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Salicylic acid inhibits the biosynthesis of ethylene in detached rice leaves

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Abstract

The effects of salicylic acid (SA) on ethylene biosynthesis in detached rice leaves were investigated. SA at pH 3.5 effectively inhibited ethylene production within 2 h of its application. It inhibited the conversion of ACC to ethylene, but did not affect the levels of ACC and conjugated ACC. Thus, the inhibitory effect of SA resulted from the inhibition of both synthesis of ACC and the conversion of ACC to ethylene.

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene-forming enzyme; SA, salicylic acid

1. Introduction

Salicylic acid (SA) was recognized as an endogenous regulator in plants after the finding that it triggers a dramatic increase in the production of metabolic heat and insect-attracting chemicals in the thermogenic inflorescences of *Arum lilies* [18, 19] and possibly other thermogenic plants [20]. Exogenous application of SA has been shown to affect a wide variety of biological processes, including stomatel function [17], disease resistance [23], flower stimulation [12], vegetative bud formation [6] and adventitious root initiation [13]. Recently, it was demonstrated that SA strongly reduced the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene in pear and apple discs, mung bean hypocotyls and pear cell suspension cultures [14, 15, 21]. However, it is not known whether SA inhibits the synthesis of ACC and/or conjugation of ACC.

Unlike other plant systems, ethylene biosynthesis in detached rice leaves was found to be promoted by polyamines [5] and inhibited by water stress [3]. Thus, rice leaves seem to be an interesting and unique system to study ethylene biosynthesis. In this study detached rice leaves were used to examine the effects of SA on ethylene biosynthesis.

2. Materials and methods

2.1 Plant material

Seedlings of rice (*Oryza sativa* cv. Taichung Native 1) were grown in hydroponic culture as described previously [4]. The apical 3 cm of the third leaves of 12-day-old seedlings were used for the experiments.

2.2 Incubation conditions

Ten randomly chosen leaf segments were placed vertically in a 14 ml test tube containing 1 ml of the treatment solutions, sealed with serum caps and incubated in darkness at 27 °C. The standard stock solution contained 0.1 M mannitol and 40 mM potassium phosphate adjusted to pH 3.5, as described by Romani et al. [21].

2.3 Determination of ethylene

Ethylene production was analyzed 6 h following treatment unless specified otherwise. A 1 ml gas

sample was withdrawn from the head space of the test tube with a hypodermic syringe, and ethylene was assayed using a gas chromatograph equipped with an alumina column and a flame ionization detector [11].

2.4 Determination of ACC and conjugated ACC

Rice leaf segments were extracted twice with boiling 80% ethanol. The ethanol was evaporated under vacuum at 40 °C. The residue was dissolved in 2 ml of water and the pigments were removed by adding 0.5 ml of chloroform. Aliquots of this extract were either analyzed for ACC content according to the method of Lizada and Yang [6], or incubated in 2 N HCl at 100 °C for 3 h before ACC analyses. The additional ACC released by hydrolysis was regarded as conjugated ACC.

2.5 Determination of ethylene-forming enzyme (EFE) activity

Leaf segments were pretreated with 10 mM of ACC for 2 h, then washed and surface dried. Leaf segments were transferred to test tubes containing treatment solution. The test tubes were capped immediately, and ethylene production was measured after 6 h incubated.

3. Results

The effect of the concentration of SA on ethylene production in detached rice leaves in darkness is presented in Figure 1. SA progressively inhibited the production of ethylene at increasing concentrations from 0.05 to 0.5 mM. The rate of production of ethylene is generally controlled by the level of ACC, the immediate precursor of ethylene [24]. The effect of SA on the level of ACC was examined and the result is shown in Figure 1. SA did not affect the level of ACC. ACC has been found to be converted into conjugated ACC [1, 9, 10] which can itself regulate the level of ACC, thus controlling the production of ethylene [24]. Therefore, it was of great interest to determine the effect of SA on the level of conjugated ACC. The result of Figure 1 clearly shows that SA did not change the level of conjugated ACC.

Since neither ACC nor the conjugated ACC level

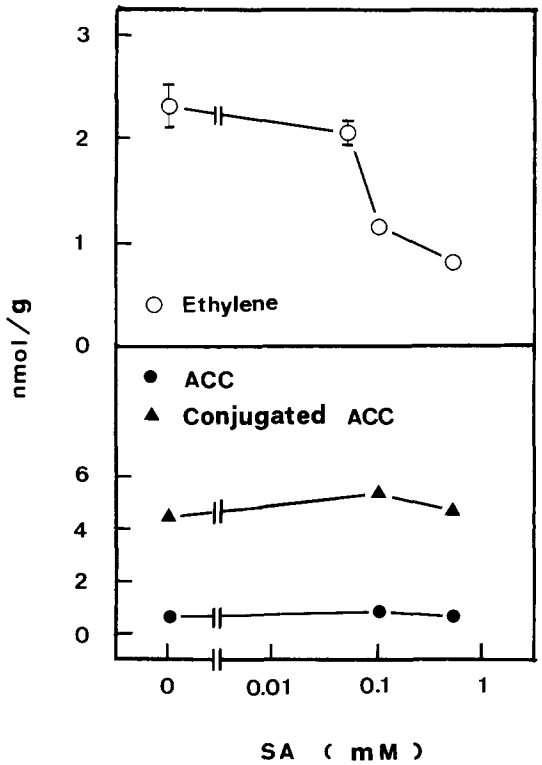

Fig. 1. Effects of salicylic acid concentration on ethylene production, ACC and conjugated ACC levels in detached rice leaves. Leaf segments were vertically placed in test tubes containing various salicylic acid concentrations. Ethylene production ACC and conjugated ACC were assayed after 6 h incubation in darkness. Bars represent standard errors (n = 4).

was affected by SA, the conversion of ACC to ethylene is possibly inhibited by SA. This possibility was tested by measuring EFE activity. As indicated in Figure 2, the conversion of ACC to ethylene was indeed inhibited by SA.

Figure 3 shows the changes with time in rates of the production of ethylene in detached rice leaves treated with 0.5 mM SA. Inhibition of ethylene production by SA was detected 2 h after the start of incubation in the dark, however, no inhibition of ethylene production by SA was observed at 5 h.

4. Discussion

SA has been reported to inhibit the production of ethylene in cultured cells, fruit discs and excised hypocotyls [14, 15, 21]. The present investigation showed that SA also inhibited ethylene production

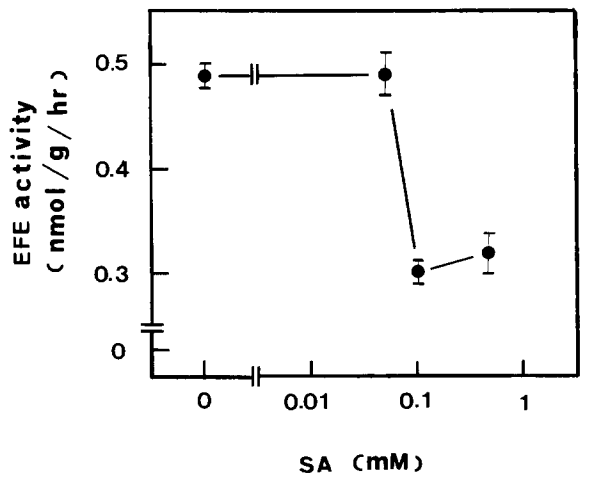

Fig. 2. Effects of salicylic acid concentration on ethylene-forming enzyme (EFE) activity in detached rice leaves. Leaf segments were pretreated with a saturating concentration of ACC (10 mM) for 2 h and then treated with various concentrations of salicylic acid. Ethylene production was assayed after 6 h incubation in darkness. Bars represent standard errors (n = 4).

in rice leaf tissues within 2 h after its application, a result that suggests SA inhibits ethylene biosynthesis directly. The question arises as to why the inhibitory effect of SA on ethylene production only lasted for 5 h. One possibility is that SA is readily conjugated and/or degraded in rice leaf cells [2, 7].

The present investigation demonstrated that SA inhibited the conversion of ACC to ethylene in rice leaves. These results are consistent with those reported by other investigators [14, 15, 21]. If the conversion of ACC to ethylene is the only step inhibited by SA, then an increase of ACC level in SA-treated rice leaves is to be expected. However, no such increase was observed. On the basis of these results, the absence of increased ACC accumulation in the presence of SA can be explained in terms of simultaneous inhibition of both the synthesis and conversion of ACC in rice leaves. For reasons not yet understood, the activity of ACC synthase in homogenates of detached rice leaves is undetectable. Thus, we were unable to present direct evidence to show that SA inhibited ACC synthase activity.

The inhibition of basal ethylene production increased with increasing SA concentration from 0.1 to 0.5 mM. However, no further inhibition of EFE activity was observed when SA concentration was increased from 0.1 to 0.5 mM. This observation suggests that the possibility that SA may inhibit transport of ACC into the vacuole, a possible control point in ethylene biosynthesis [8, 22], can not be excluded. In conclusion, SA is unlikely to be a specific inhibitor, at least in rice leaves, of the putative EFE.

Since SA was effective in inhibiting ethylene production at low pH, it should prove useful in studying ethylene biosynthesis in tissues tolerent of low pH. SA has several effects on plant development [15]. These effects could be mediated through the changes of ethylene production. Although SA or SA derivatives have been widely isolated from many plants, the possibility that endogenous SA plays a role in regulating ethylene production remains to be explored.

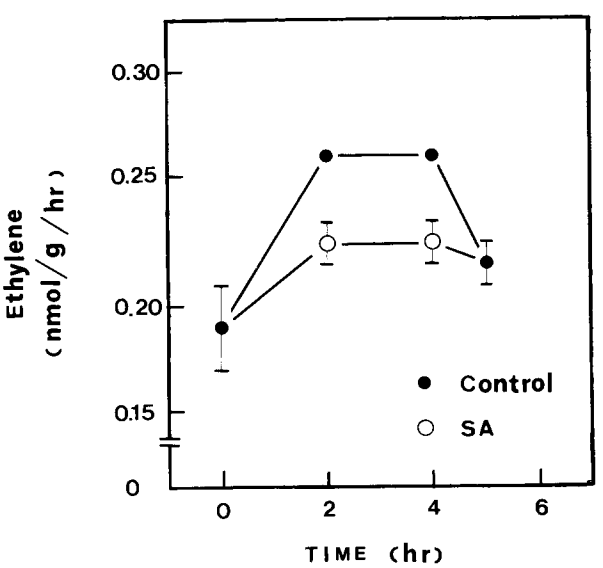

Fig. 3. Changes with time in rates of ethylene production in segments of rice leaves treated with salicylic acid. Leaf segments were treated with or without salicylic acid (0.5 mM) in darkness. Ethylene production was assayed at the time indicated.

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