Effect of 4-Thiouridine on Chloroplast Development

Sir:

The presence of 4-thiouridine (4sU) as one of minor constituents has been established in transfer RNAs from *Escherichia coli*¹⁾ and from *Mycoplasma*.²⁾ Reports regarding the effects of 4sU to organisms, however, have not yet been published, though one of us demonstrated that 4sU showed specific photochemical behaviors distinguished from other constituents of RNAs.³⁾ In this paper we wish to report the inhibitory effect of 4sU on the chloroplast development in higher plant cotyledons.

Radish seeds (*Raphanus sativus* Linn. Kaiware) obtained from Takii Shuhyo Co., Ltd., Kyoto, were allowed to germinate on 0.5 mM 4sU containing 5 mM potassium phosphate buffer (pH 7.0) in the dark at 22°C for 4 days, and etiolated seedlings obtained were illuminated with Mitsubishi fluorescent lamps (4000 lux) at 22°C for the desired time. Controls were left under the same conditions except the presence of 4sU. Germination and growth of the seeds were not affected by the presence of 0.5 mM 4sU, the rate of chlorophyll synthesis in the cotyledons on illumination, however, was significantly delayed, compared with that of the untreated plants, as shown in Fig. 1. The result indicates that the contact with 4sU in the germination stage should be primarily related to the inhibition of the greening on illumination. Cysteine, glutathione, 6-mercaptopurine, uridine, uracil, thiourea or glucose (1 mM, respectively) had no inhibitory effect

on the greening. Glutathione and plant hormones such as 6-benzyl adenine, gibberellin A₃ and indole acetic acid had no positive restoration effect against the inhibition induced by 4sU. Interestingly germination of the seeds was suppressed by 2-thiouracil (0.5 mM) almost perfectly.

FIG. 1. Comparative Study on Chlorophylls and RuDP Carboxylase in Radish Cotyledons Cultured with and without 4sU.

Radish seeds were allowed to germinate in the dark for 4 days and etiolated seedlings obtained were illuminated for 3 days. Chlorophyll content and the enzyme carboxylase activity in cotyledons were determined at 0, 24, 48 and 72 hr of illumination; chlorophyll was extracted with 80% acetone and determined according to the method of D. I. Arnon,⁴⁾ the enzyme was assayed according to the J. Hurwitz's method⁵⁾ using ¹⁴C-NaHCO₃.

○—○, RuDP carboxylase without 4sU;

△—△, RuDP carboxylase with 4sU;

●—●, Chlorophylls without 4sU;

▲—▲, Chlorophylls with 4sU.

1) M. N. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965).

2) H. Hayashi, H. Fisher and D. Söll, *Biochemistry*, **8**, 3680 (1969).

3) M. Pleiss, H. Ochiai and P. A. Cerutti, *Biochem. Biophys. Res. Comm.*, **34**, 70 (1969).

4) D. I. Arnon, *Plant Physiol.*, **24**, 1 (1949).

5) B. Filner and A. O. Klein, *Plant Physiol.*, **43**, 1587 (1968).

Determination of protochlorophyllide, a precursor of chlorophyll, after the Siegelman's method⁶⁾ indicated that the formation of protochlorophyllide in cotyledons was also prevented by the presence of 4sU. Furthermore phototransformation of protochlorophyllide holochrome, which was isolated from normal etiolated bean cotyledons after the Siegelman's method,⁶⁾ to chlorophyllide holochrome with illumination was not inhibited by the presence of 0.5 mM 4sU. Delta-aminolevulinic acid, a starting material of chlorophyll biogenesis, was demonstrated to accumulate in $40 \pm 10\%$ excess in the 4sU treated cotyledons as compared with controls.

The specific activity of glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49, D-Glucose-6-phosphate: NADP oxidoreductase),⁷⁾ a typical enzyme in the soluble cytoplasm, in the cotyledons grown on 4sU, was the same as the activity of controls. Glutamic acid dehydrogenase (GluDH, E.C. 1.4.1.3, L-Glutamate: NAD oxidoreductase),⁸⁾ a typical enzyme of mitochondria, also showed no difference in activity between the 4sU treated plants and controls as shown in Fig. 2. However the specific activity of ribulose 1,5-diphosphate(RuDP) carboxylase (E.C. 4.1.1.39, 3-Phospho-D-glycerate carboxy-lyase),⁶⁾ which is a proper enzyme of chloroplast, was low in etiolated cotyledons germinated with 4sU but significantly high in the cotyledons without 4sU. By continuous illumination the activity of the enzyme was restored to the normal value after 3 days as well as the chlorophyll content as shown in Fig. 1.

In addition, analysis by the use of polyacrylamide gel electrophoresis of cotyledon

RNAs^{9,10)} from normal or 4sU treated radish demonstrated that the rate of *de novo* biosynthesis of chloroplast RNAs induced by illumination was significantly retarded in the 4sU treated plants as compared with that of the untreated, whereas the formation of ribosomal RNAs was virtually analogous in the both cotyledons.

Fig. 2. Comparative Study on G6PDH and GluDH Activities in Radish Cotyledons Cultured with and without 4sU.

G6PDH was assayed according to the A. Kornberg's method,⁷⁾ GluDH according to the Th. Bücher's.⁸⁾ Specific activity was determined by measurement of $\Delta E_{340}/\text{mg protein}$ for the initial 3 min using NADP⁺ and NAD⁺ as cofactor, respectively.

- , G6PDH without 4sU;
- △—△, G6PDH with 4sU;
- , GluDH without 4sU;
- ▲—▲, GluDH with 4sU.

The experimental results described above indicate that 4sU must have prevented chloroplast development or formation, but not cytoplasmic and mitochondrial development during germination in the dark followed by

6) P. Schopfer and H. W. Siegelman, *Plant Physiol.*, **43**, 990 (1968).

7) A. Kornberg and B. L. Horecker, "Methods in Enzymology," Vol. I, ed. by S. P. Colowick and N. O. Kaplan, Academic Press Inc., New York, N.Y., 1955, p. 323.

8) G. Beisenherz, Th. Bücher *et al.*, *Z. Naturforsch.*, **8b**, 555 (1953).

9) J. Ingle, *Plant Physiol.*, **43**, 1850 (1968).

10) A. C. Peacock and C. W. Dingman, *Biochemistry*, **7**, 668 (1968).

growth in the light. It is interesting that the effect of 4sU disappeared by continuous illumination. Details and electron microscopic studies on these phenomena will be reported elsewhere in the near future.

Hideo OCHIAI

Hitoshi SHIBATA

*Laboratory of Biochemistry, College of Agriculture,
Shimane University, Matsue, Shimane, Japan*

Received August 31, 1970