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THE HYDROGEN EVOLUTION MEDIATED BY NITROGENASE IN HETEROCYSTS OF FILAMENTOUS *ANABAENA* HA 101

CHI-YING HUANG

Abstract: The heterocyst formations from the initiation and differentiation of vegetative cells of filamentous *Anabaena* HA 101 were inhibited by the treatment of ammonium nitrate. Besides, those heterocysts, which had already formed along the filaments, could be hastened to degenerate by the ammonium nitrate. The nitrogen fixing (acetylene reducing) and hydrogen evolving activity of nitrogenase localized in heterocysts were closely related with the level of heterocyst formations and heterocyst degenerations. The nitrogenase activity for hydrogen production was decreased while the *Anabaena* were incubated with a gas mixture containing dinitrogen, which is the substrate of nitrogenase, but the enzyme activity was increased while the *Anabaena* were grown under a dinitrogen-limited condition. The oxygen was needed for the energy production for the nitrogen fixation and hydrogen evolution mediated by the nitrogenase. Although the *Anabaena* were treated with a gas mixture containing 20% oxygen, the nitrogenase in heterocysts remained high activities for nitrogen fixation and hydrogen production. The inhibitor, KCN, but not DCMU, suppressed the nitrogenase to reduce acetylene and to evolve hydrogen. This suggests that the energy required for nitrogenase activities was derived from oxidative phosphorylation of respiratory process but was not from the photophosphorylation of photosynthetic process.

INTRODUCTION

It had been reported that cells of cyanobacteria (blue-green algae) possess an enzyme so-called "reversible hydrogenase" (Bothe *et al.*, 1977; Daday *et al.*, 1979; Daday *et al.*, 1977; Hallenbeck *et al.*, 1981; Haystead *et al.*, 1970; Houchins and Burris, 1981; Kurtz and LaRue, 1971; Peterson and Wolk, 1978). This enzyme can evolve and uptake hydrogen while the cyanobacteria are grown under an anaerobic condition, and this enzyme activity is independent of nitrogen fixing activity of nitrogenase that occurs in heterocysts and can be inhibited by carbon monoxide (Bothe *et al.*, 1977). There is an another enzyme so-called "irreversible or hydrogen uptake hydrogenase" found in the heterocysts while the filamentous cyanobacteria are grown under an aerobic condition (Almon and Boeger, 1984; Asada and Kawamura, 1986; Bothe *et al.*, 1977; Daday *et al.*, 1977; Scherer *et al.*, 1980; Schubert and Evans, 1976; Smith and Evans, 1971; Tel-Or and Stewart, 1975). This enzyme can catalyze hydrogen uptake and support the nitrogen fixing activity of nitrogenase. Therefore, the presence of hydrogen-uptake hydrogenase in the nitrogen fixing system of cyanobacteria, the nitrogen fixing efficiency of nitrogenase can be enhanced. The activity of this uptake (irreversible) hydrogenase can be inhibited by

黃啓嶺 , Botany Department, National Taiwan University, Taipei, Taiwan, ROC.

C_2H_2 but not inhibited by carbon monoxide (Bothe *et al.*, 1977; Smith and Evans, 1971). The nitrogenase is the third enzyme relates with the hydrogen metabolism (Jeffries *et al.*, 1978; Laczko, 1980; Laczko and Barabas, 1981; Weisman and Benemann, 1977). It had also been reported that both nitrogen fixation and hydrogen evolution catalyzed by nitrogenase are all ATP-dependent reactions (Fay, 1980). The total energy utilized by the nitrogenase mediated reactions is sum of the energy used by nitrogena fixation plus by hydrogen evolution (Evans *et al.*, 1977; Schubert and Evans, 1976). Therefore, hydrogen evolution by nitrogenase decreases the efficiency of nitrogen fixation. However, in the presence of a saturated concentration of acetylene, there will be no hydrogen evolution by nitrogenase. Therefore, the total energy initially used by the nitrogen fixation is equivalent to the energy used by acetylene reduction (Evans *et al.*, 1977; Fay, 1980; Schubert and Evans, 1976).

In this study, it attempted to figure-out some characteristics of hydrogen evolution catalyzed by the nitrogenase in filamentous cyanobacterium *Anabaena HA 101*.

MATERIALS AND METHODS

Algal cultures

The filamentous cyanobacterium *Anabaena HA 101* (A gift of Dr. T. C. Huang, Institute of Botany, Academia Sinica) were cultured as stock cultures on the agar slants containing SM medium (Hwang *et al.*, 1973). The *Anabaena* growth conditions were light intensity, 200 lux; photoperiod, 16 hours, day/night temperature, 28/24°C. For the experiments, part of *Anabaena* were transferred from stock to liquid medium that also contained SM medium. The *Anabaena* cultures were aerated continuously at 250 ml per minute, and kept under the environmental conditions as light intensity, 6000 lux; photoperiod, 16 hours, day/night temperature, 30/24°C. The growth rates were measured by the optical density of *Anabaena* suspension in growth medium at 660 nm (A_{660}). The A_{660} would reach to 0.6 six days after the *Anabaena* had transferred to liquid medium without containing ammonium nitrate. The *Anabaena* cultures at this density were harvested for following experiments.

Determination of nitrogen-fixing activity

Ten ml of *Anabaena* was withdrawn from the liquid culture without containing ammonium nitrate and transferred into a 25 ml serum bottle to determine the nitrogen-fixing activity by the acetylene reduction method (Huang, 1978). One tenth of acetylene was injected into a serum bottle to give an acetylene partial pressure of 0.1 atmosphere. One hour after injection of the acetylene, samples of the gas phase were withdrawn and the acetylene and ethylene were separated and quantitated by means of a gas chromatograph equipped with a glass column 0.8 m long and 3 mm i,d. packed with Porapak Q, and a hydrogen-flame ionization detector. The carrier gas was nitrogen flowing at 30 ml per minute, and the oven temperature was 65°C. For the measurements of acetylene reduction activity of nitrogenase, a gas mixture that composed of Ar, 79.94%; O_2, 20%; CO_2, 0.06% was used to incubated *Anabaena HA 101* in the serum bottle.

Induction of heterocyst formation

The *Anabaena* culture at the cell density A_{660} 0.6 were harvested by centrifugation at 500 g for 10 minutes. This concentrated *Anabaena* were then cultured in SM medium containing

5 mM ammonium nitrate. This ammonium nitrate treatment inhibited the *Anabaena* to initiate and differentiate of vegetative cells into heterocysts. After eight days, the nonheterocystous filaments could be obtained. Those undifferentiated filaments were havested, washed twice with distilled water, and re-cultured in a nitrogen-free medium to induce *Anabaena* forming heterocysts along filaments. The heterocyst frequency was counted on microscope slide under the light microscope at 600 X magnification.

Determination of hydrogen evolution

In order to obtain maximal volumes of hydrogen evolved by nitrogenase, the *Anabaena* were incubated with a gas mixture composed of CO, 1%; C_2H_2, 10%; O_2, 20%; CO_2, 0.6%; Ar, 68.94%. It has been reported that activity of uptake hydrogenase, which can re-cycle the hydrogen evolved by nitrogenase, can be inhibited by C_2H_2 (Bothe *et al.*, 1977). The nitrogenase which reduces the acetylene to ethylene and reduces proton to hydrogen can't be suppressed by carbon monoxide (Bothe *et al.*, 1977). The hydrogen evolution mediated by nitrogenase in heterocysts of filaments was measured by gas chromatographic unit (Shimadzu, GC-8A) equipped with a thermal conductivity detector. The stainless column, 5 mm o.d. 2 m long, packed with a molecular sieve 5A; detector current, 6 mamp; flowing rate of carrier gas, Ar, 56 ml per minute; column and injection port temperature, 65°C and 100°C. Hydrogen concentrations were obtained from peak heights by reference to a standard curve.

RESULTS

The heterocysts were induced to form while the filamentous blue-green algae *Anabaena* HA 101 were grown in a medium free-from the ammonium nitrate. On the contrary, the numbers of heterocyst which had already formed along the filaments of cyanobacterium *Anabaena* were decreased or heterocysts were inhibited to form while those blue-green algae were cultured in a growth medium containing ammonium nitrate (Figs. 1, 3). The longer the ammonium nitrate treated to *Anabaena*, the lower heterocyst frequency was observed. As the data shown in Fig. 1, there was 5.7% of heterocysts found on the first day of ammonium nitrate treatment, but the heterocyst frequency was decreased to 0.48% seven days after the *Anabaena* had treated with ammonium nitrate. The *Anabaena* growth was significantly promoted by the ammonium nitrate. The optical density (A_{660}) of *Anabaena* cultures increased from initially 0.05 to 0.62 within seven days of growth in medium composed of ammonium nitrate.

The nitrogenase had both the activity of nitrogen fixation (acetylene reduction) and hydrogen evolution. The nitrogen fixing activity and the hydrogen evolving activity of nitrogenase at a fixed population density, $A_{660} = 0.6$, were increased with increase in incubation time of blue-green algae. The specific activity of nitrogen fixation and hydrogen evolution were increased from 00 to 1.36 and 0.15 nmol 10 ml^{-1}, hr^{-1}, respectively, within the eight-hour measurements (Fig. 2).

The quantity of acetylene reduction and hydrogen evolution mediated by nitrogenase were positively related with the heterocyst formations. The rates of C_2H_2 formation and H_2 evolution were increased from 3.90 to 16.7, 30.5 and from 3.0 to 9.5, 13, nmol g dry-weight^{-1} hr^{-1}, respectively, when the heterocyst frequencies were increased from 0.15 to 3.3%, 5.7% (Fig. 3). The heterocyst frequency along the filaments was increased with increase in optical density of *Anabaena* cultures. The heterocyst frequency increased rapidly from 0.15% to 3.3%, 5.7% while the population density of *Anabaena* was increased from 0.2 to 0.4, 0.6. However, the heterocyst formation was slowed-down while the population density of blue-green algae was

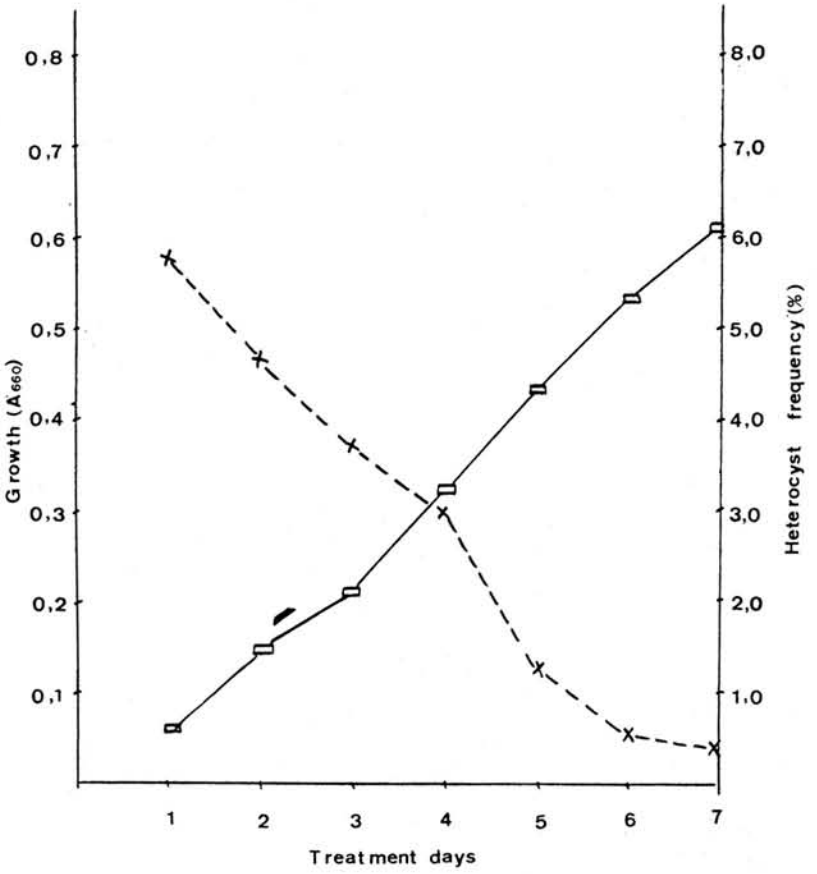

Fig. 1. The degeneration of heterocysts along the filaments of *Anabaena* HA 101 by the ammonium nitrate. The algal cultures growing in a nitrogen-free medium were harvested while their optical density were about 0.6. The heterocyst frequency was 5.7% when they were harvested. Then, the cultures were transferred to fresh growth medium containing 5 mM ammonium nitrate. This was accounted as the first day treatment and the A_{660} was 0.05. Growth, □ – □ – □; Heterocyst frequency, × – × – ×.

more concentrated than 0.6 (Fig. 3). Those *Anabaena* growing in a medium containing ammonium nitrate did not have heterocyst formation, and so they were lack of nitrogenase activity to reduce acetylene and to evolve hydrogen (Fig. 3).

The hydrogen evolution catalyzed by nitrogenase was repressed by the presence of dinitrogen gas. The nitrogenases gradually lost their activity of hydrogen evolution while those blue-green algae had transferred from a gas mixture without containing dinitrogen (Ar, 68.94%; O_2, 20%; C_2H_2, 10%; CO_2, 0.06%; CO, 1%) to a gas mixture composing dinitrogen gas, i.e. the Ar in gas mixture was replaced by N_2 (Fig. 4-a,c). As the data shown in Fig. 4, the rates of hydrogen evolution by nitrogenase were decreased from 11.2 to 1.9, and from 10.5 to 3.2 nmol g dry-weight^{-1} hr^{-1} within a day of incubation of *Anabaena* in a dinitrogen-containing gas mixture (Fig. 4-a,c). However, this inhibitory effect of dinitrogen gas on the hydrogen evolution was reversible. The nitrogenases re-gained their activity of hydrogen evolution from 1.9 to 10.5

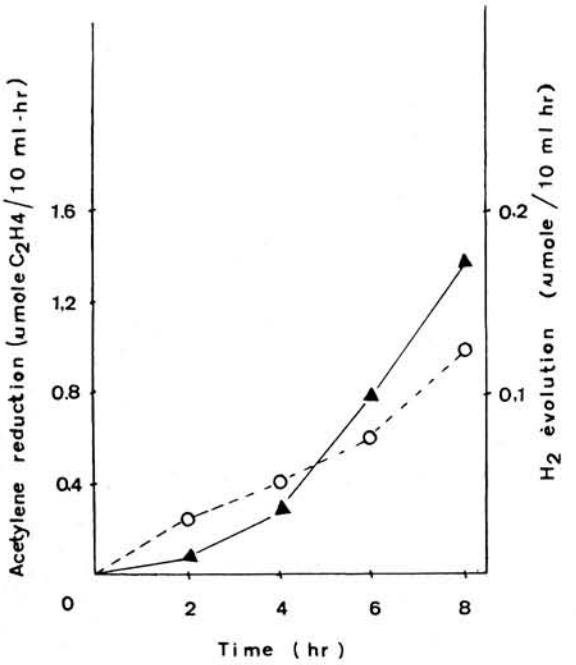

Fig. 2. Time course of acetylene reduction and hydrogen evolution by the nitrogenase. Ten ml of *Anabaena* HA 101 was withdrawn from the culture while the optical density of culture was about 0.6. Acetylene reduction, ▲ – ▲ – ▲; Hydrogen evolution, o --- o --- o.

(Fig. 4-b) or from 3.3 to 9.5 (Fig. 4-d) nmol g dry-weight^{-1} hr^{-1} when those blue-green algae had released from the dinitrogen treatment. The levels of restoration of hydrogen evolution were compatible with that of the controlled ones.

The acetylene reduction and hydrogen evolution activity of nitrogenase were inhibited while the blue-green algae were cultured under the oxygen-deficient condition. The *Anabaena* lost 70% of nitrogen fixing activity and 81% of hydrogen evolving activity two days after the blue-green algae had transferred to incubation flasks containing a gas mixture devoid of oxygen. The repressive effects caused by the oxygen-deficiency could be released when those algae were re-supplied with oxygen at a normal atmosphere pressure (Fig. 5). However, the repressive effects of oxygen-deficiency on the nitrogenase activities could not be fully restored while those blue-green algae had submitted to an oxygen-deficient growth condition for more than three days.

The acetylene reduction activity and hydrogen evolving activity of nitrogenase were also inhibited by the treatment of a respiratory inhibitor, KCN. The acetylene reduction and hydrogen evolution was reduced 74% and 77%, respectively, when those blue-green algae were treated with 100 μM of KCN, but the nitrogenase activities were not reduced by DCMU, unless higher concentration of DCMU, such as 50 μM, was used to treat *Anabaena* (Table 1).

DISCUSSION

There were about 6% of vegetative cells of filamentous *Anabaena HA 101* initiated and differentiated into heterocystous cells while those blue-green algae were grown in a medium

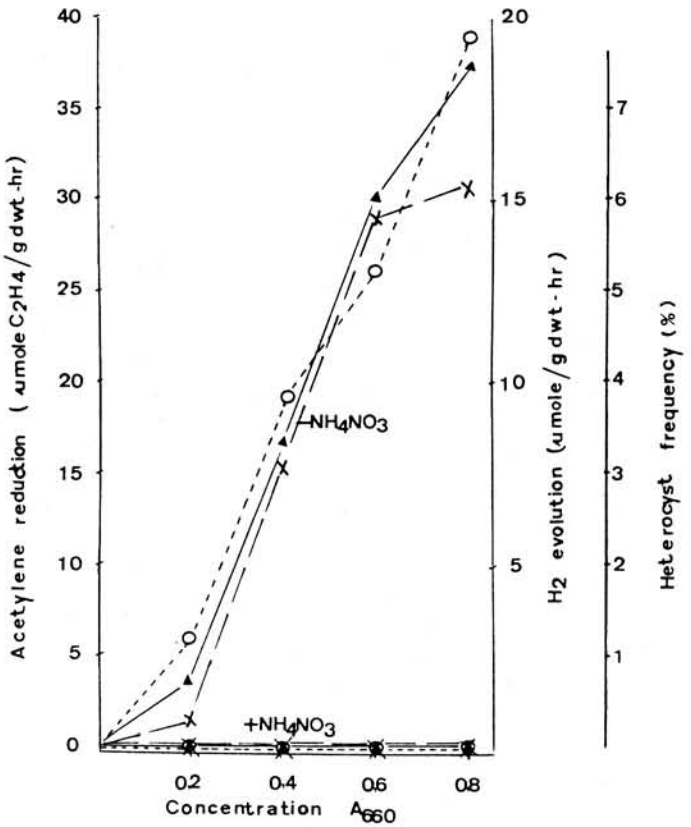

Fig. 3. The relationship between the heterocyst formation and the activity of nitrogenase for acetylene reduction and hydrogen evolution. The nonheterocystous-filamentous *Anabaena* HA 101 were obtained eight days after the algal cultures had grown in medium containing 5 mM ammonium nitrate. Part of those algal cultures were transferred to growth medium free-from ammonium nitrate. The A_{660} was 0.05. Another part of those algal cultures were grown continuously in a medium containing ammonium nitrate. The A_{660} was also 0.05. Acetylene reduction, ▲ – ▲ – ▲; Hydrogen evolution, ○ --- ○ --- ○; Heterocyst frequency, X – – X – – X.

free from ammonium nitrate. This transformation of undifferentiated cells into heterocysts was repressed when those *Anabaena* were treated with ammonium nitrate (Fig. 3). Besides, those heterocysts which had already formed were enhanced to degenerate by the ammonium nitrate (Fig. 1). This suggests that the blue-green algar might utilize more preferentially the ammonium nitrate than the fixed nitrogen by nitrogenase as the N-source for their growth and development when the ammonium nitrate and dinitrogen were present simultaneously. Therefore, the heterocyst formation and occurence for nitrogen fixation might be unnecessary or become useless when the blue-green algae were grown on medium supplying with ammonium nitrate. However, the exogenous ammonia rapidly and reversibly inhibited the nitrogenase activity in cells of *Rhodopseudomonas palustris* (Zumft and Castillio, 1978), *Azotobacter vinelandii* (Kleiner, 1975), or repressed the biosynthesis of nitrogenase in *Clostridium pasteurianium* (Daesch and

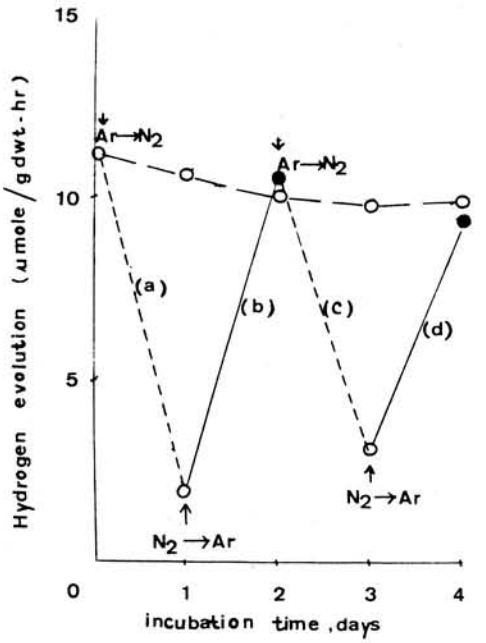

Fig. 4. The hydrogen evolution by nitrogenase in the presence or absence of dinitrogen. The *Abanaena* HA 101 was treated with a gas mixture containing dinitrogen, Ar → N_2 (a.c), or treated with a gas mixture devoid of dinitrogen, N_2 → Ar (b,d). The control, o – – o – – o.

Mortenson, 1967), *Azotobacter chroococum* (Dalton and Postgate, 1969; Drozd *et al.*, 1972) had also been reported.

The nitrogenase had both activity of nitrogen fixation (acetylene reduction) and hydrogen evolution (Figs. 2, 3, 5). However, the hydrogen evolution by nitrogenase was significantly reduced while the *Anabaena* were grown under dinitrogen condition. On the contray, the hydrogen evolution was significantly promoted while the *Anabaena* were grown under the dinitrogen-limited condition, i.e. the dinitrogen was replaced by argon (Fig. 4). This suggests that nitrogen fixation is an competitive inhibitory reaction for hydrogen evolution. Those blue-green algae grown in medium containing ammonium nitrate lost their ability of forming heterocysts, consequently, there were no activity of acetylene reduction and hydrogen evolution could be detected (Fig. 3). The nitrogenase activities were increased or inhibited closely with the increase in or decrease with heterocyst formation (Fig. 3). This suggests that nitrogen fixation and hydrogen evolution were occured in heterocysts of filamentous blue-green algae. However, some reports had been demonstrated that nitrogenase was present in all cells when filamentous blue-green algae were grown under ambient atmospheric condition (Kurtz and LaRue, 1971; Ohmori and Hattori, 1971). There were another reports shown that nitrogenase in heterocyst-forming blue-green algae was synthesized in vegetative cells as well as in heterocytous cells (Ohmori and Hattori, 1971; Rippka and Stanier, 1978; Smith and Evans, 1971; Gorkom and Donze, 1971) under the low oxygen tension.

The acetylene reducing and hydrogen evolving reaction mediated by the nitrogenase were repressed by the inhibitor of respiratory electron transport, KCN. but not inhibited by the inhibitor of photosynthetic electron transport, DCMU. Besides, the catalytic activities of

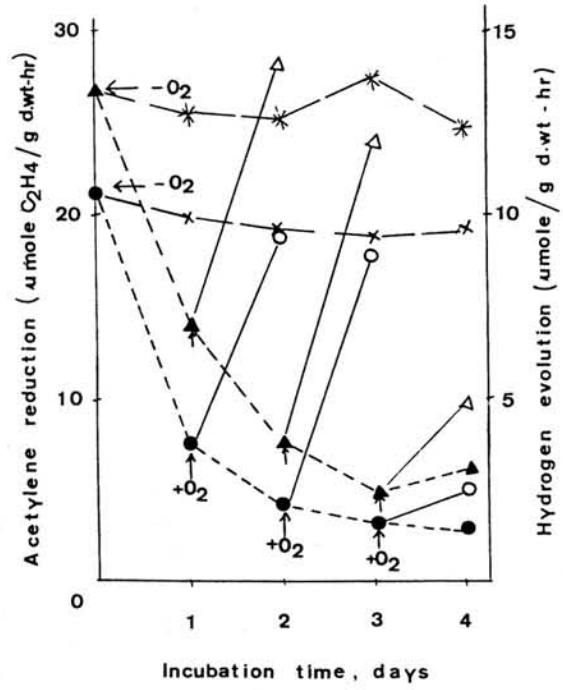

Fig. 5. The oxygen deficiency represses the acetylene reduction and hydrogen evolution mediated by nitrogenase. For the oxygen deficient treatment, the *Abanaena* HA 101 were transferred to flasks. The flasks were sealed with rubber stoppers and then flushed several times with a gas mixture devoid of oxygen. Acetylene reduction, ▲ --- ▲ --- ▲; Hydrogen evolution, ● --- ● --- ●. The upright arrow ↑ indicates that an another gas mixture containing oxygen was re-supplied to algal cultures in flasks while the algae had grown under the oxygen deficient condition for one, two, and three days, respectively. Acetylene reduction, * — — * — — *; Hydrogen evolution, X — — X — — X, in the control algal cultures. In order to prevent the vegetative cells to evolve oxygen photosynthetically, 50 μM of DCMU was added to medium.

Table 1. Effects of chemicals on the acetylene reduction (nitrogen fixation) and hydrogen evolution by nitrogenase in heterocysts of *Anabaena* HA 101

Treatment	Concentration (μM)	Acetylene reduction (%)	Hydrogen evolution (%)
1. Control		100	100
2. KCN	0.1	96	98
	10	73	85
	100	26	23
3. DCMU	1.0	100	100
	5.0	100	101
	50	60	43

nitrogenase were significantly reduced while those *Anabaena* were grown under the oxygen-deficient condition, and these inhibitory reactions could be reversed while the *Anabaena* were resupplied with oxygen (Fig. 5). This suggests that the acetylene reduction and hydrogen evolution catalyzed by the nitrogenase were ATP-dependent reactions, and this ATP was generated via the oxidative phosphorylation in respiratory process but not via the non-cyclic photo-phosphorylation in photochemical reaction of photosynthesis. It had been reported that heterocysts, unlikely the vegetative cells which contain photosystem I and II, contain photo-system I only (Alberte and Tel-Or, 1977; Donze *et al.*, 1972; Tel-Or and Stewart, 1975; Thomas, 1970). Therefore, heterocysts neither has the ability of ATP-generation nor hydrogen evolution via the process of non-cyclic electron transport, and so the ATP required for the acetylene reduction and hydrogen evolution was derived from the oxidative phosphorylation in process of respiration occurs in heterocysts.

The nitrogenase was very sensitive to oxygen (Fay and Cox, 1967; Lowe *et al.*, 1980). Therefore, all nitrogen fixing micro-organisms are obliged to protect the nitrogenase system from damaged by oxygen. In this study, the nitrogenase in heterocysts remained very active activities even if those *Anabaena* were treated with a gas mixture containing 20% of oxygen (Fig. 5). This suggests that aerobic blue-green algae which utilizes oxygen to generate ATP must simultaneously prevent oxygen from inhibition or damaging nitrogenase, and heterocyst, which loses the ability of oxygen evolution because of lack of photosystem II (Alberte and Tel-Or, 1977; Donze *et al.*, 1972; Tel-Or and Stewart, 1975; Thomas, 1970), plays an important role in regulation the oxygen-diffusion from outside into inside of heterocystous cell to keep the nitrogenase in heterocystous cell under the microaerobic environment (Granhall, 1976; Winkenbach *et al.*, 1972).

REFERENCES

ALBERTE, R. S. and E. TEL-OR, 1977. Characteristics of the photosynthetic apparatus of heterocysts and vegetative cells of *Nostoc* and *Anabaena*. Plant Physiol. (Suppl.); 129.

ALMON, H. and P. BOEGER, 1984. Nickel dependent uptake hydrogenase activity in the blue-green alga *Anabaena variabilis* 19. Z. Naturforsch **39**: 90-92.

ASADA, Y. and S. KAWAMURA, 1986. Aerobic hydrogen accumulation by a nitrogen-fixing cyanobacterium *Anabaena* sp. Appl. Environ. Microbiol. **51**: 1063-66.

BOTHE, H. J. TENNIGKEIT, G. EISHRENNER, and M. G. YATES, 1977. The hydrogenase-nitrogenase relationship in the blue-green alga *Anabaena cylindrica*. Planta **133**: 237-242.

DADAY, A., G. R. LAMBERT, and G. D. SMITH, 1979. Measurement in vivo of hydrogenase-catalyzed hydrogen evolution in the presence of nitrogenase enzyme in *Cyanobacteria*. Biochem. J. **177**: 139-144.

DADAY, A., R. A. PLATE, and G. D. SMITH, 1977. Anaerobic and aerobic hydrogen gas formation by the blue-green alga *Anabaena cylindrica*. Appl. Environ. Microbiol. **34**: 478-483.

DAESCH, G. and L. E. MORTENSON, 1967. Sucrose catabolium in *Clostridium pasteurianum* and its relation to nitrogen fixation. J. Bacteriol. **96**: 346-351.

DALTON, H. and J. R. POSTGATE, 1969. Growth and physiology of *Azotobacter chroococcum* in continous culture. J. gen. Microbiol. **56**: 307-319.

DONZE, M. J. HAVEMAN and P. SCHIERECK, 1972. Absence of photosystem II in heterocysts of blue-green alga *Anabaena*. Biochim. Biophys Acta **256**: 157-161.

DROZD, J. W., R. S. TUBB, and J. R. POSTGATE, 1972. A chemostat study of the effect of fixed nitrogen source on nitrogen fixation, membranes and free amino acids in *Azotobacter chroococcum*. J. gen. Microbiol. **73**: 221-232.

EVANS, H. J., T. RUIZ-ARQUESO, N. T. JENNINGS, and J. HANUS, 1977. Energy coupling effieiency of symbiotic nitrogen fixation. In Genetic Engineering for Nitrogen Fixation. ed., A. Holleander, Plenum Press, New York, pp. 333-354.

FAY, P., 1980. Nitrogen fixation in heterocysts. In "Recent Advances in Biological Nitrogen Fixation". ed., N. S. Subbao Rao, Edward Arnold, pp. 121-165.

FAY, P., and R. M. COX, 1967. Oxygen inhibition of nitrogen fixation in cell free preparations of blue-green

alga. Biochim. Biophys. Acta **143:** 562-569.
GRANHALL, U., 1976. The presence of cellulose in heterocyst envelope of blue-green algae and its role in relating to nitrogen fixation. Physiol. Pl. **38:** 08-216.
HALLENBECK, P. C., L. V. KOCHIAN, and J. R. BENEMANN, 1981. Hydrogen evolution catalyzed by hydrogenase in cultures of cyanobacteria. Z. Naturforsch **36:** 87-92.
HAYSTEAD, A., R. ROBINSON, and W. D. P. STEWART, 1970. Nitrogenase activity in extracts of heterocystous and nonheterocystous blue-green algae. Arch. Mikrobiol. **75:** 235-243.
HOUCHINS, J. P. and R. H. BURRIS, 1981. Occurence and localization of two distinct hydrogenase in the heterocystous cyanobacterium *Anabaena* sp. strain 7120. J. Bacteriol. **146:** 209-214.
HOWARTH, D. C. and G. A. CODD, 1985. The uptake and production of molecular hydrogen by unicellular cyanobacteria. J. gen. Microbiol. **131:** 1561-1570.
HUANG, C. Y., 1978. Effects of nitrogen fixing activity of blue-green algae on the yield of rice plants. Bot. Bull. Academia Sincia **19:** 41-52.
HWANG, J. C., C. H. CHEN, and R. H. BURRIS, 1973. Inhibition of nitrogenase in catalyzed reductions. Biochim. Biophys. Acta **292:** 256-270.
JEFFRIES, T. W., H. TIMOUVIAN, and R. L. WARD, 1978. Hydrogen production by *Anagaena cylindrica*; effects of varying ammonium and ferric ions, PH and light. Appl. Environ. Microbiol. **35:** 704-710.
KLEINER, D., 1975. Ammonium uptake by nitrogen fixing bacteria. 1. *Azotobacter vinelandii.* Arch. Mikrobiol. **104:** 163-169.
KURTZ, W. G. W. and T. A. LARUE, 1971. Nitrogenase in *Anabaena flosaque* filaments lacking heterocysts. Naturwissenschaften **58:** 417.
LACZKO, I., 1980. Mechanism of hydrogen evolution by nitrogen starved *Anabaena cylindrica.* Z. Pflanzenphysiol. **100:** 241-246.
LACZKO, E. and K. BARABAS, 1981. Hydrogen evolution by photo bleached *Anabaena cylindrica.* Planta **153:** 312-316.
LOWE, D. J., B. E. SMITH, and R. R. EADY, 1980. The structure and mechanism of nitrogenase. In Recent Advances in Biological Nitrogen Fixation. ed., N. S. Subba Rao, Edward Arnold, pp. 39-39.
OHMORI, M. and A. HATTORI, 1971. Nitrogen fixation and heterocysts in blue-green alga *Anabaena cylindrica.* Plant Cell Physiol. **12:** 961-967.
PETERSON, R. B. and C. P. WOLK, 1978. Uptake hydrogenase found only in heterocyst; localization of an uptake hydrogenase in *Anabaena.* Plant Physiol. **61:** 688-691.
RIPPKA, R. and R. Y. STANIER, 1978. The effects of anaerobiosis on nitrogenase synthesis and heterocyst development by *Nostocacean* cyanobacteria. J. gen. Microbiol. **105:** 83-94.
SCHERER, S., W. KERFIN, and P. BOEGER, 1980. Increase of nitrogenase activity in the blue-green alga *Nostoc muscorum.* J. Bacteriol. **144:** 1017-1023.
SCHUBERT, K. R. and H. J. EVANS, 1976. Hydrogen evolution; a major factor affecting the efficiency of nitrogen fixation in nodulated symbionts. Proc. Nat. Acad. Sci. **73:** 1207-1211.
SMITH, R. V. and M. C. W. EVANS, 1971. Nitrogenase activity in cell-free extracts of the blue-green alga *Anabaena cylindrica.* J. Bacteriol. **105:** 913-917.
STEWART, D. W. P. and M. LEX, 1970. Nitrogenase activity of the blue-green alga, *Plectonema boryanum* strain 594. Arch. Mikrobiol. **73:** 250-260.
TEL-OR, E. and W. D. P. STEWART, 1975. Manganese and photosynthetic oxygen evolution by algae. Nature **258:** 715-716.
THOMAS, J., 1970. Absence of the pigments of photosystem II of photosynthesis in heterocysts of a blue-green alga. Nature **228:** 181-183.
VAN GORKOM, H. J. and M. DONZE, 1971. Localization of nitrogen fixation in *Anabaena.* Nature **234:** 231-232.
WEISMAN, J. C. and J. R. BENEMANN, 1977. Hydrogen production by nitrogen-starved cultures of *Anabaena cylindrica.* Appl. Environ. Microbiol. **33:** 123-131.
WINKENBACH, F., C. P. WOLK, and M. JOST, 1972. Lipids of membrances and of cell envelope in heterocysts of a blue-green alga. Planta **107:** 69-70.
WINKENBACH, F., C. P. WOLK, and M. JOST, 1973. Activities of enzymes of the oxidative and the reductive pentose phosphate pathway in heterocysts of a blue-green alga. Plant Physiol. **52:** 480-483.
ZUMFT, W. G. and F. CASTILLIO, 1978. Regulatory properties of the nitrogenase from *Rhodopseudomonas palustris.* Arch. Microbiol. **117:** 53-60.

藍綠藻 *Anabana* HA101 其異孢中固氮酶產生氫氣活性及固氮作用之研究

黃 啓 穎

摘 要

藍綠藻異孢之形成受到硝酸銨所影響，硝酸銨不僅可抑制異孢之分化形成並且可加快已形成異孢之老化作用。固氮酶產生氫氣及固氮作用與異孢之形成有密切之關係。異孢中之固氮酶其產生氫氣之作用受到氮氣之影響，當氮氣存在時，固氮酶產生氫氣之活性減少，相對的，當以 Ar 取代 N_2 時，固氮酶產生氫氣之活性又增加。固氮酶之固氮作用及產生氫氣之作用均受氧氣所控制，當藍綠藻處於缺氧狀態時，上述固氮酶活性均受到抑制，當藍綠藻再給予氧時，具固氮活性及氫氣產生作用又可恢復。若用 KCN 處理藍綠藻，具固氮酶活性又受到抑制，但是使用 DCMU 處理藍綠藻，具固氮酶活性則不受到抑制，可見藍綠藻異孢中之固氮酶具固氮作用及氫氣產生作用均需能源，而此能源則來自異孢之呼吸作用而非來自光合作用。