Biochimica et Biophysica Acta 891 (1987) 121–128
Elsevier

BBA 42509

Regulation of electron flow to nitrogenase in a cell-free system from heterocysts of *Anabaena variabilis*

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(Received 21 December 1986)

Key words: Carbon metabolism; Nitrogen fixation; Reduction charge; Ammonium inhibition; Heterocyst; Cyanobacterium; (*A. variabilis*)

The effect of metabolic regulators on carbohydrate degradation, which supplies electrons for nitrogen fixation in heterocysts, was investigated. Measurements of nitrogenase and enzyme activities in a cell-free system showed that (1) phospho*enol* pyruvate interfered with fructose 1,6-bisphosphate aldolase activity and (2) dithiothreitol (via thioredoxin) inactivated glucose 6-phosphate dehydrogenase, whereas (3) NADPH inhibited both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in a similar way. Nevertheless, a high reductant charge (NADPH/NADP + NADPH) of more than 0.95 was maintained under nitrogen-fixing conditions. This supports the view that NADPH generation by carbohydrate degradation via the oxidative pentose-phosphate cycle serves as main electron source for nitrogen fixation. A stoichiometric relationship between substrate dissimilation and acetylene formation suggested a complete turnover to supply two electrons to nitrogenase. Addition of ammonium ions specifically inhibited electron flow to nitrogenase with ribose 5-phosphate as substrate, suggesting interference with the anaerobic rearrangement reactions of the oxidative pentose-phosphate cycle.

Introduction

The oxidative pentose-phosphate pathway is the main hexose oxidizing pathway in cyanobacteria [1–4]. The key enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are particularly active in heterocysts [5]. Moreover, in cell-free heterocyst systems, high activities of acetylene reduction have consistently been obtained only with intermediates of the pentose-phosphate pathway [6–9], which therefore has been regarded as the main route for reductant supply to nitrogenase. The generated NADPH would feed electrons into the nitrogenase system via ferredoxin/NADP : ferredoxin oxidoreductase. However, the latter enzyme required a reductant charge above 0.3, whereas glucose-6-phosphate dehydrogenase showed maximum activity only at ratios below 0.3 [10,11]. Hawkesford et al. [12] suggested the involvement of a protonmotive force (as measured across the plasmalemma membrane) in sustaining high nitrogenase activities. In permeabilized heterocysts and heterocyst homogenates, however, generation of NADPH by the oxidative pentose-phosphate pathway proceeded without participation of energized membranes [7,8].

Beyond that only little is known about the

Abbreviations: Chl, chlorophyll; Mops, 4-morpholinepropanesulfonic acid.

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regulation of electron flow to nitrogenase. Most attention has been focussed on the regulatory properties of glucose-6-phosphate dehydrogenase, as key enzyme involved in hexose dissimilation. NADPH, ATP and light (via the thioredoxin system) deactivated this hysteretic enzyme [10,13–18], the activation process, however, remained unclear. Glucose-6-phosphate (and glutamine) derived from fixed carbon reserves, such as glycogen, have been implicated to be involved in this process [17]. Whether thioredoxin-mediated inactivation of glucose-6-phosphate dehydrogenase also occurs in heterocysts is still a matter of controversy [10,17–19].

In the present communication we report on the effect of various metabolic inhibitors (derived from carbohydrate metabolism) and of ammonium on substrate-supported nitrogenase activity. Further, it will be shown that this heterocyst 'in vitro' system is able to generate a high reduction charge at the expense of hexose-phosphate dissimilation via the oxidative pentose-phosphate cycle.

Materials and Methods

Photoautotrophic growth of *Anabaena variabilis*, Kütz. (ATCC 29413) under nitrogen fixing conditions, isolation of heterocysts and subsequent French-press treatment has been described [8]. Measurements of nitrogenase activities in the presence of an ATP-generating system were performed as in Ref. 8. Partially purified nitrogenase and heterocyst ferredoxin were prepared according to Refs. 20 and 21.

Energy and reductant charge (cf. Ref. 22) was determined in heterocyst homogenates after dark incubation (20 min, 30°C) in the presence of an ATP-generating system, NADP and NAD as cofactors (20 μM) and glucose 6-phosphate as substrate (2.5 mM). First, the rate of acetylene reduction was measured (usually 32–34 μmol C_2H_4/mg Chl per h) and then the sample (containing 20 μg Chl in 250 μl reaction mixture, cf. Ref. 8) was stopped by addition of acid or alkali for extraction of either ATP/ADP or NAD(P)/NAD(P)H. For determination of adenine nucleotides 250 μl of perchloric acid (1.4 M) was added and the sample placed on ice for 20 min. Then 125 μl of potassium phosphate buffer (0.5 M, pH 6.4) were added, the sample neutralized with 5 M KOH, centrifuged and the supernatant stored in liquid nitrogen. Neutralized extracts were analyzed for ATP and ADP by bioluminescence (LKB, Luminometer 1250). To 500 μl of the luminescence reagent (ATP-CLS, Boehringer) 100 μl of phospho*enol* pyruvate (1 mM, dissolved in 100 mM potassium acetate and neutralized) and 5 μl of the diluted sample were added to determine ATP-content; subsequent addition of 5 μl pyruvate kinase (400 U/ml, Boehringer) allowed to determine ADP [23]. For measurement of reduction charge in heterocyst homogenates, the reaction was stopped by addition of 350 μl of 0.2 M KOH. Pyridine nucleotides were extracted by incubation of the samples at 60°C for 5 min. Then 50 μl of potassium-phosphate buffer (0.5 M, pH 7.0) was added, the sample neutralized by HCl and centrifuged. The clear, but greenish supernatant was stored in liquid nitrogen. To 100 μl of the NADH- or NADPH-monitoring reagent (LKB), 195 μl of potassium-phosphate buffer (0.1 M, pH 7.0) and 5 μl of the diluted sample were added and the luminescence signal recorded. For determination of total NADP/NADPH the mixture contained in addition: $MnCl_2$ (40 μM), isocitrate (2 mM), and 2 U of isocitrate dehydrogenase (Boehringer).

Enzyme activities were determined in the supernatant after centrifugation of the heterocyst homogenate for 30 min at 100000 × *g*. NADPH formation or NADH oxidation were measured with an Aminco DW2 spectrophotometer, dual-wavelength mode (340 minus 400 nm) in a thermostated cuvette at 30°C. The basic reaction mixture (300 μl) contained 100 mM Mops/NaOH buffer (pH 7.2)/4 mM $MgCl_2$/0.5% bovine serum albumin/5–20 μl of the supernatant (20–25 mg protein/ml). The reaction was started by addition of substrate (2.5 mM). 6-Phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase were measured directly by NADPH formation (2 mM NADP/assay). We found that dilution of the supernatant led to a disproportionate decrease in glucose-6-phosphate dehydrogenase activity, probably due to its hypoactive form [16], whereas 6-phosphogluconate dehydrogenase was not affected. 6-Phosphoglucose isomerase, fructose bisphosphatase and fructose-1,6-bisphosphate

aldolase were measured by coupling the reaction to glucose-6-phosphate dehydrogenase. Supplementary enzymes were added, when necessary at approx. 1–2 U of activity per assay. The dissociation reaction of aldolase and phosphofructokinase were assayed via NADH-oxidation (150 μM) by glycerophosphate dehydrogenase (2 U/assay) in the presence of triosephosphate isomerase (12 U/assay). The phosphofructokinase assay contained in addition 0.2 mM ATP.

For measurements of the stoichiometry between ethylene formation and substrate consumption, the standard reaction mixture contained in addition 10 μl nitrogenase (66 μg protein/ml) and 5 μM ferredoxin I from heterocysts to insure initial activities well over 100 μmol C_2H_4/mg Chl per h. As soon as the endogenous rate of acetylene reduction by the heterocyst homogenate approached zero (after approx. 20 min), defined amounts of substrates were added anaerobically. The reaction was followed until completion and the amount of additional acetylene formed was calculated.

Protein concentration was determined according to Bradford [24], with bovine serum albumin as standard. Chlorophyll was determined after methanol extraction [25]. Depending o the preparation used, 20–25 mg protein/ml heterocyst extract corresponded to 1 mg Chl/ml. Several heterocyst homogenate preparations were used and each experiment was repeated at least two times. Biochemicals and enzymes were purchased from Boehringer (Mannheim, F.R.G.) or Sigma (München, F.R.G.).

Results and Discussion

Metabolic inhibitors of nitrogenase activity

In heterocysts possible sources of reductant for nitrogen fixation are (1) NADPH, generated by the oxidative pentose-phosphate cycle (or by isocitrate dehydrogenase) transferring electrons to ferredoxin via NADP: ferredoxin oxidoreductase, and (2) pyruvate by direct reduction of ferredoxin via pyruvate: ferredoxin oxidoreductase.

It has been amply demonstrated [15–18] that isolated glucose-6-phosphate dehydrogenase is inhibited by NADPH. This is in accordance with the data of Fig .1, where glucose-6-phosphate-dependent nitrogenase activity in the heterocyst homogenate was inhibited by increasing concentrations of NADPH, but much less so by NADH. The data of Table I show that substrate-supported nitrogenase activity was inhibited to a certain basal level by a given concentration of NADPH. With isocitrate, nitrogenase activity was inhibited more strongly, which seems to be a specific property of isocitrate dehydrogenase. Papen et al. [26] suggested that the major role of isocitrate dehydrogenase was not to generate reducing equivalents for nitrogen fixation, but to provide oxoglutarate for glutamate biosynthesis.

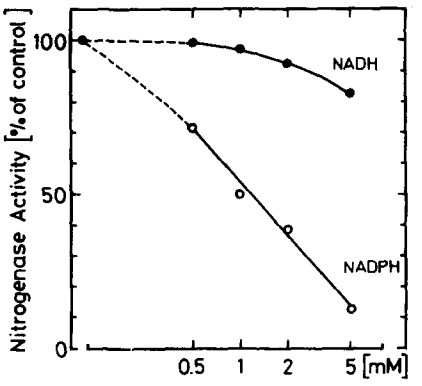

Fig. 1. Influence of reduced pyridinenucleotides on glucose-6-phosphate-dependent nitrogenase activity of a heterocyst homogenate. The asay contained: glucose 6-phosphate, 2.5 mM; NAD or NADP, 20 μM; NADH addition (●); NADPH addition (○); 100% activities were with NAD 20.7 and with NADP 18.8 μmol C_2H_4/mg Chl per h.

As shown previously [8] and also in Table I, high rates of acetylene reduction were measured only with substrates of the oxidative pentose-phosphate cycle and with dihydroxyacetone phosphate. Pyruvate added together with oxaloacetate and coenzyme A at optimum concentrations exhibited rates usually below 50% of the maximum nitrogenase activity. Other C_3-metabolites (as possible precursors of pyruvate) were even inhibitory. 3-Phosphoglycerate (5 mM) inhibited nitrogenase activity with glucose 6-phosphate, 6-phosphogluconate and ribose 5-phosphate by 25–28%. Inhibition was more pronounced with dihydroxyacetone phosphate, pyruvate/oxaloacetate and isocitrate (58–60%) as substrates.

Instead of acting as electron donor [9,27], phospho*enol* pyruvate severely inhibited glucose-6-

TABLE I

SUBSTRATE-DEPENDENT NITROGENASE ACTIVITIES OF A HETEROCYST HOMOGENATE IN THE ABSENCE AND PRESENCE OF VARIOUS METABOLIC INHIBITORS

Substrate concentrations: 2.5 mM; inhibitors: 5 mM; cofactors: NAD/NADP: 20 μM; coenzyme A: 50 μM. The basal rates (no substrate added) were 1.1–3.2 μmol C_2H_4 formed/mg Chl per h.

Substrate	Nitrogenase activity (μmol C_2H_4/mg Chl per h)			
	no inhibitor added	+ NADPH	+ phospho*enol* pyruvate	+ glyoxylate
Fructose 6-phosphate	27.0	–	9.7	–
Glucose 6-phosphate	33.5	7.5	12.3	24.8
6-Phosphogluconate	34.5	7.1	10.7	23.6
Ribose 5-phosphate	33.0	6.7	12.3	24.0
Dihydroxyacetone phosphate	20.1	7.2	0.1	10.2
Pyruvate/oxaloacetate	14.4	5.0	2.1	0.1
Isocitrate	16.0	3.1	5.4	0.1

phosphate-dependent nitrogenase activity. After an initial stimulation in rate (approx. 10%) with 0.1 mM phospho*enol* pyruvate, increasing concentrations from 0.5 to 5 mM inhibited acetylene reduction from 8% to 67%, respectively. Comparing different substrates at a given phospho*enol* pyruvate concentration (Table I), inhibition was more pronounced using the pyruvate/oxaloacetate couple; especially with dihydroxyacetone/phosphate acetylene reduction was inhibited completely by phospho*enol* pyruvate. This mechanism probably prevents wasteful degradation of C_3-metabolites. Addition of pyruvate or oxaloacetate (1–10 mM) had no effect on glucose-6-phosphate-dependent nitrogenase activity.

Taken together, these data show that the C_3-metabolism appears to be a highly regulated part of carbohydrate dissimilation. This might have partly been responsible for obtaining low or negligible nitrogenase activities upon addition of these C_3-metabolites to cell-free systems. Furthermore, the enzymes participating in this pathway might be in general less active than those of the oxidative pentosephosphate pathway.

Glyoxylate, regarded as potential electron source for nitrogen fixation [28], inhibited nitrogenase activity with metabolites of the oxidative pentosephosphate cycle by approx. 30%, with dihydroxyacetone phosphate by approx. 50%, whereas with pyruvate/oxaloacetate and isocitrate complete inhibition was observed. This is in accordance with the findings of Neuer and Bothe [9], who regarded pyruvate : ferredoxin oxidoreductase as a possible target enzyme of glyoxylate inhibition.

Enzyme activities in heterocyst extracts

In accordance with the results of Winkenbach and Wolk [5], we found very high activities of the key enzymes of the oxidative pentose-phosphate cycle (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase), whereas the activity of the key enzyme of glycolytic dissimilation of hexose phosphates, phosphofructokinase, was very low (Table II, cf. Ref. 29). By contrast, considerable activities of fructose 1,6-bisphosphate aldolase and fructose bisphosphatase were measured. This explains why dihydroxyacetone phosphate (and glyceraldehyde 3-phosphate) supported high nitrogenase activities, if aldol condensation to fructose 1,6-bisphosphate, dephosphorylation to fructose-6-phosphate and isomerisation to glucose 6-phosphate allow recycling into the oxidative pentose-phosphate cycle. This pathway would attribute to fructose bisphosphatase a function as catabolic enzyme, which was also suggested for hexose metabolism in pseudomonads [30]. NADPH, known to inhibit glucose-6-phosphate dehydrogenase, also inhibited 6-phosphogluconate dehydrogenase (Table II); the inhibition curves obtained with increasing concentrations of NADPH were almost identical for

TABLE II

ENZYME ACTIVITIES OF A CELL-FREE EXTRACT FROM HETEROCYSTS

For details, see Materials and Methods. Fructose-1,6-bisphosphate aldolase was measured with dihydroxyacetone phosphate (condensation) and fructose 1,6-bisphosphate (dissociation) as substrates (2.5 mM); phospho*enol*pyruvate: 2 mM; dithiothreitol: 0.5 mM; NADPH: 0.4 mM.

Enzyme	Activity (mU/mg protein)	(%) Activity in the presence of		
		phospho*enol* pyruvate	dithio-threitol	NADPH
6-Phosphogluconate dehydrogenase	3350.0	100.0	100.0	8.8
Glucose 6-phosphate dehydrogenase	1926.0	84.2	1.9	6.0
6-Phosphoglucose isomerase	1440.3	78.5	–	–
Phosphofructokinase	2.8	–	–	–
Fructosebisphosphatase	225.2	84.5	98.0	–
Fructose 1,6-bisphosphate aldolase (cond.)	32.8	9.3	199.4	–
Fructose 1,6-bisphosphate aldolase (diss.)	51.3	8.0	206.2	–

both enzymes (data not shown). We further tried to localize the inhibition site of phospho*enol* pyruvate. Although phosphofructokinase is considered as the classical target enzyme of phospho*enol* pyruvate inhibition (cf. Ref. 2), we found only marginal effects. The same was true for fructose bisphosphatase. As expected from our experiments on dihydroxyacetone-phosphate-supported nitrogenase activity (Table I), fructose-1,6-bisphosphate aldolase was severely inhibited by 5 mM phospho*enol* pyruvate, both the condensation reaction (with dihydroxyacetone phosphate as substrate) and the splittig reaction (with fructose 1,6-bisphosphate as substrate).

Low amounts of dithiothreitol stimulated aldolase activity twofold. Whether this effect was thioredoxin – mediated or not, we do not know yet. For *Bacillus subtilis* class II-aldolase a direct stimulation of the enzyme by SH-compounds has been reported [31]. Fructose bisphosphatase, the classical target enzyme of thioredoxin(f)- activation [32] was hardly affected by dithiothreitol. As expected from previous results, glucose-6-phosphate dehydrogenase was inhibited completely by dithiothreitol (thioredoxin), whereas the next step in hexose dissimilation, catalyzed by 6-phosphogluconate dehydrogenase was not influenced. This is in accordance with our findings on 6-phosphogluconate-supported nitrogenase activity, which initially was unaffected by dithiothreitol (see Fig. 2, preceding paper [21]). During the time-course of this experiment some inhibition became apparent, as further metabolism through the oxidative pentose-phosphate pathway would include the dithiothreitol/ thioredoxin-sensitive glucose-6-phosphate dehydrogenase (see below).

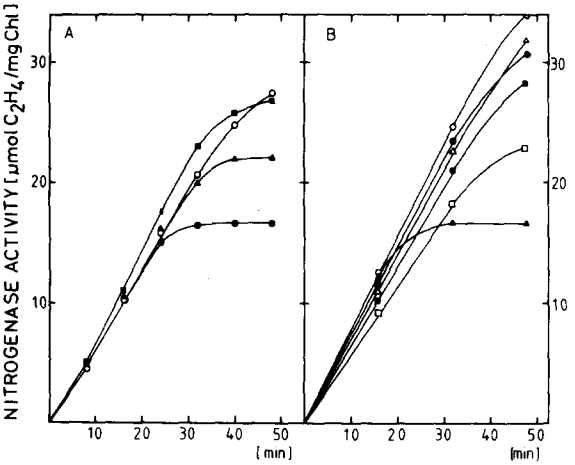

Fig. 2. Effect of ammonium chloride on substrate-supported nitrogenase activity of a heterocyst homogenate. (A) Control with glucose 6-phosphate as substrate (○); addition of NH_4Cl, 2 mM (■), 4 mM (▲), 6 mM (●). (B) Activities in the absence and presence of 2 mM NH_4Cl with 6-phosphogluconate (○, ●), ribose 5-phosphate (△, ▲) and dihydroxyacetone phosphate (□, ■). The reactions were started by anaerobic addition of heterocyst homogenate.

Stoichiometry of ethylene formation and substrate utilisation

When using limiting amounts of substrates, formation of ethylene by heterocyst homogenates ceased after some time. In such a way, the approximate stoichiometry between acetylene reduction and substrate consumption could be determined. As shown in Table III, this ratio approached the value of 6, if decreasing amounts of glucose 6-phosphate were added. For reasons not known, glucose 6-phosphate was not completely metabolized during the assay period, when added in excess. With hexose phosphates, ribose 5-phosphate and dihydroxyacetone phosphate approx. 6, 5 and 3 mol of C_2H_4 were formed, respectively, per mol of substrate added. It seems that a complete turnover of the oxidative pentosephosphate cycle (releasing one CO_2) allows formation of at least 1 mol of acetylene (in the presence of an ATP-generating system).

Inhibition of electron transport to nitrogenase by ammonium ions

NH_4Cl is known to suppress heterocyst formation and synthesis of nitrogenase [33]. Furthermore, it was suggested that ammonium ions by acting as uncouplers of phosphorylation impair energy supply to nitrogenase [34,35]. However, in recent investigations ammonium seemed to interfere more directly with nitrogenase activity. After treatment of N_2-fixing filaments with NH_4Cl at high pH, nitrogenase activity was rapidly switched off and the activity of nitrogenase, assayed in a cell-free system, remained low [36]. In isolated heterocysts addition of NH_4Cl led to immediate inhibition of nitrogenase activity, an effect which could not be attributed to possible uncoupling [37].

In Fig. 2A we show that after a slight stimulation at 2 mM, increasing concentrations of NH_4Cl inhibited glucose-6-phosphate-dependent nitrogenase activities. Notably, the rate of acetylene reduction, even at high concentrations of NH_4Cl, proceeded almost as the control for 20–30 min, in order to cease abruptly within several minutes. This is reminiscent of the switch-off phenomenon induced by ammonium ions in phototrophic bacteria, but attributed to a different mechanism, such as covalent modification of nitrogenase itself (Refs. 38–41, but see also Ref. 42). Such a short term effect of NH_4Cl is now also documented for cyanobacteria at the level of electron supply to nitrogenase through carbohydrate metabolism.

In the cell-free heterocyst system this rapid switch-off by ammonia of nitrogenase activity seems to be due to interference with certain enzymatic rearrangement reactions of the oxidative pentose-phosphate cycle. As shown in Fig. 2B, 2 mM of NH_4Cl inhibited acetylene reduction supported by glucose 6-phosphate or 6-phosphogluconate only slightly, whereas with ribose 5-phosphate as substrate nitrogenase activity was completely inhibited (after about 20–30 min). Under these conditions a 20% stimulation was observed with dihydroxyacetone phosphate, possibly caused by activation of fructose-1,6-bi-

TABLE III

STOICHIOMETRY BETWEEN SUBSTRATE UTILIZATION AND ACETYLENE FORMATION IN A HETEROCYST HOMOGENATE

The assay contained in addition: nitrogenase, 10 µl; heterocyst- ferredoxin I, 5 µM; NAD/NADP, 20 µM; coenzyme A, 100 µM.

Addition		Formation of nmol C_2H_4 per assay	Ratio (mol/mol) C_2H_4/substrate
Glucose 6-phosphate	(625 nmol)	1080	1.7
Glucose 6-phosphate	(250 nmol)	526	2.1
Glucose 6-phosphate	(100 nmol)	452	4.5
Glucose 6-phosphate	(25 nmol)	155	6.2
6-Phosphogluconate	(25 nmol)	150	6.0
Fructose 1,6-bisphosphate	(25 nmol)	165	6.6
Ribose 5-phosphate	(25 nmol)	135	5.4
Dihydroxyacetone phosphate	(25 nmol)	75	3.0

sphosphate aldolase, as documented for *B. subtilis* [31]. However, other enzymes could also be involved, since some increase in nitrogenase activity by low NH_4Cl concentrations was observed with glucose 6-phosphate and fructose 1,6-bisphosphate as well.

Reductant and energy charge under nitrogen fixing conditions

As shown above, only substrates feeding into the oxidative pentose-phosphate cycle were active in supporting nitrogenase activities of heterocyst homogenates. Despite the fact that NADPH inhibited substrate-dependent nitrogenase activity and the corresponding enzyme activities of the heterocyst extract, it was of interest to find out whether NADPH, formed by this metabolic pathway, could in principle serve as an electron source under nitrogen fixing conditions. In addition we wanted to determine possible regulatory effects exerted on nitrogenase by the ATP/ADP ratio generated by the 'artificial' creatine phosphate/creatine kinase system during acetylene reduction.

As shown in Table IV, an ATP/ADP ratio of 2.4 was generated in the cell-free system, a value quite similar to that found in intact heterocysts (Ref. 43; see also Böhme, H., unpublished data), showing high nitrogenase activities (200 μmol C_2H_4/mg Chl per h). Surprisingly, the substrate-supplemented heterocyst homogenate generated an NADPH-reductant charge of 0.95 and even higher ratios were measured in other experiments. This is more than sufficient to support maximum nitrogenase activities in heterocysts [11]. Under conditions of nitrogen fixation the cell-free system was able to reduce the entire NADP pool, when supplied with substrates of the oxidative pentosephosphate cycle. The catabolic reductant charge (NADH/NAD + NADH) was much lower (0.35). Nevertheless, this would be sufficient to support high rates of acetylene reduction by electron donation via Photosystem I/ferredoxin to nitrogenase (80% nitrogenase activity, cf. Ref. 11). In the light, however, the NADH reductant charge might be even higher.

TABLE IV

STEADY STATE CONCENTRATIONS OF NUCLEOTIDES DURING ACETYLENE REDUCTION BY A HETEROCYST HOMOGENATE

	nmol per assay	Ratio
ATP	860	ATP/ADP: 2.4
ADP	360	
NADPH	5.15	NADPH/NADP+NADPH: 0.95
NADP	0.25	
NADH	1.87	NADH/NAD+NADH: 0.35
NAD	3.48	

Our data clearly show that reductant generation through carbohydrate dissimilation via the oxidative pentose-phosphate cycle appears to be the main pathway for electron supply to nitrogenase. The main regulatory sites are at the level of glucose-6-phosphate dehydrogenase. Glycolytic degradation of hexose seems to be of minor importance, since the activity of phosphofructokinase is very low. Aldolase and fructose-bisphosphatase obviously function as 'catabolic' enzymes, providing the oxidative pentose-phosphate cycle with additional hexose phosphates.

Acknowledgements

I wish to thank Marion Oehri for expert technical assistance and Prof. P. Böger for support. This study was financed by grants from the Deutsche Forschungsgemeinschaft.

References

1 Cheung, W.Y. and Gibbs, M. (1966) Plant Physiol. 41, 731–737
2 Pearce, J. and Carr, N.G. (1969) J. Gen. Microbiol. 54, 451–462
3 Pelroy, R.A., Rippka, R. and Stanier, R.Y. (1972) Arch. Microbiol. 87, 302–322
4 Smith, A.J. (1982) in The Biology of Cyanobacteria (Carr, N.G. and Whitton, B.A., eds.), pp. 47–86, Blackwell Science Publications, Oxford
5 Winkenbach, F. and Wolk, C.P. (1973) Plant Physiol. 52, 480–483
6 Lockau, W., Peterson, R.B., Wolk, C.P. and Burris, R.H. (1978) Biochim. Biophys. Acta 502, 298–308
7 Houchins, J.P. and Hind, G. (1982) Biochim. Biophys. Acta 682, 86–96
8 Schrautemeier, B., Böhme, H. and Böger, P. (1984) Arch. Microbiol. 137, 14–20
9 Neuer, G. and Bothe, H. (1985) Arch. Microbiol. 143, 185–191
10 Apte, S.K., Rowell, P. and Stewart, W.D.P. (1978) Proc. R. Soc. Lond. B 200, 1–25

11 Schrautemeier, B. and Böhme, H. (1984) FEMS Microbiol. Lett. 25, 215–218
12 Hawkesford, M.J., Reed, R.H., Rowell, P. and Stewart, W.D.P. (1981) Eur. J. Biochem. 115, 519–523
13 Grossman, A. and McGowan, R.E. (1975) Plant Physiol. 55, 658–662
14 Duggan, J.X. and Anderson, L.E. (1975) Planta 122, 293–297
15 Pelroy, R.A., Kirk, M.R. and Bassham, J.A. (1976) J. Bacteriol. 128, 623–632
16 Schaeffer, F. and Stanier, R.Y. (1978) Arch. Microbiol. 116, 9–19
17 Cossar, J.D., Rowell, P. and Stewart, W.D.P. (1984) J. Gen. Microbiol. 130, 991–998
18 Udvardy, J., Borbely, G., Juhasz, A. and Farkas, G.L. (1984) J. Bacteriol. 157, 681–683
19 Lex, M. and Carr, N.G. (1974) Arch. Microbiol. 101, 161–167
20 Schrautemeier, B., Böhme, H. and Böger, P. (1985) Biochim. Biophys. Acta 807, 147–154
21 Böhme, H. and Schrautemeier, B. (1987) Biochim. Biophys. Acta 891, 115–120
22 Ernst, A., Böhme, H. and Böger, P. (1983) Biochim. Biophys. Acta 723, 83–90
23 Larsson, C.M. and Olsson, T. (1979) Plant Cell Physiol. 20, 145–155
24 Bradford, M. (1976) Anal. Biochem. 72 248–254
25 Mackinney, G. (1941) J. Biol. Chem. 140, 315–322
26 Papen, H., Neuer, G., Refaian, M. and Bothe, H. (1983) Arch. Microbiol. 134, 73–79
27 Bradley, S. and Carr, N.G. (1976) J. Gen. Microbiol. 96, 175–184
28 Smith, G.D., Daday, A., Newbigin, E.J. and Smith, E. (1982) FEBS Lett. 144, 219–222
29 Neuer, G. and Bothe, H. (1982) Biochim. Biophys. Acta 716, 358–365
30 Lessie, T.G. and Phibbs, P.V. (1984) Annu. Rev. Microbiol. 38, 359–387
31 Ujita, S. and Kimura, K. (1982) in Methods Enzymol. (Wood, W.A., ed.), Vol. 90, pp. 235–241, Academic Press, New York
32 Crawford, N.A., Sutton, C.W., Yee, B.C., Johnson, T.C., Carlson, D.C. and Buchanan, B.B. (1984) Arch. Microbiol. 139, 124–129
33 Haselkorn, R. (1978) Annu. Rev. Plant Physiol. 29, 319–344
34 Ohmori, K. and Hattori, A. (1978) Arch. Microbiol. 117, 17–20
35 Yoch, D.C. and Gotto, J.W. (1982) J. Bacteriol. 151, 800–806
36 Reich, S., Almon, H. and Böger, P. (1986) FEMS Microbiol. Lett. 34, 53–56
37 Böhme, H. (1986) Arch. Microbiol. 146, 99–103
38 Neilson, A.H. and Nordlund, S. (1975) J. Gen. Microbiol. 91, 53–62
39 Ludden, P.W. and Burris, R.H. (1976) Science 194, 424–426
40 Ludden, P.W. and Burris, R.H. (1978) Biochem. J. 175, 251–259
41 Zumft, W.G. and Castillo, F. (1978) Arch. Microbiol. 117, 53–60
42 Haaker, H., Laane, C., Hellingwerf, K., Houwer, B., Konings, W.N. and Veeger, C. (1982) Eur. J. Biochem. 127, 639–645
43 Ernst, A. and Böhme, H. (1984) Biochim. Biophys. Acta 767, 362–368