Studies on the Biosynthesis of Theanine in Tea Seedlings

Incorporation of Glutamic Acid-1-¹⁴C into Theanine

Sir:

Theanine (N-Ethyl- γ -glutamine) was first found in tea leaves by Sakato¹⁾. This amino acid is also present in *Xerocomus badius*²⁾. N-Ethyl- β -asparagine was detected in *Ecbalium*³⁾. The authors have intended to study the biochemical significance of ω -carboxyl groups of monoaminodicarboxylic acids and have thought it interesting to clarify the mechanism of theanine biosynthesis.

Theanine is formed in large quantities during the germination of tea seeds. Using these seedlings, the following isotopic experiments have been performed.

Seven day-seedlings were incubated in a medium (pH 5.0) containing DL-glutamic acid-1-¹⁴C (1.8×10^{-3} M, $1.6 \mu\text{C}/\text{ml}$) and ethylamine-HCl (10^{-2} M); Sample A, or DL-glutamic acid-1-¹⁴C (1.8×10^{-3} M, $1.6 \mu\text{C}/\text{ml}$) alone; Sample B, in the dark at 20°C for three days. Amino acid fractions were prepared from both incubated seedlings by a method similar to that described previously⁴⁾.

Fig. 1 shows an example of radioautograms and paper chromatograms obtained. As seen in this figure, radioactive theanine was formed remarkably from glutamic acid-1-¹⁴C.

Distribution of radioactivities in amino acids was determined by a G.M counter and is shown in Table I.

The above results clearly show that theanine is derived from glutamic acid-1-¹⁴C. Moreover, the addition of ethylamine into

FIG. 1. Incorporation of Radioactivity into Theanine from Glutamic Acid-1-¹⁴C (Sample A).

- (a) Radioautogram (time of exposure; four days)
(b) Chromatogram treated with ninhydrin after preparation of the radioautogram

1, Glutamic acid, 2, Glutamine, 3, Theanine
Solvent used: Phenol-Water (4:1, NH₃) firstly.

n-Butanol-Acetic acid-Water (4:1:1), secondly.

the incubation medium increased the incorporation of radioactivity into theanine predominantly. This result may suggest the participation of ethylamine in theanine biosynthesis, though other physiological factors should be considered. It is interesting that ethylamine was found in higher plants⁵⁾.

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TABLE I. DISTRIBUTION OF RADIOACTIVITIES

	Sample A		Sample B	
	cpm	%	cpm	%
Theanine	470	43	60	17
Glutamine	130	12	80	22
Glutamic acid	170	15	150	42
Total activity	1,110		360	

Radioactivities in amino acids were determined by a G.M. counter on two-dimensional chromatograms obtained as described in Fig. 1 (Definite amounts of each sample (25 μ l) prepared by the same way were applied). The total activity was measured at the starting point before development.

Isotopic and enzymatic experiments on this problem are in progress.

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