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Hemoglobin expression affects ethylene production in maize cell cultures

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Abstract

The formation of ethylene under different O_2 concentrations and upon addition of nitric oxide (NO) donor, sodium nitroprusside (SNP), was determined using maize (*Zea mays* L.) cell lines over-expressing (Hb+) or down-regulating (Hb–) hypoxically inducible (class-1) hemoglobin (Hb). Under all treatments, ethylene levels in the Hb– line were 5 to 6.5 times the levels in Hb+ and four to five times the levels in the wild type. Low oxygen partial pressures impaired ethylene formation in maize cell suspension cultures. 1-Amino-cyclopropane-1-carboxylic acid (ACC) oxidase (E.C. 1.14.17.4) mRNA levels did not vary, either between lines or between treatments. There was, however, significantly enhanced ACC oxidase (ACO) activity in the Hb– line relative to the wild type and the Hb+ line. ACO activity in the Hb– line increased under hypoxic conditions and significantly increased upon treatment with NO under normoxic conditions. The results suggest that limiting class-1 hemoglobin protein synthesis increases ethylene formation in maize suspension cells, possibly via the modulation of NO levels.

Keywords: ACC oxidase; Ethylene; Hemoglobin; Hypoxia/nitric oxide; *Zea mays*

1. Introduction

Various stress situations can trigger significant changes in ethylene production and accumulation in plant cells [25]. Elevated ethylene concentrations have been reported in aerial parts of flooded plants in a variety of species including *Lycopersicon esculentum* [16,32] and *Rumex palustris* [4,30]. Flooding can result in partial or complete submergence of plant organs leading to a decrease in oxygen availability. Diffusion of gases is 10,000 times slower in water compared with air; therefore, submergence of plant organs results in restricted gas exchange between plant tissues and their surrounding environment [14,23]. Under low oxygen pressures, there is a shift to anaerobic fermentation from respiration causing ATP levels to drop considerably. Plant survival requires regulation of the processes supplying the energy (e.g. carbohydrate catabolism) and those involving a consumption of ATP (e.g. substrate biosynthesis and phosphorylation) [17].

Ethylene is a gaseous hormone involved in plant maturation, aging, cell development, cell elongation and response to stress. Under low oxygen pressures, ethylene can be responsible for triggering processes such as aerenchyma formation, root and stem elongation or adventitious root growth [23] that assist the plant in avoiding the low oxygen pressure [1]. The key enzymes involved in ethylene biosynthesis have been isolated and studied in a number of higher plant species. The formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from S-adenosylmethionine is catalyzed by ACC synthase (ACS; E.C. 4.4.1.14). ACC is then oxidized to ethylene by ACC oxidase (ACO; E.C. 1.14.17.4) [33]. An increase in ethylene levels has been observed in plant tissues under hypoxia as a result of ACC-enhanced biosynthesis and accumulation in submerged tissues [19,23]. The gaseous hormone is involved in the formation of aerenchyma in the hypoxic roots of maize, facilitating the diffusion of O_2 throughout the plant [15].

Two major metabolic events have been linked to Hb expression: the first, prevention of NO accumulation [12] and the second, maintenance of energy charge [29]. In alfalfa roots and maize cell suspension cultures, nitric oxide (NO) levels increase under low oxygen pressure. A possible role for Hb in metabolizing NO in the plant cell has been suggested [22].

Abbreviations: ACC, 1-amino-cyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; SNP, sodium nitroprusside.

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Nitric oxide has been shown to act as a signal molecule in animal cells as well as in plants in response to biotic and abiotic stresses and in relation to other messengers such as reactive oxygen species (ROS) or ethylene [5,26]. Ethylene has been implicated in the programmed cell death response leading to root aerenchyma formation during hypoxia [15]. Interestingly, alfalfa roots over-expressing barley Hb exhibited almost no sign of cell disruption under 3 kPa O_2, whereas Hb– lines showed strong evidence of cell death characteristic of aerenchyma formation [12], which was correlated to a significant drop in energy charge.

We wished to further investigate the relationship between Hb expression, NO and plant cell response to low oxygen partial pressures by examining ethylene formation under the same conditions that have been used to examine energy status and NO formation. We measured the formation of ethylene in maize cells experiencing different oxygen concentrations, with or without addition of exogenous NO via sodium nitroprusside (SNP) treatment. We also determined the levels of expression of the ACO transcript, as well as the activity of the enzyme. Using maize cell lines expressing various levels of hemoglobin, we also determined the effect of Hb on the ethylene production under low oxygen.

2. Results

2.1. Varying the levels of barley class-1 hemoglobin in maize influences the ethylene levels in cell suspension cultures in oxygenic and hypoxic conditions

Hb– maize suspension cells produced significantly more ethylene after 6 h than either WT or Hb+ cells (Fig. 1), with levels being 4 to 6.5-fold higher. The Hb+ line generally had lower levels of ethylene than the WT (3–38%) but the difference between these two lines was significant only when SNP was added at 3 kPa O_2.

Low oxygen partial pressure (3 kPa O_2) caused a decrease of approximately 37–40% in ethylene production in all maize lines (Fig. 1), regardless of the presence of SNP. In most instances, the effect of treatment with SNP on ethylene levels was insignificant, although there appeared to be a slight positive effect in WT and Hb– cells.

2.2. ACC oxidase gene expression is not affected in hypoxic and SNP-treated maize cells

To determine whether the changes in the levels of ethylene where a result of variations in ACO gene expression, transcript levels of ACO were measured by RT-PCR using total RNA isolated from the cell samples. An actin probe was used as an internal control. Two sets of oligonucleotides were used in the RT-PCR reactions (see Materials and Methods). No significant changes were observed in ACO mRNA levels from any of the maize cells lines regardless of the treatments (Fig. 2).

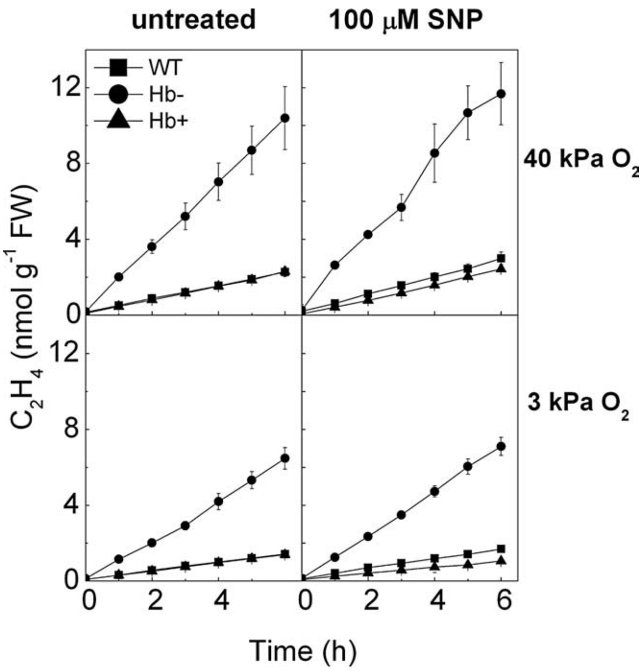

Fig. 1. Ethylene levels in headspace of vessels with maize cell cultures expressing different levels of barley hemoglobin at high and low oxygen partial pressures in the absence or presence of an NO donor (SNP).

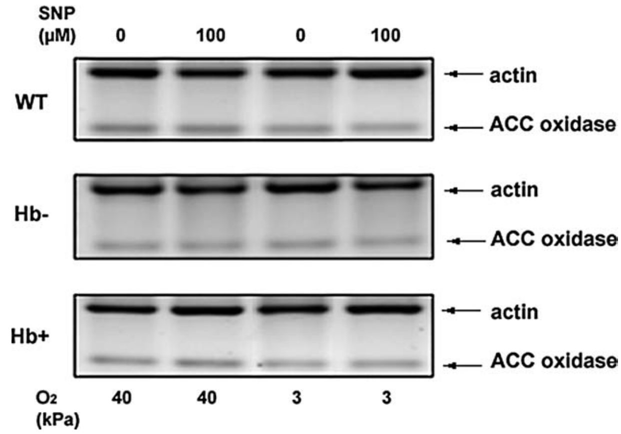

Fig. 2. ACO mRNA levels in maize cells expressing varying levels of barley hemoglobin.

2.3. ACC oxidase enzyme activity is increased in Hb– maize cell line and upon SNP treatment

Because ACO gene transcript levels were unaffected by low oxygen or SNP treatments (Fig. 3), variation in the enzyme activity was investigated as a possible explanation for the observed changes in ethylene levels. Exposure to hypoxia or addition of exogenous NO had no significant effect on ACO enzyme activities in the WT and the cells over-expressing barley hemoglobin (Fig. 3). Enzyme activities were significantly higher in the Hb– line than in the WT or Hb+ line. Hypoxia tended to increase ACO activity, while SNP treatment caused a significant 2.5-fold increase in activity in the cells down-regulating hemoglobin levels.