Nickel Control of Hydrogen Production and Uptake in Anabaena spp. Strains CA and 1F

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(Received 30 December 1983)

Net aerobic H₂ production and the induction of uptake hydrogenase activity in the nitrogen-fixing Anabaena strains CA and 1F were strictly dependent upon the Ni²⁺ concentration in the growth medium. Ni²⁺ concentrations as low as 10 nM blocked H₂ production and stimulated an uptake hydrogenase activity in whole cells. Two types of uptake hydrogenase activity were seen: a dark aerobic uptake approximately 30% as active as the H₂ production rate, and a light-dependent activity in the presence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] at low oxygen concentrations, amounting to about 50% of the H₂ production rate. Together these activities may account for the strong control of net aerobic H₂ production by nickel. A significant fraction of the Ni²⁺-stimulated uptake hydrogenase activity formed during the transition from nickel deficiency to nickel sufficiency was blocked by chloramphenicol. Nickel may be required for activation of an uptake hydrogenase, or for hydrogenase synthesis, or for synthesis of another protein which is involved in H₂ uptake.

INTRODUCTION

High aerobic H₂ production has been found in two rapidly growing isolates of marine cyanobacteria, Anabaena spp. strains CA and 1F (Zhang et al., 1983). The net rate of H₂ production was some 15% of the rate of photosynthetic O₂ evolution or about 80% of the rate of acetylene reduction. We have found that aerobic H₂ production and uptake hydrogenase activity were regulated by nanomolar levels of Ni²⁺ added to the growth medium.

METHODS

Organisms and culture conditions. Anabaena strains CA (ATCC 33047) and 1F were grown on medium ASP-2 (Van Baalen, 1962; Stacey et al., 1977; Gotto et al., 1979) free of added combined nitrogen at 39°C with continuous gassing with 1% ± 0.1% (v/v) CO₂-in-air. With strain CA the NaCl content of the medium was 5 g l⁻¹, with strain 1F 18 g l⁻¹. The growth bath was illuminated by four fluorescent lamps (F48T12/CW/1500) on each side of the bath, 14 cm from the lamp centre to the centre of the growth tubes. The intensity, as measured looking through the bath at a light bank, was 450 to 500 μE m⁻² s⁻¹ (model 185A Quantum meter, Li-Cor Inc., Lincoln, Nebr., USA). Cell dry weight and growth rates were measured as previously described (Bottomley & Van Baalen, 1978).

Analytical methods. Amperometric measurement of H₂ or H₂ and O₂ simultaneously was done as previously described (Zhang et al., 1983). The actinic beam was provided by a projector with a DAY-DAK (Sylvania Inc., Winchester, Ky., USA) 500 W lamp operated at 100 V. The projector beam was screened by a no. 34-01-2 hot mirror (Baird-Atomic Inc., Bedford, Mass., USA). The intensity incident on the electrode chamber was 1200 μE m⁻² s⁻¹ and was varied, as desired, using copper screens. Ethylene was detected and measured using a

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Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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RESULTS AND DISCUSSION

The addition of Ni\(^{2+}\) to a nitrogen-fixing culture of *Anabaena* strains CA or 1F had a profound effect on the net \(\text{H}_2\) production and on the ability of the cells to take up \(\text{H}_2\) (Fig. 1). The effect of Ni\(^{2+}\) was in the nanomolar range. This was similar to the growth response obtained with a recently isolated Ni\(^{2+}\)-requiring non-nitrogen fixing cyanobacterium, *Oscillatoria* sp. strain 3NT (Van Baalen & O’Donnell, 1978). The effect of Ni\(^{2+}\) was not mimicked by Co\(^{2+}\) added at 50 or 100 nM. Other possibly important metals such as iron, manganese or perhaps molybdenum are commonly included in a synthetic algal culture medium like ASP-2 (Van Baalen, 1962). There was also little change in the results if the major salts of medium ASP-2 were extracted with 8-hydroxyquinoline before use (Gentry & Sherrington, 1950). The suppression of aerobic hydrogen production and the stimulation of uptake hydrogenase activity were strictly dependent upon the availability of Ni\(^{2+}\) to the cell. However, neither the growth rate on N\(_2\) nor the rate of acetylene reduction were much changed in the presence of added Ni\(^{2+}\) (Table 1).

The discrepancy between the high rate of aerobic \(\text{H}_2\) production and the much lower rates of \(\text{H}_2\) uptake found for both strains CA and 1F is not easily explained. Uptake hydrogenase

![Fig. 1. Effect of Ni\(^{2+}\) on \(\text{H}_2\) production and consumption of *Anabaena* strains CA (a) and 1F (b). The cultures were grown and measured at 39 °C. The cell densities were 0.16 mg dry wt ml\(^{-1}\) for both cultures. The cultures were grown for at least five generations with the indicated Ni\(^{2+}\) concentration, then transferred directly from the growth tube to the hydrogen electrode, and bubbled for 3 min with 1% (v/v) CO\(_2\)-in-air to eliminate the background H\(_2\) always present in the cultures. H\(_2\) output or uptake was calculated from the slopes of the first 3 min of the curves. ○, Rate of hydrogen production in the light; ●, rate of hydrogen uptake measured in the dark at a saturating hydrogen level, 25 to 35 \(\mu\)M.

<p>| Table 1. Effect of added Ni(^{2+}) on growth on N(_2) and acetylene reduction by <em>Anabaena</em> strains CA and 1F |</p>
<table>
<thead>
<tr>
<th>Strain</th>
<th>Ni(^{2+}) concn (nM)</th>
<th>Growth rate (generation time, h)</th>
<th>(\text{C}_2\text{H}_4) reduced [(\mu)l (mg dry wt(^{-1}) h(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>0</td>
<td>4.9</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.1</td>
<td>40</td>
</tr>
<tr>
<td>1F</td>
<td>0</td>
<td>4.5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.4</td>
<td>25</td>
</tr>
</tbody>
</table>
activity has been noted in nitrogen-fixing cyanobacteria but the question of the number of hydrogenases present and their precise function remains controversial (Tel-Or, 1978; Eisbrenner et al., 1981). In addition to dark H₂ uptake, a light-driven uptake was discernible in nickel-sufficient cells in the presence of DCMU at a low oxygen concentration (Fig. 2). Interestingly, the H₂ uptake rate was 17 μl H₂ (mg dry wt)⁻¹ h⁻¹, nearly twice the dark aerobic rate. This suggests that there may be two sinks for H₂, one to oxygen and the other through photosystem I. If these are independent and, therefore additive, they could account for the net aerobic H₂ production falling to nearly zero in the presence of Ni²⁺.

Fig. 2. Tracings, reduced in scale, of hydrogen uptake (——), and oxygen output (-----) of Anabaena strain CA in the presence of DCMU. A culture grown in the presence of 50 nM-Ni²⁺ was transferred directly from the growth bath to the electrode chamber and gassed for 5 min with 1% CO₂-in-N₂ in the dark. The cell density was 0.2 mg dry wt ml⁻¹. Arrows indicate light on, arrows up light off. DCMU was added as an ethanolic solution (1 μl) to give a final concentration of 2 x 10⁻⁵ M at zero time.

Fig. 3. Time course of decay in H₂ production and the increase in uptake hydrogenase activity after addition of Ni²⁺ to Anabaena cultures. Ni²⁺, 50 nM, was added to a culture of strain CA (a) or strain 1F (b) at zero time and the cultures were immediately returned to the growth bath. At the indicated times samples of the culture were transferred to the H₂ electrode and the rate of H₂ production was determined as described for Fig. 1. O, H₂ production, no added Ni²⁺; ●, H₂ uptake, no added Ni²⁺; □, H₂ production with Ni²⁺ present; ■, H₂ uptake with Ni²⁺ present; ▼, H₂ uptake with Ni²⁺ and chloramphenicol (20 μg ml⁻¹) present.
Nickel has been found in several hydrogenases (Graf & Thauer, 1981; Friedrich et al., 1982) and a nickel-dependent uptake hydrogenase can explain the dramatic decay in net aerobic H₂ production (Fig. 1). Similar observations on nickel controlling H₂ uptake and aerobic H₂ production have been found in Anabaena cylindrica (Daday & Smith, 1983). However, in addition to hydrogenase, nickel is known to be a constituent of jack-bean urease (Dixon et al., 1976); of carbon monoxide dehydrogenase (Diekert et al., 1979) and of factor 430, a nickel tetrapyrrole of unknown structure, in Methanobacterium thermoautotrophicum (Diekert et al., 1980; Ellefson et al., 1982). A second function of Ni²⁺ in controlling H₂ production is, therefore, not ruled out at this time.

The time course of decay of net H₂ production and the stimulation of uptake hydrogenase activity after the addition of Ni²⁺ to cultures of strains CA or 1F was in terms of hours, and chloramphenicol blocked a significant fraction of the Ni²⁺-induced uptake hydrogenase activity formed in strain CA (Fig. 3). The availability of nickel to a cell may control the synthesis of uptake hydrogenase or the synthesis of another protein which activates uptake hydrogenase activity. We are presently examining this point and whether the increased uptake hydrogenase activity will reside in isolated, active heterocysts.

This work was supported in part by grant 83-CRCR-1-1286 from the US Department of Agriculture. We thank Rita O'Donnell for excellent technical assistance.

The University of Texas Marine Science Institute Contribution no. 607.

REFERENCES


