CHANGES OF GREGATIN A AND GRAMININ A IN A HOST PLANT

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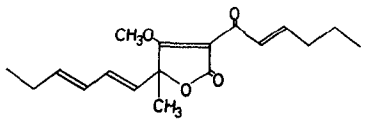
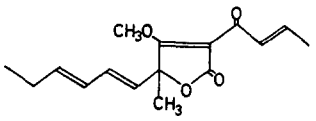
Introduction

We have already reported about chemical[1,2] and biological[3,4] properties of two toxins produced by Genus *Cephalosporium*. Gregatin A is a wilting toxin from *C. gregatum*, causal agent of brown stem rot of adzuki beans and Graminin A is that from *C. gramineum*, causal agent of stripe disease of wheat. They both induced heavy wilting against cuttings of host plants Absence of these toxins in diseased tissues and changes of them *in vivo* are described in the present paper.



Graminin A



Gregatin A

Fig. 1. Structure of Gregatin A and Graminin A.

Materials and Methods

Artificially inoculated plants were used for this study. Adzuki bean cultivar "Takara" and wheat cultivar "Haruminori" were used.

Fungus culture and inoculation techniques.

A strain of *C. gregatum* (A-13) was grown in adzuki bean stem medium as previously reported[3] and a strain of *C. gramineum* (C-3, ATCC 36969) was grown in potato dextrose agar (P. D. A), respectively. Spores were added to sterilized water and adjusted to contain 10^6 spores/ml. The plants were grown in a field. At six weeks after planting, adzuki bean and wheat plants were inoculated hypodermically with about 0.1 ml of spore suspensions of each fungus, respectively.

Extraction

Stems of diseased adzuki beans and wheats were gathered in August. Diseased adzuki bean tissues (fresh weight 1 kg) were homogenized in water, filtered through cheesecloth and centrifuged. The resulting supernatants were concentrated to one tenth the original volume and extracted three times with ethyl acetate. The ethyl acetate solutions were evaporated, leaving an oily residue (0.2 g).

By the same procedures diseased wheat tissues (fresh weight 40 g) gave 100 mg of ethyl acetate extracts. The ethyl acetate extracts were analysed for respective toxins by thin layer chromatography (T. L. C.) and gas liquid chromatography (G. L. C.). The ethyl acetate extracts from healthy tissues were also used as the controls.

Thin layer and gas liquid chromatography.

For T. L. C. ethyl acetate extracts dissolved in a small volume of acetone were spotted on T. L. C. plates (Wakogel B-5F) containing the fluorescent indicator. As a control respective toxin was spotted on it. Chromatograms were developed with chloroform and subsequently examined under ultraviolet light and/or sprayed with diazotized *o*-dianisidine. The G. L. C. instrument was a Hitachi, Model 163, equipped with dual hydrogen flame ionization detectors and fitted with glass column (1 m × 3 mm) packed with 10% SE-30 on chromosorb W (80-100 mesh).

The injection temperature was 250°C and nitrogen (flow rate 30 ml/min) served as the carrier. The instrument was programmed lineally from 170 to 250°C with increasing rate of 5°C/min. As a control each pure toxin was injected.

Toxin injecting experiments

The changes of toxins *in vivo* were studied by artificially injecting respective toxins into healthy host plants. Each toxin solution of 500 μg/ml in 2% ethanol was hypodermically injected into the stem base of each plant seedling eight weeks after planting. At three weeks after injection, plants

were cut from stem base and stems and leaves were treated by the same procedures as described above. The toxins in the resulting ethyl acetate extracts were also analysed by T. L. C. and G. L. C.

Results

Absence of Gregatin A and Graminin A in diseased tissues

On the T. L. C. plates, any compound with the same colour spots and Rf values as Gregatin A produced by *C. gregatum* and Graminin A by *C. gramineum* could not be detected.

Fig. 2 shows gas chromatograms of ethyl acetate extracts of healthy and diseased adzuki bean tissues. It can be seen that these two extracts gave almost identical chromatograms. In this system the retention time of Gregatin A was 10 min. A peak due to Gregatin A was not present in Fig. 2. Fig. 3 shows gas chromatograms of ethyl acetate extracts of healthy and diseased wheat tissues. The retention time of Graminin A was 13 min. But a peak due to Graminin A was also not detected.

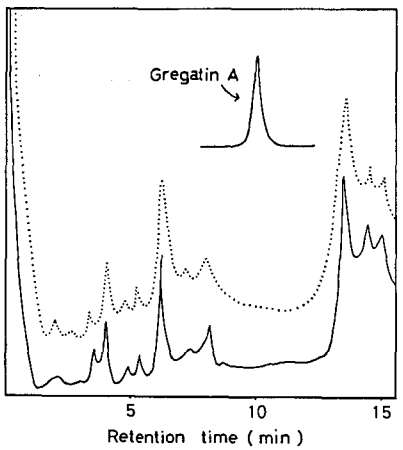


Fig. 2. Gas chromatograms of an ethyl acetate extract of healthy (·····) and diseased (——) adzuki bean tissues compared with pure Gregatin A.

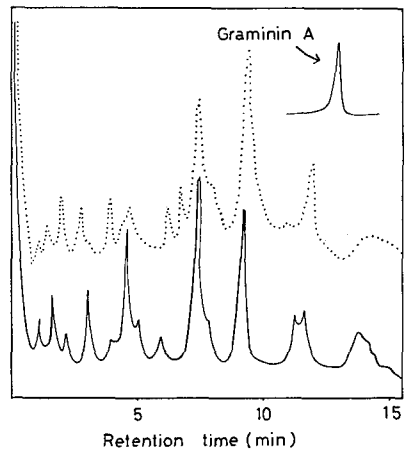


Fig. 3. Gas chromatograms of an ethyl acetate extract of healthy (·····) and diseased (——) wheat tissues compared with pure Graminin A.

From these it can be indicated that Gregatin A and Graminin A might have been changed to another compounds *in vivo*. To confirm this indication toxin injecting experiments were performed.

Toxin injecting experiments

The injection of 500 μg/ml pure Gregatin A into adzuki bean plants in

a field caused vascular browning and leaf wilting within three or four weeks. The injection of 500 μg/ml pure Graminin A into wheat plants also caused chlorosis of leaves and drying. Some of them showed typical yellowish stripes along the leaf blades similar to natural infections.

These toxin-injected tissues were homogenized in water and further extracted with ethyl acetate. The ethyl acetate extracts were analysed for each toxin by T. L. C. and G. L. C. Neither Gregatin A nor Graminin A could be detected on T. L. C. plates.

Figs. 4 and 5 show gas chromatograms of an ethyl acetate extract of toxin-injected tissues. There are no differences between toxin-treated tissues and control. No other peaks due to conversion products were detected in an ethyl acetate extract on G. L. C.

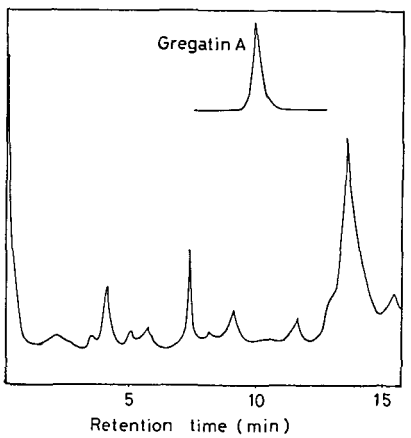


Fig. 4. Gas chromatograms of an ethyl acetate extract of Gregatin A-injected adzuki bean tissues and pure Gregatin A, showing absence of Gregatin A. Compare with Fig. 2.

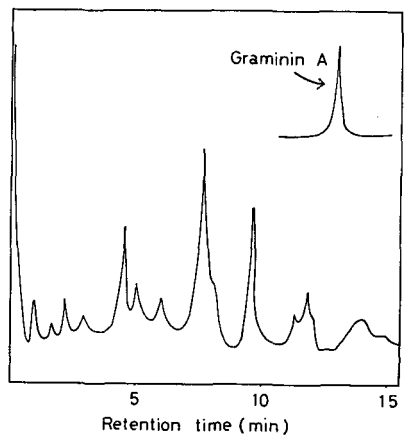


Fig. 5. Gas chromatograms of an ethyl acetate extract of Graminin A-injected wheat tissues and pure Graminin A, showing absence of Graminin A. Compare with Fig. 3.

These facts suggest that Gregatin A and Graminin A might have been converted to more polar compounds which could not be extracted with ethyl acetate in respective hosts.

Discussion

The results reported here confirm our earlier reports[3,4] that Gregatin A and Graminin A may have a role in disease development. The strongest evidence is the observation that symptoms induced by respective toxins alone

in each host plants were similar to those in natural infections. Until the present, there has been very little information about changes of Gregatin A and Graminin A *in vivo*. Gregatin A and Graminin A have more polar functions in their molecules such as conjugated double bonds, hydroxyl and ketonic groups. So some addition reactions or polymerisations or oxidations may occur in their host plants. Attempts to detect the conversion products are now in progress.

Summary

Diseased adzuki bean and wheat tissues were extracted with ethyl acetate and analysed for Gregatin A and Graminin A by T. L. C. and G. L. C., respectively. These toxins were not detected in diseased tissues. However when they were injected into healthy plants, symptoms developed in three or four weeks. From injected-tissues, Gregatin A and Graminin A could not be recovered. This indicates that these toxins may be changed to another compounds in a host plant and have a role in disease development.

Literature Cited

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