The Effects of Amino Acids on the Growth and Glutamic Acid Decarboxylase Activity of Tobacco Cell Cultures

Sir:

The content of γ -aminobutyric acid (γ -AB) in the callus tissue of tobacco is very high, and glutamic acid decarboxylase (GAD), which catalyzes the formation of γ -AB from glutamate, is very active.¹⁾ In the course of the systematic examination for the growth conditions of tobacco cell cultures, we examined the effects of each amino acid and some plant hormones on the growth and the activity of GAD.

The tobacco callus tissue used in the present experiment was obtained from the stem of *Nicotiana tabacum* L. var. *Bright Yellow*, and was subcultured for more than two years in the liquid medium. For the examination of the effects of each amino acid, Linsmaier and Skoog inorganic salt medium²⁾ supplemented with 2,4-D (0.2mg/liter), thiamine-HCl (0.4 mg/liter), and sucrose (3%), was used. The cells were grown in 500 ml Erlenmeyer flasks in the medium and inoculum with a total volume of 110 ml, dispensed in a 500 ml Erlenmeyer flask containing 1 mM of one of the amino acids to be tested. The flasks were incubated at 30°C in the dark on a reciprocal shaker. After seven-days culture, the cells were harvested by filtration through nylon cloth and weighed for the fresh weight determination. The data presented in Table I are the mean values of five replicates. Glycine, aspartic acid, asparagine, and tyrosine inhibited the growth of tobacco cells by 25~30%. On

TABLE I. EFFECTS OF AMINO ACIDS ON GROWTH AND ON GLUTAMIC ACID DECARBOXYLASE ACTIVITY OF TOBACCO CELLS

Amino acid	Fresh weight	Glutamic decarboxylase activity of the cells grown in 1 mM amino acid	
		per gram fresh weight	per culture
None added	100 ^{a)}	100 ^{b)}	100 ^{c)}
Aspartate	75	105	79
Threonine	78	123	96
Serine	80	103	82
Asparagine	76	107	81
Glutamine	99	110	109
Proline	88	108	95
Glutamate	91	112	102
Glycine	70	77	54
Alanine	85	87	74
Valine	88	105	92
Cysteine	94	89	84
Methionine	87	67	58
Isoleucine	90	83	75
Leucine	89	80	71
Tyrosine	75	100	75
Phenylalanine	98	81	79
Ornithine	94	73	69
Lysine	92	64	59
Histidine	97	100	97
γ -Aminobutyrate	100	103	103
Arginine	91	117	106

Data are presented as percentage of the control.

^{a)} The fresh weight of the control tissue was 27.4 g/110 ml culture.

^{b)} γ -Aminobutyric acid formed in 30 min per gram fresh weight was 11.3 μ moles.

^{c)} γ -Aminobutyric acid formed in 30 min per 110 ml culture was 310 μ moles.

1) A. Koiwai, M. Noguchi and E. Tamaki, *Phytochemistry*, (in press).

2) E. M. Linsmaier and Skoog, *Physiol. Plant.*, **18**, 100 (1965).

the other hand, glutamine, γ -AB, phenylalanine, and histidine had no effect on the growth.

Other investigators have examined the effects of amino acid on the cell cultures using ammonia-less nitrogen medium and showed more remarkable inhibition or acceleration of the growth by amino acids.³⁻⁵⁾ The medium used in the present experiment contained much ammonia and nitrate nitrogen, and therefore, the cells might not be so remarkably influenced by amino acids.

For the assay of GAD, cells were homogenized in a mortar in cold 0.08 M phosphate buffer, pH 5.8. The homogenate was centrifuged in a refrigerated centrifuge at $12,000 \times g$ for 15 min and the supernatant was used as the enzyme solution. The reaction mixture contained 0.5 ml of the enzyme solution and 0.5 ml of substrate solution (1% solution of sodium glutamate and 0.1 mM of pyridoxal phosphate) and 1.0 ml of phosphate buffer (0.08 M, pH 5.8). After incubation for 30 min at 37°C, 0.5 ml of 1 N HCl solution was added to the tube and the tube was immersed in boiling water for five min. The content of γ -AB formed was determined by a Beckman Model 120C Amino Acid Analyzer.

The activity of GAD of the cells grown in the presence of each amino acid (1 mM) is shown in Table I. Glycine, methionine, lysine, and ornithine showed significant repression on GAD. The inducible effect on GAD by amino acids was not certain.

The effect of each amino acid on the catalytic activity of GAD is also observed and the results are summarized in Table II. In this case, the cells were grown in the absence of amino acids, and the assay of the enzyme was performed in the presence of 3 mM of each amino acid. Aspartic acid and tyrosine inhibited the activity remarkably.

TABLE II. EFFECTS OF AMINO ACIDS ON THE CATALYTIC ACTIVITY OF GLUTAMIC ACID DECARBOXYLASE (GAD) OF TOBACCO CELLS

Amino acid	GAD activity assayed with 3 mM amino acid
None added	100
Aspartate	42
Threonine	93
Serine	98
Asparagine	88
Glutamine	95
Proline	92
Glutamate	72
Glycine	103
Alanine	108
Valine	97
Cysteine	98
Methionine	110
Isoleucine	103
Leucine	106
Tyrosine	48
Phenylalanine	104
Ornithine	100
Lysine	108
Histidine	97
γ -Aminobutyrate	97
Arginine	89

Data are presented as percentage of the control.

Other amino acids had no effect on the catalytic activity of GAD.

The effects of some plant hormones on the growth and GAD is shown in Table III. The higher level of 2,4-D content (2 mg/liter) showed lower culture yield by fresh weight basis, although no effect was observed on the yield by dry weight basis. Therefore, the cells grown in a higher level of 2,4-D seem to be compact containing a higher percentage of solid material. GAD activity was increased by high level of 2,4-D or NAA.

GAD has been reported to be present largely in non-particulate cytoplasmic fraction

3) P. Filner, *Biochim. Biophys. Acta*, **118**, 299 (1966).

4) K. W. Joy, *Plant Physiol.*, **44**, 845 (1969).

5) O. L. Gamborg, *ibid.*, **45**, 372 (1970).

TABLE III. EFFECTS OF PLANT HORMONES ON GROWTH AND ON GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY OF TOBACCO CELLS

Hormone	FW/culture (g)	DW/culture (g)	GAD activity γ -AB formed in 30 min	
			(μ moles/g FW)	(μ moles/culture)
2,4-D 0.2 mg/liter	37.9	1.52	4.2	159
2,4-D 0.2 " }	38.4	1.51	4.7	180
Kinetin 0.2 " }				
2,4-D 2 " }	29.1	1.55	7.6	221
2,4-D 2 " }	27.9	1.43	10.4	290
Kinetin 0.2 " }				
NAA 2 " }	35.5	1.53	6.3	224
NAA 2 " }	35.2	1.41	7.7	271
Kinetin 0.2 " }				

FW: Fresh weight, DW: Dry weight, γ -AB: γ -Aminobutyric acid, 2,4-D: 2,4-Dichlorophenoxy acetic acid, NAA: Naphthaleneacetic acid.

in other plant cells,^{6,7)} and we confirmed that also in tobacco cells the enzyme is present in the soluble fraction. Ohno and Okunuki⁸⁾ have investigated the inhibition of GAD systematically and shown that popular amino acids had no effect on GAD activity. In the present experiment, aspartic acid and tyrosine

showed remarkable inhibition and cells grown in a high level of 2,4-D or NAA had more increased GAD activity than those grown in a low level of 2,4-D. Further studies are in progress.

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