Nickel-Dependent Uptake-Hydrogenase Activity in the Blue-Green Alga *Anabaena variabilis*

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Z. Naturforsch. **39 c**, 90–92 (1984); received December 9, 1983

Nickel Requirement, Cyanobacterium, Blue-Green Algae, Hydrogenase, Light-Induced Hydrogen Evolution, Nitrogenase

Uptake-hydrogenase activity in the blue-green alga (cyanobacterium) *Anabaena variabilis* is dependent on the presence of nickel. Nickel depletion leads to an enhancement of net light-induced hydrogen evolution, which is catalyzed by nitrogenase. Addition of nickel chloride to the culture reverses this effect by stimulating hydrogenase activity. Methylviologen/dithionite-driven hydrogen formation of permeabilized cells is decreased by nickel depletion.

Introduction

The biological role of nickel has been underestimated for a period of time. Recent investigations have, however, demonstrated that nickel is an essential component of several enzymes, and four groups of nickel-containing proteins have been described: Urease from jack beans and soybean [1, 2], Methyl-CoM reductase from methanogenic bacteria [3], carbon-monoxide dehydrogenase from anaerobic bacteria [4–7], and uptake hydrogenases from several bacteria [8–13]. Very little is known about nickel with respect to the physiology of blue-green algae [14].

In this communication, data are presented on the effect of nickel on the hydrogen metabolism of the filamentous heterocystous cyanobacterium *Anabaena variabilis*. There is evidence that the activity of an uptake hydrogenase is dependent on the presence of nickel during cultivation.

Materials and Methods

Cultivation: The filamentous heterocystous cyanobacterium *Anabaena variabilis* (American Type Culture Collection, no. 29413) was grown in the absence of combined nitrogen in an inorganic medium modified after Arnon *et al.* [15] (= medium I), containing K_2HPO_4, 2 mM; KCl, 20 mM; $MgSO_4$, 0.5 mM; NaCl, 2 mM; $CaCl_2$, 0.1 mM; $FeSO_4$, 37 μM; EDTA, 21 μM; $MnCl_2$, 9.0 μM; $ZnSO_4$, 0.8 μM; $CuSO_4$, 2.0 μM; $Co(NO_3)_2$, 0.2 μM; $NaVO_3$, 2.0 μM; H_3BO_3, 46 μM; and $(NH_4)Mo_7O_{24}$, 0.7 μM. In medium II used for nickel depletion KCl was omitted and the concentrations of $MgSO_4$ and NaCl were reduced to 0.25 mM and 1.0 mM, respectively. For other culture conditions, see [16].

Light-induced nitrogenase-catalyzed ethylene formation and hydrogen evolution: 5 ml of the algae suspension were placed in 36-ml glass vessels, sealed with rubber stoppers, and gassed with argon for 15 min. Subsequently, 3 ml of C_2H_2 were added to those vessels which were to be used for determination of nitrogenase activity. The vessels were incubated in a Warburg apparatus under continuous illumination (160 $W \times m^{-2}$) at 28 °C. 200 μl samples were withdrawn from the gas phase at 30-min intervals and analyzed gaschromatographically (for details see [17]). Rates of C_2H_4 formation and H_2 evolution of intact cells were calculated from incubation times of 180 to 240 min.

Nickel determination: An atomic-absorption spectrophotometer (Varian, model AA75) was used, equipped with a carbon-rod atomizer.

Chlorophyll determination: After methanolic extraction of the algal filaments, the clear supernatant was measured at 665 nm using an extinction coefficient of 74.5 $ml \times mg^{-1} \times cm^{-1}$ [18].

Hydrogenase activity: Anabaena cells were permeabilized by suspending them in 20 mM MES buffer, pH 6.5, and repeated freezing and thawing. Subsequently, the filaments were sonicated for 5 min in a microsonic bath (Bandelin, model Sonorex RK 102). Hydrogenase activity was determined as hydrogen evolution in the presence of reduced methylviologen as electron donor (see Table II for details).

Abbreviations: Chl, chlorophyll *a*; methylviologen, 1,1′-dimethyl-4,4′-bipyridylium dichloride; MES, 2-(N-morpholino)ethanesulfonic acid, buffer adjusted with NaOH.

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Results

With nickel chloride added to the culture medium I in concentrations from 0.1 to 10 μM no significant variations concerning the rates of acetylene reduction and hydrogen evolution were observed (data not shown). Atomic-absorption spectroscopy showed that culture medium I contained (at least) 1.8 μM nickel, essentially due to impurities in the chemicals KCl, $MgSO_4$ and NaCl. Consequently, *Anabaena* was cultivated in a medium without KCl and with a reduced content of $MgSO_4$ and NaCl. Nickel content of this medium (= medium II) was less than 0.2 μM. Growth, measured as increase of the chlorophyll concentration in this medium, was comparable to growth in medium I. However, hydrogen evolution by cells cultivated in medium II was markedly stimulated as compared to cells cultivated in medium I, whereas the rate of acetylene reduction remained the same. Increasing the EDTA concentration in medium I from 21 μM to 50–150 μM increased the hydrogen evolution accordingly (Table I).

To examine whether the stimulation of hydrogen formation was due to nickel depletion, $NiCl_2$ was added to medium II in various concentrations. Figure 1 illustrates that addition of nickel in a concentration as low as 0.05 μM substantially reduced the rate of hydrogen evolution. Adding nickel chloride in concentrations higher than 1 μM did not result in a further reduction of hydrogen evolution.

Direct evidence for nickel affecting the activity of hydrogenase is given by experiments using permeabilized filaments of *Anabaena variabilis*. It was demonstrated that hydrogenase-catalyzed hydrogen evolution in the presence of reduced methylviologen as electron donor is significantly lower in cyanobacteria cultivated in nickel-depleted media (Table II). Although the activity of this

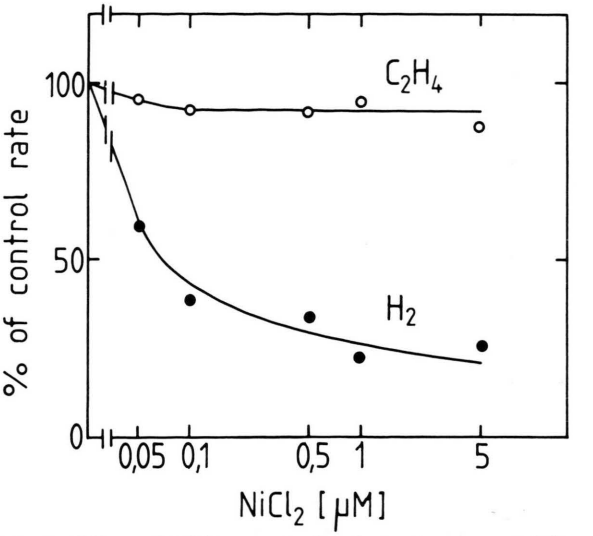

Fig. 1. Effect of $NiCl_2$ on depleted *Anabaena variabilis:* nitrogenase activity measured as C_2H_4 formation (○——○) and decrease of net H_2 evolution by counteracting hydrogenase activity (●——●). Cells were inoculated with a final chlorophyll content of 1 μg/ml culture volume and grown for 72 h in media supplemented with concentrations of $NiCl_2$ as indicated. Each value represents the mean of six experiments with three culture batches (tolerance ± 10%). Control rates (= 100%) were: 25 to 34 μmol $C_2H_4 \times$ mg $Chl^{-1} \times h^{-1}$ and 22 to 33 μmol $H_2 \times$ mg $Chl^{-1} \times h^{-1}$.

Table I. Growth (col. 1), nitrogenase activity (col. 2), and hydrogen evolution (col. 3) by *Anabaena variabilis* cultivated in media containing different concentrations of EDTA and nickel (medium I = 1.8 μM $NiCl_2$); medium II = 0.2 μM $NiCl_2$). Cells were inoculated with 1 μg Chl × ml^{-1} final culture suspension and grown for 3 days in the media indicated. Data are from a typical experiment.

Culture medium	Additions	(1) Chl (μg/ml)	(2) μmol C_2H_4 formed / mg Chl × h	(3) μmol H_2 evolved / mg Chl × h
Medium I	21 μM EDTA original medium	15.0	40	11
Medium I	50 μM EDTA	14.3	40	19
Medium I	100 μM EDTA	10.0	45	28
Medium I	150 μM EDTA	12.0	47	41
Medium II	21 μM EDTA original medium	13.1	36	43

Table II. Hydrogenase activity in *Anabaena variabilis* grown in normal medium I and in nickel-depleted medium II. Filaments were permeabilized as described in Materials and Methods. Hydrogenase was assayed under argon by H_2 evolution in the presence of methylviologen (1 mM) reduced with sodium dithionite ($Na_2S_2O_4$, 5 mM). Each value represents the mean of 4 experiments of two culture batches. Expts. were performed in the dark at 28 °C for 30 min. Tolerance was ±10%.

Culture medium	μmol H_2 evolved / mg Chl × h
Medium I (1.8 μM $NiCl_2$)	1.25
Medium II (0.2 μM $NiCl_2$)	0.55

hydrogenase is assayed as hydrogen formation, it can be safely assumed that this hydrogenase operates as an uptake enzyme in the intact cell [19].

Discussion

Under physiological conditions, nitrogenase-catalyzed hydrogen formation [20] is counteracted by an uptake hydrogenase [21]. Recently, uptake hydrogenases from several bacteria have been shown to be dependent on the presence of nickel [8–13]; the experiments presented here extend these observations to cyanobacteria. Obviously, nickel concentrations required for uptake-hydrogenase activity are low, nickel present as contaminant in chemicals of the culture medium is sufficient to secure nickel requirement(s).

Since nitrogenase activity of nickel-depleted *Anabaena* is almost unaltered as compared to cells cultivated with nickel present (at least under our culture conditions), we do not assume an essential role of the "Knallgas reaction" (oxygen-hydrogen reaction) as a mechanism to prevent inactivation of nitrogenase by oxygen. Apparently, the advantage of hydrogenase-containing cyanobacteria is an efficient energy utilization by recycling hydrogen produced during nitrogen reduction. Experiments are under way to further characterize the physiological differences between hydrogenase-containing and hydrogenase-depleted cyanobacteria.

Acknowledgement

This study was supported by the "Zentrum für Energieforschung" of this University.

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Note added in proof:

After submission of the paper a similar Ni-effect on H_2 metabolism of intact cells of *Anabaena cylindrica* (ATCC 27899) was published [22].