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Accumulation of Chlorogenic Acid as a near UV-shielding Compound in Cauliflower Grown under Enhanced UV-B Radiation

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Since solar radiation contains wavelength essential for photosynthesis accompanying with near-UV light, UV-B effects on biological parameters and acclimation mechanisms are influenced by photosynthetically active radiation (PAR). Therefore, to elucidate near-UV shielding mechanism in higher plants, we cultivated cauliflower under usual solar radiation and increased UV-B from fluorescent lamps, two- or three-fold excess over continuously estimated UV-B dose in PAR during daytime, using computer regulated systems. Increased UV-B radiation had little effect on growth expressed as fresh weigh and leaf area. Water soluble low molecular weight compounds showing absorption in near UV region were enhanced according to the irradiated UV-B dose. One of compounds in cauliflower leaves was identified as chlorogenic acid. This was found to have no near-UV photosensitizerable activity and is known to have an ability to scavenge a wide species of active oxygen. Another pro-oxidant compound that generates superoxide anion radical under near-UV irradiation was not induced by increased UV-B during cultivation, and identified as lumazine, a degradation product from folic acid.

Key words: chlorogenic acid, lumazine, near-UV, UV-shielding compound

INTRODUCTION

The enhanced exposure of UV-B wavelength (280-315 nm) of solar radiation is potentially detrimental to organisms. Preliminary effects are DNA damage by dimerization of pyrimidine bases [1] direct photosynthetic damage by destruction of the D1 protein of photosystem II [2], membrane damage, and protein and hormone inactivation [1]. Reactive oxygen species (ROS) are inducible in plants under environmental stress, including UV-B radiation [3-4]. Cellular UV-B chromophores such as amino acids, NADH, and phenolic compounds can be activated by the UV-B light and react with oxygen to form singlet molecular oxygen and superoxide anion radicals[5]. Higher plants produce a variety of metabolites that effectively absorb UV-B and prevent it from penetrating into the leaf mesophyll cells. For example flavonoid compounds are synthesized in response to UV-B, accumulate in the upper epidermal cells of leaves, and absorb in the UV range in methanolic extracts [6]. Many attentions have been paid to the importance of hydroxycinnnamic acids, another and sometimes abundant class of UV-absorptive aromatic secondary metabolites [6]. We report here the accumulation of widespread phenylpropanoid metabolite , chlorogenic acid (CGA) by the elevated level of UV-B radiation under usual PAR condition, and the properties of antioxidative properties of CGA. In other respect, we detected a photosensitaizbale compound in water soluble fraction, and identified this as lumazine, a metabolite of folic acid. The ability of ROS generation under UV-B radiation was characterized.

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MATERIALS AND METHODS

Plant. Cauliflower (var violet) seeds were obtained from Takii seed Co., Kyoto, and grown at Fukuyama, Hiroshima. Two-weeks –old seedlings were transferred to the enhanced UV-B radiation apparatus, where PAR and UV-B radiation energies were continuously measured respectively, and UV-B, from fluorescent lamps covered with acetate film to eliminate UV-C, was enhanced 2- and 3-fold over the measured UV-B level contained in solar radiation by computer regulated system. The enhanced UV-B radiation was continued for two weeks before harvesting plant to analyze growth and UV-B shielding or sensitizable compounds.

Preparation and separation of water soluble fraction containing low molecular weight compound. Tissue (2 g) was homogenized in 10 mM K-phosphate, pH 7.0, and squeezed. The extract was centrifuged at 144,000 x *g* for 30 min, and the fraction containing pale yellow low molecular compound in the supernatant was separated on Sephadex G-25 column by eluting with distilled water. After lyophylization, the fraction was suspended in 10 mM K-phosphate, pH 7.0, and insoluble material was removed by centrifugation. The soluble fraction was applied to C_{18}Sep-Pak cartridge (Waters), equilibrated with phosphate buffer, eluted successively with phosphate buffer (passed through fraction), 16% acetonitrile, pH 1.5 (acetonitrile fraction), and 100% ethyl acetate. The HPLC conditions for HPLC analysis for acetonitrile fraction were as follows: ODS column, 0-10 min linear gradient of 0-100% of solvent A (methanol: water: acetic acid, 20: 75: 5) and B (100% methanol), detection at 330 nm and 0.8 ml/min

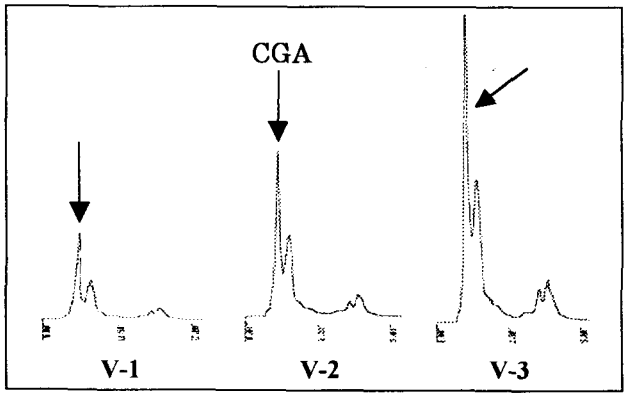

Fig. 1. HPLC analysis of the acetonitrile eluted fraction. PAR (V-1), 2-fold excess UV-B(V-2) , and 3-fold excess UV-B(V-3).

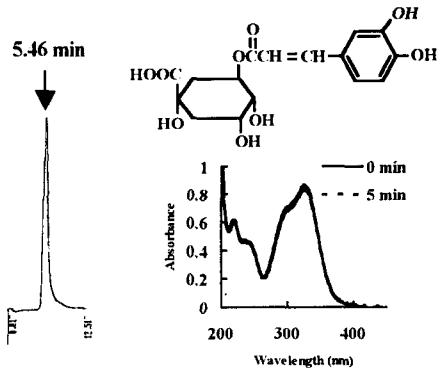

Fig. 2 Structure, HPLC elution profile , and absorption spectra of Chlorogenic acid and stability under UV-B radiation

Detection of O_2^- and ESR spectra. The generation of O_2^- was evaluated by measuring the reduction of Cyt *c* under irradiation of near-UV at 2.5 mW/cm^2. The source and measurement of near-UV were described previously [7]. The experimental conditions for ESR measurement were described previously [8].

RESULTS AND DISCUSSION

Cauliflower was cultivated from June 1st to 14th 2001 at Fukuyama, Hiroshima, Japan. The average PAR and UV-B energies were 5.3 MJ m^{-2} day^{-1} and 5.3 KJ m^{-2} day^{-1}, respectively, thus, the additional average UV-B energies were estimated to be 10.1 KJ m^{-2} day^{-1} (2-fold) and 14.8 KJ m^{-2} day^{-1} (3-fold). The 2- and 3-fold excess UV-B radiation for 2 weeks had little effect on the growth expressed as the dry weight of whole plant (about 5.1 ~ 5.3 g/ plant).

The acetonitrile eluted fraction from C_{18}Sep-Pak cartridge

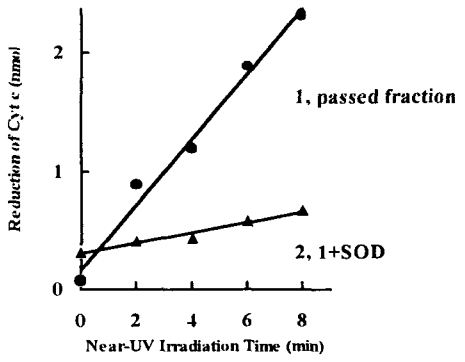

Fig. 3. Reduction of Cyt *c* by the Sep-pak passed through fraction under near-UV radiation

prepared from water soluble fraction was analyzed by HPLC, monitoring near UV absorption at 330 nm. At least 4 peaks were detected (Fig. 1), and the compound eluted at 5.46 min was identified as CGA by the same retention time and absorption properties in near UV region (200-400 nm) as those of authentic CGA. The contents of CGA appeared to increase according to the elevated UV-B energy. CGA has an absorption in near-UV region (Fig. 2), with molecular absorption coefficient of 19,200 at 325 nm in ethanol (Merk Index). Spectrum of CGA in 50 mM phosphate buffer, pH 7.4 did not change by the high energy UV-B irradiation at least for 5 min (Fig. 2).

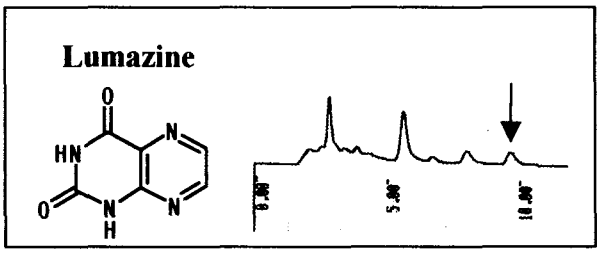

Fig. 4 Detection of lumazine in the passed through fraction by HPLC

In the separated experiments for near-UV sensitizable compounds in water soluble low molecular weight fraction, we found that the passed through fraction showed a distinct reduction of cyt *c* under near-UV irradiation (Fig. 3). The reduction was inhibited by superoxide dismutase, indicating the generation of O_2^- via near-UV sensitizable reaction. A compound that works as near-UV sensitizer was isolated from the passed through fraction, and identified as lumazine by comparing the retention time and absorption spectrum of those of the authentic compound (Fig. 4). ESR experiments performed in the presence of lumazine and DMPO showed the distinct spectra of DMPO-OH during the irradiation of high energy of near-UV, indicating that lumazine is a near-UV sensitizer. However, CGA gave no DMPO-OH signal, indicating that it does not work as near-UV sensitizer. In the presence of equi-molar both compounds, the signal of DMPO-OH derived from lumazine decreased to about a half intensity observed in the absence of CGA. These results suggested that CGA works not only as the near-UV shielding as well as a scavenger for the generated ROS, because CGA is reported to work as an antioxidant against a wide variety ROS [7]. CGA is synthesized via phenylpropanoid pathway: PAL , cinnamate hydroxylase , *p*-coumaroyl-CoA ligase and *p*-coumaroyl-CoA quinate transferase. Lumazine is metabolized from folic acid, via biopterin and pterin.

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