Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 194-199

CHITOSANS AND PECTIC POLYSACCHARIDES BOTH INDUCE THE ACCUMULATION OF THE ANTIFUNGAL PHYTOALEXIN PISATIN IN PEA PODS AND ANTINUTRIENT PROTEINASE INHIBITORS IN TOMATO LEAVES[1]

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Received November 22, 1982

Summary. The Proteinase Inhibitor Inducing Factor, PIIF, a pectic polysaccharide that induces synthesis and accumulation of proteinase inhibitor proteins in tomato and potato leaves, is an effective elicitor of the phytoalexin pisatin in pea pod tissues. The levels of pisatin induced by PIIF, and the time course of elicitation, are similar to those induced by chitosans, β-1,4 glucosamine polymers, which are potent elicitors of pisatin in pea pods. Similarly, the chitosans, found in both insect and fungal cell walls, are the most potent inducers yet found of proteinase inhibitor accumulation in excised tomato cotyledons. The similarity in the induction of synthesis of proteinase inhibitors in tomato cotyledons and of pisatin in pea pods by pectic polysaccharides and chitosans suggests that the two polysaccharide types may be triggering a similar fundamental system present in pea and tomato plants that regulates the expression of genes for natural protection systems.

The wound-induced synthesis and accumulation of proteinase inhibitors in tomato and potato leaves is apparently initiated by the release of pectic polysaccharides, fragmented from the cell walls following injury (1,2). A highly pure pectic fragment, MW ∿ 5000, called the proteinase inhibitor inducing factor, or tomato PIIF (3,4), isolated from tomato leaves, has potent proteinase inhibitor inducing activity when supplied to excised young tomato leaves (4,5).

Tomato PIIF has also been shown to elicit the accumulation of a diterpene phytoalexin, casbene in castor bean seedlings (6), indicating that the same

[1] College of Agriculture Research Center Paper No. 6383 , Project 1791 and 1884. Supported in part by National Science Foundation grants #PCM-8023285, PCM-8203176 and CSRS grant 5921-0410-9-0244-0.

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0006-291X/83/010194-06$01.50/0

pectic polysaccharides can be responsible for initiating the synthesis of both the protein proteinase inhibitors in tomato leaves and of the enzymes involved with synthesis of casbene. Additionally, pectic polysaccharides have recently been shown to elicit the accumulation of an isoflavanoid phytoalexin from soybeans, called glycinol (7).

In this communication we report that tomato PIIF is an elicitor of the isoflavanoid phytoalexin pisatin, in pea pods. More importantly we find that chitosans, β-1,4 glucosamine homopolymers found in fungal and insect cell walls, which are potent elicitors of pisatin in pea pods (8), are the most potent inducers of proteinase inhibitor synthesis in tomato leaves found to date.

METHODS AND MATERIALS

Tomato leaf PIIF was prepared as described previously (1,2). This water soluble polysaccharide, when supplied to young tomato plants at concentrations of 0.5 to 2.0 mg/ml in 0.05 M sodium phosphate, pH 6.0, for 60 min, will ordinarily induce the leaves to accumulate Inhibitors I or II for over 50 h at steady state rates of accumulation of about 5 μg and 2 μg of the inhibitors respectively per h (cf Figure 2).

Chitosans were prepared as described previously (8) from shrimp chitosan by nitrous acid hydrolysis. For assays, chitosans were dispersed in 0.05 M sodium phosphate buffer, pH 6 and dissolved by adding 1-2 drops of glacial acetic acid and adjusting the pH to 6.0 with 1 N NaOH.

Solutions containing PIIF or chitosans were assayed for inducing activity by supplying to young tomato plants through the cut petiole for 60 min and incubating the plants in CO_2 free air for 24 h under 1000 ft c of light (2). Inhibitors I and II concentrations in the juice expressed from cotyledons were determined immunologically by radial diffusion in agar gels containing rabbit anti-potato Inhibitor I or II antibodies (9,10).

Pisatin concentrations in pea pods were assayed spectrophotometrically at 309 nm as described by Hadwiger and Beckman (8). Solutions to be assayed for elicitor activity were applied to the exposed exocarp surface of freshly excised immature pea pods. Pisatin present in each 0.5 g lot of pods was extracted overnight with 10 ml hexane and the pisatin quantified.

Phenylalanine ammonia lyase activity was determined with [^{14}C]phenylalanine by the method of Loschke et al (11).

RESULTS AND DISCUSSION

The observations that the pectic polysaccharides (PIIF), which are active in inducing proteinase inhibitors in excised tomato leaves, were also active in eliciting synthesis of the terpenoid phytoalexin casbene in castor bean seedlings (6) led us to explore whether PIIF could also induce the isoflavanoid

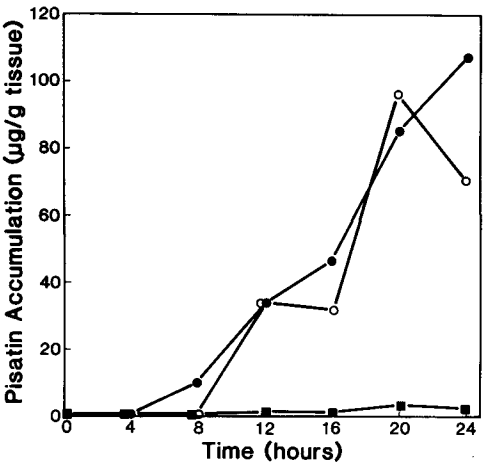

Figure 1. Pisatin accumulation in pea pods after treatment with tomato PIIF, 2 mg/ml in .05 M Na-phosphate, pH 6.0 (O—O); chitosan, 2 mg/ml in buffer (●—●); and buffer alone (■—■).

phytoalexin pisatin in pea pods when supplied at levels that induce proteinase inhibitors in tomato leaves (about 1-2 mg/ml). In Figure 1 pisatin is shown to be strongly elicited by PIIF. This elicitation is compared with the powerful natural elicitor of pisatin (8) called chitosan, a β-1,4 glucosamine

TABLE 1

Accumulation of Tomato Leaf Proteinase Inhibitor I Induced by Tomato Leaf Polysaccharides and Shrimp Chitosans

Additions*	Accumulation of Proteinase Inhibitor I in Tomato Cotyledons (µg/g leaf tissue)
Tomato PIIF (mg/ml)	
2.0	123
1.0	143
0.5	60
Chitosans (mg/ml)	
2.0	135
0.4	143
0.08	144
0.016	115
0.003	98
Buffer Alone	23

* Tomato cotyledons were supplied with tomato PIIF, chitosans, or buffer (0.05 M Na-phosphate, pH 6.0) for one h, followed by incubation in water for 24 h in water and assayed immunologically.

polymer derived from shrimp chitin by deacetylation. Chitin and chitosans are both found in fungal and insect cell walls and have been postulated as possible signals to plants to initiate resistance toward pest attacks (12). As can be seen in Figure 1 PIIF elicits pisatin with a rate of accumulation similar to that induced by chitosans.

The positive results of this experiment logically led to the testing of chitosans in simulating PIIF as an inducer of proteinase inhibitors in excised tomato leaves. In Table 1 chitosans are shown to be potent inducers of proteinase Inhibitor I in cotyledons of excised tomato plants. They induce maximal quantities of Inhibitor I, even when supplied at levels less than one-tenth of tomato PIIF. The time course of accumulation of both Inhibitor I and Inhibitor II induced by chitosans and PIIF are compared in Figures 2A and 2B. Again both the temporal course and quantitative levels of inhibitors that accumulate are identical when PIIF or chitosans are inducers.

Wagoner et al. (16) have previously shown that chitosans induced the de novo synthesis of phenylalanine ammonia lyase (PAL), the first enzyme for the synthesis of pisatin from phenylalanine. The synthesis of this enzyme, as well as the synthesis of several other enzymes involved in isoflavanoid biosynthesis, have been shown to be induced in parsley suspension cultures in response to fungal elicitors (13,14). A similar induction is probably elicited

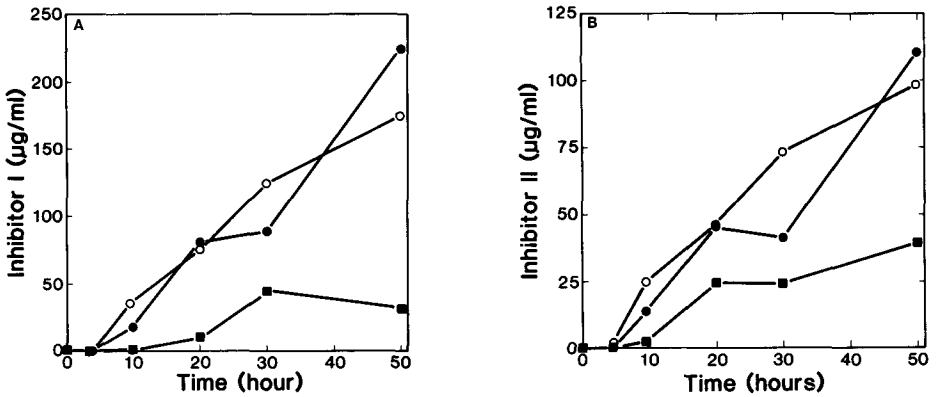

Figure 2. Accumulation of proteinase Inhibitors I (A) and Inhibitor II (B) in cotyledons of young excised tomato plants supplied with tomato PIIF, 2 mg/ml in 0.05 M Na-phosphate buffer, pH 6.0 (○-○) ; chitosan, 0.2 mg/ml in buffer (●-●) ; and buffer alone (■-■) for one h, followed by incubation in water for 24 h.

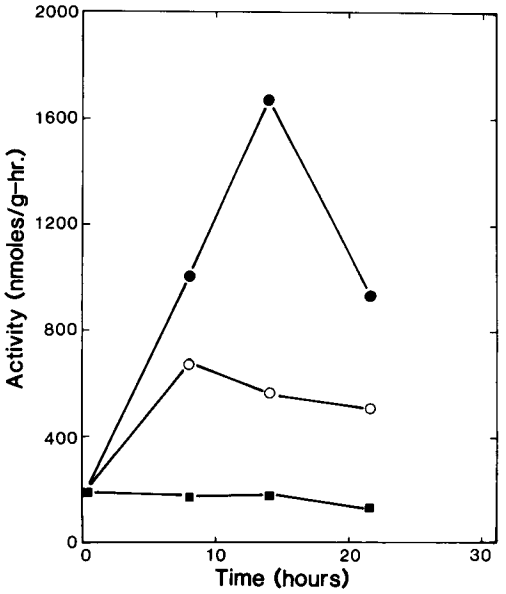

Figure 3. Changes in PAL activity after treatment of pea pod tissue with tomato PIIF, 2 mg/ml in buffer (O—O); chitosan, 2 mg/ml in buffer .05 M Na-phosphate, pH 6.0 (●—●); and buffer alone (■—■)

in pea pods with chitosans. The effects of PIIF on PAL activity in pea pods was assayed since it was eliciting the synthesis of pisatin. In Figure 3 the induction of PAL activity by PIIF in pea pods is shown compared with that by chitosans. Although the amount of PAL induced by PIIF was only about one-half that induced by chitosans, the results clearly demonstrate that PIIF is inducing PAL activity, suggesting that perhaps the entire pathway of phenylalanine to pisatin is being elicited by tomato PIIF.

The possibility that phenylalanine ammonia-lyase activity was induced in tomato leaves by chitosans or PIIF was also considered. Neither chitosans nor PIIF elicited an increase in PAL activity in excised tomato leaves. PAL activity actually decreased when the leaves were detached and neither the addition of PIIF nor chitosans affected the rate or magnitude of decrease.

The similarities of induction of proteinase inhibitors in tomato leaves and of pisatin in pea pods by both chitosans and PIIF suggests that a common mechanism of gene activation may be operating in peas and tomato plants. This same mechanism may also be present in castor beans where casbene synthesis is induced by pectic polysaccharides (6). This would imply that different

polysaccharide molecules including chitosans and pectic polysaccharides, as well as β-glucans, which also elicit phytoalexins in beans (15), may be activating a common system which has evolved in various plants to regulate the expression of different genes involved in a variety of natural plant defenses.

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