THE SYNTHESIS OF O-ACETYLSERINE BY EXTRACTS PREPARED FROM HIGHER PLANTS[1]

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Received May 19, 1969

SUMMARY: This communication describes a novel plant enzyme, which catalyzes the formation of O-acetylserine from serine in the presence of S-acetylCoA. The enzyme activity was shown to be present in both the particulate and soluble fractions of a range of plant seedlings and leaves.

The enzymatic synthesis of cysteine from O-acetylserine and sulfide (reaction 1) has been reported in Escherichia coli, yeast, and Neurospora (Wiebers and Garner, 1967).

O-acetylserine + sulfide ⟶ cysteine + acetate (1)

Cysteine synthase present in higher plant extracts also apparently utilizes O-acetylserine more readily than serine as a sulfide acceptor (Giovanelli and Mudd, 1967, 1968; Thompson and Moore, 1968).

An enzyme, serine acetylase, which catalyzes the acetylation of serine (reaction 2) has been reported in bacteria (Kredich and Tomkins, 1966).

S-acetylCoA + serine ⟶ O-acetylserine + CoASH (2)

The present communication reports the occurrence of serine acetylase in higher plant extracts.

EXPERIMENTAL: Seeds were soaked overnight in distilled water and grown in vermiculite at 30°C. Seedlings (50 g.) were ground in a mortar with

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[1]This work was supported in part by the Sulphur Institute, Washington, D. C., and London, England.

[2]Department of Agronomy, Paper No. 856.

sand using two volumes of 0.3 M mannitol containing 0.1% bovine serum albumin, 0.05% cysteine, and 1 mM ethylenediaminetetracetate, adjusted to pH 7.2. The extract was separated into supernatant and mitochondrial fractions using the procedure of Bonner (1965). The supernatant fraction was concentrated by the addition of solid ammonium sulfate (final concentration 70% saturation); the precipitate was collected by centrifugation and dissolved in 0.002 M potassium phosphate buffer, pH 7.5, (20 ml) containing 0.1 mM dithioerythritol. The mitochondrial pellet was suspended in 10 ml of the same buffer. Both fractions were dialyzed overnight against 0.002 M potassium phosphate buffer, pH 7.5 (2 x 3 liters) containing 0.1 mM dithioerythritol.

The assay of serine acetylase activity (reaction 2) is based on the observation that the product of the reaction, O-acetylserine, is readily converted to N-acetylserine by treatment with alkali (Nagai and Flavin, 1967). Radioactive serine was used as a substrate and was separated from N-acetylserine by absorption on sulfonic acid resin in the hydrogen form. The complete reaction mixture contained the following components (in μmoles) in a final volume of 1.1 ml, potassium phosphate, pH 7.5 (60), S-acetylCoA (1), 0.5 μc U-^{14}C-serine (0.5), and protein equivalent to 1 g. fresh weight of tissue.

Reaction mixtures were incubated for 1 hour at 30°C in stoppered tubes, and the reaction terminated by the addition of three volumes of 90% ethanol. Precipitated protein was sedimented by centrifugation and discarded. The supernatant solution was evaporated to dryness at room temperature, and the residue was redissolved in either 0.3 ml of water (control) or 0.3 ml of 3 N ammonium hydroxide and left at room temperature for 1 hour. An aliquot of this solution was applied to a column of sulfonic acid resin (0.9 x 6 cm) in the H^+ form. N-acetylserine was washed from the column with distilled water (20 ml) and an aliquot counted by liquid scintillation.

The product of the reaction was identified by paper chromatography of the reaction mixture with authentic compounds. Duplicate descending chromatograms of each treatment were developed for 16 hours in butanol: acetic acid:water (12:3:5, by volume). Serine and O-acetylserine were located by spraying one chromatogram with ninhydrin (1% in 95% ethanol). The second chromatogram was sprayed lightly with 0.1 N HCl, placed in a closed jar containing a beaker of 3 N HCl, and incubated for 1 hour at 80° C. This procedure hydrolyzed N-acetylserine to serine. The chromatogram was air dried and briefly placed in an ammonia atmosphere to neutralize residual HCl. N-acetylserine (as serine) was located by spraying with ninhydrin. To determine the radioactivity of chromatographed products, the compounds were located using a strip counter. The radioactive spot was excised and eluted with water in a counting vial; radioactivity was determined by liquid scintillation.

Table I. Assay of Serine Acetylase Activity Present in a Supernatant Fraction of Kidney Bean

	Production of OAS* and NAS* from Serine (mμmoles/g. fresh wt./hr.)					
	Determined by Paper Chromatography				Determined by Ion-Exchange Chromatography	
	Minus NH_4OH		Plus NH_4OH**		Minus NH_4OH	Plus NH_4OH**
Reaction Mixtures	OAS	NAS	OAS	NAS	NAS	NAS
Complete	39.6	2.0	3.8	34.8	1.8	39.8
" With Boiled Enz.	-	0.3	-	0.4	1.8	2.0
" Minus Enzyme	-	2.0	-	3.8	7.4	7.4
" Minus AcetylCoA	-	0	-	0	0	0

Incubation conditions are given in text.
*OAS = O-acetylserine; NAS = N-acetylserine.
**NH_4OH used to shift O-acetylserine to N-acetylserine.

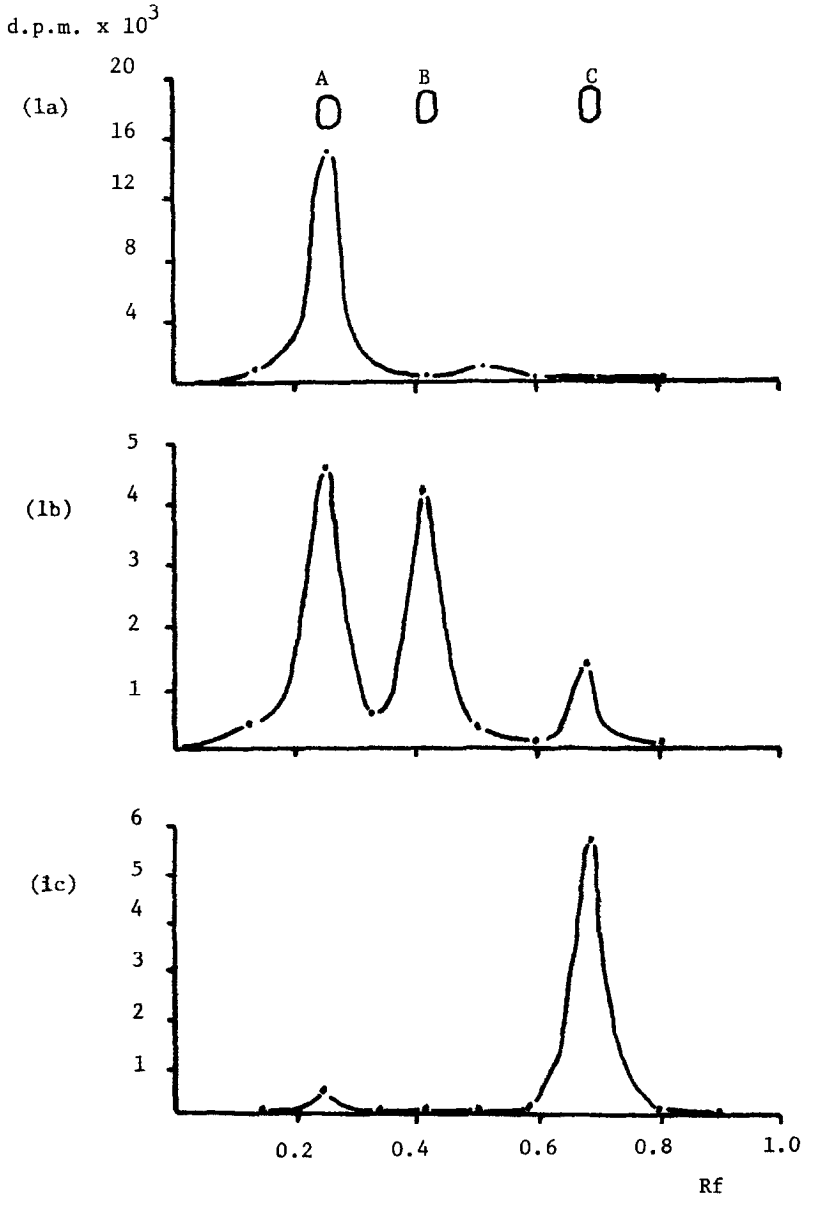

Figure 1. Paper chromatographic separation of serine, O-acetylserine, and N-acetylserine.

Compounds A, B, and C are authentic samples of serine, O-acetylserine and N-acetylserine, respectively.

1a. Complete reaction mixture minus S-acetylCoA.

1b. Complete reaction mixture.

1c. Complete reaction mixture treated with NH_4OH and material not retained by Dowex 50 H^+ chromatographed.

RESULTS: Extracts from 5-day-old kidney bean seedlings proved particularly suitable for the demonstration of serine acetylase activity, because they contain no serine deaminase in the supernatant fraction. In the presence of the standard incubation mixture, extracts catalyzed the acetylation of serine. O-acetylserine was identified as the product of the reaction by paper chromatography; the Rf's of serine, O-acetylserine, and N-acetylserine, were 0.25, 0.41, and 0.68, respectively. The major radioactive reaction product co-chromatographed with O-acetylserine (Table I, Fig. 1-b); a small quantity of N-acetylserine was also present due to O to N shifting of the acetyl group. Treatment of the same reaction mixture with alkali (NH_4OH) resulted in a greater than 90% conversion of O-acetylserine to N-acetylserine (Table I, Fig. 1-c). Acetylation was routinely assayed using ion exchange chromatography; the material not retained by a sulfonic acid resin (H^+ form) co-chromatographed with authentic N-acetylserine (Table I). There is some evidence for a non-enzymatic acetylation of serine, which is favored by high pH.

Serine acetylase activity was demonstrated in a variety of seedlings and leaves (Table II) and was present in both the particulate and supernatant fractions; whereas serine deaminase activity was always associated with the particulate fraction. The distribution of serine acetylase may indicate either that it is loosely bound to the mitochondria or is soluble and is adsorbed by the mitochondria during extraction. Since acetylCoA is a substrate, the former explanation would seem more logical. The apparent decrease in serine acetylase activity in 6-day-old barley seedling mitochondria (Table II) is probably due to substrate competition from serine deaminase, which is particularly high in this fraction.

DISCUSSION: Although serine acetylase has been described in bacteria (Kredich and Tomkins, 1966), this is the first report of its presence in higher plants. Evidence that O-acetylserine was a more active substrate for cysteine synthase than serine raised the possibility that

Table II. Acetylation of Serine Catalyzed by Extracts from Various Higher Plant Sources

Plant Material	O-Acetylserine Produced (mμmoles/g. fresh wt./hr.)	
	Particulate Fraction	Supernatant Fraction
Seedlings		
3-Day-Old Barley	6.3	9.6
5-Day-Old Barley	20.5	1.6
6-Day-Old Barley	7.4	0
5-Day-Old Lupin	14.6	9.5
5-Day-Old Pea	10.9	17.6
5-Day-Old Kidney Bean	37.9	51.9
Leaves		
Swiss Chard	4.7	15.7
Turnip	5.5	1.3
Radish	5.4	3.0

Reaction carried out as indicated in the text.

O-acetylserine may be formed in higher plants (Giovanelli and Mudd, 1967, 1968; Thompson and Moore, 1968). The demonstration of serine acetylase provides a mechanism for O-acetylserine production and indicates that this compound may be a constituent of plants. Furthermore, O-acetylserine may be the normal or principal precursor of cysteine, although there is no direct evidence.

ACKNOWLEDGMENT: The authors wish to acknowledge the excellent technical assistance of Miss Anna-Marie Muenster.

REFERENCES

Bonner, W. D. (1965) In Plant Biochemistry, Eds. J. Bonner and J. E. Varner, Academic Press, N. Y., p. 97.

Giovanelli, J., and Mudd, S. H. (1967) Biochem. Biophys. Res. Commun. 27, 150.

Giovanelli, J., and Mudd, S. H. (1968) Biochem. Biophys. Res. Commun. 31, 275.

Kredich, N. M., and Tomkins, G. M. (1966) J. Biol. Chem. 241, 4955.

Nagai, S., and Flavin, M. (1967) J. Biol. Chem. 242, 3884.

Thompson, J. F., and Moore, D. P. (1968) Biochem. Biophys. Res. Commun. 31, 281.

Wiebers, J. L., and Garner, H. R. (1967) J. Biol. Chem. 242, 5644.