Hypothesis

The coupling of ethylene biosynthesis to a transmembrane, electrogenic proton flux

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It is proposed that the reactions which lead to the generation of ethylene from 1-aminocyclopropane–1-carboxylic acid are arranged asymmetrically in the plasma membrane of plant cells so that ethylene biosynthesis is coupled to an inwardly directed, electrogenic flow of protons. According to this model a membrane potential (outside positive) is required for ethylene biosynthesis. This proposed requirement is indicated by previous observations of a marked sensitivity of ethylene biosynthesis to the protonophore 2,4-dinitrophenol, and by its unusually strict dependence on membrane integrity.

Ethylene biosynthesis *ACC* *Membrane potential* *Proton flux*

1. INTRODUCTION

I propose here that the ethylene-forming enzyme is asymmetrically organised in the plasma membrane of plant cells so that the generation of ethylene from 1-aminocyclopropane-1-carboxylic acid is coupled to an electrogenic flow of protons into the plant cell.

In plants the most likely route of C_2H_4 synthesis from ACC is via the sequence of reactions shown in fig.1. This sequence has been inferred from studies of the non-enzymatic generation of C_2H_4 from ACC [1]. In vivo these reactions are catalysed by an enzyme (or enzyme-complex) called the ethylene-forming enzyme [2]. The catalytic activity of this enzyme has not yet been demonstrated in vitro, and almost nothing is known of its properties, except that it is almost certainly located in the plasma membrane [3,4].

2. HYPOTHESIS

In the proposed model (fig.2) ACC, synthesized by the ACC synthase in the cytosol, is oxidised at the inner face of the plasma membrane, so that

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; DNP, 2,4-dinitrophenol

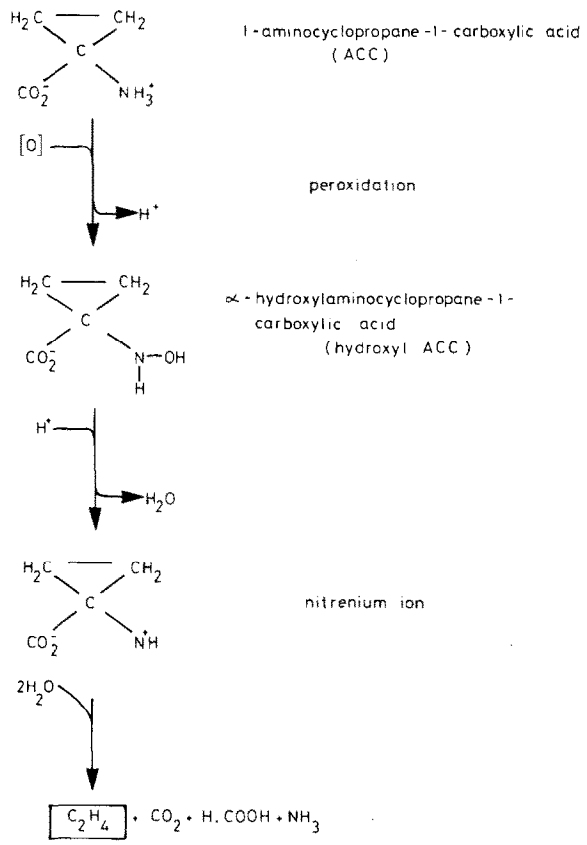

Fig.1. The mechanism of C_2H_4 synthesis from ACC [1].

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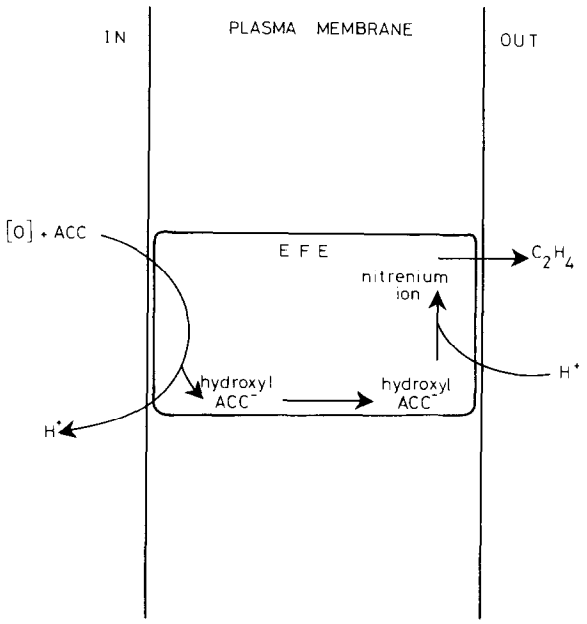

Fig.2. The proposed topological organisation of the ethylene-forming enzyme (EFE) in the plasma membrane such that the generation of C_2H_4 from ACC is coupled to a transmembrane flow of protons from the outside to the inside of the plant cell.

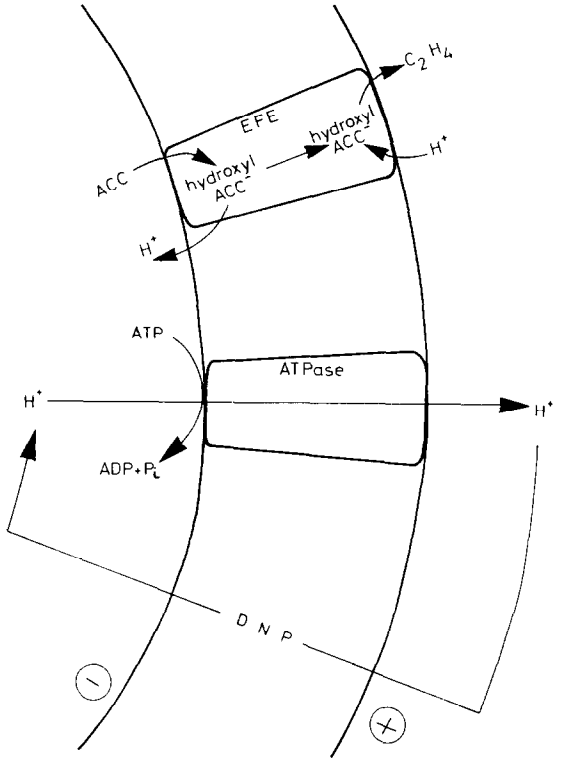

Fig.3. Normally the electrogenic, proton translocating ATPase helps to maintain a membrane potential (positive outside) which draws the negatively charged intermediate, hydroxyl ACC across the membrane. DNP discharges the potential by allowing protons to return to the cytosolic phase, and thus DNP inhibits the activity of the ethylene forming enzyme (EFE).

protons are released into the cytosol. The oxidised intermediate, α-hydroxylaminocyclopropane-1-carboxylic acid, then crosses the membrane and is converted to the nitrenium ion near the outer face so that protons are consumed from the free space outside the plasma membrane. Finally, the nitrenium ion is broken down to release C_2H_4.

The negatively charged intermediate, α-hydroxylaminocyclopropane-1-carboxylic acid, is drawn across the membrane by the potential difference (positive outside) known to be maintained across the plasma membrane [5]. The dependence of the activity of the ethylene forming enzyme on the potential difference explains why ethylene biosynthesis has a strict requirement for membrane integrity [4,6] and why the activity of this enzyme (unlike the activities of other enzymes involved in C_2H_4 biosynthesis) fails to survive cell breakage [3]. This dependence on a potential difference also explains why the synthesis of C_2H_4 from ACC in vivo should be sensitive to inhibition by low concentrations of the protonophore DNP [7]. In hypocotyls 50 μM DNP inhibits by about 50% the conversion of ACC to C_2H_4 but has no significant effect on the synthesis of the C_2H_4 precursor *S*-adenosylmethionine, which requires ATP [7]. DNP at 100 μM is known [8] to depolarise the plasma membrane without causing irreversible damage. The depolarising effect of DNP on a plasma membrane bearing the postulated proton-translocating EFE is shown in fig.3.

The model presented here allows for a simple interaction between C_2H_4 biosynthesis and the action of both phytochrome and auxin. Thus red light, which suppresses C_2H_4 production [9], causes a rapid depolarisation of the cell membrane [10]. While auxin, which stimulates C_2H_4 production [3], has as its most rapid effect the hyperpolarisation of the cell membrane [11]. Additional support is provided by the recent suggestion that preserva-

tion of the plasma membrane is required not only for ethylene biosynthesis but also for the interaction of auxin with the ethylene-forming enzyme [4].

The validity of the model presented here can be tested most unambiguously by determining whether, as predicted, the production of C_2H_4 from ACC responds directly to an experimental modulation of the potential difference across the plasma membrane.

REFERENCES

[1] Yang, S.F. and Adam, D.O. (1980) in: The Biochemistry of Plants – A Comprehensive Treatise, vol.4 Lipids: Structure and Function (Stumpf, P.K. ed) pp.163–175, Academic Press, New York.
[2] Hoffman, N.E., Yang, S.F., Ichihara, A. and Sakamura, S. (1982) Plant Physiol. 70, 195–199.
[3] Lieberman, M. (1979) Annu. Rev. Plant Physiol. 30, 533–591.
[4] Lieberman, M. and Yang, S.F. (1982) Plant Physiol. 69, 1150–1155.
[5] Etherton, B. and Higinbotham, N. (1960) Science 131, 409–410.
[6] Field, R.J. (1981) Ann. Bot. (London) 48, 33–40.
[7] Yu, Y.B., Adams, D.O. and Yang, S.F. (1980) Plant Physiol. 66, 286–290.
[8] Higinbotham, N., Graves, J.S. and Davis, R.F. (1970) J. Membr. Biol. 3, 210–222.
[9] Goeschl, J.D., Pratt, H.K. and Bonner, B.A. (1967) Plant Physiol. 42, 1077–1080.
[10] Racusen, R.H. (1976) Planta 132, 25–29.
[11] Hanson, J.B. and Trewavas, A.J. (1982) New Phytol. 90, 1–18.