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CONTROL OF HYDROGEN-DEPENDENT NITROGENASE ACTIVITY BY ADENYLATES AND ELECTRON FLOW IN HETEROCYSTS OF *ANABAENA VARIABILIS* (ATCC 29413)

ANNELIESE ERNST and HERBERT BÖHME *

Lehrstuhl für Physiologie und Biochemie der Pflanzen, Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz (F.R.G.)

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Hydrogen-dependent nitrogenase activity was studied in heterocysts, isolated from the filamentous cyanobacterium *Anabaena variabilis* (ATCC 29413). Hydrogen provides reductant and ATP for nitrogenase via linear electron flow through Photosystem I. This allows for regulation of nitrogenase activity by controlling the turnover of the photosystem. When nitrogenase activity was varied by changing either the light intensity or the supply of reductant (i.e., hydrogen) or by inhibition of photosynthetic electron transport by DBMIB, no rate-dependent changes in cellular ATP concentrations were observed. This homeostasis of ATP was perturbed by addition of metronidazole, acting as alternative electron sink to nitrogenase, and by uncoupling agents like FCCP, gramicidin and nigericin. Valinomycin (in presence of KCl) exerted little effect on nitrogenase activity and adenylate pool composition. Metronidazole increased and uncoupling agents decreased cellular ATP concentration, ATP/ADP ratio and energy charge. Inhibition of nitrogenase activity by metronidazole was caused by reductant limitation; inhibition by uncoupling agents was due to energy limitation. Control exerted on nitrogenase activity by ATP (energy limitation) was more pronounced at high rates of electron flow to nitrogenase than during reductant limitation. When cellular ATP synthesis was suboptimal due to partial uncoupling, the connection of phosphorylation and nitrogenase activity by electron transport allowed for homeostasis of ATP also at a lowered cellular concentration.

Introduction

In some filamentous cyanobacteria 3–10% of the cells exhibit morphological and biochemical differentiation by forming heterocysts upon exhaustion of combined nitrogen during cultivation. Heterocysts are the major site of aerobic fixation of dinitrogen (for a review, see Ref. 1).

Nitrogen assimilation by the enzyme nitrogenase requires ATP and a low potential reductant [2]. Studies of the effect of ATP/ADP ratios or energy charge values on the activity of purified components of nitrogenase revealed a nonlinear relationship between these parameters [3,4]. These results strengthened the hypothesis of a major participation of energy metabolism in nitrogenase regulation in vivo [5].

However, Haaker et al. [6] rejected this hy-

* To whom correspondence should be addressed.

Abbreviations: ATTC, American Type Culture Collection; Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole; Tes, 2-{[2hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulphonic acid.

pothesis based on the observation that the intracellular ATP/ADP ratio did not change when nitrogenase was inhibited by uncouplers. Instead nitrogen fixation in Azotobacter [7,8], Rhizobium [9] and Rhodopseudomonas [10] was suggested to be controlled by the transfer of low potential electrons, driven by the membrane potential (reversed electron flow).

In cyanobacteria similar results were reported from the comparison of nitrogenase activity and the components of the protonmotive force generated at the plasmalemma membrane [11,12]. However, cyanobacteria differ from aerobic heterotrophs and Rhodospirillaceae by possessing a photosystem which allows for generation of reductant (reduced ferredoxin) at a redox-potential effective to supply nitrogenase with electrons (compare also the chloroplast-nitrogenase system [13]). Furthermore, cell-free extracts of heterocysts exhibit high rates of nitrogenase activity not only in presence of dithionite, but also when several physiological reductants were used in the presence of an ATP generating system [14]. Neither of these activities was found to be sensitive to uncouplers [14,15]. This contrasts substantially with the findings in aerobic heterotrophs [6,16].

Isolated heterocysts of *Anabaena variabilis* exhibit high rates of light induced, hydrogen-dependent nitrogenase activity [17,18]. Recent experiments using freeze-thawed heterocysts [14] or cell-free homogenates of heterocysts [15] showed that Photosystem I is an essential part of the pathway of electrons from hydrogen to acetylene (nitrogen). A stoichiometric coupling of photophosphorylation and nitrogenase activity by linear electron flow through Photosystem I might regulate nitrogenase activity by the rate of electron flow without changing the cellular ATP concentration. Thus, a homeostasis of ATP will be possible (compare Kacser and Burns [19] for detailed discussion). To corroborate the homeostasis preserving nature of the energy-converting system in heterocysts, we compared acetylene reducing activity of nitrogenase and steady-state levels of ATP in presence of various inhibitors of phosphorylation. For comparison also ATP/ADP ratios and energy charge values are given in some experiments. However, cellular ATP concentrations sufficiently characterize the metabolic state of heterocysts, as they contain an active adenylate kinase with an apparent equilibrium constant around 1 [18].

Materials and Methods

A. variabilis (ATCC 29413) was grown and harvested as described previously [18]: heterocysts were isolated according to Ref. 18 (preparation A, comprising lysozyme treatment, osmotic shock and cavitation in an ultrasonic cleaning bath) or according to Ref. 17 (preparation B, lysozyme treatment and sonification). Whereas both preparations yielded heterocysts largely impermeable to hydrophilic substances (compare also Ref. 17), preparation A generally was accessible to membrane soluble substances like gramicidin, valinomycin and nigericin. In preparation B, nitrogenase activity was only inhibited by FCCP, but not by the other ionophores. Due to the osmotic shock used in preparation A, nitrogenase activity and adenylate content was lower and exhibited more variation. Therefore preparation B was used in most experiments.

Assays were performed in 7.8 ml vessels closed by rubber stoppers in a gas phase of H_2/C_2H_2 (87/13%; v/v) at 30°C. The reaction mixture contained: Tes/NaOH, 10 mM; $MgCl_2$, 10 mM; phosphate, 5 mM; sorbitol, 300 mM and bovine serum albumin (0.5%, w/v). The heterocyst-suspension (0.5 ml) contained 25 μg chlorophyll. Nitrogenase activity was determined by measuring ethylene formation [18]; chlorophyll, adenylate concentrations and energy charge values were determined as reported in Ref. 18. The intensity of white light was varied by neutral density filters (Oriel) and calibrated in the waterbath using a quantum sensor (Li-cor).

Metronidazole was dissolved in the assay mixture, other inhibitors in methanol. The methanol concentration never exceeded 2% of the assay volume. Inhibitors were added 5–10 min before the activity test was started. DBMIB was reduced by borohydride prior to use, since the reduced form is more resistant to debromation, causing inactivation, which is usually observed in intact cells [20].

Chemicals were obtained from following sources: FCCP and Gramicidin (Boehringer, Mannheim), Valinomycin (Serva, Heidelberg),

Metronidazole (May and Baker, Dagenham, Essex). Nigericin was kindly made available by Eli Lilly Laboratories, Indianapolis, IN, U.S.A.

Results

In a previous study [18] we showed, that light intensity controls nitrogenase activity, while little changes were found in the energy charge down to 10% of maximum nitrogenase activity. A similar relationship of nitrogenase activity to cellular ATP concentration, ATP/ADP ratio or energy charge was observed when photosynthetic electron transport was inhibited at the oxidizing site of Photosystem I by DBMIB (20–200 μM, Fig. 1).

Metronidazole, acting as an electron acceptor at the reducing site of Photosystem I [21] strongly inhibited nitrogenase activity. However, the steady-state concentration of ATP increased, as well as ATP/ADP ratio and energy charge values. This was observed in short time experiments (15 min) using low concentrations of metronidazole (Fig. 2). Prolonged incubation and enhanced levels of inhibitor (above 1.5 mM) diminished the increase in ATP concentration, probably due to secondary effects of reduced metronidazole [22].

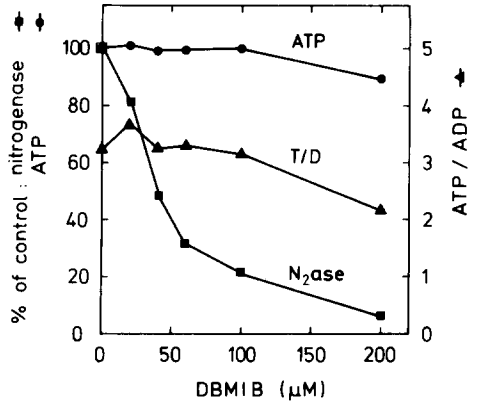

Fig. 1. Effect of DBMIB (20–200 μM) on nitrogenase activity, cellular ATP and the ATP/ADP ratio. Heterocysts were prepared according to method B. Assays were conducted in the light for 25–35 min and stopped by perchloric acid; adenylates were determined from the same sample. Activity (100%) of nitrogenase (N_2ase) was 129 μmol C_2H_4/mg Chl per h (■———■) and ATP content (100%) was 214 nmol/mg Chl (●———●), ATP/ADP (T/D) ratio (▲———▲). The energy charge values showed little variation (from 0.79 to 0.74).

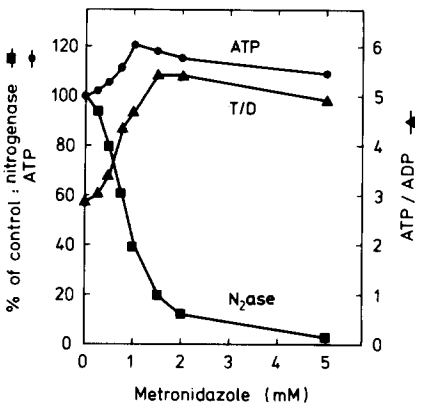

Fig. 2. Effect of metronidazole (0.2–5 mM) on nitrogenase (N_2ase) activity, cellular ATP concentration and the ATP/ADP ratio. The experiment was carried out as described in Fig. 1 but adenylates were extracted after 15 min. Nitrogenase activity (100%) was 135 μmol C_2H_4/mg Chl per h (■———■), ATP content (100%) was 227 nmol/mg Chl (●———●), ATP/ADP ratio (▲———▲). The energy charge increased from 0.80 to 0.89.

Uncoupling agents inhibit ATP synthesis by dissipating the energy stored in the chemiosmotic gradient [23]. A decline of steady-state levels of ATP, of the ATP/ADP ratio and of energy charge values was observed when FCCP was added to illuminated heterocysts (Fig. 3). Concomitantly, an inhibition of ATP-dependent nitrogenase activity was found. The ionophore nigericin showed a similar pattern (Fig. 4 left). Valinomycin, by increasing membrane permeability to potassium but also to ammonia, acts as a potent inhibitor of phosphorylation in mitochondrial and bacterial systems. No significant effect on either ATP pool or nitrogenase activity was found in a concentration range of 0.1–10 μM in the presence of KCl (50 mM, Fig. 4 right). The inefficiency of valinomycin did not result from impermeability of the ionophore, as addition of ammonium chloride (1 mM) caused inhibition of phosphorylation and nitrogenase activity (Fig. 4 right; compare also Ref. 24).

In Fig. 5 data obtained with several inhibitor concentrations are summarized as a plot of nitrogenase activity versus ATP pool concentration. Results of a series of experiments comprising

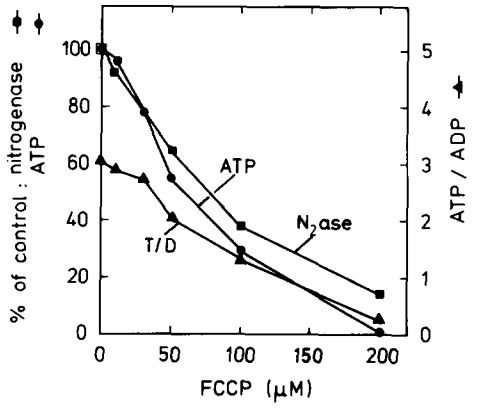

Fig. 3. Effect of FCCP (10–200 μM) on nitrogenase activity, cellular ATP concentration and the ATP/ADP ratio. The experiment was carried out as described in Fig. 1. Nitrogenase (N_2ase) activity (100%) was 101 μmol C_2H_4/mg Chl per h (■———■), ATP content (100%) was 239 nmol/mg Chl (●———●), ATP/ADP ratio (▲———▲). The energy charge was inhibited from 0.81 (control) to 0.21 at highest concentration of FCCP.

decreasing light intensities and decreasing hydrogen concentrations were included. When the rate of photosynthetic electron flow was varied either

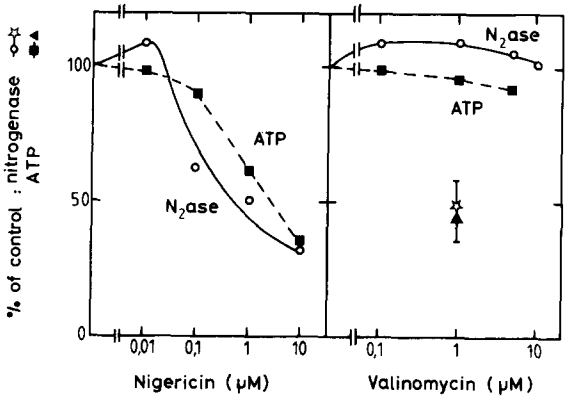

Fig. 4. Effect of nigericin (0.01–10 μM) and valinomycin (0.1–10 μM) on nitrogenase (N_2ase) activity and cellular ATP concentration. Heterocysts were prepared according to method A, assay conditions as in Fig. 1. Nitrogenase activity (100%) was 39 μmol C_2H_4/mg Chl per h (○———○), adenylate content (100%) was 180 nmol/mg Chl (■———■). The effect of valinomycin was tested in the presence of 50 mM KCl. At one concentration of valinomycin (1 μM) ammonium chloride was added to a final concentration of 1 mM; (nitrogenase activity (☆), ATP (▲)). Controls showed no inhibition of nitrogenase activity or ATP level by 1 mM ammonia alone.

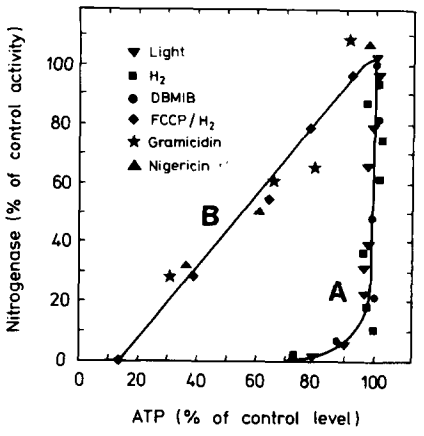

Fig. 5. Nitrogenase activity as a function of cellular ATP concentration in isolated heterocysts. Data obtained from Fig. 1 (DBMIB, ●), Fig. 3 (FCCP, ◆) and Fig. 4 (nigericin, ★) were supplemented by data obtained from variation of light intensity (▼) (3–300 μEinstein·m^{-1}·s^{-1}), variation of hydrogen concentration (■) (0.1–20%, v/v; balanced by Ar/C_2H_2), and gramicidin (★) (0.1–10 μg/ml). ATP concentrations and nitrogenase activity are given as percent of controls without inhibitor. Preparation method A was used in two experiments (gramicidin and nigericin), the other data were obtained with preparation B.

by light intensity, or by DBMIB a largely ATP-independent mode of regulation of nitrogenase was obtained (curve A).

The uncoupling agents FCCP, gramicidin and nigericin decreased the steady-state concentration of ATP followed by a decline of nitrogenase activity (curve B). Experimental variation was observed when results from different methods of heterocyst-preparation were compared (see Materials and Methods). However, the characteristic relationship (curve A and curve B) of nitrogenase activity to adenylate concentration was consistently observed, irrespective of the heterocyst preparation method used and irrespective of both maximum activity and adenylate content (comp. Fig. 3 and Fig. 4).

In order to characterize intermediate stages of regulation of nitrogenase by ATP and reductant supply we combined two inhibitory treatments. When electron transport was restricted by a constant amount of inhibitor (DBMIB, 40 μM, or metronidazole, 1 mM, Fig. 6A), no effect on steady-state concentration of ATP was seen when light intensity was decreased, except for low light

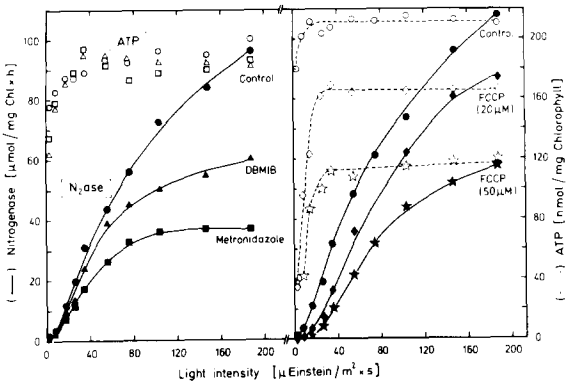

Fig. 6. Light dependency of nitrogenase activity and cellular ATP concentration in presence of constant amounts of inhibitors. Light intensity was varied by neutral density filters. Preparation and assay conditions were described in Fig. 1, nitrogenase activity (closed symbols) and ATP concentration (open symbols) are shown. (A) Control values (nitrogenase ●, ATP ○) without inhibitors are compared to experiments in presence of 40 µM $DBMIBH_2$ (▲, △) and in presence of 1 mM metronidazole (■, □). (B) Control values (nitrogenase (N_2ase) ●, ATP ○) are compared to experiments in the presence of 20 (◆, ◇) and 50 µM (★, ☆) FCCP.

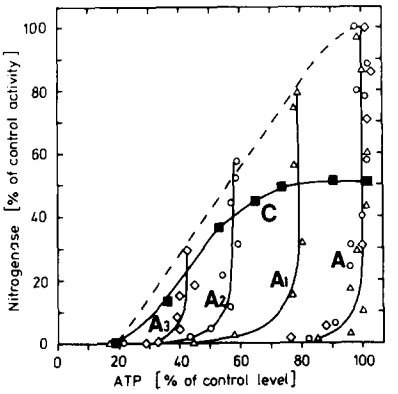

Fig. 7. Nitrogenase activity as a function of cellular ATP concentration from double inhibition experiments. Nitrogenase activity obtained by decreasing light intensity from 190 to 3 µEinstein·m^{-2}·s^{-1} in the presence of a fixed amount of FCCP is shown as a function of steady-state ATP concentration determined in the same sample. Control values without inhibitors accumulate in curve A. Addition of FCCP (20 µM in A1 (△), 40 µM in A2 (○), 100 µM in A3 (◇) establish parallel curves at lowered ATP levels. Another set of data (■) (curve C) was obtained by increasing concentrations of FCCP (10–200 µM) similar to the experiment described in Fig. 3, however, the gas phase consisted of H_2/C_2H_2/Ar (4.4/13/82.6%, v/v) thereby limiting nitrogenase activity to 50% of the control.

intensities (e.g., 3 µEinstein · m^{-2} · s^{-1}) when ATP concentration approached the dark value (compare Ref. 18). On the other hand, if nitrogenase activity was restricted by the presence of DBMIB and metronidazole, the amount of light necessary to saturate nitrogenase activity was reduced significantly (Fig. 6, left).

In a different set of experiments, light intensity was varied in the presence of fixed amounts of uncoupler (FCCP). Addition of FCCP diminished the steady state level of ATP and concomitantly inhibited ATP dependent nitrogenase activity in strong light. Lowering the light intensity further decreased nitrogenase activity without additional effect on the ATP pool (Fig. 6, right). In contrast to the effect of electron transport inhibitors, as shown in Fig. 6 (left), an increased fractional inhibition of nitrogenase activity by the uncoupler was observed when light intensity was lowered. Simultaneous analysis of ATP concentrations revealed, that the maximum ATP-level was reached at 8 µEinstein · m^{-2} · s^{-1} in the controls, whereas in the presence of FCCP (20 and 50 µM) ATP-homeostasis appeared above 20 and 30 µEinstein · m^{-2} · s^{-1}, respectively (Fig. 6, right).

In an ATP-versus-activity plot a series of nearly parallel curves was obtained by using fixed amounts of FCCP (20, 50 and 100 µM) while decreasing light intensity (Fig. 7) indicating that ATP was not limiting nitrogenase activity, when electron transport was suboptimal. A direct proof of this hypothesis was obtained by addition of increasing amounts of FCCP (10–200 µM) to a reductant limited assay. In this experiment, reductant limitation was caused by a suboptimal concentration of hydrogen (4.4% v/v hydrogen, balanced by argon/acetylene) in the assays. While decreasing the steady-state level of ATP, FCCP had little effect on nitrogenase activity, until energy limitation prevailed reductant limitation (Fig. 7, curve C).

Discussion

ATP requirement and the need for a low-potential reductant renders nitrogen fixation an expensive biosynthetic process. Numerous data obtained with the isolated nitrogenase complex suggest a

minimum requirement of 2 Mg · ATP hydrolysed per electron transferred to substrate during turnover [25], thus exceeding ATP demand of CO_2 fixation.

Studies on the electron transport in freeze-thawed [14] and cell-free [15] extracts from heterocysts revealed that electrons from hydrogen are transferred via Photosystem I and ferredoxin towards nitrogenase. Thus in cyanobacteria, the generation of the terminal reductant (ferredoxin) is coupled to photophosphorylation. When the ratio of ATP synthesized per electron transported through Photosystem I and the ratio of ATP consumed per electron transferred to a substrate of nitrogenase are constant, then nitrogenase activity is coupled to electron flow without variation of the cellular ATP-concentration [19]. A homeostasis of ATP was indeed observed at high values of energy charge (0.7–0.8) when nitrogenase activity in isolated heterocysts was varied by light intensity, hydrogen concentration or by addition of DBMIB (Figs. 1 and 5) or by a combination of these treatments (Fig. 6A). Though the rate of electron transport and therefore of the protonmotive force sensed by the ATPase is changed by any of these treatments, the ratio of ATP synthesized per electrons delivered to nitrogenase appeared not to be affected. At flow rates of electrons smaller than 10% of maximum activity, however, steady states of ATP were established at concentrations lower than the homeostatic level (Fig. 6A). This indicates that either photophosphorylation or nitrogenase or both changed their ATP/e ratios at low activities.

We applied several inhibitors (metronidazole, uncouplers) to impair photophosphorylation and nitrogenase activity. Metronidazole inhibits nitrogenase activity by oxidizing ferredoxin [21]. This changed the relative activities of the ATP-producing and the ATP-consuming system. Fig. 2 demonstrates that ATP-concentration, energy charge, and the ATP/ADP ratio raised upon treatment with metronidazole. Despite the highly favorable effect of an increased ATP/ADP ratio on nitrogenase [4,5], its activity was severely inhibited, underlining the limitation of nitrogenase by electron supply.

Uncouplers dissipate the proton gradient necessary for ATP synthesis, but stimulate the flow of electrons, when an electron acceptor is available. Since acetylene, the final electron acceptor, is not rate-limiting in our assay system, its reduction is controlled by the catalytic activity of nitrogenase, which in turn is limited by the cellular concentration of ATP. In the presence of an uncoupler, enhanced electron flow to nitrogenase lowered the steady-state concentration of ATP by nitrogenase consuming ATP. Thus linear electron transport from hydrogen to acetylene via nitrogenase was inhibited by uncouplers through a lower ATP/ADP ratio (Figs. 3 and 4, and curve B in Fig. 5).

When uncouplers were added, a decreased range of ATP-homeostasis and enhanced inhibition of nitrogenase was observed at low light intensities (Fig. 6B). In line with the discussion above, a diminished efficiency of photophosphorylation at low rates of electron flow in presence of uncouplers [26] caused energy limitation of nitrogenase resulting in a lag-phase of its activity.

Electron-flow limitation (Fig. 5, curve A) and energy limitation (Fig. 5, curve B) represent two limiting cases of regulation of nitrogenase. We used double inhibition experiments to demonstrate intermediate stages of regulation. The results of Figs. 5, 6 and 7 can be summarized as follows. (1) Heterocysts are able to establish steady states of cellular ATP concentrations at any level of energy charge between 0.35 and 0.9 (compare also Ref. 18); (2) in addition they are able to establish a homeostasis of ATP not only at high, but at any cellular ATP concentration (Fig. 6B); (3) maximum activity of nitrogenase is limited by the phosphorylation capacity of the photosystem, which artificially can be decreased by uncouplers (Fig. 3 and curve B in Fig. 5); (4) submaximal activity is caused by reductant limitation, when a light activated reductant (hydrogen) is used (Fig. 7, curves A, A1, A2 and A3). In this study we found no evidence of membrane potential influencing nitrogenase activity directly, since addition of valinomycin was without effect (Fig. 4). Rather the observed effects are explained by the stoichiometric connection of phosphorylation and nitrogenase activity via the photosynthetic electron flow.

All experiments were carried out with intact heterocysts, however, using hydrogen, an electron donor of limited importance in nature. The yet unknown reductant of nitrogenase, ultimately de-

rived from photolysis of water, must be transported into heterocysts, since these cells lack Photosystem II and ribulose-bisphosphate carboxylase [27]. If this reductant is metabolized and feeds into Photosystem I like hydrogen and reduced pyridine nucleotides [14,15,28], nitrogenase activity in the light will be regulated by electron flow coupled to phosphorylation. A homeostasis of ATP will be established. On the other hand, if the reductant supplies nitrogenase in a light-independent pathway, e.g., via reduced ferredoxin generated by glycolysis [28,29], homeostasis of ATP, generated by cyclic phosphorylation, might be impaired, thus allowing additional control of nitrogenase by the ATP/ADP ratio.

Acknowledgements

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References

1 Wolk, C.P. (1982) in The Biology of Cyanobacteria (Carr, N.G. and Whitton, B.A., eds.), pp. 359–386, Blackwell, Oxford
2 Mortenson, L.E. and Thorneley, R.N.F. (1979) Annu. Rev. Biochem. 48, 387–418
3 Upchurch, R.G. and Mortenson, L.E. (1980) J. Bacteriol. 141, 274–284
4 Mortenson, I.E. and Upchurch, R.G. (1981) in Current perspectives of nitrogen fixation (Gibson, A.H. and Newton, W.E., eds.), pp. 75–78, Elsevier/North-Holland, Amsterdam
5 Thorneley, R.N.F. and Cornish-Bowden, A. (1977) Biochem. J. 165, 255–262
6 Haaker, H., Laane, C. and Veeger, C. (1980) in Nitrogen Fixation (Stewart, W.D.P. and Gallon, J.R., eds.), pp. 113–138, Academic Press, Cambridge
7 Haaker, H., De Kok, A. and Veeger, C. (1974) Biochim. Biophys. Acta 357, 344–357
8 Haaker, H. and Veeger, C. (1978) Eur. J. Biochem. 77, 1–10
9 Laane, C., Krone, W., Konings, W.N., Haaker, H., Veeger, C. (1979) FEBS Lett. 103, 328–332
10 Veeger, C., Haaker, H. and Laane, C. (1981) in Current Perspectives of Nitrogen Fixation (Gibson, A.H. and Newton, W.E., eds.), pp. 101–104, Elsevier/North-Holland/Biomedical Press, Amsterdam
11 Hawkesford, M.J., Reed, R.H., Rowell, P. and Stewart, W.D.P. (1982) Eur. J. Biochem. 127, 63–66
12 Hawkesford, M.J., Reed, R.H., Rowell, P. and Stewart, W.D.P., (1981) Eur. J. Biochem. 115, 519–523
13 Yoch, D.C. and Arnon, D.I. (1970) Biochim. Biophys. Acta 197, 180–184
14 Houchins, J.P. and Hind, G. (1982) Biochim. Biophys. Acta 682, 86–96
15 Schrautemeier, B., Böhme, H. and Böger, P. (1984) Arch. Microbiol. 137, 14–20
16 Scherings, G., Haaker, H. and Veeger, C. (1978) Eur. J. Biochem. 77, 621–630
17 Peterson, R.P. and Wolk, C.P. (1978) Proc. Natl. Acad. Sci. USA 75, 6271–6275
18 Ernst, A., Böhme, H. and Böger, P. (1983) Biochim. Biophys. Acta 723, 83–90
19 Kacser, H. and Burns, J.A. (1973) in Rate Control of Biological Processes (SEB Symposia XXVII) (Davies, D.D., ed.), pp. 65–104, University Press, Cambridge
20 Reimer, S. and Trebst, A. (1975) Z. Naturforsch. 31c, 103
21 Edwards, D.I., Mathison, G.E. and Platt, D.I. (1974) Z. Pflanzenphysiol. 71, 424–427
22 Guikema, J.A. and Sherman, L.A. (1980) J. Bioenerg. Biomembranes 12, 277–295
23 Mitchell, P. (1977) Annu. Rev. Biochem. 46, 996–1005
24 Almon, H. and Böhme, H. (1982) Biochim. Biophys. Acta 679, 279–286
25 Burgess, B.K. (1984) in Advances in Nitrogen Fixation Research (Veeger, C. and Newton, W.E., eds.), pp. 103–114, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
26 Clark, A.J., Cotton, N.P.J. and Jackson, J.B. (1983) Biochim. Biophys. Acta 723, 440–453
27 Tel-Or, E. and Stewart, W.D.P. (1977) Proc. R. Soc. Lond. B, 198, 61–86
28 Schrautemeier, B., Böhme, H. and Böger, P. (1984) in Advances in Photosynthesis Research (Sybesma, C., eds.), Vol. II, pp. 703–706, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
29 Neuer, G. and Bothe, H. (1982) Biochim. Biophys. Acta 716, 358–365