Transcriptional Regulation of Nostoc Hydrogenases: Effects of Oxygen, Hydrogen, and Nickel

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The transcription of structural genes encoding two hydrogenases in N2-fixing cultures of the cyanobacteria Nostoc muscorum and Nostoc sp. strain PCC 73102 were examined by reverse transcription-PCR. A low level of oxygen and addition of nickel induce higher transcript levels of both hydrogenases, whereas molecular hydrogen has a positive effect on the transcription of the genes encoding only the uptake hydrogenase.

Two distinct NiFe hydrogenases have been described in cyanobacteria: an uptake and a bidirectional hydrogenase (3, 7, 10, 18). The uptake hydrogenase is a dimeric enzyme consisting of a large subunit (HupL) and a small subunit (HupS). Cyanobacterial hupSL genes have been sequenced in Anabaena sp. strain PCC 7120 (3), Nostoc sp. strain PCC 73102 (15), and Anabaena variabilis (8). The physiological role of the uptake hydrogenase appears to be coupled to N2 fixation (9, 14, 23). Transcriptional studies have shown that hupSL is a transcript unit (8, 13) present in N2-fixing cultures (1, 6, 8). The bidirectional hydrogenase has the in vivo and in vitro capacity to both evolve and take up hydrogen. It consists of a diaphorase (HoxFU) and a hydrogenase moiety (HoxHY) (18) and is not a universal cyanobacterial enzyme (21, 22). The in situ role of the bidirectional hydrogenase is not clearly defined, but an involvement in fermentation has been suggested (20). As part of a larger molecular characterization of cyanobacterial hydrogenases with the ultimate goal of examining the potential of photobiological hydrogen production, we have further examined the transcriptional regulation of cyanobacterial hydrogenases in Nostoc muscorum, a strain containing an uptake and a bidirectional hydrogenase, and Nostoc sp. strain PCC 73102, a strain containing only an uptake hydrogenase.

Cells of N. muscorum Agardh CCAP 1453/12 and Nostoc sp. strain PCC 73102 were grown in BG11 (17) as described previously (14, 21). All cultures were sparged with air. In cultures supplemented with H2, 9% of the air was replaced with H2. Nickel shifts were performed by adding 0.5 μM NiSO4 to the culture (14), and anaerobic conditions were obtained by replacing the air with a gas mixture of 98% argon and 2% CO2. In vivo hydrogen uptake activity was determined as described previously (14).

Total RNA from N. muscorum was isolated as described previously (1) with the following modifications. The cells were disrupted with 1 volume of phenol (65°C) followed by the addition of 1 volume of phenol-chloroform (1:1) until no or only a minor interphase could be detected. Total RNA from Nostoc sp. strain PCC 73102 was isolated by washing a cell pellet once in 500 μl of resuspension buffer (20 mM Na-acetate and 1 mM EDTA, pH 5.5) before resuspending it in 500 μl of resuspension buffer and 50 μl of 10% sodium dodecyl sulfate. One volume of a 1:1 mixture of phenol (equilibrated with resuspension buffer) and chloroform was added and incubated for 5 min at 60°C followed by quick cooling on ice and centrifugation (7 min; 4°C). The aqueous phase was transferred to a new tube and mixed with phenol-chloroform as described above. DNase treatment and quantification of obtained RNA were performed as detailed previously (1).

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FIG. 1. (A) Agarose gel visualizing the plateau effect of an RT-PCR with hoxH primers, using reverse-transcribed RNA from air-grown N. muscorum (t = 0 h) shifted to anaerobic conditions (t = 24 h). Samples were taken after 30, 32, 34, 36, 38, and 40 PCR cycles. dh2O (distilled H2O), PCR negative control; DNA, PCR positive control. (B) Air-grown culture of N. muscorum divided (t = 0 h) in two, either bubbled with air or shifted to anaerobic conditions. An agarose gel visualizing the hoxH transcript is shown. Lanes: 1, 100-bp ladder; 2, t = 0 h; 3, t = 24 h in air; 4, t = 24 h under anaerobic conditions; 5 to 7, corresponding RT-PCR negative controls; 8, PCR negative control; 9, genomic DNA positive control.
FIG. 2. Cells of *N. muscorum* and *Nostoc* sp. strain PCC 73102 shifted from aerobic to anaerobic conditions (A), from air to air supplemented with 9% H\textsubscript{2} (B), and with the addition of 0.5 μM Ni to the cultures (C). Shown are the in vivo hydrogen uptake activities prior to and after the shift or addition (nanomoles of H\textsubscript{2} oxidized per hour and micrograms of chlorophyll a [chl a]) and the relative mRNA abundances of *hupL* and *hoxH*. M, marker (100-bp ladder); dH\textsubscript{2}O (distilled H\textsubscript{2}O), PCR negative control; DNA, PCR positive control.
Total RNA (0.6 to 1.0 µg) was reverse transcribed into cDNA, followed by PCR (25 µl) as described previously (1). The PCR program profiles used were as follows: hupL, 1 min at 94°C followed by repetitive cycles of 45 s at 94°C, 45 s at 50°C, and 45 s at 72°C and finishing with 7 min at 72°C; hoxH, 1 min at 94°C followed by repetitive cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C and finishing with 7 min at 72°C. The PCR products obtained were analyzed on a 1% agarose gel. To ensure that the plateau effect was avoided, all PCRs were performed with different numbers of cycles (Fig. 1A). Representative samples from the reverse transcription (RT)-PCR experiments are presented in Fig. 2. To exclude changes in the expression of hupL and hoxH and in hydrogen uptake activity due to time and growth, experiments were performed in which a culture was split in two at zero hour and either bubbled with air or shifted to the conditions described above. The results demonstrated that in logarithmic growth phase there are no significant changes in the expression of hupL (Fig. 1B) or hoxH or in the in vivo hydrogen uptake activity (data not shown).

We used RT-PCR to examine differences in the relative amounts of transcripts of hoxH and hupL in cultures with shifts in key environmental conditions. Each experiment was performed at least three times. Samples from a specific shift were treated identically, and a change in the intensity of a specific PCR-generated DNA fragment was interpreted as a change in the relative amount of the specific transcript as a result of the changed environmental condition. Comparisons of relative quantities between different experiments (e.g., data presented in different figures in the present study) are invalid due to differences in, e.g., the starting amount of RNA in the reverse transcription and the number of PCR cycles.

In cells of N. muscorum transferred to anaerobic conditions, an increase of the in vivo hydrogen uptake activity was observed concomitantly with an increase of the relative amount of both hupL and hoxH transcripts (Fig. 2A). Similarly, in Nostoc sp. strain PCC 73102, the mRNA abundance of hupL transcript as well as the in vivo hydrogen uptake activity increased (Fig. 2A). It has previously been demonstrated that a hoxH transcript is present in vegetative cells of aerobically grown N. muscorum with no specific regulation in respect to a shift from non-N₂-fixing to N₂-fixing conditions (1). Similarly, hox transcripts are present in both vegetative cells and heterocysts of A. variabilis (2). Both cyanobacterial hydrogenases may be regulated by the O₂ partial pressure, and by reducing the external levels of O₂, both enzymes are induced (10, 19, 21) and regulated at the level of transcription. In support of these results, induction of hupSL transcription by reduced levels of O₂ has been reported in Rhizobium leguminosarum (16) and Bradyrhizobium japonicum (11).

The mRNA abundance of hupSL was raised by adding 9% H₂ to the air in both N. muscorum and Nostoc sp. strain PCC 73102. The increased level of hupL transcript was followed by a higher in vivo hydrogen uptake activity. No change in the mRNA abundance of hoxH transcript could be detected (Fig. 2B). Addition of H₂ has previously been shown to induce higher activity of the uptake hydrogenase (10, 14). However, the activity of the bidirectional hydrogenase was unaffected by addition of H₂ (10). Transcriptional studies of the uptake hydrogenase in *Rhodobacter capsulatus* (4) and *B. japonicum* (11) demonstrated a stimulation of the transcription of hupSL in the presence of H₂.

Nickel is a central and essential component of an NiFe hydrogenase. Addition of external nickel to the growth medium increased the hydrogenase uptake activity in several cyanobacteria (5, 12, 14, 24). These results are in agreement with the results obtained in the present study, where addition of 0.5 µM external nickel increased the in vivo hydrogen uptake in both *N. muscorum* and *Nostoc* sp. strain PCC 73102. RT-PCR demonstrated an increase in the mRNA abundances of hupL and hoxH (Fig. 2C). Further supporting these conclusions, stimulatory effects of nickel on the transcription of hupSL have been observed in *B. japonicum* (11).

Transcriptional factors binding to the promoter regions of cyanobacterial hydrogenases remain to be identified. However, a putative Fnr-binding motif upstream of hupSL has been identified in *A. variabilis* (8). Sequence analyses identified putative binding sites for an integration host factor, a transcriptional factor involved in activation of nif genes in purple bacteria, and NtcA, a known global nitrogen regulator in cyanobacteria, upstream of the hupSL (NtcA and integration host factor) and ORFhypFCDEAB (NtcA) genes in Nostoc sp. strain PCC 73102 (6, 13). The transcription start sites have been identified 103 and 259 bp upstream of the hupS start codon in *A. variabilis* and *Nostoc* sp. strain PCC 73102, respectively (8, 13). However, only limited information is available on the transcription of the bidirectional hydrogenase. Analysis of the promoter region of the bidirectional hydrogenase is meager, and no transcription start site or promoter motifs have been identified.

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**REFERENCES**