%) and the microsomal fraction (4.5%) (Table 1). When expressed on a unit protein basis, the tomatine concentration was highest in the final supernatant, but greater in the microsomal fraction than in the tissue residue (Table 1).

Parallel extractions of tomatine from whole pericarp of small and large green fruits revealed alkaloid levels (Table 2) similar to calculated values in fractionation experiments (Table 1). A concentration of tomatine of the same order was also found in sap expressed from whole

Table 1. Tomatine content of fractionated pericarp tissue*

| | Tomatine (mg) | Protein (g) | Tomatine concn (mg/g protein) | No. fruits used | Tissue fr. wt(g) | Tomatine concn (mg/fruit) |
|---|---|---|---|---|---|---|
| Large fruits | | | | | | |
| 105000*g* supernatant | 6.1 | 1.01 | 6.0 | — | — | — |
| Total | 6.1 | — | — | 8 | 520 | 0.8† |
| Small fruits | | | | | | |
| Tissue residue | 33.9 | 10.15 | 3.3 | — | — | — |
| 105000*g* pellet | 9.0 | 0.13 | 69.2 | — | — | — |
| 105000*g* supernatant | 158.0 | 1.12 | 141.1 | — | — | — |
| Total | 200.9 | — | — | 48 | 381 | 4.2† |

* Only those fractions which contained the alkaloid are shown. † Based on pericarp tissue only.

Table 2. Tomatine content of whole pericarp tissue and expressed sap

| | No. fruits | Total tissue fr. wt (g) | Sap vol. (ml) | Tomatine (mg) | Tomatine concn (mg/fruit) | Tomatine concn (mg/ml) |
|---|---|---|---|---|---|---|
| Large fruits | 5 | 334 | — | 3.5 | 0.7* | — |
| Small fruits | 9 | 82 | — | 27.1 | 3.0* | — |
| Sap from small fruits | 26 | 200 | 170 | 68.8 | 2.6† | 0.40 |

* Based on pericarp tissue only. † Based on sap from pericarp tissue.

pericarp tissue of small green fruits (Table 2), although it is by no means claimed that all the sap present in the tissue was expressed or collected.

## DISCUSSION

The results presented here suggest that tomatine is not bound to, or a constituent of, cell or organelle membranes, but is located in the soluble phase of the cell. The possibility that the alkaloid was released from a particulate phase by the homogenization/fractionation procedures tends to be over-ruled by the high concentration of tomatine in sap obtained by the relatively mild method of applying pressure. The high alkaloid concentration of sap (0.4 mM) further points to the vacuoles as being the main site of accumulation, although a certain amount may remain in the soluble phase of the cytoplasm. In view of the hydrophobic nature of the aglycone, the above findings may suggest that glycosylation of tomatidine is, at least in part, a solubilization process.

The absence of tomatine from the 500, 2500 and 16000 *g* pellets, and the fact that all pellets were washed (resuspended) in fresh medium render it unlikely that the alkaloid in the microsomal fraction was carried over from the supernatant. Its presence in this fraction may, on the other hand, reflect its site of synthesis. There is already evidence [10, 11] that plant sterols are synthesized in microsomes, and sterols have further been shown to be involved in the biosynthesis of tomatine [12]. Microsomes have also been suggested as the site of synthesis of the steroidal sapogenin, diosgenin [13]. However, it remains to be ascertained whether or not the small fruits used here were actually capable of tomatine synthesis. The low tomatine levels in large green fruits are probably the result of degradation of the alkaloid, which is known to occur during fruit development [14], but it is not known at what stage biosynthesis ceases or degradation begins.

It is possible that the failure to detect tomatine in the microsomal fraction from large green fruits was due to cessation of alkaloid biosynthesis, but in view of the absence of tomatine from the tissue residue (which contained more total alkaloid than the microsomal fraction in small fruit tissue) and the very small amounts extracted from whole tissue, it is equally possible that tomatine may have been present, but at levels below the limits of detection.

The conclusions drawn here relating to the intracellular distribution of tomatine contrast to some extent with those of Akahori *et al.* [13] for steroidal sapogenins and saponins. These compounds, which resemble steroidal alkaloids in structure and properties, were found to be present mainly in the organelle fractions, and particularly associated with their membranes, with little in the supernatants. Since there are indications that the sterols of membranes may be important in the stability of the lipid bilayer [15] and membrane permeability [16–18], Akahori *et al.* [13] have raised the question of steroidal sapogenins and their glycosides possibly playing a similar role. As yet however, no such functions for these compounds have been demonstrated. The role of tomatine is also uncertain, but of the suggestions which have been put forward [8], almost all are concerned with protection against pathogens and predators by high alkaloid concentrations, and none involve membrane function. To this extent at least, the findings of this work are not incompatible with other reports relating to tomatine.

## EXPERIMENTAL

*Plant material.* Tomato fruits were obtained from glasshouse-grown plants of the cv. Best of All. Small green fruits were normally 20–30 mm diam. and 5–10 g fr. wt and large green fruits, 50–60 mm diam. and 60–80 g fr. wt.

*Fractionation of pericarp tissue.* Tissue from large fruits (520 g) and from small fruits (380 g) was cooled to 4° then cut into small pieces and homogenized in a blender with 300 and 200 ml respectively of ice-cold medium (0.1 M Pi buffer pH 7.4 containing 0.5 M D-mannitol, 0.001 M diNa-EDTA and 0.1% dithiothreitol) for 1 × 1 min and 2 × 30 sec periods. Homogenates were strained through 2 muslin layers and residues washed with 2 × 300 and 2 × 200 ml of medium respectively. Filtrates were centrifuged at 500 *g* for 10 min. The 500 *g* supernatant was then spun at 2500 *g* for 20 min, the 2500 *g* supernatant at 16 000 *g* for 30 min, and the 16 000 *g* supernatant at 105 000 *g* for 90 min. All pellets were resuspended in fresh medium, and a small amount removed for protein analysis. Of the remaining vol. of the resuspended 2500 and 16 000 *g* pellets, half was retained for sonication. The remainder of all resuspended pellets was then centrifuged at previous speeds and these supernatants discarded. All procedures were carried out at 0–4°.

*Sonication of the* 2500 *and* 16000 *g fractions.* The resuspended pellets were treated in a sonic disintegrator at peak output (22 kHz) for 3 min. The suspensions were centrifuged at original speeds and then both supernatants spun at 105000 *g* for 90 min. All pellets were resuspended as before, a small vol. removed for protein analysis, and re-centrifuged. Secondary supernatants were discarded.

*Expressed sap.* Pericarp tissue (200 g fr. wt) from small green fruits was placed in a deep-freeze overnight then cut into small pieces and pressed in a hand-operated screw press. The pH of the sap collected (170 ml) was adjusted to 3 with 0.1 N HCl, after which it was filtered through paper and centrifuged at 105 000 *g* for 90 min to remove particulate matter. The pellet (very small) was discarded and the supernatant retained.

*Extraction of tomatine.* Pellets, still in centrifuge tubes, were extracted with 10 ml 94% MeOH containing 2% HOAc for 16 hr. Tubes were centrifuged at 27 000 *g* for 20 min, supernatants removed and pellets reextracted 2× more for a total

of 24 hr with 10 ml 64% MeOH. Supernatants were combined, reduced to aqueous under vacuum at 45° and the pH adjusted to 10 with conc. $NH_3$. After cooling overnight in a refrigerator, extracts were centrifuged at 27 000 *g* for 30 min. Supernatants were discarded and the pellets washed with 1% $NH_3$ and centrifuged as before. When all traces of $NH_3$ had evaporated, pellets were dried in a $CaCl_2$ desiccator and extracted with 3 × 10 ml MeOH. MeOH extracts were combined, reduced to dryness under vacuum at 35° and taken up in 3 × 2 ml hot EtOH. EtOH extracts were added to a 10 ml volumetric flask which was made to the mark with $H_2O$ and EtOH to give a final EtOH conc of of 96%. A small vol. of all supernatants was retained for protein analysis. Supernatants from sonicated extracts were then adjusted to pH 10, cooled and centrifuged, and pellets extracted as above. The final (105 000 *g*) nonsonicated supernatant was similarly extracted, except that before addition of $NH_3$, the vol. was reduced to *ca* 200 ml under vacuum at 45°. A small amount of the tissue residue was also retained for protein analysis after which the material was extracted as described above for pellets, except that the vol. of extractant used was in the ratio of 2 ml per g fr. wt of tissue. Intact pericarp tissue was extracted by the same method used for homogenized tissue residue. Tomatine was extracted from expressed sap by the method employed for the final supernatant, except that the vol. was reduced to 40 ml.

*Assay of protein and tomatine.* Protein content of pellets and supernatants was determined by the Folin–Ciocalteau method of Lowry *et al.* [19], and tomatine content of extracts by the radioligand method of Heftmann and Schwimmer [20]. The tomatine-precipitating soln was prepared using cholestrol-[4-$^{14}$C] (sp. act. 149 μCi/mg) and the radioactivity remaining in soln was determined by liquid scintillation counting using a dioxan-based liquid scintillator. Counting efficiency was 88% and background 28 cpm. Tomatine was quantified by reference to a calibration graph.

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