The Effect of Indole-3-Acetic Acid on Ethylene Formation in Wheat Seedlings

IVANA MACHÁČKOVÁ, V. NAŠINEC** and Z. ZMRHAL

Research Institute of Crop Production, Department of Plant Nutrition*

Abstract. Isoperoxidase B 1 isolated from winter wheat (Triticum aestivum L., cv. Jubilar) seedlings was shown to catalyze ethylene formation from α-keto, γ-methylmercaptobutyric acid (KMBA). In the presence of Mn²⁺, indole-3-acetic acid (IAA), and p-coumaric acid, the kinetics by isoperoxidase B 1 catalyzed conversion of KMBA into ethylene and other products was similar to that of IAA oxidation. The reaction rate was therefore controlled by IAA through its electron-donating properties.

Exogenous IAA induced ethylene formation in the segments of etiolated wheat coleoptiles. IAA-induced ethylene production was enhanced by L-methionine and mitomycin C. Aminoethoxy-analogue of rhizobitoxine, ferulic acid, sodium benzoate, cycloheximide and actinomycin D exhibited significant inhibitory effects. These data indicate that the overall reaction mechanism in coleoptile segments involves RNA and protein synthesis.

The site of IAA action is not specific; 2,4-dichlorophenoxyacetic, α-naphthylacetic and indole-3-butyric acids, respectively, possessed comparable inductive effect as IAA. Indole-3-propionic acid, indole, L-tryptophan and glucobrassicin had only low inductive efficiency, and moreover indole and L-tryptophan slowed down IAA-induced ethylene formation.

Although the effect of IAA on ethylene formation is well known and has been described in several plant organs, e.g. bean hypocotyls (Sakai and Imaseki 1971), pea epicotyls (Kang et al. 1971), pea roots (Steen and Chadwick 1973) and sorghum mesocotyls (Franklin and Morgan 1978), its mode of action is still not quite clear. In most of the above tissues, the inductive effect of IAA on ethylene biosynthesis has been found. Comparable IAA effect was not found in ageing tissues, e.g. in apples (Lieberman and Kunishi 1975).

L-methionine is the precursor of ethylene in plant cells and two enzymes are believed to catalyze the overall reaction: transaminase and peroxidase (Yang 1975). From our previous results (Macháčková et al. 1975, Zmrhal and Macháčková 1978) we know that IAA serves as an electron donor in some oxidase reactions catalyzed by wheat isoperoxidase B 1. The purpose of the present study was:
1. to determine whether isoperoxidase B 1 is able to catalyze the production of ethylene in vitro;
2. to test the influence of IAA and related compounds on ethylene formation in vivo.

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* Adresse: Dronová 507, 161 06 Praha 6, Czechoslovakia.

** Present address: Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha; Ks dvoru 16, 166 30 Praha 6, Czechoslovakia.
MATERIAL AND METHODS

Isolation and Characterization of Isoperoxidase B 1

The following steps were involved in the isolation procedure: homogenization of the upper parts of wheat seedlings, centrifugation, ammonium sulphate precipitation, column chromatography on Sephadex G 25, Sephadex G 100 and DEAE-cellulose, followed by freeze-drying of isoenzyme B 1 fraction. Pure isoperoxidase B 1 was chemically characterized (amino acids, sugars).

Details of the isolation procedure as well as those of chemical composition of the isoenzyme molecule were published elsewhere (ZMRHAL and MACHÁČKOVÁ 1978).

Preparation of KMBA

α-keto, γ-methylmercaptobutyric acid (KMBA) was used as substrate for isoperoxidase B 1.

The mixture of 0.5 M L-met, 0.05 M KCl, catalase (1 μg ml⁻¹) and L-amino acid oxidase (100 μg ml⁻¹) in 0.1 M Tris—HCl buffer pH 7.8 was incubated for 1 h at 37 °C. Reaction was terminated by the addition of solid m-phosphoric acid to provide pH ~ 3. The product was extracted three times with diethyl ether, ether evaporated in vacuum (MARPSON et al. 1969) and the product identified by the melting point of its 2,4-dinitrophenylhydrazone (149 °C).

Ethylene Formation from KMBA

Ethylene formation was assayed in stoppered 10 ml vials containing air at 10⁵ Pa pressure and 3 ml of the following reaction mixture: 0.2 mM IAA, 1 mM Mn²⁺ (MnCl₂), 0.05 mM HCA, 50 μg KMBA and 50 μg isoperoxidase B 1 in 0.05 M potassium phosphate buffer pH 5.6. The reaction was initiated by the addition of enzyme to the reaction mixture. The incubation period was 30 min at 30 °C on a Dubnoff incubator. The reaction was terminated by adding TCA to the concentration of 6%. Samples of the gas were withdrawn using 1 ml disposable plastic syringes (Becton Dickinson Co. Ltd.). Ethylene was determined by gas chromatography of the samples on Perkin-Elmer F 30 chromatograph using Porapak N column and nitrogen as the carrier.

Ethylene Formation in Wheat Coleoptile Segments

Surface sterilized (70% ethanol, 5 min) and imbibed (6 h in redistilled water) seeds of winter wheat (cv. Jubilar) were placed on the wet filter paper discs in Petri dishes and allowed to germinate 4 days in the darkness at 25 °C. Coleoptiles 30—35 mm in length were selected and segments 10 mm in length were cut of them starting 3 mm below the apex. The first leaves

Abbreviations used: IAA = indole-3-acetic acid; KMBA = α-keto, γ-methylmercaptobutyric acid; HCA = 4-hydroxyeinnamic(p-coumaric) acid; FA = 3-methoxy, 4-hydroxyeinnamic (forulic) acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; NAA = α-naphthylacetic acid; IBA = indole-3-butyrice acid.