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Increase in phenylalanine ammonia-lyase activity in bean leaves infected with tobacco necrosis virus

G. L. FARKAS and J. SZIRMAI

Institute for Plant Physiology, Eötvös University, Budapest, Hungary
Institute for Plant Protection, Budapest, Hungary

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Abstract

In bean leaves infected with tobacco necrosis virus the phenylalanine ammonia-lyase activity dramatically increases parallel with the necrotization process. At the same time the amount of proteins decreases, and the total amount of phenolics increases in the diseased tissues.

Introduction

Ever since the discovery of phenylalanine ammonia-lyase by Koukol and Conn in 1961 the enzyme attracted considerable interest among plant pathologists. The enzyme is involved in aromatic biosynthesis and it appears to be a candidate for the explanation of increased aromatic biosynthesis in infected or injured tissues. It occurs in normal tissues generally at a low level but upon infection its activity greatly increases. Such an increase in the level of phenylalanine ammonia-lyase has been demonstrated to occur in sweet potato infected by *Ceratostomella fimbriata* (Minamikawa and Uritani, 1965) and in soybean inoculated with fungi non-pathogenic to soybean (Biehn et al., 1967). The increase in enzyme activity paralleled the increase in polyphenol concentration. The present paper reports data on the level of phenylalanine ammonia-lyase in virus-infected leaves.

Materials and methods

Bean seedlings (*Phaseolus vulgaris*) of the Hungarian variety 'Fürj' were grown under ordinary greenhouse conditions. The primary leaves were inoculated mechanically with tobacco necrosis virus (TNV) and on the 2nd or 3rd day after inoculation the tissues were used for the biochemical analyses. Brown, necrotic lesions developed on the infected leaves and extensive vein necrosis was also observed. Lesions started to develop, depending on the prevailing conditions, on the 2nd or 3rd day after inoculation. Lesion development was soon followed by yellowing of the leaf areas between the lesions. Uninfected opposite leaves served as control.
Phenylalanine ammonia-lyase activity was measured essentially as described by Rissland and Mohr (1967). The tissues were extracted in 0.1 M borate buffer, pH 8.8, in the presence of 3.7 mg glutathione/100 ml buffer. The extracts were centrifuged in the cold at 16,000 g for 20 min and then mixed with charcoal in a ratio of 50 mg/ml to

remove UV-absorbing material which interferes with the assay. After thorough stirring the extracts were centrifuged again for 10 min at 16,000 g to remove the adsorbent, and the supernatants were used for the enzyme assays. The assay consisted of incubating the extracts with 200 μmoles of borate buffer at pH 8.8 and 60 μmoles of L-phenylalanine. The increase in absorbance at 290 mμ (formation of trans-cinnamic acid) was measured in a Beckman DU-2 spectrophotometer against a blank containing no phenylalanine. Results were calculated by using the molar absorption coefficient of trans-cinnamic acid under the given conditions.
Protein content was determined according to Lowry et al. (1951). Total phenolics were determined in alcoholic extracts colorimetrically by using the Folin-Ciocalteu reagent (Farkas, 1962).

Results and discussion

Results are summarized in Table 1. They show that healthy bean leaves exhibit fairly low phenylalanine ammonia-lyase activity. An about 8–10 fold increase in the level of phenylalanine ammonia-lyase was detected when the lesions appeared. The large increase in enzyme activity occurred simultaneously with lesion formation. There was a trend for a further increase in phenylalanine ammonia-lyase activity when yellow areas developed between the necrotic lesions. The protein content of the infected tissues decreased dramatically during the 2nd to 3rd day of infection .Thus even more striking values are obtained by expressing the results on a protein basis.
Lesion formation and the associated ageing phenomena are accompanied by an increase in total phenolics. In the present experiments the increase in phenolics was relatively low. Thus, in contrast to earlier reports (Minamikawa and Uritani, 1965;

Table 1. Effect of infection with tobacco necrosis virus on the activity of phenylalanine ammonia-lyase (PAL) in bean leaf tissues

Sample		*PAL-activity*[1]	*Protein content mg/g fresh wt.*	*Total phenolics mg/g fresh wt.*[2]
Healthy	Expt. No. 1	10.2	14.0	0.9
	2	15.0	13.0	1.2
	3	12.3	11.4	1.3
2 days after infection	Expt. No. 1	80.5	10.0	1.4
	2	95.0	9.3	1.5
	3	110.0	9.0	1.5
3 days after infection	Expt. No. 1	130.5	7.2	1.6
	2	115.0	7.8	1.4
	3	150.7	6.5	1.9

[1] PAL-activity is expressed as μmoles of trans-cinnamic acid produced/g fresh weight of leaf tissue/h
[2] Expressed in chlorogenic acid equivalents

Tabel 1. Invloed van infectie met tabaksnecrosevirus op de activiteit van fenylalanine ammonia-lyase (PAL) in weefsel van boneblad

Biehn et al., 1967) no proportionality between phenylalanine ammonia-lyase activity and polyphenol formation was observed. This may be due to the rapid death of the leaf after the lesions are formed. Apparently there is no time for the accumulation of larger amounts of phenolics before the tissues die. By contrast, enzyme activation (synthesis) appears to be a very fast process. This was also shown in those cases in which the formation of phenylalanine ammonia-lyase was induced by light treatment (Rissland and Mohr, 1967;Attridge and Smith, 1967; Engelsma, 1967a, b). If the death of the tissue occurs extremely fast upon infection, there is no increase in phenylalanine ammonia-lyase and, as expected, there is no increase in phenolics at all. Such a case has been described recently in connection with the hypersensitive reaction of tobacco leaves infected by *Pseudomonas syringae*, a bacterium non-pathogenic to tobacco (Németh et al., 1969).
By contrast, the hypersensitive reaction induced by *Helminthosporium carbonum* in soybean is a slower process (Biehm et al., 1967) just like the metabolic alterations induced by *Monilia fructicola* spores in bean pods which lead to phytoalexin formation (Hadwiger, 1967). In both cases the increase in phenylalanine ammonia-lyase parallels (or precedes) the formation of phenolics and pisatin respectively. The participation of phenylalanine metabolism in pisatin production is well documented (Hadwiger, 1967). Thus, the results obtained support the idea that tissue necrosis in some way triggers the formation of phenylalanine ammonia-lyase which is perhaps the most important enzyme controlling polyphenol biosynthesis in plants. If the tissues do not die very fast the increase in enzyme activity is followed by polyphenol accumulation.

Samenvatting

Toeneming in activiteit van fenylalanine ammonia-lyase in bonebladeren geïnfecteerd met tabaksnecrosevirus

In bonebladeren geïnfecteerd met tabaksnecrosevirus neemt tegelijk met het afstervingsproces de activiteit van fenylalanine ammonia-lyase drastisch toe. Gelijktijdig neemt in de zieke weefsels de hoeveelheid eiwitten af en de totale hoeveelheid fenolen toe.

References

Attridge, T. H. and Smith, H., 1967. A phytochrome-mediated increase in the level of phenylalanine ammonia-lyase in the terminal buds of Pisum sativum. Biochim. biophys. Acta 148: 805–807.

Biehn, W. L., Kuc', J. and Williams, E. B., 1967. Accumulation of phenols in soybean after inoculation with fungi nonpathogenic to soybean. Phytopathology 57: 804.

Engelsma, G., 1967a. Effect of cycloheximide on the inactivation of phenylalanine deaminase in gherkin seedlings. Naturwissenschaften 54: 319–320.

Engelsma, G., 1967b. Photoinduction of phenylalanine deaminase in gherkin seedlings. I. Effect of blue light. Planta 75: 207–219.

Farkas, G. L., 1962. Probleme der parasitogen stimulierten Biosynthese aromatischer Verbindungen. Ber. dt. bot. Ges. 74: 382–388.

Hadwiger, L. A., 1967. Changes in phenylalanine metabolism associated with pisatin production. Phytopathology 57: 1258–1259.

Koukol, J. and Conn, E. E., 1961. The metabolism of aromatic compounds in higher plants. IV.

Purification and properties of phenylalanine deaminase of Hordeum vulgare. J. biol. Chem. 236: 2692–2698.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. J. biol. Chem. 193: 265–275.
Minamikawa, T. and Uritani, J., 1965. Phenylalanine ammonia-lyase in sliced sweet potato roots. J. Biochem., Tokyo 57: 678–688.
Németh, J., Klement, Z., and Farkas, G. L., 1969. An enzymological study of the hypersensitive reaction induced by Pseudomonas syringae in tobacco leaf tissues. Phytopath. Z. (in press).
Rissland, I. und Mohr, H., 1967. Phytochrom-induzierte Enzymbildung (Phenylalanin-desaminase), ein schnell ablaufender Prozess. Planta 77: 239.