Studies of the Effect of Tenuazonic Acid on Plant Cells and Seedlings

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The biological and biochemical studies of the effect of tenuazonic acid on plant cells and seedlings were carried out. Tenuazonic acid exhibited a conspicuous stunting effect on the seedling-growth of rice plant, mung bean, radish and turnip, and on the growth of suspension cultured cells of soybean and rice plants. Tenuazonic acid exhibited no effect on the O_2 -uptake and the activity of SH-enzyme of the plant, but inhibited the incorporation of ^{14}C -leucine into the protein fraction and that of ^{14}C -adenine into nucleic acid fraction of suspension cultured soybean cells as well as these uptake into the cells. And then it has been proved that these incorporation-inhibitions were not merely due to the inhibition of ^{14}C -leucine and ^{14}C -adenine uptake into the cells but based on the intrinsic inhibition of protein and nucleic acid syntheses, respectively.

The symptom of blast disease suggests that the blast fungus (*Piricularia oryzae* Cavara) produces some effective toxic substances. In 1954, Tamari and Kaji¹⁾ isolated piricularin and α -picolinic acid from the culture broth of blast fungus and demonstrated their presence in the stunted rice plants affected with blast disease. They also isolated coumarin^{2,3)} from severely blast-diseased rice plants and revealed that these three toxins might play an important role in disease development.

The authors⁴⁾ and Iwasaki *et al.*⁵⁾ have recently isolated tenuazonic acid (T.A.) from the culture broth of blast fungus. The authors⁶⁾ have also isolated this acid from blast-diseased rice plants and demonstrated that this acid was one of the principal vivotoxins of blast disease. Application of T.A. to a small mechanically wounded area of rice leaf, made with punchlike instrument, caused a necrotic spot similar to that of blast disease.⁴⁾ On the other hand, Mikami *et al.*⁷⁾ isolated T.A. as a vivotoxin of tobacco brown-spot disease.

T.A. was first isolated as an antitumor agent from the culture broth of *Alternaria tenuis* by Rosett *et al.*⁸⁾ Shigeura and Gordon⁹⁾ report-

ed that T.A. inhibited the incorporation of amino acid into protein with Ehrlich ascites tumor, rat liver and then with rat (*in vivo*). On the other hand, very meager information on the plant-growth inhibition mechanism of T.A. is available.

From this view point, this paper deals with the biological and biochemical studies of the effect of T.A. on plant cells and seedlings.

MATERIALS AND METHODS

Plant material and growth-inhibition test. Rice plant, mung bean (*Phaseolus aureus*), radish and turnip (*Sazanami*) were used for the seedling-growth inhibition test. The seeds were sterilized with 0.1% $HgCl_2$ for 10 min, washed with water and then placed on wet filter paper, being allowed to germinate. The seedlings of uniform size were then selected and transferred onto wet absorbent cotton in a glass dish (3.0 cm in diameter, 2.0 cm in depth) containing 4.0 ~ 8.0 ml of test solution. The glass dishes were then placed in a growth cabinet under the dark for 3 ~ 7 days.

Radioactive chemicals and reagent. The labelled compounds were obtained from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan.

T.A. was synthesized from L-isoleucine and diketene according to the method of Harris *et al.*¹⁰⁾ The synthesized T.A. ($[\alpha]_D^{20} -105^\circ$, $c=2.0$ in methanol) is mainly composed of L-tenuazonic acid.¹¹⁾ In order to prevent

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from a change of optical rotation, T.A. was preserved as a cupric salt.

Suspension culture of isolated plant cells. Cells from the root tissue of soybean and rice plant were provided by Dr. K. Ojima, Tohoku University. The suspension culture of soybean cell was carried out using the method of Gamborg *et al.*¹²⁾ The cells were cultured under sterile conditions in a 250 ml Erlenmyer flask containing 50 ml of B₅ medium¹²⁾ (consisted of sucrose, *m*-inositol, 2,4-D, vitamins and inorganic salts) with or without T.A., with gentle shaking for 2~12 days at 27°C under continuous light (300~400 lux) from white fluorescent lamps. The suspension culture of rice plant cell was carried out similarly in a 250 ml Erlenmyer flask containing 50 ml of the R₂ medium¹³⁾ (consisted of sucrose, 2,4-D, vitamins B and inorganic salts) with or without T.A.

Measurement of O₂-uptake of plant materials. Rice seedlings of uniform size (age: 5 days, length: 3.0~4.0 cm) were vacuum-immersed in T.A. solution of each concentration for 5~24 hr. Control sample was similarly vacuum-immersed in distilled water. After vacuum-immersion, each of the samples was transferred into the modified Warburg vessels and the O₂-uptake during 20 min was measured.

Suspension cultured soybean-cells, 500 mg cells in 3.0 ml of B₅ medium containing T.A. of each concentration, were transferred into Warburg vessels, containing 0.2 ml of 20% KOH in the center well, and after pre-incubation period, the O₂-uptake of the cells during 20 min was measured.

Incorporation test of radioactive precursors into protein and nucleic acid fractions of suspension cultured soybean cells. Suspension cultured soybean cells were incubated at 27°C under shaking in a 250 ml Erlenmyer flask with 60 ml of the B₅ medium containing ¹⁴C-leucine or ¹⁴C-adenine and each 10 ml aliquot (0.3~0.5 g of cells in fresh weight) was pipetted with predetermined intervals into a test tube containing 10 ml of chilled B₅ medium. The test tube was allowed to stand in an ice bath and the resulted precipitate (cells) was collected on Miracloth, followed by washing with the medium. The filtrate was filled up to 25 ml and the radioactivity was measured.

The procedure to determine the incorporation of ¹⁴C-leucine into the protein fraction is as follows: The collected cells were homogenized in 1 ml of 0.02 M Tris-HCl buffer (pH 7.6) for 3 min at 0°C. The homogenate was transferred into a centrifuge tube, being added with 2 ml of the chilled buffer solution, and centrifuged at 1000×*g* for 10 min. This washing procedure was repeated three times. The supernatant and washings were combined and cold trichloroacetic acid (TCA) was added to bring the final concentration of 5%

and allowed to stand overnight. The resulted precipitate was separated by centrifugation (1100×*g*, 10 min) and was suspended again in 2 ml of cold 5% TCA, followed by the same procedures being repeated three times. The supernatant and washings were combined and designated as the "TCA soluble fraction." The precipitate was dissolved in 2 N NH₄OH and designated as the "protein fraction." Protein contents in the protein fraction was determined by Lowry's method.¹⁴⁾

The extraction of nucleic acid from the cells was run after the modified method of Schmidt-Thannhauser.¹⁵⁾ The procedure to determine the incorporation of ¹⁴C-adenine into the nucleic acid fraction is as follows: The cells were ground in a mortar containing 1 ml of 0.02 M Tris-HCl buffer (pH 7.6) for 3 min at 0°C and transferred into centrifuge tube, washing with another 1 ml of the buffer solution. Two milliliters of cold 5% perchloric acid (PCA) was added to bring the final concentration to 2.5%, followed by centrifugation at 8000×*g* for 15 min. The precipitate thus obtained was suspended in 2 ml of 2.5% PCA and centrifuged at 8000×*g* for 15 min, being repeated once again. The supernatant and washings were combined and designated as the "PCA soluble fraction." The precipitate was suspended in 2 ml of 5% PCA and hydrolyzed at 95°C for 15 min, followed by centrifugation at 8000×*g* for 15 min. The precipitate was then washed twice with 2 ml of 5% PCA by centrifugation at 8000×*g* for 15 min. The supernatant and washings were combined and designated as the "nucleic acid fraction." Nucleic acid contents in the nucleic acid fraction was determined by the absorbance at 260 nm.

Measurement of radioactivity. Each 1 ml aliquot of the above mentioned TCA soluble fraction, the PCA soluble fraction and the nucleic acid fraction was dissolved in 6 ml of dioxan scintillator fluid. And then, 1 ml aliquot of the protein fraction was dissolved in 0.2 ml of hyamine plus 6 ml dioxane scintillator fluid.

In the case of cells, about 10 mg of each sample was placed in the counting vials with 6 ml of toluene scintillator fluid. The radioactivities of these specimens were counted with a liquid scintillation counter.

RESULTS

Growth inhibiting effect of T.A. on seedlings

T.A. inhibited the growth of rice seedlings perfectly at 2.5×10^{-4} M and by 50~100% at 1.25×10^{-4} , and then inhibited that of mung bean seedlings (70% at 5.0×10^{-4} M), that of radish seedlings (90% at 1.0×10^{-3} M and 50% at 5.0×10^{-4} M) and that of turnip seedlings (90~100% at 5.0×10^{-4} M and 50% at 1.25×10^{-4} M). Root growth-inhibition of all seed-

FIG. 1. Effect of T.A. on the Growth of Suspension Cultured Cells of Rice Plant and Soybean.

a, rice plant; b, soybean.

Each 5 ml aliquot of seed cultures (6~8 days age) was added into 250 ml Erlenmyer flask containing 50 ml of R₂ (for rice plant) and B₅ media (for soybean) with or without T.A. and incubated at 27°C. Cell growth is expressed as fresh weight per flask. ○—○, control (incubated in R₂ or B₅ media); ▲—▲, treated with 1.25×10^{-4} M of T.A.; ■—■, treated with 2.5×10^{-4} M of T.A.; ×—×, treated with 1.0×10^{-3} M of T.A.

lings tested was observed to be more severe.

It is noteworthy that the growth-inhibition of rice seedlings by T.A. was conspicuous among those of the seedlings tested.

Effect of T.A. on the growth of suspension cultured cells of rice plant and soybean

The growth of suspension cultured cells of rice plant and soybean was inhibited by T.A. As shown in Fig. 1-a, the growth of rice plant cells was inhibited by T.A. about 60~70% at 2.5×10^{-4} M and almost perfectly at 1.0×10^{-3} M. The growth inhibition of soybean cell was more severe (Fig. 1-b), 40~50% at 1.25×10^{-4} M and almost perfectly at 2.5×10^{-4} M.

Effect of T.A. on O₂-uptake of plant materials

The effect of T.A. on the O₂-uptake of rice

seedlings and suspension cultured soybean-cells has been investigated. As shown in Table I, T.A. in a concentration range of 2.5×10^{-4} ~ 1.0×10^{-3} M revealed no significant effects on the O₂-uptake of rice seedlings in 5~24 hr period. And then, the O₂-uptake was scarcely inhibited under such a high T.A. concentration of 1.0×10^{-3} M, which inhibited the rice-seedling-growth perfectly.

The same is also the case with the experimental results obtained with suspension cultured soybean-cells.

In conclusion, T.A. manifested no effects on the O₂-uptakes of rice seedlings and suspension cultured soybean-cells.

Effect of sulfhydryl compounds on the inhibitory action of T.A. on seedling-growth

The effect of cysteine and thioglycolate on the growth inhibition of test seedlings induced by T.A. (1.25×10^{-4} M) was investigated. Test rice grains (*Fujiminori*) were grown with or without addition of T.A. (1.25×10^{-4} M) at 27°C for 6 days on wet absorbent cotton in a glass dish containing cysteine or thioglycolate (each 5.0×10^{-4} M). The experimental results demonstrated that the inhibition of root-elongation of rice seedlings by T.A. was hardly reversed by the simultaneous addition of the cysteine or thioglycolate. From this observation, it may be concluded that T.A. has no

TABLE I. EFFECT OF T.A. ON THE O₂-UPTAKE OF RICE SEEDLINGS

Concentration of T.A. (M)	O ₂ -uptake after each vacuum-immersion period		
	5 hr	10 hr	24 hr
0	89.6 ^{a)}	64.1 ^{a)}	56.2 ^{a)}
2.5×10^{-4}	81.8	68.8	50.6
5.0×10^{-4}	70.4	56.3	46.2
1.0×10^{-3}	72.5	57.7	53.0

a) μ l/vessel/20 min.

FIG. 2. Time Course of the Effect of T.A. on the Uptake of Amino Acid and Purine Base into Suspension Cultured Cells.

a, uptake of leucine; b, uptake of valine; c, uptake of adenine.

Soybean cells (3.0 g) were pre-incubated for 1 hr in a 250 ml Erlenmyer flask containing 60 ml of B₅ medium with or without T.A., after which ¹⁴C-labelled leucine or valine or adenine (each 0.5 μ Ci) was added respectively to bring the final concentration of 5.0×10^{-6} M and incubated for 20~180 min. At predetermined intervals, 10 ml aliquots of the culture broth were pipetted and radioactivities absorbed into the cells were measured. $\circ$ — $\circ$, control; $\blacktriangle$ — $\blacktriangle$, treated with 1.25×10^{-4} M of T.A.; $\blacksquare$ — $\blacksquare$, treated with 2.5×10^{-4} M of T.A.

effect on sulfhydryl radicals.

Time course of the effect of T.A. on the uptake of amino acid and purine base into suspension cultured cells

Our preliminary study has demonstrated that an uptake of ¹⁴C-leucine into suspension cultured soybean cells (decrease in radioactivity in the medium), as well as the incorporation of it into the protein fraction, was remarkably inhibited by T.A. Therefore, the effect of T.A. on the uptake of ¹⁴C-labelled leucine, valine and adenine into the cells has been studied in detail.

The effect of T.A. on the time course of the uptake of ¹⁴C-labelled leucine, valine and adenine into the cells was shown in Fig. 2-a, b, c, respectively. T.A. inhibited the uptake of all labelled compounds into the cells in 20~180 min incubation period at concentrations of 1.25×10^{-4} M and 2.5×10^{-4} M. This finding was also confirmed by simultaneous measurement of the decrease in radioactivity in the medium. It is noteworthy that T.A. remarkably inhibited the uptake of all labelled compounds into the cells in the early stage of incubation (at 20~40 min), but this inhibition gradually decreased. This phenomenon may be elucidated that most of the radioactivity in the medium was absorbed into control cells

during the early incubation period, whereas the uptake of that into the cells treated with T.A. was delayed.

Time course of the effect of T.A. on the incorporation of ¹⁴C-leucine into the protein fraction

In order to investigate the effect of T.A. on protein synthesis, the effects of T.A. on the incorporation of ¹⁴C-leucine into the protein fraction and the TCA soluble fraction were examined. ¹⁴C-Leucine-incorporation into these fractions of the cells was affected by delicate fluctuation of the experimental conditions, that is, concentration of leucine added in the medium as a carrier, cultural temperature and especially cell age. Remarkable difference in the uptake velocity of ¹⁴C-leucine between the cells of early exponential stage and those of late exponential stage was observed in our preliminary experiment. Therefore, the experiment was carried out with the cells of different ages.

a) Experiment-I

The experiment was run with the cells of late exponential stage (8~10 days age). The decrease in radioactivity in the medium, which represents an uptake of ¹⁴C-leucine into the cells (Fig. 3-a), and its incorporation into the protein and TCA soluble fractions (Fig. 3-

FIG. 3. Time Course of the Effect of T.A. on the ^{14}C -Leucine-uptake into the Cells and its Incorporation into the TCA Soluble and Protein Fractions Using the Cells in Late Exponential Stage (Experiment I).

a, decrease in ^{14}C -leucine in the medium which represents an uptake of ^{14}C -leucine into the cells; b, radioactivity of TCA soluble fraction; c, incorporation of ^{14}C -leucine into the protein fraction.

Soybean cells (2.0 g) in the late exponential stage were pre-incubated for 1 hr in a 250 ml Erlenmeyer flask containing 60 ml B_5 medium with or without T.A., after which ^{14}C -leucine ($0.5 \mu\text{Ci}$) was added to bring the final concentration of $5.0 \times 10^{-6} \text{ M}$ and incubated for 20~180 min. At predetermined interval, 10 ml aliquots of the culture broth were removed and with each of them the radioactivity in the medium and the incorporation of ^{14}C -leucine into the TCA soluble and protein fractions were measured. $\circ$ — $\circ$, control; $\blacktriangle$ — $\blacktriangle$, treated with $7.5 \times 10^{-5} \text{ M}$ of T.A.; $\blacksquare$ — $\blacksquare$, treated with $1.25 \times 10^{-4} \text{ M}$ of T.A. Each TCA soluble fraction was filled up to 20 ml and radioactivity was indicated for one milliliter.

b, c) proceeded lineary with incubation period during 20~180 min. T.A. of $7.5 \times 10^{-5} \text{ M}$ and $1.25 \times 10^{-4} \text{ M}$, inhibited the uptake of ^{14}C -leucine by about 20% and 35%, respectively and then inhibited its incorporation into the protein fraction about 50% and 70%, respectively. The experimental result that T.A. inhibited the incorporation of leucine into the protein fraction more severe than the uptake of leucine into the cell reflects that T.A. inhibited the biogenesis of protein in soybean.

Table II, which shows the distribution of radioactivity after 180 min incubation, also supported this conception. The ratio of radioactivities incorporated into the protein to that absorbed in the cells with or without the effect of T.A. was 12.7% (control), 8.8% ($7.5 \times 10^{-5} \text{ M}$ of T.A.) and 6.5% ($1.25 \times 10^{-4} \text{ M}$ of T.A.), respectively. In conclusion, $7.5 \times 10^{-5} \text{ M}$ and $1.25 \times 10^{-4} \text{ M}$ of T.A. inhibited the incorporation into protein of the leucine, absorbed into the cell, by about 30% and

TABLE II. EFFECT OF T.A. ON THE INCORPORATION OF ^{14}C -LEUCINE INTO EACH FRACTION AFTER 180 MIN INCUBATION IN EXPERIMENT I

	Radioactivity (cpm/10 ml of culture broth)		
	Control	$7.5 \times 10^{-5} \text{ M}$ of T.A.	$1.25 \times 10^{-4} \text{ M}$ of T.A.
Activity supplied	145,350 cpm	145,350 cpm	145,350 cpm
Activity recovered in			
(I) Medium at end of incubation	36,075	60,325	79,075
(II) TCA soluble fraction	64,500	67,540	49,660
(III) Protein fraction	13,930	7,520	4,340
Uptake	109,275	85,025	66,275
Activity in protein fraction as percentage of uptake	12.7%	8.8%	6.5%

FIG. 4. Time Course of the Effect of T.A. on the ^{14}C -Leucine-uptake into the Cells and its Incorporation into the TCA Soluble and Protein Fractions Using the Cells in Early Exponential Stage (Experiment II).

a, decrease in ^{14}C -leucine in the medium; b, radioactivity of TCA soluble fraction; c, incorporation of ^{14}C -leucine into the protein fraction.

The experiment was carried out using the soybean cells (3.0 g) in the early exponential stage under the same condition as in Fig. 3. $\circ$ — $\circ$, control; $\blacktriangle$ — $\blacktriangle$, treated with 7.5×10^{-5} M of T.A.; $\blacksquare$ — $\blacksquare$, treated with 1.25×10^{-4} M of T.A.

50%, respectively.

b) Experiment-II

The experiment was run with the cells of early exponential stage (4~5 days age). The radioactivity in the medium almost disappeared during 20~40 min incubation period with or without T.A. and uptake-inhibition of ^{14}C -leucine by T.A. into the cells was not observed apparently, as shown in Fig. 4-a. On the other hand, the incorporation of ^{14}C -leucine into protein fraction was inhibited with 7.5×10^{-5} M and 1.25×10^{-4} M of T.A. about 30% and 45%, respectively as shown in Fig. 4-c. This finding is also concordant with Fig. 4-b, which demonstrates the fluctuation of the radioactivity in

the TCA soluble fraction. T.A. of 7.5×10^{-5} M and 1.25×10^{-4} M largely increased the radioactivity in the TCA soluble fraction during 40~120 min incubation by incorporation-inhibition of leucine into protein. In this experiment, the inhibition ratio of leucine-incorporation into the protein fraction means that of the intrinsic leucine-incorporation into the protein fraction.

As shown in Table III, the ratio of radioactivity incorporated into the protein fraction in 120 min incubation to that absorbed into the cells was 25.6% (control), 17.7% (with 7.5×10^{-5} M of T.A.) and 14.2% (with 1.25×10^{-4} M of T.A.), respectively. This means that T.A. of these concentrations inhibited the

TABLE III. EFFECT OF T.A. ON THE INCORPORATION OF ^{14}C -LEUCINE INTO EACH FRACTION AFTER 120 MIN INCUBATION IN EXPERIMENT-II

	Radioactivity (cpm/10 ml of culture broth)		
	Control	7.5×10^{-5} M of T.A.	1.25×10^{-4} M of T.A.
Activity supplied	136,550 cpm	136,550 cpm	136,550 cpm
Activity recovered in			
(I) Medium at end of incubation	2,200	3,625	6,075
(II) TCA soluble fraction	55,420	99,900	102,040
(III) Protein fraction	34,390	23,560	18,560
Uptake	134,350	132,925	130,475
Activity in protein fraction as percentage of uptake	25.6%	17.7%	14.2%

FIG. 5. Time Course of the Effect of T.A. on the ^{14}C -Adenine-uptake into the Cells and its Incorporation into the PCA soluble and Nucleic Acid Fractions.

a, decrease in ^{14}C -adenine in the medium which represents an uptake of ^{14}C -adenine into the cells; b, radioactivity of PCA soluble fraction; c, incorporation of ^{14}C -adenine into the nucleic acid fraction.

Soybean cells (3.0 g) in exponential stage were pre-incubated for 1 hr in a 250 ml Erlenmeyer flask containing 60 ml of B_5 medium with or without T.A., after which ^{14}C -adenine ($0.5 \mu\text{Ci}$) was added to bring the final concentration of $5 \times 10^{-6} \text{ M}$ and incubated for 20~180 min. At predetermined interval, 10 ml of aliquots of the culture broth were removed and with each of them the radioactivity in the medium and the incorporation of ^{14}C -adenine into the PCA soluble and nucleic acid fractions were measured. $\bigcirc$ — $\bigcirc$, control; $\blacktriangle$ — $\blacktriangle$, treated with $7.5 \times 10^{-5} \text{ M}$ of T.A. Each PCA soluble fraction was filled up to 8 ml and the radioactivity was indicated for one milliliter.

intrinsic leucine-incorporation into the protein fraction by about 30% and 45%, respectively. These inhibition ratio was in good agreement with those obtained by Experiment-I.

Time course of the effect of T.A. on the incorporation of ^{14}C -adenine into the nucleic acid fraction

Our experimental results demonstrated that T.A. inhibited the ^{14}C -leucine incorporation into protein fraction of suspension cultured soybean cells. However, there is a possibility that T.A. may inhibit protein synthesis indire-

ctly by interfering with nucleic acid synthesis. So the investigation of the effect of T.A. on the incorporation of ^{14}C -adenine into the nucleic acid fraction has been carried out using suspension cultured soybean cells of exponential stage (7 days).

The decrease in radioactivity in the medium, which represents an uptake of ^{14}C -adenine into the cells (Fig. 5-a), and the incorporation of ^{14}C -adenine into the nucleic acid fraction (Fig. 5-c) and that into the PCA soluble fraction (Fig. 5-b) proceeded lineary with incubation period during 20~180 min. T.A. of $7.5 \times$

TABLE IV. EFFECT OF T.A. ON THE INCORPORATION OF ^{14}C -ADENINE INTO EACH FRACTION AFTER 120 MIN INCUBATION

	Radioactivity (cpm/10 ml of culture broth)	
	Control	$7.5 \times 10^{-5} \text{ M}$ of T.A.
Activity supplied	156,486 cpm	156,486 cpm
Activity recovered in		
(I) Medium at end of incubation	46,134	77,792
(II) PCA soluble fraction	69,480	47,295
(III) Nucleic acid fraction	7,550	3,850
Uptake	110,352	78,694
Activity in nucleic acid fraction as percentage of uptake	6.48%	4.89%

10^{-5} M inhibited the uptake of ^{14}C -adenine by about 30~40% and the incorporation of it into the nucleic acid fraction by about 50~80%. The experimental result that T.A. inhibited the incorporation of adenine into the nucleic acid fraction more severe than the uptake of adenine into the cell, reflects that T.A. also inhibited the biogenesis of nucleic acid as well as protein by some way.

As shown in Table IV, the ratio of radioactivity incorporated into the nucleic acid fraction in 120 min incubation to that transported into the cells was 6.8% (control) and 4.89% (with 7.5×10^{-5} M of T.A.), respectively. This means that T.A. inhibited the intrinsic adenine-incorporation into the nucleic acid fraction by about 30%.

DISCUSSION

T.A. exhibited a marked stunting effect on the seedling-growth of rice plant, mung bean, radish and turnip and on the growth of suspension cultured cells of soybean and rice plants. It is noteworthy that the growth-inhibition of rice seedling by T.A. was conspicuous among those of the seedlings tested.

Shigeura and Gordon⁹⁾ have reported that T.A. inhibited the protein synthesis of rat and *in vitro* of Ehrlich ascites tumor and rat liver cells. And then, several reports^{16,17)} have been presented which dealt with the effect of T.A. on the protein synthesis of yeast or animal cells. But, on the other hand, very meager information on the plant-growth inhibition mechanism is available. From this view point, the effect of T.A. on the respiration, the activity of SH-enzyme, protein synthesis and nucleic acid synthesis of plants has been studied.

Our experimental results demonstrated that T.A. exhibited no effect on the O_2 -uptake and the activity of SH-enzyme of plants, but inhibited the incorporation of ^{14}C -leucine into the protein fraction as well as its uptake into the cells. And then it was also proved that the inhibition of ^{14}C -leucine incorporation into protein fraction was not merely due to that of ^{14}C -leucine uptake into the cell but based on

the intrinsic inhibition of protein synthesis.

However, there is a possibility that T.A. may inhibit protein synthesis indirectly by interfering with nucleic acid synthesis. Contrary, there is also a possibility that the inhibition of protein synthesis secondarily results a inhibition of nucleic acid synthesis. The experimental results demonstrated that T.A. also inhibited the incorporation of ^{14}C -adenine into the nucleic acid as well as its uptake into the cell, and then the inhibition of ^{14}C -adenine incorporation into nucleic acid fraction was not merely due to that of ^{14}C -adenine uptake into the cell but based on the intrinsic inhibition of nucleic acid biogenesis. The other experimental result which will be presented in another paper in detail demonstrated that more than 90% of the incorporated ^{14}C -adenine into total nucleic acid was into RNA fraction.

As the inhibition ratio of nucleic acid synthesis was almost the same as that of protein synthesis, whether or not protein synthesis is inhibited directly or indirectly by interfering with nucleic acid synthesis, remained unknown. These problem will be elucidated in future by cell-free studies on protein and nucleic acid syntheses.

Rees and Bryant¹⁸⁾ reported that cycloheximide inhibited the protein synthesis of carrot tissue and also the uptake of leucine into the cells. T.A. remarkably inhibited the uptake of ^{14}C -labelled valine and adenine as well as leucine into the plant cells. This inhibition may be due to various causes, that is, secondary effect of the inhibition of protein synthesis, inhibition of the permeability of cell membrane and so on. Even in our case, the uptake-inhibition may be due to the inhibition of protein synthesis.

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