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Glycogen content and nitrogenase activity in *Anabaena variabilis*

Anneliese Ernst [1], Heide Kirschenlohr [2], Jesus Diez [3], and Peter Böger [1]

[1] Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany
[2] Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany
[3] Departamento de Bioquímica, Faculdad de Veterinaria, Universidad de Córdoba, Córdoba, Spain

Abstract. Nitrogenase (= acetylene-reducing activity) was followed during photoautotrophic growth of *Anabaena variabilis* (ATCC 29413). When cell density increased during growth, (1) inhibition of light-dependent activity by DCMU, an inhibitor of photosynthesis, increased, and (2) nitrogenase activity in the dark decreased. Addition of fructose stabilized dark activity and alleviated the DCMU effect in cultures of high cell density.

The resistance of nitrogenase towards oxygen inactivation decreased after transfer of autotrophically grown cells into the dark at subsequent stages of increasing culture density. The inactivation was prevented by addition of fructose. Recovery of acetylene-reducing activity in the light, and in the dark with fructose present, was suppressed by ammonia or chloramphenicol. In the light, also DCMU abolished recovery.

To prove whether the observed effects were related to a lack of photosynthetic storage products, glycogen of filaments was extracted and assayed enzymatically. The glycogen content of cells was highest 10 h after inoculation, while light-dependent nitrogenase activity was at its maximum about 24 h after inoculation. Glycogen decreased markedly as growth proceeded and dropped sharply when the cells were transferred to darkness. Thus, when C-supply (by photosynthesis or added fructose) was not effective, the glycogen content of filaments determined the activity of nitrogenase and its stability against oxygen. In cells lacking glycogen, nitrogenase activity recovered only when carbohydrates were supplied by exogenously added fructose or by photosynthesis.

Key words: Photosynthesis – Glycogen – Fructose – Reductant supply – Nitrogenase – Oxygen protection – Cyanobacteria

Cyanobacteria are able to accumulate a variety of putative reserve materials. Polysaccharides like glycogen, however, represent the main energy and reductant storage in these organisms (for review see Smith 1982). Accumulation of products of photosynthesis has been observed in batch cultures (Giesy 1964; Sutherland et al. 1979) and, when grown on a diurnal cycle, in continuous cultures (van Liere et al. 1979). Glycogen was isolated and characterized from *Nostoc muscorum* (Chao and Bowen 1971) and *Anacystis nidulans* (Weber and Wöber 1975). The interaction of glycogen content with nitrogenase activity, nitrogenase synthesis and stability has, however, not yet been studied.

In heterocystous cyanobacteria, aerobic nitrogenase activity is located in heterocysts (for review see Wolk 1982). Conceivably, nitrogenase activity as well as respiration rely on vegetative cells for reductant supply from photosynthesis or transient storage compound(s). With *Anabaena variabilis*, however, fructose can substitute for photosynthesis (Wolk and Shaffer 1976). Nitrogenase activity is under metabolic control through availability of low-potential reductant and ATP. In intact filaments, reductant limitation can be achieved by inhibition of photosynthesis by DCMU (Weare and Benemann 1973; Lex and Stewart 1973) or by darkening of cultures (Fay 1976; Ohmori 1984). In the dark, however, additional ATP limitation may occur in heterocysts (Ernst et al. 1983). In isolated heterocysts, light controls nitrogenase activity via reductant supply (Ernst and Böhme 1984).

Nitrogenase is highly labile in the presence of oxygen. Protection of the enzyme against oxygen deterioration, achieved by confining the enzyme to heterocysts, as well as by the absence of water photolysis in these cells may further be supported by enhanced respiratory activity (see e.g. Peterson and Burris 1978; Scherer and Böger 1982). Nevertheless, variable degrees of oxygen sensitivity of nitrogenase are observed in heterocystous cyanobacteria (Stewart and Pearson 1970; Bone 1972). As shown by immunological studies, nitrogenase is continuously inactivated by oxygen, degraded and resynthesized during growth under aerobic conditions (Murry et al. 1983, see also for further references).

This study has be performed to closer investigate the relationship of nitrogenase activity and stability to the glycogen pool. In contrast to previous studies in the literature, glycogen was extracted and specifically determined by an enzymatic method to overcome possible interference by intra- and extracellular carbohydrates different from glycogen.

Materials and methods

Anabaena variabilis (ATCC 29413; American Type Culture Collection, Rockville, Maryland) was cultivated autotrophically without combined nitrogen in a thermostated waterbath (Kniese-Edwards, Marburg, FRG) at a temperature of 29° C. Culture vessels, 4 cm in width, 46 cm in height, contained 220 ml of mineral medium according to Arnon et al. (1974) and were illuminated by fluorescent light (12 W/

Offprint requests to: P. Böger

Abbreviations. Chl, chlorophyll a; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

m^2, as measured by a radiometer of YSI-Kettering, model 65A). Agitation was maintained by bubbling air, enriched with 1.5% CO_2 (v/v) through the culture at a flow rate of 60 ml/min. When cultures were darkened as indicated, no extra CO_2 was supplied. The inoculum (110 µg of chlorophyll equivalent to 10 mg dry weight) was taken from a 48-h culture. However, similar results as shown in Figs. 4 and 5 were obtained with cells grown under light limitation in 250-ml Erlenmeyer flasks (2 W/m^2) or in a 5-l fermenter (Brunswick, Microferm) illuminated by 30 W/m^2 fluorescent white light.

To measure nitrogenase activity, acetylene reduction was performed in 7.8-ml glass vessels closed by Suba Seal rubber stoppers (Barnsley, UK) in a water-bath at 29°C either in the light (100 W/m^2) or in the dark. Unless mentioned otherwise, air was the gas phase of all assays, supplied with C_2H_2 (87/13%, v/v). Aliquots of the gas phase were analyzed every 15 min over a 45-min period. For short-term assays, the reaction was stopped by addition of 0.25 ml of trichloroacetic acid (30% w/v) after 15 min of incubation. Analysis of gases and determination of chlorophyll have been described previously (Ernst et al. 1983). Dry weight was determined according to Fay (1976).

For glycogen extraction, the cells were concentrated by two centrifugations (5 min, 1,500 × *g*) to yield 100 to 300 µg of chlorophyll per ml. Then, glycogen was extracted and determined by a method modified after Good et al. (1933) and Marshall and Whelan (1970), as follows: 50 µl of a concentrated cell suspension was added to 200 µl of KOH (30% w/v) and hydrolyzed for 90 min at 100°C in a water-bath. Cell homogenation by ultrasonication (Branson Sonifier C30) prior to KOH treatment did not increase the glycogen yields. To precipitate glycogen, 600 µl of absolute ethanol was added to the cooled extracts. Samples were kept on ice for 1 to 2 h, before glycogen was collected by centrifugation (5 min) in a microcentrifuge (Eppendorf, model 5413). Pellets were washed twice with ethanol. The pellets were dried under air for 5–10 min at 60°C and resuspended in 300 µl of acetate buffer (100 mM, pH 4.75). Thereafter, glycogen was digested by amyloglucosidase (4 units/assay; 1 unit converts 1 µmol of substrate/min under optimum conditions) and amylase (8 units/assay) for 1 h at room temperature, or 25 min at 55°C. Following digestion, insoluble membrane fragments were removed by centrifugation. Aliquots of the supernatant were assayed by a standard hexokinase/glucose-6-phosphate dehydrogenase assay coupled to $NADP^+$ reduction (Bergmeyer et al. 1974). The procedure was checked (1) by incubating cell material without enzymes to ensure that no soluble glucose had been left in the pellets after preparation and washing, and (2) by using commercial glycogen to control complete digestion. More than 95% of the original glycogen was recovered in the final assay. All data were obtained from duplicate determinations.

Chemicals. Enzymes and glycogen (from molluscs) were purchased from Boehringer, Mannheim; other compounds used were of the highest analytical grade available.

Results

1. *Growth of Anabaena variabilis.* Growth rate and growth yield of autotrophically grown cyanobacteria depend on illumination and carbon supplies (compare Sutherland et al. 1979; Haury and Spiller 1981). Growth is shown as the increase of chlorophyll (Fig. 1). After a 30-h exponential growth period, the increase in cell density decelerated (= post-log phase growth) (Sutherland et al. 1979). The stationary phase was not reached within the time of this

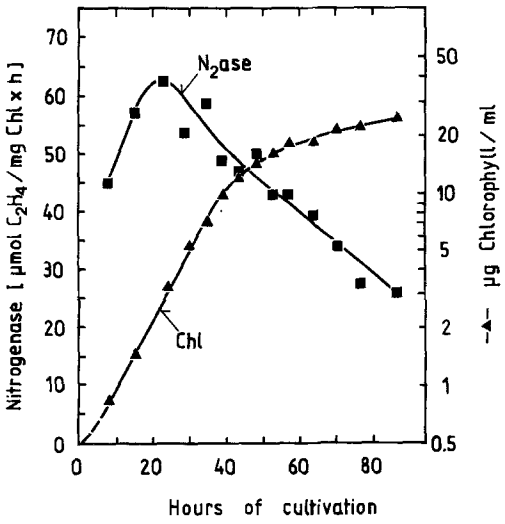

Fig. 1. Growth of *Anabaena variabilis* in batch culture. Eight cultures were grown in parallel as described in Materials and methods. Chlorophyll, referred to ml culture volume, is given as a growth parameter to be compared with nitrogenase (acetylene-reducing activity) in the light on a chlorophyll basis (indicating "specific" activity of nitrogenase)

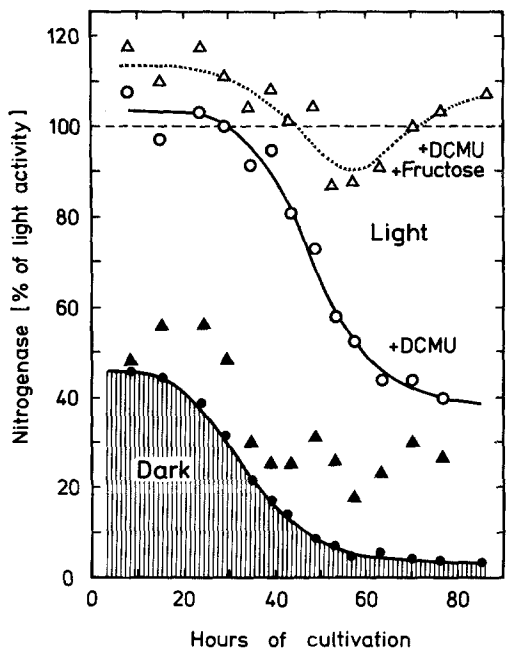

Fig. 2. Decrease of nitrogenase activity in the dark and in the light with DCMU present and its relief by fructose. Aliquots of an autotrophic culture taken after various hours of cultivation as indicated were assayed for nitrogenase activity. Rates were calculated by assuming linearity over a 45-min assay period. Nitrogenase activity in the dark (●——●); dark activity in the presence of 10 mM fructose (▲——▲); nitrogenase activity in the light with 5 µM DCMU present (○——○); activity in the presence of 5 µM DCMU and 10 mM fructose (△——△). Rates are percent of control-sample activities in the *light*, as measured after the particular cultivation period indicated, without any additions as shown in Fig. 1

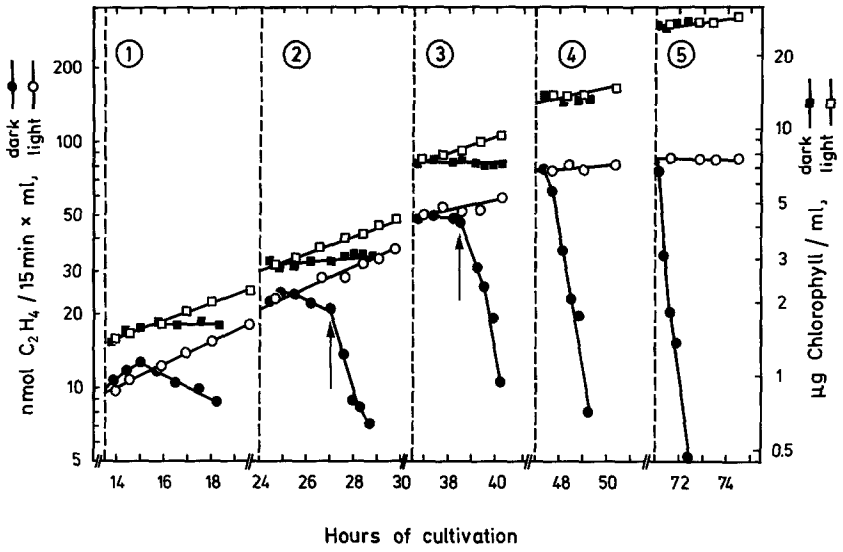

Fig. 3
Comparison of nitrogenase activity and chlorophyll content after dark incubations of autotrophic cells at various stages of growth. Five autotrophic cultures were grown in duplicates for different periods of time, namely 13.5, 24, 36.5, 47, 71 h in the experiments 1 to 5, respectively. After these cultivation periods, one culture vessel was withdrawn and placed into the dark, while growth in the others continued in the light. Aliquots were taken every 30 min from a dark and a light culture vessel (●——●, ○——○) and assayed for nitrogenase activity by a short-term assay of 15 min (see Materials and methods). Chlorophyll contents of the illuminated and darkened cultures are shown by square symbols (□——□, ■——■). The arrows in parts 2, 3 indicate the end of the slow and the start of the fast decay. The latter is observed exclusively in older cultures (parts 4, 5)

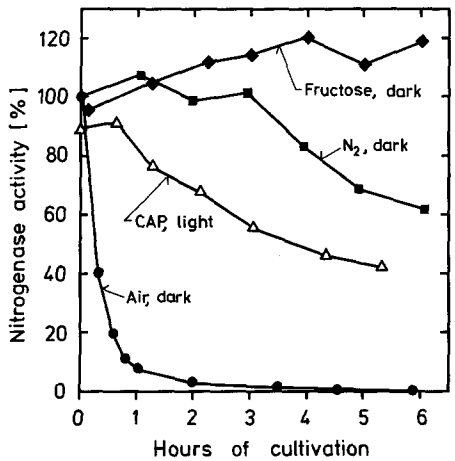

Fig. 4. Effect of the atmosphere and of fructose on nitrogenase activity in darkened post-log phase cultures. An aliquot of an autotrophic culture pregrown for 72 h (with nitrogenase activity of 110 nmol C_2H_4/ml × 15 min = 100%) was supplemented with chloramphenicol (CAP, 25 µg/ml), kept in the light, and nitrogenase activity assayed every hour (△——△). Three other aliquots of this culture were placed in the dark and sparged either with air (●——●), dinitrogen (■——■), or kept under air with 10 mM fructose present (◆——◆). All assays for nitrogenase activity were performed in the light over a 15-min period

experiment (85 h). The doubling time (for chlorophyll content) during exponential growth was 8.5 h. Nitrogenase activity developed exponentially with a doubling time of 6.9 h and became stationary after 50 h of growth (data not shown). With 60 µmol C_2H_4/mg Chl × h (equivalent to 0.78 µmol C_2H_4/mg dry weight × h), maximum "specific" activity was attained after about 24 h (Fig. 1).

2. *Nitrogenase activity.* Acetylene reduction in the light as shown in Fig. 1 was compared either to nitrogenase activity with DCMU present or to its activity in the dark (Fig. 2). Fructose, known to support growth of *Anabaena variabilis* (Wolk and Shaffer 1976), was added during the assay as a possible substitute for endogenous reductant when photosynthesis did not occur.

As demonstrated by Fig. 2, no inhibition by DCMU was observed in young cultures during a 45-min incubation period; inhibition became apparent in the late log-phase. After 80 h of cultivation, light-dependent nitrogenase activity was inhibited up to 60% by DCMU. However, this inhibition was alleviated by fructose. Data obtained in the presence of fructose scattered more than other data. This is explained by the fructose-uptake process being partly inducible in *A. variabilis* (Haury and Spiller 1981). Calculated over a 45-min period, dark nitrogenase activity in young cultures was 46% of that in the light. No stimulation by fructose was seen. Eventually, dark activity declined to 5% (Fig. 2) of the light control (shown in Fig. 1); fructose was able to restore about 25% of light activity (Fig. 2). This demonstrates that limitations occurring in the dark were only partly relieved by fructose under our conditions (comp. Wolk and Shaffer 1976).

3. *Oxygen-sensitivity of nitrogenase.* When aliquots of autotrophic cultures were transferred into the dark at various times during growth, oxygen sensitivity of nitrogenase increased markedly. In the following experiments, nitrogenase was assayed in the light over 15 min only, to overcome the limitation of activity observed in the dark (see preceding section) and to avoid induction of nitrogenase (see below). In cells grown for 13, 24 and 36 h, nitrogenase activity decreased very little during 6, 3 and 2 h after start of the dark incubation (see parts 1, 2, 3 of Fig. 3). This slow decay (Bone 1971; Murry 1983) is followed by a fast one as can be seen e.g. in parts 2, 3 of Fig. 3. The half-life of the slow decay was found to be 6 h, that of the fast one was less than 1 h. It should be noted that in cells from the early log phase (part 1 of Fig. 3), nitrogenase activity as well as chlorophyll content even increased initially, similar to the sample kept in the light. During post-log phase growth, only the fast decay was observed (parts 4, 5).

The inactivation of nitrogenase observed after prolonged dark treatment and in old cultures was more rapid than the enzyme turnover in the light. This was shown by addition of chloroamphenicol (CAP) to an illuminated culture of the post-log phase (Fig. 4). The rapid activity decay did not occur when darkened cultures were sparged with dinitrogen

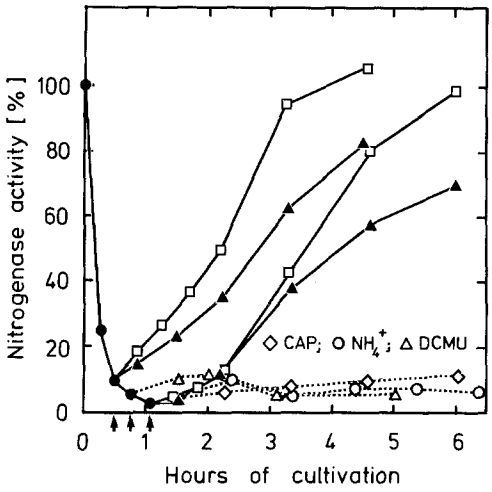

Fig. 5. Inactivation and reactivation of nitrogenase activity in a post-log phase culture. Nitrogenase was inactivated by aerobic dark treatment for 1 h (●——●). Arrows indicate the time when different treatments for reactivation were started: (a) by light (□——□), or (b) by addition of 10 mM fructose to continuously darkened cultures (▲——▲). Reactivation was inhibited by chloramphenicol (25 μg/ml), ammonia (10 mM), or DCMU (5 μM); see symbols ◇, ○, △, respectively. With DCMU present, the atmosphere of the sample consisted of hydrogen

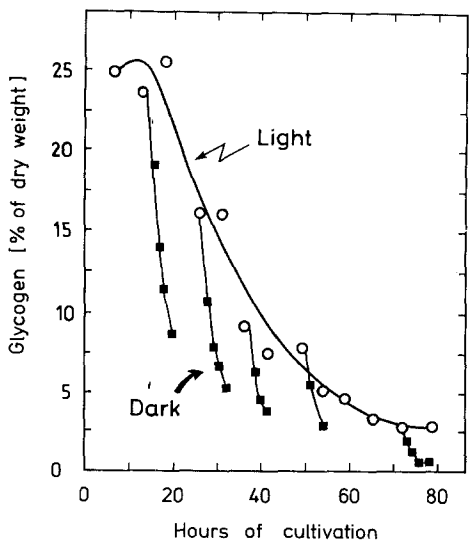

Fig. 6. Glycogen content of continuously illuminated batch cultures and glycogen degradation during dark treatment starting after different growth periods. Ten cultures were grown in parallel. Glycogen was extracted and assayed from illuminated cultures (○——○) and from culture samples transferred into darkness at various stages of growth as indicated (■——■). Glycogen degradation was followed over a 6-h period

(Fig. 4), indicative of oxygen being responsible for the loss of activity. Addition of fructose (10 mM) prevented inactivation of nitrogenase (Fig. 4, upper curve).

4. *Recovery.* Cells of the post-log phase with inactivated nitrogenase (curve ●——●, Fig. 5) contained less than 2% (w/w) of glycogen. Nitrogenase activity recovered when cells were illuminated (comp. also Bone 1971). This reappearance of nitrogenase activity was prevented by addition of DCMU, even in an anaerobic atmosphere (Fig. 5). In the dark, recovery was induced by addition of fructose. Dark and light recovery was prevented by addition of ammonia or chloramphenicol.

5. *Glycogen content.* Glycogen is a possible source of reductant for respiration as well as nitrogenase activity. During the first 10 h of growth, glycogen was accumulated up to more than 27% of the dry weight (Fig. 6). Noteworthy, the amount of glycogen dropped, (1) before maximum specific activity of nitrogenase was attained (about 24 h after inoculation, Fig. 1), and (2) before the growth rate decelerated, as judged by increase in dry weight or chlorophyll (30 h after inoculation, Fig. 1). At the end of the exponential growth phase, the glycogen content had decreased to 10% of the dry weight, and further diminished to 3% after 72 h of cultivation. Of course, the chlorophyll content per dry weight increased, when the glycogen content decreased (not documented here). The maximum of the *absolute* amount of glycogen in the culture (=62 μg as referred to *ml* culture volume) was attained at the end of the exponential growth phase.

Darkening of cultures after different period of light cultivation resulted in degradation of glycogen (Fig. 6, full symbols). The dark period (6 h for each aliquot) was not sufficient to completely deplete cells of glycogen, when these were taken from the exponential or early post-lag phase. In old cultures (late post-log phase), however, glycogen was dissimilated to a final content of 0.6% of dry weight.

Discussion

Little is known about the accumulation of glycogen, a storage product of photosynthesis. In contrast to former assumptions (Giesy 1964; Sutherland et al. 1979), it is shown here that cellular glycogen content reached a maximum shortly after inoculation and decreased during exponential growth. Net degradation of glycogen was observed in the dark. Therefore, old and darkened cultures of photoautotrophically grown *Anabaena variabilis* exhibited a decreased supply for reductant- and energy-requiring processes, like nitrogenase activity, biosynthesis and respiration. This was shown with batch cultures of post-log phase growth, when nitrogenase activity was assayed: a continuous decline of dark activity was paralleled by an increasing sensitivity of light-induced activity towards DCMU (Fig. 2). Both effects were alleviated by fructose yielding nitrogenase activity as high as in young cultures (Fig. 2). This indicates exhaustion of reductant supply for nitrogenase in cultures of high cell density and little glycogen storage (comp. Fig. 6).

Old cultures, which had lost nitrogenase activity during dark treatment, contained less than 2% (w/w) of glycogen. Recovery of activity can be suppressed by DCMU (and, of course, by ammonia or chloramphenicol, Fig. 5). Hydrogen was not able to substitute for lacking endogenous reductant in the presence of DCMU. Thus, carbohydrates, derived either from photosynthesis or from added fructose, are necessary for biosynthesis of nitrogenase, extending similar observations of Rippka and Stanier (1978) on organisms exhibiting anaerobic nitrogen fixation.

Little is known about oxygen protection of cyanobacterial nitrogenase. Besides a diffusion barrier formed

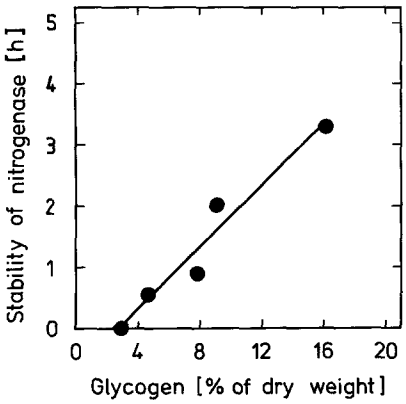

Fig. 7. Stability of nitrogenase in the dark at different levels of glycogen content. Glycogen content of cells taken after different hours of growth as shown in Fig. 6 was compared with stability of nitrogenase during dark incubation of filaments according to Fig. 3

by the heterocyst-cell wall (Walsby 1982), the oxyhydrogen reaction as a means of respiratory protection was demonstrated with isolated heterocysts (Peterson and Burris 1978). Additionally, it was reported that respiratory CO_2 release in the light had been found highest in dinitrogen-fixing strains of five cyanobacteria (Scherer and Böger 1982), and that CO_2-release accompanied induction of nitrogenase in a non-heterocystous *Phormidium* (Weisshaar and Böger 1983). In this study, oxygen sensitivity caused a rapid loss of nitrogenase activity in old cultures (Fig. 3, part 5, Fig. 4) which contained less than 3% (w/w) of glycogen, while in younger cultures, having more glycogen (Fig. 6) a period of relative stability preceded the rapid inactivation (Fig. 3, parts 2, 3). No inactivation was seen, when photosynthesis was active (Fig. 3, open symbols) or in the presence of fructose (Fig. 4). Apparently, a supply of carbohydrates for respiration is a prerequisite for oxygen resistance of nitrogenase.

It was shown that oxygen protection in heterocystous cyanobacteria is not complete (Stewart and Pearson 1970; Weare and Benemann 1973). A detailed study of Bone (1972) documented that oxygen above air saturation leads to a fast decay of nitrogenase activity (half-life less than 1 h), while at air concentration of oxygen a slow decay (half-life time of about 6 h) was apparent. In our experiments, both types of decay were observed at 20% (v/v) of oxygen, however, they showed up *subsequently,* after the cultures had been placed into the dark (Fig. 3, parts 2, 3). With increasing cell density and decreasing glycogen content, the slow decay disappears and only the fast one is seen (Fig. 3, part 5; see Fig. 6 for glycogen content). A slow decay of nitrogenase activity with similar kinetics as observed by us was shown by Murry et al. (1983), when protein synthesis was artificially inhibited. The rapid dissimilation of glycogen as found in our darkened cultures (Fig. 6), however, indicates a continuous respiratory activity, maintaining a certain stability of nitrogenase (i.e. slow decay). We conclude that this stability is lost when respiration ceases (comp. Ohmori 1984) due to exhaustion of glycogen. Then, the fast decay will start.

Although the level of glycogen in heterocysts is not yet known, the carbohydrate reserves present in filaments at any time during growth can be correlated with the period of slow decay. Apparently, a linear relationship is obtained (Fig. 7) which allows to estimate a level below which no further protection by respiration is possible. This is 3% glycogen on a dry-weight basis. This threshold amount is lower than the minimum concentrations observed in non-nitrogen fixing *Oscillatoria agardhii* during dark/light cycles (van Liere et al. 1979). A similar cycle present in *Anabaena variabilis,* with glycogen levels as found in our study, would imply that nitrogenase activity is not rapidly lost during the night. In natural habitats, changing conditions may cause insufficient regeneration of the glycogen pool and limit resynthesis of nitrogenase (Fig. 5; comp. also Bone 1972). This may lead to a complete loss of the enzyme and to destabilization of a nitrogen-fixing cyanobacterial bloom. The ecological implication will be a matter of further investigation.

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References

Arnon DI, McSwain BD, Tsujimoto HT, Wada K (1974) Photochemical activity and components of membrane preparations from blue-green algae, I. Biochim Biophys Acta 357:231–245

Bergmeyer UH, Bernt E, Schmidt F, Stork H (1974) D-Glucose: Bestimmung mit Hexokinase und Glucose-6-phosphat-Dehydrogenase. In: Bergmeyer HU (ed) Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim, pp 1241–1246

Bone DH (1971) Kinetics of synthesis of nitrogenase in batch and continuous culture of *Anabaena flos-aquae.* Arch Mikrobiol 80:242–251

Bone DH (1972) The influence of canavanine, oxygen and urea on the steady-state levels of nitrogenase in *Anabaena flos-aquae.* Arch Mikrobiol 86:13–24

Chao L, Bowen CC (1971) Purification and properties of glycogen isolated from a blue-green algae, *Nostoc muscorum.* J Bacteriol 105:331–338

Ernst A, Böhme H (1984) Control of hydrogen-dependent nitrogenase activity by adenylates and electron flow in heterocysts of *Anabaena variabilis* (ATCC 29413). Biochim Biophys Acta (in press)

Ernst A, Böhme H, Böger P (1983) Phosphorylation and nitrogenase activity in isolated heterocysts from *Anabaena variabilis* (ATCC 29413). Biochim Biophys Acta 723:83–90

Fay P (1976) Factors influencing dark nitrogen fixation in a blue-green alga. Appl Environ Microbiol 31:376–379

Giesy RM (1964) A light and electron microscopic study of interlamellar polyglucoside bodies in *Oscillatoria chalybea.* Am J Bot 51:388–396

Good CA, Kramer H, Somogyi M (1933) The determination of glycogen. J Biol Chem 100:485–491

Haury JF, Spiller H (1981) Fructose uptake and influence on growth of and nitrogen fixation by *Anabaena variabilis.* J Bacteriol 147:227–235

Lex M, Stewart WDP (1973) Algal nitrogenase, reductant pools and photosystem-I activity. Biochim Biophys Acta 292:436–443

Liere L van, Mur LR, Gibson CE, Herdman M (1979) Growth and physiology of *Oscillatoria agardhii* Gomont cultivated in continous cultures with a light-dark cycle. Arch Microbiol 123:315–318

Marshall JJ, Whelan WJ (1970) Incomplete conversion of glycogen and starch by cristalline amyloglycosidase and its importance

in the determination of amylaceous polymers. FEBS Lett 9:85–88

Murry MA, Hallenbeck PC, Estiva D (1983) Nitrogenase inactivation by oxygen and enzyme turnover in *Anabaena cylindrica*. Can J Microbiol 29:1286–1294

Ohmori M (1984) Effects of preillumination on dark nitrogen fixation and respiration by *Anabaena cylindrica*. Plant Cell Physiol 25:125–130

Peterson RB, Burris RH (1978) Hydrogen metabolism in isolated heterocysts of *Anabaena* 7120. Arch Microbiol 116:125–132

Rippka R, Stanier RY (1978) The effects of anaerobiosis on nitrogenase synthesis and heterocyst development by *Nostocacean* cyanobacteria. J Gen Microbiol 105:83–94

Scherer S, Böger P (1982) Respiration of blue-green algae in the light. Arch Microbiol 132:329–332

Smith AG (1982) Modes of cyanobacterial carbon metabolism. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, London, pp 47–86

Stewart WDP, Pearson HW (1970) Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. Proc R Soc London B 175:293–311

Sutherland JM, Herdman M, Stewart WDP (1979) Akinetes of the cyanobacterium *Nostoc* PC 7524: Macromolecular structure and control of differentiation. J Gen Microbiol 115:273–287

Walsby AE (1982) The permeability of gas vesicles, vegetative cells and heterocysts of *Anabaena flos-aquae* to oxygen and nitrogen, in: IV Int Symp Photosynthetic Prokaryotes, Bombannes, France, abstract C33

Weare NM, Benemann JR (1973) Nitrogen fixation by *Anabaena cylindrica*. II. Nitrogenase activity during induction and ageing of batch cultures. Arch Microbiol 93:101–112

Weber M, Wöber G (1975) The fine structure of the branched α-D-glucan from the blue-green alga *Anacystis nidulans*: comparison with other bacterial glycogens and phytoglycogen. Carbohydrate Res 39:295–302

Weisshaar H, Böger P (1983) Nitrogenase activity of the non-heterocystous cyanobacterium *Phormidium foveolarum*. Arch Microbiol 136:270–274

Wolk CP (1982) Heterocysts. In: Carr NG, Whitton BA (eds) The Biology of Cyanobacteria. Blackwell Scientific Publications, London, pp 359–387

Wolk CP, Shaffer (1976) Heterotrophic micro- and macrocultures of a nitrogen-fixing cyanobacterium. Arch Microbiol 110:145–147

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