Hydrogen uptake by Azolla-Anabaena*

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Summary Experiments under laboratory conditions were carried out with the objective of studying hydrogen uptake in the Azolla-Anabaena system. Tritium was used as tracer and plants were incubated under different atmospheric composition: a) Air + 3H2; b) Air + 3H2 + CO; c) Air + 3H2 + CO2; d) Argon + 3H2 + CO2 + CO and in presence and absence of light, to study hydrogen uptake via hydrogenase and nitrogenase activity.

Azolla-Anabaena showed greater hydrogen uptake under argon atmosphere than under air. Carbon monoxide decrease hydrogen uptake as well as nitrogenase activity. Under dark conditions, nitrogenase activity was smaller under argon than air atmosphere. Carbon monoxide decreased hydrogen uptake. H2-uptake was not affected by light, as it occurs under dark conditions. There are evidences of recycling of the hydrogen evolved through nitrogenase.

Introduction

The Azolla-Anabaena system has a nitrogenase-catalysed hydrogen evolution activity9,13,14,15 which is greater under an argon atmosphere than in air19. This suggests that the system has an uni-directional hydrogenase activity and is extremely efficient16. An increase of nitrogenase activity was observed in Anabaena azollae cells free from Azolla which is similar to other cyanobacteria in the presence of hydrogen gas and especially under dark conditions if oxygen (5%) was added11,12. Thus, one of the initial electron donors for Anabaena azollae nitrogenase can apparently be molecular hydrogen3. These authors3 also observed hydrogen absorption by Anabaena azollae cells in dark, which increased with oxygen addition. However, in the light, oxygen inhibited this reaction.

Significant benefit of the H2-uptake can be achieved by symbiont partner to the overall plant productivity1,4,7,18 since energy is one of the limiting factors in nitrogen fixation2,6. In this work, H2-uptake and nitrogenase activity by Azolla-Anabaena was studied under different atmospheric composition.

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Material and methods

Plants of *A. filiculoides* were cultivated in a greenhouse in the nutrient solution. The composition of the nutrient solution was $\text{K}_2\text{SO}_4 \ 0.5\text{M}, \ 5\text{ml}; \ \text{MgSO}_4.\text{H}_2\text{O} \ 1.0\text{M}, \ 2\text{ml}; \ \text{CaHPO}_4 \ 0.5\text{M}, \ 10\text{ml}; \ \text{CaSO}_4 \ 0.01\text{M}, \ 200\text{ml}; \ \text{FeEDTA} \ 0.50\ \mu\text{mol}; \ \text{H}_3\text{BO}_3 \ 2.86\ \text{mg}, \ \text{MnCl}_2.4\text{H}_2\text{O} \ 1.81\ \text{mg}, \ \text{ZnSO}_4.7\text{H}_2\text{O}, \ 0.22\ \text{mg}, \ \text{CuSO}_4.5\text{H}_2\text{O}, \ 0.08\ \text{mg}, \ \text{H}_2\text{MoO}_4.\text{H}_2\text{O}, \ 0.09\ \text{mg}$. The nutrient solution was changed every two weeks.

Three experiments were carried out to determine the hydrogenase and nitrogenase activities in different atmospheres. In the first experiment, the following treatments were assayed with two replications: a) normal air atmosphere (NA); b) NA + carbon monoxide (CO, 2.8% v/v), c) Argon + CO$_2$ (1% v/v), d) Argon + CO$_2$ (1% v/v) + CO (2.8% v/v), and e) Dead Azolla.

Fresh Azolla (1.0 g) was transferred to 35 cc glass flasks, which were hermetically sealed with subba seal. In the flasks correspondent to treatment (b), 1 cc of CO was added, and in those correspondent to treatment (c) and (d), a flow with argon (2 min) was done to eliminate the normal atmosphere into the flasks. After 0.35 cc was evacuated and an equal amount of CO$_2$ was injected into the flasks. Finally 1 cc of CO was added into the flasks correspondent to treatment (d). The dead Azolla used in treatment (e), was obtained by immersion of the plants in liquid nitrogen for approximately 30 min. This procedure killed the plants, remaining plants which intact physical characteristics. To the flasks containing Azolla 1 ml of tritium ($^3\text{H}_2 \ 1.9\ \mu\text{Ci}$), was injected and incubated at ambient temperature (25°C), under light intensity of 1.5-4.0 W.m$^{-2}$ approximately. After 1 hour incubation, the flasks were opened and the non-absorbed tritium was eliminated. Approximately 0.30 g of Azolla were transferred to the oxidizer for combustion of plant material into CO$_2$ and $^3\text{H}_2\text{O}$. The tritiated water formed was then transferred to scintillation flasks containing liquid scintillation cocktail solution samples being analysed in a Beckman LS-230 liquid scintillation counter.

Another series of samples with the same treatments already cited, were incubated for a period of 30 min. under 10% v/v acetylene for the nitrogenase activity assay. The ethylene formed was measured in a gas chromatograph Beckman GC-65 equipped with porapak N column.

In the second experiment the effect of light on nitrogenase (atm air) activity was studied, with the following treatments: a) atmospheric air under light conditions; b) atmospheric air under dark; c) Argon atmospheric + 0.3% CO$_2$ (light; d) idem c (dark). Plants were grown as in Expt. 1. Again 35 ml flasks were used. Flasks for dark treatment were covered with aluminum foil and incubated under dark conditions for two hours before the experimental was started and then the treatments were applied.

In the third experiment the effect of light on hydrogenase activity was studied, with the following treatments with four replications: a) plants under light conditions; b) died plants under light conditions; c) plants under dark conditions, and d) died plants under dark conditions. Died plants were used to eliminate $^3\text{H}_2$ diffusion on plant material.

*Azolla-anabaena* was grown as in Expt. 1. Plants of treatments under dark conditions were previously incubated in the dark for two hours before the hydrogen (Tritium) has been added in the vessels (1.0 of Azolla/35 ml flasks) as explained in Expt. 2. Evaluation of hydrogenase was determined under argon (Ar) atmosphere plus 0.3% hydrogen and 0.3% CO$_2$ (v/v) after one hour incubation. Nitrogenase activity (C$_2$H$_2$-reduction), H$_2$-(Tritium)-uptake and weight of plants were determined as described in the Experiment one.

Results and discussion

Hydrogen ($^3\text{H}_2$) absorption and nitrogenase activity by *Azolla-Anabaena* (A.A.) were studied under different atmospheres (atmospheric air, Ar and combination of those with carbon monoxide) as well as the effect of light on these activities (Table 1). It was observed that Azolla has hydrogen uptake activity, since under air or argon atmosphere, $^3\text{H}_2$ was absorbed, being higher with Ar than in air, indicating that probably there was a recycling of hydrogen evolved via nitrogenase.