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Specific Inhibition of Cell Wall-Bound ATPases by Fungal Suppressor from *Mycosphaerella pinodes*

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Activities of phosphatases were found in the fractions which were solubilized from cell walls of both pea and cowpea seedlings with 0.5 M NaCl. These phosphatases hydrolyzed triphosphonucleotides in the order: UTP=CTP>GTP>ATP; and UTP=GTP>CTP=ATP, respectively. The activities of a pyrophosphatase and a *p*-nitrophenylphosphatase were also detected in these fractions. The suppressor in the spore germination fluid of a pea pathogen, *Mycosphaerella pinodes*, inhibited all of these phosphatase activities in the fraction solubilized from pea cell walls, but it rather enhanced only the activity of the ATPase among those phosphatases from the cowpea cell wall. Hydrolysis of ATP by a cell wall fraction of pea was also markedly inhibited by the suppressor, while hydrolysis of ATP by similar fractions from cowpea, kidney bean and soybean were rather enhanced by the suppressor, as well as by the elicitor. Thus, the cell wall-bound ATPases responded to the suppressor species-specifically. These cell wall-bound ATPases seemed to be different from the plasma membrane ATPases in several respects. The results suggest that plants recognize the fungal signals not only on their plasma membranes but also on their cell walls and, moreover that putative receptors for the fungal signals might be located close to cell wall-bound ATPases or might even be these ATPases themselves.

Key words: Cell wall-bound ATPase — Elicitor — *Mycosphaerella pinodes* — *Pisum sativum* L. — Suppressor — *Vigna sinensis* Endl.

A pea pathogen, *Mycosphaerella pinodes*, secretes an elicitor and a suppressor of the defense responses of pea in its pycnospore germination fluid (Oku et al. 1977, Shiraishi et al. 1978, Thanutong et al. 1982). The elicitor induces active defense reactions, such as the production of a major phytoalexin, pisatin (Shiraishi et al. 1978, Yamada et al. 1989) and an as yet-unidentified infection inhibitor (Yamamoto et al. 1986), and the activation of endochitinase and β-1,3-glucanase (Yoshioka et al. 1992b) in pea plants. The concomitant presence of the suppressor blocks or delays these defense responses that are induced by treatment with the elicitor. The action of the suppressor is species-specific since it inhibits the production of phytoalexin and induces susceptibility only in host plants of *M. pinodes* among various plant species tested (Oku et al. 1980, Shiraishi et al. 1991b).

Recently, it was reported that the suppressor inhibits the ATPase activity in both isolated pea plasma membranes and in pea cells, with an effect similar to orthovanadate, an inhibitor of P-type ATPase (Kato et al. 1993, Shiraishi et al. 1991a, Yoshioka et al. 1990).

On the other hands, orthovanadate suppresses pea defense responses, in acting similarly to the suppressor (Yoshioka et al. 1990, 1992b). These results suggest that inhibition of the ATPase is closely correlated with suppression of the defense responses of pea plants. However, the suppressor was found to inhibit non-specifically the ATPase activities in plasma membranes isolated from seedlings of pea, cowpea, kidney bean, soybean and barley, whereas cytochemical observations showed that the suppressor inhibited the ATPase activity in only pea cells from among these plant species (Shiraishi et al. 1991a). That is, the action of the suppressor appears to be species-specific in vivo.

Abbreviations: BHT, butylated hydroxytoluene; BSA, bovine serum albumin; DTT, dithiothreitol; EGTA, *O,O'*-Bis(2-aminoethyl)ethyleneglycol-*N,N,N'-N'*-tetraacetic acid; MOPS, 3-(*N*-morpholino)propane sulfonic acid; NTPase, nucleotide 5'-triphosphatase; PMSF, phenylmethylsulfonyl fluoride; pNPP, *p*-nitrophenylphosphate; pNPPase, *p*-nitrophenylphosphatase; PPase, pyrophosphatase; RuBP, ribulose bisphosphate; SHAM, salicyl hydroxamic acid.

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These findings suggested the possibility that cell walls include certain target molecules of the suppressor and that these putative molecules affect the plasma membrane ATPase and play an important role in the determination of plant-parasite specificity.

Plant cell walls, which include diverse proteins, are thought to play an important role in the recognition and modification of external or internal signals, such as osmotic stress, plant hormones and metabolites of pathogens (for reviews, see Ralton et al. 1986, Showalter 1993, Varner and Lin 1989). With respect to microbial stress, proline-rich glycoprotein (Bradley et al. 1992) and β-fructosidase in cell walls (Benhamou et al. 1991) have been suggested to participate in resistance to pathogens. However, it is unclear whether plant cell walls also respond to fungal signals, such as the suppressor from *M. pinodes*, that induce susceptibility in host plants. In an early study, it was demonstrated that activities of phosphatases, including ATPase, were associated with cell walls prepared from corn coleoptiles (Kivilaan et al. 1961). However, the relationship between these phosphatases and external signals is unknown. In this study, therefore, we examined whether cell wall fractions from several leguminous plants might include the phosphatase activities. We also examined whether the elicitor and suppressor from *M. pinodes* could affect the function of the cell wall by analyzing their actions on these phosphatases, especially, on ATPases.

Materials and Methods

Chemicals—Tris, 5′-AMP, ATP, CTP, glutathione, α-glycerophosphate dehydrogenase-triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, GTP, ribulose diphosphate, UDP-glucose and UTP were purchased from Sigma Chemical Co. Ltd., St. Louis, MO. UDP-[C^{14}]glucose was obtained from Amersham Life Science, Tokyo, Japan. Other chemicals were from Wako Pure Chemical Inc., Osaka, Japan.

Plant materials—Seeds of *Pisum sativum* L., cv. Midoriusui; *Vigna sinensis* Endl., cv. Sanjakusasage; *Glycine max* Mer., cv. Greenhomer; and *Phaseolus vulgaris* L., cv. Ceremony were soaked in water overnight and sown on vermiculite in plastic containers. Seedlings were grown at 20–24°C for 8–10 days in the darkness.

Elicitor and suppressor from Mycosphaerella pinodes—The elicitor and suppressor were prepared from the pycnospore germination fluid of *Mycosphaerella pinodes* (Berk. et Blox) Vestergren, strain OMP-1 (IFO-30342, ATCC-42741), by the method of Yoshioka et al. (1990). In this report, we used a partially purified preparation of suppressor, which included supprescins A and B (Shiraishi et al. 1992) and at least four other, as yet-unidentified suppressor molecules (our unpublished data), for an examination of the overall effect of the suppressor fraction on the phosphatase activities in cell walls.

Fractionation of cell walls and solubilization of proteins from cell walls—Preparation of the cell wall fraction and solubilization of cell wall-bound phosphatases were carried out at 0–4°C by the method of Hayashi and Ohsumi (1994) with slight modifications. About 100 g of etiolated seedlings were homogenized with 200 ml of 75 mM MOPS-KOH (pH 7.6) that contained 0.25 M sucrose, 5 mM EDTA, 5 mM EGTA, 2 mM PMSF, 4 mM SHAM and 10 μg ml^{-1} BHT with a homogenizer (Polytron, model K; Kinematica AG, Switzerland). The homogenate was filtered through three layers of cheesecloth. The tissue fibrils remaining on the cheesecloth, were further homogenized in the same buffer with a mortar and pestle, and the filtered and pooled homogenate was centrifuged at $800 \times g$ for 15 min. This step was repeated at least three times. The pellet was then homogenized with four volumes of 5 mM MOPS-KOH (pH 6.5) that contained 0.25 M sucrose, 1 mM EDTA, 0.2 mM PMSF, 10 mM KCl and 1 mM DTT, and the homogenate was centrifuged at $800 \times g$ for 15 min. After these manipulations had been repeated at least three times, the protein and ATPase activity were scarcely detectable in the final supernatant. The final pellet was collected as the cell wall fraction.

Solubilization of cell wall proteins with NaCl—The cell wall fraction was incubated with an equal volume of 1 M NaCl at room temperature for 15 min and then centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatant was filtered through a membrane filter (Centricell 20; cut-off 10,000 Da; Polyscience Inc., Warrington, PA.). The inner solution was washed twice with 30 mM Tris-MES (pH 6.5) to remove NaCl. About 18% of the protein and more than 99% of the ATPase activity, that were tightly bound to the cell wall fraction, were collected in the inner chamber by this method. The materials in the inner chamber was used as the solubilized enzyme fraction.

Assessment of the purity of the cell wall fraction—To assess the purity of the cell wall fraction, the activities of several marker enzymes were examined. The activities of seven enzymes were assayed as described below by the methods of Hodges and Leonard (1974) with slight modifications. The activities of 5′-nucleotidase and glucose-6-phosphatase, marker enzymes of nuclear membranes, were determined in 30 mM Tris-MES buffer at pH 8.5 and pH 6.5 that contained 3 mM $MgSO_4$ with 3 mM 5′-AMP and 3 mM glucose-6-phosphate, respectively. Activities of acid phosphatase and alkaline phosphatase, marker enzymes of the tonoplasts, were determined using pNPP as the substrate at pH 5.5 and 9.5, respectively. The assays of NADH-cytochrome *c* reductase and NADPH cytochrome *c* reductase, marker enzymes of the endoplasmic reticulum, were carried out in 30 mM Tris-MES buffer (pH 7.5) that contained 0.5 mM cytochrome *c* with 0.5 mM NADH and 0.5 mM NADPH, respectively. The activity of cytochrome

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c oxidase, a marker enzyme of mitochondria, was assayed in 30 mM Tris-MES buffer (pH 7.5) that contained the reduced form of cytochrome *c* at 5 mM. Activities of glucan synthases I and II, marker enzymes of golgi bodies and plasma membranes, respectively, were determined by the method of Kauss et al. (1983). The activity of fumarase, a marker enzyme of mitochondria, was determined in 30 mM Tris-MES buffer (pH 7.3) as described by Massey (1953). The activity of ribulose bisphosphate carboxylase, a marker enzyme of chloroplasts, was measured spectrophotometrically by the method of Racker (1962). The amount of protein in each fraction was determined by the method of Bradford (1976) with BSA as the standard.

Assays of NTPase, pNPPase and PPase activities—The activity of NTPase in the fractions solubilized from cell walls was determined by the method of Perlin and Spanswick (1981). Assays of NTPases were carried out at 25°C for 20 min in 30 mM Tris-MES buffer (pH 6.5) that contained 3 mM Mg-NTP in the absence or presence of 1 mM Na_3VO_4, 50 mM $NaNO_3$, 1 mM NaN_3, 1 mM Na_2MoO_4, 100 μg ml^{-1} (glucose equivalents) of the elicitor or 100 μg ml^{-1} (BSA equivalents) of the suppressor from *M. pinodes*. The assay of pNPPase or PPase was carried out at 25°C for 20 min in 30 mM Tris-MES buffer (pH 6.5) that contained 3 mM $MgSO_4$ plus pNPP or PP_i at a concentration of 3 mM.

Results

Purity of cell wall fractions from seedlings of pea and cowpea—To examine the purity of each cell-wall fraction, the activities of several marker enzymes in these fractions, after solubilization from cell walls with 0.5 M NaCl, were determined. As shown in Table 1, almost all of these enzymes, with the exception of acid phosphatase and glucan synthase II, were undetectable in the fractions solubilized from cell walls of both pea and cowpea seedlings, indicating that there was no contamination by nuclei, chloroplasts, golgi bodies, mitochondria and endoplasmic reticulum in the cell wall fractions. These fractions included acid phosphatase but not alkaline phosphatase activity, indicating that tonoplasts had also been excluded from the cell wall fractions of both plants. The activity of glucan synthase II was not detectable in the fraction solubilized from cowpea cell walls, but the specific activity of this enzyme was 0.40 mM (mg protein)$^{-1}$ h^{-1} in the fraction from pea cell wall (Table 1). However, this activity was markedly lower than that in plasma membranes; the specific activities in plasma membranes from pea and cowpea seedlings were 15.16 and 18.95 mM (mg protein)$^{-1}$ h^{-1}, respectively. Thus, contamination by plasma membranes of the pea cell walls seemed to be below 3% at with respect to glucan synthase II, if all the activity of the enzyme originated from pea plasma membranes. These fractions were used for subsequent experiments.

Presence of NTPase, pNPPase and PPase activities in the fractions solubilized from cell walls, and the effects of the suppressor on these activities—As shown in Figure 1, since UTP, GTP, ATP, CTP, pNPP and PP_i were hydrolyzed by the fractions solubilized from cell walls of both pea and cowpea, NTPase, PPase and pNPPase were all present or tightly bound to these cell wall fractions. The

Table 1 Activities of several marker enzymes in the fractions solubilized from cell walls of pea and cowpea

Enzyme	Pea	Cowpea
	Specific activity [mmol (mg protein)$^{-1}$ h^{-1}]	Specific activity [mmol (mg protein)$^{-1}$ h^{-1}]
Acid phosphatase[a]	0.008±0.001	0.005±0.002
Alkaline phosphatase[a]	ND[e]	ND
Cytochrome *c* oxidase[a]	ND	ND
Fumarase[b]	ND	ND
Glucan synthase I[c]	ND	ND
Glucan synthase II[c]	0.40 ±0.15	ND
Glucose-6-phosphatase[a]	ND	ND
NADH cytochrome *c* reductase[a]	ND	ND
NADPH cytochrome *c* reductase[a]	ND	ND
5-Nucleotidase[a]	ND	ND
RuBP carboxylase[d]	ND	ND

[a] The activities of these marker enzymes were determined by the methods of Hodges and Leonard (1974).
[b] Fumarase activity was determined by the method of Massey et al. (1953).
[c] Activities of glucan synthases I and II were determined by the method of Kauss et al. (1983).
[d] Ribulose bisphosphate carboxylase activity was determined by the method of Racker (1962).
[e] The activity was not detected in the fractions solubilized from cell walls with 0.5 M NaCl.

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substrate-specificities of these phosphatases of pea and cowpea were in the order: UTP=CTP>GTP>ATP>PP_i>pNPP; and UTP=GTP>CTP=ATP>PP_i=pNPP, respectively (Fig. 1). These specificities are very different from those of enzymes in plasma membranes, since the NTPases in pea plasma membranes hydrolyze these substrates in the order: ATP>>CTP>ADP>GTP=UTP, and the activity of PPase was undetectable (Yoshioka et al. 1990 and our unpublished data). The comparison of specific activities of these solubilized phosphatases with that of glucan synthase II (Table 1) showed that almost all of the phosphatase activities in the solubilized fractions seemed to have originated from the cell walls but not from the plasma membranes.

The suppressor from *M. pinodes* significantly but to varying degrees inhibited the activities of CTPase, GTPase, UTPase PPase and pNPPase in the fraction solubilized from cell walls of pea. The activities of GTPase, UTPase, PPase and pNPPase in the cell walls from cowpea were also inhibited, but the CTPase activity was barely inhibited by suppressor. When ATP was used as the substrate, the suppressor also inhibited hydrolysis of ATP in the fraction solubilized from pea cell walls. However, the activity in the analogous fraction from cowpea was inversely enhanced by the suppressor (Fig. 1). These results show the species-specific suppression by the suppressor remarkably revealed on the activity of ATPase, among the various NTPases, PPases and pNPPase in the cell walls.

Some properties of the cell wall-bound ATPases— As described above, only the cell wall-bound ATPase responded to the suppressor in a species-specific manner. Therefore we examined several properties of this enzyme. As shown in Figure 2, the activities of the ATPases that were solubilized from cell wall fractions of pea and cowpea were inhibited by orthovanadate, an inhibitor of P-type ATPases, but they were not significantly affected by nitrate and azide. These results indicate that the cell wall-bound ATPases were P-type ATPases and not V-type or F-type. Since the cell wall-bound ATPase of cowpea was inhibited by molybdate, an inhibitor of acid phosphatases (Lanfermeijer and Prins 1994), the fraction might also have included another type of phosphatase.

Divalent cations such as magnesium ions, calcium ions and manganese ions, enhanced the activity of the cell wall-bound ATPase above 25% compared to those in the absence of these cations (Fig. 3). The effects of cations on the cell wall-bound ATPases of pea and cowpea were in the order: $Mn^{2+}=Mg^{2+}\geqq Ca^{2+}$ and $Ca^{2+}\geqq Mn^{2+}=Mg^{2+}$, respectively, but no very marked differences in the effects of

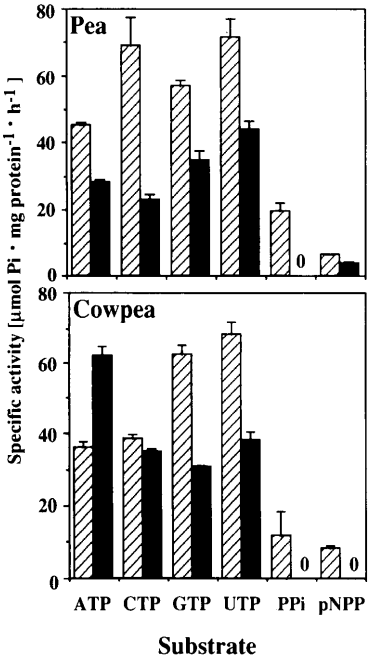

Fig. 1 Effects of the suppressor from *Mycosphaerella pinodes* on the activities of NTPases, PPases and pNPPases solubilized of the cell wall fractions from pea and cowpea. The assays for respective phosphatase activities were carried out at 25°C for 20 min in 30 mM Tris-MES (pH 6.5) with 3 mM $MgSO_4$ in the absence (water control; ▨) or presence (■) of the suppressor at 100 $\mu g\ ml^{-1}$. Respective substrates were used at a concentration of 3 mM. Each value represents the mean with standard deviation (SD) of results from triplicate experiments.

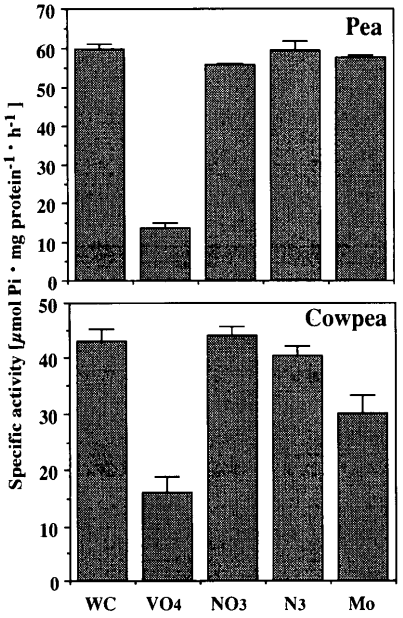

Fig. 2 Effects of several inhibitors on ATPase activities solubilized from cell wall fractions of pea and cowpea. The assay for ATPase activity was carried out at 25°C for 20 min with 3 mM Mg-ATP in 30 mM Tris-MES (pH 6.5) in the absence (water control; WC) or presence of 1 mM Na_3VO_4 (VO_4), 50 mM $NaNO_3$ (NO_3), 1 mM NaN_3 (N_3) or 1 mM Na_2MoO_4 (Mo). Data are presented in the same way as in Figure 1.

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these cations were observed. The ATPase activities were slightly inhibited by zinc ions (Fig. 3). The absence of these cations did not severely reduce the activities of the cell wall-bound ATPases. By contrast the activities of plasma membrane ATPases of pea and cowpea were reduced about 90% in the absence of divalent cations such as Mn^{2+} and Mg^{2+} (our unpublished data).

The effect of pH on these ATPase activities is shown in Figure 4. Both ATPases were at high level in a broad range of pH values. The maximal activities of ATPases of pea and cowpea were observed at pH 6.0 and 8.0 and at pH 6.5 and 9.0, respectively. This result showed that there were several ATPases in the fractions solubilized from cell walls of both pea and cowpea. Since the maximal activities of plasma membrane ATPases from pea and cowpea were observed between pH 6.0 and 7.5, there seemed to be another types of ATPase in the cell wall fractions.

It seems likely that the cell walls of pea and cowpea also contain certain ATPases that are very different from the plasma membrane ATPase and, moreover, that the cell wall-bound ATPase of pea is different from that of cowpea in several respects, as shown in Figures 1, 2 and 4.

Effects of elicitor and suppressor from M. pinodes on ATPase activities in the fractions solubilized from cell walls—As shown in Figure 5, the elicitor from *M. pinodes* significantly enhanced the ATPase activities in the fractions

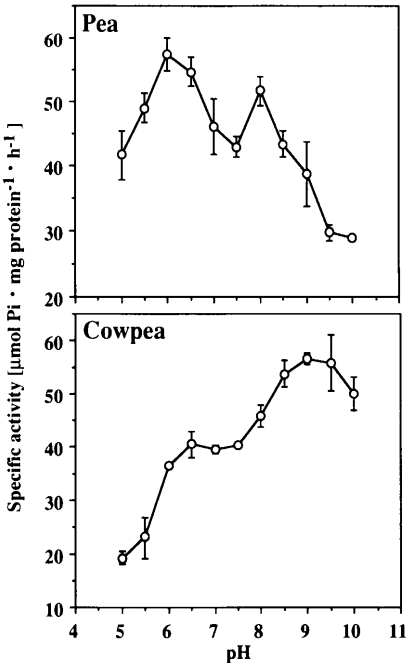

Fig. 4 Effects of pH on the ATPase activity solubilized from cell wall fractions of pea and cowpea. The assay for ATPase activity was carried out at 25°C for 20 min with 3 mM Mg-ATP in 30 mM Tris-MES (pH 5.0–10.0) by the method described in the text. The open circles and bars indicate means and SD, respectively, of results from triplicate experiments.

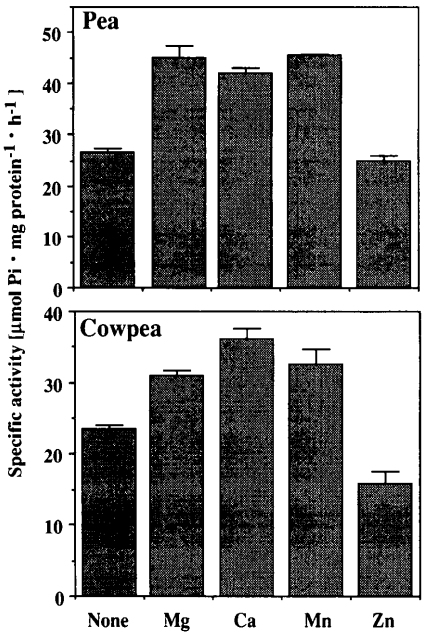

Fig. 3 Effects of several divalent cations on ATPase activities solubilized from cell wall fractions of pea and cowpea. The assay for ATPase activity was carried out at 25°C for 20 min with 3 mM ATP in 30 mM Tris-MES (pH 6.5) in the absence (water control; None) or presence of 3 mM $MgSO_4$ (Mg), 3 mM $CaCl_2$ (Ca), 3 mM $MnCl_2$ (Mn) or 3 mM $ZnCl_2$ (Zn). Data are presented in the same way as in Figure 1.

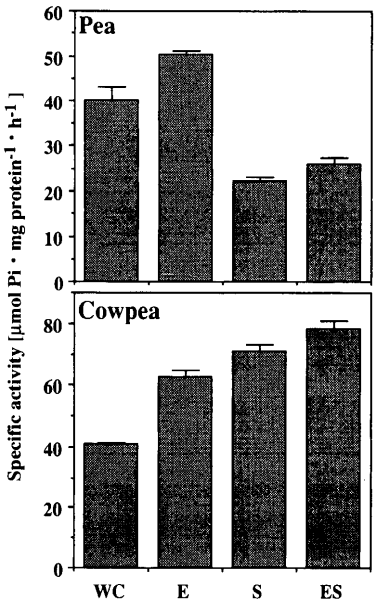

Fig. 5 Effects of the suppressor and elicitor from *Mycosphaerella pinodes* on the activities of ATPases solubilized from the cell wall fractions of pea and cowpea. The assay for ATPase activity was carried out at 25°C for 20 min in 30 mM Tris-MES (pH 6.5) that contained 3 mM Mg-ATP in the absence (water control; WC) or presence of 100 $\mu g\ ml^{-1}$ elicitor alone (E), 100 $\mu g\ ml^{-1}$ suppressor alone (S) or a mixture of the suppressor and the elicitor (ES). Data are presented in the same way as in Figure 1.

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solubilized from cell walls of both pea and cowpea. The suppressor by itself inhibited the activity of cell wall-bound ATPase of pea, the host of *M. pinodes*, but rather enhanced that of cowpea, which dose not serve as a host for the fungus (Fig. 5). In the presence of the suppressor and the elicitor together, enhancement of the ATPase activity by the elicitor was markedly blocked only in the fraction from pea cell walls. By contrast, the cell wall-bound ATPase activity of cowpea was rather enhanced by the concomitant presence of the suppressor. Thus, the suppressor acted on the cell wall-bound ATPase in a species-specific manner.

Effects of the elicitor and the suppressor on the ATPase activities in cell wall fractions from pea, cowpea, kidney bean and soybean—As described above, the ATPase activities in fractions solubilized from cell walls of both pea and cowpea were significantly activated by the elicitor. Moreover, the suppressor affected the activities in both fractions in a species-specific manner. However, it remained to be determined fungal signals could directly affect the activities of ATPases in cell walls. Therefore, the effects of the suppressor and elicitor on the ATPase activities of the cell wall fractions from pea, cowpea, kidney bean and soybean were examined. As shown in Figure 6, the elicitor enhanced the ATPase activities by more than 20% in cell wall the fractions of all leguminous species tested. However, the suppressor inhibited the ATPase activity of the cell walls only in the case of pea, and conversely, the suppressor rather enhanced the ATPase activities of cowpea, kidney bean and soybean, as did the elicitor (Fig. 6). Thus, the ATPases in these cell wall fractions were also able to responded to fungal signals, and the suppressor acted on these ATPases in a species-specific manner, as we observed above with the fractions solubilized from cell walls pea and cowpea (Fig. 1, 5).

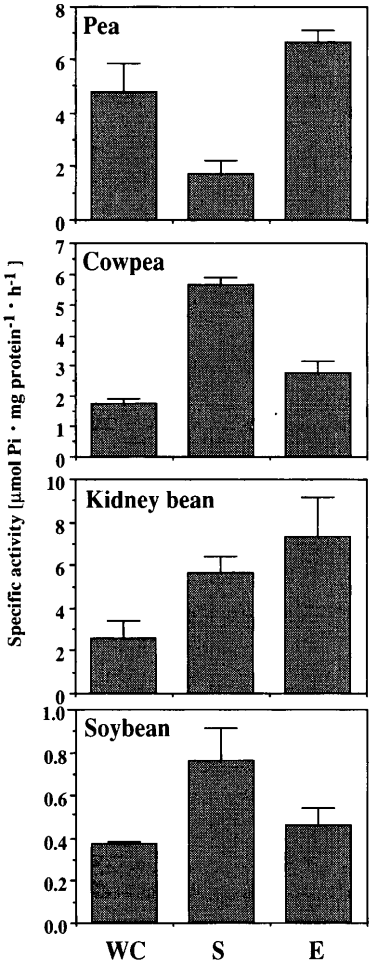

Fig. 6 Effects of the suppressor and elicitor from *Mycosphaerella pinodes* on ATPase activities in cell wall fractions from pea, cowpea, kidney bean and soybean. The ATPase assay was carried out at 25°C for 20 min in 30 mM Tris-MES (pH 6.5) that contained 3 mM Mg-ATP in the absence (water control; WC) or presence of 100 μg ml^{-1} elicitor alone (E) or 100 μg ml^{-1} suppressor alone (S). The protein content of cell wall fractions from pea, cowpea, kidney bean and soybean were 4.0, 14.6, 15.0 and 12.3 mg (g dry wt)$^{-1}$, respectively. Data are presented in the same way as in Figure 1.

Discussion

In this study, we demonstrated that certain phosphatases, such as NTPases, pNPPases and PPases, were tightly bound to cell wall fractions prepared from pea and cowpea seedlings, as Kivilaan et al. (1961) found in their early work with corn coleoptiles. The role of the cell wall-bound phosphatase in cell functions has been in question for many years but these phosphatases have been shown to respond to the fungal signals. The elicitor and suppressor from *M. pinodes* affected the activities of phosphatases in cell wall fractions from several leguminous species tested (Fig. 1, 5, 6). The activities of phosphatases in the fractions solubilized from cell walls of both pea and cowpea were inhibited by the suppressor when GTP, UTP, PP_i and pNPP were used as substrates. The suppressor also markedly inhibited the CTPase activity in the fraction from pea, but only slightly affected that from cowpea (Fig. 1). However, ATP-hydrolyzing activities were enhanced nonspecifically by the elicitor but they were inhibited by the suppressor in a species-specific manner (Figs. 1, 5, 6). The suppressor also inhibited the ATPase activity in the cell wall fraction prepared from pea plants only, and it rather enhanced the ATPase activity in cell wall fractions from cowpea, kidney bean and soybean, which are unable to serve as hosts for *M. pinodes*. The inhibition of cell wall-bound ATPase in vitro by the suppressor seems to reflect our previous cytochemical observation that the suppressor inhibited ATPase activities in vivo only in pea cells and not in cells of cowpea, kidney bean, soybean and barley (Shiraishi et al. 1991a). It was reported that the suppressor inhibited the

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ATPase activities in plasma membranes isolated from all plant species tested, namely, pea, cowpea, kidney bean, soybean and barley (Shiraishi et al. 1991a). In other words, the inhibitory effect of the suppressor on plasma membrane ATPases is not species-specific in vitro. Taken together with other features, such as substrate specificity (Fig. 1) and the dependence both on cations (Fig. 3) and pH (Fig. 4), the results indicate that the cell wall-bound ATPases are very different from the plasma membrane ATPases and, moreover, that the specific inhibition of cell wall-bound ATPases by the suppressor seems to be attributable to difference in the properties of cell wall-bound ATPases from the respective plant species, as shown in Figures 1, 2, 4 and 5. That is, the inhibition of cell wall-bound ATPases by the suppressor seems to be closely related to the specificity of interactions between leguminous species and *M. pinodes*.

It seems very likely that plant cell walls participate in resistance to the pathogen. There are recent reports that suggest that several cell wall-bound proteins, such as peroxidase (Apostol et al. 1989, Bradley et al. 1992), β-fructosidase (Benhamou et al. 1991), β-1,3-glucanase (Benhamou et al. 1989, Mauch and Staehelin 1989) and chitinase (Benhamou et al. 1990), might play important roles in plant defense responses. In pea plants, even when the tissues were treated with elicitor without prior injury, local resistance was induced within 1 h that was accompanied by the production of an as yet unidentified infection-inhibitor. The suppressor also blocked this defense response (Yamamoto et al. 1986) and conditioned pea cells to be susceptible even to avirulent pathogens (Oku et al. 1980, Shiraishi et al. 1978). In this case, the suppressor and elicitor seemed to affect pea cells via their cell walls. Orthovanadate, placed on the surface of cells, also inhibited the ATPase activities associated with all membrane systems in the epidermal cells of five plant species tested (Shiraishi et al. 1991a). As mentioned above, the suppressor inhibited in vitro the ATPase activities in plasma membranes isolated from both host and non-host plants of *M. pinodes*, whereas the ATPase activities of non-host cells were never inhibited in vivo by the suppressor as well as those of cell walls isolated from non-hosts in vitro. It is likely that the cell wall-bound ATPases might affect the ATPases of other organella, such as the plasma membrane and vacuole. Inhibition of cell wall-ATPases might result in a decrease in the activity of plasma membrane ATPases with subsequent by suppression of defense responses, as described previously (Shiraishi et al. 1991b, Yoshioka et al. 1990, 1992a, b). If so, the cell walls might also participate in acceptance of a virulent pathogen as well as in rejection of an avirulent pathogen. The tight connections between cell walls and cytosolic microtubles via plasma membranes was reported to exist (Akashi and Shibaoka 1991, Shibaoka 1993), we assume the above concept may not distance from the facts.

The plant cell wall is thought to be the primary receptor site from which the secondary signals are generated (Ralton et al. 1986). In fact, fragments of plant cell wall such as pectic polysaccharides and oligosaccharides have been reported to induce the accumulation of phytoalexins or the expression of defense genes (Hahn et al. 1981, Darvill and Albersheim 1984, Ryan 1988). While plant hormone-binding proteins have been reported to exist in cell walls of maize and hyoscyamus (Jones and Herman 1993, MacDonald et al. 1991), there are no reports of cell wall-bound receptors for fungal signals, and for suppressors in particular. From our present observations that cell wall-bound ATPases are stimulated non-specifically by the elicitor but are inhibited by the suppressor in a species-specific manner, it seems probable that the cell wall-bound ATPases might acts as a receptor and/or a modifier to recognize and change these fungal signals. In other words, the putative receptor for the fungal signals might bind tightly to and affect the cell wall-bound ATPases. Alternatively, the cell wall-bound ATPases might be the receptors of the signals from *M. pinodes*. The establishment of host-parasite specificity might be mainly regulated by the suppressor as follows: (1) the suppressor inhibits cell wall-bound ATPase activity of host (pea) plants but rather activates those of non-host plants, in the presence or in the absence of the elicitor; (2) putative secondary signals generated in cell walls inhibit the ATPase activity and polyphosphoinositide metabolism in plasma membranes of host (pea) plants (Toyoda et al. 1992, 1993, Yoshioka et al. 1990, 1992a) but those of non-host plants are activated; (3) defense responses, including the expression of defense genes, are suppressed (delayed) in the host (pea) plants but rather elicited in non-host plants (Yamada et al. 1989, Yamamoto et al. 1986, Yoshioka et al. 1992b); and (4) finally host (pea) cells are conditioned such that may become susceptible even to avirulent pathogens but cells of non-host plants exhibit resistance to the pathogens (Oku et al. 1980, Shiraishi et al. 1978). Thus, the cell wall, a structure that is specific to plant cells, plays a key role in the determination of plant-microbe specificity.

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