PYRIDOXALPHOSPHATE-DEPENDENT ETHYLENE PRODUCTION FROM METHIONINE BY ISOLATED CHLOROPLASTS

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1. Introduction

The biosynthetic pathway of the plant hormone ethylene has not yet been established. Methionine is the most likely precursor for ethylene production in tissue slices from higher plants [1,2]. 2-Keto-4-*S*-methyl butyric acid (KMB) and 3-*S*-methyl propanal (methional, MMP), in contrast to methionine, are precursors in ethylene forming model reactions, but are less effective as precursors in tissue slices [3].

In vivo ethylene production is dependent on oxygen [1]. For model reactions with KMB or MMP as substrates, the active oxygen species has been identified as the OH-radical [4–6]. Like other systems [7,8], the unsupplemented chloroplast model system produces negligible amounts of ethylene from methionine as compared to KMB of MMP [5]. In this paper we show that chloroplasts produce ethylene from methionine in the presence of pyridoxal phosphate, ferredoxin and an electron donor.

2. Materials and methods

Ferredoxin [9], NADP-ferredoxin-reductase [10] and chloroplast lamellae [11] were isolated from spinach. Methional was synthesized from acrolein and methylmercaptane [5]. Superoxide dismutase was isolated from dried green peas [12].

The reactions were carried out with 10 ml Fernbach flasks in a photo-Warburg thermostate at 20°C with illumination from the bottom. The vessels were closed with rubber stoppers and the reactions were terminated with 0.2 ml 0.5 N H_2SO_4. Ethylene was determined as earlier described [5].

Catalase and pyridoxalphosphate were from

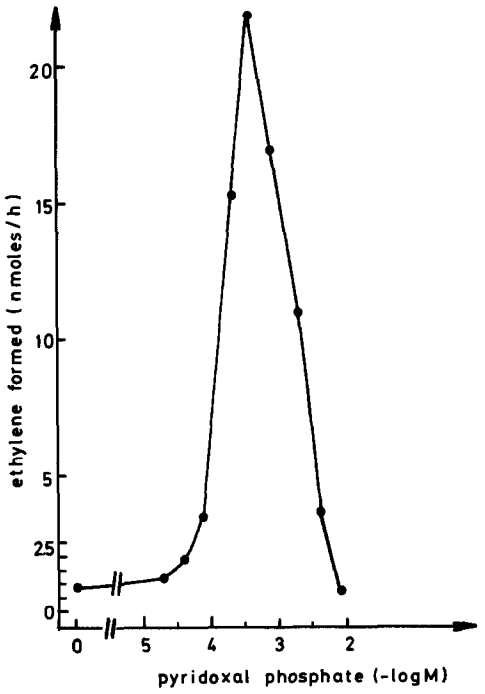

Fig.1. Dependence on pyridoxal phosphate of ethylene formation from methionine by illuminated chloroplast lamellae. The reaction system contained in 1 ml (mM): Phosphate buffer, pH 6.3 (25); $MgCl_2$ (2.5); NH_4Cl (2.5); azide (0.3); L-methionine (6.5); ferredoxin (0.02); chloroplast lamellae with 50 μg chlorophyll. The reaction was conducted for 45 min at 20°C; Light source: 5×10^4 erg/cm^{-2}·s^{-1} (red light, 610 nm).

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Boehringer, Mannheim. L-Canaline was obtained from Sigma, St. Louis.

N,N-dimethyltryptophan methylester was a generous gift from Dr J. A. Lamberton, CSIRO, Div. of appl. org. Chemistry, Melbourne.

3. Results and discussion

The rate of ethylene formation from methionine in the presence of illuminated chloroplasts and ferredoxin is greatly enhanced by the addition of pyridoxal phosphate (fig.1). The pH-optimum is between 6.9 and 7.4, and the rate of the reaction is linearly increased by increasing D- or L-methionine concentrations up to 10 mM.

Water as electron donor for ferredoxin-dependent oxygen reduction by isolated chloroplast in the light [13] (table 1, reaction A) can be substituted for by NADPH in the dark (table 1, reaction B). In the dark reaction, chloroplasts can be substituted for by NADP-fd-reductase (table 1, reaction C).

Ethylene formation from methionine in the presence of pyridoxal phosphate is inhibited by two classes of inhibitors:

(a) Superoxide dismutase and catalase as inhibitors of $O_2^{\cdot -}$ and H_2O_2-dependent reactions, and

(b) L-Canaline [14] and dimethyltryptophan

Table 1
Dependence on pyridoxal phosphate and light (or NADPH) of ethylene formation from methionine by chloroplast lamellae

Reaction	System	Ethylene formed (nmoles/mg Chl./h)
A	complete	930
	minus pyridoxal phosphate	43
	minus light	18
B	complete	132
	minus pyridoxal phosphate	2
	minus electron donor	1
		(nmoles/h)
C	complete	17
	minus pyridoxal phosphate	0
	minus electron donor	0

The reaction systems contained in 1 ml (mM):

Reaction A: (light reaction)
Phosphate buffer pH 7.0 (25); $MgCl_2$ (2.5); NH_4Cl (2.5) azide (0.3); L-methionine (13); pyridoxalphosphate (0.32); ferredoxin (0.02); chloroplast lamellae with 50 μg chlorophyll. Light source: 5×10^4 erg (red light, 610 nm)·cm^{-2}·sec^{-1}. The reaction was conducted for 30 min at 20°C.

Reaction B: (dark reaction)
Phosphate buffer pH 7.6 (25); $MgCl_2$ (2.5); azide (0.3); chloroplast lamellae with 25 μg chlorophyll; Electron donor: 20 μmol glucose 6-phosphate; 25 μg glucose 6-phosphate dehydrogenase (Boehringer) and 1 μmol NADP; Ferredoxin (0.04); L-methionine (20); pyridoxal phosphate (0.16). The reaction was conducted for 45 min at 20°C.

Reaction C: (dark reaction)
Conditions identical to reaction B, except that the chloroplast lamellae were substituted for by 1.8 nmol isolated NADP-ferredoxin reductase, and 10 mM methionine and 0.1 mM pyridoxal phosphate were present.

Table 2
Inhibitors of ethylene formation from methionine in the presence of pyridoxal phosphate by chloroplast lamellae

Reaction	Inhibitor[a]	I_{50}[b]	
A	Catalase	20	U[c]
	Superoxide dismutase	200	U
	L-Canaline	250	μM
	N,N-dimethyltryptophan-methylester	600	μM
B	Catalase	0.8	U
	Superoxide dismutase	25	U
	L-Canaline	60	μM
	N,N-dimethyltryptophan-methylester	180	μM

The reaction conditions were the same as described for reactions A and B in table 1.
Reaction A: light reaction.
Reaction B: dark reaction with NADPH as electron donor.

[a] The inhibitors show no influence on photosynthetic electron transport at the applied concentrations.
[b] I_{50} = concentration of inhibitor, yielding a 50% inhibition of ethylene formation as compared to the complete systems described in table 1, reactions A and B.
[c] U = Enzyme units: catalase, according to H. U. Bergmeyer, Meth. der enzymat. Analyse (1970) Verlag Chemie, Weinheim: superoxide dismutase, according to Mc Cord and Fridovich, (1969) J. Biol. Chem. 244, 6049.

methylester [15] as inhibitors of in vivo ethylene production by plant tissue (table 2).

The inhibition by both superoxide dismutase and catalase indicates that ethylene formation from methionine in the presence of pyridoxalphosphate involves the OH-radical (OH·), formed by dismutation of $O_2^{\cdot -}$ and the Haber-Weiss reaction according to:

$$O_2^{\cdot -} + H_2O_2 \longrightarrow OH^{\cdot} + OH^- + O_2 \quad [16].$$

Ferredoxin as an autoxidizable electron acceptor has been shown to stimulate ethylene formation from methional in the presence of illuminated chloroplasts [6]. Substitution of ferredoxin by other autoxidizable electron acceptors of photosystem I like methylviologen (MV) result in the loss of ethylene production (fig.2), although the production of $O_2^{\cdot -}$ (measured as NO_2^- – formation from NH_2OH, cf. [17]) in the presence of either one of these acceptors is almost identical (fig.3).

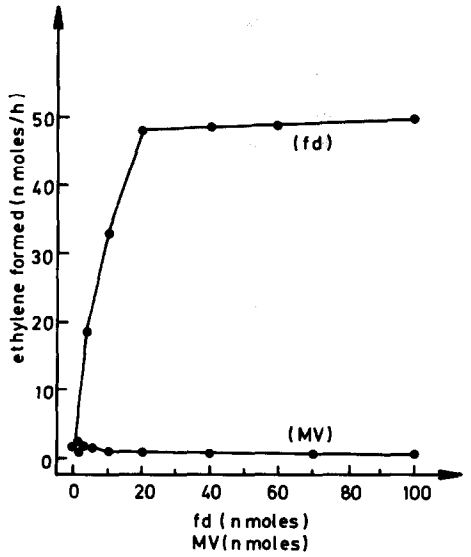

Fig.2. Dependence on ferredoxin of ethylene formation from methionine in the presence of pyridoxal phosphate by illuminated chloroplast lamellae. The reaction conditions were as described for table 1, reaction A.

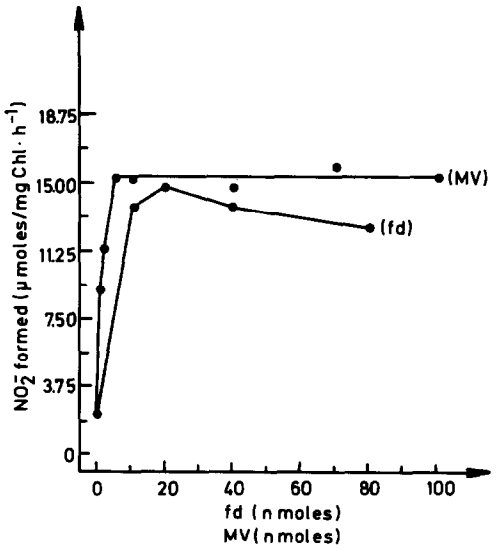

Fig.3. Stimulation by methylviologen (MV) or ferredoxin (fd) of hydroxylamine oxidation by illuminated chloroplast lamellae. The reaction system was essentially as described in table 1, reaction A, except that L-methionine, pyridoxal phosphate and ferredoxin were omitted and 0.4 μmoles NH_2OH were present. The reaction was conducted for 15 min at 20°C.

We therefore conclude that ferredoxin catalyzes not only the production of $O_2^{\cdot -}$, but is also active in further steps involved in ethylene formation from methionine.

The results show that an oxygen activating system as represented by chloroplasts with an appropriate electron donor and ferredoxin generate ethylene from methionine in the presence of pyridoxal phosphate. The system described is in many respects in good agreement with in vivo ethylene producing systems [13,14,15].

Acknowledgements

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