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SHORT COMMUNICATION

DATURA TISSUE CULTURES: ARGINASE, TRANSAMINASE AND ESTERASE ACTIVITIES

ANTONIN JINDRA* and E. JOHN STABA

Department of Pharmacognosy, College of Pharmacy, University of Nebraska, Lincoln, Nebraska, U.S.A.

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Abstract—In enzyme preparations from *Datura stramonium* tissue cultures arginase, transaminase and esterase activities were detected using arginine, ornithine, phenylalanine, tropine and tropic acid as appropriate substrates for the specific reactions.

INTRODUCTION

THE biosynthetic scheme of hyoscyamine and other tropane alkaloids in *Solanaceae* plants involves several reaction steps.[1–3] As some of these reactions are assumed to be enzymatically catalyzed, enzyme studies have been reported on the hydrolysis of arginine,[4] the transformation of ornithine to a pyrroline derivative[5–8], and the esterification of basic and acidic portions of alkaloid molecules.[9] In several plant tissue cultures enzyme activities have been detected.[10,11] We now report that preparations of *Datura stramonium* L-(USDA-5450)[12] seed callus grown as suspension cultures contain arginase, transaminase and esterase activities.

RESULTS AND DISCUSSION

Arginase Activity

In *Datura* suspension cell extracts the arginase activity was detected *in vitro* by the amount of urea hydrolyzed from the substrate arginine. The enzyme unit is defined as the milimicromoles of urea released in 60 min from 1.0 ml 0·1 M L-arginine hydrochloride at pH 9·5 and 40°.

* NSF Senior Foreign Scientist Fellow (YF-1965) from Department of Biochemistry and Microbiology, Faculty of Pharmacy, Bratislava, Czechoslovakia.

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TABLE 1. ARGINASE ACTIVITY IN *Datura* SUSPENSION CELL EXTRACTS*

	Hours of incubation					
	6	12	18	24	36	48
Enzyme unit†	0	0	194±0·016	223±0·012	236±0·009	217±0·018

* Tissue age: 15 days.
† Average of five experiments.

Urea first appeared after 18 hr of incubation and the concentration present was studied for 48 hr. The extract alone, and the arginine solution without extract, did not contain urea after 48 hr of incubation. Extracts denatured by heat (100°) did not show any activity.

The arginase activity values found in suspension cells (Table 1) correspond to the activities reported previously for *D. stramonium* roots.[4] Frozen extracts retained the activity for two weeks.

Transaminase Activity

Dialyzed extracts from *Datura* suspension cells fortified with pyridoxal phosphate and L-ketoglutaric acid demonstrated ornithine-2-oxoglutarate and phenylalanine-2-oxoglutarate transaminase activities. The enzyme unit is defined as the milimicromoles of glutamic acid formed in 120 min from 1 g wet weight of tissue. The dialyzed extracts did not contain any endogenous glutamic acid after 2-hr incubation. In experiments with dialyzed extracts and cofactors, but without exogenous amino acids, no formation of glutamic acid could be detected. The incubation of cofactors and exogenous amino acids, but without extracts, formed only traces of glutamic acid which could not be evaluated quantitatively. Extracts denaturated by heat (100°) did not show any activity.

The transaminase activities found in suspension cells (Table 2) were much lower than those described for *Datura* plants.[13] Frozen extracts lost their activity so that after one week only 50–60 per cent of the original transaminase activity were present.

TABLE 2. TRANSAMINASE ACTIVITY IN *Datura* SUSPENSION CELL EXTRACTS*

Substrate	Enzyme unit† (mμmoles Glu/1g wet wt./120 min)
Phenylalanine	140±0·011
Ornithine	120±0·014

* Tissue age: 28 days.
† Average of three experiments.

Esterase Activity

Concentrated *Datura* suspension cell extracts catalyzed the formation of hyoscyamine from tropine and tropic acid. The *in vivo*[14] and *in vitro*[9] formation of tropane alkaloids from

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such components in plants has been previously reported. The *in vitro* synthesis from *Datura* cell extracts occurred only in the presence of the essential cofactors adenosine triphosphate and coenzyme A. After 24, 36, 48-hr incubation at pH 7 120, 260, 315 μg of hyoscyamine respectively were found. Samples incubated at pH 5·3, heat denaturated preparations, or those without cofactors showed no alkaloid formation. Recently the *in vivo* transformation of tropine and tropic acid precursors into hyoscyamine and scopolamine by *Datura* suspension cultures were proven.[15]

EXPERIMENTAL

Arginase Activity

Fourteen and 27 days old *Datura stramonium* L. (USDA-5450) seed callus cultures grown on modified Murashige's and Skoog's tobacco medium[16] were used to prepare the extract. The tissue was filtered from the medium and washed with ice-cold water on a Buchner funnel. The tissue was then removed and ground with purified sand. The resultant material was squeezed through a nylon cloth and the extract obtained used for the experiments immediately or was frozen. The procedure was performed at 0–4° and 50–55 ml was obtained from 100 g wet weight of tissue. In each experiment the mixture contained 1·0 ml of extract and 1·0 ml of 0·1M arginine solution (2·1067 g of L-arginine hydrochloride was dissolved in 11·54 ml of 1 N NaOH; sufficient Sørensen's glycine-NaOH buffer solution pH 9·5 was added to prepare 100 ml solution) and was incubated from 6 to 48 hr at 40° in glass tubes closed by cotton. After the incubation 2·0 ml of meta-phosphoric acid solution (24 per cent) was added and the samples frozen. For the analytical procedure the material was thawed, centrifuged (approx. 3,000 rev/min), and 2·0 ml of the clear supernatant used. The urea content was determined by a modified photometric method[4, 17] in which a color developed with α-isonitrosopropiophenone in an acidic milieu was measured at 530 nm. The standard curve for urea was determined with known concentrations of urea in non-incubated extracts.

Transaminase Activity

The extracts were prepared from 28 days-old *Datura* suspension cultures as described for the arginase activity experiment. They were then dialyzed for 48 hr at 0–4° against Sørensen's phosphate buffer solution (pH 8·0). The dialyzed extract was used immediately or frozen. In each experiment the mixture contained 2·5 ml extract, 0·5 ml L-ketoglutaric acid (20 μmoles), 0·5 ml ornithine or phenylalanine (20 μmoles), 0·5 ml pyridoxal phosphate (1 μmole) and 1·0 ml phosphate buffer solution (pH 8·0). The mixture was incubated for 120 min at 40° in glass tubes closed by cotton. The clear portion of the incubation mixture (200 μl) was analyzed for glutamic acid by quantitative paper chromatography and photometry.[18]

Esterase Activity

Extracts from 27 or 31 day-old *Datura* suspension cultures were prepared in the same way as described for the arginase activity experiment. The extracts were then centrifuged for 3 min at approx. 3000 rev/min and lyophylized. The lyophylized residue was dissolved in

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redistilled cold water to obtain a concentrate ten times greater than the original extract and used immediately. The incubation mixture contained 1·0 ml enzyme preparation, 0·25 ml 0·03M tropic acid (1,240 μg), 0·25 ml 0·03M tropine (1,050 μg), 0·5 ml citrate-phosphate buffer solution pH 7·0 and 10 mg ATP and 0·3 mg coenzyme A. In each glass two drops of toluene were added as a preservative and the contents incubated at 30°. After 6, 12, 24, 36, 48 hr the samples were analyzed for hyoscyamine by thin layer chromatography[12] and quantitative paper chromatography.[19]

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