

# Physiology of Nitrogen Fixation in Two New Strains of *Anabaena*

Pei-Chung Chen

Department of Botany, National Chung Hsing University, Taichung, Taiwan, Republic of China

Z. Naturforsch. **40c**, 406–408 (1985); received December 30, 1984

Nitrogenase, Dark Effect, *Anabaena*

Two different cyanobacteria, *Anabaena* CH1 and CH2, were isolated from Taiwan paddy soils. Both strains can grow well with daily dilution method. *Anabaena* CH1 shows a blue-green color and *Anabaena* CH2 a green brownish one. Nitrogenase activity decreased as cultures were transferred from light to dark. When a darkened culture was placed again into the light, nitrogenase activity recovered within two hours, but not in the presence of chloramphenicol. Energy supply for nitrogenase within both strains was different. Nitrogenase activity of *Anabaena* CH1 was light-dependent and oxygen in heterocyst was exhausted through oxyhydrogen reaction. Except photosynthesis, respiration may be used as energy source for nitrogenase in *Anabaena* CH2. Respiration was the major one to protect nitrogenase against oxygen.

## Introduction

Nitrogen fixation in cyanobacteria, reduction of nitrogen to ammonia, is an energy consuming process. Solar energy captured by the light reaction of photosynthesis therefore is the most direct energy source for nitrogen fixation in heterocystous cyanobacteria. Nitrogenase activity will increase at the start of and keep higher during light period. At transfer from light to dark nitrogenase activity will decrease rapidly [1–3]. There are reports demonstrating ATP and reductant requirement for nitrogen fixation being provided by photosynthesis and respiration [2, 4]. Dark nitrogenase activity was reported to depend on reserve substances [5].

There are many different strains of cyanobacteria existing in the Taiwan paddy soils [6, 7]. Seven strains of nitrogen-fixing cyanobacteria were isolated and collected in our laboratory. Three of them can grow well in liquid culture medium. One strain of which, *Anabaena* CH2, was reported to exhibit its brownish color even when growing under conditions of relatively, high temperature and light intensity [6]. In this study, some physiological properties of nitrogenase of these two new strains (*Anabaena* CH1 and CH2) are reported.

## Materials and Methods

The cyanobacteria used in this study, *Anabaena* CH1 and CH2, were isolated from Taiwan paddy

soils. *Anabaena* CH1 is a shorter one, each filament consists of about 30 to 40 cells, with tapered terminal cells; vegetative cells contain gas vesicle. The cell size is about 2.5 to 3.5  $\mu\text{m}$  in diameter. *Anabaena* CH2 consists more than 50 cells in each filaments. The cell size is about 5  $\mu\text{m}$ . They were cultivated at 32 °C, in modified BG11 medium [8], bubbled with air, and continuously illuminated with 6 fluorescence lamps. Light intensity was 240  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ . Each batch culture was diluted every day to a chlorophyll concentration of 0.2  $\mu\text{g}/\text{ml}$  in order to keep the cells in a logarithmic growth state. The doubling times were 7 and 18 h for CH1 and CH2 respectively.

Nitrogenase assays were performed as previously described [6]. 5 ml cyanobacterial suspension was transferred to a 25 ml serum bottle; 30% acetylene was injected to each bottle, shaking with hand once per 5 min. The incubation condition was the same as in culture. After exactly 60 min, 0.5 ml 2N  $\text{H}_2\text{SO}_4$  were injected into the serum bottle to stop the reaction. 0.5 ml gas samples were taken for determination of ethylene by gas chromatography (Varian Model 3700). Oven temperature was 85 °C and a 9 feet long column filled with porapak N was used. The flow rate of carrier gas  $\text{N}_2$  was 30 ml/min.

## Results

When cyanobacterial cultures were transferred from light to dark, rates of nitrogenase activity (assayed in the light for 1 h) of *Anabaena* CH1 and CH2 declined in the dark (Fig. 1). When cultures were placed again into the light after 1 h dark incubation, recovery of nitrogenase activities took place immediately in both strains, but not in the presence

**Abbreviation:** CAP, chloramphenicol; pcv, packed cell volume.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen  
0341–0382/85/0500–0406 \$ 01.30/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

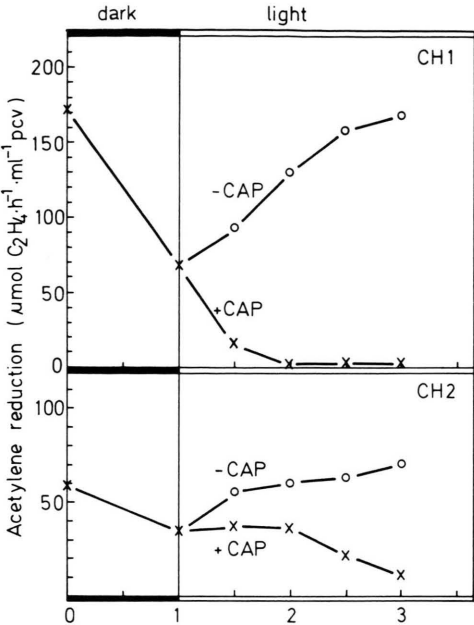


Fig. 1. Nitrogenase activity of *Anabaena* CH1 and CH2 which had been incubated in the dark for 1 h and then treated again in the light with (x) or without (o) chloramphenicol (50  $\mu\text{g/ml}$ ).

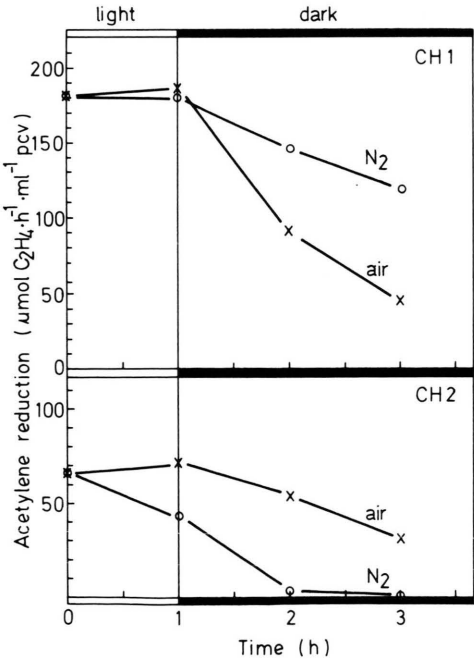


Fig. 2. Nitrogenase activity of *Anabaena* CH1 and CH2 incubated under air or  $\text{N}_2$ , 1 h in the light and then in the dark (assayed in the light). (x) under air; (o) under  $\text{N}_2$ .

of chloramphenicol (50  $\mu\text{g/ml}$ ). The loss of nitrogenase activity in *Anabaena* CH1 was less under  $\text{N}_2$  than under air. On the contrary, in CH2 loss of nitrogenase activity was complete within one hour under anaerobic conditions (Fig. 2). Otherwise, nitrogenase activity of CH2 also declined in the light and anaerobic conditions.

In Table I the results show that the rate of nitrogenase activity in the dark, aerobic conditions (assayed also in the dark) was only 5% (*Anabaena* CH1) or 50% (*Anabaena* CH2) as compared to those in the light. When put under anaerobic conditions nitrogenase activities of both strains was not altered significantly in the light. In the presence of DCMU and in the light, nitrogenase activity of *Anabaena* CH1 was totally inhibited under aerobic condition, but it was still remaining 25% activity when incubated under anaerobic condition. In contrast, DCMU inhibited only 15% of nitrogenase activity of *Anabaena* CH2 (Table I). Inhibitor KCN would inhibit totally nitrogenase activity of *Anabaena* CH1 no matter what incubated conditions. In the presence of KCN, *Anabaena* CH2 showed 25 of 50% activities under aerobic or anaerobic conditions respectively (Table I).

Table I. Nitrogenase activities of *Anabaena* CH1 and CH2 incubated with or without DCMU, KCN, under aerobic or anaerobic conditions. Activity was assayed in the light or in the dark for 1 h.

Spezies and Nitrogenase activity [ $\mu\text{mol C}_2\text{H}_4 \cdot \text{h}^{-1} \cdot \text{ml}^{-1} \text{pcv}$ ] conditions	Control DCMU [10 $\mu\text{M}$ ] KCN [10 mM]		
	Control	DCMU [10 $\mu\text{M}$ ]	KCN [10 mM]
<i>Anabaena</i> CH1			
light, air	160	2	0
light, $\text{N}_2$	167	32	0
dark, air	6	0	0
<i>Anabaena</i> CH2			
light, air	75	64	18
light, $\text{N}_2$	70	65	40
dark, air	39	34	18

Discussion

Energy for nitrogenase activity expressed in heterocystous cyanobacteria comes mainly from light. The rate of nitrogen fixation under heterotrophic conditions does not exceed 50 to 70% of the light rate [2, 9]. When using stored carbohydrates as energy supply cyanobacteria can fix nitrogen in the dark with only 20 to 30% of the activity measured in

the light [10]. The loss of nitrogenase activity after transferring cultures to the dark was reported to occur in *Anabaena* [1, 2]. Similar phenomena were measured in *Anabaena* CH1 and CH2 cultures. But their activities did not recover in the light when chloramphenicol was added (Fig. 1). It was different from that stated by Weare and Benemann (1973). It indicates that the declination of nitrogenase activity of *Anabaena* CH1 or CH2 in the dark is related to the biosynthesis of nitrogenase. When cyanobacterial cells were put under anaerobic conditions, dark declination of nitrogenase activity of *Anabaena* CH1 could be protected (Fig. 2). On the contrary, nitrogenase activity of *Anabaena* CH2 under anaerobic conditions was less than that under aerobic conditions whether CH2 was incubated in the light or in the dark (Fig. 2). It can be interpreted as that each heterocystous cyanobacteria might serve individual way to protect nitrogenase against oxygen in nature. Such as in *Anabaena* CH1 uptake hydrogenase will participate in oxygen protection mechanism. In *Anabaena* CH2 respiration is the major one to protect nitrogenase against oxygen and meanwhile supply energy and reductant to nitrogenase (Table I). Under anaerobic condition nitrogenase activity of *Anabaena* CH2 in the light decreased due to stopping the oxidative respiration (Fig. 1). In the dark and anaerobic conditions, the rapidly turnover rate of nitrogenase may be the reason for declination of nitrogenase activity (assayed in the light) in *Anabaena* CH2.

Nitrogen fixation in cyanobacteria requires much energy and reductant. The energy may be supplied in heterocyst by cyclic photophosphorylation or oxidative phosphorylation [2, 3], the oxyhydrogen reaction included [11, 12]. Reductant may be generated via the oxidative pentosephosphate pathway using fixed carbon transferred from vegetative cells [1, 3, 4], photosystem, or  $H_2$  [11, 13]. From the results shown in Table I, dark nitrogenase activity of *Anabaena* CH1 (assayed in the dark) was only 5% of that in the light and light activity would be totally inhibited by DCMU or KCN. It indicates that energy and reductant supply for nitrogenase is mainly dependent on photosynthesis. When CH1 cells put under light and anaerobic conditions, the oxyhydrogen reaction which used to exhaust oxygen existing in the heterocysts might be used as energy supplier for nitrogenase [11]. In *Anabaena* CH2, energy supply for nitrogenase might have two different ways. First, photosynthesis was not the only way to supply energy for nitrogenase, because DCMU inhibited only 15% of light activity. Second, using reserve substances through respiration [5] was possible, because *Anabaena* CH2 cells showed relative higher dark nitrogenase activity.

#### Acknowledgement

This study was supported by National Science Council, Republic of China.

- [1] N.M. Weare and J.R. Benemann, Arch. Mikrobiol. **90**, 323–332 (1973).
- [2] P.J. Bottomley and W.D.P. Stewart, New Phytol. **79**, 625–638 (1977).
- [3] R.H. Rowell, M.J. Reed, M.J. Hawkesford, A. Ernst, J. Diez, and W.D.P. Stewart, Proc. 4th Int. Symp. Nitrogen Fixation, Canberra. pp. 186–189.
- [4] M. Lex and W.D.P. Stewart, Biochim. Biophys. Acta **292**, 436–443 (1973).
- [5] A. Ernst, H. Kirschenlohr, J. Diez, and P. Boeger, Arch. Microbiol. **138**, (1984) in press.
- [6] P.C. Chen, Proc. Natl. Sci. Coun. B. ROC **7**, 317–321 (1983).
- [7] T.C. Huang, Natl. Sci. Coun. Monthly, ROC **10**, 619–631 (1983), in chinese.
- [8] G.W. Stratton and C.T. Corke, Can. J. Microbiol. **25**, 1094–1099 (1979).
- [9] P.J. Bottomley and W.D.P. Stewart, Arch. Microbiol. **108**, 249–258 (1976).
- [10] N.G. Carr and B.A. Whitton, The Biology of Cyanobacteria. Blackwell Scientific Publications, London 1982.
- [11] R.B. Peterson and R.H. Burris, Arch. Microbiol. **116**, 125–132 (1978).
- [12] W. Kerfin and P. Boeger, Physiol. Plant. **54**, 93–98 (1982).
- [13] B. Schrautemeier, H. Boehme, and P. Boeger, Arch. Microbiol. **137**, 14–12 (1984).