A-5.1.2 Defense and Repair Mechanisms at the Cellular and Molecular Levels

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Abstract

We have studied the mechanism of UV-B retardation of growth in irradiated plants. Growth of the first leaves of fertilized cucumber seedlings was markedly retarded by UV-B radiation. A decrease in Photosynthetic activity and an increase in ascorbate peroxidase activity in these plants were observed, suggesting that active oxygen might be involved in the growth retardation. Growth promoting activity normally present in the extracts of the first leaves, decreased upon UV-B irradiation.

Ultraviolet-B induced lesions in DNA, namely cyclobutane pyrimidine dimer (CPD) and pyrimidine (6,4) pyrimidone (6-4PP), in several plants were determined. In cucumber and sorghum, induction of these photolesions was dependent on temperature and their extent was reduced by simultaneous irradiation with white light. Dark repair of both types of photolesions was undetectable in cucumber. Light dependent removal of 6-4PP was very slow with 50% removal in 4 h. In contrast, 50% of initial CPDs were removed within 15 min. Both photorepair processes were dependent on the intensity of white light and were sensitive to temperature. Inhibition of plant anthocyanin synthesis was correlated with the amount of CPD present. CPD and 6-4PP were also detected in chloroplast DNA isolated from UV-B irradiated spinach leaves. No photorepair activity of these lesions was detected in the chloroplasts.

Thirteen *Arabidopsis thaliana* mutants were isolated to identify the genes involved in UV-B sensitivity.

Key Words: Growth retardation, Photorepair, Chloroplast, Mutant, DNA

Introduction

Partial depletion of the stratospheric ozone layer is resulting in an enlargement of the earth receiving solar radiation at wavelengths from 290 to 315 nm (UV-B). It is feared that increase in UV-B radiation will have some deleterious effects on plants and ecosystem because of its potential to destroy biological compounds. UV-B radiation could cause lesions in nucleic acids and photosynthetic apparatus, resulting in severe damage to physiological function. It has been reported that UV-B radiation retards the rate of growth of various plant species. Despite these reports, plants are expected to have some defense mechanisms to mitigate against the harmful effects of UV-B radiation. However, the mechanisms of plant growth retardation and defense are not well understood.

In this study, we investigated the mechanism of UV-B retardation of growth and defense reactions to UV-B. We show that some growth regulators participate in the UV-B retardation of the growth of cucumber first leaves. To study the defense reaction, indices of DNA lesion repair activity were measured and some UV-B sensitive mutants were isolated.

Materials and methods

Seeds of cucumber (*Cucumis sativus* L. cv. Hokushin) were germinated in wet soil and grown for a week at 25℃ with a relative humidity of 70% under natural light in a greenhouse. The plants were then transferred to an artificially illuminated growth chamber, preconditioned for 2 days with a 12:12 h light dark cycle at 20℃ during the light periods and at 15℃ during the dark periods. UV-B radiation was supplemented to the white light provided by fluorescent sun lamps (Toshiba FL20SE, Tokyo, Japan). Polyvinylchloride film (Cutting Sheet 000C, Nakagawa Chemical Inc., Tokyo, Japan) was used as a filter to cut off the UV light at wavelengths shorter than 290 nm. Fertilized plants received fifty milliliter of Hyponex™ (The Hyponex Co. Inc. Ohio, USA) was every other day. UV-B irradiated leaves were cut off and then immediately frozen in liquid nitrogen until extraction of their DNA[1)].

The activity of growth-promoting substances in leaves was measured as described below. Primary leaves were extracted in 80% ethanol. The alcohol was then dried in an evaporator. The dried residue was dissolved in water. The growth-promoting activity in these solution, in terms of fresh weight increase, were assayed with of cucumber leaf disk[2)].

Sorghum (*Sorghum bicolor*) or cucumber seeds were germinated, and then grown for 5 (cucumber) or 7 days (sorghum) in darkness to obtain etiolated seedlings. Spinach

(*Spinacea oleracea*) leaves were obtained from a commercial market and irradiated vertically above the leaves in the darkness with UV-B from the same lamp described above. UV-B irradiated plants were frozen in liquid nitrogen until subsequent extraction of their DNA [3].

Seeds of *Arabidopsis thaliana* L. Henyh. were mutated with ethyl methanesulfonate (EMS). The mutants resulting were screened for UV-B sensitive phenotypes.

The activity of ascorbate peroxidase was determined by the method of Tanaka et al.[4] The photosynthetic activity of the cucumber leaves was measured by the oxygen electrode method. Isolation of DNA from the plants was done according to the method of Lichtenstein et al.[5] Cyclobutane pyrimidine dimers and pyrimidine (6,4) pyrimidone (6-4PP) in the DNA were measured according to the method of Mori et al. [6]

Results and discussion

The growth of cucumber seedling first leaves was promoted by fertilization. However, the growth of first leave of fertilized seedlings was suppressed by UV-B irradiation to a level similar to that of unfertilized seedlings. The growth of first leaves of unfertilized plants did not decline significantly upon UV-B irradiation. It has been suggested that UV-B radiation causes the generation of active oxygen, which destroys photosynthetic activity. We found that the photosynthetic activity of first leaves was decreased by UV-B irradiation, especially, in the case of fertilized plants. The activity of ascorbate peroxidase, an enzyme involved in detoxification of active oxygen, in first leaves was increased by UV-B irradiation, suggesting that active oxygen was generated by UV-B irradiation in first leaves. Active oxygen was probably the cause of the observed decrease in photosynthetic activity.

Exogenous benzyladenine, a synthetic cytokinin, promoted the growth of disks excised from the first leaves of plants without exposure to UV-B, especially of those supplied with supplemental nutrients. However, the cytokinin did not promote the growth of leaf disks from plants UV-B irradiated. The growth-promoting activity of extracts from first leaves was examined. The activity of the extracts from fertilized, unirradiated plants was high, but the activity of extracts from UV-B irradiated plants was not significant, irrespective of fertilization. These results suggest that growth-promoting substances in first leaves were increased by fertilization, but inactivated by UV-B irradiation. In addition UV-B induced decrease in the sensitivity of first leaves to cytokinin could be related to sensitivity to plant hormones and the amounts of hormones present in first leaves.

It is well known that UV-B induces DNA damage and activity in DNA repair. We tried to produce an impaired DNA standard to examine the DNA damage induced in plants by UV-B. Such a standard was produced by irradiation of λ DNA with a UV lamp or monochromatic light of 260 nm from a large spectrograph. Then we examined the effects of UV-B irradiation on DNA in cucumber first leaves. Irradiation with UV-B induced cyclobutane pyrimidine dimers in the leaves, but the amounts of accumulated dimers were not proportional to the UV-B dose. Furthermore, the dimers, which were induced by UV-B irradiation and accumulated in the leaves, rapidly decreased under illumination without UV-B-free light. These results suggest some repair mechanisms in plants. Irradiation with 290 nm light induced much more of the dimers than did the wavelengths of 300 and 310 nm. Thus, the effect of UV wavelength on the induction of pyrimidine dimers resembles its inhibitory effect on leaf growth.

We have also measured the fluctuations in CPD and 6-4PP level in other plant materials. Both products were formed in etiolated cucumber and sorghum seedlings, and green leaves of spinach. These products increased with UV-B dose and with rising temperature. Both products were repaired even in darkness at different rates depending on plant species. In etiolated sorghum seedlings, CPD decreased to half, and 6-4PP declined by 80% of the respective original amounts in 2 h, while both products remained substantially unchanged through 24 h in etiolated cucumber cotyledons. Under white light containing UV-A, CPD was quickly repaired. The time for decay of CPD to 50% of original levels was observed to be between 10 and 15 min in sorghum and cucumber. 6-4PP was repaired more slowly than was CPD. Spinach chloroplast DNA also formed CPD and 6-4PP, but no photorepair activity was detected. Purification of photolyase from spinach leaves is underway.

We studied induction of anthocyanin, a major UV absorbing pigment in plants, in UV-B irradiated etiolated sorghum seedlings. UV-B induced anthocyanin synthesis at low flux whereas it inhibited anthocyanin synthesis at high flux. The amounts of CPD and 6-4PP were not correlated with anthocyanin induction, but were closely correlated with its inhibition, suggesting that either CPD or 6-4PP may have some inhibitory effect on the induction of anthocyanin synthesis. To identify the genes involved in UV-B sensitivity, *Arabidopsis thaliana* mutants were screened for development under UV-B irradiation. Thirteen strains which showed different UV-B damage symptoms were isolated.

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