Pathways of hydrogen uptake in the cyanobacterium *Nostoc muscorum*

Hans Weisshaar and Peter Böger

Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany

**Abstract.** Two pathways of hydrogen uptake in *Nostoc muscorum* are apparent using either oxygen or nitrogen as electron acceptor. Hydrogen uptake (under argon with some oxygen as electron acceptor assayed in the dark; oxyhydrogen reaction) is found to be more active in dense, light-limited cultures than in thin cultures when light is not limiting. Addition of bicarbonate inhibits this hydrogen uptake, because photosynthesis is stimulated. In a cell-free hydrogenase assay, a 10-fold increase of the activity can be measured, after the cells having been kept under light-limiting conditions. After incubation under light-saturating conditions, no hydrogen uptake is found, when filaments are assayed under argon plus some oxygen. Assaying these cells under a nitrogen atmosphere, a strong hydrogen uptake occurs. The corresponding cell-free hydrogenase assay exhibits low hydrogenase activity. Furthermore, the hydrogen uptake by intact filaments under nitrogen in the light apparently is correlated with nitrogenase activity. These studies give evidence that, under certain physiological conditions, hydrogen uptake of heterocysts proceeds directly via nitrogenase, with no hydrogenase involved.

**Key words:** Hydrogen uptake - Hydrogenase and nitrogenase activity (cellular, cell-free) - Photosynthetic oxygen evolution - Cyanobacteria

**Introduction**

There is general accordance that nitrogenase catalyzes hydrogen evolution, whereas hydrogen uptake is mediated by hydrogenase. Three different enzymes involved in hydrogen metabolism of heterocystous cyanobacteria have been reported (Tel-Or et al. 1977; Hallenbeck and Benemann 1978; Daday et al. 1979; Houchins and Burris 1981a, b), namely uptake hydrogenase, reversible hydrogenase, and nitrogenase. The existence of only one hydrogenase type (uptake hydrogenase) is suggested by Eisbrenner et al. (1981). For a recent review of hydrogen metabolism in cyanobacteria see Houchins (1984).

Apparently, hydrogen evolution and hydrogen uptake are localized in the heterocysts (Peterson and Wolk 1978; Houchins and Burris 1981a). Within the heterocysts, the oxygen protection of nitrogenase is thought to occur essentially through respiration (Jensen and Cox 1983; Ernst et al. 1984; Murry et al. 1984), a strong oxyhydrogen reaction is not assumed.

Dinitrogen is thought to inhibit H₂-formation due to the competition of N₂-reduction with proton reduction (Jones and Bishop 1976). However, it is also found that proton reduction is independent of N₂-reduction (Chatt 1980). The absence of hydrogen evolution in the presence of nitrogen can be due to recycling of H₂. Benemann and Weare (1974) presented data for hydrogen-stimulated nitrogenase activity.

Hydrogenase is an inducible enzyme as was shown by growth in the presence of hydrogen (Tel-Or et al. 1977). An inhibition of hydrogenase activity (hydrogen uptake) by low sulfide concentrations leads to higher amounts of hydrogen evolution in the heterocystous cyanobacteria *Nostoc muscorum* (Weisshaar and Böger 1983a). When hydrogen uptake is missing, as found in the non-heterocystous cyanobacterium *Phormidium foveolarum*, hydrogen evolution is optimized (Weisshaar and Böger 1983a). In the absence of nickel, hydrogenase activity is decreased (Daday and Smith 1983; Almon and Böger 1984).

In this study, we present evidence for two different pathways of hydrogen uptake, either via hydrogenase or directly by nitrogenase. Apparently, these pathways are regulated by photosynthetic activity, i.e. availability of reductant supply to nitrogenase.

**Materials and methods**

**Culture conditions.** The cyanobacterium *Nostoc muscorum* PPC 7119 was grown in a mineral medium according to Allen and Arnon (1955) with bound nitrogen. *Nostoc* was cultivated in 2-l Fernbach flasks containing 1.3 l of algal suspension according to Spiller et al. (1978) with an initial chlorophyll concentration after inoculation of 0.5 µg/ml. All experiments were performed with filaments grown for 4 days at 24°C under continuous illumination (5 W/m² = 50 µeinsteins/m² measured with a quantum meter from Licor Inc., Lincoln, NB, USA: LT-185B and quantum sensor LJ-190SB, 400 – 700 nm). A chlorophyll content between 5 and 6 µg/ml was obtained. The percentage of heterocysts was 5% and did not change during cultivation time. For growth on ammonia, the medium was supplemented with 2 mM NH₄Cl and (in addition) 8 mM K₂HPO₄ to adjust the buffering capacity. For growth on nitrate, the medium contained 20 mM KNO₃.
Fig. 1. Simultaneous measurement of hydrogen and oxygen uptake and carbon dioxide evolution in the dark with Nostoc muscorum after cultivation for 24 and 96 h. Open symbols, namely Δ Δ hydrogen uptake, ○ ○ oxygen uptake, □ □ carbon dioxide evolution, stand for the 24-h culture, the closed symbols indicate the 96-h old culture. Chlorophyll was 4 μg/ml. Both hydrogen and oxygen content was 2% (v/v) at start. The 24-h culture was in the exponential growth phase and the 96-h culture in the linear growth phase. Both culture stages differ in glycogen content (see Table 1).

Table 1. Different DCMU sensitivity of light-induced nitrogenase-catalyzed ethylene and hydrogen evolution dependent on glycogen content in intact Nostoc muscorum. Rates are given in μmol/ml pcv × h

<table>
<thead>
<tr>
<th>Culture time (Days)</th>
<th>Glycogen content (μg/μg Chl)</th>
<th>Ethylene evolution</th>
<th>Hydrogen evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+DCMU</td>
<td>-DCMU</td>
<td>+DCMU</td>
</tr>
<tr>
<td>1</td>
<td>13.6</td>
<td>44.3</td>
<td>41.9</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>8.6</td>
<td>61.2</td>
</tr>
</tbody>
</table>

Measurements were performed after a 10-h incubation at 25°C using a light-intensity of 60 W/m². The Chl content was 4 μg/ml, DCMU 5 μM. The light intensity used for growth (5 W/m²) was increased to 60 W/m² during incubation to obtain the highest possible nitrogenase activity.

Assay conditions. Intact cells were incubated in the light at 5 W/m² (except for the assay of Table 1) using 36-ml glass vessels containing 10-ml aliquots of cell suspension, for a period as indicated. The samples were sealed with rubber stoppers (Suba seal, Freeman and Co., Barnsley, England), flushed with argon or nitrogen, and incubated at 25°C in a Warburg shaker illuminated by tungsten bulbs (7 W/m²). For determination of hydrogen uptake 2% H₂ (v/v) was added; acetylene reduction was measured in the presence of 10% C₂H₂ (v/v).

Gas-exchange measurements. Hydrogen, oxygen, ethylene, and carbon dioxide were detected by gas-chromatography. For details see Weisshaar and Büger (1983 b).

Cell-free hydrogenase assay. Cell-free hydrogenase activity was measured in the dark as described by Hallenbeck et al. (1978) using Na₂S₂O₄ (dithionite)-reduced methylviologen as e⁻-donor. The Nostoc cells were disrupted by repeated freezing and thawing and by an additional ultrasonic treatment (Weisshaar and Büger 1983 b).

Glycogen measurements. The glycogen content of Nostoc cells was determined by a method of Ernst et al. (1984). The glycogen was digested with amyloglucosidase and amylase. Then, the glucose was assayed with hexokinase/glucose-6-phosphate dehydrogenase coupled to NADP reduction.

Results and discussion

In reductant-limited filaments of Nostoc muscorum, those having a low glycogen content, nitrogenase activity was decreased by addition of DCMU. As shown in Table 1, nitrogenase-catalyzed ethylene and hydrogen evolution is dependent on the glycogen content of the cyanobacterium, which is correlated with the culture age or more precisely with cell density (light-intensity per cell). With glycogen in excess, as found in a 24-h culture, DCMU had no effect on ethylene and hydrogen evolution. To exclude the influence of photosynthetic oxygen evolution on oxygen-uptake activity...