Stimulation of ethylene production in detached rice leaves by vanadate

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Abstract

The effect of vanadate on ethylene biosynthesis in detached rice leaves was investigated. Vanadate at pH 5.0–7.0 effectively enhanced ethylene production within 3 h of its application. It promoted the conversion of ACC to ethylene. Treatment with vanadate did not decrease ACC level until late stage of incubation, i.e. at 12 h after incubation. Molybdate, an inhibitor of phosphatase had no or much less stimulatory effect on ethylene production than did vanadate at comparable concentrations. Azide, an inhibitor of F_1 -ATPase, inhibited ethylene production in detached rice leaves. FC and vanadate were observed to be synergistically increased ethylene production in detached rice leaves. In conclusion, plasma membrane H^+ -ATPase does not seem to be involved in ethylene biosynthesis in detached rice leaves.

Abbreviations: ACC = 1-Aminocyclopropane-1-carboxylic acid; FC = Fusicoccin

1. Introduction

Zoglauer *et al.* [15] proposed that changes in the activity of plasma membrane H^+ -ATPase may be involved in the induction of biosynthesis of ethylene. An effect of transmembrane proton gradients in the conversion of ACC to ethylene has been observed *in vivo* and in isolated vacuoles [7, 11]. In our previous work, we have shown that FC, which is known to induce the secretion of proton via plasma membrane H^+ -ATPase [10], stimulated ethylene production in detached rice leaves [3]. Vanadate is recognized to act as an inhibitor of the plasma membrane H^+ -ATPase [13] and has been shown to inhibit ethylene production in tomato hypocotyls [9]. In order to examine the possibility that changes in the activity of the plasma membrane H^+ -ATPase may be involved in the induction of ethylene synthesis, we characterized the effects of vanadate on the biosynthesis of ethylene in detached rice leaves.

2. Materials and methods

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. Ten segments of rice leaves, weighing about 45 mg, were floated on 10 mL of test solution in a Petri dish. All samples were kept at 27 °C in the dark. The standard sodium vanadate solution contained 20 mM potassium phosphate adjusted by KOH (1 N) to pH 5.0.

After various periods of incubation, the leaf samples were transferred to a 14-mL test tube. The tubes were sealed with serum caps. After 1 h of incubation at 27 °C in the dark, a 1 mL gas sample was withdrawn from the headspace of each test tube. Ethylene was assayed with a gas chromatograph equipped with an alumina column and a flame ionization detector. Absolute levels of ethylene varied among experiments because of seasonal effects. However, the patterns of responses to vanadate were reproducible.

ACC was extracted and quantitated as described previously [8].

All experiments were repeated at least three times. Similar results and identical trends were obtained on each occasion. The data obtained here are all from a single experiment.

3. Results

The effect of the concentration of vanadate on ethylene production in detached rice leaves in darkness is presented in Fig. 1. Increasing concentration of vanadate from 0.1 to 1 mM progressively enhanced the production of ethylene. Vanadate-promoted ethylene production by detached rice leaves was affected by pH (Fig. 2). Vanadate buffered to pH values ranging from 5 to 7 was effective in enhancing ethylene production. However, vanadate at pH 4.0 and 8.0 had no and less effect, respectively, on ethylene production.

Figure 3 shows the changes with time in rates of the production of ethylene in detached rice leaves treated with 1 mM vanadate. Promotion of the production of ethylene by vanadate was detected at 3 h after the start of incubation in the dark. Increased duration of treatment with vanadate increased the production of ethylene by detached rice leaves. Incubation periods longer than 6 h did not result in higher rate of ethylene production.

The rate of production of ethylene is generally controlled by the level of ACC, the immediate precursor of ethylene [14]. In control leaves levels of ACC increased with increasing time of incubation (Fig. 3). Treatment with vanadate had no effect on ACC levels within 3 h and 9 h, but resulted in lower levels of ACC than the controls at 12 h after incubation (Fig. 3). These results suggest that an enhanced rate of production of ethylene in vanadate-treated segments of rice leaves is unlikely regulated by the promotion of the synthesis of ACC. To test if the conversion of ACC to ethylene is promoted by vanadate, we measured the production of ethylene in the presence of a saturating concentration of ACC (10 mM). As indicated in Fig. 4, the conversion of ACC to ethylene was promoted by vanadate during a 12-h incubation.

Molybdate, an inhibitor of acid phosphatase (which may also be inhibited by vanadate) but not of H^+ -ATPase [5, 6, 12], had no or much less stimulatory effect than did vanadate at comparable concentrations (Fig. 5). Azide is among several potent inhibitors of mitochondrial type F_1 -ATPase [1]. Contrary to the effect of vanadate, azide inhibited ethylene production by detached rice leaves (Fig. 5). All these results

Fig. 1. Effect of vanadate concentration on ethylene production in detached rice leaves. Ethylene production was assayed after 6 h incubation in darkness. Bars represented standard errors ($n = 4$).

Fig. 2. Effect of pH on vanadate-induced ethylene production in detached rice leaves. Leaf segments were incubated in either water or 1 mM vanadate contained 20 mM potassium phosphate adjusted to various pH. Ethylene production was assayed after 6 h incubation in darkness. Bars represented standard errors ($n = 4$).

indicate that the vanadate effect on ethylene production was not a consequence of nonspecific phosphohydrolyase inhibition.

Figure 6 shows the effect of fusicoccin (FC) on vanadate-enhanced ethylene production in detached rice leaves. A synergistic effect on the increase of ethylene production was observed when FC and vanadate were applied together.

Fig. 3. Changes with time in rates of ethylene production (upper) and levels of ACC (lower) in segments of rice leaves treated with vanadate. Segments were treated with either water or 1 mM vanadate in darkness. Production of ethylene and levels of ACC were assayed at the times indicated (n = 4).

4. Discussion

Vanadate is known to be a potent inhibitor of the plasma membrane H^+ -ATPase [13] and has been reported to inhibit the production of ethylene in tomato hypocotyls [9]. Unexpectedly we found that vanadate stimulated the production of ethylene in detached rice leaves. Since the increase of ethylene production in rice leaves is rapid following excision, one may argue that the effect of vanadate on ethylene production is probably a wound or other stress response. When detached leaves were used to determine ethylene production, wounding is always a problem. However, in the present study, each long and narrow rice leaf was cut once transversely, the area of wounding was very small. No loss of turgidity in detached rice leaves treated with vanadate was observed. Therefore, the increase in ethylene production by vanadate could not result from water

Fig. 4. Effect of vanadate on the conversion of ACC to ethylene. Segments of rice leaves were pretreated with a saturating concentration of ACC (10 mM) for 2 h and then treated with either water or 1 mM vanadate in darkness. Rates of ethylene production were determined at the times indicated. Bars represented standard errors (n = 4).

Fig. 5. Effect of molybdate and azide concentrations on ethylene production in segments of rice leaves. Ethylene production was assayed after 6 h incubation in darkness. Bars represented standard errors (n = 4).

stress. The effects of vanadate on the production of ethylene seem to be complex. At present, it is impossible to propose a general explanation for the stimulation by vanadate of the production of ethylene in rice leaf tissues and inhibition in other tissues. The discrepancy

Fig. 6. Effect of FC on ethylene production and of FC on vanadate-induced ethylene production. Detached rice leaves were incubated in the dark for 6 h in vanadate (1 mM), FC (1 μ M) + vanadate (1 mM). Bars represented standard errors ($n = 4$).

might be because different species respond to identical vanadate treatment in different ways.

Results from the present study clearly showed that vanadate induced a significant increase in the production of ethylene shortly (within 3 h) after application. This observation suggests that vanadate stimulates biosynthesis of ethylene directly. Since senescence of rice leaves in darkness occurred at 2 days after incubation [2]. The vanadate-enhanced production of ethylene in detached rice leaves is unlikely to be mediated via a mechanism associated with senescence.

Why is the extent of the stimulatory effect of vanadate on the production ethylene quite small, usually two-fold? One possibility is that vanadate is not readily transported in rice leaf cells. It should be noted that, in the present study, segments of unpeeled leaves were used.

The present investigation demonstrated that the increased production of ethylene in vanadate-treated leaves is regulated via promotion of the conversion of ACC to ethylene. However, it is unclear whether the increase in the activity of ACC oxidase is regulated by the de novo synthesis of ACC oxidase and/or by the activation of preexisting ACC oxidase.

FC has been shown to enhance ethylene production in detached rice leaves [3]. The present investigation showed that FC and vanadate synergistically increased the production of ethylene. Since FC and vanadate have opposite effects on H^+ -ATPase activity, it is unlikely

that H^+ -ATPase is involved in the biosynthesis of ethylene in detached rice leaves.

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