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Role of β-1, 3-Glucanase in Defense Responses of Cultured Carrot Cells

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Induction of glycolytic enzymes such as β-1, 3-glucanase and chitinase has been postulated as one of the defense responses of plants because the activities of these enzymes are induced by the treatment of the cells with ethylene, mycelial walls and some glycans related to the wall components[1,2]. These enzymes have been shown to prevent microbial growth *in vitro*, and in some cases, liberate elicitors of phytoalexin and phenolic acid production from invading fungi[2-4]. However, these findings have been reported from a very limited plant species, and possible defense function of β-1, 3-glucanase was demonstrated only in endo-hydrolases. Recently, we have purified two isoforms of β-1, 3-glucanases (glucanase I and II) from cell wall-bound fraction of cultured carrot[5]. These isoenzymes were found to be exo-glucanases which liberated glucose as a sole product from laminarin. In the present experiments, we examined possible induction of the glucanases and their lytic activities to mycelial walls in order to understand physiological roles of the exohydrolases in plant defense responses.

Changes in wall-bound β-1, 3-glucanase activity of cultured carrot were tested by the treatment of the cells with several substances related to microbial infection. Carrot cells (10-day-old) grown in Murashige and Skoog's medium[6] were transferred to test tubes (5 m*l*), and various chemicals (10 mg each) dissolved or suspended in Na-citrate buffer (0.1 M, pH 6.0) were added. For ethylene treatment, the gas was introduced to the cell culture in screw-capped test tubes at a final concentra-

Table 1. Changes in cell wall-bound β-1, 3-glucanase activity of cultured carrot cells by the treatment with various chemicals.

	β-1, 3-Glucanase activity (%)
Control	100
Mycelial walls	68
Laminarin	51
Lichenan	53
Chitin	67
Chitosan	69
Control for ethylene	118
Ethylene	124

β-1, 3-Glucanase activities were expressed as percentage to control (1.36 nkat/g fresh weigh cells) as 100%.

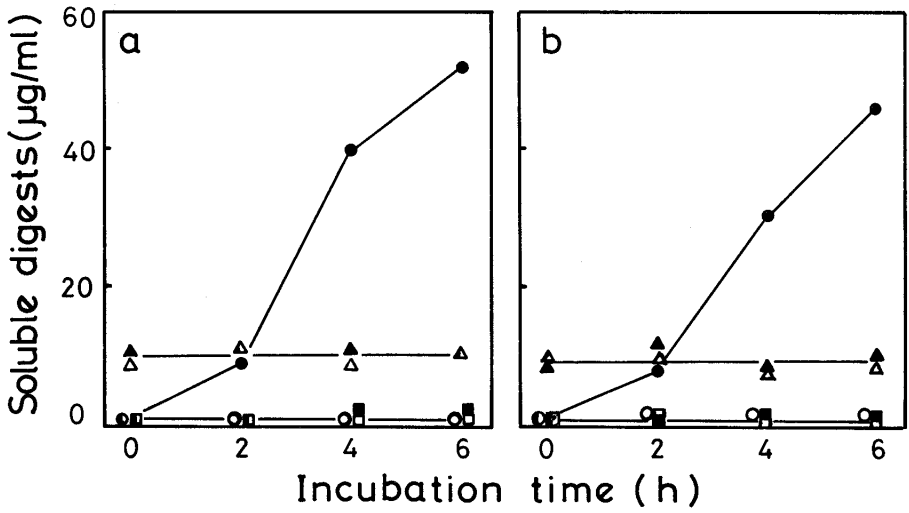

Fig. 1 Hydrolytic activity of β-1, 3-glucanase to mycelial walls of *C. globosum*. Insoluble mycelial walls (10 mg) were incubated with 50 μg of purified glucanase I (a) or II (b) in Na-citrate buffer, and at regular intervals, neutral sugars (●), N-acetylamino sugars (■) and proteins (▲) solubilized from the walls were determined. Open symbols represent the control runs which were incubated with heat denatured enzymes.

Table 2. Effect of mycelial wall fragments hydrolyzed by purified β-1, 3-glucanases on PAL activity and phenolic acid contents in cultured carrot cells.

	PAL activity (nkat/culture)	Caffeic acid (μg/culture)	Ferulic acid (μg/culture)
Control	0. 2	2. 5	1. 7
Mycelial walls	2. 4	48. 2	22. 7
Glucanase I digests	0. 3	3. 1	0. 8
Glucanase II digests	0. 3	2. 6	2. 1

Mycelial walls of *C. globosum* (10 mg) were incubated with 50 μg of purified β-1, 3-glucanase isoenzymes for 6 h at 37° in Na-citrate buffer, and solubilized fragments of the walls (50 μg glucose equivalent) were incubated with 5 m*l* of carrot cell culture, respectively. Insoluble mycelial walls (10 mg) were added to carrot culture as a positive control, and control received only the buffer. PAL activities were determined after 8 h incubation while contents of phenolic acids were after 24 h by the methods described previously[2].

tion of 10 nl/m*l* air. These cultures were incubated on a reciprocal shaker (120 strokes/min) at 26°, and after 24 h, β-1, 3-glucanase activity in cell wall-bound fraction was determined[7]. As shown in Table 1, the enzyme activity decreased by the incubation of the cells with mycelial walls of a fungus, *Chaetomium globosum*. Laminarin, lichenan, chitin and chitosan also showed no stimulative but a negative effect. By contrast, treatment with ethylene resulted in a slight increase in the activity. However, this enhancement was also observed in control culture incubated in screw-capped test tubes without exogenous ethylene suggesting that the apparent increase in the activity was not a direct effect of the hormone.

In the next experiments, we examined hydrolytic activity of the purified glucanases to the mycelial walls of *C. globosum, in vitro*. Mycelial walls (10 mg) were suspended in 5 m*l* of 0. 1 M Na

-citrate buffer (pH 6.0), and were mixed with 50 μg of purified glucanase I and II, respectively. Controls received heat denatured enzymes. They were incubated at 37°, and at regular intervals, 1 m*l*-aliquots were removed and solubilized products were recovered by centrifugation (750 g, 10 min). Hexoses in the digests were determined by phenol-sulfuric acid method[8], and proteins were determined by the method of Bradford[9]. N-Acetylamino sugars were determined according to the method described previously[10]. Carrot glucanases liberated neutral sugars from insoluble walls, while proteins and N-acetylamino sugars were not solubilized (Fig. 1). We reported previously[10] that partial hydrolysates of mycelial walls obtained by carrot chitinase exhibited a stimulating activity of phenylpropanoid pathway in cultured carrot cells. However, glucanase digests of fungal walls obtained here did not enhance phenylalanine ammonia-lyase (PAL) activity, and contents of phenolic acids in carrot cells were comparable to those in the control culture (Table 2). These results, together with the non-inducible nature of this activity, suggested that wall-bound β-1, 3-glucanases of cultured carrot cells play a minor role in defense responses.

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《和文要約》

ニンジン培養細胞の防御反応における β-1, 3-グルカナーゼの役割

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ニンジン培養細胞の壁結合画分に存在する β-1, 3-グルカナーゼは糸状菌の歯糸壁やその構成成分のグルカンの添加によっても活性に変化が認められなかった．精製した二つのグルカナーゼイソ酵素はエキソ型の分解活性を示し不溶性の歯糸壁から中性糖を遊離したが，この部分加水分解物は宿主であるニンジンのフェニルプロパノイド系を賦活する活性を示さなかった．