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HYDROGEN UPTAKE BY THE NITROGEN FIXING ROOT NODULES AND NODULE HOMOGENATES OF *CORIARIA NEPALENSIS*

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Abstract

Nitrogen fixing root nodules of *Coriaria nepalensis* consumed hydrogen on incubation with a gas mixture containing hydrogen, indicating an uptake hydrogenase activity. The H_2 uptake by the excised nodules varied throughout the year and was highest (2.16 μ mole H_2 consumed g fresh nodule wt.$^{-1}$ hr.$^{-1}$) in May. The enzyme activity was detected in vesicle cluster suspensions of the endophyte (20 μm residue) as well as in the 20 μm filterate fractions of the nodule homogenate.

Introduction

During reduction of N_2 by the nitrogenase system in microorganisms, considerable amount of energy is lost in the simultaneous process of reduction of protons to H_2 (Evans *et al.,* 1979). Several N_2-fixers posses an uptake hydrogenase which recycles this H_2, thus regenerating energy and reducing power which may be reutilized by nitrogenase (Schubert *et al.,* 1978; Emerich *et al.,* 1979; Evans *et al.,* 1979, 1981). This also provides a means of protection of nitrogenase from oxygen (Dixon 1972; Emerich *et al.*, 1979).

Uptake hydrogenase activity has also been reported in the *Frankia* induced N_2 fixing root nodules of non-legumes (Roelofsen & Akkermans, 1979; Hafeez *et al.,* 1984). The endophyte (*Frankia*) inhabits cortical cells of the nodule in the form of "Vesicle Clusters" that can be easily isolated from the host cells (Roelofsen & Akkermans, 1979). Isolated vesicle clusters are relatively pure endophyte tissue and can be used for study of various physiological processes such as nitrogenase activity, hydrogenase activity and other metabolic processes of the endophyte (Akkermans *et al.,* 1979; Benson *et al.,* 1979; Roelofsen & Akkermans, 1979; Hafeez *et al.,* 1984).

The occurrence of hydrogenase activity and its seasonal variation in the root nodules and nodule homogenates of *Coriaria nepalensis* is presented here.

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Materials and Methods

H_2-uptake by excised root nodules: Root nodules of *C. nepalensis* were collected from their natural habitat at Lower Topa, Murree every month for one year. Collection time was always between 11 a.m. to 2 p.m. The nodules were washed with water, dried on a filter paper and 1 g nodules incubated at ambient temperature in air tight bottles (7.5 ml) with a gas mixture containing 1% H_2. Control (a) contained only 1% H_2 in air, while control (b) contained only nodules and air for the detection of any H_2 evolution from the nodules. After preincubation for 10 minutes, the gas samples were taken at different time intervals to determine hydrogen uptake by the nodules using gas chromatograph (Hitachi, Model 163), equipped with a 1 m long molecular sieve column and a thermal conductivity detector.

Preparation of nodule homogenate for H_2 uptake: The homogenates, from green house collected nodules, were prepared according to Roelofsen & Akkermans (1979). Six g washed nodule lobes were homogenized anaerobically in N_2 atmosphere in 50 ml buffer with Virtis mixer (45-HI speed) for 3 min at 3000 rpm. The homogenization buffer (50 mM, P-buffer, pH 7.0) contained sucrose (0.3 M), soluble polyvinyl pyrolidine (4%), sodium dithinonite (10 mM) and dithioerythritol (10 mM). Under continuous flow of nitrogen the homogenate was passed through 100 μm and 20 μm filters, respectively to separate the vesicle clusters of the endophyte. The 20 μm residue was washed with phosphate buffer (dithioerythritol and sodium dithionite free) and suspended in 6 ml of the buffer (vesicle suspension). The 20 μm filtrate was centrifuged for 10 min @ 12,000 rpm. The pellet was resuspended in 6 ml of P-buffer (20 μm filtrate suspension) and the supernatant was also saved for the assay.

Fractions of vesicle suspension in 1.8 ml quantities was injected in 13.5 ml vials with 0.2 ml solution of 10 mM electron acceptor. Electron acceptors used were: 2, 6-dichlorophenol indophenol (DCPIP), methylene blue (MB) and phenazine metasulfate (PMS). The gas phase was nitrogen with 1% hydrogen uptake was measured on TCD system of gas chromatograph (Hitachi Model-163) after preincubation for 15 min.

Results

Root nodules of *C. nepalensis* did not evolve any detectable amount of hydrogen throughout the year. However, nodules consumed hydrogen on incubation with a gas mixture. The enzyme activity was low during winter and fluctuated considerably during different seasons (Table 1). With the emergence of leaves during early spring, the activity progressively increased with a peak of 2.16 μ mole H_2 consumed, g fresh nodule wt.$^{-1}$ hr.$^{-1}$ in May.

Table 1: H_2 uptake by nodules of *Coriaria nepalensis* excised during different months of the year.

Months	Ambient temperature °C	μ mole H_2 consumed.* g fresh nodule $wt.^{-1}$ $hr.^{-1}$
January	0.5	0.23
February	1.4	0.28
March	1.4	0.28
April	8.5	0.74
May	11.5	2.16
June	19.0	1.79
July	16.3	1.64
August	15.4	1.68
September	13.3	0.95
October	10.1	1.23
November	6.4	0.86
December	1.5	0.73

*Each value is an average of 3 determinations.

Hydrogen uptake by nodule homogenate: The hydrogenase activity noted in both fractions of the nodule homogenate, was higher in 20 μm residual fraction than 20 μm filterate (Fig. 1). No activity could be detected in the supernatant of 20 μm filtrate fraction. Intact nodules consumed H_2 @ 1.7 μ moles H_2. g fresh nodule $wt.^{-1}$ $hr.^{-1}$ and the rates in case of nodule homogenate (20 μm residue + filtrate) in the presence of PMS, MB and DCPIP were, respectively, 1.3, 1.2 and 0.7 μ moles H_2. g fresh nodule $wt.^{-1}$ $hr.^{-1}$.

Under the microscope, the 20 μm residue mainly consisted of intact vesicle clusters of the endophyte while 20 μm filterate contained only broken parts of host cells and of the endophyte.

Discussion

Like many other actinorhizal nodules e.g. *Alnus, Datisca,* (Roelofsen & Akkermans, 1979; Hafeez *et al.*, 1984) an uptake hydrogenase is also present in nodules *C. nepalensis.* The nodules always consumed H_2 from the gas phase while no net H_2 evolution was detected in control (nodule + air) during any season. Thus *Coriaria* nodules behave differently from those of *Alnus* that evolve H_2 during autumn (Roelofsen & Akkermans, 1979). The low activity of H_2 consumption rate in winter may be due to old, degenerate nodules, low temperatures and less availability of photosynthates since the plants survive cold winter in leafless dormant state. The enzyme hydrogenase is more active during

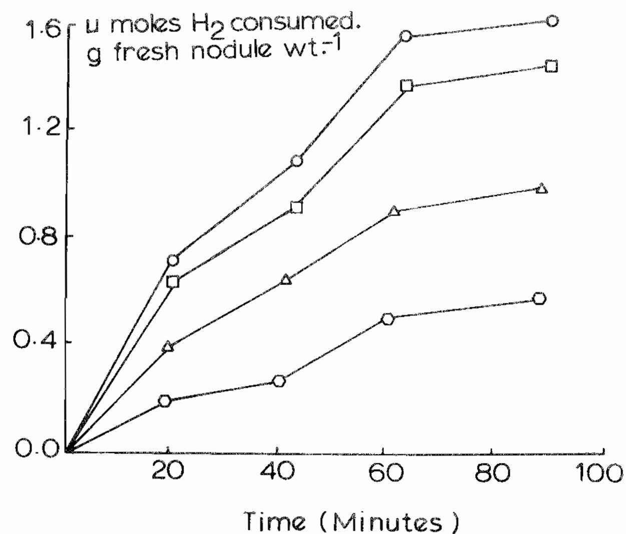

Fig. 1. H_2 uptake by intact nodules and nodule homogenate of *Coriaria nepalensis* in the presence of PMS. Intact nodules (○); nodule homogenate (20 μm residue + filtrate) (□); 20 μm residue (△); 20 μm filterate (◇).

summer when the conditions for vegetative growth of the host plant are favourable or possibly due to stimulation by the increase in H_2 production by the nitrogenase. The nitrogenase activity of *Coriaria* nodules also shows seasonal variations and is highest in summer (Mirza *et al.*, 1986).

Hydrogenase activity is present in vesicle cluster suspension as well as filterate fractions of the *C. nepalensis* nodule homogenate. High enzyme activity in 20 μm residue is obviously due to the presence of intact vesicle clusters of the endophyte, as confirmed under the microscope. The activity in the filterate, too is particle bound as exhibited in the pellet fraction while the activity was totally missing in the supernatant. The fact that hydrogenase activity of the nodule homogenate (20 μm residue + filterate) is less than that of the intact nodules may be due to partial inactivation of the enzyme or alternately all of the vesicle clusters may not be released from the host cells during homogenization. The results with nodule homogenates of *Coriaria* indicate that the uptake hydrogenase is associated with the endophyte. However, its confirmation awaits successful isolation and use of pure endophyte.

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