Short Communication

Ethylene Production from Peptides and Protein Containing Methionine

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It has been reported that the C4-C6 carbons of methionine are converted to ethylene in FMN-light (23) and Cu+-ascorbate (10) model systems. Yang (22) has shown that C4-C6 carbons of 2-keto-4-methylthiobutyrate and the C3-C6 carbons of 3-methylthiopropionaldehyde (i.e., methional) are converted to ethylene in a model peroxidase system. When methionine was fed to apple tissue, the C4-C6 carbons were apparently converted to ethylene (1, 11). These reports demonstrate that the C4-C6 carbons of methionine or its related derivatives are converted to ethylene in model systems and in plant tissue. It was thus of interest to demonstrate whether or not methionine residues in protein and peptides could be converted to ethylene. Using a peroxidase system described by Yang (21), it was found that peptides containing a C-terminal methionine residue, but not an N-terminal or internal residue, produced ethylene at initial rates two to four times greater than the small but significant rate from methionine. Purified egg albumin did not produce any ethylene; however, after limited proteolysis of egg albumin, significant ethylene was formed. Of particular importance was the finding that N-acetyl-p,D,L-methionine produced ethylene at a rate about equal to methional.

The methods used were similar to those described by Yang (21). The incubation mixture contained the following in a total volume of 1 ml: 0.05 μmole of MnSO4, 0.02 μmole of resorcinol, 50 μmoles of KH2PO4 (pH 7.8), 3.0 μg of peroxidase, 2.0 μmoles of NaHSO3, and 0.5, 1.0, or 2.0 μmoles of substrate. The NaHSO3 was added to start the reaction, and the ethylene concentration was determined with a gas chromatograph after 5-min incubation at room temperature. In experiments with crystallized egg albumin, 12 μmoles of H2O2 was substituted for the MnSO4. The peptides and N-formyl-p,D,L-methionine were purchased from Mann, New York, New York. Horseradish peroxidase (Rz = 3) was obtained from Worthington, Freehold, New Jersey. Crystallized egg albumin was obtained from Pierce, Rockford, Illinois. Methional was purchased from Eastman, Rochester, New York.

The results of ethylene production from peptides and other derivatives of methionine in the peroxidase system are shown in Figure 1. Peptides which contained an N-terminal (L-Met-Gly-Gly) or internal residue (Gly-L-Met-Gly and N-F-L-Met-Gly-Gly) of methionine produced little or no detectable ethylene, respectively. This finding was unchanged even for incubation times as long as 120 min. On the other hand, peptides which contained a C-terminal methionine residue (Gly-L-Met and L-Phe-L-Met) produced ethylene at initial rates as high as 5 mIul per min. In addition, N-acetylated derivatives of methionine, N-F-D,L-Met and N-Ac-D,L-Met, were equally and much more active, respectively, in producing ethylene than the peptides with a C-terminal methionine residue. In particular, N-Ac-D,L-Met was very active and produced ethylene at rates comparable to methional. D,L-Methionine produced ethylene at initial rates two to four times lower than the rates
for peptides with C-terminal methionine, but after 15 to 120 min of incubation, methionine produced from two to three times more ethylene than these peptides. Yang (23) found that N-Ac-D,L-Met and L-Met-Gly produced 7.7 and 1.7% ethylene, respectively, of that produced from D,L-methionine in a FMN-light system. The finding that peptides with C-terminal L-methionine are more active than D,L-methionine is probably not due to greater activity of the L-isomer since D- and L-methionine are equally active in the FMN-light (23) and Cu²⁺ ascorbate (10) systems.

In other experiments it was found that crystallized egg albumin, which had been fractionated from low molecular weight substances by column chromatography on Sephadex G-25, did not produce any detectable ethylene after 24 hr of incubation in the peroxidase system at levels from 0.5 to 2.0 mg protein per ml. Since egg albumin contains 16 residues of methionine (3) and a C-terminal proline residue (13), these results are consistent with the findings in Figure 1 which demonstrated that peptides with an internal residue of methionine did not produce ethylene. In a separate experiment, it was found that when 25% of the peptide bonds of egg albumin had been hydrolyzed with pronase, 3.0 ml of ethylene was produced in the peroxidase system in 24 hr from 2.0 mg of egg albumin.

A peroxidase system similar to the one used in these experiments may be operative in plant tissue. Mapson et al. (12) isolated a peroxidase system from cauliflower florets which produced ethylene from methional, and Takeo and Lieberman (18) have isolated a methional peroxidase from apples. Ku et al. (8) have shown a direct correlation between peroxidase and ethylene production during ripening of tomato.

The results presented in this communication suggest two possibilities as to the nature of ethylene biogenesis in plants. First, the data suggest that N-acetylated derivatives of methionine may be precursors to ethylene in plant tissue. N-Ac-D,L-Met was very active in producing ethylene in the peroxidase system. It is known that acylase activity towards N-Ac-D,L-Met is high and ubiquitous in plant seeds (13) and in various plants (2) compared with acylase activity towards other N-acetylated and N-acetylated amino acids. Keglevic et al. (7) have found by radioactive tracer experiments that N-malonyl-methionine, and not 2-keto-4-methylthiobutyrate, is the major acidic metabolite of D-methionine in intact tobacco plants. Thus, N-acetylated derivatives of methionine are involved in the metabolism of methionine in some plants and may be precursors to ethylene.

Secondly, the results presented here suggest that proteolysis in the plant may increase ethylene production by producing peptides with a C-terminal methionine residue. Since peptides with N-terminal and internal methionine residues produced very little or no ethylene in the peroxidase system, whereas peptides with C-terminal methionine produced ethylene at significant rates, it would be expected that proteolysis of proteins containing methionine would result in an increase in ethylene production due to the release of peptides with a C-terminal methionine residue. This possibility was confirmed by the finding that limited proteolysis of crystallized egg albumin resulted in significant ethylene production by the peroxidase system.

Apparently there are no reports which have demonstrated a direct relationship between proteolytic enzymes and ethylene production in plants. But it has been reported that mechanical injury to carnations (16), sweet potato roots (5), and fruits (14) causes an increased production of ethylene in those tissues. It is conceivable that autolysis in the area of the wound might have produced peptides with a C-terminal methionine residue. It should also be mentioned that cutting of some plant tissue increased peroxidase activity around the wound (19) and increased synthesis of particular peroxidase isozymes (6).

It has been also reported that fungal-infected plants (16, 17, 20) produced more ethylene than healthy plants. This ethylene production may have been due to an increase in proteolytic activity during infection since an increase in proteolysis has been observed in some fungal infections such as broad bean rust and peach leaf curl (15), apple rot (9), Southern anthracnose, and Stemphylium leaf spot of alfalfa (4). However, further experiments with radioactive tracers should be done before it can be definitely concluded that proteins or peptides containing methionine or N-acetylated derivatives of methionine are precursors to ethylene in plants.

LITERATURE CITED