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Ethylene-Enhanced Ethylene Oxidation in *Vicia faba*

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Abstract. The alkene oxygenase (AO) of fababean (*Vicia faba* L.) converts ethylene to ethylene oxide. Treatment of fababeans with 10 μl/liter ethylene increases the activity of this enzyme within 2 hours of ethylene treatment. Though other alkenes were taken up by fababean seedlings, ethylene was the most active in inducing AO activity. The ability of ethylene to increase AO was blocked 60% by cycloheximide, an inhibitor of protein synthesis, and 35% by $AgNO_3$, an inhibitor of ethylene action.

Alkene oxygenase (AO) oxidizes ethylene to ethylene oxide. Similar enzymes occur in the actinomycete, *Mycobacterium paraffinicum* (De Bont 1975, De Bont 1976, De Bont and Albers 1976, De Bont et al. 1979, De Bont and Harder 1978, De Bont et al. 1980, Wiegant and De Bont 1980), animals (Ehrenberg et al. 1977, Filser and Bolt 1983), and plants (Beyer 1975, Beyer 1977, Beyer 1979a, Beyer 1979b, Beyer 1980, Beyer and Blomstrom 1979, Beyer and Sundin 1978, De Bont 1976, Dodds and Hall 1982, Dodds et al. 1979, Jerie and Hall 1978). In *M. paraffinicum,* the function of ethylene oxidation is to provide energy and carbon for the growth of this soil-inhabiting organism. Since this enzyme lacks substrate specificity and removes a number of alkenes from the surrounding gas phase, the generalized name of alkene oxygenase is used in this paper. However, ethylene is probably the major alkene available to plants or actinomycetes. The description of *in vivo* alkene consumption as an oxygenase is based on the reports that ethylene oxide is the end product of the reaction (Dodds et al. 1979) and that the activity of the enzyme was lost in a nitrogen gas phase (Abeles 1984). However, we have not shown that molecular oxygen was incorporated directly into the alkenes used in this study. The term AO then is used here with this understanding and as a convenient way to describe the ability of *V. faba* to metabolize ethylene and other alkenes.

Table 1. Correlation between oxidation and action of ethylene analogs applied to fababeans at a concentration of 10 μl/liter. Seedlings were placed in 4-liter plastic containers and the gases indicated introduced by injection through a rubber vaccine stopper in the lid. After 24 h the level of each hydrocarbon was measured and the rate of uptake expressed as nl/g h. The seedlings were then removed, and the length of epicotyls and their ability to consume ethylene measured. Concentration of ethylene in the subsequent ethylene consumption assay system was 10 μl/liter.

Treatment	Hydrocarbon uptake (nl/g h)	Epicotyl length, mm after 24 h	Epicotyls, ethylene uptake after 24 h (nl ethylene/g h)
Initial, 0 h	nd	17 B	nd
Air control	nd	27 A	1 ± 2
Ethylene	6.2	18 B	27 ± 4
Acetylene	4.2	24 A	6 ± 2
Vinyl fluoride	3.9	28 A	5 ± 1
Vinyl bromide	36.0	26 A	5 ± 1
1,3-Butadiene	22.0	23 A	6 ± 2

nd = No data recorded.

The role of AO in animal systems is unknown. However, the AO from all organisms may be similar because diethyldithiocarbamate is an inhibitor of the enzyme from animals (Filser and Bolt 1983) as well as plants and *M. paraffinicum* (Abeles 1984).

Possible functions for plant AO include: a method of synthesizing ethylene oxide, a regulator of internal ethylene levels, an enzyme system capable of forming epoxides from a variety of alkenes, and a consequence of ethylene interacting with its site of action.

The purpose of the experiments described here was to evaluate the possibility that ethylene, acting in a hormonal capacity, was capable of regulating the levels of AO. The basis for this concept stems from the observation by Beyer that both ethylene production and ethylene oxidation increase during floral senescence (Beyer 1977) and tomato ripening (Beyer and Blomstrom 1979). Beyer also demonstrated an increase in AO during abscission (Beyer 1979b). In the case of abscission, ethylene is thought to act as a result of an increase in sensitivity of the tissue to the ethylene already there, as opposed to an increase in the rate of production (Abeles 1973).

Materials and Methods

Fababean (*Vicia faba* L. 'Diana', a gift of Northern Sales Co. Ltd., Winnipeg, Canada) was washed for 20 min with dilute Clorox (commerical NaOCl, 1 part Clorox:4 parts water), rinsed with running tap water for 10 min, and coated with Captan. Seeds were then placed on a 5-cm thick layer of water saturated vermiculite, covered with dry vermiculite treated with Captan (0.5 g/1.5 dm^3), and grown in the dark at 22–28°C for 4 d. In the experiments shown in Table 1, 15 seeds were planted in 11 × 8 cm plastic containers and treated as undisturbed growing seedlings. In the experiments described in Tables 2–4, 4-

Table 2. Dose response curve for the induction of ethylene uptake by fababean epicotyls after a 24-h exposure to ethylene.

μl/liter Ethylene for 24 h	AO activity (nl ethylene/g h)
0	9 ± 2
1	25 ± 2
10	32 ± 2
100	30 ± 2

day-old seedlings were removed from the vermiculite, rinsed with deionized water, and placed horizontally in 4-liter gas-tight plastic containers. In the experiment described in Table 5, 30 seedlings were placed in 9-cm Petri plates containing 6 ml of the solutions indicated, and then placed in the 4-liter containers. After 24 h, the seedlings were removed, 2.5-cm sections excised from below the cotyledons, and then placed in 25-ml flasks for the measurement of subsequent AO activity. Ethylene and other hydrocarbon gases were introduced into the containers by means of a syringe through a rubber vaccine stopper fitted in the lid. The leak rate of these containers was approximately 10% in 24 h. Hydrocarbon levels were measured by gas chromatography.

The AO activity was followed by measuring the rate of ethylene uptake by 1 g (approximately seven 2.5 cm hypocotyl sections) of epicotyl tissue in a 25-ml flask sealed with a silicon rubber stopper and incubated for 2 to 4 h at room temperature. The initial concentration of ethylene in the gas phase was 10 μl/liter. Loss of ethylene from the gas phase by leakage was measured by two methods. The first was to include flasks which did not contain fababean epicotyl tissues. The second was to add saturated hydrocarbon gases such as methane or ethane, which are not metabolized by the tissue, to the gas phase to serve as internal standards. The data shown are corrected for the reduction of ethylene levels by leakage, normally about 10% in 24 h. The removal of ethylene from the gas phase requires physiologically competent tissue. As reported earlier (Abeles 1984), no ethylene, or other alkene is consumed by the tissue when the air is replaced with nitrogen, when the flasks are incubated at 0°C, when the tissue was killed by heating, when the enzyme was inhibited by treatment with CS_2 or COS, or when seedling tissues from other plants such as pea (*Pisum sativum*) or bean (*Phaseolus vulgaris*) were placed in the flasks.

Dodds et al. (1979) reported that ethylene oxide is the product of AO activity when ethylene is the substrate. They also noted that it is metabolized to eth-

Table 3. The effect of ethylene oxide on the induction of ethylene uptake by fababean epicotyls. Fababeans were pretreated with the gases shown for 24 h before assaying the epicotyls for ethylene uptake activity.

Treatment (10 μl/liter)	Ethylene uptake (nl ethylene/g h)
Air control	3 ± 1
Ethylene	22 ± 1
Ethylene oxide	1 ± 1
Ethylene oxide + ethylene	25 ± 2

Table 4. Induction of ethylene uptake activity in fababean epicotyls as a function of the time that they were treated with ethylene.

100 μl/liter ethylene (hours)	Ethylene uptake (nl ethylene/g h)
0	8 ± 1
2	12 ± 1
12	18 ± 1
17	23 ± 2
24	25 ± 2

ylene glycol, glycollic acid, and ethanolamine and not released from the tissue unless it was heated to 70°C. Because of this and the fact that the oxidation products of the other alkenes used in the experiments reported here are not known, the end products of enzyme activity were not measured.

The ± values shown are standard deviations and the letter following data are the mean separation by Duncan's multiple range test, 5% level.

Results

A comparison of the relative rates of alkene uptake by fababean seedlings is shown in Table 1. Of the various hydrocarbons tested, vinyl bromide was the most rapidly removed from the gas phase. Ethylene, on the other hand, had the greatest effect on inhibiting epicotyl growth and increasing subsequent AO activity of epicotyl tissue.

One feature of ethylene action is its characteristic dose response curve. Typically, 0.1 μl/liter is the concentration with a half maximal effect and 10 μl/liter is the saturating dose (Abeles 1973). As shown in Table 2, 1 μl/liter had more than a half maximal effect and 10 μl/liter was a saturating dose.

The increase in epicotyl AO activity may represent enzyme activation (i.e., the substrate ethylene, or the product ethylene oxide, acting as an allosteric effector) as opposed to a hormone stimulating protein synthesis. One approach used to test these alternatives was to see if ethylene oxide, the end product of the reaction, would inhibit or promote the induction of AO activity. As shown in Table 3, ethylene oxide did not inhibit the ability of ethylene to increase AO activity.

A time course experiment on the induction of epicotyl AO is shown in Table 4. An increase in AO was observed 2 h after ethylene treatment.

A preliminary method used to assess the role of RNA and protein synthesis in the increase of AO activity is to measure the effect of actinomycin D and cycloheximide on the ability of ethylene to increase AO activity. Because inhibitor's studies on their own are not conclusive, the data shown in Table 5 are presented as preliminary evidence that protein, but not RNA synthesis, is needed for the increase in AO activity. Additional experiments with labeled intermediates are needed to validate this interpretation.

Silver ions have been used as a competitive inhibitor or antagonist of ethylene action (Beyer 1979a). The effect of 590 μM $AgNO_3$ on ethylene induced

Table 5. The effect of 36 μM cycloheximide, 40 μM actinomycin D, and 590 μM $AgNO_3$ on ethylene-enhanced ethylene uptake activity.

Treatment	Ethylene uptake (nl ethylene/g h)		
	Cycloheximide	Actinomycin D	$AgNO_3$
Air control	3 ± 1	3 ± 1	4 ± 1
Inhibitor	2 ± 2	6 ± 1	5 ± 1
Ethylene	16 ± 6	18 ± 1	30 ± 4
Ethylene + inhibitor	7 ± 3	21 ± 4	21 ± 1

AO activity is shown in Table 5. We observed that this concentration of Ag^+ ions reduced AO induction by 35%.

Discussion

The results presented here indicate that ethylene acts both as a substrate for, and an inducer of, AO activity in *V. faba* hypocotyls. While other hydrocarbons were more readily oxidized by AO, ethylene had the greatest effect on inducing enzyme activity. As yet, the end products of the oxidation of acetylene, vinyl fluoride, vinyl bromide, and 1,3-butadiene are unknown.

The ability of ethylene to increase AO activity in fababean hypocotyls is more consistent with its role as a hormone than as an allosteric effector for a number of reasons. A characteristic feature of some allosteric enzymes is the observation that end products inhibit subsequent enzyme activity. As shown in Table 3, ethylene oxide had little or no effect on AO activity. Another observation in support of the hormonal hypothesis is the observation that silver ions, a competitive inhibitor of ethylene action, caused a partial inhibition of ethylene induced AO activity. Finally, support for a hormonal role for ethylene action arises from a comparison between the dose response curves for ethylene consumption and AO induction. As Beyer (1980) and Abeles (1984) reported earlier, there was a straight line relationship between the amount of ethylene in the system and the rate of oxidation up to a concentration of 1,000 μl/liter. On the other hand, the inductive action of ethylene saturates at two orders of magnitude below this value (Table 2, 10 μl/liter).

The observations reported here suggest that the increase in AO activity during ripening (Beyer and Blomstrom 1979), abscission (Beyer 1979b), and senescence (Beyer 1977) may be due to an effect of ethylene increasing levels of AO in plant tissue. However, the fababean system with its high AO activity may be atypical, and not representative of other plant systems.

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