Allixin Induction and Accumulation by Light Irradiation

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Received June 20, 2001; accepted August 24, 2001

Allixin, a phytoalexin isolated from garlic, was induced by irradiating fresh garlic cloves with sunlight or UV light. Induced allixin was analyzed by HPLC, and the accumulated amounts of allixin were 3.1—6.3 μg/g under experimental conditions.

Key words allixin; phytoalexin; induction; UV light irradiation

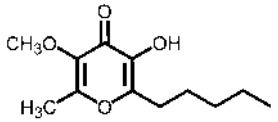
Plants have several defense systems against the invasion of microorganisms. One of these systems is the accumulation of newly produced chemicals which are called phytoalexins.[1,2] Allixin, 3-hydroxy-5-methoxy-6-methyl-2-penthyl-4*H*-pyrane-4-one, is the first phytoalexin compound isolated from garlic, and this compound showed several unique biological properties,[3] such as anticancer promoting activity,[4] inhibition of aflatoxin B1 DNA binding activity[5] and neurotrophic activity.[6]

It is said that phytoalexin is produced by continuous stress conditions which are induced by chemical or biological agents, such as mercury chloride, hydrogen peroxide, or the invasion of microorganisms.[1,2] A number of criteria have been proposed to consider the roles played by phytoalexin in plants through the above experimental conditions.[2] Allixin was induced by standard induction methods for phytoalexins, such as treatment with mercury chloride, hydrogen peroxide, or enzymes (cellulase and pectinase), then this compound was confirmed to phytoalexin by these experimental results, as shown in a previous report.[3]

Phytoalexins can be induced not only by chemical or biological agents, but also by physical agents such as wounding, partial freezing and light irradiation.[1,2,7—10] Evidence of allixin induction was not clearly observed upon cutting or wounding treatment for garlic, but another kind of physical agent, burn treatment, induced allixin.[3] This report presents the possibility of allixin induction by another physical stress, *i.e*. light irradiation.

Result and Discussion

The induction of allixin was performed under sunlight irradiation and UV light irradiation. The results of allixin induction are summarized in Table 1. Although allixin was not detected in the control group, it was detected in the light irradiated groups, except for the stored group after short wavelength irradiation (UV-2 group) and the group of long wavelength irradiation (UV-3 group). The amount of allixin which accumulated after sunlight irradiation treatment was not different between the sun light exposed side (SLI-1) and the side opposite the sunlight exposed side (SLI-2). The group exposed to sunlight irradiation produced higher amounts of allixin than the group exposed to short wavelength UV light irradiation (SWUVL). SWUVL irradiation produced almost the same amount of allixin produced by sunlight irradiation (90% against sunlight groups). The UV-1 group of SWUVL was exposed to stress for a longer period of time than the SLI group, however the UV-1 group also received only half of the UV light energy, lower temperatures and no visual light irradiation compared to the sunlight group. Previously, we investigated the effect of temperature on the induction of allixin at 25 and 37 °C using the chemical elicitor, mercury chloride (not published, experimental methods were the same as in a previous report[3]). The amount of allixin induced at 37 °C was a trace amount compared with that at 25 °C (accumulated range of allixin at 10 d: 2—15 μg/g). Thus, it might be suggested that UV light irradiation from sunlight is a more



Structure of allixin

Table 1. Amount of Allixin Produced by Light Irradiation

Sample	Specimen No.	Produced amount (μg/g)	Average
Control	1	ND	ND
	2	ND	
	3	ND	
SLI-1[a,e,h)]	1	5.0	5.1
	2	6.3	
	3	4.0	
SLI-2[b,e,h)]	1	4.8	5.0
	2	5.7	
	3	4.5	
UV-1[c,f)]	1	5.5	4.5
	2	3.1	
	3	4.9	
UV-2[d,f)]	1	ND	ND
	2	ND	
	3	ND	
UV-3[g)]	1	ND	ND
	2	ND	
	3	ND	

a) SLI-1: part of irradiated side exposed to sunlight irradiation sample. *b*) SLI-2: part of non-irradiated side exposed to sunlight irradiation (opposite side of SLI-1). *c*) UV-1: UV light irradiated sample. *d*) UV-2: UV light irradiated garlic stored 4 d at 25 °C in a dark place. *e*) Irradiation condition: energy of UV: 8.22 mW·h/cm^2, brightness of visual ray: 900—10700 lux, temperature of irradiated period: 30—32 °C, irradiation time: 3.2 h (afternoon in midsummer). *f*) Irradiation condition: main wavelength: 254 nm, energy of UV light: 3.82 mW·h/cm^2, time and temperature: 20 h at 25 °C. *g*) Irradiation condition: main wavelength: 366 nm, energy of UV light: 9.41 mW·h/cm^2, time and temperature: 22 h at 25 °C. *h*) Specimen number on SLI-1 and SLI-2 corresponding to the same garlic clove sample. ND: not detected.

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important factor than heat energy from sunlight in the induction of allixin.

Generally, sunlight is deficient in radiation of wavelengths below around 300 nm because UV lights below these wavelengths are scattered/absorbed in the atmosphere and do not reach to the earth's surface. Therefore, induction experiments of phytoalexin were succeeded using the epigeal part of plant by irradiation of UV-C, below a wavelength of 280 nm.[8—11] Allixin induction was not observed in the group of long wavelength light irradiation (LWUVL), although allixin induction was observed at SWUVL irradiation. This result was similar to the result of resveratrol induction by irradiation of UV light in grapevines.[8] However, sunlight irradiation induced allixin. The end region of UV-B in the long wavelength side has shown the potency of phytoalexin induction ability in the experiment of grapevines, although the amount of phytoalexin produced was small.[8] It might be suggested that garlic cloves are the hypogeal part, and this part may have sensitivities against UV light in the UV-B wavelength range, or perhaps the end region of UV-B in the long wavelength side elicited allixin induction.

Hadwiger and Schwochau reported the induction of pisatin by UV light irradiation, where pisatin was induced by short wave length UV light irradiation for 30 s to 30 min, and pisatin was detectable within 6 to 8 h after irradiation.[7] The results of Hadwiger and Schwochau were similar to allixin case in which allixin was not detectable in UV-2 samples. This result suggests that allixin is not the final secondary metabolite because the amount of accumulated allixin decreased in our previous time course study of allixin accumulation.[3] There were also some reports about the metabolism of phytoalexins by plants.[2] Hadwiger mentioned that the induction of pisatin is related to DNA lesions by UV light,[7] and other researchers mentioned the induction of enzymes related to phytoalexin synthesis by UV irradiation.[8,12] However, we still have not confirmed such evidence of allixin induction, further investigation has to be performed regarding DNA analysis of allixin induction.

Experimental

Garlic (*Allium sativum* L.) for the experiments was cultivated in-house on Wakunaga's experimental field. All chemicals purchased for analysis were of analytical grade and came from Wako Pure Chemical Industries (Osaka, Japan).

Sunlight Irradiation Garlic cloves were peeled carefully and placed on white paper, then irradiation treatment was performed outside for 3.2 h on a sunny day in midsummer. An illuminance meter (Minolta Digital Mater T-1H, Minolta, Osaka, Japan) was placed beside the tested garlic cloves and measured the energy received from sunlight.

UV Light Irradiation The wavelength of UV light for this irradiation experiment was decided according to reports by Hadwiger and Schwochau,[7] Langcake and Pryce[8] and Rakwal *et al*.[12] The skins of the garlic cloves were carefully removed and the garlic was placed in a dish. This dish was wrapped with a polyvinylidene chloride sheet to prevent dryness and these test materials were irradiated by short wavelength UV light at 25 °C for 20 h (main wave length: 254 nm, irradiated UV light energy: 3.82 $mW \cdot h/cm^2$). The energy of UV light was measured using the illuminant meter placed beside the test dish. Some of the irradiated garlic samples were stored at 25 °C for 4 d in a dark place after UV irradiation. The experiment on the irradiation of long wavelength UV light was performed in the same manner as the experiment of short UV light (main wavelength: 366 nm, irradiated UV light energy: 9.41 $mW \cdot h/cm^2$, time and temperature: 22 h, 25 °C).

Preparation of Sample Solution for HPLC Analysis Garlic samples were immediately treated after light irradiation. The root was removed by cutting, then the irradiated side was separated from the non-irradiation side. Each part was weighed and homogenized with about 30 ml of methanol. The resulting homogenate was transferred to a 50 ml volumetric flask with methanol added to fill the flask. A part of this mixture was centrifuged at 15000 rpm for 5 min using a microcentrifuge, then the supernatant was used for the analytical sample solution. Intact garlic (non-irradiated garlic) was used as a control sample, and a test solution of the control was prepared the same as the above. Sample solutions irradiated by UV light were prepared in the same manner as sunlight irradiation.

HPLC Analysis The allixin content of the prepared sample solution was analyzed using HPLC under the following conditions. Column: TSK gel ODS 80 TM (4.6 mm i.d.×150 mm), detection: UV 280 nm (0.02 Aufs), mobile phase: a mixture of methanol and 0.1% phosphate aqueous solution (72 : 28), flow: 0.8 ml/min, HPLC system: Shimadzu LC10A (Shimadzu, Kyoto, Japan). Standard allixin for analysis was prepared according to a previous report.[13]

Acknowledgements We greatly thank Miss Brenda Lynn Petesch, M.S., Nutritionist, Supervisor, Research and Development, Wakunaga of America Co., Ltd., for suggestions for our presented study.

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