Biochimica et Biophysica Acta 807 (1985) 147–154
Elsevier

BBA 41735

Reconstitution of a light-dependent nitrogen-fixing and transhydrogenase system with heterocyst thylakoids

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(Received January 9th, 1985)

Key words: Heterocyst; Photosystem I, Electron transport; Transhydrogenase; Nitrogen fixation; (*A. variabilis*)

Reconstituted photosynthetic membranes obtained from heterocysts of *Anabaena variabilis* are shown to support acetylene reduction with a partially purified, ferredoxin-free nitrogenase preparation from the same heterocysts. H_2 and NADH, but not NADPH, serve as electron donors to Photosystem I of heterocyst membranes washed free of ferredoxin-$NADP^+$ oxidoreductase. H_2 and NADH as electron donors to nitrogenase via Photosystem I share the same electron transport pathway as demonstrated by identical reconstitution with cytochrome *c*-553 (or plastocyanin) and ferredoxin from *A. variabilis*. Using clostridial hydrogenase for dark ferredoxin reduction by H_2, additional evidence is obtained for ferredoxin serving as direct electron donor to nitrogenase. Furthermore, $NADP^+$-photoreduction with H_2, as well as by a light-dependent transhydrogenase reaction ($NADH \rightarrow NADP^+$) is measured with heterocyst membranes, using cytochrome *c*-553/plastocyanin, ferredoxin and ferredoxin-$NADP^+$ oxidoreductase as reconstituting components.

Introduction

Integration of nitrogen fixation into cyanobacterial metabolism still is a matter of controversy. This is especially true for the relative importance of electron-transport pathways to nitrogenase and their regulation (for reviews see Refs. 1 and 2). A main role of the pentose-phosphate pathway in electron donation to nitrogenase via ferredoxin-$NADP^+$ oxidoreductase has been questioned, because glucose-6-phosphate dehydrogenase is drastically inhibited by high NADPH/NADP ratios [3,4], which were shown to be necessary for ferredoxin-$NADP^+$ oxidoreductase-dependent nitrogenase activity [5]. The recent discovery of glycolytic enzymes in vegetative cells and heterocysts of *Anabaena* [6], as well as detection of NADH-supported, light-dependent nitrogenase activity in heterocysts [5,7] indicated a possible role of glycolysis as electron source for nitrogen reduction. With the enzymes of the tricarboxylic acid cycle, to form oxoglutarate and glutamate synthase occurring in heterocysts [8,9], glycolysis could also provide carbon skeletons for subsequent nitrogen assimilation. Accordingly, we obtained high dark rates of acetylene reduction in heterocyst homogenates with glycolytic substrates and NAD^+ (or $NADP^+$) as obligate cofactors. Additionally, both pyridine nucleotides feed into photosystem I in the light [5], indicating a possible functional coupling of glycolysis to photosynthetic electron transport for nitrogen fixation.

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Abbreviations, Chl, chlorophyll, DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mops, morpholinepropanesulfonic acid

Only little information is available on the nature of interaction of pyridine nucleotides with thylakoids of heterocysts and their relation to nitrogen fixation.

With a partially purified, ferredoxin-free nitrogenase preparation and washed thylakoids from heterocysts of *A. variabilis*, we have been able to reconstitute H_2- and NADH-supported, photosystem I-mediated nitrogenase activity, as well as $NADP^+$ photoreduction (see also Refs. 11 and 12). Reconstitution was shown to be absolutely dependent on the addition of ferredoxin and cytochrome *c*-553 or plastocyanin from the same organism. Evidence is presented that only ferredoxin-$NADP^+$ oxidoreductase functions as apparent, NADPH-specific, membrane-associated dehydrogenase in heterocysts. The results are discussed in terms of interaction of photosynthesis and nitrogen fixation in cyanobacteria.

Materials and Methods

Growth conditions. A. variabilis (ATCC 29413) was grown autotrophically under nitrogen-fixing conditions and heterocysts were prepared as described in [5].

Preparation of heterocyst thylakoids. Heterocyst homogenates (16 ml; 1 mg chlorophyll/ml) were prepared by French-press treatment (two passages at 138 MPa under H_2; Ref. 5) and centrifuged at $1500 \times g$ for 10 min to remove unbroken cells and cell-wall fragments. The crude extract was centrifuged at $48\,000 \times g$ for 30 min. Both centrifugation steps were performed under strictly anaerobic conditions (with H_2 present), in order to preserve high nitrogenase activity of the resulting supernatant (1) (approx. 10 ml). It was immediately frozen in liquid N_2 for nitrogenase preparation (see below). The subsequent steps were performed under aerobic conditions. The chlorophyll-containing pellet (1) was resuspended in 30 ml containing 30 mM Hepes–NaOH-KOH (pH 7.8), 10 mM NaCl and 5 mM sodium/potassium phosphate, and was centrifuged at $48\,000 \times g$ for 30 min, the supernatant (2) was saved for assay purposes, and the pellet (2) was resuspended. This procedure was repeated, yielding supernatant 3 and pellet 3. The latter (i.e., washed membranes) was resuspended in the same buffer containing 10 mM $MgCl_2$ instead of NaCl (referred to as membrane buffer), to give a chlorophyll concentration of 0.6–1 mg/ml. This suspension was stored in liquid N_2 until use.

Partial purification of nitrogenase from heterocysts of A. variabilis. All preparation steps were performed under an O_2-free argon atmosphere. The nitrogenase-containing supernatant 1 was centrifuged for 1 h at $100\,000 \times g$, and the pellet was discarded. The resulting supernatant was again centrifuged for 5 h at $350\,000 \times g$. The light-brown (ferredoxin-containing) supernatant was free of nitrogenase activity, which was recovered from the pellet. It was resuspended in 16 ml of membrane buffer (corresponding to the heterocyst-homogenate volume) and stored in liquid N_2.

Purification of plastocyanin, cytochrome c-553 and ferredoxin. For preparation of plastocyanin, *Anabaena* was grown in the presence of 1 μM $CuSO_4$, for cytochrome *c*-553 preparation, it was grown in the absence of $CuSO_4$. Additionally, the culture bottles were rinsed with 12 M HCl, then with 0.1 M EDTA. Distilled water, passed through a Chelex-100 column (Bio-Rad), and suprapure chemicals were used to keep the $[Cu^{2+}]$ of the growth medium below $1 \cdot 10^{-8}$ M. Purification of the redox proteins followed the initial steps as described [13], comprising ammonium sulfate precipitation (40–90%) and chromatography on a DE52-cellulose column (Whatman). The basic cytochrome *c*-553 or plastocyanin passed through the column, whereas ferredoxin and phycobiliproteins remained adsorbed. The column was washed with 20 mM Tris-HCl (pH 8.0)/0.2 M NaCl, until all phycobiliproteins had been removed. Ferredoxin was eluted with 20 mM Tris (pH 8.0)/0.4 M NaCl. Ferredoxin-containing fractions were dialyzed and rechromatographed on a small DE-52 column (1.5×5 cm), and the whole procedure was repeated. The ferredoxin concentration was determined at 420 nm ($\varepsilon = 9.7\ mM^{-1} \cdot cm^{-1}$) and stored at $-30°C$.

The cytochrome or plastocyanin eluate was adsorbed on a CM-52 cellulose column (1.5×5 cm) equilibrated with 1 mM Mops–NaOH (pH 7.0). The column was washed with 20 mM Mops, and the respective redox protein was eluted with the same buffer, containing 0.1 M NaCl. After dialysis, the whole procedure was repeated. Plastocyanin concentration was determined at 597 nm

($\varepsilon = 4.9\ mM^{-1} \cdot cm^{-1}$) and cytochrome *c*-553 at 553 nm ($\varepsilon = 25.3\ mM^{-1} \cdot cm^{-1}$). Both proteins were stored at −30°C.

Purification of ferredoxin-NADP$^+$ oxidoreductase. Ferredoxin-NADP$^+$ oxidoreductase from *A. variabilis* was prepared as described [14], using Red Sepharose 4B (Pharmacia) instead of ADP-Sepharose for affinity chromatography. Ferredoxin-NADP$^+$ oxidoreductase was eluted as a sharp peak at 0.3 M NaCl. Its concentration was determined at 456 nm ($\varepsilon = 10.7\ M^{-1} \cdot cm^{-1}$), and the enzyme was stored at −30°C.

Hydrogenase. Ferredoxin-free hydrogenase from *Clostridium pasteurianum* was prepared as described [15] and stored in liquid N_2.

Nitrogenase activity. This was measured in 8-ml vials, closed with septum stoppers. The reaction mixture, buffered with 20 mM Hepes–NaOH-KOH (pH 7.5), contained, in a total volume of 0.25 ml: 5 mM ATP, 5 mM $MgCl_2$, 15 mM creatine phosphate and 25 μg creatine kinase, as ATP-regenerating system. Prior to addition of 1 ml acetylene (approx. 12.5% (v/v) of the gas phase), the vials were flushed for 5 min with Ar or H_2, depending on the electron donor used in subsequent assays. The reaction was started by addition of 25 μl partially purified nitrogenase and carried out in a Warburg apparatus at 30°C. For light-dependent activities, white light was used at an intensity of 700 $\mu E/m^2$ per s. Acetylene reduction was detected by gas chromatography (Varian M 940, equipped with a 2-m Porapack-R column and a flame-ionization detector) and quantitated using a Hewlett Packard integrator (HP 3385).

Note: For better comparison, the activity of the nitrogenase fraction was also related to chlorophyll, since the nitrogenase-containing pellet was resuspended in the original volume of the heterocyst homogenate (i.e., 16 ml, see above).

Ferredoxin-NADP$^+$ oxidoreductase activities. Transhydrogenase activity (NADPH → thio-NADP$^+$) was determined following reduction of thioNADP$^+$ photometrically at 400 nm ($\varepsilon = 11.3\ mM^{-1} \cdot cm^{-1}$, see Ref. 16). The assay was performed in membrane buffer containing in addition 0.25 mM NADPH, 0.05 mM thioNADP$^+$, 1–10 μl of the ferredoxin-NADP$^+$ oxidoreductase fraction. Ferredoxin-dependent NADP$^+$-reduction by H_2 and hydrogenase in the dark (see Table II) was measured anaerobically in cuvettes closed with septum stoppers and flushed with H_2 for 5 min. The absorbance change at 340 nm was measured in 1 ml of membrane buffer containing 1 mM NADP$^+$, 4.3 μM ferredoxin from *A. variabilis*, 10 μl of clostridial hydrogenase, and 1–10 μl of the ferredoxin-NADP$^+$ oxidoreductase fraction.

(Thio)NADP$^+$-photoreduction by heterocyst membranes. This was measured in an Aminco-DW2 spectrophotometer in the dual-wavelength mode (see also Fig. 3). The instrument was equipped for side-illumination, temperature control (30°C) and magnetic stirring; light intensity, defined by a red filter (Balzers KG65), was 980 $\mu E/m^2$ per s. The photomultiplier was protected by a red-absorbing filter (B12, 3 mm, Schott). Measuring wavelength was 340 nm (for thioNADP$^+$, 395 nm), reference wavelength 400 nm (for thioNADP$^+$, 460 nm) and slit-width 5 nm [17]. For measurements 3-ml cuvettes were used, closed with septum stoppers, and flushed for 5 min with either H_2 or Ar, when NADH was used as electron donor.

Chlorophyll. Chlorophyll was determined after methanol extraction according to MacKinney [18].

All results show typical activities of double determinations, with several membrane and nitrogenase preparations.

Biochemicals. Biochemicals were purchased from Sigma (München, F.R.G.) or Boehringer (Mannheim, F.R.G.).

Results

Reconstitution of a native thylakoid-bound electron-transport system to nitrogenase has not been attempted so far. With both partially purified, ferredoxin-free nitrogenase and washed reconstituted thylakoids from heterocysts of *A. variabilis* this was achieved at significant rates.

The following data characterize the nitrogenase preparation in terms of activity with different electron sources. We compared an artificial (dithionite) and a biological one (H_2/hydrogenase) with reconstituted native electron-donating systems (Table I, Fig. 1). No dark or light activity was seen with H_2, NADH or NADPH as electron donors for the ferredoxin-supplied nitrogenase preparation, showing that it did not contain any contaminating ferredoxin-reducing enzyme(s) or

TABLE I

ACTIVITIES OF A PARTIALLY PURIFIED NITROGENASE FROM *A VARIABILIS* WITH ARTIFICIAL AND PHYSIOLOGICAL ELECTRON SOURCES

Assay mixtures (0.25 ml) contained 25 μl of nitrogenase (related to 1 mg of chlorophyll/ml; see Materials and Methods) Reconstituted heterocyst thylakoids comprise: 25 μl of washed thylakoids (0.64 mg Chl/ml), 4.6 μM cytochrome *c*-553 and 20 μM ferredoxin. H_2 concentration was 0.64 mM in solution (87.5%, v/v, of the gas phase). Light intensity: 700 $\mu E/m^2$ per s, white light. FNR, ferredoxin-$NADP^+$ oxidoreductase.

Electron donor	Other additions	Nitrogenase activity (μmol C_2H_4 produced/mg Chl per h)	
		light	dark
5 mM dithionite	–	–	53.0
0.64 mM H_2	+ ferredoxin (20 μM)	0	0
1 mM NADH		0	0
1 mM NADPH		0	0
1 mM NADH	+ ferredoxin + FNR (0.33 μM)	0	0
1 mM NADPH		10.5	12 1
0.64 mM H_2	+ reconstituted heterocyst thylakoids	16.2	0
1 mM NADH		4 5	0
1 mM NADPH		0	0

thylakoids. Dark activity was observed with NADPH, when ferredoxin-$NADP^+$ oxidoreductase was present. Addition of ferredoxin-$NADP^+$ oxidoreductase allows activity only with NADPH, but not with NADH, demonstrating $NADP^+$-specificity of this enzyme.

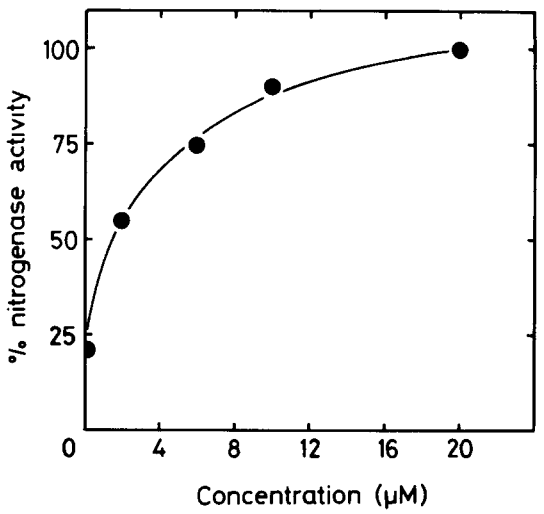

Fig. 1. Reconstitution of dark nitrogenase activity with ferredoxin reduced by clostridial hydrogenase. The assay mixtures contained 10 μl hydrogenase, 25 μl nitrogenase (related to 1 mg chlorophyll/ml; see Materials and Methods); and increasing concentrations of ferredoxin Concentration of H_2 in solution. 0.64 mM; 100% activity = 25 μmol C_2H_4 produced/mg Chl per h

In the light, thylakoids reconstituted with ferredoxin and cytochrome *c*-553 lead to nitrogenase activity with NADH or H_2 as electron donor, but not with NADPH. Obviously, washed heterocyst thylakoids are devoid of any $NADP^+$-specific dehydrogenase (c.f. Ref. 12). Consequently, the apparent NADPH-dehydrogenase function, as measured in heterocyst homogenates [5] was only fulfilled by ferredoxin-$NADP^+$ oxidoreductase. As shown in Table II, the bulk ferredoxin-$NADP^+$ oxidoreductase activity remains already in the first supernatant during successive washing steps of thylakoid membranes. Only little activity was detectable in the membrane fraction after the second washing step.

Clostridial hydrogenase, reducing plant-type ferredoxins as well, is a suitable tool for measuring ferredoxin-dependent enzyme activities [15]. This hydrogenase was able to reconstitute nitrogenase activity in the dark upon addition of increasing amounts of *A. variabilis* ferredoxin (Fig. 1).

In analogy to results with aerobic, nitrogen-fixing bacteria, the membrane potential was claimed to regulate (membrane-bound) electron transport

TABLE II

ACTIVITIES OF FERREDOXIN-NADP$^+$ OXIDOREDUCTASE IN DIFFERENT FRACTIONS OBTAINED FROM A HETEROCYST HOMOGENATE OF *ANABAENA VARIABILIS*

Assays and designation of fractions are described in Materials and Methods, second section.

Fraction	Activity (μmol substrate reduced/ml per min)	
	NADPH → thioNADP$^+$ transhydrogenase	H_2 → NADP$^+$ via clostridial hydrogenase + ferredoxin
Supernatant 1	29 9	121.0
Pellet 1	1.5	8.8
Supernatant 2	0.8	4.6
Pellet 2	0.3	3.4
Supernatant 3	0.3	2.9
Pellet 3	0.05	2.2

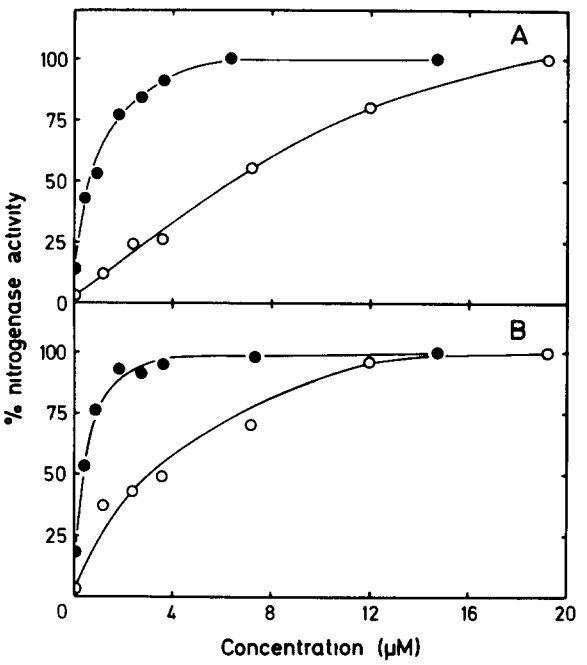

Fig 2 Reconstitution of an H_2- (A) and NADH- (B) supported, light-dependent nitrogen fixing system, comprising heterocyst thylakoids, nitrogenase, cytochrome *c*-553 and ferredoxin. The reaction mixture (pH 7.8) consisted of 30 mM Hepes–NaOH-KOH, 5 mM sodium/potassium phosphate and 2 mM $MgCl_2$. The assay contained in addition 25 μl washed thylakoids (0.64 mg Chl/ml), 25 μl partially purified nitrogenase and increasing concentrations of cytochrome *c* (●——●, with 20 μM ferredoxin), or ferredoxin (○——○, with cytochrome *c* kept at 4.6 μM). Substrate concentrations. H_2,; 0.64 mM; NADH, 1 mM. Light intensity: 700 μE/m^2 per s. Maximum activities (100%) with saturating concentrations of cytochrome *c* plus ferredoxin were 16.2 (A) and 4.5 (B) μmol C_2H_4 produced/mg Chl per h.

to nitrogenase in cyanobacteria [19,20]. This finding was questioned later [5,7]. Our data give additional evidence as to why the original hypothesis of Haaker et al. [10] does not apply for the thylakoid-bound electron-transport system to nitrogenase in heterocysts. With H_2 (Fig. 2A) and NADH (Fig. 2B) as electron donors to Photosystem I only the soluble components cytochrome *c*-553 (or plastocyanin) and ferredoxin are necessary to restore light-dependent electron transport to nitrogenase.

NADP$^+$ competes effectively with nitrogenase for electrons from photosystem I [5]. Accordingly, heterocyst thylakoids exhibited two further activities: H_2-supplied NADP$^+$-photoreduction (Fig. 3A) and light-dependent transhydrogenase reaction (NADH → NADP$^+$ (Fig. 3B; for filaments see Ref. 21). Both activities were reconstituted with the same components as was light-dependent nitrogenase activity. Plastocyanin from *A. variabilis* was equivalent to cytochrome *c*-553 for reconstitution (Fig. 3A). Low amounts of ferredoxin-NADP$^+$ oxidoreductase from *A. variabilis* stimulated H_2- and NADH-dependent photoreduction of NADP$^+$. Obviously, residual membrane-bound ferredoxin-NADP$^+$ oxidoreductase (see Table II), while not able to support NADPH-dependent nitrogenase activity (Table I), had to account for the initial approx. 50% activity in NADP$^+$-photoreduction; 20–30% activity was retained when ferredoxin was omitted, whereas no activity was found when either cytochrome *c*-553 or plastocyanin were absent.

Note that the 100% values as depicted in Figs. 1–3 are not meant to represent maximum in vivo nitrogenase activity or maximum electron-transport capacity of the thylakoids (Figs. 2 and 3). Limitation in acetylene reduction was caused mainly by the amount of added nitrogenase, showing a drastic dilution effect (cf. Ref. 5). Some depletion or loss of activity of NADH-dehydrogenase and hydrogenase has to be assumed as well, limiting photosynthetic electron-transport rates.

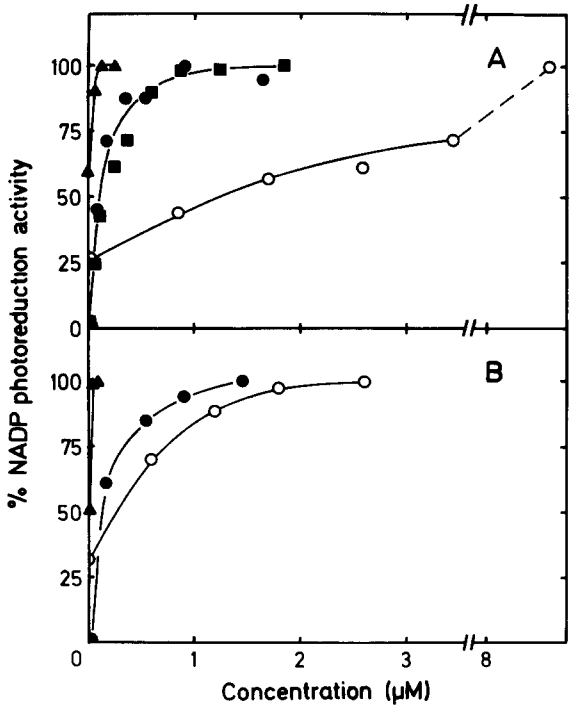

Fig 3. Reconstitution of $NADP^+$-photoreduction by heterocyst thylakoids with H_2 (A) and NADH (B) as electron donors. The reaction mixture (pH 7.8) consisted of 30 mM Hepes–NaOH-KOH, 5 mM sodium/potassium phosphate and 2 mM $MgCl_2$. The assay contained in addition 20 μl washed thylakoids (0.88 mg Chl/ml), 1 mM NADP (A) or 1 mM thioNADP$^+$ (B), and increasing amounts of ferredoxin-NADP$^+$ oxidoreductase (▲———▲), cytochrome *c*-553 (●———●), plastocyanin (■———■) or ferredoxin (○———○), while the complementary components were kept constant at saturating concentrations. 100%-activities were 60 (A) and 10.6 (B) μmol of (thio)NADP$^+$ reduced/mg Chl per h, and represent maximum rates of washed thylakoids reconstituted with saturating concentrations of ferredoxin-NADP$^+$ oxidoreductase (0.13 μM), cytochrome *c*-553 or plastocyanin (1.8 μM), and ferredoxin (8.6 μM).

Discussion

The membrane-bound electron-transport pathway to nitrogenase

As shown in this study, a thylakoid-bound electron-transport system to nitrogenase in heterocysts comprises hydrogenase or NADH-dehydrogenase, the plastoquinone pool, the cytochrome b_6/f complex, cytochrome *c*-553 or plastocyanin, photosystem I (P700), and ferredoxin (cf. Ref. 22). After earlier provisional data [23,24] concerning the role of ferredoxin in cyanobacterial N_2-fixation, there remains no doubt, now, that it can serve as direct electron donor to nitrogenase.

In cyanobacteria, regulation of thylakoid-bound electron transport to nitrogenase is not accomplished by the membrane-potential component of the generated proton-motive force, as claimed earlier [10,19,20]. Rather, membrane energization is a consequence of photosystem I-mediated ferredoxin reduction, requiring only cytochrome *c*-553 or plastocyanin as reconstituting components for light-dependent nitrogen- or $NADP^+$-reduction (Figs. 2,3). In the dark, membrane energization (cf. Ref. 7) may play a limited role in 'reversed' electron flow from NADH (or H_2) to ferredoxin, the components and mechanisms of which remain to be elucidated. For heterocyst membranes, being functional in both respiration and photosynthesis (compare results from vegetative cells [25–27]), an apparent NADPH-dehydrogenase function can be assigned to ferredoxin-$NADP^+$ oxidoreductase only (cf. Ref. 12). Ferredoxin-$NADP^+$ oxidoreductase obviously is able to feed electrons directly into the cytochrome b_6-*f* complex, as supported by studies with chloroplasts [28] and heterocyst homogenates [5]. An additional NADPH-dehydrogenase as concluded by others [29–32] seems not to be present on these heterocyst thylakoids (see Tables I and II).

A light- (i.e., energy-) dependent transhydrogenase system

Energy-dependent transhydrogenase reactions are widespread among organisms and have been described for mitochondria [33,34], heterotrophic [35] and phototrophic [36] bacteria. Despite their probable central role in metabolism, the mechanisms of these reactions remained obscure (for review see Ref. 37). The assignment of a transhydrogenase function to ferredoxin-$NADP^+$ oxidoreductase in chloroplasts [38] does not have a physiological meaning per se, as it represents a ferredoxin-stimulated [39,40] equilibrium reaction between NADPH and NAD(P)$^+$ at the nucleotide-binding site of ferredoxin-$NADP^+$ oxidoreductase (cf. Table II).

The occurrence of a light-dependent transhydrogenase reaction was already shown by studies with homogenates from vegetative cells [21]. As

demonstrated here, this reaction involves the same thylakoid-bound components as described above for light-dependent nitrogenase activity and, additionally, ferredoxin-$NADP^+$ oxidoreductase. Here, the reductase is part of a metabolically meaningful transhydrogenation, as energy input from light allows formation of a high $NADPH/NADP^+$ ratio, a prerequisite for Photosystem I-mediated nitrogen reduction [5]. Furthermore, a special property of reductase can be observed in heterocyst homogenates [41]. With Photosystem I-electron transport blocked by DBMIB, NADPH-dependent ferredoxin reduction via ferredoxin-$NADP^+$ oxidoreductase is drastically inhibited already at low light intensities. This 'light-switch' confirms the view that ferredoxin-$NADP^+$ oxidoreductase acts as a regulatory component of cyclic electron flow (cf. Refs. 5, 28): in the light, ferredoxin-$NADP^+$ oxidoreductase-mediated backflow of electrons from NADPH directly to ferredoxin is inhibited and diverted via the energy-conserving (and DBMIB-sensitive) cyclic pathway to ferredoxin.

The described features of the transhydrogenase reaction are in line with a general rule in metabolism, as emphasized by Atkinson [42], suggesting that $NADP^+$ is energetically equivalent to NADH plus ATP (or light, as shown here). Accordingly, our results point to a role for NADH in energy-generating catabolism and for NADPH in energy-requiring anabolism also in cyanobacteria.

A concept for interaction of photosynthesis and nitrogen fixation in cyanobacteria

The described characteristics of heterocyst thylakoids should allow one to conceive a hypothesis of how nitrogen fixing, especially non-heterocystous, cyanobacteria could accommodate O_2-evolving photosynthesis and O_2-sensitive nitrogenase activity. Temporal separation of both processes seems to operate in *Gloeocapsa* [43] with nitrogenase activity mainly confined to the dark. However, nitrogen fixation also occurred in continuous-light cultures of *Gloeocapsa* and *Plectonema* [44,45]. Under micro-aerobic conditions, high rates of oxygen evolution preceded induction of nitrogen fixation in *Plectonema*, whereas during maximum nitrogenase activity oxygen-evolution rates were low [45]. From these results we assume priority of photosynthetic carbon accumulation over nitrogen assimilation, which is also reflected by competition of $NADP^+$ (via ferredoxin-$NADP^+$ oxidoreductase) with nitrogenase for photosystem I-reduced ferredoxin, favoring $NADP^+$ reduction [5]. This priority is also seen in extensive glycogen accumulation preceding high nitrogenase activities in early log-phase cultures of *Anabaena variabilis* [46]. Interestingly even heterocysts accumulate glycogen under conditions of nitrogen starvation [47].

With the crucial enzyme, NADH-dehydrogenase, present on cyanobacterial thylakoids, electrons from glycolytic carbon degradation [5] could then generate a high $NADPH/NADP^+$ ratio via the described, light-dependent transhydrogenase reaction (Fig. 3B). A high $NADPH/NADP^+$ ratio has three advantageous effects: (1) repression of O_2-evolving photosystem-II activity, and (2) concomitant increase in cyclic photophosphorylation (cf. Refs. 48,49), (3) provision of a low redox potential for nitrogen reduction and related biosynthetic reactions. Accordingly operation of an energy-dependent transhydrogenase has early been related to nitrogen assimilation in mitochondria [50] and *Escherichia coli* [51].

In cyanobacteria, an energetically balanced anabolism could thus proceed temporarily without electrons from water oxidation by photosystem II, but with glycolysis and part of the tricarboxylic-acid cycle providing for reductant and carbon skeletons, and (cyclic) photophosphorylation providing for ATP. To support this concept, further experiments are needed, taking into account adaptational effects to changing light conditions and oxygen concentrations in situ.

Acknowledgments

This work was supported by a grant from the Deutsche Forschungsgemeinschaft and the Zentrum für Energieforschung (Universität Konstanz). We wish to thank M. Eisenhauer for expert technical assistance.

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