A-5. 4 Research on the effect of increased UV-B radiation on the wild plants.

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Total Budget for FY1996-FY1998 28,583,000Yen (FY1997 9,514,000Yen)

Abstract Accumulation of anthocyanins in UV-B irradiated *Solidago virgaurea* seedling were investigated. Population which is growing in higher altitude accumulated high amount of anthocyanins by UV-B irradiation. They seem to be more sensitive to UV-B in anthocyanin production than that is growing in lower altitude. We have also focus on the UV-B induced lesions in DNA, namely cyclobutane pyrimidine dimer (CPD) and pyrimidine (6,4) pyrimidone in plants. Solar radiation in Iriomote Island accumulated more dimers in cucumber cotyledons than that did in Sapporo. CPD photolyase activity decreased when the leaves maturated. This enzyme activity was induced by UV-A. The wavelength-dependence of the CPD photolyase activities in partially purified enzyme from *Sorgum bicolor* was determined. Maximum activity showed between 380 and 420 nm. Changes in the amount of transcripts of AcetylCoA carboxylase genes in *Arabidopsis thaliana* were investigated. The gene encoding acc1 was induced by UV-B irradiation.

Key Words: Wild plants, flavonods, Solar UV, DNA lesion, Photolyase

1. Introduction

Plants use sunlight for photosynthesis and, as a consequence, they are exposed to the UV radiation that is present in sunlight. The UV-B region of sunlight has received much attention in recent years because it has been predicted that a decrease in the ozone layer, as a result of contamination of the atmosphere by chlorofluorocarbons[1], will lead to an increase in the UV-B radiation (290-320 nm) that reaches the earth's surface.

Effect of increased UV-B radiation on higher plants were extensively investigated recent 15 years. By the supplemental UV-B radiation, inhibition of leaf expansion, accumulation of flavonoids and increase in leaf thickness were observed in many plant species [2], [3], [4]. As the most of these studies used crop plants, results of the effect of UV-B on the wild plants are limited[5]. In the this study, we investigated the effect of increase UV-B radiation on the *solidago virgaurea* seedlings which seeds were collected from Mt. Hakusan.

UV radiation directly alters the structure of DNA, in addition to damaging both proteins and membranes. Two major products of the direct damage to DNA are dimers of adjacent pyrimidines, namely, cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6-4)-pyrimidone photoproducts (6-4PP)[6]. Photorepair directly reverses the dimerization of pyrimidines. In this study we determined how much the solar radiation damages plants DNA *in situ*.

The enzyme involved in photorepair of DNA damages, called photolyase, uses energy from light at wavelengths from 300 to 500 nm. Photolyases from bacteria and insect have been purified and well characterized[7,8]. Photorepair has been reported in many plant species[9], but few biochemical and molecular biological investigations of this process have been reported. In this report, we characterize photorepair of CPD and (6-4)PP in dark grown *Sorghum bicolor* seedlings using *in vivo* and *in vitro* assays.

In response to UV irradiation, higher plants are known to produce flavonoids, which absorb UV light and act as a UV protector. Biosynthesis of flavonoids is regulated by cytosolic acetyl-CoA carboxylases (ACCase) which were encoded by genes designated acc1 and acc2 in *Arabidopsis thaliana*. To investigate the mechanism of regulation, changes in the amount of transcripts of acc genes in *A. thaliana* were compared with that of the enzyme as well as that of flavonoids during UV irradiation.

2. Research Methods

To investigate effects of increased UV-B radiation on the wild plants, seeds of *Solidago virgaurea* plants growing at different altitudes of Mt. Hakusan area were collected *in situ* and cultivated at artificial conditions in NIES's phytotrone. Twenty-one days old seedlings were irradiated by supplemental UV-B using fluorescent lamps for 12hr a day. Fresh weight of seedlings, area of 3rd leaves and amount of antocyanins were measured.

Cyclobutane pyrimidine dimers and (6-4)PP, in cucumber cotyledons that had been exposed to solar radiation at two points of different latitude, Sapporo (43 ° N) and Iriomote Is. (24 ° N) were quantitated by enzyme-linked immunosorbent assays (ELISAs) with monoclonal antibodies specific to each type of photolesion.

Using dark grown 3 d-old *Sorghum bicolor* seedlings, CPDs and (6-4)PPs were studied *in vivo* and *in vitro* assays. Twenty of UV irradiated *S. bicolor* seedlings were extracted and activity of photolyases in the extracts were determined with UV-irradiated salmon sperm DNA as a substrate. Dimers remained in the substrate were measured by ELISA with monoclonal antibodies specific for these two types of DNA photoproducts.

For determination of transcript level from different acc genes in *A. thaliana*, partial cDNA for acc1 and acc2 which have different nucleotide sequences were amplified with Polymerase chain reaction (PCR). Level of transcripts for acc1 and acc2 were determined by RNase

protection assay with PCR amplified DNA as probes.

3. Results

(1) Effects of UV-B radiation on *S. virgaurea* seedlings.

When the seedlings were irradiated for a week, population which is growing in higher altitude (ON) accumulated more anthocyanins than population growing lower altitude (BD) (Fig.1). The amount of antocyanins in ON was highly correlated with dose of UV-B radiation (Fig.2). Growth of 3rd leaves was retarded by UV-B radiation in both populations. Extent of growth retardation was also correlated with dose of UV-B radiation in both populations (Fig.3). When the seedlings were irradiated two weeks, extent of growth retardation and the amount of antocyanins were correlated with the dose of UV-B radiation in both populations. However, no difference between ON and BD in these dose response was observed.

(2) Induction of DNA Photolesions in Plant Cells by Solar UV Radiation

The amounts of photolesions increased as the dose of total solar radiation increased. There was a tendency that the amounts of photolesions at Iriomote Is. were more than those at Sapporo in the same dose of total solar radiation (Fig. 4).

(3) Formation of DNA Photolesions and Their Repair in Plants

CPD photolyase formation was induced with maximal efficiency in the UV-A spectral range, whereas (6-4)PP photolyase formation was not affected by light (Fig.5). The monochromatic light source of the Okazaki Large Spectrograph was used to study the wavelength-dependence of the photolyase activities in partially purified enzyme preparations. CPD photolyase spectral sensitivity of activity showed a peak between 380 and 420 nm (Fig.6).

(4) Induction of Acetyl-CoA Carboxylase by UV Irradiation

Before irradiation, the amount of mRNA from both acc1 and acc2 were kept low (Fig 7c). Especially, the acc1 transcripts increased to 4-5 times, at 3-4 hr and 20 hr, after the irradiation began. The change in the level of ACCase and flavonoids showed significant delay in comparison of the level of mRNA (Fig .7a and b). This phenomena suggests that the level of the enzyme is regulated post-transcriptionally and that synthesis of flavonoids is regulated by several other enzymes as well as ACCase.

Amount of flavonoids reached to a plateau level at 24-48 hr after irradiation (Fig.7 a), showing *Arabidopsis* can acclimate to UV within a few days. However, even after the synthesis of flavonoids almost stopped, both ACCase and their transcripts did not come down

to the initial level (Fig.7and c). ACCase might serve the product for the other secondary metabolism.

4. Discussion

S. virgaurea population that was growing higher altitude accumulated more antocyanins than that accumulated in lower altitude population. These results indicated that there populations differentially respond to UV-B radiation. As the flavonoids, such as antocyanins, play an important role in the resistance against UV-B radiation in many plants, population growing higher altitude may adapt themselves to the environment condition of their habitat.

Amount of DNA damages in cucumber cotyledons which irradiated solar radiation seems to depend on the ratio of UV-B in the radiation. This results suggesting the likely effects on plants of the increased UV radiation that will be a consequence of depletion of the ozone layer.

This study showed that activity of CPD photolyase in *S. bicolor* was induced by UV-A and this enzyme was activated with around 400nm monochromatic light. These results indicated that CPD photolyase has different chromophore from that of other organisms.

ACCases are participated to biosynthesis of fatty acids and flavonoids. In this study, transcript level of acc1 is specifically increased precedent to flavonoids accumulation by UV-B radiation. These results suggest that acc1 regulates the level of flavonoids in *A. thaliana*

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