ENZYMIC CONVERSION OF LINOLENIC ACID TO ETHYLENE BY EXTRACTS OF APPLE FRUITS

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1. Introduction

The origin of ethylene, the ripening hormone of many fruits, has yet to be defined. A recent review [1] described possible sources of ethylene and from recent studies with both plant preparations and model systems, methionine (*via* methional) [2–4], acetaldehyde [5], propionaldehyde [6] and linolenic acid [7] have emerged as possible precursors of ethylene.

The present paper describes the enzymic conversion of linolenic acid to ethylene by cell-free extracts of apple fruits in the presence of oxygen and ascorbic acid. Metal chelators, thiols and ferrous ions inhibit ethylene production. Lipoxidase and a copper enzyme appear to be essential components of the enzyme system.

2. Methods

Apple fruits (Sturmer variety imported from Australia) were used in the post-climacteric condition when the fruits were producing ethylene *in vivo*. Peel tissue was washed with water and homogenized (Ultra-turrax homogenizer, Janke and Kunel KG) with three volumes of an ice-cold solution containing mannitol (0.4 M), potassium maleate (0.1 M) and soluble polyvinyl-pyrrolidone (MW = 24 000) (1%) at pH 7.2. The homogenate was filtered through cheesecloth and the pH of the extract adjusted carefully from approximately 6.2 to 5.6 with HCl.

Fatty acids were converted to ammonium salts in solution and excess ammonia removed by warming under a stream of nitrogen. Ethylene and ethane production was assayed by analysis of the gas phase above incubation mixtures in 25 ml flasks. Extract (4 ml) and 10 mM ammonium linolenate (2 ml) were pre-incubated for 15 min at pH 5.6 by gentle shaking at 25° in open flasks. At the end of the pre-incubation period, 120 mM potassium ascorbate (0.5 ml) was added together with other additions, if any, and then the flasks were closed with rubber stoppers each of which was fitted with a glass tube connected to a piece of silicone-rubber tubing and sealed with a clip. After incubation by shaking at 25° for one hour, a 5 ml sample of the gas phase was taken for analysis of ethylene and ethane content by gas-chromatography using modifications [8] of a method previously described [9].

Table 1
Enzymic conversion of linolenic acid to ethylene and ethane by a cell-free extract from apple.

	nl/hr/g tissue	
	Ethylene	Ethane
Complete system *	90	160
Complete system (boiled extract)	3	10
omit extract	0	8
omit linolenic acid	1	2.5
omit ascorbate	2.5	7.7

* See sect. 2. Methods.

3. Results

Both ethylene and ethane were produced in the

Table 2
Substrate and co-factor specificities

Incubation system *		Relative rates of gas production	
		Ethylene	Ethane
Extract + ascorbate + linolenic acid	1 mM	100	100
Extract + ascorbate + linoleic acid	1 mM	2	0
Extract + ascorbate + oleic acid	1 mM	0	10
Extract + ascorbate + methionine	1 mM	0	0
Extract + ascorbate + methional	1 mM	10	13
Extract + ascorbate + propionaldehyde	1 mM	15	4
Extract + ascorbate + hydrogen peroxide	1 mM	2	2
Extract + ascorbate + glucose + glucose oxidase	1 mM	2	2
Extract + linolenic acid + ascorbate	10 mM	100	100
Extract + linolenic acid + cysteine	10 mM	4	62
Extract + linolenic acid + metabisulphite	10 mM	8	120
Extract + linolenic acid + dithionite	10 mM	8	65
Extract + linolenic acid + dehydroascorbate	10 mM	0	7
Extract + linolenic acid + α-tocopherol	10 mM	0	0

* See sect. 2. Methods.

complete system with extract, linolenic acid and ascorbate as indicated in table 1. Boiling the extract or omission of any of the components of the complete system almost eliminated the production of ethylene and ethane. Under the incubation conditions used, the production of ethylene was proportional to the amount of extract present; the pH optimum of the reaction was 5.6.

Table 2 shows that linolenic acid could not be replaced by linoleic or oleic acids in the ethylene-producing system and that methional and propionaldehyde gave less ethylene than did linolenic acid in this system. Furthermore, hydrogen peroxide or peroxide-generating systems would not replace linolenic acid (cf. [7]). Preliminary experiments in which uniformly labelled ^{14}C-linolenic acid was used as substrate confirmed that this fatty acid was the source of the carbon atoms in both ethylene and ethane (unpublished results).

Dehydroascorbate would not substitute for ascorbate and no other reducing conditions investigated would replace ascorbate in ethylene production although ethane was produced under more general reducing conditions (table 2). Oxidized and reduced pyridine and flavine nucleotides also had no effect in these systems.

Ethylene production was dependent upon oxygen tension; pure oxygen increased ethylene and reduced ethane production whereas anaerobic conditions completely inhibited ethylene formation (table 3). Thiols, ferrous ions and metal chelators also inhibited ethylene production. Addition of EDTA to the extract, followed by dialysis against buffer solution, completely inhibited the ethylene system which could be restored by the addition of copper ions (Cu^{+} in the presence of ascorbate). Addition of ferrous ions at this stage stimulated the ethane system. Thiol-binding reagents partially reduced ethane formation but had no effect on the ethylene system. Nordihydroguaiaretic acid, an inhibitor of lipoxidase [10] decreases the formation of ethylene and ethane.

4. Discussion

The enzymic system from apple fruits which converts linolenic acid to ethylene appears to employ a similar mechanism to the model system described by Lieberman and Mapson [7]. The results are compatible with a sequence of reactions in which linolenic acid is first converted by the lipoxidase present in the extracts [11] to its hydroperoxide(s) the dismu-

Table 3
Factors affecting the enzymic conversion of linolenic acid to ethylene and ethane

Incubation conditions *		Relative rates of gas production	
		Ethylene	Ethane
Control (aerobic)		100	100
anaerobic		0	60
100% oxygen		185	37
Control + cysteine	(1 mM)	0	17
+ mercaptoethanol	(1 mM)	3	15
+ $FeSO_4$	(1 mM)	4	100
+ $CuSO_4$	(1 mM)	100	6
+ EDTA	(0.1 mM)	2	100
+ diethyldithiocarbamate	(1 mM)	3	9
+ *o*-phenanthroline	(0.1 mM)	2	40
+ KCN	(1 mM)	0	7
+ iodoacetamide	(10 mM)	100	40
+ *p*-chloromercuribenzoate	(0.1 mM)	100	80
+ nordihydroguaiaretic acid	(0.1 mM)	60	60
Extract dialysed against buffer		77	51
Extract dialysed against EDTA	(5 mM)	2	24
Extract dialysed against EDTA then added $CuSO_4$	(5 mM) (1 mM)	170	17
Extract dialysed against EDTA then added $FeSO_4$	(5 mM) (0.1 mM)	8	170

* Control incubations contained peel extract, linolenic acid and ascorbate as described in sect. 2. Methods.

tation of which takes place by an unknown series of steps in the presence of oxygen and a copper enzyme to give ethylene as a final product. The present work supports the observations of Meigh [12] that ethane is produced by the anaerobic breakdown of peroxidized linolenic acid in the presence of ferrous ions and reduced thiols. The production of ethane may represent a disruption of the natural ethylene-forming system since, although the *in vivo* ethane production by fruits is low in relation to the ethylene evolved, subcellular preparations of fruit have been shown to evolve relatively more ethane [12].

Support for the physiological significance of ethylene production from linolenic acid in apples has been presented earlier [9]; further support for this view is obtained from evidence (to be presented elsewhere) that the rate of conversion of linolenic acid to ethylene by extracts is related to the ethylene production *in vivo* of the tissue from which the extracts were prepared.

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