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BIOCHEMICAL EFFECTS OF TOBACCO ETCH VIRUS INFECTION ON TOBACCO LEAF TISSUE—II.

POLYPHENOL OXIDASE ACTIVITY IN SUBCELLULAR FRACTIONS

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Abstract—The effect of tobacco etch virus infection on polyphenol oxidase (O-diphenol: O-quinone oxidoreductase) activity in subcellular fractions of tobacco leaf tissue was investigated. Activity was found in the chloroplast fraction (1000 × *g* fraction), in the "mitochondrial" fraction (12,000 × *g* fraction), and in the "soluble" fraction (12,000 × *g* supernatant). Enzyme activity increased in all fractions, but not equally. The highest increase, based on specific activity, was in the chloroplast and soluble fractions. Total protein of all fractions and chlorophyll of the chloroplast fraction were decreased by infection, but both protein and chlorophyll per chloroplast were unchanged.

INTRODUCTION

ONE of the common biochemical changes occurring in plant tissues following virus infection is an increase in polyphenol oxidase (O-diphenol: O-quinone oxidoreductase) activity. The intracellular distribution of this enzyme has been investigated in several species and has been reported to be associated with chloroplasts and mitochondria and also to occur in a soluble form.[1–3] In previous investigations on the influence of disease on this enzyme, no distinction was made between soluble and particulate activity. Rather, only total activity in tissue homogenates was measured. The present investigation was undertaken to determine the relationship between the intracellular distribution and increased activity of this enzyme following virus infection.

RESULTS AND DISCUSSION

Nicotiana tabacum L. (var. Ky 26) leaves systemically infected with tobacco etch virus were harvested 19–20 days after inoculation of a lower leaf. Control leaves were obtained from uninoculated plants which were of the same age and grown under the same conditions. The tissue was fractionated by centrifugation to yield a chloroplast fraction (1000 × *g* fraction) a "mitochondrial" fraction (12,000 × *g* fraction), and a soluble fraction (12,000 × *g* supernatant). All fractions were adjusted to the original volume of the homogenate. The method of homogenization employed[4] gave a high yield of intact chloroplasts. Oxidation of catechol and chlorogenic acid by each fraction was determined and activity was expressed on the basis of volume of homogenate, protein content, and, in the chloroplast fraction, chlorophyll content. Tables 1 and 2 summarize the results of a typical experiment. With

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catechol as a substrate, specific activity of the enzyme (μl O_2/mg protein/min) increased 2·6 fold in the chloroplast fraction, 1·5 fold in the mitochondrial fraction, and 5 fold in the soluble fraction. With chlorogenic acid, the increase in specific activity was 4·8 fold in the chloroplast fraction, 1·7 fold in the mitochondrial fraction, and 3·6 fold in the soluble fraction. These values are somewhat lower when calculated on the basis of homogenate volume because of a lower protein content in the fractions from infected tissue.

Table 1. Polyphenol oxidase activity of fractions from etch infected and healthy tobacco leaves

Fraction and substrate	Mg protein/ml extract		μl O_2/ml		μl O_2/mg protein		μl O_2/mg chlorophyll/min	
	I*	C*	I	C	I	C	I†	C‡
Chloroplast	1·20	1·60						
Chlorogenic acid			13·9	3·8	11·5	2·4	100·6	17·5
Catechol			9·7	4·9	8·1	3·1	70·3	22·7
Mitochondrial	0·55	0·63						
Chlorogenic acid			4·5	3·0	8·2	4·8		
Catechol			3·0	2·3	5·5	3·6		
Soluble	2·50	3·60						
Chlorogenic acid			54·9	19·1	21·9	6·1		
Catechol			22·5	5·6	9·0	1·8		

* I, infected leaves; C, healthy leaves. † 137·7 μg/chlorophyll per ml of extract; ‡ 213·7 μg/chlorophyll per ml of extract.

Table 2. Chlorophyll content of chloroplasts from etch infected and healthy tobacco leaves

Tissue	No. chloroplasts/ml	Chlorophyll/ml	Chlorophyll/10^6chloroplasts
Infected	$9{\cdot}4 \times 10^7$	252 μg	2·8 μg*
Control	$1{\cdot}16 \times 10^8$	367 μg	3·0 μg*

* Difference not significant.

The chloroplast fraction exhibited a 3·1 fold increase against catechol and a 5·7 fold increase against chlorogenic acid when calculated on the basis of chlorophyll content. Since actual counts of the number of chloroplasts showed that the chlorophyll content per chloroplast was the same in healthy and infected plants (Table 2) these values reflect the extent of increase in enzyme activity per chloroplast. However, since the infected plants contained only approximately 60 per cent as many chloroplasts as did healthy plants on a fresh weight basis, the contribution of the chloroplast fraction to the total enzyme activity would not be this large. Judging from the protein content of the mitochondrial fraction, the same is probably true of this fraction although particle counts were not made.

The well-known increase in polyphenol oxidase activity in plant tissues following virus infection results, at least in this instance, from a significant but non-uniform increase in activity in all cellular components in which the enzyme is known to occur. Of particular interest is the marked increase in the activity in the chloroplast fraction. This seems to be a

selective effect of infection of these organelles because the total chlorophyll, total protein, rate of amino acid incorporation[4] and the Hill reaction[5] are unaffected by infection with this virus. However, the number of chloroplasts per unit fresh weight of tissue is lower in infected tissue (Table 2). The question of whether the increased polyphenol oxidase activity observed in diseased plants results from synthesis of new protein or from activation of proenzymes has often been raised. In the present case, since the protein per chloroplast and the apparent rate of protein synthesis by chloroplasts were unchanged,[4] it would seem more likely that inactive forms of the enzyme are activated by the infection, although this cannot be concluded from our data.

A second interesting observation is that activity against catechol and chlorogenic acid are not affected to the same extent by infection. For example, the ratio of catechol oxidation to chlorogenic acid oxidation in chloroplasts from healthy plants is 1·3 and in chloroplasts from infected plants is 0·7 (based on specific activity in these fractions). These values are apparently in disagreement with the suggestion that the same enzyme catalyses the oxidation of both substrates, unless the infection in some way alters the structure of the enzyme to selectively alter its activity against the two substrates.

EXPERIMENTAL

Seeds of *Nicotiana tabacum* L. (var. Ky 26) were sown in vermiculite in plastic tubes of approximately 50 ml capacity which had a hole in the bottom for subirrigation. The plants were grown under a bank of fluorescent lights which produced 16,000 lux. at the level of the plants. A photoperiod of 18 hr light and 6 hr darkness was employed and the plants were irrigated daily with Hoaglands complete nutrient solution. The plants were inoculated on the two lowest leaves 25 days after sowing the seeds and the three top leaves were harvested 19–20 days after inoculation. The harvested leaves were in the stage of rapid expansion at this time and infected plants exhibited pronounced mottling, leaf distortion, and stunting.

Tissue homogenates were prepared by grinding 20 g leaf tissue from which the midveins had been removed in a prechilled mortar with 30 ml cold 0·36 M sucrose in 0·05 M tris (hydroxymethyl) aminomethane-HCl buffer at pH 7·5 and 20 g of 4 mm diameter glass beads.[4] The tissue homogenate was fractionated according to the scheme described previously.[4] All steps were carried out at 0–3°.

Polyphenol oxidase activity against catechol and chlorogenic acid was determined with an oxygen electrode (Yellow Springs Instrument Company, Cleveland, Ohio). The reaction vessel initially contained 3 ml of tissue fraction, diluted with sucrose-tris as required and maintained at 36°. The reaction was started by injecting 0·3 ml of 0·01 M catechol or chlorogenic acid with a hypodermic syringe. After mixing, 0·3 ml of liquid was withdrawn from the vessel to maintain a constant volume. Autoxidation of the substrates at this pH was very slight compared with the rate of enzymatic oxidation and therefore no correction was made for this.

Protein was measured by the method of Lowry *et al.*[7] using trichloroacetic acid precipitates of the samples, and total chlorophyll was determined by the method of Arnon.[8]

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