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Ethylene Production and 1-Aminocyclopropane-1-Carboxylic Acid Content of Lichen Bionts

P. SCHIELEIT and S. OTT*
Botanisches Institut, Universität Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany. Tel. +49-211-8113535, Fax. +49-211-8112881, E-mail. otts@mail.rz.uni_duesseldorf.de

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Abstract

This study reports on ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) content of isolated lichen bionts (mycobiont and photobiont) in comparison with lichen thalli. All bionts investigated show ethylene producing activity and contain ACC, the ethylene precursor in higher plants. The level of ACC is nearly identical in the mycobiont and in the lichen thalli. Ethylene production of bionts was only enhanced after treatment with ACC while α-oxoglutarate a known ethylene precursor in ascomycetic fungi gives no reaction. The results presented here indicate that in the complex system of the lichen thallus both partners are involved in the production of the regulative substances and seem to use identical biochemical pathways.

Keywords: Ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC), lichen, isolated bionts, mycobiont, photobiont

1. Introduction

The organization, development and reproduction of lichens depends on a sensitive balance between two or more bionts. Differentiation and growth processes are regulated by a system of complicated interactions (Honegger,

*The author to whom correspondence should be sent.

1992; Jahns and Ott, 1990). The development of the thallus is probably influenced by phytohormones. Several phytohormones have been discovered in lichens (Epstein et al., 1986; Hartung and Gimmler, 1994) of these the gaseous phytohormone ethylene has been examined in some detail (Epstein et al., 1986; Ott and Zwoch, 1992; Lurie and Garty, 1991). The influence of exogenous ecological factors such as air pollution (Garty et al., 1995) and the relationship with water content, temperature and light on the production of ethylene have been described previously (Ott and Schieleit, 1994; Ott, 1993).

The lichen material used in experiments on ethylene production is neither homogenous nor sterile and the developmental stage of the thalli varies, as it cannot be grown in the laboratory. These aspects complicate the interpretation of results achieved in these experiments compared with investigations made in higher plants. The results have to be replicated many times but even after that the specific role of the mycobiont or the photobiont remains questionable. Measurements with intact lichen thalli indicate the existence of the biosynthetic pathway which is typical for higher plants. The presence of 1-aminocyclopropane-1-carboxylic acid (ACC) which is known as a direct precursor of ethylene in higher plants has been shown by HPLC/MS in several lichen species (Schieleit et al., in prep.). However different biosynthetic pathways may exist for the ethylene production in the bionts and the production is either controlled by all organisms, separately by the individual or in mutual interdependence between the bionts. For this reason it is necessary to investigate ethylene production of the bionts separately. It is possible to cultivate the algae and the fungi at sufficiently high amounts and under sterile conditions in the laboratory. A comparison of the ethylene production in different isolated fungi and algae can show possible differences between the bionts and prove indirectly the reliability of experiments with intact lichen thalli. Production of ethylene and ACC by the green algae *Coccomyxa* and *Trebouxia* and by the ascomycetes which form the fungal component of lichens has not been attempted previously. The comparison between the properties of the ethylene producing system in the isolated bionts and of the intact thallus may elucidate also the abiotic influences on ethylene production in lichens as it is not obscured by possible undetectable enhancing or neutralizing reactions of the symbiotic partners.

2. Materials and Methods

Experimental lichen material

Cladonia rangiferina (L.) Weber ex Wigg (1780) and *Cetraria islandica* (L.)

Ach. (1803) were sampled in a natural habitat of a forest near Mullsjö (Sweden). Air dried material was transported to the laboratory as soon as possible (one day) and stored in a growth chamber under constant conditions (Ott and Schieleit, 1994) until it was used for investigations.

Cultivation of the mycobiont

Apothecia of the mycobionts of *C. rangiferina* and *C. islandica* were removed from the thallus, washed with deionized water and fixed to the top cover of Petri dishes. The spores discharged on malt-yeast medium in the lower half of the Petri dishes. Mycelia which grew from germinated spores were transferred to solid and liquid malt-yeast medium. Cultivation was essentially carried out as described by Ahmadjian (1993). The cultured mycobiont of *C. rangiferina* was used for the determination of ACC and ethylene, and cultures of *C. islandica* were used only for the determination of ACC.

Cultivation of algae

The photobionts *Trebouxia irregularis* and *Coccomyxa* sp. were a kind gift from T. Friedl (Botanisches Institut, Universität Bayreuth, Germany). They were cultivated according to the instructions of the donor (Friedl, 1989), in liquid medium (Trebouxia– organic – medium), *T. irregularis* at 11°C and 20–25 µmol photons/m^2s; *Coccomyxa* sp. at 25°C and 30 µmol photons/m^2s.

Preparation of lichen thalli for the ethylene determination

Lichen thalli were cleaned from adhering particles of soil and organic materials and cut into small squares (1 mm). The effect of stress ethylene due to cutting can be excluded (Ott and Schieleit, in preparation). Small sections of dried material (0.05 g) were placed into 2.5 ml glass vials and treated with 0.5 ml deionized water (control) or an aqueous solution of 1-aminocyclopropane-1-carboxylic acid (ACC) or α-oxoglutarate. The solutions were infiltrated for 1–2 minutes.

Preparation of the mycobiont for ethylene determination

The *C. rangiferina* mycobiont from the cultures in liquid malt-yeast medium was harvested by filtration, washed 4 times with deionized water and all remaining water was carefully removed. The mycelium (0.05 g) was placed into 2.5 ml glass vials and treated with 80 µl deionized water (control), an aqueous

solution of 1-aminocyclopropane-1-carboxylic acid (ACC) or α-oxoglutarate. The solutions were infiltrated for 1–2 minutes.

Preparation of the photobiont Trebouxia irregularis for ethylene determination

The algal cells were harvested after 3–4 weeks of cultivation, by gentle centrifugation at room temperature washed with 25 mM MOPS-buffer (pH 6) three times and resuspended in 6 ml MOPS-buffer. The algal suspension (200 µl) was pipetted into a 2.5 ml glass vial and imbibed with 150 µl MOPS-buffer (50 mM, pH 6) alone as a control and with 50 mM 1-aminocyclopropane-1-carboxylic acid (ACC) or α-oxoglutarate. The solutions were infiltrated under vacuum for 1–2 minutes. For reference purposes the dry weight of the algal cells in 200 µl suspension was determined.

Ethylene determination

The prepared vials were sealed with rubber septa and placed in the dark at 30°C. Two independent experiments with 4 replicates in each experiment were sampled after 3 h and after 24 h incubation periods. For this a 1 ml sample was withdrawn from the top of the vials and analyzed immediately by gas chromatography (Ott and Schieleit, 1994). To exclude ethylene production by the solution it was tested for ethylene without the presence of a sample.

ACC extraction of lichen thallus and mycobiont

ACC extraction from lichens was essentially carried out as described by Ott and Zwoch (1992). Fresh lichen material (2–3 g) was used. For ACC determination of the pure mycobiont, 2–3 g of the mycelial mass was carefully removed, washed with deionized water several times and remaining surface water blotted off. The mycelium was pulverized in liquid nitrogen with a mortar and pestle, according to Ott and Zwoch (1992).

ACC extraction from algae (photobiont)

Algal cells from 300 ml of algal cultures were centrifuged at room temperature, washed three times and resuspended in 25 mM MOPS-buffer (pH 6). After renewed centrifugation (5000 U/min, 5 minutes at room temperature) the algal cells were suspended in 15 ml 100% ethanol for 1 h and glass beads (0.17–0.18 mm) supported the breaking of the cells by sonication and vortex. After extraction the supernatant was evaporated to dryness at 50°C. The various steps of that treatment were described by Ott and Zwoch (1992).

Determination of ACC

ACC was determined according to a method developed by Lizada and Yang (1979) and modified by Rohwer and Schierle (1984). ACC analysis was carried out in 2 or 3 independent experiments.

3. Results

Ethylene production was measured in the intact thallus of *C. rangiferina* and its isolated mycobiont and photobiont. This permits comparative estimates and clarifies the role of the bionts during the production of this phytohormone.

Ethylene production of the isolated photobiont

Trebouxia irregularis shows only slight ethylene production activity in deionized water (control). Treatment with 1-aminocyclopropane-1-carboxylic acid (ACC) clearly induces ethylene production but α-oxoglutarate has no effect on its synthesis in the algal cells (Table 1). This suggests a participation of ACC in the ethylene forming system in *T. irregularis*. The green alga *Coccomyxa* sp. showed similar results (data not shown).

Ethylene production of the isolated mycobiont

Endogenous ethylene production in the mycobiont is low and is detectable in the controls only after 24 h. Treatment with α-oxoglutarate, a common precursor of ethylene in many ascomycetic fungi, did not stimulate ethylene production. The exogenous application of ACC which is the precursor of ethylene in higher plants resulted in a significant rise of ethylene production. This effect can only be observed after an incubation period for at least 24 h and increases are related to the amount of the applied ACC (Table 1).

Ethylene production of the intact lichen

Ethylene production of the entire lichen thallus corresponds to the results of the isolated bionts. In the thallus it is stimulated by ACC only and the application of α-oxoglutarate again shows virtually no stimulation. Here increasing ACC concentrations (from 10 mM to 50 mM) do not raise the ethylene production (Table 1). This observation suggests that the response to ACC is probably saturated around 10 mM. Compared with the ethylene production by the alga and the mycobiont the synthesis values for the deionized water

Table 1. Ethylene production of the thallus of *Cladonia rangiferina* and the isolated bionts after treatment with special ethylene percursors.

Treatment	Lichen thallus 3 h x (SD)	Lichen thallus 24 h x (SD)	Mycobiont 3 h x (SD)	Mycobiont 24 h x (SD)	Photobiont 3 h x (SD)	Photobiont 24 h x (SD)
Control	2.10 (0.14)	0.40 (0.10)	n.d.	0.26 (0.02)	n.d.	0.17 (0.01)
ACC						
10 mM	**3.65 (0.18)**	**0.65 (0.11)**		**0.33 (0.05)**		n.d.
50 mM	**3.33 (0.37)**	**0.61 (0.07)**	n.d.	**0.52 (0.01)**	**5.83 (0.40)**	**1.03 (0.19)**
α-oxoglutarate						
10 mM	2.16 (0.12)	0.39 (0.03)		0.29 (0.01)	0	0
50 mM	1.78 (0.22)	0.37 (0.05)	n.d.	0.26 (0.03)	n.i.	n.i.

Ethylene production is given as nl/g/h; x = mean values; SD = standard deviation; n.d. = not detectable; n.i. = not investigated. Bold figures are significant increases when compared with the water controls ($p = 0.05$).

activated lichen is remarkably high (control). The ACC-dependent ethylene production of the lichen shows interesting similarities with that of the algae. Here ethylene production is markedly increased after the addition of ACC and a short incubation period of 3 h. After 24 h ACC-dependent ethylene production in the lichen is lower than in the alga but of the same order as that of the mycobiont (Table 1).

ACC content

The above results suggest that ACC plays a major role for ethylene synthesis in the lichen thallus and in both isolated bionts. These results could be confirmed by the presence of ACC in the lichen and its individual components. For this reason the thalli of the lichen species *C. rangiferina* and *C. islandica*, the isolated mycobionts and the photobionts – *Trebouxia irregularis* and *Coccomyxa* sp., respectively – were investigated. The results are shown in Table 2. In all these organisms this precursor of ethylene was present. The

amount of ACC in each lichen and in its mycobiont is nearly identical but the content in the algae is variable.

Table 2. ACC content of lichen thalli, the mycobionts and some photobionts.

	ACC content
Lichen	
Cladonia rangiferina	0.34
Cetraria islandica	0.13
Mycobiont	
Cladonia rangiferina	0.36
Cetraria islandica	0.08
Photobiont	
Trebouxia irregularis	0.26
Coccomyxa sp.	0.43

ACC content is given in nmol/g dry weight for the lichen thallus as well as for the mycobionts. The ACC content of the photobionts is given in nmol/g fresh weight.

4. Discussion

The results show unequivocally that the lichen *C. rangiferina*, its mycobiont and the photobiont *Trebouxia irregularis* are capable to synthesize ethylene in measurable amounts. It has been established here that only 1-amino-cyclopropane-1-carboxylic acid (ACC) serves as substrate for ethylene in all investigated organisms and that its ethylene production is not influenced by α-oxoglutarate, a typical precursor for ethylene in other ascomycetes (Chou and Yang, 1973). ACC, the immediate precursor of ethylene in higher plants was detected in all investigated organisms and the lichen and isolated bionts indicate that the biosynthetic pathway is similar. As both organisms may be involved in the production of ethylene in the lichen thallus the organism-specific effects can be superimposed upon one another. Such interactions are to be expected in the thallus as the symbiotic system is known to be influenced by both organisms. The special role of each constituent organism and its nature is still a matter of speculation and is currently investigated. Additionally, the

complexity of the interactions between the bionts will be enhanced by the influence of external factors, e.g. light, water content and temperature. The complexities of internal and external factors have not been elucidated. With respect to the biosynthesis of ethylene the mutual influence of the bionts is probably regulated by the activity of enzymes which catalyse the various stages of the biosynthetic pathway. The current measurements have shown that ACC dependent ethylene production is not solely dependent upon the concentration of ACC as the lichen thallus and the mycobiont contain similar levels of ACC (Tables 1 and 2) but also upon the activity of the ethylene forming enzyme (ACC-oxidase). The differences between ethylene production in the lichen thallus and the mycobiont are remarkable (Table 1).

The different consistency of the lichen thallus and the cultured mycobiont probably influences diffusion of the applied solutions which may be of relevance for the ethylene production. This could explain the observation that the ACC level in the lichen thallus and the isolated mycobiont are nearly identical while ethylene production differs. Probably the two bionts in the lichen influence one another and the enhancement of ethylene synthesis is caused by symbiotic interactions whereas the production of ACC by the mycobiont seems to be uneffected by symbiosis. Presumably the ACC content measured in the lichen thallus is due to the mycobiont. As can be seen in Table 2, the photobiont is able to produce ACC, too, but the ratio of mycobiont/ photobiont favoures the mycobiont which represents the major part of the biomass.

The first report on ethylene production by the intact lichen thallus as well as both its isolated bionts is of major importance. To date ethylene synthesis has been observed neither in ascomycetes belonging to the Lecanorales nor in the green algae *Trebouxia* and *Coccomyxa* which occur as photobionts in lichens. The synthetic pathway seems to be identical in all these organisms which belong to unrelated taxa. The results indicate that in the complex lichen thallus both partners are probably involved in the production of the regulative substances and seem to use identical biochemical pathways. This may be important for a balanced interaction between the bionts. The exact nature of the interactive processes and the mutual influences between fungus and alga are still unknown. Regulation may include participation of both partners as well as restriction of processes in one of them. However, the results show that a balanced interaction does exist and further research on these regulating processes may continue on the basis of these results.

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