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Antigenic relationships between petunia peroxidase a and specific peroxidase isoenzymes in other Solanaceae

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Summary. A highly specific rabbit antiserum raised against peroxidase (PRXa) from petunia (*Petunia hybrida*) was used to investigate the antigenic relatedness of peroxidases in the Solanaceae. After SDS-PAGE of crude leaf extracts from a large number of species of this family, immunoblotting revealed that cross-reacting protein bands were present in all species tested. In order to determine whether these protein bands represent peroxidases, the peroxidase isoenzymes in thorn apple (*Datura stramonium* L.), tobacco (*Nicotiana tabacum* L.), sweet pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* Mill.) were further analyzed. Immunoblots obtained after native PAGE revealed that the antiserum only recognized fast-moving peroxidase isoenzymes that are localized in the apoplast. Despite their serological relatedness, these peroxidases differed with respect to heat stability and apparent molecular weight. Differences in avidity for the petunia PRXa antiserum were suggested by immunoprecipitation with antibodies bound to protein A-Sepharose. The antiserum did not react with peroxidases from horseradish (*Armoracea rusticana* Gaertn., Mey and Scherb), turnip (*Brassica napus* L.), African marigold (*Tagetes cresta* L.), maize (*Zea mays* L.), and oats (*Avena sativa* L.). Apparently, the Solanaceae contain orthologous genes encoding the fast-moving anionic peroxidases homologous to petunia PRXa.

Key words: Immunology – Peroxidase isoenzymes – Petunia – Solanaceae

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Introduction

The peroxidase system in plants consists of multiple isoenzymes thought to play a role in growth, differentiation, and defense. Based on differences in substrate specificities, it has been assumed that anionic peroxidases are involved in cell wall fortification, whereas basic peroxidases are involved in the oxidation of the auxin indoleacetic acid (Gaspar et al. 1982; Greppin et al. 1986).

Amino acid sequence data indicate that peroxidases from different plant species may be about 40%–60% homologous (Welinder 1985; Lagrimini et al. 1987; Roberts et al. 1988; Roberts and Kolattukudy 1989). Much higher homologies (up to 90% or more) are found around the two histidine residues involved in the binding of the heme group, as well as in some other regions of the protein. Alignment of the amino acid sequences of the peroxidases from horseradish (Welinder 1979), turnip (Mazza and Welinder 1980), tobacco (Lagrimini et al. 1987), potato (Roberts et al. 1988), and tomato (Roberts and Kolattukudy 1989) shows conserved carbohydrate attachment sites and disulphide bridge-forming cysteine residues, indicative of similar tertiary structures (Welinder 1985). Antisera raised against peroxidase isoenzymes from horseradish (Bakardjieva and Georgiev 1977; Conroy et al. 1982; Clark and Conroy 1984; Conroy 1986; Bernardini et al. 1986), peanut (Cairns et al. 1980; Van Huystee and Maldonado 1982; Chibbar et al. 1984), flax (Gaudreault and Tyson 1986), and tobacco (Lagrimini et al. 1987) have been found to cross-react with various peroxidases from the same or other plant species.

In petunia (*Petunia hybrida*) the fast-moving anionic peroxidase isoenzyme, PRXa, seems to be a representative of a group of fast-moving anionic peroxidases present in the Solanaceae family. In petunia, tobacco, thorn apple, potato, and tomato, these enzymes have similar

electrophoretic mobilities, display similar heterogeneous patterns and variation therein on zymograms, as well as biochemical properties, and show corresponding developmental expression, organ specificity, and cellular location (cf. Van den Berg and Wijsman 1981; Van den Berg et al. 1983; Van den Berg and Van Huystee 1984; Hendriks et al. 1985; Wijsman and Hendriks 1986, and literature cited therein). These similarities prompted us to further investigate the extent of homology of these peroxidases in the Solanaceae by determining their antigenic relatedness to petunia PRXa.

Materials and methods

Plant material

The various plant species used were grown either in a greenhouse or in the field (Table 1). Mature, fully expanded leaves were harvested and used either immediately or after storage at −80 °C.

Protein extraction

Water-soluble extracellular proteins were extracted from leaves (or leaf parts) by the vacuum infiltration method, as described in detail elsewhere (Hendriks 1989). For potato only, the infiltrated demineralized (demi) water was centrifuged from the leaflets at $750 \times g$ rather than the usual $200 \times g$.

To extract total water-soluble proteins, leaves or remaining leaf tissue (after vacuum infiltration) were ground with acid-purified sea sand in a mortar with a pestle in 1–2 ml of demi water per gram of fresh weight of tissue. The homogenate was filtered through three layers of gauze and the filtrate was centrifuged for 15 min at $16{,}800 \times g$.

Table 1. Plant species tested for proteins cross-reacting with an antiserum against petunia PRXa by means of immunoblotting

Species (inbred line)	Growth conditions	Cross-reaction
Petunia Juss.:		
P. × *hybrida* Hook.,		
cv Roter Vogel (R27)	greenhouse	+
cv Blauzwerg (V23)	greenhouse	+
P. axillaris Lam.		
ssp. *axillaris* (S1)	greenhouse	+
P. integrifolia Hook.		
ssp. *integrifolia* (S12)	greenhouse	+
ssp. *inflata* R. E. Fries (S9)	greenhouse	+
ssp. *inflata* R. E. Fries (S15)	greenhouse	+
Nicotiana tabacum L. cv Samsun NN	greenhouse	+
Solanum tuberosum L. cv Ukama	field	+
Lycopersicon esculentum Mill. cv Sonato	greenhouse	+
Capsicum annuum L.	greenhouse	+
Datura stramonium L.	greenhouse	+
Brassica napus L.	field	−
Tagetes cresta L. cv Harmony Boy	field	−
Zea mays L.	field	−
Avena sativa L. cv Victory	greenhouse	+[a]

[a] See text

Proteins in the extracellular extracts and in the supernatants of crude or remaining tissue extracts were precipitated overnight in 80% acetone at −20 °C. After low speed centrifugation, pellets were washed with 100% acetone and solubilized in 50 m*M* sodium acetate buffer, pH 5.0.

Horseradish peroxidase (HRP) was purchased from Calbiochem.

Determinations of peroxidase activity and protein content

Peroxidase activity was determined by following the increase in the absorbance at 470 nm in the presence of 2.2 m*M* hydrogen peroxide and 4.5 m*M* guaiacol in 50 m*M* sodium acetate buffer, pH 5.0, and is expressed as the number of moles of hydrogen peroxide consumed per second (katals) (Hendriks 1989). Protein content was determined according to Bradford (1976), using bovine gamma globulin as a standard.

Gel electrophoretic analysis

Starch gel electrophoresis was performed as described for the separation of the anionic and cationic peroxidase isoenzymes of petunia (Van den Berg and Wijsman 1981). In subsequent experiments, the anionic peroxidases were separated in 12.5% polyacrylamide gels in the absence (native PAGE; Davis 1964) or in the presence of 0.1% sodium dodecyl sulphate (SDS-PAGE; Laemmli 1970).

Peroxidase isoenzymes on gels were visualized by activity staining using hydrogen peroxide and either 3-amino-9-ethylcarbazole (Van den Berg and Wijsman 1981) or guaiacol as the hydrogen donor, at pH 5.0. Proteins were fixed and stained in a mixture containing 45% methanol, 9% acetic acid, and 0.25% Coomassie Brilliant Blue G 250 (Serva), after which the gels were destained in 5% methanol, 7% acetic acid.

Preparation of antiserum against petunia PRXa

Petunia PRXa1 was purified from the leaves of the petunia inbred line R27 (Hendriks 1989). A New Zealand white rabbit was injected subcutaneously with 100 µg of the purified enzyme emulsified in complete Freund's adjuvant. Two more injections with 100 µg PRXa in incomplete Freund's adjuvant were given at 2-week intervals. One week after the last injection the rabbit was bled by cutting the marginal ear vein, and serum was prepared. The antiserum did not react with proteins other than PRXa in extracts from petunia leaves. As with the antiserum against PRXa1 obtained previously (Van den Berg et al. 1984), peroxidase activity was still expressed in the presence of the antiserum as evidenced after immunodiffusion and rocket immunoelectrophoresis (not shown).

Immunoblotting

Proteins separated on native or SDS-containing polyacrylamide gels were electrotransferred for 1 h at maximum voltage to nitrocellulose membranes (Millipore) using a LKB 2117 Multiphor unit (LKB, Bromma, Sweden) and a blot buffer containing 25 m*M* TRIS, 0.19 *M* glycine, pH 8.8, and 20% methanol. Blots obtained from native gels were heated at 80 °C for at least 6 h to inactivate endogenous enzyme activities.

The blots were then incubated for 1 h in 10 m*M* TRIS-HCl, pH 7.4, 0.15 *M* NaCl, and 0.5% Tween 20 (TBS-Tween), containing 1% bovine serum albumin (BSA), followed by 1 h in TBS-Tween containing 1% BSA and a 1 : 2,000 dilution of the antiserum against PRXa. Subsequently, the blots were washed for 18 h in TBS-Tween with several changes, and incubated for 1 h in TBS-Tween containing 1% BSA and a 1 : 1,000 diluted goat-antirabbit serum conjugated with either horseradish peroxidase (Nordic, Tilburg, The Netherlands) or alkaline phos-

phatase (Sigma, St. Louis, USA). Finally, the blots were thoroughly rinsed in TBS-Tween and stained for enzyme activity. Peroxidase activity was visualized with 3-amino-9-ethylcarbazole as described for peroxidase isoenzymes on gels. Staining for alkaline phosphatase activity was performed by immersing the blot in 75 m*M* TRIS, 25 m*M* diethylbarbituric acid buffer, pH 8.6 (LKB), containing 40 m*M* $MgCl_2$, 0.1 m*M* *p*-toluidine-5-bromo-4-chloro-3-indolylphosphate (BioRad, Richmont, USA) and 60 μ*M* nitro blue tetrazolium chloride (BioRad). After staining, the blots were rinsed in demi water and dried on filter paper.

Immunoprecipitation

Immunoprecipitation of extracts with the antiserum was performed with protein A (Prot A)-Sepharose. Prot A-Sepharose (Pharmacia, Uppsala, Sweden) was swollen and washed in 6.5 m*M* K_2HPO_4, 1 m*M* NaH_2PO_4, 135 m*M* NaCl, 3 m*M* KCl, pH 7.4 (PBS). After spinning 1 ml suspension of Prot A-Sepharose (0.15 mg dry weight) for 10 min at 10,000 × *g*, 0.6 ml of the supernatant was replaced by 0.6 ml antiserum. Nonbound antibodies were removed by washing five times with PBS. To estimate binding of peroxidase in the extracts from the various plant species, 0.1 vol. of 10 × PBS was added prior to incubation with excess Prot A-Sepharose. After incubation overnight at 4 °C, the Prot A-Sepharose was spun down, and the peroxidase activity remaining in the supernatant was determined.

Results

Characterization of the antiserum against petunia PRXa

Petunia peroxidase a consists of at least three molecular forms of slightly different molecular weight, generated posttranslationally from a single transcript (Hendriks and Van Loon 1990). The locus encoding PRXa, *prxA*, exists in several allelic forms, giving rise to variants of PRXa that differ in electrophoretic mobility on native gels (Fig. 1 A). Upon SDS-PAGE of extracts from leaves of the lines expressing PRXa1 (*Petunia hybrida* R27), PRXa2 (*P. hybrida* V23), PRXa3 (*P. integrifolia* ssp. *inflata* S9), PRXa4 (*P. integrifolia* ssp *inflata* S15), and PRXa5 (*P. integrifolia* ssp. *integrifolia* S12) and immunoblotting, in every case the three or four bands corresponding to PRXa were recognized (Fig. 1 B). Under these conditions the relative electrophoretic mobilities of the PRXa variants were rather similar. Thus, the different electrophoretic mobilities upon native PAGE are due mainly to different charges, related to differences in isoelectric points (data not shown).

In preliminary experiments crude extracts from leaves of the various Solanaceae were subjected to SDS-PAGE and immunoblotting. In all cases, cross-reacting bands were apparent (Table 1). In order to characterize these bands in those Solanaceae for which peroxidase isoenzyme patterns have been sufficiently well documented, leaf extracts from thorn apple, tobacco, sweet pepper, potato, and tomato were further analyzed. For comparison, in some experiments extracts from non-Solanaceae,

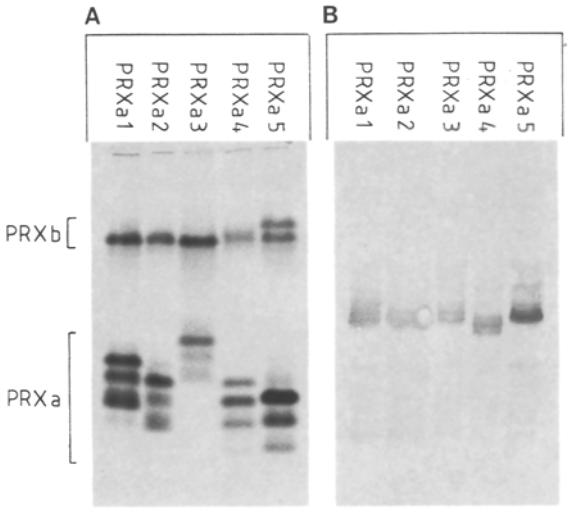

Fig. 1 A and B. Reaction of the antiserum against PRXa1 with electrophoretic variants of PRXa. **A** Native polyacrylamide gel stained for peroxidase activity with 3-amino-9-ethylcarbazole. **B** SDS-PAGE gel after immunoblotting with the antiserum. Soluble protein extracts from leaves containing 25 nkat peroxidase activity from the petunia lines R27 (PRXa1), V23 (PRXa2), S9 (PRXa3), S9 (PRXa4), and S12 (PRXa5) were applied

Table 2. Peroxidase activities in leaves from selected Solanaceae. Extracellular extracts from fully expanded leaves were obtained by the vacuum-infiltration method ("Materials and methods"). Thereafter, the remaining tissue was extracted in water. The specific activity (SA) of the extracts was determined after acetone precipitation. The activities given are means ± SD

Species (no of extracts)	Extracellular extract			Remaining tissue extract	
	μkat · g^{-1}FW	%	SA	μkat · g^{-1}FW	SA
Thorn apple (4)	0.070 ± 0.013	22 ± 6	2.34	0.271 ± 0.142	0.04
Tobacco (3)	0.042 ± 0.003	50 ± 2	0.91	0.041 ± 0.001	0.10
Petunia (4)	0.245 ± 0.014	19 ± 1	10.85	1.061 ± 0.152	0.44
Pepper (4)	0.014 ± 0.003	13 ± 4	3.28	0.099 ± 0.036	0.01
Potato (4)	0.020 ± 0.010	4 ± 2	0.20	0.556 ± 0.222	0.05
Tomato (4)	0.005 ± 0.002	5 ± 1	0.02	0.111 ± 0.045	0.04

two dicots, African marigold and turnip, and two monocots, maize and oats, as well as a preparation of horseradish peroxidase were likewise tested.

Analysis of peroxidase activity in selected Solanaceae

Total water-soluble peroxidase activities in the solanaceous species are listed in Table 2. In both extracellular and remaining tissue extracts, widely varying peroxidase activities were encountered in the different species. The percentage of the peroxidase activity present in the extracellular extracts varied between 4% in potato and 51% in tobacco (Table 2), whereas the percentage protein in the extracellular extracts varied between only 1% and 3%

for any species (data not shown). In all cases peroxidase-specific activity in the extracellular extract was higher than that in the extract from the remaining leaf tissue, indicating that at least part of the peroxidase activity is specifically localized in the apoplast.

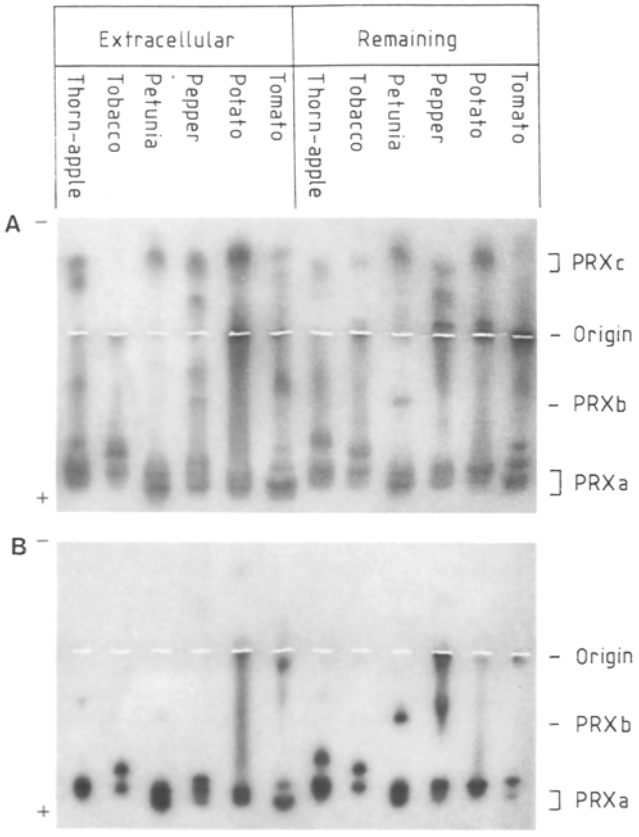

Fig. 2 A and B. Starch gel zymograms of extracellular and remaining tissue peroxidases of six selected solanaceous species. After starch gel electrophoresis, the same gel was sliced horizontally and stained for peroxidase activity using 3-amino-9-ethylcarbozole **A** and guaiacol **B**. Positions of PRXa, b, and c in petunia are indicated

Acetone precipitation of the extracts followed by resuspension of the protein pellets in acetate buffer resulted in some cases in considerable (up to 40%) losses of peroxidase activity, particularly from the remaining tissue extracts. This step thus constituted an enrichment of peroxidases similar to petunia PRXa, which is soluble under these conditions. The specific activities of the peroxidase preparations after the acetone precipitation step are given in Table 2. These preparations were used in following experiments.

Comparison of peroxidase zymograms of selected Solanaceae

Soluble proteins extracted from leaves of the selected Solanaceae were subjected to starch gel electrophoresis. The similarity of the peroxidase zymograms (Fig. 2 A) was obvious, particularly with respect to the fast-moving anionic and cationic groups of peroxidase bands (cf. Wijsman and Hendriks 1986). These two groups were the peroxidases predominantly present in extracellular extracts, except for the fast-moving cationic peroxidases in tobacco, which seemed to be present in the remaining tissue extract only. None of the cationic bands was visualized when guaiacol was used as the hydrogen donor instead of 3-amino-9-ethylcarbazole (Fig. 2 B), suggesting that the cationic peroxidases hardly contribute to the activity measured spectrophotometrically (cf. Hendriks et al. 1985). Native PAGE gave a much higher resolution for the anionic peroxidase bands (Fig. 3 A), confirming that a group of fast-moving anionic peroxidases is present in the apoplast of leaves of all the selected Solanaceae.

Performing native PAGE at room temperature rather than at 4 °C resulted in a considerable loss of activity of the PRXa-like bands in the extracts from thorn apple, tobacco, and petunia, whereas the tomato and potato peroxidases seemed to be less affected (Fig. 3 B). Appar-

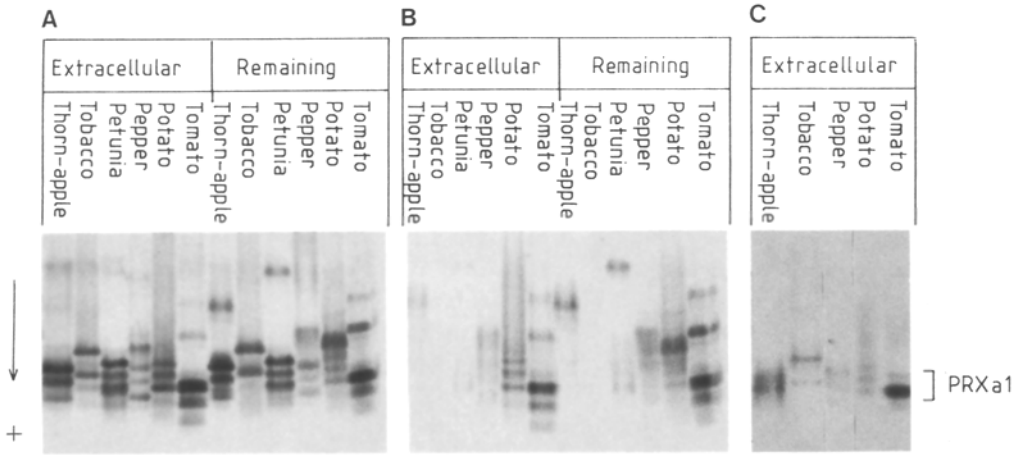

Fig. 3 A–C. Electrophoretic patterns of anionic peroxidases in extracellular and remaining tissue extracts from six selected solanaceous species. Native polyacrylamide gels run at 4 °C **A** and room temperature **B** were stained for peroxidase activity using 3-amino-9-ethylcarbazole, and a gel **C** equivalent to **A** was subjected to immunoblotting using the antiserum against petunia PRXa1

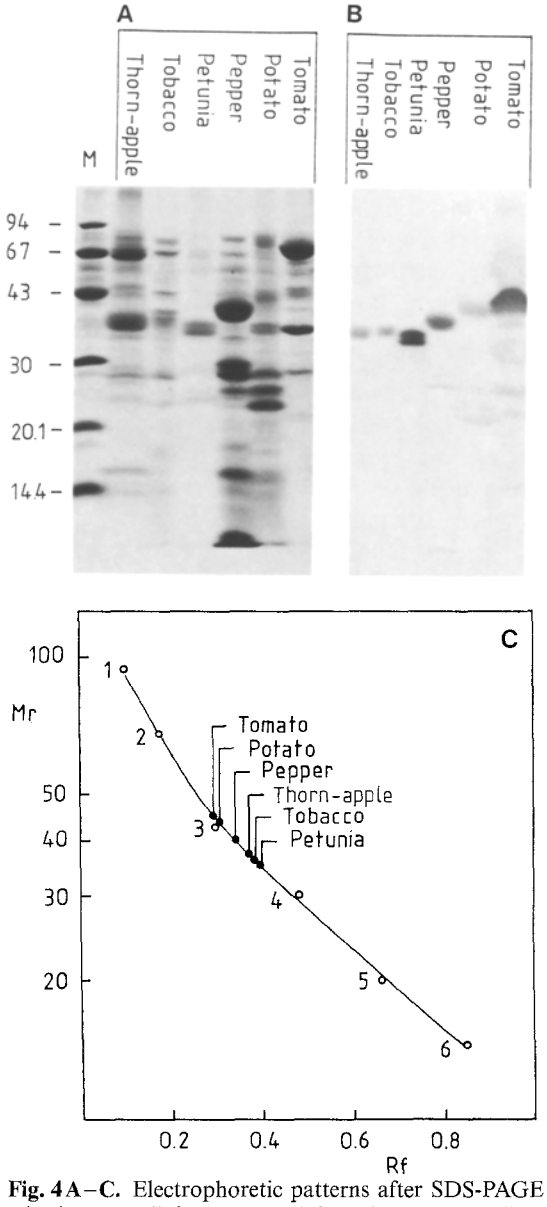

Fig. 4 A–C. Electrophoretic patterns after SDS-PAGE of proteins in extracellular extracts. **A** Samples corresponding to 40 µg protein were applied and proteins were stained with Coomassie Blue. *Lane M*: marker proteins phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), and α-lactalbumin (14.4 kD). **B** Samples corresponding to 50 nkat peroxidase activity were applied and the gel was subjected to immunoblotting using the antiserum against petunia PRXa. **C** Molecular weight determination of the bands in **B**

ently, the fast-moving anionic peroxidases in the former four species are rather heat labile. In contrast, the more slowly moving PRXb-like isoenzymes within the remaining tissue were stable under these conditions (Fig. 3 B).

Immunological relationships

Upon immunoblotting of native PAGE gels with the antiserum against petunia PRXa, in all other five Solanaceous species cross-reacting bands were present among the fast-moving peroxidases in extracellular extracts (Fig. 3 C). Some bands in this group, which were barely revealed by activity staining, did not or only hardly reacted with the antiserum. Similar patterns were obtained with extracts from the remaining tissue. Notably, the additional peroxidases present within the tissue did not react with the antiserum. Except for the presence of a band cross-reacting in remaining leaf tissue from oats, no specific reaction occurred with extracts from the four non-Solanaceae or with a preparation of horseradish peroxidase. Similar results were obtained whether the second antiserum was conjugated with peroxidase or with alkaline phosphatase (data not shown).

Immunoblotting of the denatured proteins after SDS-PAGE (Fig. 4 A) indicated that in each species the

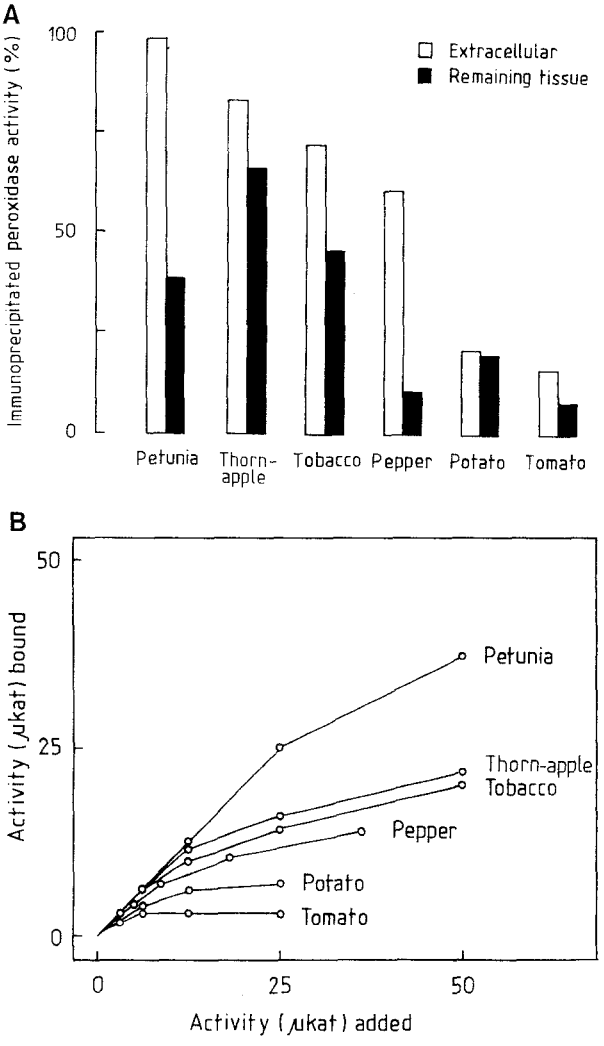

Fig. 5 A and B. Immunoprecipitation of peroxidase activity from extracellular and remaining tissue extracts. **A** Percentage activity precipitated with Prot A-Sepharose-conjugated antiserum against petunia PRXa1. **B** Peroxidase activity in the extracellular extracts bound by the Prot A-Sepharose-conjugated antiserum as a function of peroxidase activity added

antiserum recognized a set of extracellular proteins with apparent molecular weights that ranged from 37 kD for petunia PRXa to about 44 kD for the corresponding peroxidase in tomato (Fig. 4B).

The extent of homology between the peroxidases in the various species was tested by immunoprecipitation using antibodies coupled to Prot A-Sepharose. In petunia, all extracellular peroxidase and about 40% of the enzyme activity in the remaining tissue extract was precipitated. Since the antiserum is specific for PRXa, it can be concluded that the total amount of PRXa in leaves accounts for about 60% of the guaiacol peroxidase activity. In extracts from thron apple, tobacco, and pepper, a large fraction of the peroxidase activity was precipitated, in particular from the extracellular extracts. Much less peroxidase activity was precipitated from extracts of potato and tomato. However, again a larger percentage cross-reacted in the extracellular than in the remaining tissue extracts (Fig. 5A). Thus, in the selected solanaceous species, the antiserum against PRXa recognizes particularly those peroxidases that are localized in the apoplast.

The relative affinities of the antiserum for the peroxidases from the six species were confirmed in an experiment in which increasing amounts of the extracellular extracts were incubated with a given amount of Prot A-Sepharose-bound antiserum, and the extent of binding of the peroxidase activity was determined (Fig. 5B).

Zymograms of the supernatants after immunoprecipitation revealed that petunia PRXa had been removed completely from the supernatant. The activity of the fast-moving group of peroxidases in the other species was decreased to various extents. A stronger reduction in the staining intensities of the various molecular forms was apparent for thorn apple, tobacco, and sweet pepper than for potato and tomato, suggesting that the former are more homologous to petunia PRXa than the latter. None of the peroxidase bands other than the fast-moving group was removed by the antiserum (Fig. 6). In tomato the situation was complicated. As can be seen in Fig. 6, the activities of only some of the bands in the fastest-moving group were reduced. No peroxidase isoenzymes were immunoprecipitated from extracts of marigold, turnip, maize, or oats, nor was cross-reaction observed with horseradish peroxidase. These results again demonstrate that only the group of the fast-moving anionic peroxidases in the selected Solanaceae reacted with the antiserum against petunia PRXa. PRXa-like enzymes were present in all five species investigated but, as shown in Fig. 5, their extent of homology to petunia PRXa appeared to decrease in the order petunia > thorn apple = tobacco > pepper > potato = tomato.

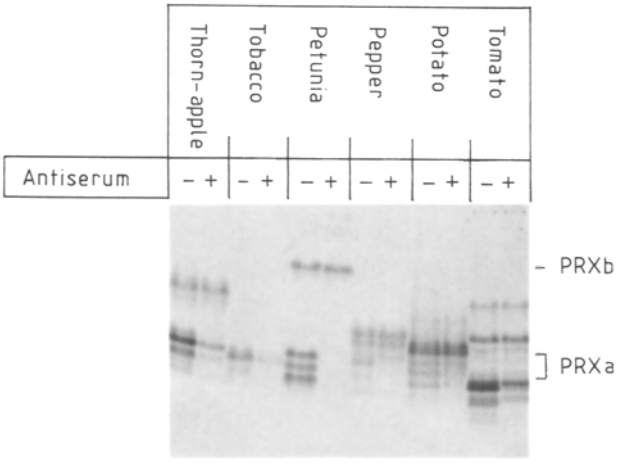

Fig. 6. Electrophoretic patterns of anionic peroxidases from remaining tissue before and after removal by immunoprecipitation with the antiserum against petunia PRXa1 (cf. Fig. 5). A native polyacrylamide gel was stained for peroxidase activity with 3-amino-9-ethylcarbazole. Positions of petunia PRXa and b are indicated

Discussion

Fast-moving anionic peroxidase isoenzymes structurally homologous to petunia PRXa are present in various solanaceous species. Even though we only demonstrated this for thorn apple, tobacco, pepper, potato, and tomato, cross-reacting bands were detected in immunoblots after SDS-PAGE of leaf extracts from a large number of Solanaceae (Hendriks 1989). The antiserum proved to be specific for petunia PRXa and reacted only with homologous peroxidase isoenzymes in the selected species. Thus, the epitopes recognized are not the highly homologous amino acid sequences present in all plant peroxidases (Welinder 1985; Lagrimini et al. 1987; Roberts et al. 1988; Roberts and Kolattukudy 1989). Although varying amounts of the immunoreactive peroxidases were retained in the remaining leaf tissue, these constituted the same fast-moving anionic peroxidases as were present in the extracellular extracts.

In spite of their homology, the fast-moving anionic peroxidases of, on the one hand, petunia, tobacco, thorn apple, and pepper and, on the other hand, potato and tomato, showed some distinguishing characteristics. SDS-PAGE indicated that the PRXa-like peroxidases in petunia, tobacco, thorn apple, and pepper have lower molecular weights than those in potato and tomato. They are more heat labile and are more readily precipitated by the antiserum against PRXa. These observations suggest that the antiserum has a higher affinity for the peroxidases of the first group than for those in the second group, or that the peroxidases in the second group have a substantially lower specific activity in our spectrophotometric peroxidase assay, or both. The relatively small reduction in the staining activity of the peroxidase bands of

tomato and potato after immunoprecipitation points to a low antiserum affinity; the strong immunoreaction of particularly the tomato proteins upon immunoblotting is suggestive of a low specific peroxidase activity. In contrast, the ummunoblotting experiments suggest that the antiserum may have a higher affinity for the tomato and potato peroxidases after denaturation than in their native state. The differences between the peroxidases in both groups could then be explained by differences in glycosylation patterns. The presence of extra carbohydrate groups in the potato and tomato peroxidases would increase their stability and molecular weights, and might even be responsible for the much lower recovery of these peroxidases in extracellular extracts. Carbohydrate groups covering an epitope could prevent immunoprecipitation of the peroxidases in their native form; denaturation might cause the epitopes to be exposed. Similarly, the stronger affinity of some of the molecular forms of the tomato peroxidase (cf. Fig. 6) may be caused by partial deglycosylation (cf. Gaudreault and Tyson 1988; Hendriks and Van Loon 1990).

Genetic studies on peroxidase isoenzymes in petunia and tomato have revealed that the group of fast-moving anionic bands, PRXa and Prx-1, are coded for by single structural genes, designated *prxA* and *Prx-1*, respectively (Van den Berg and Wijsman 1982; Rick and Tanksley 1980). Our results suggest that this is also the case in potato, thorn apple, pepper, and tobacco, since all bands of the fast-moving anionic peroxidases cross-reacted with the antiserum. This conclusion is in accordance with the observed variation of the electrophoretic mobility of these bands as a group (Smith et al. 1970; Sheen 1970; Conclin and Smith 1971). The monogenic inheritance of a structural gene for the fast-moving anionic peroxidase isoenzyme in tobacco has been demonstrated in progenies of crosses between *Nicotiana langsdorffii*, *N. alata*, and *N. sanderae* (Hoess et al. 1974; Labroche et al. 1983). In *N.tabacum*, an amphiploid, two sets of these bands are present, indicative of the expression of both homoeologous structural genes derived from its ancestors, *N. sylvestris* and *N. tomentosiformis* (Lagrimini et al. 1987). Little is known about the genetics of the fastest-moving anionic peroxidase isoenzymes in thorn apple (Conclin and Smith 1971; Smith and Conclin 1975), potato (Oliver and Martinez-Zapater 1985; Quiros and McHale 1985), and pepper (McLeod et al. 1983).

A common nomenclature for peroxidase genes in tomato, potato, and pepper has been advanced by Quiros and McHale (1985). Our results suggest that the genes designated *Prx-1* according to this nomenclature, as well as the gene Px_1 in tobacco (Hoess et al. 1974; locus $P_{2,3,4}$ according to Labroche et al. 1983) and *prxA* in petunia (Van den Berg and Wijsman 1981, 1982) are orthologous genes. As to the evolution of an ancestral peroxidase gene coding for the PRXa-like peroxidases, this question can only be investigated properly once the sequences of the present-day genes are known. cDNA clones obtained for fast-moving anionic peroxidases from tobacco (Lagrimini et al. 1987), potato (Roberts et al. 1988), and tomato (Roberts and Kolattukudy 1989) are already available. It has been shown that the potato and tomato cDNAs are highly homologous (Roberts et al. 1988; Roberts and Kolattukudy 1989). Alignment of the amino acid sequences of the tobacco and potato and tomato cDNAs (Lagrimini et al. 1987; Roberts et al. 1988; Roberts and Kolattukudy 1989) indicates that these proteins are only about 44% homologous, similar to the homology of both peroxidases with horseradish peroxidase C (Welinder 1979) and turnip peroxidase P7 (Mazza and Welinder 1980). The tobacco clone was obtained by screening a cDNA library with an oligodeoxynucleotide probe synthesized on the basis of an amino acid sequence of two fast-moving anionic peroxidases (Lagrimini et al. 1987). These two peroxidases as a group were characterized previously (Mäder et al. 1986), and correspond to both groups of bands cross-reacting with the petunia PRXa antiserum.

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