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Short communication

Differential effects of oxygen on N_2 fixation and C_2H_2 reduction by *Anabaena cylindrica*

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Both nitrogen fixation and acetylene reduction by intact cells of *Anabaena cylindrica* were inhibited by oxygen, but nitrogen fixation was invariably less sensitive than acetylene reduction. The C_2H_2/N_2 ratio ranged from 6 to 8 in the absence of oxygen, and it decreased with increase in partial pressure of oxygen to 2 at a pO_2 of 0.3 atm.
Key words: Acetylene reduction — *Anabaena cylindrica* — Nitrogen fixation.

Acetylene reduction technique has been widely used to investigate biological nitrogen fixation because of its simplicity, high sensitivity and inexpensiveness (*6*, *15*, *16*). Nitrogen fixation ($N_2 \rightarrow NH_3$) requires 6 electrons, whereas acetylene reduction ($C_2H_2 \rightarrow C_2H_4$) requires 2. Therefore, a C_2H_2/N_2 conversion factor of 3 is often used.

However, features of acetylene reduction are not always idenitcal with those of nitrogen fixation. Working with natural populations of blue-green algae, Peterson and Burris (*12*) have shown that the C_2H_2/N_2 ratio varies in a range from 3 to 7. A much larger variation has been reported by Mague et al. (*9*). We observed that the rate of acetylene reduction by *Anabaena cylindrica* increases initially with time and attains a steady level after about 30 min. This lag phase was not observed for nitrogen fixation (*10*). In this paper, we report that nitrogen fixation is significantly less sensitive to oxygen than is acetylene reduction, evidence for the operation of an internal protecting mechanism to oxygen in nitrogen fixation.

Three to four day-old cells of *A. cylindrica*, grown under nitrogen-fixing conditions (*11*) were used. Three milliliter portions of the algal suspension were placed in serum bottles (13 ml vol) with Venoject rubber caps, then they were incubated for 30 min in the light (10,000 lux) under an argon atmosphere. DCMU (5×10^{-5} M) was added to each bottle to prevent photosynthetic oxygen evolution. The gas space was filled with gas mixtures of the indicated compositions. Incubation was conducted at 28°C in the light (10,000 lux) with shaking. At 15 min intervals, 0.2 ml portions of the gas were removed by a Hamilton gas tight syringe, then ethylene production was monitored by gas chromatography (*14*). For the nitrogen fixation assay, 0.2 ml portions of the algal suspension were filtered through Reeve Angel 984H glass fiber filters. The collected algal cells were dried in a vacuum desiccator, after which cellular nitrogen was converted to N_2 by the modified Dumas method

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Table 1 *N_2 fixation and C_2H_2 reduction by* A. cylindrica *under anaerobic conditions*

Exp.	N_2 fixation nmoles (mg prot.)$^{-1}$ hr^{-1}	C_2H_2 reduction nmoles (mg prot.)$^{-1}$ hr^{-1}	C_2H_2/N_2
1	92	687	7.5
2	100	610	6.1
3	72	570	7.9
4	101	722	7.2

A. cylindrica cells were incubated under $^{15}N_2$ (95 atom%)-Ar (0.5 : 0.5) or C_2H_2-Ar (0.2 : 0.8) atmosphere.

of Wada et al. (*19*). ^{15}N content was determined by emission spectroscopy in a JASCO NIA-1 ^{15}N analyzer. Details of this method will appear elsewhere. Protein content was determined by the method of Lowry et al. (*8*).

Values of 6.1 to 7.9 (average 7.2) were obtained for the C_2H_2/N_2 ratio in the absence of oxygen (Table 1). These values are two times or more the theoretical value of 3. In the nitrogenase system, electrons flow simultaneously from the reductant to N_2 and H^+ to yield NH_3 and H_2 (*17*). In the presence of acetylene at a partial pressure of 0.17 atm, however, hydrogen evolution is completely inhibited (*13*). Therefore, under our assay conditions for acetylene reduction, electrons from the reductant exclusively flow to acetylene. Values higher than 3 that were observed for the C_2H_2/N_2 ratio thus can be, partly, explained.

The oxygen sensitivity of the acetylene-reducing activity in intact cells of *A. cylindrica* varies depending on their physiological state (*1*). We observed that a preliminary incubation of *A. cylindrica* under an argon atmosphere in the light raises sensitivity (data not shown). When algal cells of the same culture age were used, nitrogen fixation was invariably less sensitive to oxygen than was acetylene reduction (Fig. 1). At a pO_2 of 0.2 atm nitrogen fixation was reduced by only 20%, but acetylene reduction was reduced by 50%. This is another factor which might be responsible for the variation in the C_2H_2/N_2 ratio. In the absence of oxygen the ratio was 7, and at the pO_2 of 0.1, 0.2 and 0.3 the ratios were 6, 5 and 2, respectively. The mode of oxygen inhibition differs somewhat from that in the detached soybean nodules reported by Bergersen (*2*).

Oxygen-induced inhibition of acetylene reduction in the light was removed by the introduction of hydrogen (Fig. 2). At a pH_2 of 0.2 atm the rate of acetylene reduction in the presence of oxygen (pO_2=0.2 atm) was almost the same as that in the absence of oxygen. A similar H_2-dependent acetylene reduction in the presence of oxygen has been reported by Bothe et al. (*3*). Less sensitivity of nitrogen fixation to oxygen would result from the evolution of the hydrogen associated with nitrogen fixation. Benemann and Weare (*1*) and Bothe et al. (*4*) suggested that the hydrogen evolved by nitrogenase is utilized in the oxyhydrogen reaction mediated by the uptake hydrogenase to reduce oxygen tension at the site of nitrogenase. Two additional functions have been considered for the uptake hydrogenase: (i) production of ATP for nitrogenase coupled to the oxyhydrogen reaction, (ii) supply of a reductant for nitrogenase in the light in the absence of oxygen (*5*). However, our experimental conditions showed that the hydrogen serves primarily as an oxygen scavenger.

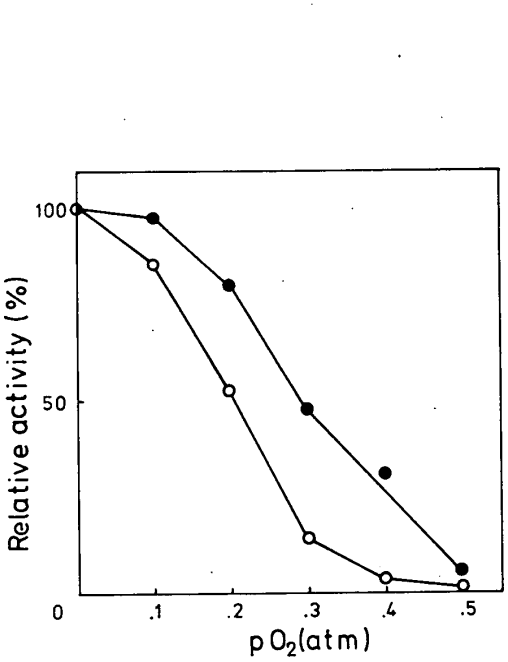

Fig. 1.

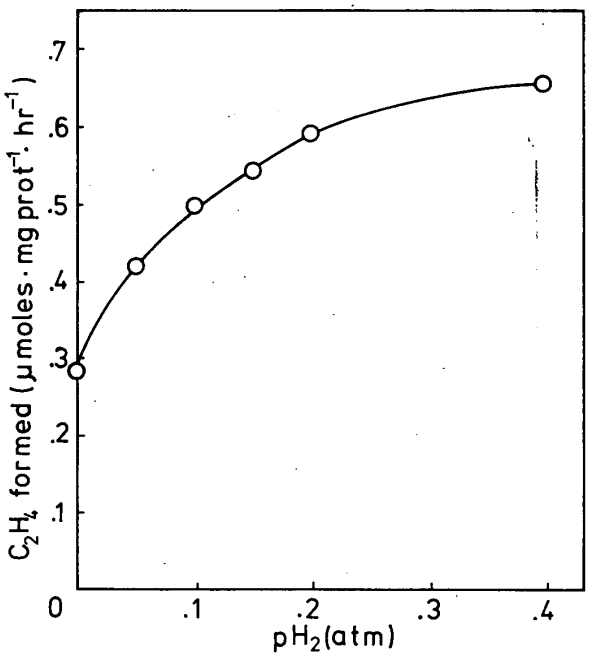

Fig. 2.

Fig. 1. *Effects of the partial oxygen pressure (pO_2) on nitrogen fixation and acetylene reduction by* A. cylindrica. The gas space was filled with a mixture of $^{15}N_2$ (95 atom%) : O_2 : Ar=0.5 : p : (0.5−p) for the N_2 fixation assay (●) or with mixtures of C_2H_2 : O_2 : Ar=0.2 : p : (0.8−p) for the C_2H_2 reduction assay (○). p: partial pressure of O_2.

Fig. 2. *Effect of the partial hydrogen pressure (pH_2) on acetylene reduction by* A. cylindrica *under aerobic conditions.* The gas space contained 0.2 atm O_2, 0.2 atm C_2H_2 and the indicated amounts of H_2; it was balanced with Ar.

The uptake hydrogenase is tightly bound to the cellular membrane (*18*), but nitrogenase is easily solubilized when algal cells are disrupted (*7*). In *A. cylindrica* cells, nitrogenase is probably associated with the uptake hydrogenase. This structural association is beneficial to a rapid recycling of hydrogen and an efficient removal of oxygen.

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