BIOLOGIA PLANTARUM 39 (3): 387-394, 1997

Accumulation of stress-proteins in intercellular spaces of barley leaves induced by biotic and abiotic factors

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Abstract

Accumulation of the extracellular proteins localized in intercellular spaces of barley primary leaves was examined after inoculation with powdery mildew (*Erysiphe graminis* f. sp. *hordei*) as biotic stress factor and after abiotic stresses such as heat shock, low temperature and heavy metal (Mg, Zn, Cu, Al, Cd and Co) treatment using native polyacrylamide gel electrophoresis. Six to eight major pathogen-induced proteins (bands on native gel) have been identified. Their accumulation at host-parasite incompatibility was more expressive than at compatibility interaction. Elevated temperature did not induce pathogenesis-related (PR) proteins while low temperature induced three of them. Cu, Al, Cd and Co induced accumulation pattern of extracellular proteins was very similar to that in powdery mildew inoculated leaves. Mg and Zn had no effect on the induction of protein accumulation in the intercellular spaces of leaves. Induction of PR proteins by different stresses indicated their general function in the resistance of plants to changing environment.

Additional key words: chilling, heat shock, heavy metals, *Hordeum vulgare*, intercellular washing fluid, pathogenesis-related proteins, powdery mildew.

Introduction

The extracellular matrix with a system of intercellular spaces plays a central role in plant defence response. The amount of proteins dissolved in the water of these spaces or weakly bound to the cell wall is rapidly affected by environmental changes and the pathogen attack. The intercellular spaces contain a large group of so called pathogenesis-related (PR) proteins, which are strongly induced by infection (Carr and Klessig 1989, Boll and Linthorst 1990), cutting injury, high osmotic pressure (Ohashi and Ohshima 1992), heavy metals (Cruz-Ortega and Ownby 1993), freezing (Hon *et al.* 1995) and several chemicals (Van Loon 1985).

Received 26 August 1996, accepted 6 January 1997.
Acknowledgements: The authors thank Mrs. A. Grycová for the excellent technical assistance. This work was supported by Grant Agency VEGA, projects No. 1176 and 478.
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The common characteristics of PR proteins are selective extractibility at low pH, high resistance to proteases, low molecular mass and localization in intercellular spaces (Van Loon 1985). Recently, they have been classified into five groups and in addition the basic vacuolar counterparts to some acidic extracellular forms have been identified (Carr and Klessig 1989). In contrast to the genes encoding acidic PR proteins which are induced mostly upon infection and by salicylic acid treatment, the genes encoding basic PR proteins are induced by ethylene and wounding (Bol *et al.* 1996). Their role in plant defence is only partially known. Glucanase and chitinase are the well-known members of pathogenesis-related proteins with specific function in defence reaction (Mauch and Staehelin 1989).

The aim of the present study was to compare the influence of infection as biotic stress and low temperature, heat shock and heavy metal treatments as abiotic stresses on accumulation of extracellular proteins in barley leaves.

Materials and methods

Barley (*Hordeum vulgare* L. cv. Ricardo) with *Mla3* powdery mildew resistance gene was grown in growth chamber at dark/light temperature of 16/20 °C and a photoperiod (irradiance 70 μmol(PAR) m^{-2} s^{-1}) 16 h. Stress conditions were applied to 7-d-old seedlings. Leaves were inoculated with an avirulent and a virulent race of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) or sprayed with 10 mM solutions of heavy metals ($MgCl_2 . 6 H_2O$, $ZnSO_4 . 7 H_2O$, $CuSO_4 . 5 H_2O$, $AlCl_3 . 6 H_2O$, $CdCl_2 . 2 H_2O$, $CoCl_2 . 6 H_2O$) containing 0.05 % Tween 20 to increase the permeability of the leaf surface. Low temperature stress took 6 d at 6 °C and recovery from low-temperature stress took another 2 d at 20 °C. Two-hour-exposure of plants to 40 °C induced heat shock that was sub-lethal for barley. Leaves exposed to heat shock were infiltrated 24 h after the treatment.

Intercellular washing fluid (IWF) was isolated by the technique described by Rohringer *et al.* (1983). The cut leaves were rinsed with distilled water and infiltrated for one minute with three different infiltration media in desiccator using a vacuum pump. Either 100 mM Tris-HCl buffer, pH 8.0, containing 15 mM mercaptoethanol, or 100 mM citrate-phosphate buffer, pH 2.8, containing 15 mM mercaptoethanol, or only 15 mM mercaptoethanol were used for infiltration. The IWF was recovered after centrifugation of infiltrated leaves at 750 *g* for 10 min. After passing through *Sephadex G25* the IWF was lyophilized. The proteins were separated under nondenaturating conditions on 15 % slab polyacrylamide gels using the discontinuous buffer system (Laemmli 1970) and visualized by silver staining (Heukesloven and Dernick 1985). Proteins were quantified with *Bovine Serum Albumin* as a standard by the method of Bradford (1976).

Results

Using the three different solutions for infiltration of the barley leaves did not show qualitative differences in protein patterns of the intercellular spaces (Fig. 1). Only

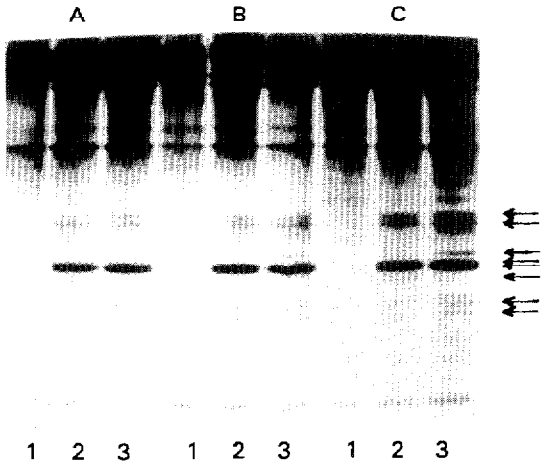

Fig. 1. Anodic PAGE patterns of barley extracellular proteins, 72 h post-inoculation with an avirulent and a virulent races of powdery mildew infiltrated with different solutions. *A* - 100 mM citrate-phosphate buffer, pH 2.8, containing 15 mM mercaptoethanol; *B* - 15 mM mercaptoethanol; *C* - 100 mM Tris-HCl buffer, pH 8.0, containing 15 mM mercaptoethanol; *lane 1* - control plants; *lane 2* - virulent race; *lane 3* - avirulent race.

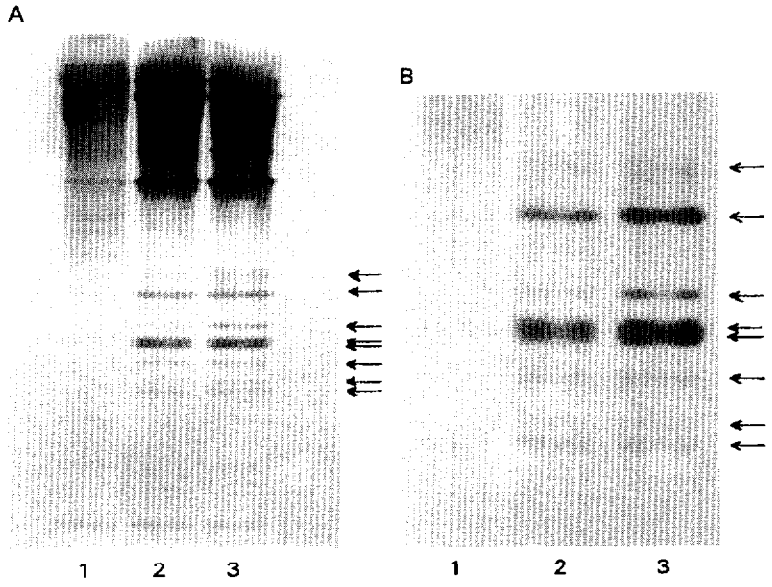

Fig. 2. *A* - anodic PAGE patterns of barley extracellular proteins, 72 h post-inoculation with an avirulent and a virulent races of powdery mildew; *B* - detail of PR proteins. *Lane 1* - control plants; *lane 2* - virulent race; *lane 3* - avirulent race. The pathogen-induced proteins are indicated by an *arrow*.

quantitative differences were detected (data not shown). The highest concentration of proteins in IWF was obtained by the application of 100 mM Tris-HCl buffer, pH 8.0, containing 15 mM mercaptoethanol, therefore only this buffer in the following experiments was used. The accumulation of PR proteins at host-parasite incompatibility was more expressive than at compatibility interaction. Six to eight major pathogen-induced proteins (bands on native gel) have been identified 72 h after inoculation (Fig. 2).

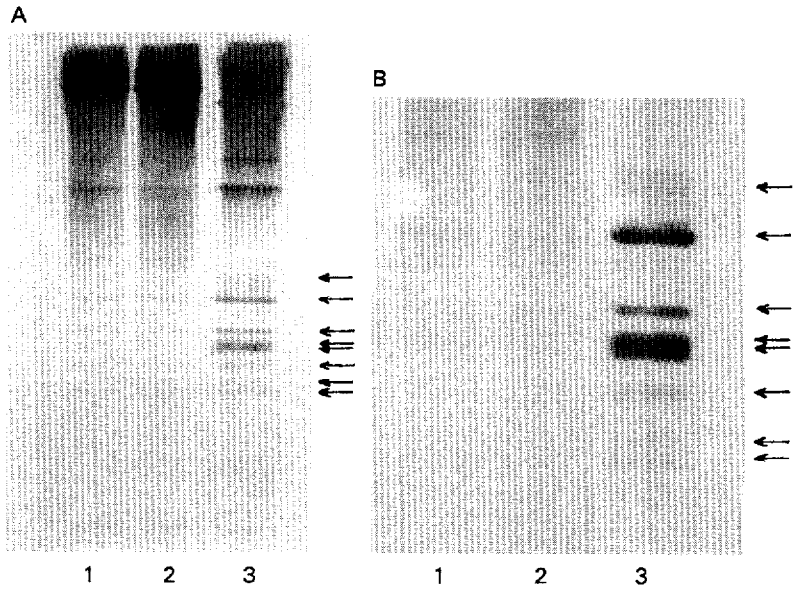

Fig. 3. *A* - anodic PAGE patterns of barley IWF proteins induced by heat shock and avirulent race of powdery mildew; *B* - detail of PR proteins. *Lane 1* - control plants; *lane 2* - heat shock treated plants; *lane 3* - plants 72 h post-inoculation. The pathogen-induced proteins are indicated by an *arrow*.

The heat shock did not induce the accumulation of any proteins in the intercellular spaces of barley leaves (Fig. 3). In contrast, low-temperature stress induced accumulation of three pathogen-induced proteins (Fig. 4). However, this induction was much lower than after the infection. Seedlings, recovering for 2 d under control conditions following the low temperature stress retained increased level of only two proteins (Fig. 4).

Magnesium and zinc treatments did not affect the protein composition of IWF (Fig. 5). Copper, aluminium, cadmium and cobalt induced accumulation of the similar proteins as the powdery mildew infection (Fig. 5). Copper, aluminium and cadmium did not cause any visible changes on the leaf surface. Accumulation of PR proteins induced with these elements was lower than after the inoculation with avirulent races of powdery mildew. Cobalt caused large necrotic lesions like those in incompatible powdery mildew-barley interaction which are the consequence of hypersensitive response of cells. Accumulation of PR proteins induced by Co has always been more intensive than after the infection (Fig. 5).

Discussion

The accumulation of PR proteins is a ubiquitous reaction of monocot and dicot plants to pathogen attack and to several abiotic stresses (Carr and Klessig 1989, Ohashi and Ohshima 1992). In incompatible as well as in compatible barley-powdery mildew interactions the appearance of PR proteins in intercellular spaces is as early as in the preparasitic phase (up to 24 h) of host-parasite interaction (Tamás and Frič 1995).

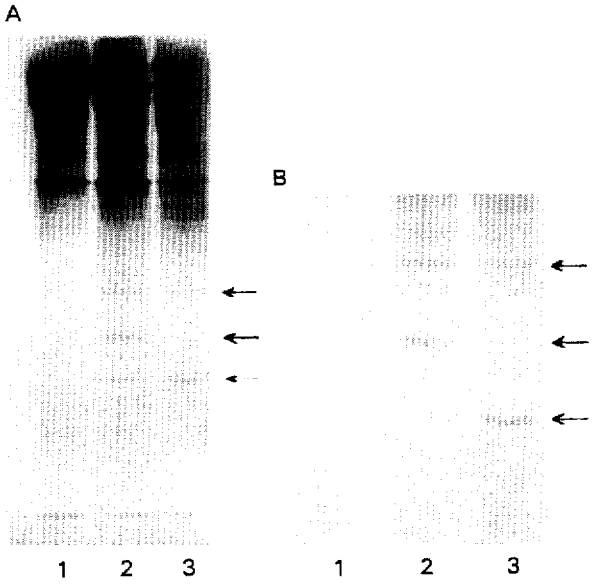

Fig. 4. *A* - anodic PAGE patterns of barley IWF proteins induced by low temperature (6 °C) and after recovery; *B* - detail of low temperature-induced proteins. *Lane 1* - control plants; *lane 2* - low temperature; *lane 3* - recovery. The low temperature-induced proteins are indicated by an *arrow*.

It is a frequent observation, that heavy metals induce some members of the PR proteins. Mercuric chloride induced the PR proteins such as glucanase, chitinase and thaumatin-like protein in maize (Nasser *et al.* 1990, Frendo *et al.* 1992). The induction of PR proteins has also been reported in tomato leaves, where silver nitrate induced ten PR proteins (Granell *et al.* 1987). However, only one of these was acidic while the other nine were basic. In our experiment we detected six to eight main acidic protein bands after infection that were also induced by Cu, Al, Cd and Co treatment. These proteins are similar to tobacco PR proteins by their extractability at low pH and their localization in intercellular spaces (Van Loon 1985). Extraction of these proteins from intercellular spaces of leaves with 0.1 mM Tris-HCl buffer, pH 8.0, gave the same electrophoretic pattern of proteins as extraction with 0.1 mM citrate-phosphate buffer, pH 2.8, but with greater amount of proteins. Magnesium and zinc treatments did not affect the protein composition of IWF. However, excessive zinc in the rooting medium strongly affected the polypeptide composition of the barley leaf apoplasm (Brune *et al.* 1994). A protein similar to PR proteins was

elicited by aluminium chloride, copper chloride and cadmium chloride in wheat roots (Cruz-Ortega and Ownby 1993).

Recent observation, that antifreeze proteins of winter rye are similar to the members of three classes of PR proteins (1.3-glucanase, chitinase and thamatin-like proteins) which have antimicrobial activity, has extended their function in defense

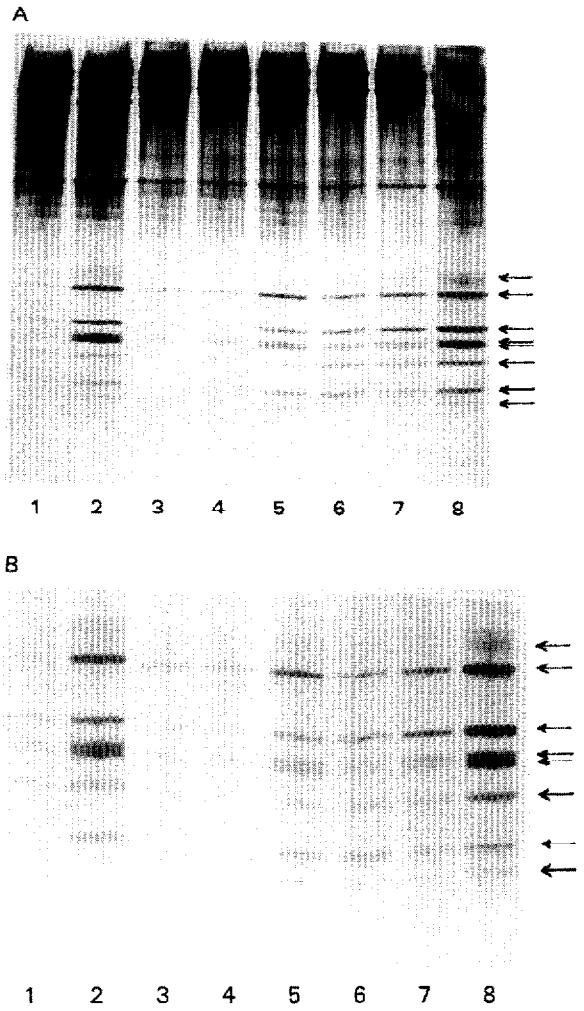

Fig. 5. *A* - anodic PAGE patterns of barley IWF proteins, induced by inoculation and heavy metal treatment, *B* - detail of PR proteins. *Lane 1* - control plants; *lane 2* - plants 72 h post-inoculation with an avirulent race of powdery mildew; *lane 3* - Mg; *lane 4* - Zn; *lane 5* - Cu; *lane 6*- Al; *lane 7* - Cd; *lane 8* - Co. The stress-induced proteins are indicated by an *arrow*.

response (Hon *et al.* 1995). However, the authors detected, that chitinase and glucanase induced by pathogens in freezing-sensitive tobacco did not exhibit

antifreeze activity. In our experiments three proteins, which were accumulated in intercellular spaces after pathogen attack, were accumulated also during low temperature stress. This result suggests their function in cold acclimation process of barley. Moreover, two of these proteins remained accumulated also during recovery period after low temperature stress. The heat-shock did not induce the accumulation of extracellular pathogen-induced proteins in barley leaves which is in conformity with the property of PR proteins that they are not induced by elevated temperature (Carr and Klessig 1989). Absence of such proteins might correspond with the finding that heat induced susceptibility of barley to the powdery mildew (Ouchi *et al.* 1975).

Our results confirmed that heavy metals induce pathogen-induced proteins, some of which are also induced by low temperature. Induction of PR proteins with heavy metals and another stresses indicates their general function in the resistance of plants to changing environment.

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