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Ethylene Production during Lipid Peroxidation in Cowpea Leaves Infected with Cucumber Mosaic Virus

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加藤 盛*: キュウリ・モザイク・ウイルス感染ササゲ葉の
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It is well established that the production of ethylene markedly increases as a result of virus infection in a local lesion host. Nakagaki *et al.*¹⁾, working with TMV-infected local lesion hosts, postulated that the enhanced ethylene production reflects necrotization of the cells invaded by the virus. Gáborjanyi *et al.*^{2,3)} have reported that the increased production of ethylene perhaps was a consequence but not a cause of tissue necrosis. Although numerous papers^{4,5)} have been published on the evolution of ethylene from injured or infected tissues, little information has been available concerning the biogenesis of ethylene.

The present paper deals with the time course study of ethylene production in CMV-infected leaves and the effects of various inhibitors on ethylene production.

The yellow strain of CMV was used. Cowpea leaves (*Vigna sinensis* Endl. var. *sequipedalis*, cultivar Kurodane-sanjaku) grown in a greenhouse at 25 C, were inoculated with virus suspension using Carborundum by the opposite leaf method. Lesion numbers produced per leaf were 600 or more. Control leaves were rubbed with 0.1 M phosphate buffer solution (pH 7.0). Both infected and uninfected cowpea leaves were harvested immediately after inoculation for determining the evolution of ethylene. The detached leaves were placed in the flask (100 ml) sealed with rubber cap, which contained filter paper and 15 ml of distilled water. Gas samples were withdrawn using a gas tight syringe at various times during incubation, and immediately analyzed by a modification of the procedure of Shimokawa and Kasai⁶⁾ in the following manner: Gas sample was injected into a gas chromatograph (Shimadzu model IV) with a hydrogen ionization detector, fitted with 0.2×150 cm column containing 40-60 mesh activated alumina coating silicon oil, carrier gas, N₂, the gas flow rate, 35 ml/min and column temp., 75 C. Quantitative determination of ethylene was made by measuring peak areas. An aliquot of inhibitor solution (10⁻³ M) was administered into the leaves by using a vacuum-infiltration technique immediately and 20 hr after inoculation, and the treated leaves were incubated in an incubation flask containing 15 ml of inhibitor solution. The inhibitors used in the experiment were propyl gallate, cystein, sodium diethylthiocarbamate (DIECA) and

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1) Nakagaki, Y., Hirai, T. and Stahmann, M. A. (1970). Virology 40: 1-9. 2) Gáborjanyi, R., Balázs, E. and Király, Z. (1971). Acta Phytopath. Hung. 6: 51-55. 3) Balázs, E., Gáborjanyi, R., Tóth, A. and Király, Z. (1969). Acta Phytopath. Hung. 4: 355-358. 4) Ross, A. F. and Williamson, C. E. (1951). Phytopathology 41: 431-438. 5) Williamson, C. E. (1950). Phytopathology 40: 205-208. 6) Shimokawa, K. and Kasai, Z. (1966). Plant & Cell Physiol. 7: 1-9.

disodium ethylenediaminetetraacetate dihydrate (EDTA). As shown in Fig. 1, time course changes of ethylene production in CMV-infected leaves showed a very

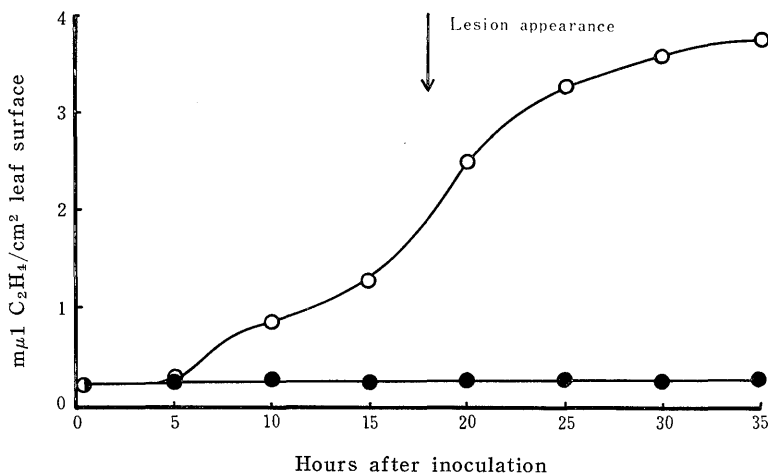


Fig. 1. Time course of ethylene production in CMV-inoculated cowpea leaves. Average of 3 independent experiments, each of which consisted of 3 incubation flasks, 2 leaves each. Total accumulation of ethylene during the interval indicated. ○ : Inoculated leaves. ● : Uninoculated leaves.

characteristic pattern. A small but significant increase in ethylene production was observed after about 8 hr, but an impressive increase in ethylene production occurred at the beginning of tissue necrosis. Ethylene production was increased as the necrotic area increased. The increased period of ethylene production, before the appearance of visible lesions, coincided in time with active period of lipid peroxidation in infected tissues⁷⁾. In uninfected leaves the rate of ethylene production remained unchanged.

Although peroxidized linolenate has been suggested as a precursor of ethylene, no enzymatic mechanism has been found. On the other hand, Demorest and Stahmann⁸⁾ proposed a model for ethylene production in plants from peptides and protein containing methionine. According to their suggestion, it is possible that proteolysis in plants enhanced ethylene production by producing peptide with a C-terminal methionine residue. However, an increase in proteolytic activity has not yet been observed, although one can suppose that local yellowing or local senescence is related to a certain degree of proteolysis. Working with tomato fruit, Ku *et al.*⁹⁾ demonstrated that peroxidase catalyzed ethylene production *in vitro*, and both the activity of peroxidase and ethylene production increased during fruit ripening. But, in CMV-infected cowpea, the peroxidase activity was never increased in the enhanced period of ethylene production before the lesion became visible¹⁰⁾.

Studies with CMV-infected cowpea favor the possibility that peroxidized linolenate is a precursor of ethylene in the early stages of infection. When various

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inhibitors were applied shortly after inoculation, ethylene production was markedly inhibited by propyl gallate which tends to counteract lipid peroxidation in the tissues, but other inhibitors were almost ineffective (Table 1). The number of local lesions caused by CMV was decreased about 25 per cent by propyl gallate, while DIECA, EDTA and cystein did not inhibit the lesion formation. These data

Table 1. The effects of various inhibitors on ethylene production in CMV-inoculated cowpea leaves

Inhibitor	0 hr ^{a)}		20hr ^{a)}	
	Ethylene ($\mu\text{l}/\text{cm}^2$)	Lesions (no/leaf)	Ethylene ($\mu\text{l}/\text{cm}^2$)	Lesions (no/leaf)
Non-inoculated	0.21	0	0.28	0
Inoculated				
Untreated	1.90(0)	822(0)	2.21(0)	850
Propyl gallate	1.09(57.4)	621(24.5)	1.98(10.4)	818
DIECA	1.61(15.3)	794(3.5)	2.09(5.4)	821
EDTA	1.50(21.1)	771(6.2)	1.41(36.2)	860
Cystein	1.79(5.8)	778(5.4)	2.15(2.7)	815

a) Hours of treatments after inoculation.

Figures in parentheses show the percentage of inhibition.

Figures represent average of 3 independent experiments, each of which consisted of 3 incubation flasks, 2 leaves each. Total accumulation of ethylene in 10 hr after treatment.

obtained with cowpea leaves support the hypothesis that peroxidation of unsaturated fatty acids is related to the formation of ethylene. However, it is unlikely that the products of peroxidized lipid are converted to ethylene by a copper mediated enzyme¹¹⁾, because DIECA was almost ineffective to the ethylene production. Conceivably, conversion of products of peroxidized lipid to ethylene may be due to the nonenzymatic reaction.

On the other hand, when these inhibitors were applied 20 hr after inoculation, the inhibitory effect of various inhibitors on ethylene production markedly differed from the above facts. In the leaves with local lesions, EDTA was most effective in inhibiting ethylene production, and propyl gallate was less effective. These results indicate that the sensitivity of ethylene-forming system to various inhibitors was altered with time after infection.

Based on these findings, it was considered that the precursors of ethylene *in vivo* and a pathway of ethylene biosynthesis was altered before and after the appearance of local lesions. Before the appearance of lesions, the precursor of ethylene is some of the products of peroxidized linolenate and conversion of the products to ethylene may be due to the non-enzymatic radical reaction. On the other hand, after the lesions appeared, although the precursor of ethylene remains still unidentified, ethylene production seems to be mediated by some EDTA-sensitive enzymes, such as peroxidase.

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