LIGHT-DEPENDENT ETHYLENE PRODUCTION BY ISOLATED CHLOROPLASTS

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1. Introduction

Ethylene is a plant hormone with effects on plant growth and development [1]. Its biosynthesis has been studied by many investigators, and methionine, 2-keto-4-mercaptomethylbutyrate (KMB) or 3-mercaptomethylpropanol (methional) have been proposed as the most likely precursors [2]. The absolute requirement for oxygen in the synthesis of ethylene has been pointed out (cf. [1]).

Yang et al. [3–4] and Mapson and Wardale [6] described model reactions, which proposed the involvement of oxygen radicals in ethylene production. Beauchamp and Fridovich [7] could show, that the reactive species in the formation of ethylene from methional in the presence of xanthin and xanthinoxidase was probably the OH-radical.

Evidence was provided recently, that illuminated chloroplasts are able to reduce oxygen yielding both H_2O_2 [8–10] and the superoxide free radical ion $O_2^{\cdot -}$ [11–14]. This communication describes the formation ethylene from KMB or methional by illuminated chloroplast lamellae. The results provide evidence, that the OH-radical is active in generating ethylene from the above substrates.

2. Materials and methods

Ferredoxin was isolated from spinach leaves [16] and chloroplast lamellae were prepared from spinach [17] or sugar beet leaves [18]. Methional was synthesized from acrolein and methylmercaptane [19], from methionine by oxidative desamination [20,21]. The purity of the substrates was shown by paper chromatography (Whatman 3 MM) and cellulose thin layer chromatography, developed in 1-butanol–acetic acid–H_2O (4:1:4, v/v) and 1-propanol–formic acid–H_2O (40:2:10, v/v). Catalase was purchased from Boehringer, Mannheim, and was purified of thymol [7]. Superoxide dismutase was isolated from dried green peas [12,22] and the specific activities were determined as described [23]. The catalase used in the experiments had no detectable superoxide dismutase activity and the prepared superoxide dismutase had no detectable catalase activity at the applied concentrations. The reactions were carried out with 10 ml Fernbach flasks (with a side arm) in a Photo-Warburg thermostat at 20°C with illumination (20 000 lux) from the bottom. Chloroplast lamellae with 0.1 mg chlorophyll were used in the tests. The vessels were closed with rubber stoppers and the reactions were terminated with 0.1 ml of 0.5 N H_2SO_4, Ethylene was determined in a Varian Aerograph model 1400, with a flame ionization detector, equipped with a Poropak R column (1/8″, 80/100). The temperature of the column oven was 50°C.

3. Results

By incubation of either (KMB) or methional with chloroplast lamellae in the light, a generation of ethylene from both substrates can be observed. This ethylene production is enhanced by ferredoxin (table 1). Incubation of either methionine or 2-ketoglutarate failed to produce ethylene (fig. 1).

The requirement of this system for an intact electron transport was confirmed by heat treatment of the chloroplast lamellae and by blocking the electron transport with *o*-phenanthroline.

Heat treated chloroplasts, which have lost the ac-

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Table 1
Photosynthetic ethylene production from KMB and methional

	$CH_2 = CH_2$ (pmoles) formed	
Complete	0.0	
	KMB[1]	Methional[2]
Complete	531 (700)[3]	530
Without ferredoxin	350 (400)	224
Without chloroplast lamellae	103 (142)	0.0
Complete (dark)	0.0 (0.0)	0.0

[1] Standard reaction mixture + 3.3 μmoles KMB.
[2] Standard reaction mixture + 3.3 μmoles methional.
[3] With chloroplasts lamellae isolated from sugar beet leaves.
The reaction mixture contained: 40 μmoles Tris–HCl pH 8.0; 5 μmoles $MgCl_2$, 30 nmoles ferredoxin; chloroplast lamellae with 0.1 mg chlorophyll; H_2O ad 1 ml; the reaction was carried out for 40 min in the light.

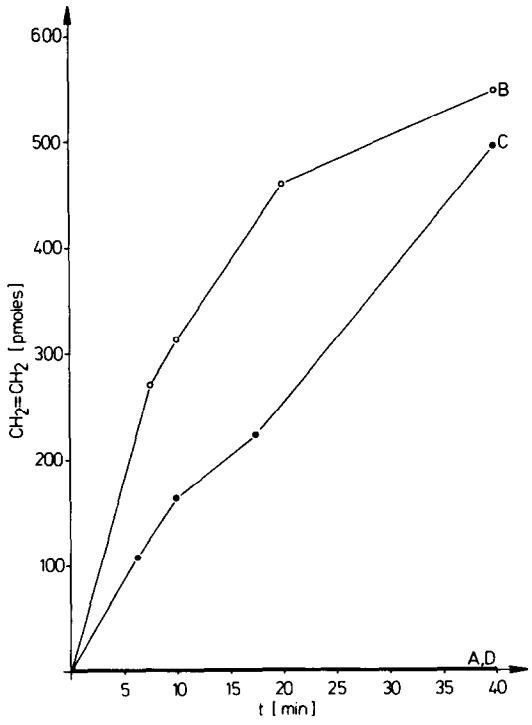

Fig. 1. Ethylene production from KMB and methional and lack of ethylene production from 2-ketoglutarate and methionine. For experimental conditions see table 1; A = + 3.3 μmoles methionine, B = 3.3 μmoles KMB, C = 3.3 μmoles methional, D = 3.3 μmoles 2-ketoglutarate.

tivity of the water splitting system [24] have also lost their ability to generate ethylene from both KMB and methional (table 2). The decrease of ethylene production in the presence of *o*-phenanthroline, an inhibitor of photosystem II is also shown in table 2.

Although H_2O_2 itself is not able to generate ethylene from either methional (cf. [7]) or KMB in the dark, ethylene production by isolated chloroplast lamellae in the light is inhibited both by addition of catalase or superoxide dismutase (table 3).

4. Discussion

The intention of this communication is to: a) provide evidence that isolated chloroplast lamellae under certain conditions are able to produce OH-radicals in addition to H_2O_2 and $O_2^{\cdot -}$; b) to introduce a new and simple system for testing potential ethylene precursors, and c) to provide evidence for the possibility that chloroplasts might be involved in the synthesis of ethylene.

As shown under Results, the formation of ethylene from KMB or methional by isolated chloroplast lamellae is dependent on light, an intact electron transport system and ferredoxin as an auto-oxidizable electron acceptor.

Methionine and 2-ketoglutarate on the other hand, which have been shown to stimulate ethylene production in plant tissues (cf. [2]) or *Penicillium digitatum*

Table 2
Effect of heat treatment of chloroplast lamellae and *o*-phenanthroline on photosynthetic ethylene production

	$CH_2 = CH_2$ (pmoles) formed KMB[1]
With untreated lamellae	471
With untreated lamellae (dark)	0.0
5′ 55°C	90
3′ 80°C	84
With untreated lamellae + 10^{-3} M *o*-phenanthroline	50

[1] See table 1.
Assay: see table 1.
5′ 55°C = isolated chloroplasts heated for 5 min at 55°C [24].
3′ 80°C = isolated chloroplasts heated for 3 min at 80°C.

Table 3
The influence of catalase (A), superoxide dismutase (B) an H_2O_2 (C) on the enthylene production from KMB

	$CH_2 = CH_2$ (pmoles) formed
A) Complete	470
Complete + catalase (50 U)	85
B) Complete	526
Complete + superoxide dismutase (50 U)	266
C) Without chloroplast lamellae and ferredoxin + 3 μmoles H_2O_2 (dark)	0.0

For experimental conditions see table 1 + 3.3 μmoles KMB as substrate.

[25] respectively, show no activity in isolated chloroplast lamellae.

Several authors could show, that the oxidizing agent in ethylene formation from the above substrates is most likely an oxygen radical species [3–6]. The inhibition of ethylene formation by catalase as well as by superoxide dismutase (table 3) is taken as evidence, that both H_2O_2 and $O_2^{\cdot -}$ are necessary for the oxidation of the KMB. Since H_2O_2 alone is not active in the production of ethylene, one can assume, that H_2O_2 together with $O_2^{\cdot -}$ result in the formation of the oxidizing species, i.e. the OH-radical [7,15]. This result is in good agreement with the findings of Beauchamp and Fridovich [7], who suggested OH-radicals as the active species in ethylene formation form methional.

According to the above findings we have to assume that isolated chloroplast lamellae under certain conditions are able to produce OH-radicals in addition to H_2O_2 and $O_2^{\cdot -}$, as previously shown [10,12]. These results might also explain earlier findings of Trebst and Eck [26], who observed a light dependent *p*-hydroxylation of salicylaldehyde by isolated chloroplasts, a reaction which has been shown to involve O_2 [27], and/or OH-radicals [28].

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