Peroxidase Activity in Tobacco Plants with Polyanion-Induced Interference to Tobacco Mosaic Virus

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ABSTRACT

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Peroxidase activity (PA) in tobacco cultivar Samsun NN leaves increased after injection of ethylene-maleic anhydride (EMA) 31, vinyl methyl ether/maleic anhydride (VME/MA), or VME/MA 0.5 methylester (VME/MAes), but not after that of polyacrylic acid (PAA). No correlation was established between increase in PA and induction of interference to TMV: interference induced by EMA 31 became evident before increases in PA; PA increased in plants treated with VME/MAes, a polyanion that does not induce resistance; actinomycin D inhibited the development of EMA 31-induced interference, but did not affect the increase in PA; and in a systemic host, PA increased after application of EMA 31 without reducing virus titre. Therefore, in the polyanion system at least, induced interference is not caused by enhanced PA.

Formation of necrotic lesions is accompanied by an increase in peroxidase activity which is greater than that in systemic infections and increases with the severity of symptoms (1, 4). Simons and Ross (8) suggested that high peroxidase activity kills infected cells, and that subsequent changes in advance of infection form a barrier to virus spread. Induction of systemic resistance in upper uninoculated leaves of Samsun NN tobacco by inoculation of lower leaves with tobacco mosaic virus (TMV) also was accompanied by a parallel increase in peroxidase activity (7).

Previously we reported that when certain polyanions, especially copolymers with a maleic acid component, were injected intercellularly into leaves of Samsun NN tobacco they induced resistance to TMV, causing reductions both in lesion number and lesion size (9). The resistance developed gradually after application of the inducer, reducing lesion number to 20-25% of those on control leaves. The development of the resistance was sensitive to actinomycin D, suggesting that the transcription mechanism of the cell has to operate. Subsequently, Gianinazzi and Kassanis (3) found that polyacrylic acid also induced resistance.

We therefore studied peroxidase activity in leaves with polyanion-induced resistance, especially as the development of resistance in this system is not associated with apparent necrotization prior to inoculation of the challenge virus.

MATERIALS AND METHODS

Plants of *Nicotiana tabacum* L. 'Samsun' and 'Samsun NN' were grown in 15-cm diameter pots in a screened greenhouse for 5-6 weeks following transplanting. One to 2 days before use they were trimmed to three expanded leaves and transferred to a greenhouse chamber maintained at 21 C. The respective polyanion, or sterile double-distilled water as a control, was injected into the opposite halves of 12-15 tobacco leaves, on four or five plants, as described previously (6). In additional control plants, sterile water was injected into one side of the leaves, and the opposite sides were left uninjected.

The following polyanions were used: ethylene/maleic anhydride (EMA) 31, vinyl methyl ether/maleic anhydride (VME/MA), VME/MA 0.5 methylester (VME/MAes), and polyacrylic acid (PAA). The compounds were donated by Monsanto Co., St. Louis, Mo.; data on their structure and molecular weight were given previously (9). EMA 31 and VME/MA were potent inducers of interference, whereas VME/MAes and PAA did not induce interference under our conditions.

Actinomycin D (Lyovac, Cosmogen, Merck, Sharp and Dohme) was used as an inhibitor of induced interference. One to three injections were given, the first one together with the polyanion.

Peroxidase activity (PA) was determined as described previously (5), by adding an aliquot of leaf tissue homogenate to a pyrogallol reagent solution. The change in optical density at 420 nm per 60 seconds (Δ_{OD}) after addition of H_2O_2 was determined with a Bausch and Lomb colorimeter. Relative peroxidase activity (RPA) was expressed as the ratio of Δ_{OD} treated/Δ_{OD} control. Five replicates per treatment were sampled from different half-leaves, each consisting of four 3-cm disks.

Induced interference was measured in parallel leaves as percent decrease in lesion number.

RESULTS

Injection of deionized sterile water into tobacco NN leaves did not affect peroxidase activity. Following injection of EMA 31 (0.5 mg/ml), RPA reached a peak after 7-8 days and then decreased (Fig. 1), but this was not correlated with the development of induced interference. Induced interference became evident after 2 days, whereas RPA increased only from the 4th day onward; and while induced interference remained at 70-80% between day 8 and day 15, RPA decreased markedly. When a low concentration of EMA 31 was used (0.25 mg/ml), RPA was between 1.1 and 1.2 at 9-13 days after injection, whereas induced interference remained around 50%.

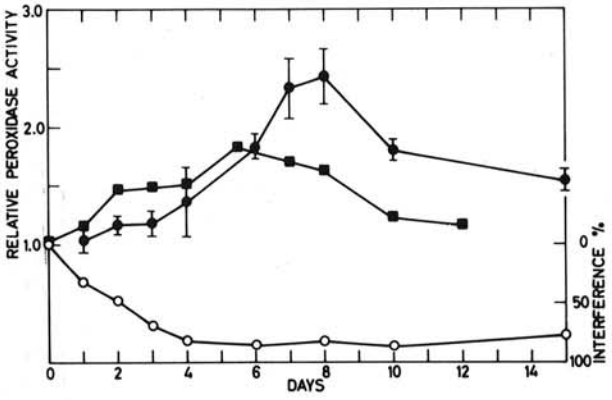

Fig. 1. Relative peroxidase activity (RPA) in Samsun (■——■) and Samsun NN (●——●) tobacco leaves, at different time intervals after injection with ethylene-maleic anhydride (EMA) 31 (0.5 mg/ml); and induced interference (○——○) in EMA 31-treated Samsun NN leaves. RPA from water-injected controls = 1.0. Averages from two to four experiments. Error bars represent ± standard error.

Furthermore, no correlation between increases in RPA and induction of interference was observed when VME/MA, which induces interference, was compared with VME/MAes, which does not. Similar patterns of RPA were obtained with both polyanions (Fig. 2). No increase in RPA was observed after injection of PAA, which under our conditions did not induce interference.

Actinomycin D, which partially inhibited the development of induced interference following injection of Samsun NN leaves with EMA 31, did not inhibit the increase in RPA (Fig. 3).

Injection of EMA 31 into leaves of tobacco cultivar Samsun (a systemic host for TMV) also increased RPA (Fig. 1), although no effects on TMV titre were observed in previous work (9).

DISCUSSION

A causal relation between peroxidase activation and

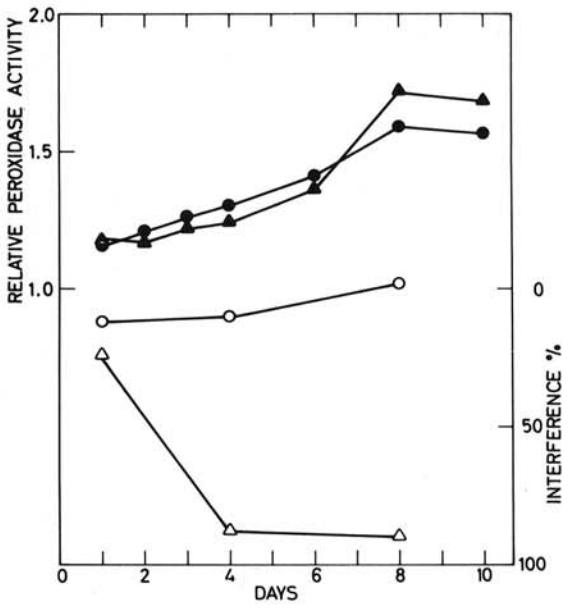

Fig. 2. Relative peroxidase activity (RPA) and induced interference in Samsun NN tobacco leaves injected with vinyl methyl ether/maleic anhydride (VME/MA) [RPA (▲——▲); induced interference (△——△)] or VME/MA 0.5 methylester [RPA (●——●); induced interference (○——○)]. RPA from water-injected controls = 1.0. Averages from two to four experiments.

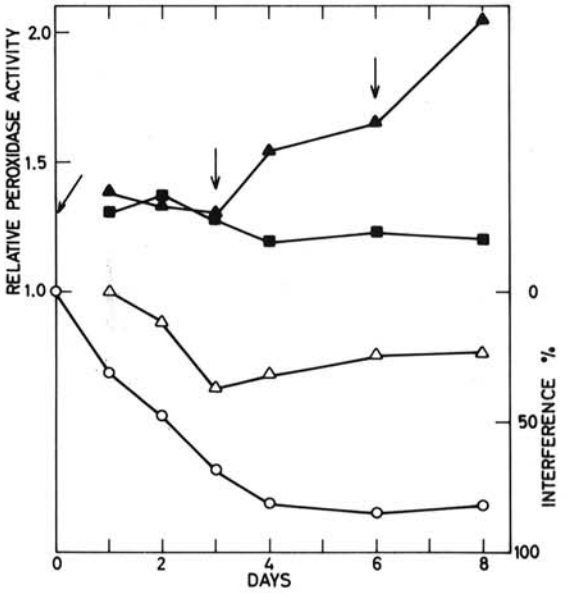

Fig. 3. Effect of actinomycin D on relative peroxidase activity (RPA) and induced interference in Samsun NN leaves injected with ethylene-maleic anhydride (EMA) 31 (0.5 mg/ml). With one to three injections of actinomycin D (each at 10 μg/ml) given as indicated by arrows (RPA: ▲——▲; induced interference: △——△). Induced interference in leaves injected only with EMA 31: ○——○. Relative peroxidase activity in leaves without EMA 31 injected once (0 time) with actinomycin D: ■——■.

virus localization and systemic induced resistance has been suggested (7, 8). Van Loon and Geelen (11) also associated increases in peroxidase activity with a decrease in lesion size. This suggestion was based on experiments with actinomycin D, applied 1 day before inoculation with TMV. However, actinomycin D applied at various intervals before inoculation decreases TMV multiplication and lesion size in several hosts (G. Loebenstein, *unpublished*); and even in noninfected tissue, peroxidase activity increases considerably after injection with actinomycin D. Furthermore, it was later reported that application of indoleacetic acid (IAA), which decreased lesion size, counteracted the virus-induced increase of peroxidase (10). Cabanne et al. (2) came to the conclusion that increases in peroxidase activity found during the hypersensitive reaction are a consequence, and not a cause, of the death of the cells.

No correlation was established between induction of interference by several polyanions and peroxidase activity. Increased peroxidase activity following injection with polyanions is probably due to release of cell wall-bound peroxidases from damaged tissues and not to de novo synthesis, because RPA remains high also in the presence of actinomycin D.

Therefore, it seems that, at least in the polyanion system, development of interference is not a result of enhanced peroxidase activity.

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