Plant Physiol. (1970) 46, 172–174

Short Communication

Induction of Peroxidase Activity by Ethylene in Sweet Potato[1]

Received for publication February 17, 1970

Hidemasa Imaseki
Institute for Biochemical Regulation, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464

Metabolic activation in sweet potato root tissues injured by mechanical, chemical, or fungal damage has been extensively investigated (1, 2, 8, 14–16). Previously, it was shown that metabolic activation, particularly peroxidase and phenylalanine-ammonia lyase activities, developed when sweet potato roots were sliced. These activities were significantly enhanced by a strikingly low level of ethylene exogenously supplied to the slices (11). Incubated root slices produced enough ethylene to enhance metabolic activation before any increase in enzyme activity occurred (9). It was also found that ethylene production is greatly stimulated when tissue is injured (10, 12, 16). A comparison of polyacrylamide gel electrophoretic patterns for peroxidase isoenzymes extracted from slices incubated with or without ethylene revealed identical qualitative patterns (9). Those results suggest that the metabolic activation which occurs in sliced tissues in the absence of exogenously added ethylene is regulated by endogenous ethylene produced in response to the injury caused by slicing the tissue. In the present communication, the author wishes to present additional evidence in support of the above view. Since the increase in peroxidase activity after slicing was particularly sensitive to exogenous ethylene, peroxidase activity is mainly dealt with in the present paper.

MATERIALS AND METHODS

Sweet potato roots (*Ipomoea batatas* Lam., cultivar Norin 1) were harvested in late October and stored at 10 C until used. Storage at this temperature until the next June caused no apparent cold injury although there was a slight change during the storage in the degree of biochemical response of the roots to slicing. Most of the experiments were performed with roots cultivated in Aichi Prefecture; however, the experiment presented in Table I was done with the roots cultivated in Chiba Prefecture. The roots were cut into 2-mm-thick slices, and discs 18 mm in diameter were prepared with a cork-borer from the inner parenchymatous tissue. The discs were thoroughly washed with water and placed in Petri dishes containing a sheet of filter paper, and then the Petri dishes were set in large desiccators. Desired gas mixtures were obtained by first evacuating dessicators then introducing a desired volume of oxygen, carbon dioxide, or ethylene, and finally refilling the desiccator with ethylene-free air to atmospheric pressure. In the case of experiments under reduced pressure, the top and bottom parts of a desiccator were tightly clamped to minimize gas leakage, and pressure was monitored by a manometer. Oxygen and carbon dioxide were obtained in tanks from local sources and used without prior washing; the air to be introduced into desiccators was passed through a brominated charcoal column. Ethylene of 99.9% purity was purchased from Takachiho Chemical Industry, Shibuya, Tokyo.

Five discs weighing 2.8 g were sampled at intervals during incubation at 27 C, and were homogenized in a glass homogenizer with 5 ml of pH 7.0 potassium phosphate buffer (0.05 M) containing 0.5% sodium isoascorbate. The resultant homogenate was centrifuged at 12,000 *g* for 15 min, and 2 ml of the supernatant was charged onto a column of Sephadex G-25 (16 mm by 10.5 cm, bed volume 20 ml), equilibrated with the same phosphate buffer but without isoascorbate. The column was eluted with the above mentioned buffer, and total protein was quantitatively collected in a 5-ml eluate. All of the procedures were carried out below 5 C.

Peroxidase activity was measured as described previously (9) and was expressed in figures and tables as absorbance at 450 nm/g of tissue under standard conditions.

RESULTS AND DISCUSSION

Figure 1 shows that the peroxidase increase stimulated by ethylene was inhibited by 5% carbon dioxide. Similarly, the increase without ethylene was also inhibited by carbon dioxide at the same level. Inhibition of the increase without added ethylene was always partial, and complete inhibition has not been obtained even with 10% carbon dioxide in an incubation atmosphere. Furthermore, the extent of the inhibition varied with length of the storage period of sweet potato roots after harvest; inhibition by carbon dioxide decreased as the storage period lengthened. Carbon dioxide is known to be an inhibitor of ethylene action in plant tissues. For example, by using etiolated pea stem sections, Burg and Burg (4) demonstrated that carbon dioxide is a competitive inhibitor of ethylene action. Similar results were obtained in the regulation of plumular hook opening in etiolated pea seedlings by light and ethylene (13). Therefore, a reduced enhancement of peroxidase activity in sweet potato root slices treated with carbon dioxide suggests participation of endogenous ethylene in the induction of peroxidase.

Storage of banana fruits under subatmospheric pressure has been reported to minimize the effect of endogenous ethylene on the ripening processes, resulting in a delay of yellowing (5). When sweet potato slices were incubated under reduced pressure, peroxidase increase during the incubation period was always lower than the control level. As is shown in Figure 2, incubation at one-fifth atmospheric pressure in pure oxygen, which provided roughly the same oxygen level as incubation in air, resulted in a reduced enhancement of enzyme activity, but the presence of exogenous ethylene under these conditions gave rise to enzyme formation at the rate observed with ethylene-air at atmospheric pressure. Therefore, it is assumed that the inhibition observed under reduced pressure is not a physical effect of reduced pressure, but rather is due to a lowered level of endogenous ethylene.

Incubation of slices in pure oxygen at atmospheric pressure also lowered the rate of enzyme increase compared to an air control, and addition of ethylene to the pure oxygen resulted in an in-

[1] This work was supported in part by Grant No. 0803 from Ministry of Education of Japan.

Table I. *The Effect of Ethylene on Peroxidase Increase in 2 mm Discs and Thick Blocks of Sweet Potato Root*

Experiment 1: Round root blocks, 3-cm-thick with peels attached, were incubated in various concentrations of ethylene for 48 hr and were cut parallel to the original cut surface into 3-mm slices. Peroxidase activity in the first and third layer slices was assayed. Experiment 2: Peroxidase activity was compared in 2-mm discs and the third layer of 3-cm-thick blocks treated for 48 hr with various concentrations of ethylene.

Concentrations of Ethylene	Peroxidase Activity (A_{450}/g Fresh Weight) in Tissues Located at			
	Experiment 1		Experiment 2	
	0–3 mm (1st layer)	6–9 mm (3rd layer)	2-mm discs	6–9 mm (3rd layer)
µl/liter				
0	294	237	333	208
1	551	391	...	...
10	1277	842	930	713
100	1455	1000	985	1483
1000	1525	1072	1125	2155

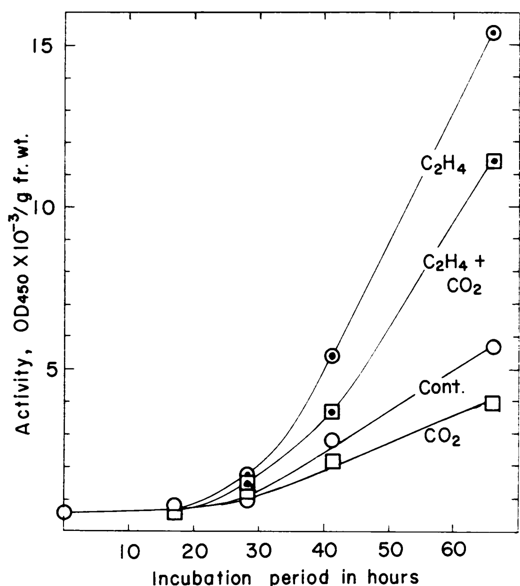

FIG. 1. The effects of ethylene and carbon dioxide on peroxidase increase in sweet potato root slices during aerobic incubation at 27 C. Discs, 2-mm-thick and 18-mm in diameter, were incubated in desiccators in atmospheres containing 10 µl/liter of ethylene, 10 µl/liter of ethylene plus 5% carbon dioxide, or 5% carbon dioxide alone. In the control without carbon dioxide or ethylene, the desiccator contained 20% KOH and 0.25 M mercuric perchlorate to remove carbon dioxide and ethylene generated by the slices. The gas mixture was renewed whenever samples were withdrawn.

crease to the ethylene-air level. Thus, high oxygen tension itself was not inhibitory to the enzyme increase mechanism. Previously, it was found that a high oxygen tension in an incubation atmosphere inhibited ethylene production in diseased sweet potato roots (10). Therefore, the lowered enzyme increase observed here might be due to reduced ethylene production under high oxygen levels. When air was employed as an incubation atmosphere, the presence of ethylene under reduced pressure caused some increase in enzyme formation, but did not restore the increase to the ethylene-air level at atmospheric pressure, presumably resulting from shortage of oxygen.

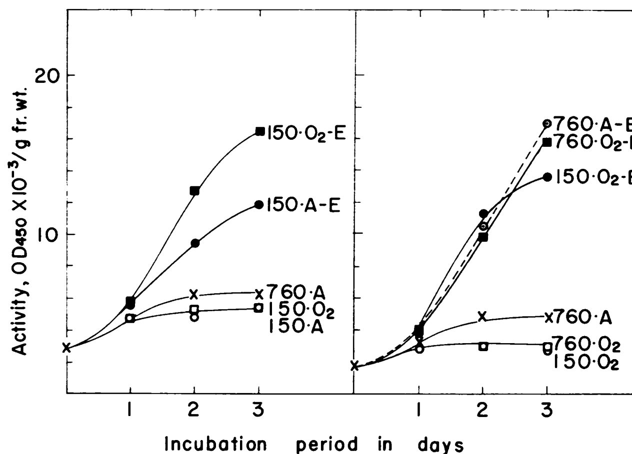

FIG. 2. The effects of reduced pressure and oxygen on peroxidase increase in sweet potato root slices. After flushing with ethylene-free air or oxygen, the desiccators containing the discs evacuated to the desired pressure before 10 µl/liter of ethylene were added as indicated. A: Air; O_2: oxygen; E: ethylene; 760 and 150: atmospheric pressure in mm of Hg.

In order to examine the effect of ethylene on sweet potato tissue in relation to degree of injury caused by cutting, 3-cm-thick blocks of roots were incubated for 48 hr with various levels of ethylene, and peroxidase activity in tissue between the cut surface and the 3-mm depth, and also in the inner 6 to 9 mm from the surface was examined. As shown in Table I, when activities are plotted against ethylene concentration, levels of peroxidase activity in the inner part are always slightly lower than those at the surface area, and increase with ethylene concentration in the incubation atmosphere. A similar comparison was made between discs and the inner part of thick blocks. In discs, enzyme formation was nearly maximal with about 10 µl/liter of ethylene. The enzyme activity in the inner part of thick blocks was lower than that of discs at low ethylene concentrations, but it increased as the ethylene concentration was raised. Because incubation in ethylene was carried out for periods as long as 48 hr, exogenously applied ethylene must be equilibrated within the tissue block so that no declining gradient in ethylene level from the surface to inner part should be expected. Lower peroxidase levels in the inner part at a given ethylene concentration must then be interpreted to mean that the effect of cut injury is greatest at the surface area that is injured, i.e., different tissues may show a differential response to the same ethylene level. Tissue in the inner part was only 6 to 9 mm from the cut surface and hence may receive some influence from cutting, possibly in the form of an enhanced production of ethylene. It is still not clear whether cells become sensitive to ethylene only after receiving some effect of injury or whether they are always sensitive to exogenous ethylene and injury simply causes cells to produce ethylene.

Slices or excised segments of other plant tissues, such as potato tuber, burdock, and radish root, increase in peroxidase activity during an incubation, and this increase seems to be regulated by ethylene, since the increase was affected by exogenous ethylene (unpublished data).

The results described in this paper indicate that endogenous ethylene may regulate enzyme formation in injured plant tissues. Production of ethylene by plant tissues is nearly a universal phenomenon (3), which is affected by many environmental factors, such as light (6, 13) or physical and chemical stimuli (7, 17) and also by such internal factors as growth regulators (3). Inasmuch as most physiological phenomena induced by ethylene are also regulated by other growth regulators, ethylene may play an important role in the regulation of cellular metabolisms which are

related not only with morphological changes, but also with basic cell processes.

Acknowledgment—The author wishes to express his sincere thanks to Dr. I. Uritani for his helpful discussions.

LITERATURE CITED

1. Akutsu, K., H. Imaseki, and I. Uritani. 1966. Induced respiration of sweet potato roots infected by the black rot fungus. Bot. Mag. (Tokyo) 70: 644-653.
2. Asahi, T., Y. Honda, and I. Uritani. 1966. Increase of mitochondrial fraction in sweet potato root tissue after wounding or infection with *Ceratocystis fimbriata*. Plant Physiol. 41: 1179-1184.
3. Burg, S. P. 1962. The physiology of ethylene formation. Ann. Rev. Plant Physiol. 13: 265-302.
4. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Science 148: 1190-1196.
5. Burg, S. P. and E. A. Burg. 1966. Fruit storage at subatmospheric pressure. Science 153: 314-315.
6. Goeschl, J. D., H. K. Pratt, and B. A. Bonner. 1967. An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. Plant Physiol. 42: 1077-1180.
7. Goeschl, J. D., L. Rappaport, and H. K. Pratt. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol. 41: 877-885.
8. Hyodo, H. and I. Uritani. 1966. A study on increase in a diphenol oxidase activity during incubation of sliced sweet potato tissue. Plant Cell Physiol. 7: 137-144.
9. Imaseki, H., T. Asahi, and I. Uritani. 1968. Investigations on the possible inducers of metabolic changes in injured plant tissues. *In:* T. Hirai, J. Hidaka, and I. Uritani, eds., Biochemical Regulation in Diseased Plants and Injury. Phytopathology Society of Japan, Tokyo. pp. 189-201.
10. Imaseki, H., T. Teranishi, and I. Uritani. 1968. Production of ethylene by sweet potato roots infected by the black rot fungus. Plant Cell Physiol. 9: 769-781.
11. Imaseki, H., M. Uchiyama, and I. Uritani. 1968. Effect of ethylene on the inductive increase in metabolic activities in sliced sweet potato roots. Agr. Biol. Chem. 32: 387-389.
12. Imaseki, H., I. Uritani, and M. A. Stahmann. 1968. Production of ethylene by injured sweet potato root tissue. Plant Cell Physiol. 9: 757-768.
13. Kang, B. G., C. S. Yocum, S. P. Burg, and P. M. Ray. 1967. Ethylene and carbon dioxide. Mediation of hypocotyl hook-opening response. Science 156: 958-959.
14. Kawashima, N. and I. Uritani. 1963. Occurrence of peroxidase produced in sweet potato infected by the black rot fungus. Plant Cell Physiol. 6: 247-265.
15. Minamikawa, T. and I. Uritani. 1964. Phenylalanine deaminase and tyrosine deaminase in sliced or black rot infected sweet potato roots. Arch. Biochem. Biophys. 108: 573-574.
16. Stahmann, M. A., B. G. Clare, and W. Woodburg. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by black rot infected sweet potato tissue. Plant Physiol. 41: 1505-1512.
17. Williamson, C. E. 1950. Ethylene, a metabolic product of diseased or injured plants. Phytopathology 40: 205-208.