Radiolysis of Active Sites of Gibberellic Acid Molecule in Dilute Solution[†]

Itsuo KUROBANE and Hikoyuki YAMAGUCHI

Laboratory of Radiation Genetics, Faculty of Agriculture,
The University of Tokyo, Tokyo 113, Japan

Received June 10, 1975

1. Introduction

Gibberellic acids are very significant as inducers of synthesis of enzymes essential for germination of cereal seeds. Sideris et al.^{1,2)} demonstrated that the biological activity of gibberellic acid was destructed when its aqueous solutions were irradiated with relatively low doses of gamma rays. And, the protection experiments²⁾ with OH radical scavengers, such as potassium iodide and *p*-aminobenzoic acid, suggested that the inactivation of the gibberellic acid by gamma-irradiation might be attributed to an indirect effect through reactions with OH radicals originating presumably from the radiolysis of water. Furthermore, they speculated that the reduced absorbance of the gibberellic acid molecule at the γ -lactone ring coupled with its reduced biological activity, following irradiation.

This communication presents further evidence for the *in vitro* destruction of γ -lactone ring of gibberellic acid by gamma irradiation, leading presumably to reduction of the biological activity of the gibberellic acid.

2. Materials and Methods

Dwarf rice (*Oryza sativa* cv. Tanginbozu) and barley (*Hordeum vulgare* cv. Fuji 2-Jyo) seeds were used for gibberellic acid bioassay as described by Ogawa³⁾ and Jones and Varner⁴⁾, respectively.

Gibberellic acid (90% GA₃, the rest, main-

ly other gibberellins) was kindly given by Kyowa Hakko Co., Ltd. Prior to experiments, gibberellic acid was purified by crystallization. Solutions of 100 ppm GA₃, which were dissolved into the twice distilled water, were irradiated at 25°C with 0, 50, 100, 150, 250, 500 and 1,000 kR of gamma rays from ¹³⁷Cs source. The exposure time was 6 $\frac{2}{3}$ hours.

After irradiation, the pH of GA₃ solution was adjusted to 7.0 and then the solution was partitioned three times against equal volumes of ethyl-acetate to obtain the neutral ethyl-acetate fraction. The pH of the water phase was adjusted to 3.0 and then it was partitioned again against ethyl-acetate. Thus, the acidic ethyl-acetate fraction was obtained. The remaining water phase was then partitioned against *n*-butanol to obtain the acidic butanol fraction. Each fraction was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure at 40°C.

Each fraction was spotted on thin layer of silica gel G (0.3-0.4 mm in thickness), and developed in a solvent system of chloroform, ethyl acetate and acetic acid (60:40:5, v/v) for the neutral and acidic ethyl-acetate fractions, as well as in a solvent system of chloroform, methanol, acetic acid and water (40:15:3:2, v/v) for the acidic butanol fraction. Chromatograms were sprayed with 70%

[†] 希薄溶液におけるジベレリン分子活性部位の放射線分解。黒羽徹夫，山口彦之：東京大学農学部放射線遺伝学教室（東京都文京区弥生1-1-1）

sulfuric acid vapor, heated for about 10 minutes at 100°C and separated into several fractions using UV-absorption. Each fraction was extracted with 60% acetone-water solution and evaporated to dryness under reduced pressure at 40°C.

Infrared and mass spectra were determined after methylation of the fractions separated by TLC and authentic GA_3 with diazomethane.

3. Results

Five fluorescent spots in addition to that of GA_3 were detected on the chromatogram of the acidic ethyl-acetate fraction (Fig. 1),

Fig. 1 TL chromatogram of the acidic ethyl-acetate fraction partitioned from the 100 ppm GA_3 solution after gamma irradiation.

while no fluorescent spot on the chromatograms of the other fractions. Newly found spots were termed F-I, II, III, IV and V in the order of R_f value. Because F-II was produced even with the lowest dose used, 50 kR, and the other fractions did not appear until using the higher doses, it seemed to be the primary radiolytic product of GA_3 .

The fluorescent colors of F-II, IV and V were very similar to that of GA_3 , namely light blue. However, those of F-I and III were a little more greenish than that of GA_3 .

After one litre of 100 ppm GA_3 solution, dissolved 100mg GA_3 in one litre of the twice distilled water, was irradiated with 100 kR of gamma rays, the amount of the products

in each fraction, partitioned against ethyl-acetate or *n*-butanol, was weighed. The result showed that the yields of the neutral and acidic ethyl-acetate fractions, and the acidic butanol fraction were 0.8, 85.5 and 3.6 mg, respectively.

The biological activities of the products, separated by TLC, in the acidic ethyl-acetate fraction was completely destroyed by gamma-irradiation with 100 kR.

The absorption peak at 1770 cm^{-1} in the infrared spectrum of F-II was strongly reduced after irradiation of 100 kR.

Mass spectrum of F-II revealed the highest molecular ion peak at m/e 330 (Fig. 3).

Fig. 2 Infrared spectra of F-II (dotted line) separated from irradiated and non-irradiated GA_3 (solid line).

Fig. 3 Mass spectrum of F-II separated from irradiated GA_3 .

F-II together with the GA_3 derivatives such as gibberic, allogibberic and epiallogibberic acids were spotted on one and the same thin layer and developed using several different solvent systems. No derivative was correspondent to F-II.

4. Discussion

The results of the TLC of the acidic ethylacetate fraction indicated that at least five fluorescent spots were yielded as the radiolytic products of GA_3 , and suggested that F-II might be the primary product (Fig. 1).

Infrared (Fig. 2) and mass (Fig. 3) spectra of F-II suggested that the γ -lactone ring of gibberellic acid was destructed by gamma radiation. Namely, F-II failed to show any significantly strong ion peak at M-44 (CO_2), M-46 ($HCOOH$) or M-62 (H_2O , CO_2) presumably due to the elimination of the γ -lactone ring⁵⁾.

On the other hand, the basic structure of GA_3 molecule, that is gibberellane, seems to be more resistant to gamma rays, because the absorbance peaks at 1670 and 880-900 cm^{-1} were not appreciably affected (Fig. 2).

In conclusion, the present investigation suggests that GA_3 molecule in aqueous solution is inactivated by gamma rays, primarily

through degradation of the γ -lactone ring, and that, then, the loss of the biological activities of the radiolytic products is ascribable to the initial destruction of the γ -lactone ring with gamma radiation.

Acknowledgement

Valuable discussion of Professors S. Tamura and N. Takahashi is greatly acknowledged.

References

- 1) E.G. Sideris, A. Kleinhofs and R.A. Nilan: *Radiation Botany*, **9**, 349 (1969)
- 2) E.G. Sideris, M.M. Nawar and R.A. Nilan: *Radiation Botany*, **11**, 209 (1971)
- 3) Y. Ogawa: *Plant and Cell Physiol.*, **4**, 227 (1967)
- 4) R.L. Jones and J.E. Varner: *Planta*, **72**, 155 (1967)
- 5) N. Takahashi: in "Gibberellins" (ed. S. Tamura), Univ. of Tokyo Press, Tokyo, p. 59 (1969)