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EVIDENCE FOR *O*-ACETYLSERINE IN *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; isolation; amino acid; *O*-acetylserine; cysteine biosynthesis.

Abstract—*O*-Acetylserine, a precursor of cysteine in plants, was isolated from cells of *Nicotiana tabacum* cultured in liquid medium.

In plants, the biosynthesis of cysteine from L-serine apparently involves the intermediary formation of *O*-acetylserine [1–4]. The evidence for this is derived from two facts. First, serine transacetylase, which catalyses the synthesis of *O*-acetylserine from serine and acetyl CoA, is present in a variety of plants [5]. Second, *O*-acetylserine is more readily utilized than serine as a substrate for *O*-acetylserine sulphydrylase, which in the presence of sulphide catalyses the formation of cysteine [1, 4]. Additionally, an enzyme which cleaves *O*-acetylserine to pyruvate, ammonia and acetate has been reported in plants [6]. However, there is no direct evidence that *O*-acetylserine occurs in plants. This paper presents some direct evidence for *O*-acetylserine in higher plant cells.

PC of an extract from cells fed serine-[U-^{14}C] for 1.5 hr reveals that considerable metabolism of serine has occurred (Fig. 1a). The majority of the radioactivity was recovered in serine (Section 6, 7, Fig. 1a) but appreciable radioactivity was present in Section 9 coincident with *O*-acetylserine. *O*-Acetylserine is readily converted to *N*-acetylserine under alkaline conditions, greater than 90% conversion in 5 min at room temperature [5]. Treatment of the original extract with an equal volume of 3M NH_4OH prior to chromatography resulted in the loss of radioactivity from the *O*-acetylserine spot (section 9) and the appearance of additional radioactivity in the position of *N*-acetylserine (sections 15 and 16, Fig. 1b). Because of the metabolism of serine to a variety of compounds the chromatograms of the original extract (Fig. 1a, 1b) did not provide unequivocal evidence for the presence of *O*-acetylserine. Therefore material in section 9 was eluted with acidified ethanol and an aliquot was rechromatographed, when 70% of the radioactivity cochromatographed with authentic *O*-acetylserine, 20% cochromatographed with serine and the remaining 10% ran slightly faster than *O*-acetylserine. A second aliquot of the eluate was mixed with an equal volume of 3 M NH_4OH and rechromatographed, when 65% of the radioactivity cochromatographed with authentic *N*-acetylserine, 25% cochromatographed with serine and the remaining 10% ran slightly faster than *O*-acetylserine. The above evidence for the presence of *O*-acetylserine in these cells was supported by the use of ion exchange chromatography reported in the next section.

The extensive metabolism of serine necessitated that the routine quantitation of *O*-acetylserine be done by treatment of the original extract with sulphonic acid resin wherein *O*-acetylserine is converted to *N*-acetyl-

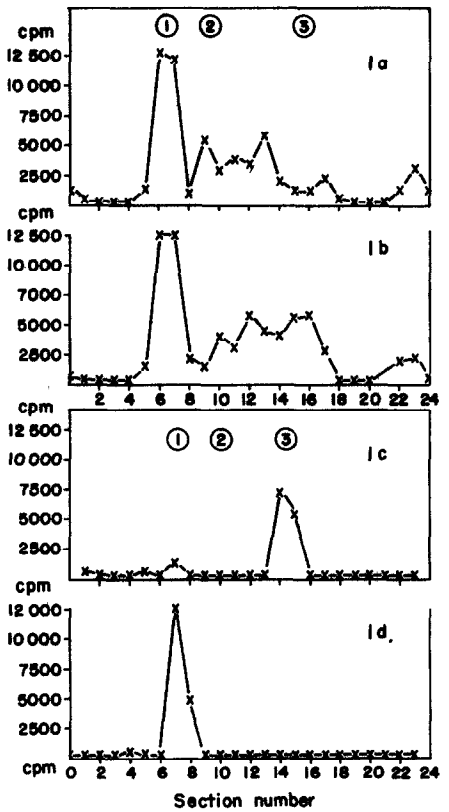

Fig. 1. Paper chromatography of compounds isolated from cultured tobacco cells. Cells were incubated with L-serine-[U-^{14}C] for 1.5 hr; chromatograms were cut into 2 cm sections and radioactivity/section determined. Representation: 1 serine, 2 *O*-Acetylserine, 3 *N*-Acetylserine. 1a. Chromatogram of original extract, 1b. Chromatogram of original extract treated with 3M NH_4OH. 1c. Chromatogram of material reported as *N*-acetylserine in Table 1 (see Experimental). 1d. Chromatogram resulting from hydrolysis of material in 1c.

Table 1. Ion-exchange separation of compounds isolated from cultivated tobacco cells. Cells were incubated with L-serine-[U-^{14}C] for the periods indicated; results obtained from 4 separate experiments are expressed in cpm/mg fr. wt of tissue and S.D. is shown

Fraction	Incubation time (hr) 1.5	3	6
Cell residue	105±13	375±76	876±173
Extract			
Not retained by Dowex 50 H^+	48±8	243±29	904±76
Retained by Dowex 50 H^+	311±51	1176±22	3170±292
Dowex 50 eluate			
Not retained by Dowex 50 H^+	15±2*	22±3*	44±6*
Retained by Dowex 50 H^+	297±49†	1082±17†	3080±256†

* *N*-acetylserine derived from *O*-acetylserine. † Primarily serine.

serine (see Experimental). Cells which had transported serine for either 1.5, 3 or 6 hr were used to isolate *N*-acetylserine (Table 1). A time dependent incorporation of radioactivity into the cell residue was observed, possibly indicating serine incorporation into protein or its conversion to ethanolamine and incorporation into membranes. More than 75% of the transported serine was recovered in the cell extract and most of this material was retained by Dowex-50 H^+ and is serine (Fig. 1a). A considerable metabolism of serine to anionic or neutral compounds is indicated by the radioactivity not retained by the cation exchange resin. The fraction eluted from Dowex 50-H^+ was passed through a second cation exchange column and a small fraction was not retained; this material cochromatographed with authentic *N*-acetylserine (Fig. 1c). Hydrolysis of this material with 3 M HCl produced a single compound which cochromatographed with serine (Fig. 1d).

Based on a counting efficiency of 66% and a sp. act. of 0.25 μCi/μmol of the original serine, the 44000 cpm/g fr. wt (Table 1) represents an *O*-acetylserine content of at least 120 nmol/g fr. wt. If the *O*-acetylserine is restricted to the cytoplasm in these highly vacuolated cells and if some serine was originally present in the pool from which the *O*-acetylserine was synthesized the above figure indicates a relatively high concentration of this compound in the cytoplasm of the cells.

In conclusion a variety of plants contain an enzyme for the synthesis of *O*-acetylserine [2–4], *O*-acetylserine is present in tobacco cells in substantial amounts and *O*-acetylserine is preferred to serine as a substrate for *O*-acetylserine sulphydrylase in the synthesis of cysteine [1, 4].

EXPERIMENTAL

Absorption of ^{14}C-serine. Tobacco XD-cell line (*Nicotiana tabacum* L. var. Xanthi) were grown in B-5 medium [7] for 6 days, when the cell density was 10 g cells/80 ml of medium. Cells were harvested by vacuum filtration and washed with 100 ml of 1% sucrose. The washed cells (0.4–0.6 g fr. wt) were placed in a 250 ml conical flask containing 80 ml of medium composed of 30 mM sucrose, 0.5 mM $CaCl_2$, and 0.1 mM L-serine-[U-^{14}C] (sp. act. 0.25 μCi/μmol). The flasks were stoppered with cotton plugs and placed on a rotary shaker at 25°. Following incubation cells were harvested by vacuum filtration and washed with 1 mM L-serine. These cells were placed in 20 ml of 70% EtOH containing 0.1 mM HCl and stored at −15° for up to 3 weeks without detectable changes in cellular components.

Isolation of O-acetyl serine. Cells were disrupted ultrasonically in acidified EtOH and the cellular debris sedimented by centrifugation. To demonstrate the presence of *O*-acetylserine, an aliquot of the supernatant was chromatographed on paper with *n*-BuOH–HOAc–H_2O (12:3:5) [5]. For routine quantitation *O*-acetylserine was converted to *N*-acetylserine. An aliquot of the supernatant was passed through a column (1 × 3 cm) of Dowex 50-H^+ which had been equilibrated with 70% EtOH containing 0.1 mM HCl; the column was washed with 10 ml of acidified EtOH and the cation fraction eluted with 10 ml of 3M NH_4OH in 70% EtOH. Serine and *O*-acetylserine are absorbed by the resin. However, elution with NH_4OH converts *O*-acetylserine to *N*-acetylserine [5], which is no longer absorbed by Dowex 50-H^+. The eluate was air dried at 30°, the residue was dissolved in 5 ml of acidified EtOH and column chromatography repeated. An aliquot of the fraction that was not retained on the resin was chromatographed on paper to demonstrate the presence of *N*-acetylserine. *N*-acetylserine was hydrolyzed to serine with 3M HCl in an autoclave at 1 kg/sq cm for 2 hr. The radioactive material on chromatograms was quantified by liquid scintillation; the chromatogram was cut into 2 cm sections and placed in a scintillation vial with 2 ml of acidified EtOH. After 4 hr, 15 ml of liquid scintillation cocktail was added and the vials vigorously shaken.

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