Uptake hydrogenase in cyanobacteria: novel input from non-heterocystous strains

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Abstract

Most of the research to date on cyanobacterial uptake hydrogenases has been performed on filamentous heterocystous strains. However, recent results on the hup gene cluster organization and its transcriptional regulation in non-heterocystous strains has contributed to the widening of knowledge in this field. In the present study, we outline the recent findings on uptake hydrogenases from non-heterocystous cyanobacteria, comparing it with the presently available data from heterocystous strains, and draw attention to potential areas for future research.

Introduction

Cyanobacteria show a considerable morphological diversity and many strains are capable of N₂ fixation. Since the enzyme complex responsible for N₂ fixation, nitrogenase, is very oxygen labile, cyanobacteria have evolved diverse mechanisms/strategies to protect it (e.g. spatial or temporal separation of N₂ fixation and O₂ evolution; see Figure 1). The molecular hydrogen produced during nitrogen fixation is recaptured by a NiFe-uptake hydrogenase, encoded by the structural genes hupSL [1]. Additionally, another NiFe-hydrogenase (encoded by boxEFUYH, bi-directional hydrogenase) may be present that, depending on the growth conditions, has the capacity of both producing and consuming H₂ [1,2]. The uptake hydrogenase has been found in all N₂-fixing strains examined so far [1,3], but its cellular/subcellular localization is still not clear. However, available results suggest that the enzyme is membrane-bound, and possibly localized on the cytoplasmic side of either the cytoplasmic or thylakoid membrane [1]. Immunolocalization using cyanobacterial specific antibodies will help to clarify the exact location of the enzyme.

Uptake hydrogenase structural genes and the deduced proteins

Like the majority of NiFe-hydrogenases, the structural genes encoding the cyanobacterial uptake hydrogenase are clustered, and part of a transcriptional unit, in which the gene for the smaller subunit (hupS) is upstream from the gene for the larger one (hupL) [1,4]. In some heterocystous strains, e.g. Anabaena/Nostoc sp. PCC 7120, the excision of a DNA element by site-specific recombination occurs within hupL during the differentiation of a vegetative cell into a heterocyst, indicating that HupL is expressed exclusively in the latter cell type [1,5]. Analysis of the predicted proteins demonstrates that HupS (∼35 kDa) has the same number of residues in all cyanobacteria (320 amino acids), whereas HupL (∼60 kDa) generally has 531 amino acids with the exception of the filamentous non-heterocystous strains: Trichodesmium erythraeum IMS101 (three extra) and Lyngbya majuscula CCAP 1446/4 (six extra). Overall, the cyanobacterial uptake hydrogenases are highly conserved with >80% identity, yet parsimony analysis of combined small and large subunit amino acid sequences show that non-heterocystous and heterocystous strains form two separate clusters (Figure 2). The enzyme contains all the conserved cysteine residues putatively involved in the formation of the [FeS] clusters, and the Ni-binding sites in HupS and HupL, respectively. Moreover, HupL contains the C-terminal region that is presumably cleaved off during the maturation process of the large subunit [1,4]. In contrast with other organisms, the cyanobacterial HupS lacks both the twin-arginine signal peptide at the N-terminal, and the hydrophobic motif at the C-terminal proposed to be involved in the translocation and anchorage to the membrane respectively. Furthermore, Kyte-Doolittle hydropathy plots demonstrated that no transmembrane segments are present within HupS and HupL of cyanobacteria (results not shown). In contrast, physiological and biochemical results strongly indicate that the cyanobacterial uptake hydrogenase is membrane-bound, therefore it is probable that an additional membrane anchoring polypeptide exists. Supporting this hypothesis, analysis of cyanobacterial genomes revealed the presence of open reading frames that potentially constitute this missing subunit HupC [6].

Although the general features of the cyanobacterial uptake hydrogenases cluster them together with the H₂-sensing enzymes [7], the production of uptake deficient mutants [8–11] clearly demonstrates that cyanobacterial hupSL encode an uptake hydrogenase rather than a regulatory enzyme.

Key words: cyanobacteria, hup, intergenic region, repetitive sequence, uptake hydrogenase.

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Figure 1 | Schematic representation of spatial versus temporal separation of N₂ fixation/H₂ uptake and photosynthesis in cyanobacteria

<table>
<thead>
<tr>
<th>SPATIAL</th>
<th>TEMPORAL</th>
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<tbody>
<tr>
<td>Heterocystous strains</td>
<td>Non-heterocystous strains</td>
</tr>
<tr>
<td>Vegetative cells: Photosynthesis H₂ uptake</td>
<td>Unicellular Light H₂ fixation H₂ uptake</td>
</tr>
<tr>
<td>Heterocysts: Photosynthesis N₂ fixation H₂ uptake</td>
<td>Filamentous Dark</td>
</tr>
</tbody>
</table>

+ non-heterocystous Trichodesmium (with diazocytes)

Figure 2 | Unweighted, unrooted maximum parsimony analysis of combined small and large subunit amino acid sequences of cyanobacterial uptake hydrogenases (100 replicate heuristic search with TBR branch-swapping)

Numbers by nodes indicate bootstrap support (1000 replicates). GenBank® accession nos. AF030525 (Nostoc punctiforme PCC 73102), U08013 (Anabaena sp. PCC 7120), Y13216 (A. variabilis ATCC 29413), NZ_AABK03000001 (T. erythraeum IMS101), AF368526 (L. majuscula CCAP 1446/4) and AY260103 (Gloeothece sp. ATCC 27152). [N.B. Corresponding sequences for the unicellular Crocosphaera watsonii WH 8501 are now available (NZ_AADV01000239); highest amino acid identity with Gloeothece 27152].

hupSL intergenic region

The cyanobacterial hupSL differ from those of other microorganisms in being separated by longer intergenic regions [1]. Most of these regions contain repetitive sequences. A screening of seven filamentous heterocystous cyanobacteria revealed that all, except one, contained STRRs (short tandemly repeated repetitive) sequences, belonging to different families of STRRs (GenBank® accession nos. Y13216, U08013, AF030525 and AF455565–68). In the unicellular Gloeothece sp. ATCC 27152 (AY260103) and in the filamentous non-heterocystous T. erythraeum (NZ_AABK03000001) no repeats could be discerned. In contrast, the unicellular Crocosphaera watsonii WH 8501 (NZ_AADV01000239) contains inverted repeats, whereas in the filamentous non-heterocystous L. majuscula two sets of long repeated repetitive sequences (30 and 58 bp LRRs (long repeated repetitive sequences)) were identified (AF368526). Since the repeats in the cyanobacterial hupSL intergenic regions are highly variable or even absent, it is unlikely that they play a direct role in gene expression regulation. However, in all strains tested, a putative stem-loop structure derived by two-dimensional computer modelling might occur in the transcribed mRNA [1,12]. It has been suggested that the hairpin may increase the stability of the transcript, and/or confer a translational coupling between hupS and hupL, ensuring the synthesis of the two subunits in equal amounts [12]. However, only the construction of specific mutants will help to clarify the physiological and molecular significance of the hairpin structure and/or the repetitive sequences of the hupSL intergenic region.

Transcriptional regulation and maturation of the enzyme

The first transcriptional data on cyanobacterial uptake hydrogenases arose from reverse transcriptase–PCR experiments on Anabaena 7120, revealing that hupL transcription coincides with the formation of heterocysts [5]. Subsequently, studies on other filamentous strains, as well as on the unicellular Gloeothece 27152, have confirmed the induction of transcription under nitrogen-fixing conditions only [1,4]. One exception is Anabaena variabilis ATCC 29413, where a low level of hupL expression has been detected in vegetative cells grown with the addition of ammonia [13]. In the unicellular Gloeothece 27152, an evident light/dark regulation of the uptake hydrogenase transcription was recently demonstrated. The highest transcript levels were detected during the light cycle, whereas the peak of the hydrogen uptake activity occurred in dark [4]. Until recently, cyanobacterial hupSL was shown to constitute a single transcript, containing no additional open reading frames [8,12]. However, novel results from Gloeothece 27152, revealed the presence of hupW (encoding an uptake hydrogenase-specific endopeptidase) directly downstream of hupL, and co-transcription of the three genes [4]. Recently, it was proposed that NtcA (transcriptional regulator mediating nitrogen control in cyanobacteria) is probably involved in the regulation of uptake hydrogenase transcription [4,6].

The maturation of hydrogenases is a highly complex process (requiring a number of accessory proteins), which has...
been fairly well characterized in a few microorganisms [7,14]. Although little is known about the biosynthesis/maturation of cyanobacterial NiFe-hydrogenases, several genes required for this process in other microorganisms have also been identified in cyanobacteria (e.g. hypA-F) [1]. However, no results are available on the specific role of cyanobacterial Hyp proteins. In addition, the presence and expression of a C-terminal endopeptidase, specific for HupL maturation (HupW), was recently reported, see above [4,15]. Once more, the construction of specific mutants will provide accurate information for the involvement/function of these proteins in the biosynthesis of cyanobacterial hydrogenase(s).

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References

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