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Identification and characterization of maize pathogenesis-related proteins. Four maize PR proteins are chitinases

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Abstract

Eight pathogenesis-related proteins extractable at pH 2.8 were found to accumulate in maize leaves after mercuric chloride treatment or brome mosaic virus infection. These proteins were called PRm (pathogenesis-related maize) proteins. Seven PRm proteins were purified to homogeneity by preparative polyacrylamide gel electrophoresis and their amino acid compositions determined. Estimated molecular weights in SDS-containing gels were: PRm 1 14.2 kDa; Prm 2 16.5 kDa; PRm 3 and PRm 4 25 kDa; PRm 6b 30.5 kDa; PRm 6a 32 kDa; PRm 7 34.5 kDa. Antisera raised against either PRm 3 or PRm 4 reacted specifically each with PRm 3 or PRm 4. Antisera raised against PRm 6b reacted with PRm 6b as well as with PRm 6a and antisera against PRm 7 reacted with PRm 7 and PRm 5. Tobacco anti-PR 1b antisera reacted with maize PRm 2.

Chitinase (poly[1,4-(N-acetyl-β-D-glucosamide)]glycanhydrolase, EC 3.2.1.14) activity was found for PRm 3, PRm 4, PRm 5, and PRm 7.

Introduction

Pathogenesis-related (PR) proteins are induced in plants infected with various pathogens: viruses [1, 10, 14, 22, 34, 40, 44], viroids [10], bacteria [2] and fungi [1, 10, 16]. They are also produced after treatment with chemicals such as polyacrylic acid [4, 17], acetylsalicylic acid [4, 45], salicylic acid [40, 46], or mercuric chloride [1, 36], during plasmolysis [44] or during the flowering process of healthy plants [15]. First identified in tobacco [18, 42], PR proteins have since been detected and studied in several dicotyledonous plants with respect to physical, biochemical and serological properties [21, 27, 28, 36, 41], relationships to the corresponding mRNAs [11, 12, 20, 37], gene activation [11, 35] and gene structure [13]. These proteins have characteristic properties: they are extractable at low pH [19, 39], are predominantly localized in the intercellular spaces [24, 30, 36], are resistant to proteolytic enzymes [41] and have relatively low molecular weights. More recently, the biological functions of several tobacco and potato PR proteins have been demonstrated. It has been shown that among the ten major tobacco PR proteins [33] referred to as PR 1a, 1b, 1c, 2, N, O, P, Q, R and S, proteins PR P and PR Q are chitinases [27] and proteins PR O, N and 2 are 1,3-β-glucanases [23]; in addition to the two acidic chitinases (PR P and Q) and the three 1,3-β-glucanases (PR O, N and 2) two basic chitinases and one basic 1,3-β-glucanase which can also be considered as tobacco PR proteins have been found [23, 26]. Among the potato PR proteins six show a chitinase activity and two a 1,3-β-glucanase activity [24].

In spite of the considerable amount of data available on PR proteins in dicotyledonous plants, to our

knowledge, there is only one report giving some informations on PR proteins in monocotyledonous plants. White *et al.*, [46], using an immunoelectroblotting technique, have recently shown the presence of tobacco PR 1-type proteins in mildew-infected barley and in brome mosaic virus (BMV)-infected maize.

Here we report on the identification of eight proteins induced in maize leaves upon mercuric chloride treatment or BMV infection. Seven of these eight proteins were purified to homogeneity and characterized by some of their biological and serological properties, four of them were found to be chitinases.

Materials and methods

Plant material

Maize seeds (*Zea mays* cv. INRA 258) were seeded in trays and grown in a greenhouse under standard conditions. After 10 to 12 days, plants were transferred to a growth chamber at 22 °C and the leaves were vaporized with a solution containing 0.2% mercuric chloride or inoculated with a suspension of BMV using celite as abrasive. Control plants were treated with distilled water or remained undisturbed. BMV was maintained on maize leaves and an inoculum was obtained by griding 1 g of infected leaf tissue in 1 ml of 50 mM sodium acetate buffer pH 4.8 according to Pfeifer and Hirth [32].

Protein extraction

Mercuric chloride-treated leaves were harvested three to four days after treatment and virus-inoculated leaves were taken five days after inoculation. Leaves were either immediately processed or stored at -80 °C. Batches of 800 g leaf tissue were ground for 2 min at high speed in a Waring Blendor in the presence of phosphate-citrate buffer pH 2.8, using 1 ml buffer per g leaf tissue, and the crude PRm protein extracts were prepared as previously described for bean PR proteins [36]. The protein content of the crude extracts was analysed by subjecting aliquots to polyacrylamide gel electrophoresis (PAGE). Protein concentration was measured according to Bradford [8] using the Bio Rad (München) dye reagent and bovine serum albumin (BSA) as a standard.

Polyacrylamide gel electrophoresis

PAGE was performed onto composite 1 mm analytical or 5 mm preparative slab gels with a 5% stacking gel and a 12% separating gel [36]. For molecular weight estimations polyacrilamide gels were run in the presence of 0.1% SDS according to Laemmli [25] with a 5% stacking gel and a 15% resolving gel.

Gels were stained for 1 hour with 0.25% Coomassie Brillant Blue (Serva) in a methanol/acetic acid/water (40/10/50) mixture and destained 2 hours in the same mixture. Protein bands were localized either directly after staining or from densitometer tracings at A_{280} in a Transidyn 2955 scanning densitometer.

Antiserum production and immunoblotting

Sera were collected from rabbits following a course of five injections (at intervals of two weeks) using 20 μg of proteins for each of the purified PRm 3, PRm 4, PRm 6b and PRm 7 for the first injection and 10 μg of proteins for the subsequent booster injections. Tobacco PR 1b antiserum was a kind gift of M. Legrand and B. Fritig.

Immunoblots were prepared essentially according to the basic procedure of Towbin *et al.* [38]. After incubation in rabbit antiserum and subsequent washings the nitrocellulose membranes were placed, for immunodetection of the reactive proteins, in the presence of [^{125}I] protein A, and the serological reactions detected by autoradiography [36], or incubated for 4 hours at room temperature in phosphate-buffered saline (20 mM phosphate, 150 mM NaCl, 3 mM KCl, pH 7.4) containing 0.05% Tween 20, 0.5% BSA and phosphatase-conjugated goat anti-rabbit IgG (Sigma) at 1 μg/ml. Serological reactions were detected by the coloric method using 0.005% 5-bromo-4-chloro-3-indoyl phosphate (Aldrich), 0.01% nitro blue tetrazolium

chloride, 1 mM $MgCl_2$, 0.1 M diethanolamine, pH 9.6 described by Blake *et al.* [5].

Amino acid analysis

Freeze-dried protein samples (50–70 μg) were dissolved in 6 N HCl and hydrolysed under nitrogen for 24 h at 110 °C. In order to determine the cysteine and methionine residues, the protein samples were first subjected to a performic acid oxydation and then hydrolysed at 110 °C in 6 M HCl according to Moore [29]. The samples were analysed using either an amino acid analyser Durrum D 500 or by HPLC-WISP 712 system (Waters).

Chitinase assay

Endo- and exochitinase activity was determined by a colorimetric assay according to published procedures [6, 26]. Each enzyme activity was measured using at least 6 different enzyme dilutions (each dilution in duplicate). The activity was estimated for an enzyme concentration approaching zero [6]. The enzyme activity catalysing the formation of 1 mol GlcNAc per second was defined as a Katal (kat).

Results

Identification of PR proteins

Mercuric chloride-treated leaves showed necrotic symptoms increasing in severity with the concentration of the solution used (0.1 to 0.3%). Most of the treatments were done with a 0.2% solution because this concentration yielded the highest amounts of maize PR proteins. The necrotic leaves from BMV-inoculated maize wer removed for extraction 5 days after inoculation.

The crude extracts of control, mercuric chloride-treated, and virus-infected leaves were subjected to gel electrophoresis under native conditions (Fig. 1). As compared to extracts from control plants, seven additional protein bands are present in treated or infected leaves. Proteins corresponding to these bands showing some common properties with PR proteins

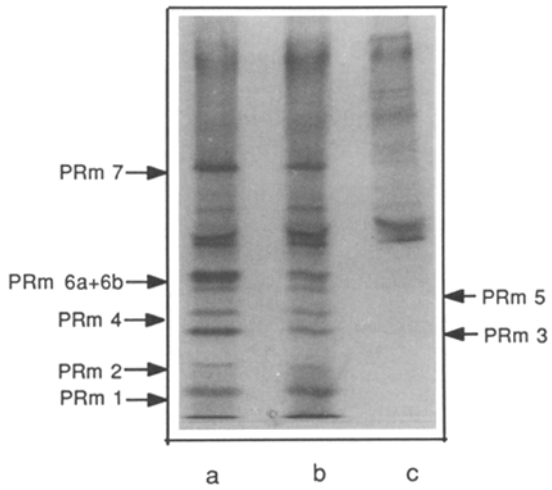

Fig. 1. Electrophoretic patterns in 12% native gels of soluble proteins extracted at pH 2.8 from 0.2% mercuric chloride-treated (a) and BMV-infected (b), three days after treatment or five days after inoculation. Lane (c) shows undisturbed of distilled water-treated leaves.

from other plants (solubility at low pH, low molecular weight, resistance to proteases) were called PRm (m for maize) proteins and referred to as PRm 1 to PRm 7 in order of decreasing mobility.

The protein band corresponding to PRm 6 contains, in fact, two proteins hardly separated on 12% polyacrylamide gels under native conditions. These two PRm 6 proteins are referred to as PRm 6a and PRm 6b. Variations in the relative amounts of PRm 1 and PRm 2 proteins were sometimes observed from one crude extract to the other: in some extracts PRm 1 was present in normal amounts whereas PRm 2 could hardly be detected. The opposite situation was found in other extracts, but usually, by using severely stressed plants, PRm 1 and PRm 2 were both present in relatively high amounts. Other types of stresses such as salicylic acid treatment or a mild plasmolysis induce most of the PRm proteins (results not shown); however under these conditions PRm 1 and PRm 2 proteins are usually undetectable or present in very low amounts.

Purification and characterization of PRm proteins

Since the PRm proteins were well separated on native

analytical polyacrylamide gels apparently as a result of differences in net charge, preparative polyacrylamide gel electrophoresis was employed to separate and purify these proteins. The bulk of PRm proteins in crude extracts prepared at pH 2.8 from 800 g batches of leaf tissue were precipitated at 80% ammonium sulphate saturation. The proteins were recovered by centrifugation and the pellet dissolved in Tris-HCl buffer, pH 8 (50 mM Tris, 1 mM EDTA, 3 mM mercapto-ethanol). The ammonium sulphate step removed some of the pigments present in the crude extract. The protein solution was passed through a Sephadex G 50 column (4.5×100 cm) equilibrated with the same buffer. This step removed material of higher molecular weight as well as a large proportion of the remaining yellow colour. The PRm protein-containing fractions were pooled, concentrated in an Ultrafiltration Cell 202 (Amicon) using YM 5 membranes, loaded onto a 12% preparative polyacrylamide gel and electrophoresed at 25 mA for 24 h. After electrophoresis the gels were scanned at A_{280} and the areas containing the individual PRm proteins cut out and electroeluted from the gel using an Electrophoretic Concentrator Isco 1750.

Since after electroelution, the individual PRm proteins were contaminated by non-protein material from the polyacrylamide gel [9] additional purification was required. Each individual protein was subjected to DEAE-cellulose chromatography and eluted with Tris-HCl containing 200 mM NaCl in order to remove polyacrylate contaminations. Finally the purified protein was passed through a small Sephadex G 25 column to remove the remaining salts.

Each individual protein fraction was analysed by electrophoresis on SDS-containing polyacrylamide gels (Fig. 2). When stained PRm 1, 2, 3, 4, 5, 6b and 7 proteins appeared homogeneous. While PRm 6b protein could be purified to homogeneity, PRm 6a remained contaminated by PRm 6b. This protein could not be purified so far, neither by gel electrophoresis nor by chromatography on TSK-phenyl 5PW column (Beckman) nor on a QMA column (Waters) in a high-performance liquid chromatography system. Yields of about 0.15 to 0.5 mg protein (depending on which PRm protein is considered) were routinely obtained from 800 g of fresh leaf tissue.

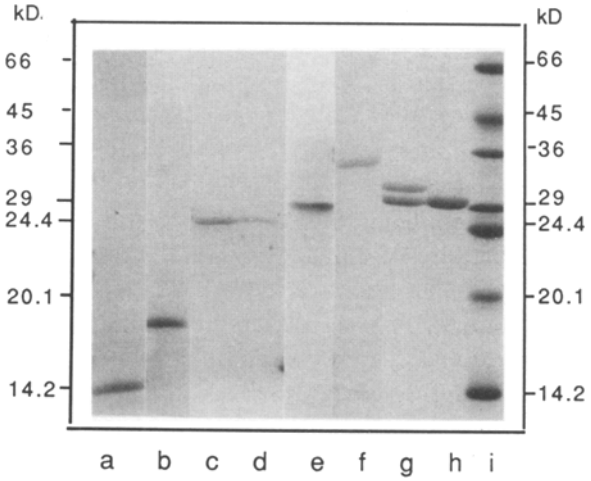

Fig. 2. Electrophoretic patterns of purified PRm proteins on a 15% polyacrylamide gel containing 0.1% SDS. PRm 1 (a); PRm 2 (b); PRm 3 (c); PRm 4 (d); PRM 5 (e); PRm 7 (f); mixture of PRm 6a + PRm 6b (g); PRm 6b (h); molecular weight markers (i). The numbers indicated the molecular weight of the various marker proteins.

The molecular weights of the various PRm proteins were estimated by PAGE in the presence of SDS, by comparing their mobility with that of proteins of known molecular weight (Fig. 2). Under denaturing conditions PRm 3 and PRm 4 ran together whereas all other proteins gave distinct bands. The mean molecular weights from five experiments conducted with different preparations were: PRm 1 14.2 kDa: PRm 2 16.5 kDa; PRm 3 and PRm 4 25 kDa; PRm 5 29 kDa; PRm 6b 30.5 kDa; PRm 6a 32 kDa; PRm 7 34.5 kDa, PRm proteins 6a and 6b, hardly separated by gel electrophoresis under native conditions, showed slightly different molecular weights when run on SDS-containing polyacrylamide gels; PRm 6a which migrated somewhat faster under native conditions had the highest molecular weight when estimated on SDS-containing polyacrylamide gels.

The amino acid compositions of the purified PRm 1, 2, 3, 4, 5 and 6b are given in Table 1; for each amino acid the value shown represents the average of three determinations. Since tobacco PR 1-type proteins have been found in Gramineae [46], the composition of the low-molecular-weight PRm 1 and PRm 2 proteins were compared to those of tobacco PR 1 proteins determined by Antoniw *et al.*

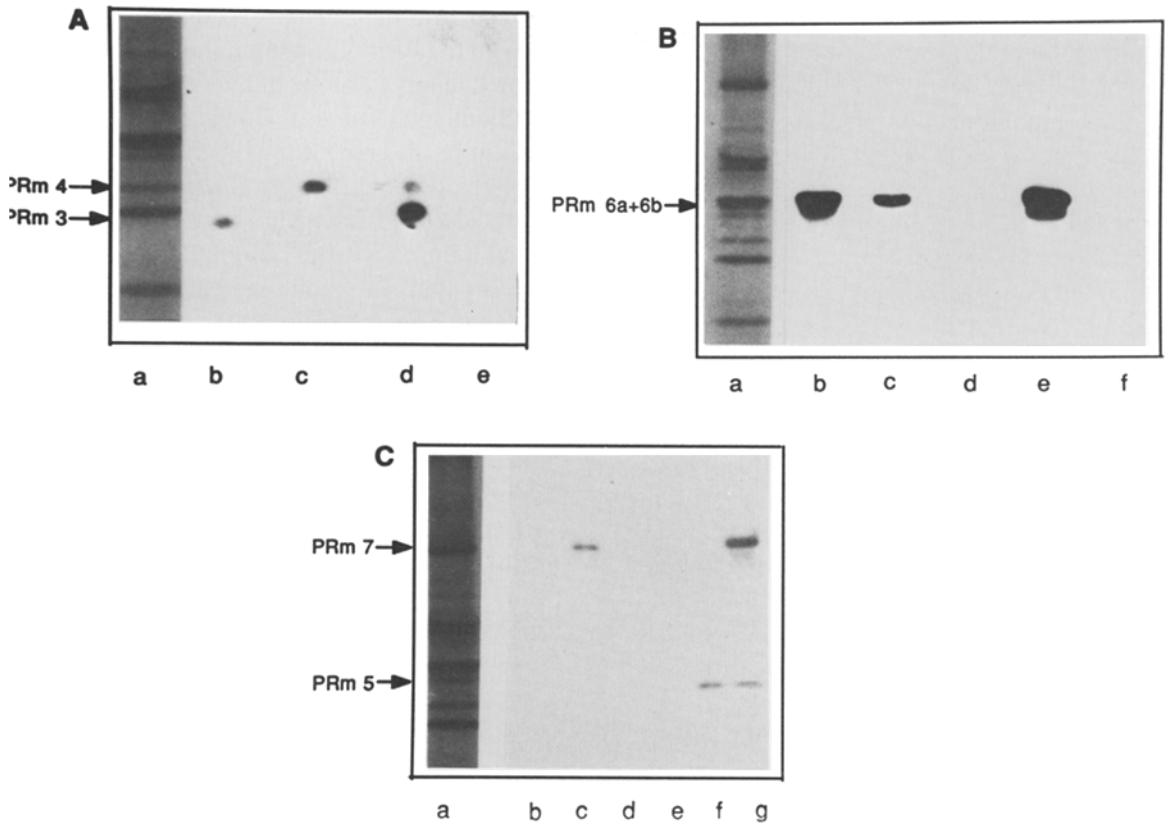

Fig. 3. Immunological reactions with various anti PRm antibodies. Pure proteins or crude extracts from mercuric chloride treated leaves were electrophoresed under native conditions, transferred onto nitrocullulose membranes and reacted with antisera. Reactive proteins were detected by [^{125}I] protein A and autoradiography. In each panel lane (a) shows a crude extract after electrophoresis under native conditions and Coomassie Blue staining.

Panel A: immunological reactions with anti PRm 3 antiserum and pure PRm 3 (b); pure PRm 4 (c); crude extracts from mercuric chloride-treated leaves (d) and from untreated leaves (e). The same patterns are obtained by using anti PRm 4 antiserum instead of anti PRm 3 antiserum.

Panel B: immunological reactions with anti PRm 6b antiserum and a mixture of PRm 6a + PRm 6b (b); pure PRm 6b (c); pure PRm 5 (d); crude extracts from mercuric chloride-treated leaves (e) and from untreated leaves (f).

Panel C: immunological reactions with anti PRm 7 antiserum and a crude extract from untreated leaves (b); pure PRm 7 (c); PRm 6a + 6b (d); pure PRm 4 (e); pure PRm 5 (f); crude extract from mercuric chloride-treated leaves (g).

[3] and van Loon *et al.* [43]. Protein PRm 1 differs completely in amino acid composition from any of the members of tobacco RPm 1 group. In contrast the composition of PRm 2 was similar to that given for PR 1b from tobacco [43] differing only in a higher content of serine and proline and a lower content of tyrosine and histidine. PRm 3 and PRm 4, which have a higher molecular weight than tobacco PR 1 proteins, differ substantially in amino acid composition from those proteins. However they resemble each other to such an extent that they appear to be closely related (see also Serological properties).

The amino acid compositions of PRm 5 and PRm 6b show some noticeable differences from each other, mainly in the content of aspartic acid, proline, glycine, alanine and isoleucine. They also differ from the amino acid compositions determined for the other maize PR proteins.

Table 1. Amino acid composition expressed as mol/100 mol.

Amino acid	PRm 1	PRm 2	PRm 3	PRm 4	PRm 5	PRm 6b
Aspartic acid	7.5	17.2	17	16.3	17.8	14.5
Threonine	8	3.8	5.2	5.5	5.5	5
Serine	4.5	10.3	7.5	7.1	7.3	6.7
Glutamic acid	13.5	12	6.4	6	7.2	8.6
Proline	7.3	6.4	4.8	5	5.5	8.4
Glycine	13.2	12.2	15.3	16	13.7	11.1
Alanine	6.7	11.5	9.8	9	10.5	13.8
Cysteine	1.7	1.3	2.4	2.8	ND	ND
Valine	10.2	8.4	5.5	5.1	6	7.7
Methionine	1.5	0.7	0.4	0.4	0.5	1.7
Isoleucine	4.5	1	5.6	5.5	5.9	2.7
Leucine	5.2	3.5	9	8.5	9.5	7.4
Tyrosine	3.7	3.8	4.4	3.8	4.9	4
Phenylalanine	5.2	1.3	2.7	3	2.9	4.3
Histidine	2.1	2	0.8	1	0.7	0.7
Lysine	4.5	2.7	2	2	1	2
Arginine	0.7	2.2	1.2	3	1.1	1.4

ND, not determined.

Serological properties

Immunological blotting test revealed that none of the antisera raised against purified PRm 3, PRm 4, PRm 6b and PRm 7 proteins reacted specifically with proteins present in healthy plants (Fig. 3A, B and C). The antisera raised against PRm 3 or PRm 4 reacted specifically with PRm 3 and PRm 4 in a crude extract from mercuric chloride-treated plants as well as with purified PRm 3 and PRm 4 proteins (Fig. 3A), thus confirming again the close relationship between PRm 3 and PRm 4 which not only have the same molecular weight and a similar amino acid composition but also show common antigenic sites.

The immunological reactions with antiserum raised against pure PRm 6b is shown in Fig. 3B. The antiserum reacted with two barely separated proteins present in the crude extract from chemically treated leaves (lane e), a strong reaction also occurred with a mixture of PRm 6a and PRm 6b (lane b) and with purified PR 6b (lane c), but none with purified PRm 5 (lane d). This result suggests that antibodies raised against pure PRm 6b cross-reacted with PRm 6a which is the closest to PRm 6b and that the two proteins are serologically related.

The antiserum raised against purified PRm 7 reacted with pure PRM 7 protein (Fig. 3C) as well as with the PRm 7 present in the crude extract from treated leaves (lane g). In addition the anti-PRm 7 antibodies reacted also with a second PRm protein present in the crude extract. Among the three PRm proteins located in this ara of the polyacrylamide gel (PRm 6, PRm 5 and PRm 4), only pure PRm 5 reacted with the anti-PRm 7 antibodies (lane f), demonstrating that the second reacting protein is PRm 5 and showing a serological relationship between PRm 5 and PRm 7.

Using antibodies raised against tobacco PR 1b, an immunological reaction occurred with crude extracts from BMV-infected (Fig. 4 lane c) or mercuric chloride-treated leaves (lane e). Among the low-molecular-weight proteins (PRm 1 and PRm 2) only PRm 2 (lane f) reacted with tobacco PR 1b antiserum. This result is in good agreement with the similarity shown in the amino acid composition of tobacco PR 1b and PRm 2. These two proteins have also serological relationships.

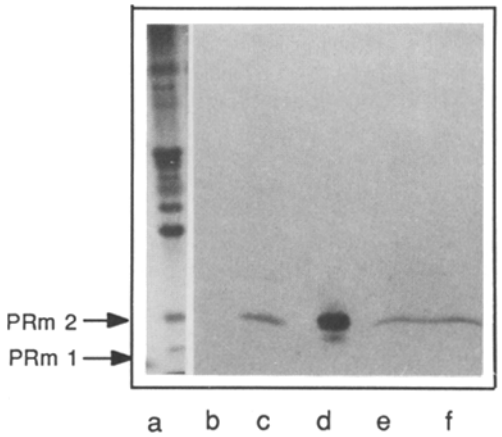

Fig. 4. Immunological reactions with anti tobacco PR 1b antiserum. Pure proteins or crude extracts electrophoresed under native conditions and transferred onto nitrocellulose membranes with antiserum (b, c, d, e, f). Immunodetection was done by a colorimetric method [5]. Crude extract from mercuric chloride-treated leaves after Coomassie Blue staining (a). Immunological reactions with crude extracts from untreated leaves (b); from BMV-infected leaves (c); from mercuric chloride-treated leaves (e); pure PRm 2 (f). Lane (d) shows the immunological reactions with a crude extract from tobacco mosaic virus-infected tobacco leaves, a positive reaction can be seen with tobacco PR 1a and PR 1b.

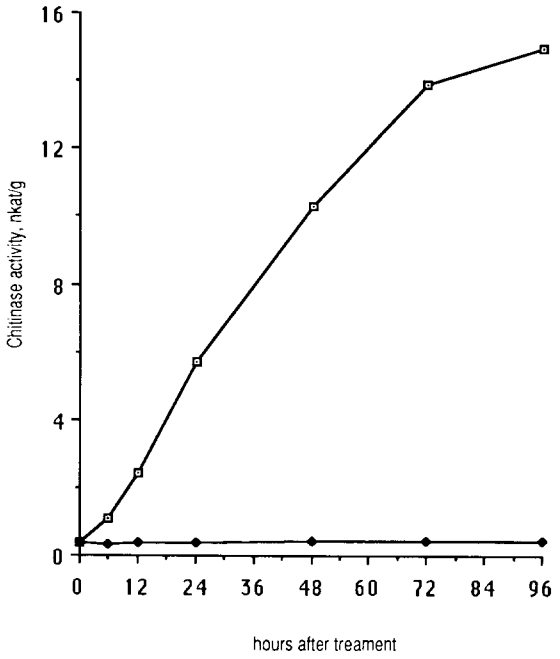

Fig. 5. Time course curve of mercuric chloride-induced chitinase activity. After mercuric chloride treatment (open symbols) or distilled water vaporization (black symbols) of maize leaves. Enzymic activity was assayed on void volume fractions after Sephadex G25 filtration of the crude extract and expressed on a fresh-weight basis.

Chitinase activity of maize PR proteins

The chitinase activity was low in untreated leaves but increased strongly after mercuric chloride treatment (Fig. 5). The chitinase activity increased soon after onset of treatment and reached an about 40-fold increase four days later. The time course of induction is shorter than that described for tobacco chitinase activity upon tobacco mosaic virus infection [26] and similar to that of potato chitinase activity induced by treatment with *P. infestans* elicitors [24].

Since in tobacco [26] and in potato [24] several PR proteins are chitinases, each purified PRm protein was tested for chitinase activity. The results given in Table 2 show that PRm 3, PRm 4, PRm 5 and PRm 7 have chitinase activity. No exochitinase activity could be detected for any of the four proteins showing that these enzymes are endochitinases.

Table 2. Chitinase activity and yields of the maize PR proteins.

Proteins	Specific activity, nkat/mg	Yield µg/100 g
PRm 1	0	30
PRm 2	0	20
PRm 3	50	40
PRm 4	80	25
PRm 5	80	20
PRm 6a + 6b	0	65
PRm 7	60	30

Chitinase activity was measured as described. Of the initial activity about 20% was recovered in the purified proteins. Yields are expressed on a fresh-weight basis.

Discussion

Eight PRm proteins with molecular weights ranging between 14200 and 34500 were identified in leaves from mercuric chloride-treated or BMV-infected maize, and seven of them were purified to homogeneity. As far as we know this is the first report on purification and biological functions of monocotyledonous plant PR proteins. These PRm proteins are usually undetectable in untreated leaves; nevertheless in a few experiments, extracts of control leaves contained trace amounts of PRm 7 protein suggesting that this protein might normally be present in low amounts in healthy plants or that it might reflect the senstivity of maize to small uncontrolled stresses occurring in the greenhouse or in the growth chamber.

The variations in the relative amounts of PRm 1 and PRm 2 proteins could be explained if PRm 1 is degradation product of PRm 2 or in relation with the severity of the stresses. That PRm 1 could be a degradation product of PRm 2 seems most unlikely because of the important differences in amino acid compositions between the two proteins and the absence of serological relationships. It seems more likely that the induction of these two proteins might be more or less related to the severity of the stresses. This hypothesis is supported by results obtained upon application of salicylic acid for several days on maize plants or after weak plasmolysis. In our experimental conditions these stresses seem less drastic

than mercuric chloride treatments or BMV infections, as no necrotic symptoms appear. After these treatments PRm 1 and PRm 2 are usually undetectable or present in very low amounts. As can be judged from the variaiton of the number of necrotic lesions borne by the leaves from one treatment or infection to the other or, in the same experiment, from one plant to the other, even if always the same concentration of mercuric chloride or viruses is used, one can conclude that the severity of a stress is not easily reproducible. Some modulations in the stresses, depending on experimental conditions, may occur from one experiment to the other and could be responsible for the variations observed in the amounts of PRm 1 and PRm 2. Differences in the time course of induction and in the amount of PR proteins synthesized in relation with the severity of the stresses have also been described in bean [36].

White *et al.* [46] by immunoblotting tests with tobacco anti-PR 1a antibodies have identified a tobacco PR 1-type protein in maize with a molecular weight of about 17 000. This protein is very likely the PRm 2 protein, also cross-reacting with tobacco anti-PR 1b antiserum, which we have purified and whose amino acid composition is very similar to that of PR 1b from Samsun NN tobacco leaves [43].

The amino acid analysis of six of the eight PRm proteins show a relatively high proportion of acidic residues (21–29%), which is a common feature shared with tobacco PR proteins even if, except in PRm 2, the proportion is not as high as in tobacco PRm proteins. In addition to the high similarity of the amino acid composition of PRm 3 and PRm 4, the relatively low proportion of aspartic acid in PRm 1 compared to that of all other maize PR proteins and the high proportion of glutamic acid in comparison with that of PRm 3, 4, 5 and 6b are noticeable.

In view of the results obtained in the study of their serological properties and their biological functions, maize PR proteins can be divided into at least tree families. First, the family of the chitinases includes four members, PRm 3, 4, 5 and 7 which can again be divided into two groups. The first group includes PRm 3 and 4 which have the same molecular weight (25 kDa), have a high similarity in their amino acid composition and are serologically related. The second group contains PRm 5 and 7, chitinases with higher molecular weights (29 and 34.5 kDa respectively). They are serologically related to each other but have no serological relationships with PRm 3 and PRm 4. In addition, PRm 5 and PRm 7 cross-reacted with tobacco PR P antiserum (W. Nasser & G. Burkard, manuscript in preparation) indicating serological relationships with tobacco chitinases [26].

To the second family of maize PR proteins belong PRm 6a and PRm 6b because of their serological relationships. In addition, it has been found that PRm 6b as well as the mixture of PRm 6a and 6b show a 1,3-β-glucanase activity (W. Nasser, unpublished results). As PRm 6a and 6b are serologically related, it is likely that the enzymic activity found in the mixture of PRm 6a and 6b is not only due to the presence of PRm 6b but that PRm 6a might also have a 1,3-β-glucanase activity. So, this second family could be constituted by the maize 1,3-β-glucanases.

Protein PRm 2 belongs to the group of tobacco PR 1 proteins as shown by its serological relationships with these proteins and also the similarities in the amino acid compositions of PRm 2 and tobacco PR 1b. The remaining PRm 1 with the lowest molecular weight (14.2 kDa) is somewhat apart, as this protein has a quite different amino acid composition compared to those of all other maize PR proteins and no serological relationship with either maize PR proteins or tobacco PR 1 proteins.

In tobacco all four chitinase isoforms possess similar antigenic sites [26] and the same is true for potato chitinase isoforms [24]. However in maize PRm 3 and PRm 4 chitinases which share common antigenic sites possess no similar antigenic sites with the isoforms PRm 5 and PRm 7. The two latter chitinases cross-reacted with antisera from other plants (W. Nasser unpublished data) as do all six chitinase isoforms from potato with the bean anti-chitinase antiserum [24]. Maize PRm 3 and PRm 4 chitinase show a lower molecular weight (25 kDa) than those of tobacco or potato chitinases (ranging from 27.5 kDa to 38.5 kDa), whereas PRm 5 chitinase (29 kDa) was a molecular weight similar to those of tobacco acidic chitinases, and the molecular weight of PRm 7 chitinase (34.5 kDa) is similar to those of the basic tobacco chitinases and of two of the six potato chitinases. As judged from their

molecular weight and their serological properties maize PRm 3 and PRm 4 chitinases seem to be different from those described in dicotyledonous plants and can therefore be considered to be specific for this plant (or this family of monocotyledons).

The predominant extracellular localization of PR proteins [30, 36] and especially of some of the potato chitinases [24] has been described, On the other hand, Boller and Vögeli [7] established the vacuolar localization of a chitinase in bean leaves which accumulated upon ethylene induction [6]. Intra- or extracellular localization of maize chitinases has not yet been established, and to know whether the presence of two groups of maize chitinases is in relation with different localizations requires further investigations.

The production of PR proteins increase the resistance of plants to viruses [17, 22, 41], but how these proteins act against viral multiplication remains unknown. It is easier to understand the role of chitinases or 1,3-β-glucanases in defence of plants against pathogen attack, as it has been observed that chitinase, for example, can restrict the growth of a fungus [6] and that many fungi or bacteria contain 1,3-β-glucans or chitin in their cell walls. The production of PR proteins is not only restricted to infection by pathogens but it occurs also under various stresses and during flowering [15] or senescence [31], which raises the quetion of their possible role under these conditions.

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