Suppression of Oxyfluorfen Activity and Protoporphyrin IX Accumulation in Intact Cucumber Plants by Tetrapyrrole Synthesis Inhibitors*

Hiroshi Matsumoto** and Kozo Ishizuka**

Abstract : Effect of tetrapyrrole biosynthesis inhibitors on herbicidal activity of oxyfluorfen [2-chloro-1(3-ethoxy-4-nitrophenoxy)-4-trifluoromethylbenzene] and protoporphyrin IX accumulation was determined in intact cucumber seedlings. Oxyfluorfen induced rapid light dependent bleaching and desiccation in the plant. The tetrapyrrole biosynthesis inhibitors, gabaculine and 4,6-dioxoheptanoic acid, strongly suppressed oxyfluorfen activity when the roots were treated with these chemicals for 15 hr prior to the herbicide treatment to the shoots. A large accumulation of protoporphyrin IX occurred in oxyfluorfen-treated cotyledons in advance of the appearance of herbicidal symptoms. The inhibitors also reduced protoporphyrin IX accumulation and the pattern of reduction was very similar to that of herbicidal activity. These data indicate that protoporphyrin IX plays a critical role in the mode of action of oxyfluorfen in intact cucumber plants.

Key words : oxyfluorfen, protoporphyrin IX, gabaculine, 4,6-dioxoheptanoic acid, mode of action

Introduction

It is well demonstrated that photobleaching herbicides such as diphenyl ethers, cyclic imides and oxadiazoles cause protoporphyrin IX (PPIX) accumulation in leaf disks or cultured cells of many plant species^{1,2,6,15~17,20,25}. Accumulation of PPIX in plant tissues begins immediately after the herbicide treatment in the light. Since strong correlation has been found between the amount of PPIX accumulated in response to the herbicide treatment and the amounts of ensuing herbicidal damage^{1,2,20,24}, PPIX is considered to be the responsible compound for the action of photobleaching herbicides. PPIX is known to be a generator of singlet oxygen through photodynamic action^{4,7,9}. The toxic oxygen species is thought to induce peroxidative degradation of plant membranes which also has been amply demonstrated^{5,12,14,23}.

The accumulation of PPIX is caused by inhibition of protoporphyrinogen oxidase (Protox) that converts protoporphyrinogen (Protogen) to PPIX, and by the subsequent autooxidation of Protogen to PPIX^{10,18,19,26} in plant cells. Although the molecular target of the action of the herbicides appears to have been discovered, there is less published work on the relationship between PPIX accumulation and the herbicidal activity in intact plants. Matsumoto and Duke²⁰ studied the effect of acifluorfen-methyl on porphyrin accumulation in an aquatic higher plant, *Lemna paucicostata*. They found that PPIX alone accumulated to

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** Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

Abbreviations: PPIX, protoporphyrin IX; Protogen, protoporphyrinogen; Protox, protoporphyrinogen oxidase; DHA, 4,6-dioxoheptanoic acid; ALA, δ -aminolevulinic acid

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significantly high levels and that the amount correlated well with phytotoxic activity. There has not, however, been a study of porphyrin accumulation by photobleaching herbicides and their herbicidal activity in other intact higher plants.

Several inhibitors of porphyrin synthesis pathway such as gabaculine and 4,6-dioxoheptanoic acid (DHA) have been used to demonstrate the involvement of accumulated PPIX for the action of photobleaching herbicides. Great reductions of the herbicidal activity in inhibitor-treated excised tissues or cultured cells have been reported^{15-17,25)}. The objectives of the study were to determine effect of the porphyrin synthesis inhibitors on herbicidal activity of a diphenyl ether herbicide oxyfluorfen [2-chloro-1(3-ethoxy-4-nitrophenoxy)-4-trifluoromethylbenzene] and on PPIX accumulation caused by the herbicide in intact cucumber plants.

Materials and Methods

Plant materials and treatment with chemicals

Cucumber (*Cucumis sativus* L. [cv. Shimoshirazu-jibai]) seeds were planted in flats in vermiculite and watered with distilled water or one-tenth strength of Hoagland solution. Plants were grown in a growth chamber with 25/20°C, day (12 hr)/night for 7 days under 150 $\mu\text{E}/\text{m}^2/\text{s}$ PAR. They were then transferred to a nutrient solution culture. A day after transfer to the solution, roots of the plants were soaked in gabaculine or DHA solution for 15 hr in darkness. Shoots were subsequently soaked in 1 μM of oxyfluorfen solution for 1.5 hr, then washed with distilled water and immediately exposed to the light.

Measurement of herbicidal damage

After 6-hr exposure to the light, one cotyledon of the plants was excised from shoots and the cut end was covered with vaseline. The excised cotyledon was placed adaxial surface down in a polystyrene petri dish containing 10 ml of distilled, deionized water. The dishes were then

shaken slowly for 1 hr in darkness. Electrolyte leakage into the water from the cotyledon was determined with a conductivity meter. The other cotyledons were excised after 8-hr light exposure and their fresh and dry (oven-dried for 24 hr at 90°C) weights were determined.

PPIX assay

Two cotyledons from one plant were homogenized with methanol/0.1 N NH_4OH (9/1, v/v) under a dim, green light source. PPIX in the extracts was determined by HPLC with a fluorescence detector (Ex 400 nm, Em 630 nm) as described elsewhere²⁰⁾.

Results

Effect of tetrapyrrole synthesis inhibitors on herbicidal activity of oxyfluorfen

Shoots of intact cucumber plants were treated with oxyfluorfen for 1.5 hr in darkness; then the plants were exposed to light. Wilting and bleaching of the cotyledons were initiated by the herbicide after 4- and 6-hr of light exposure,

Fig. 1. Effect of pre-treatment with gabaculine on oxyfluorfen-induced desiccation and electrolyte leakage from cucumber cotyledons.

Gabaculine treatment was made on root of intact cucumber plant for 15 hr followed by oxyfluorfen (1 μM) treatment to shoots for 1.5 hr and then exposure to light (150 $\mu\text{E}/\text{m}^2/\text{S}$). Electrolyte leakage and water content were determined at 6 and 8 hr after light exposure, respectively. Bars represent SD of the mean.

(C: untreated control, G: gabaculine, O: oxyfluorfen)

Fig. 2. Effect of pre-treatment with DHA on oxyfluorfen-induced desiccation and electrolyte leakage from cucumber cotyledons.

Experimental conditions are shown in Fig. 1. Bars represent SD of the mean.

(C: untreated control, D: DHA, O: oxyfluorfen)

respectively. Water content of oxyfluorfen-treated plants was reduced drastically within 8 hr and a large quantity of electrolytes leaked from the cotyledons after 6-hr light exposure (Figs. 1 and 2). However, pre-treated tetrapyrrole biosynthesis inhibitors reduced this oxyfluorfen-induced action. Gabaculine (100 μ M–1 mM) completely suppressed desiccation and electrolyte leakage (Fig. 1). Increasing concentration of DHA also reduced the herbicidal action and it was completely arrested by 1 mM of DHA treatment (Fig. 2). The two inhibitors themselves showed no phytotoxic effect at the highest concentration used in the experiment.

Effect of tetrapyrrole synthesis inhibitors on PPIX accumulation

PPIX content in oxyfluorfen treated cucumber cotyledon rose rapidly under light exposure, peaking at 2 hr and then rapidly decreasing (Fig. 3). Only a small accumulation occurred during the 1.5 hr of oxyfluorfen treatment in darkness. Effect of pre-treated gabaculine or DHA on oxyfluorfen-induced PPIX accumulation was surveyed at 2 hr after the light exposure (Table 1). Gabaculine completely suppressed its accumulation at higher than 333 μ M. Increasing concentration of DHA also reduced accumulation of

Fig. 3. Time course of accumulation of PPIX in oxyfluorfen-treated cucumber cotyledons during 6 hr of light exposure (150 μ E/m²/s).

Shoots of the plant were soaked in oxyfluorfen (1 μ M) solution for 1.5 hr in darkness and then exposed to light. Bars represent SD of the mean.

Table 1. Effect of pre-treatment with tetrapyrrole synthesis inhibitors on oxyfluorfen-induced PPIX accumulation in cucumber cotyledons*.

Inhibitor	PPIX content (nmol/g FW)**			
	0	Inhibitor concentration		
		100 μ M	333 μ M	1 mM
gabaculine	2.60 $\pm$ 0.43	0.04 $\pm$ 0.02	<0.02	<0.02
DHA		2.13 $\pm$ 0.33	0.83 $\pm$ 0.14	0.31 $\pm$ 0.10

* Roots of intact cucumber plants were treated by inhibitors for 15 hr followed by oxyfluorfen (1 μ M) treatment to shoots for 1.5 hr and then exposure to light (150 μ E/m²/s). Cotyledons were sampled after 2-hr light irradiation.

** Values are mean of three replications $\pm$ SD.

PPIX and only a small quantity was accumulated at 1 mM.

Discussion

A diphenyl ether herbicide oxyfluorfen induced light dependent phytotoxic action on intact cucumber seedlings. Water content of the plant decreased rapidly and electrolyte leaked from the

cotyledons (Figs. 1 and 2). It is considered that membrane disruption and chlorophyll degradation caused by the herbicide are responsible for the bleaching and desiccation.

Gabaculine and DHA are inhibitors of aminotransferase activity which produces δ -aminolevulinic acid (ALA) from glutamate-1-semialdehyde in a five-carbon pathway^{3,11)} and of ALA dehydratase which produces porphobilinogen from ALA^{8,22)}, respectively. Treatment to roots of cucumber plant by inhibitors prior to oxyfluorfen treatment suppressed the herbicidal activity (Figs. 1 and 2). Similar effects have been reported in detached tissues, leaf disks or cultured cells. However, we found that the suppression of diphenyl ether's action by the tetrapyrrole synthesis inhibitors was also present in intact cucumber plant.

A large amount of PPIX was accumulated rapidly in cucumber cotyledons by oxyfluorfen treatment in the light (Fig. 3). The amount of PPIX reached the maximum 2 hr after light irradiation and then rapidly decreased. The pattern of PPIX accumulation and dissipation in intact cucumber cotyledons was very similar to that in *L. paucicostata*²⁰⁾. Photodegradation and metabolism of PPIX in plants have been suggested as one of the main factors of the decrease^{1,13,20)}. PPIX accumulation appeared to be enhanced by the light irradiation. Mayer and Beale²¹⁾ reported that ALA synthesizing enzymes from glutamate were light dependent. Thus, enhancement of ALA synthesis by light may be involved in this high level of PPIX accumulation.

PPIX accumulation during 2-hr light irradiation was almost arrested by any concentration of gabaculine used in the experiment (Fig. 3 and Table 1). Significant decrease by DHA was detected at higher than 333 μ M. This reducing pattern of PPIX accumulation paralleled that of oxyfluorfen activity.

All above data strongly suggest that PPIX is directly involved in the photodynamic herbicidal

action of oxyfluorfen on intact cucumber plants.

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キュウリ無傷植物体でのオキシフルオルフェン活性とプロトポルフィリン IX 蓄積のテトラピロール合成阻害剤による抑制

松本 宏*・石塚皓造*

摘 要

オキシフルオルフェンは光の存在下で、感受性植物を短時間内に枯殺する作用をもつ。この殺草作用発現へのテトラピロール蓄積の関与をより明らかにするために、テトラピロール合成阻害剤を前処理したキュウリ無傷植物に対するオキシフルオルフェンの作用と、プロトポルフィリン IX の蓄積への影響を調べた。

キュウリの茎葉部を暗条件下で $1\mu\text{M}$ のオキシフルオルフェンに1.5時間浸漬した後に光($150\mu\text{Em}^{-2}\text{s}^{-1}$)を照射すると、茎葉部に速やかな白化と乾燥がおこった。テトラピロール合成阻害剤である

gabaculine と 4,6-dioxoheptanoic acid をオキシフルオルフェンの処理前15時間にわたって根から吸収させると、それぞれ0.1 mM と 1 mM の濃度でオキシフルオルフェンの作用をほぼ完全に抑制した (Fig. 1, Fig. 2)。これらの前処理をせずにオキシフルオルフェンのみを処理したキュウリ体内には、可視症状の発現に先んじて急激なプロトポルフィリン IX の蓄積がおこり、蓄積量は光照射後2時間でピークに達した (Fig. 3)。阻害剤を前処理した場合はプロトポルフィリン IX の蓄積が抑制され、蓄積と作用の抑制の程度はよく一致した (Table 1)。

これらのことからキュウリの無傷植物体でおこるオキシフルオルフェンの枯殺作用に、それによって蓄積されるプロトポルフィリン IX が直接に関与しているものと考えられた。

キーワード: オキシフルオルフェン, プロトポルフィリン IX, gabaculine, 4,6-dioxoheptanoic acid, 作用機構

* 筑波大学応用生物化学系