Arch Microbiol (1998) 169:267–274

ORIGINAL PAPER

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Hydrogen uptake in *Nostoc* sp. strain PCC 73102. Cloning and characterization of a *hupSL* homologue

Received: 20 August 1997 / Accepted: 24 November 1997

Abstract Structural genes encoding an uptake hydrogenase of *Nostoc* sp. strain PCC 73102 were isolated. From partial libraries of genomic DNA, two clones (pNfo01 and pNfo02) were selected and sequenced, revealing the complete sequence of both a *hupS* (960 bases) and a *hupL* (1,593 bases) homologue in *Nostoc* sp. strain PCC 73102. A comparison between the deduced amino acid sequences of HupS and HupL of *Nostoc* sp. strain PCC 73102 and *Anabaena* sp. strain PCC 7120 showed that the HupS proteins are 89% identical and the HupL proteins are 91% identical. However, the noncoding region between the genes in *Nostoc* sp. strain PCC 73102 (192 bases) is longer than that of *Anabaena* sp. strain PCC 7120 and of many other microorganisms. Southern hybridizations using DNA from both N_2-fixing and non-N_2-fixing cells of *Nostoc* sp. strain PCC 73102 and different probes from within *hupL* clearly demonstrated that, in contrast to *Anabaena* sp. strain PCC 7120, there is no rearrangement within *hupL* of *Nostoc* sp. strain PCC 73102. Indeed, 6 nucleotides out of 16 within the potential recombination site are different from those of *Anabaena* sp. strain PCC 7120. Furthermore, we have recently published evidence demonstrating the absence of the bidirectional/reversible hydrogenase in *Nostoc* sp. strain PCC 73102. The present knowledge, in combination with the unique characteristics, makes *Nostoc* sp. strain PCC 73102 an interesting candidate for the study of deletion mutants lacking the uptake-type enzyme.

Key words Cyanobacteria · Uptake hydrogenase · *hupSL* · *Nostoc*

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Introduction

In nitrogen-fixing cyanobacteria, H_2 production is mainly catalyzed by a nitrogenase during the reduction of N_2 to NH_3 and is quickly metabolized by a unidirectional uptake hydrogenase. In addition, a bidirectional/reversible enzyme may also be present and oxidize some of the molecular hydrogen [for general reviews, see Smith (1990), Rao and Hall (1996), and Schulz (1996)].

Hydrogenases have been characterized in many microorganisms representing different taxonomic groups, and all enzymes studied to date have subunit structures ranging from one to four polypeptides. Most of the membrane-bound (NiFe) uptake hydrogenases are heterodimeric enzymes with a large subunit (α-subunit) in the range of 46–72 kDa, and a small subunit (β-subunit) in the range of 23–38 kDa. The large subunit contains nickel in the active site, whereas the small subunit plays a major role in electron transfer to the large subunit. The structural genes coding for both subunits are part of a transcriptional unit in which the gene for the smaller one is located upstream from the gene coding for the larger one (Przybyla et al. 1992; Voordouw 1992; Wu and Mandrand 1993; Albracht 1994; Hahn and Kück 1994; Vignais and Toussaint 1994).

At present, only a few sequences/molecular studies concerning hydrogenases from cyanobacteria are available, and most of them concern the bidirectional/reversible enzyme (*hox* genes; Schmitz et al. 1995; Appel and Schulz 1996; Boison et al. 1996; see also the complete genome sequence of *Synechocystis* sp. strain PCC 6803, http://www.kazusa.or.jp./cyano/cyano.html). For the filamentous, heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120, Carrasco et al. (1995) described a developmental genome rearrangement from within *hupL*, a gene that exhibits sequence similarity to genes encoding the large subunit of membrane-bound uptake hydrogenases. This rearrangement occurs late during the heterocyst differentiation process and involves the excision of a 10.5-kb DNA element by site-specific recom-

bination, indicating that the product HupL is expressed only in heterocysts in *Anabaena* sp. strain PCC 7120 (Carrasco et al. 1995).

Previously, we examined *Nostoc* sp. strain PCC 73102, a filamentous heterocystous cyanobacterium, for the presence of hydrogenases by using antisera directed against several proteins purified from other microorganisms, and demonstrated that the antigens are present in both the nitrogen-fixing heterocysts and the photosynthetic vegetative cells. Moreover, a common polypeptide with a mol. mass of approximately 58 kDa was observed (Tamagnini et al. 1995). The effects of nickel, hydrogen, carbon, and nitrogen on in vivo hydrogen uptake have also been studied. This uptake is stimulated by light and is positively regulated by the substrate H_2 (either added directly from an external source or produced through the action of nitrogenase). Furthermore, the in vivo nitrogenase and uptake hydrogenase activities appear to be co-regulated when nitrogen-fixing cells are exposed to either combined nitrogen or organic carbon sources (Oxelfelt et al. 1995). Recently, using both molecular and physiological techniques, we have found no evidence for either the *hox* genes or the corresponding bidirectional/reversible enzyme activities in *Nostoc* sp. strain PCC 73102, making this strain an interesting candidate for future biotechnological applications (Tamagnini et al. 1997).

The present study was carried out in order to continue our characterization of H_2 metabolism in *Nostoc* sp. strain PCC 73102 and to identify and sequence potential structural genes encoding an uptake hydrogenase – *hupSL*. Moreover, we were able to demonstrate that in this strain, when a photosynthetic vegetative cell differentiates into a nitrogen-fixing heterocyst, there is no rearrangement occurring within the *hupL* gene. We believe that it is very important to examine one particular strain in detail because only with this knowledge can further molecular experiments, e.g., construction and physiological studies of specific deletion mutants lacking a functional uptake hydrogenase, be performed and correctly evaluated.

Materials and methods

Organisms and growth conditions

Nostoc sp. strain PCC 73102, a free-living, filamentous, heterocystous cyanobacterium originally isolated from coralloid roots of the cycad *Macrozamia* sp., was obtained from the Pasteur Culture Collection (PCC; Paris, France) (Rippka et al. 1979). *Nostoc* sp. strain PCC 73102 has been proposed as the type strain of the species *Nostoc punctiforme* in the PCC classification. Axenic, N_2-fixing, and non-N_2-fixing cultures were grown in $BG11_0$ and BG11 media, respectively (Stanier et al. 1971), in continuous light (Thorn Polylux 4000 and Osram Warmtone Warm White 400–700 nm; 40 μmol photons m^{-2} s^{-1}), at 26° C with the use of a magnetic stirrer to obtain a homogeneous cell suspension (Lindblad 1992). The plasmid used in the ligation steps in this study was pBluescript II SK (+) (Stratagene), and the bacterial strain *Escherichia coli* XL1-Blue (Stratagene; La Jolla, Calif., USA) was used in the transformations. The *E. coli* strain was grown aerobically at 37° C in liquid Luria Bertani (LB) medium (Sambrook et al. 1989). Solid LB medium for plates contained 1.5% agar. α-Carboxylenzylpenicillin (carbenicillin; 80 μg/ml; Sigma, St. Louis, Mo., USA) was used as the antibiotic in both liquid and solid medium. For subcloning of smaller fragments of the two clones pNfo01 and pNfo02, the Erase-A-Base system (Promega; Madison, Wis., USA) was used.

Isolation of DNA and agarose gel electrophoresis

Genomic DNA from cells of *Nostoc* sp. strain PCC 73102 was extracted according to the method used in Tamagnini et al. (1997). Plasmid DNA was obtained using the Wizard Plus Miniprep DNA Purification System (Promega). Recovery of DNA from 1% agarose gels was performed as in Tamagnini et al. (1997). Agarose gel electrophoresis was performed following standard protocols and using 0.5 × TBE (44.5 mM Tris-borate, 44.5 mM boric acid and 1 mM EDTA) (Sambrook et al. 1989). The DNA was visualized by using the fluorescent dye ethidium bromide and by direct examination of the gel in UV light.

Sequence comparisons

Amino acid sequences from several bacteria [*Desulfovibrio gigas*, GeneBank accession no. P12944; *Desulfovibrio vulgaris*, accession no. P21852; *Azotobacter chroococcum*, accession no. P18191; *Alcaligenes hydrogenophilus*, accession no. P33374; *Bradyrhizobium japonicum*, accession no. P12636; *Pseudomonas hydrogenovora*, accession no. D1013912; *Rhodobacter capsulatus*, accession no. P15284; *Rhodocyclus gelatinosus*, accession no. P17632; *Rhizobium leguminosarum*, accession no. P18636; *Thiocapsa roseopersicina*, accession no. 349577; and the cyanobacterium *Anabaena* sp. strain PCC 7120 (*hupS* – Carrasco and Golden, personal communication; *hupL* – accession no. U08013, Carrasco et al. 1995)], were aligned (Clustal W 1.6.1), and conserved regions within the uptake hydrogenases were identified.

PCR and DNA sequencing

PCR was carried out in a thermal cycler Gene Amp PCR System 2400 (Perkin Elmer) with *AmpliTaq* DNA polymerase (Perkin Elmer) following the protocol used by Tamagnini et al. (1997). The PCR products were separated on a 1.5% agarose gel along with either 123- or 100-bp ladders (Gibco BRL) as length markers. The primers used were H4A and H6B [see Tamagnini et al. (1997)] for amplifying the fragment hup2, which was then used for screening for the clone pNfo01 in the initial partial library. DNA sequencing reactions were performed in a thermal cycler Gene Amp PCR System 2400 (Perkin Elmer) using an ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) according to the instructions of the manufacturer. The sequences of the samples were determined with an automated DNA sequencer (ABI Model 373 DNA sequencer); the comparisons of obtained sequences were determined using the National Center for Biotechnology Information (USA) via the BLAST e-mail server, and the programs SeqEd 1.0.3 and MacVector 4.1.4. The 3,940-bp sequence encompassing the *hupSL* homologue is available from GenBank under accession no. AF030525.

Southern hybridization

Genomic DNA was isolated as described above and was digested with the restriction endonucleases (*Hin*dIII, *Eco*RI, *Xba*I and/or *Hin*dIII and *Eco*RI). One-half to one microgram of each digested DNA was separated electrophoretically in a 1% agarose gel. After denaturation and neutralization steps [see Tamagnini et al. (1997)], the DNA was transferred to a nylon membrane (Hybond-N; Amersham) by the capillary method described by Sambrook et al. (1989) and was fixed onto the membrane by exposure to UV light using a UV Stratalinker 1800 (Stratagene). The DNA-loaded membranes were treated for 1–2 h at 63° C in prehybridization so-

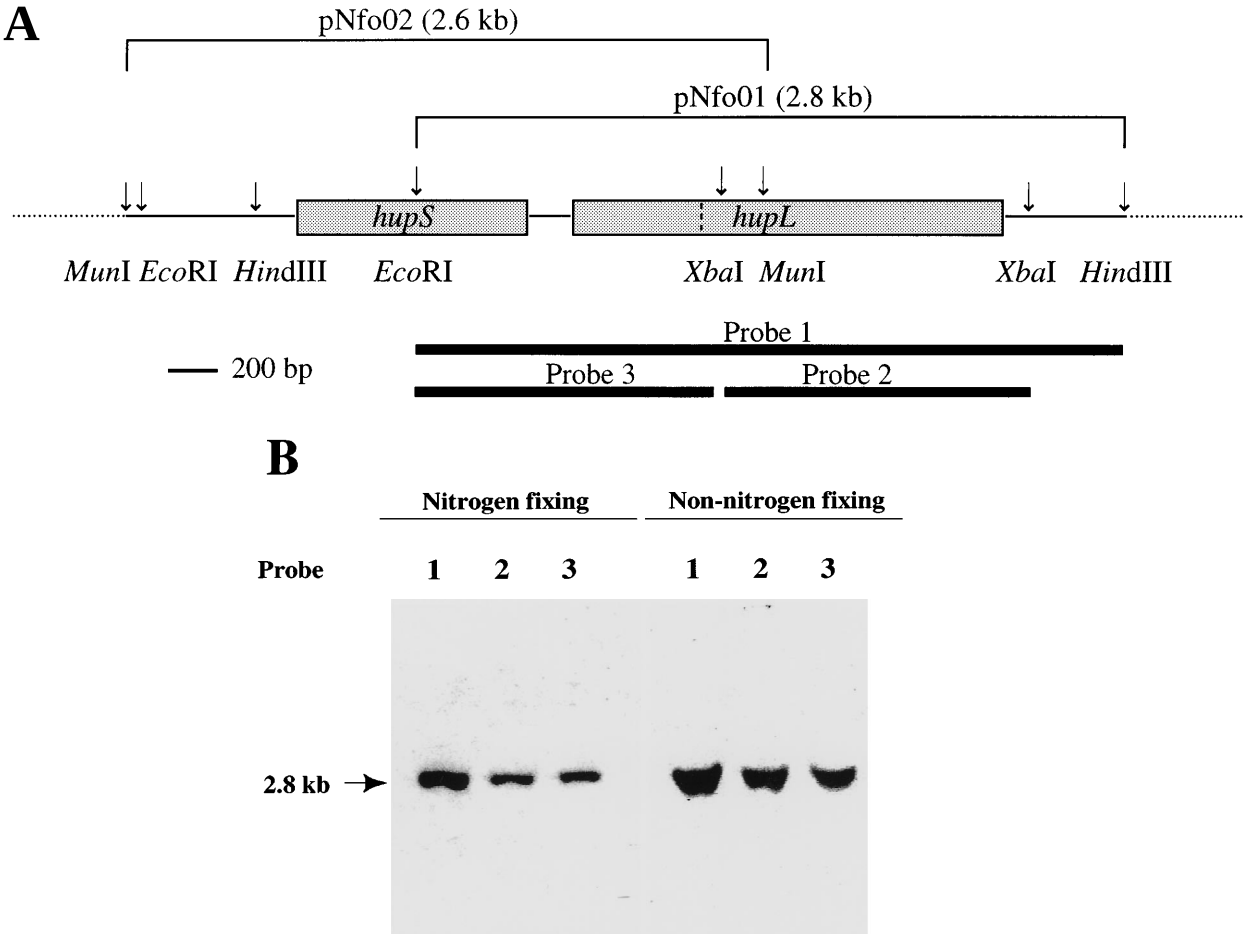

Fig. 1 **A** Physical map of the two clones pNfo01 and pNfo02 of genomic DNA of *Nostoc* sp. strain PCC 73102 containing the complete *hupSL* homologue. Restriction sites in the cloned *hupSL* homologue of *Nostoc* sp. strain PCC 73102 (↓) and the position of the recombination site in *Anabaena* sp. strain PCC 7120 (┆) are indicated. Fragments used as probes in Southern hybridizations (obtained by digestion with restriction endonucleases *Eco*RI, *Xba*I, and *Hin*dIII) are labeled *1*, *2*, and *3*, respectively. The 3,940-bp sequence is available from GenBank under accession no. AF030525. **B** Autoradiographs demonstrating the presence of a contiguous *hupL* gene in *Nostoc* sp. strain PCC 73102. Genomic DNA was extracted from cells grown under both nitrogen-fixing and non-nitrogen-fixing conditions and digested with the restriction endonucleases *Eco*RI and *Hin*dIII. Separation of the DNA on an agarose gel and transfer to a nylon membrane were followed by hybridization with the ^{32}P-labeled probes 1, 2, and 3 described in **A**. All probes recognized a single 2.8-kb DNA fragment

lution (Tamagnini et al. 1997). The probes used for Southern hybridizations were prepared either from DNA fragments obtained by PCR [hup2; see Tamagnini et al. (1997)] or cloned fragments from the partial genomic library (probes 1, 2, and 3; see Fig. 1 A). The ^{32}P-labeling of the probes, the separation of labeled DNA from unincorporated ^{32}P-labeled nucleotides, and the hybridization were performed following the protocol described by Tamagnini et al. (1997) with the exception that the hybridization and the washing steps were performed at 63° C. The membranes were air-dried, mounted for autoradiography, and exposed for up to several hours at –70° C to an X-ray film (Hyperfilm-MP; Amersham).

Construction of a partial genomic library of *Nostoc* sp. strain PCC 73102 and isolation of a *hupSL* homologue

Genomic DNA was hydrolyzed by restriction endonucleases (a combination of *Hin*dIII and *Eco*RI or *Mun*I) and separated on a 1% agarose gel. A region between approximately 2.3- and 3.3-kb (for clone pNfo01) and 2.0- and 3.0-kb (for clone pNfo02) was cut out, and the DNA was extracted from the gel piece according to the method described above. Extracted DNA fragments were ligated with linearized plasmid vectors as specified by the manufacturer (Stratagene). After the ligation, the hybrid DNA was introduced into supercompetent *E. coli* cells by transformation. Transformed *E. coli* cells were selected on LB plates in the presence of carbenicillin, with 30 µl 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (20 mg/ml) and 30 µl isopropyl-β-D-thiogalactopyranoside (0.1 M) distributed on the plates.

Colony hybridization

White colonies were selected and grown overnight on LB plates with carbenicillin. Replicas were made on Hybond-N$^+$ nylon membranes (Amersham). The membranes were immediately placed colony-side-up on an SDS (10%)-impregnated 3MM Whatman paper for 3 min. Colonies were lysed by transfering the membranes to Whatman papers presoaked in denaturating solution (0.5 N NaOH and 1.5 M NaCl) and incubated for 5 min. The nylon membranes with alkaline-solubilized cells were neutralized by blotting onto a Whatman filter paper prewetted with neutralizing solution [1.5 M NaCl and 0.5 M Tris-HCl (pH 7.4)] for 5 min. A Whatman paper was saturated with 2 × SSC [20 × 3 M NaCl and 0.3 M $C_6H_5Na_3O_7$ (pH 7.0)], to which the membranes were transferred and incubated for 5 min before being air-dried and exposed to UV

(as above). The hybridization was then performed as mentioned under Southern hybridization.

Results and discussion

To identify the structural gene encoding the large subunit of an uptake hydrogenase (*hupL*) in *Nostoc* sp. strain PCC 73102, oligonucleotide primers were designed from conserved sequences within the *hupL* gene of *Anabaena* sp. strain PCC 7120. These primers were used in PCR with genomic DNA from *Anabaena* sp. strain PCC 7120 as template, and the expected PCR product obtained was subsequently used as a probe in Southern hybridization experiments. The probe "hup2" hybridized with genomic DNA from *Nostoc* sp. strain PCC 73102 digested with *Eco*RI, *Hin*dIII, or both the restriction endonucleases recognizing DNA fragments of approximately 3.2 and 2.8 kb [see Tamagnini et al. (1997)]. A partial genomic library (to clone the 2.8-kb *Eco*RI/*Hin*dIII fragment of *Nostoc* sp. strain PCC 73102) was constructed, and 1,200 white colonies were screened by hybridization with the hup2 fragment used as probe. Four colonies were identified, picked, and purified, and their inserts were analyzed by digestion with the combination of the restriction endonucleases *Eco*RI/*Hin*dIII. They all showed the same pattern of restriction, and the clone pNfo01 was chosen for further studies. Sequencing revealed that this clone contained a DNA sequence similar to that of the *hupL* structural gene and part of the *hupS* structural gene from *Anabaena* sp. strain PCC 7120 (Carrasco et al. 1995). The restriction endonuclease map of clone pNfo01 is shown in Fig. 1 A.

An *Eco*RI/*Xba*I fragment containing the downstream region of *hupS* and the upstream region of *hupL* was used to probe DNA sequences in genomic DNA of *Nostoc* sp. strain PCC 73102 digested with the restriction endonuclease *Mun*I. A 2.6-kb fragment hybridized with the probe (data not shown). A second partial genomic library was constructed to obtain the remaining part of *hupS*. Fragments from the 2.6-kb region of *Mun*I-restricted genomic DNA from *Nostoc* sp. strain PCC 73102 were cloned into the *Eco*RI unique site of the vector pBluescript SK (+). A hybridization screening of 800 white colonies with the above-mentioned *Eco*RI/*Xba*I fragment as probe was performed. Only one positive colony was obtained. This clone showed the expected restriction pattern, and together with sequence data from PCR-based automated sequencing we could show that the insert contained the remaining part of *hupS*. The clone was given the name pNfo02 (Fig. 1 A). Together, the clones pNfo01 and pNfo02 revealed the complete sequence of a *hupSL* homologue with upstream and downstream regions in *Nostoc* sp. strain PCC 73102 (Fig. 1 A). The 3,940-bp sequence encompassing the *hupSL* homologue is available from GenBank under accession no. AF030525. *hupS* and *hupL* in *Nostoc* sp. strain PCC 73102 have exactly the same size as in *Anabaena* sp. strain PCC 7120. However, one difference is that the noncoding region between the two genes in *Nostoc* sp. strain PCC 73102 (192 bases) is longer than that of *Anabaena* sp. strain PCC 7120 and of many other microorganisms.The nucleotide sequences of *hupS* and *hupL* show 84% identity with their respective sequences in *Anabaena* sp. strain PCC 7120. The deduced amino acid sequences of HupS and HupL of *Nostoc* sp. strain PCC 73102 and *Anabaena* sp. strain PCC 7120 show that the HupS proteins are 89% identical (93% similar) and the HupL proteins are 91% identical (95% similar) (Figs. 2 and 3). However, the noncoding regions show no similarities. The genes encoding the small and the large subunit (*hupSL*) in *Nostoc* sp. strain PCC 73102 encode two proteins with calculated mol. masses of 34,917 and 60,157 Da, respectively. Interestingly, by using SDS-PAGE followed by immunoblotting, we have previously shown in *Nostoc* sp. strain PCC 73102 that one polypeptide with a mol. mass of approximately 58 kDa is immunologically related to hydrogenases purified from *Bradyrhizobium japonicum*, *Azotobacter vinelandii*, *Methanosarcina barkeri*, and *Thiocapsa roseopersicina*. In addition, another polypeptide (with a mol. mass of approximately 34 kDa) is immunologically related to a hydrogenase purified from *T. roseopersicina* (Tamagnini et al. 1995).

The HupS protein of *Nostoc* sp. strain PCC 73102 contains 11 Cys residues, 8 of which clearly correspond to the residues that are proposed to be involved in the formation of Fe-S clusters. In comparison with the HupS protein from *Desulfovibrio gigas*, the second Cys residue in the first four cluster ligands binding the proximal [4 Fe-4 S] cluster (Volbeda et al. 1995) is missing in *Nostoc* sp. strain PCC 73102. The His at the first position of the second four-ligand cluster, binding the distal [4 Fe-4 S] cluster, is not present in *Nostoc* sp. strain PCC 73102, and the second Cys residue in this cluster is differently positioned, similar to the one observed in hupU of *Rhodobacter capsulatus* (Elsen et al. 1996), according to our alignment studies (Fig. 2). The three Cys residues that bind the [3 Fe-4 S] cluster in *D. gigas* are all present in *Nostoc* sp. strain PCC 73102. HupS in *Nostoc* sp. strain PCC 73102 also lacks the signal peptide at the N-terminus present in many other organisms with membrane-bound or periplasmic hydrogenases. This signal peptide could, according to its structural characteristics to form an amphipathic helix, presumably be involved in translocation of the protein (Wu and Mandrand 1993). Moreover, the motif located at the C-terminus of the small subunit of Class I [NiFe] hydrogenases (Wu and Mandrand 1993), the unique feature of membrane-bound hydrogenases for anchoring the protein to the membrane, was not found in the HupS protein of *Nostoc* sp. strain PCC 73102. However, the role of that motif in membrane anchoring is not clear because some of the membrane-bound hydrogenases lack it [see Maier and Triplett (1996)]. The HupL protein of *Nostoc* sp. strain PCC 73102 contains the putative Ni-binding site present in [NiFe] hydrogenase large subunits at the N-terminal end ($R \times CG \times C$). At the C-terminal end, the Ni-binding site is also present, but the second amino acid (Pro) is exchanged for a Ser (Wu and Mandrand 1993; Albracht 1994). The structural *hupL* gene shows a considerable se-

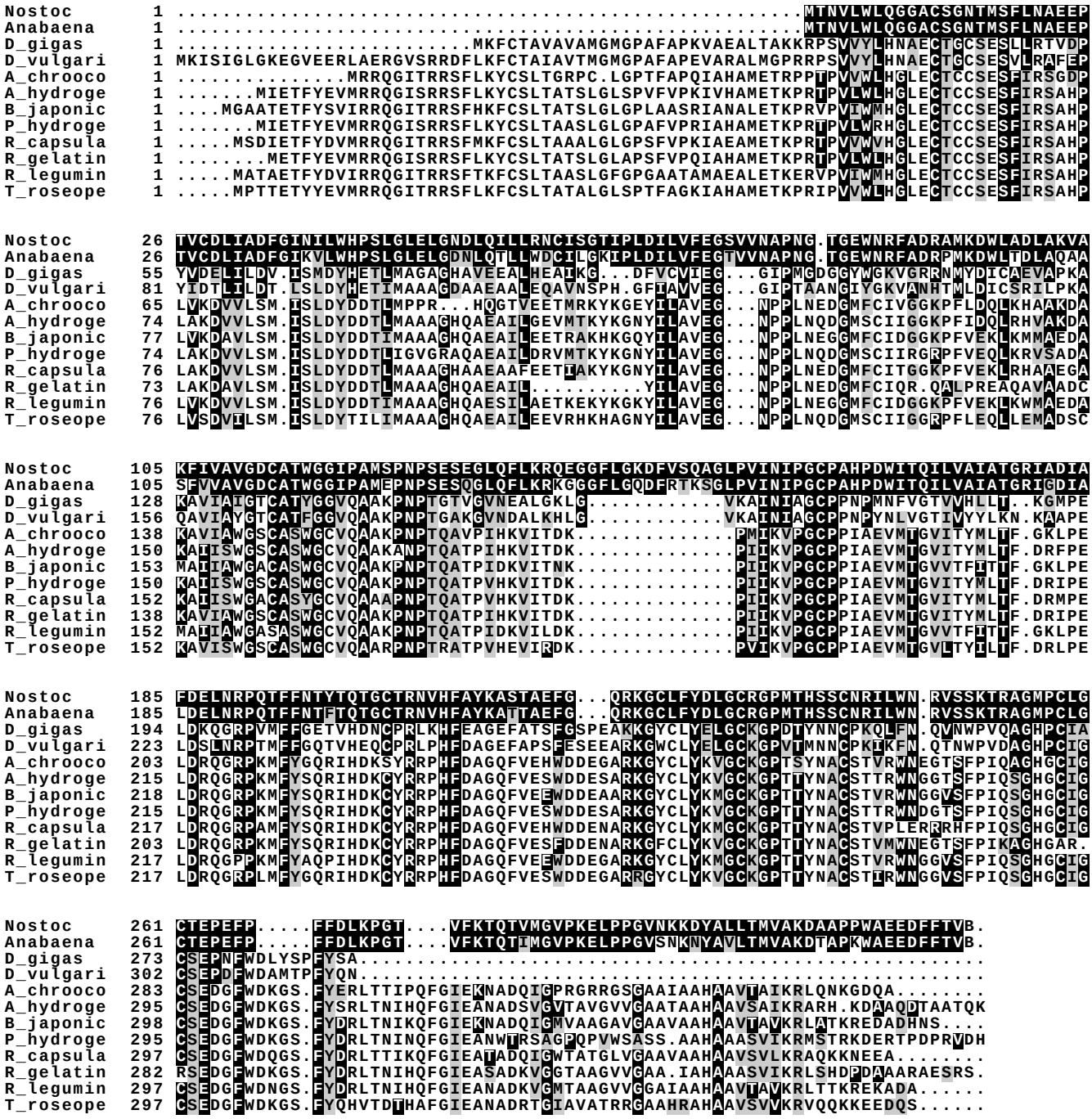

Fig. 2 Alignment of the deduced HupS sequence of *Nostoc* sp. strain PCC 73102 with the corresponding sequences of the cyanobacterium *Anabaena* sp. strain PCC 7120 and the bacteria *Desulfovibrio gigas*, *Desulfovibrio vulgaris*, *Azotobacter chroococcum*, *Alcaligenes hydrogenophilus*, *Bradyrhizobium japonicum*, *Pseudomonas hydrogenovora*, *Rhodobacter capsulatus*, *Rhodocyclus gelatinosus*, *Rhizobium leguminosarum*, and *Thiocapsa roseopersicina*. Amino acid identities in the alignments are indicated by *black boxes with white letters*. Similar amino acids are indicated by *grey boxes*

quence similarity to the regulatory *hupV* recently described in *R. capsulatus* (Elsen et al. 1996), in *B. japonicum* (Black and Maier 1994), and earlier in *D. baculatus* (Menon et al. 1987; Voordouw et al. 1989). A feature present in HupL – but not in HupV – and also present in *Nostoc* sp. strain PCC 73102 (Fig. 3) is the amino acid sequence that is removed proteolytically when the protein undergoes maturation [Menon and Robson 1994; see also reviews by Friedrich and Schwartz (1993) and Vignais and Toussaint (1994)]. Some of the above-discussed features of the *hupSL* homologues characterized in the present study might make it necessary to take into consideration that the genes could correspond to *hupUV*, genes encoding proteins involved, for example, in sensing H_2 (Elsen et al. 1996). Specifically, *hupS* in *Nostoc* sp. strain PCC 73102 shows some characteristics similar to those

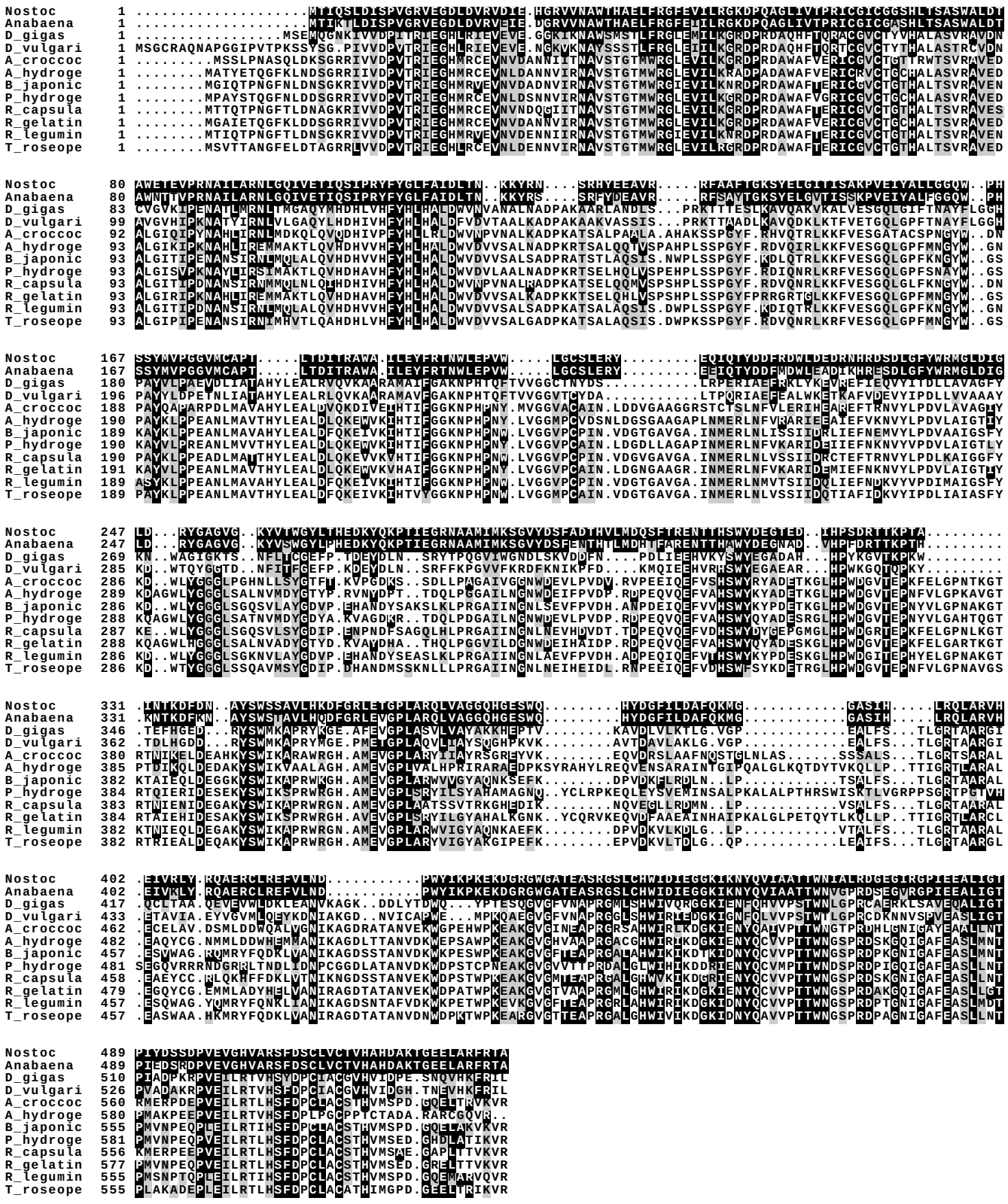

Fig. 3 Alignment of the deduced HupL sequence of *Nostoc* sp. strain PCC 73102 with the corresponding sequences of the cyanobacterium *Anabaena* sp. strain PCC 7120 and the bacteria *Desulfovibrio gigas*, *Desulfovibrio vulgaris*, *Azotobacter chroococcum*, *Alcaligenes hydrogenophilus*, *Bradyrhizobium japonicum*, *Pseudomonas hydrogenovora*, *Rhodobacter capsulatus*, *Rhodocyclus gelatinosus*, *Rhizobium leguminosarum*, and *Thiocapsa roseopersicina*. Amino acid identities in the alignments are indicated by *black boxes with white letters*. Similar amino acids are indicated by *grey boxes*

found in *hupU*. It would then be expected to find another gene, *hupT*, directly upstream of the presumptive *hupU*; the product of *hupT*, together with hupU and hupV, participates in sensing H_2 [Elsen et al. 1996; see also Maier and Triplett (1996)]. However, the sequence of approximetaly 500 bp upstream of the *hupS* homologue in *Nostoc* sp. strain PCC 73102 did not contain any identifiable ORF or part of an ORF similar to any gene. Furthermore, sequence comparisions using available databases showed a higher degree of sequence similarity between the presented *Nostoc* sp. strain PCC 73102 sequences and genes encoding structural proteins (*hupSL*) than did genes encoding regulatory/sensing proteins (*hupUV*).

A third ORF, *hupC*, has been identified and is located just downstream of the *hupSL* genes in a variety of bacteria capable of H_2 uptake (Hidalgo et al. 1992; Van Soom et al. 1993; Vignais and Toussaint 1994). It has been proposed that HupC could play a role in the cytochrome-mediated electron transport to the terminal acceptor oxygen (Cauvin et al. 1991; Vignais and Toussaint 1994). In the sequence just downstream of *hupL* in *Nostoc* sp. strain PCC 73102, we could find no evidence of an ORF similar to *hupC* (Fig. 1 A).

Genomic DNA of nitrogen-fixing and non-nitrogen-fixing cells of *Nostoc* sp. strain PCC 73102 was digested with the combination of the restriction endonucleases *Eco*RI and *Hin*dIII. High-stringency Southern hybridizations using three different probes covering or flanking the potential recombination site in *Nostoc* sp. strain PCC 73102 revealed the presence of a contiguous *hupL* gene – encoding the large subunit of an uptake hydrogenase – in nitrogen-fixing and non-nitrogen-fixing cells of *Nostoc* sp. strain PCC 73102 (Fig. 1 B). This together with sequence data shows that *Nostoc* sp. strain PCC 73102 does not exhibit the same type of rearrangement within the structural *hupL* gene as has previously been shown in *Anabaena* sp. strain PCC 7120 (Carrasco et al. 1995). A potential rearrangement site was found in *Nostoc* sp. strain PCC 73102, but the sequence differs in 6 positions out of 16 on the nucleotide level (i.e., only 62.5% identical) in comparison with *Anabaena* sp. strain PCC 7120 (Fig. 4). Interestingly, in our earlier immunological experiments, antigens with the same molecular masses were recognized when nitrogen-fixing or non-nitrogen-fixing cells and antisera directed against hydrogenases purified from *T. roseopersicina* or *M. barkeri* were used (Tamagnini et al. 1995).

In conclusion, we identified and sequenced structural genes (*hupSL*) encoding an uptake hydrogenase homologue in *Nostoc* sp. strain PCC 73102. They show a high degree of similarity to corresponding sequences in *Anabaena* sp. strain PCC 7120. A longer, noncoding region between the genes and the absence of a rearrangement within *hupL*, which occurs when a photosynthetic vegetative cell differentiates into a nitrogen-fixing heterocyst, are specific characteristics of the *Nostoc* sp. strain PCC 73102 *hupSL* homologue. Furthermore, we have recently published evidence demonstrating the absence of both the *hox* genes and the corresponding activities of the bidirectional/reversible enzyme in the same organism (Tamagnini et al. 1997). The particular characteristics of *Nostoc* sp. strain PCC 73102 make this strain an interesting candidate for the study of deletion mutants lacking the uptake-type enzyme. At present, efforts are being made to create such mutants in our laboratory.

Anabaena PCC 7120 CACAGCAGTTATATGG
Nostoc PCC 73102 CATTCTAGCTACATGG

Fig. 4 Nucleotide sequence of the recombination site within the *hupL* gene in *Anabaena* sp. strain PCC 7120 in comparison with the corresponding sequence in *Nostoc* sp. strain PCC 73102. Six nucleotides out of 16 are different (i.e., 62.5% identity) in *Nostoc* sp. strain PCC 73102

Acknowledgements This work was financially supported by a European scholarship from the Swedish Institute, the Fundação Calouste Gulbenkian, and Praxis XXI (P. Tamagnini) and by grants from the Swedish Natural Science Research Council, the Carl Tryggers Foundation and the OK Environment Foundation (P. Lindblad).

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