On the role of membrane integrity in the conversion of 1-aminocyclopropane 1-carboxylic acid to ethylene in carnation petals

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Received 29 April 1984; revised version received 22 May 1984

The reaction converting 1-aminocyclopropane 1-carboxylic acid (ACC) to ethylene in plants is independent of cell or membrane integrity. Both intact and detergent-solubilised membrane fractions can readily convert ACC to ethylene. The reaction catalysed by the solubilised enzyme is inhibited by 100 μM 2,4-dinitrophenol. ATP, even at very low concentrations, totally inhibits the reaction. The results would appear to invalidate a previously hypothesised model for this reaction.

Ethylene *1-Aminocyclopropane 1-carboxylic acid* *Ethylene-forming enzyme*

1. INTRODUCTION

The mechanism by which the last step of ethylene biosynthesis is carried out in plants is not yet known. The immediate precursor has been identified as 1-aminocyclopropane 1-carboxylic acid (ACC) [1,2], and this is converted to ethylene by a membrane-bound enzyme [3], which has been designated ethylene-forming enzyme (EFE) [4]. A model suggesting a mechanism for this reaction was recently proposed in [5]. According to this model, EFE 'is asymmetrically organised in the plasma membrane of plant cells so that the generation of ethylene from ACC is coupled to an electrogenic flow of protons into the plant cell'. The model is based on in vivo observations that the reaction is dependent on cell and membrane integrity [6], and that 2,4-dinitrophenol (DNP), acting as a protonophore, inhibits the reaction [7].

The purpose of this work was to test the validity of this model.

2. MATERIALS AND METHODS

Flowers of carnation (*Dianthus caryophyllus* L. cv. White Sim) were grown in a greenhouse, cut at a fully open stage and immediately processed. Membranes were isolated and solubilised as in [8]. The isolated membrane fraction consisted of closed vesicles, as shown in [9].

Protein was determined by the modified Lowry method [10].

The conversion of ACC to ethylene (EFE activity) was determined essentially as in [8]. The protein source in the reaction mixture was either the intact membrane or the solubilised fraction. In some experiments DNP or ATP were also included in the reaction mixture.

ATPase activity in the membrane fraction was determined as in [11].

3. RESULTS AND DISCUSSION

At least 3 criteria should be met experimentally to validate the model proposed in [5]. (i) The activity converting ACC to ethylene should not survive cell breakage and loss of membrane integrity. (ii) Moreover, if the activity can be preserved in vitro, it should not be affected by DNP. (iii) ATP, via ATPase activity, should increase the apparent activity of EFE.

The results presented in table 1 show that EFE

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Table 1

EFE activity in intact and detergent-solubilised membrane fractions

Enzyme	EFE activity (nl/mg protein per h)
Membrane-bound	188 ± 12
Solubilised	194 ± 8
Solubilised + 100 μM DNP	155 ± 3

To the standard reaction mixture as in [8], either a membrane-bound enzyme or a solubilised enzyme with or without 100 μM DNP was added, and EFE activity was assayed. Values ± SE are presented

Table 2

Effect of ATP on ATPase and EFE activities

[ATP] (mM)	ATPase activity (μmol P_i/mg protein per h)	EFE activity (nl/mg protein per h)
0	*	324 ± 15
1	6.7 ± 0.3	*
2	12.1 ± 0.3	*
3	16.5 ± 0.1	*

Various concentrations of ATP were added to the reaction mixtures for determination of ATPase and EFE activities. ATPase activity was assayed as in [11] and EFE activity as in [8]. Values ± SE are presented. * Not detectable

activity survives cell breakage. A membrane fraction prepared from petals capable of producing ethylene from ACC [3] readily converts ACC to ethylene at a high rate, as demonstrated elsewhere [3,12]. Moreover, solubilisation of the membranes with a non-ionic detergent preserves the activity at the same level (table 1). It has been shown that the characteristics of the solubilised enzyme are similar to those of the membrane-bound one [8]. The solubilisation treatment also caused destruction of membrane integrity, as shown by the massive release of [^{14}C]sucrose (~75%), encapsulated in vesicles during membranes preparation [9].

Inclusion of 100 μM DNP in the reaction mixture with the solubilised enzyme led to a reduction in the activity (table 1) to the extent of ~20%. Lower concentrations of DNP employed with either the intact membrane vesicles or the solubilised fraction had no effect on the activity. Since the solubilised enzyme is associated with detergent micelles rather than with an organised membrane, we infer that DNP affects the enzyme directly.

According to the proposed model [5], introducing ACC and ATP into the same side of a membrane vesicle should induce ATPase activity, leading to a flow of protons to the opposite side of the membrane, thus creating a transmembrane potential. This would consequently facilitate the conversion of ACC to ethylene. To test this hypothesis, we included membrane vesicles, ACC and ATP in the same reaction mixture and tested for ATPase and EFE activities. As expected, ATPase activity was proportional to ATP concentration (table 2). However, the activity of EFE was totally inhibited upon addition of ATP; indeed, inhibition of more than 90% of EFE by ATP was observed even at 250 μM ATP (not shown).

Clearly, none of the 3 suggested criteria has been met experimentally. The EFE activity is preserved in a membrane fraction. Loss of membrane integrity as a result of detergent-induced solubilisation of the membrane had no effect on EFE activity. ATP was found to be a potent inhibitor rather than a stimulator of EFE activity. The effect of DNP, which is an important element in the rationale of the hypothesis in [5], appears to be exerted through a direct interaction with the enzyme rather than with the membrane lipids. In addition, it was previously shown that ethylene production by petals is correlated negatively with ATPase activity [11] but positively with a loss of cell semipermeability [13].

The results of this work, supported by evidence from other studies, do not support the model in [5]. However, we suggest that in vivo transmembrane potential may play a major role, albeit an indirect one in the process of conversion of ACC to ethylene. Being an amino acid, ACC is probably transported actively into the cell compartment in which it accumulates like other amino acids. This process might be coupled to proton transport, as reported for other amino acids [14]. Actually, a similar coupling mechanism was recently proposed for ethylene biosynthesis by plant mitochondria [15]. Such a transport mechanism might explain the inhibition in vivo of EFE activity by DNP.

ACKNOWLEDGEMENT

The authors wish to thank Professor S. Mayak for helpful discussions.

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