Hydrogen-supported Nitrogenase Activity in Two Cyanobacteria

R. Rao, M. Banerjee, A. Kumar, and H.D. Kumar
Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India

Abstract. The effects of hydrogen gas on nitrogenase activity of nitrogen-fixing cyanobacteria *Aulosira fertilissima* and *Chlorogloeopsis fritschii* were studied. Addition of as little as 10% hydrogen either in air, N₂, or N₂ + CO₂ markedly stimulated acetylene reduction. It has been shown that hydrogen can act as an alternate source both under reductant-saturating and reductant-deficient conditions. The results indicate the presence of a strong oxyhydrogen reaction in both species.

The process of nitrogen fixation in nitrogen-fixing microorganisms is affected by a variety of physical and physiological processes [5, 17]. In cyanobacteria, regulation of nitrogenase synthesis and/or activity by assimilable combined nitrogen sources has been studied [17, 19]. It has been shown that ammonia or a product of its metabolism inhibits nitrogenase synthesis and may also inactivate the nitrogenase already present in the cells in many nitrogen-fixing cyanobacteria [19].

Recently the role of hydrogen in regulating nitrogenase activity has been demonstrated [2, 11, 15, 16]. It has been shown that hydrogenase activity can increase nitrogen fixation in many nitrogen-fixing microorganisms [11]. As early as 1974, Bene mann and Weare reported hydrogen-supported nitrogenase activity in *Anabaena cylindrica*. They concluded that this activity in reductant-depleted cultures was due to the presence in the heterocysts of an ‘uptake’ hydrogenase that can link up with an electron transport chain to nitrogenase. The above findings were confirmed when hydrogen-supported acetylene reduction was demonstrated even in isolated heterocysts [9, 10, 13, 14].

The uptake hydrogenase enzyme has been isolated, purified, and shown to be present in the heterocysts [6, 7, 11]. Most previous studies have been carried out in *Anabaena cylindrica* or *Anabaena* strain 7120 [2, 4, 11].

The aim of the present investigation was to seek answers to the questions: (a) Does hydrogen support acetylene reduction in cyanobacteria other than *Anabaena*? (b) Is H₂-supported acetylene (C₂H₂) reduction higher in N₂, in N₂ + CO₂, or in air, and (c) Can recovery of partially inactivated nitrogenase be mediated by H₂ treatment? The study has been carried out in the genera *Aulosira* and *Chlorogloeopsis*.

Materials and Methods

The test organisms *Aulosira fertilissima* Ghose and *Chlorogloeopsis fritschii* Mitra were isolated from a wetland paddy field and a polluted pond in Varanasi respectively. Clonal cultures were raised from single spores and made axenic by standard microbiological techniques. Both the test algae showed optimum growth in Hughes’ medium devoid of any combined nitrogen sources [8]. Cultures were routinely grown in a culture room at 27 °C ± 1 °C and illuminated with daylight fluorescent tubes (14.4 W/m²) for 14 h per day.

Nitrogenase activity. Nitrogenase activity was measured by an acetylene reduction technique [18]. Unless otherwise stated, 2-ml cultures were taken in 7-ml vacutainer tubes (Becton, Dickinson, Rutherford, New Jersey, USA) and were then thoroughly evacuated, and the desired concentration of any gas (viz. N₂, CO₂, H₂, or C₂H₂) was injected by a hypodermic syringe. Acetylene concentration was kept at 10%, and all the assays were performed at 27°C and 2400 lx light intensity. Dark treatment was given by wrapping the vials with black aluminum foil. The ethylene formed was determined in a CIS (Baroda) gas chromatograph fitted with a Porapak R column and flame ionization detector. Chlorophyll was extracted in 80% acetone, and the chlorophyll a concentration was calculated from 663 nm absorbance according to Mackinney [12]. All the gases of high purity were obtained from Indian Oxygen Limited (Bombay). DCMU (3,3-dichlorophenyl-1,1-dimethyl urea) was a gift from late Prof. C. Van Baalen. It was dissolved in a minimum volume of ethanol and was added in culture suspension to obtain a final concentration of 1 × 10⁻³ M.

Address reprint requests to: Dr. Ashok Kumar, Department of Botany, Banaras Hindu University, Varanasi-221005, India.
Results and Discussion

Under aerobic conditions, actively growing cultures of *Aulosira* and *Chlorogloeopsis* show a typical C$_2$H$_2$ reduction rate of 4–5 nmol C$_2$H$_4$/µg chlorophyll $a$ per h. This rate is dependent on the normal endogenous source of reductant which ultimately comes through CO$_2$ fixation. If hydrogen is to act as an alternative source of reductant, it is imperative first to block the generation of endogenous source of reductant, i.e., CO$_2$ fixation. To achieve this, we incubated the algae under N$_2$ atmosphere, and thereafter the desired concentration of hydrogen was added. Figure 1 shows that addition of as little as 5% H$_2$ in N$_2$ stimulated the acetylene reduction rate. Maximum stimulation of about ten-fold was obtained with 10% H$_2$ in N$_2$ set. This activity (C$_2$H$_2$ reduction) is dependent on H$_2$ because the absence of CO$_2$ in N$_2$ gas phase will block CO$_2$ fixation, and ultimately the reductant pool is limited. Thus the exogenous addition of H$_2$ under such reductant-limited condition is utilized to drive nitrogenase activity. Addition of H$_2$ in N$_2$ + CO$_2$-supplemented sets also showed a similar trend of stimulation, and also the basal activity in N$_2$ + CO$_2$ alone was almost 25% higher than in the N$_2$ set. These data suggest that hydrogen is taken up by both the algae, and this in turn stimulates acetylene reduction activity. The increase in activity is not due to anaerobic conditions because 100% N$_2$ or 1% CO$_2$ + 99% N$_2$ shows one-tenth of the activity shown by the H$_2$-supplemented sets (Fig. 1).

To test whether such stimulation of nitrogenase activity also occurs under air, the effect of hydrogen on C$_2$H$_2$ reduction was tested under aerobic conditions with 10% or 15% H$_2$ + air; there was significant stimulation of C$_2$H$_2$ reduction in both algae, although it was one-half as much as in the N$_2$ + H$_2$ sets (Table 1). These findings suggest that exogenous hydrogen may act as an alternative source of reductant in both the test algae. This was further confirmed by another set of experiments in which PS-II activity was blocked by the addition of DCMU. Addition of DCMU to N$_2$-fixing cultures led to rapid loss of acetylene reduction activity. The loss of C$_2$H$_2$ reduction activity by DCMU addition was more pronounced and rapid under aerobic condition than in the N$_2$ gas phase (see Fig. 2 and Table 1). This might be owing to a differential effect of DCMU on PS-II activity under aerobic and anaerobic conditions. Alternatively, the N$_2$ atmosphere somehow protects the nitrogenase from being rapidly inactivated. However, when DCMU and H$_2$ were added together, there was no decline in nitrogenase activity, and the acetylene reduction rate was almost equal to that in H$_2$-supplemented aerobic cultures. The above findings are in agreement with earlier observations made on *Anabaena cylindrica* and *Nostoc muscorum* (*Anabaena* 7119), in which hydrogen-dependent C$_2$H$_2$ reduction activity was shown [1, 11, 15]. It is interesting that in both the algae, the acetylene reduction rates were supported considerably by hydrogen supplementation in the dark (Table 1). The dark rates with H$_2$ were about 65% and 75% in *Aulosira* and *Chlorogloeopsis*, respectively, of those in light H$_2$-supplemented aerobic cultures. It is pertinent to mention that both these algae showed prolonged C$_2$H$_2$ reduction activity in the dark even in the absence of any exogenous reductant sources. This might be due to the pres-