Effect of Tenuazonic Acid on *In Vitro* Amino Acid Incorporation by Rice Embryo Ribosomes

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Received May 21, 1974

The symptom of blast disease suggests that the blast fungus (*Pyricularia oryzae* Cav) must produce some toxic substances. As one of the vivotoxin of blast fungus, tenuazonic acid (T. A.) has been isolated from the culture broth of blast fungus¹⁾ and blast-diseased rice plants^{2,3)} by the authors. Shigeura and Gordon⁴⁾ reported that T.A. inhibited the incorporation of amino acid into protein with Ehrlich ascites tumor, rat liver (*in vitro*) and with rat (*in vivo*). However, still, very meager information on the plant-growth inhibition mechanism of T.A. is available.

In the previous paper,⁵⁾ we have reported the biological and biochemical studies of the effect of T.A. on plant cells and seedlings. T.A. 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. Then, T.A. inhibited the incorporation of ¹⁴C-leucine into protein fraction as well as that of ¹⁴C-adenine into nucleic acid fraction of suspension cultured soybean cells. Since the inhibition ratio of protein synthesis was almost the same as that of nucleic acid synthesis, it remained unknown whether protein synthesis is inhibited directly or indirectly by interfering with nucleic acid synthesis.

In this paper, we preliminarily report the effect of T.A. on *in vitro* amino acid incorporation by rice embryo ribosomes.

The preparation of cell free polyuridylic acid (poly U) directed phenylalanine incorporating system from rice embryo was carried out by

the modified method of A.A. App and M. M. Gerosa.⁶⁾ The dry rice (*Oryza sativa* L. Sasaminori) embryos (7.0 g) were ground for 2 min with quartz sand in a cold mortar containing 25 ml of homogenating buffer consisted of 0.5 M sucrose, 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 25 mM KCl and 5 mM 2-mercaptoethanol. The homogenate was strained through cheesecloth, centrifuged for 20 min at 24,000×g, and the supernatant was again centrifuged for 2 hr at 100,200×g. The upper half of the supernatant was collected, and chromatographed on a Sephadex G-25 column. The early eluted fraction was collected and designated as the "supernatant enzyme." The ribosomal pellet was suspended in 2.5 ml of resuspending buffer containing 50 mM Tris-HCl (pH 7.6), 1 mM MgCl₂, 25 mM KCl and 5 mM 2-mercaptoethanol and centrifuged for 20 min at 24,000×g. The supernatant was designated as the "ribosome." The optimum concentration of magnesium ion, potassium ion, guanosine triphosphate and poly U of the amino acid incorporating system was approxi-

FIG. 1. The Time Course for the Transfer of ¹⁴C-Phenylalanine from ¹⁴C-Phenylalanyl-sRNA into Protein with or without Tenuazonic Acid.

The transfer reaction was carried out at 30°C with or without T.A. and stopped at predetermined interval by adding trichloroacetic acid to bring a final concentration of 5% (w/v) and 1.0 mg casein was added. The precipitates thus obtained were hydrolyzed at 90°C for 15 min, followed by standing for 30 min in ice bath. The precipitates were collected on filter-paper disks (Whatman 3 MM, ϕ 2.3 cm), washed 3 times with 5% trichloroacetic acid, once with ethanol-ether (1:1, v/v), then dried and the radioactivities of the precipitates were counted with a liquid scintillation counter. ●—●, control; ▲—▲, treated with 1.0 mM of T.A.

FIG. 2. Effect of Tenuazonic Acid and Cycloheximide on the Incorporation of ^{14}C -Phenylalanine into Protein with Various Concentrations.

The experimental methods are the same as in Fig. 1, except for 10 min incubation. ●—●, T.A.; ▲—▲, cycloheximide.

mately the same as the data by A. A. App and M. M. Gerosa.⁶⁾

Therefore, the cell free amino acid incorporating system in a total volume of 0.5 ml was determined as follows: 50 mM Tris-HCl (pH 7.6), 6 mM MgCl_2 , 65 mM KCl, 1.0 mM GTP, 6 mM 2-mercaptoethanol, 30 μg poly U, 4,000 cpm ^{14}C -phenylalanyl-sRNA* (specific activity of phenylalanine: 395 $\mu\text{Ci}/\mu\text{mole}$), 0.8 mM ^{12}C -phenylalanine, 113 μg of the ribosome as ribosomal RNA and 337 μg of protein of the supernatant enzyme.

The time course for the transfer of ^{14}C -phenylalanine from ^{14}C -phenylalanyl-sRNA into protein with or without T. A. is shown in Fig. 1. The incorporation of ^{14}C -phenyl-

alanine into protein proceeded linearly with 10 min incubation period. T.A. inhibited this reaction about 80% at the concentration of 1.0 mM. Cycloheximide, which is widely known to inhibit protein synthesis of plants,⁷⁻¹⁰⁾ also inhibited this incorporation reaction about 80% at the concentration of 0.5 mM. Figure 2 shows the effect of T.A. and cycloheximide on the incorporation of ^{14}C -phenylalanine into protein after 10 min reaction period. The incorporation-inhibition apparently depended on the concentration of T.A., and the incorporation was perfectly inhibited at the concentration of 3.0 mM.

Detailed studies on the acting site of T.A. on protein synthesis by rice embryos are now in progress.

REFERENCES

- 1) N. Umetsu, J. Kaji and K. Tamari, *Agr. Biol. Chem.*, **36**, 859 (1972).
- 2) N. Umetsu, J. Kaji and K. Tamari, *ibid.*, **37**, 451 (1973).
- 3) N. Umetsu, J. Kaji, K. Aoyama and K. Tamari, *ibid.*, **38**, 1867 (1974).
- 4) H. T. Shigeura and C. N. Gordon, *Biochemistry*, **2**, 1132 (1963).
- 5) N. Umetsu, T. Muramatsu, H. Honda and K. Tamari, *Agr. Biol. Chem.*, **38**, 791 (1974).
- 6) A. A. App and M. M. Gerosa, *Plant Physiol.*, **41**, 1420 (1966).
- 7) T. AP Rees and J. A. Bryant, *Phytochemistry*, **10**, 1183 (1971).
- 8) R. J. Ellis and I. R. Macdonald, *Plant Physiol.*, **42**, 1297 (1967).
- 9) W. J. Jachymczyk and J. H. Cherry, *Biochim. Biophys. Acta*, **157**, 368 (1968).
- 10) E. Davies and G. A. MacLachlan, *Arch. Biochem. Biophys.*, **129**, 581 (1969).

* sRNA was prepared from baker's yeast.