Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8395–8399, November 1988
Biochemistry

Nucleotide sequence of the genetic loci encoding subunits of *Bradyrhizobium japonicum* uptake hydrogenase

LUIS A. SAYAVEDRA-SOTO*†, GARY K. POWELL‡§, HAROLD J. EVANS*, AND ROY O. MORRIS‡§

*Laboratory for Nitrogen Fixation and ‡Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331

Contributed by Harold J. Evans, July 15, 1988

ABSTRACT **An indispensable part of the hydrogen-recycling system in *Bradyrhizobium japonicum* is the uptake hydrogenase, which is composed of 34.5- and 65.9-kDa subunits. The gene encoding the large subunit is located on a 5.9-kilobase fragment of the H_2-uptake-complementing cosmid pHU52 [Zuber, M., Harker, A. R., Sultana, M. A. & Evans, H. J. (1986) *Proc. Natl. Acad. Sci. USA* 83, 7668–7672]. We have now determined that the structural genes for both subunits are present on this fragment. Two open reading frames are present that correspond in size and deduced amino acid sequence to the hydrogenase subunits, except that the small-subunit coding region contains a leader peptide of 46 amino acids. The two genes are separated by a 32-nucleotide intergenic region and likely constitute an operon. Comparison of the deduced amino acid sequences of the *B. japonicum* genes with those from *Desulfovibrio gigas*, *Desulfovibrio baculatus*, and *Rhodobacter capsulatus* indicates significant sequence identity.**

The H_2 evolution that accompanies biological N_2 fixation represents a large energy expenditure. At least 25% of the available electron flux is utilized in H_2 production, thus diminishing the potential for N_2 fixation (1). However, some strains of rhizobia possess an active H_2-uptake (Hup) system that catalyzes H_2 oxidation, which results in increased ATP production and decreased levels of dissolved O_2, thus providing benefits for the nitrogenase system (1).

Lambert *et al.* (2) isolated a cosmid, pHU52, from an *Eco*RI library of *Bradyrhizobium japonicum* DNA prepared in pLAFR1 (3). It encoded all determinants for H_2 recycling in *B. japonicum* and conferred Hup activity and autotrophic growth capability to Hup$^-$ strains of rhizobia. Zuber *et al.* (4) cloned a 5.9-kilobase (kb) *Hin*dIII fragment of pHU52 in a plasmid, pMZ550, and determined by immunological methods that the gene encoding the large subunit of the hydrogenase was located on this fragment. The gene for the small subunit originally was thought to reside on a separate 2.9-kb fragment of pHU52 but was found subsequently to be present on the same 5.9-kb insert in pMZ550 (4).

We now report the nucleotide sequence of the relevant region of this insert.¶ It encodes structural genes for both the large and small subunits of the *B. japonicum* hydrogenase. The deduced amino acid sequence of this hydrogenase from *B. japonicum* is compared to the sequences of hydrogenases from *Rhodobacter capsulatus* (5), *Desulfovibrio gigas* (6), *Desulfovibrio baculatus* (7), and *Desulfovibrio vulgaris* (8).

MATERIALS AND METHODS

Bacterial Strains. *B. japonicum* 122DES (9) was used to nodulate soybean [*Glycine max* (L.) Merr., cultivar Wilkin] for bacteroid RNA preparation. *Escherichia coli* strains DC646 (4), MV1304 (10), HB101 (11), and JM103 (12) were grown as described.

Plasmids and Cloning. The plasmid vectors utilized were pTZ19U (10) and M13mp18 and M13mp19 (13). The source of the hydrogenase genes was the cosmid pHU52, which was maintained in HB101. The 5.9-kb *Hin*dIII fragment from pHU52 was recloned from pMZ550 (4) into the plasmid pTZ19U to give the plasmid pLAS1. Fragments were generated from the 5.9-kb insert by use of *Hin*dIII, *Pst* I, *Sst* I, or *Sph* I and were subcloned into M13mp18 and M13mp19 for sequencing (14).

Enzymes and Chemical Reagents. Restriction and modification enzymes were purchased from Bethesda Research Laboratories or from United States Biochemical (Cleveland) and used according to the supplier's directions. Reagents for DNA sequencing were purchased from Pharmacia or as a kit from United States Biochemical. 2′-Deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate (1400 Ci/mmol; 1 Ci = 37 GBq) for sequencing and adenosine 5′-[γ-^{32}P]triphosphate tetra-(triethylammonium) salt (6000 Ci/mmol) for 5′-end labeling were purchased from NEN.

Nucleotide Sequencing. Overlapping subclones were generated by exonuclease III/nuclease S1 digests (14) and sequenced by the dideoxy chain-termination method (15). The large fragment of DNA polymerase I or the modified T7 DNA polymerase (Sequenase; United States Biochemical) was used.

Oligonucleotide Probes and Hybridization. Probes were synthesized by Agrigenetics (Madison, WI) or by the Gene Research Center at Oregon State University. Fragments of pHU52 or the 5.9-kb DNA insert from pLAS1 were separated by electrophoresis in 0.75% agarose gels and transferred to GeneScreen*Plus* (NEN) (16). Prehybridization (7 hr) and hybridization (16 hr) were at 42°C (17). Probes were 5′-end-labeled with ^{32}P by polynucleotide kinase and used at 2.0 pmol (1.5×10^6 cpm) per reaction. Tetramethylammonium chloride washes were performed at 42°C and autoradiographs were prepared by exposure to Kodak X-Omat film for 24 hr at −80°C. *Hin*dIII-digested λ phage DNA fragments, 5′-end labeled with ^{32}P, served as size markers.

Transcript Mapping. Total RNA was isolated from 3 g of 30-day-old soybean nodule bacteroids or 0.5 g of autotrophically grown *B. japonicum* cells (18). Two probes were employed: the first extended from the *Mlu* I site (nucleotide position 69; Fig. 2) to the left *Hin*dIII site of pLAS1. The second probe extended from the *Not* I site (position 304; Fig. 2) to the same *Hin*dIII site. Both probes were 5′-end-labeled by using [^{32}P]ATP and polynucleotide kinase. Transcript mapping was performed as described by Corbin *et al.* (19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: ORF, open reading frame.

†Present Address: Department of Plant Molecular Genetics, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071.
§Present Address: Department of Biochemistry, 322A Chemistry Building, University of Missouri, Columbia, MO 65211.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J04114).

RESULTS

Hydrogenase Gene Location. Hybridization (data not shown) using two oligonucleotide probes whose sequences were based on N-terminal peptides from both subunits (L. Seefeldt and D. Arp, personal communication) indicated that the hydrogenase genes were located on pHU52. Both probes hybridized to the 5.9-kb *Hin*dIII fragment of pHU52. To precisely locate the large-subunit structural gene, the 5.9-kb *Hin*dIII fragment of pHU52 was recloned in pLAS1 (Fig. 1*a*). After digestion of pLAS1 with *Sst* I, *Sst* I/*Hin*dIII, *Bam*HI, or *Bam*HI/*Hin*dIII (Fig. 1*b*), fragments were separated and hybridized with a labeled probe whose sequence was derived from an internal peptide (Met-Asp-Tyr-Phe-Gln-Asp-Lys-Leu) fragment of the large subunit (H. Paaren, personal communication). The pattern of hybridization to the *Sst* I and *Bam*HI fragments of pLAS1 indicated that the probe was homologous to part of the internal 450-nucleotide *Bam*HI–*Sst* I fragment (Fig. 1 *b–d*).

Nucleotide Sequencing. A restriction map of the 5.9-kb *Hin*dIII insert of pLAS1 was generated by use of *Bam*HI, *Bgl* II, *Pst* I, *Sph* I, and *Sst* I (Fig. 1*b*) and used as a guide for preparation of subclones in M13mp18 and M13mp19 for sequencing. The data (Fig. 2) revealed that structural genes for the two hydrogenase subunits were located between the left *Bam*HI site and the *Bgl* II site of the pLAS1 insert. Two open reading frames were present in this region. The leftmost, ORF1 (1089 nucleotides), was capable of encoding a protein of 39.5 kDa (363 amino acids). This was somewhat larger than expected, since the hydrogenase small subunit has an experimentally determined molecular mass of 35 kDa (20). However, the small-subunit N-terminal peptide (Leu-Glu-Thr-Lys-Pro-Arg-Val-Pro-Val; L. Seefeldt and D. Arp, personal communication) was specified within ORF1 at nucleotides 152–178. Thus, ORF1 encodes the small subunit and a leader peptide of 46 amino acids. The calculated size of the ORF1 peptide excluding the leader was 34.5 kDa, in excellent agreement with the experimentally determined value of the small subunit (20). A consensus Shine–Dalgarno sequence (GGAGG) was present 4 bases upstream from the leader peptide start codon. No such sequence was present in the 75 nucleotides upstream from the start of the mature peptide. It appears that a leader peptide of 46 amino acids is present in the primary translation product of this gene and is removed during subunit maturation.

ORF2 (1788 nucleotides) was capable of encoding a peptide of 65.9 kDa (596 amino acids), a value in agreement with the experimentally determined value of 65 kDa (20). In addition, ORF2 encoded an N-terminal peptide, Met-Gly-Ile-Gln-Thr-Pro-Asn-Gly-Phe, identical to that present in the hydrogenase large subunit (L. Seefeldt and D. Arp, personal commu-

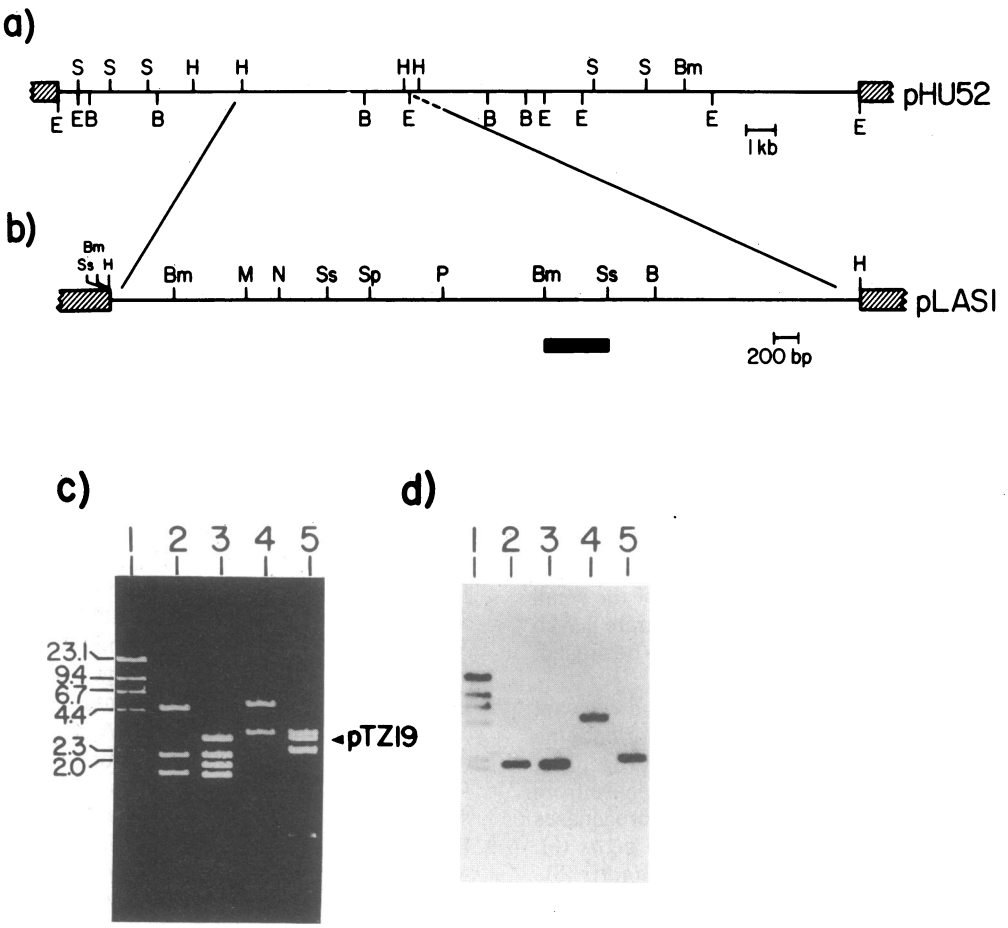

FIG. 1. Cloning of the *B. japonicum* hydrogenase genes and localization of the large-subunit gene. (*a* and *b*) Construction of pLAS1. Starting material was the cosmid pHU52 (*a*), which confers H_2-uptake activity and autotrophic growth on Hup^- strains of *Rhizobium* (4). The physical map of the 35-kb *Eco*RI DNA insert in pHU52 is illustrated. The 5.9-kb *Hin*dIII subfragment containing the hydrogenase genes was subcloned into pTZ19U to give pLAS1 and mapped. To localize the large-subunit gene, fragments from pLAS1 (*b*) were hybridized with a 24-base-pair (bp) oligonucleotide probe whose sequence was derived from an internal peptide of the hydrogenase large subunit. B, *Bgl* II; Bm, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sma* I; Sp, *Sph* I; Ss, *Sst* I. (*c*) Endonuclease digest of pLAS1. Lanes: 1, size markers; 2, *Sst* I; 3, *Sst* I–*Hin*dIII; 4, *Bam*HI; 5, *Bam*HI–*Hin*dIII. Faint bands at 4.4 kb in lane 4 and 1 kb in lane 5 are due to *Bam*HI "star" activity. Fragments were visualized with ethidium bromide. (*d*) Autoradiograph of the fragments that hybridized to the probe. Hybridization to the 2.2-kb *Sst* I fragment (lane 2) and the 3.0-kb *Bam*HI–*Hin*dIII fragment (lane 5) indicated that a region homologous to the probe (solid bar in *b*) was located on the rightmost 450-bp *Bam*HI–*Sst* I fragment.

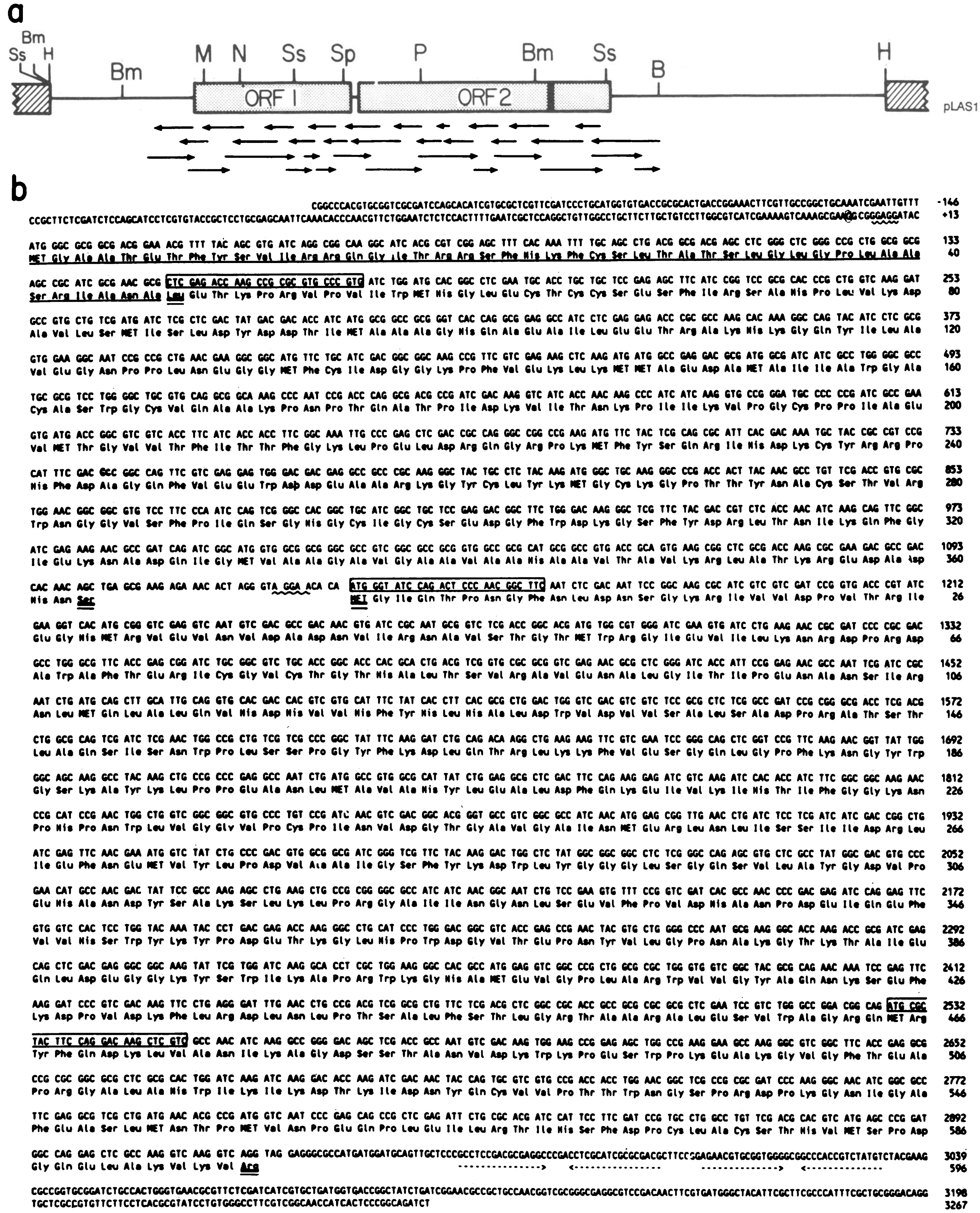

FIG. 2. Restriction map, sequencing strategy, and nucleotide sequence of the *B. japonicum* uptake hydrogenase genes. (*a*) Restriction map of pLAS1. Sequencing strategy is illustrated by horizontal arrows showing the orientation and extent of sequences obtained from overlapping fragments cloned in M13mp18 and M13mp19. Hydrogenase subunit open reading frames ORF1 and ORF2 are represented by shaded boxes. The point of hybridization of probe used in Fig. 1 is indicated by a vertical bar in ORF2. Transcription is from left to right. (*b*) Nucleotide sequence. Deduced amino acid sequences of ORF1 and ORF2 are indicated. Wavy underlining (nucleotides 5–9 and 1126–1130) indicates putative ribosome binding sites. First and last amino acids of the mature proteins are doubly underlined. Putative leader peptide is underlined. Transcription start site (position 1) is circled. Horizontal arrows located after the stop codon of the large subunit (positions 2955–2991 and 2999–3031) show inverted repeats that may be transcription termination signals. Sites of hybridization of the oligonucleotide probes are boxed.

nication). It also contained the internal peptide Met-Arg-Tyr-Phe-Gln-Asp-Lys-Leu, which was identical in all but one amino acid (arginine instead of asparagine) to that isolated from the large subunit and on which the original probe was based. A ribosome binding site (AGGAA) was present 4 bases before the start codon. The two ORFs were separated by a 32-nucleotide intergenic region. Inverted repeats were located after ORF2 (nucleotides 2955–2991 and 2999–3031), but no such potential transcription terminators were present in the intergenic region. No other ORFs of any significant size were present in either orientation.

The calculated isoelectric points of 6.1 for the small and 6.09 for the large subunit may be compared to the experimentally determined value of 6.30 for the holoenzyme (20). Similarly, the deduced amino acid composition of the two subunits agreed closely with that of the purified hydrogenase (20). One cysteine residue was present in the leader peptide sequence, 13 in the small subunit, and 6 in the large subunit.

Transcript Mapping. The position of transcription initiation was determined by S1 nuclease mapping (data not shown). The protected fragments were 69 and 304 nucleotides long, respectively, indicating that transcription initiation is at the guanine located 13 nucleotides prior to the start codon of the small-subunit leader sequence. The nucleotide sequences at −10 and −35 resembled neither the prokaryotic consensus promoter sequence (21) nor the sequences involved in initiation of *nod* gene transcription (22). The position of transcription initiation was the same with mRNA from autotrophic or bacteroid cells.

Sequence Comparison. The Needleman–Wunsch algorithm (23) was used to compare the amino acid sequence homology of the small and large subunits of *B. japonicum, R. capsulatus, D. gigas*, and *D. baculatus*. The coding regions for the small subunits of *B. japonicum* and *R. capsulatus* exhibited striking similarity (Fig. 3). Overall identity was 70%, with major areas of complete identity. Significant divergence was present only at residues 280–286. In *R. capsulatus* this region was very basic, whereas in *B. japonicum* it was not. The degree of similarity between the *B. japonicum* and *R. capsulatus* large subunits was even greater (i.e., 75%).

Alignment of the small subunits of *D. baculatus* and *D. gigas* indicated that these two proteins were similar neither to each other nor to the small subunits of *B. japonicum* or *R. capsulatus*. Overall identity was only 23% and the two sequences were characterized by isolated islands of similarity, for example at *D. baculatus* positions 50–56 and 262–274. All four subunits exhibited strong conservation of a few amino acid clusters in relation to their overall sequence. All mature small subunits contained 12–14 cysteine residues of which 8 were strongly conserved, and all contained the consensus sequence $GXCX_4GCKGPXTYXXCX_{16-17}CIXC$ (*B. japonicum* positions 259–298). The positions of proline and arginine were also strongly conserved. In the large subunits there was little conservation of cysteine number or position. The cysteine content ranged from 6 to 18 residues and, of these, 2 were aligned.

Sequence similarity between small-subunit pairs extended to the leader peptides. *R. capsulatus* and *B. japonicum* are membrane-bound and their leader peptides were almost identical. Both were fairly hydrophobic and contained two separate arginine-rich clusters. The hydrogenases of *D. gigas* and *D. baculatus* are periplasmic enzymes (6, 7). Their leader peptides were much shorter than those of *B. japonicum* and were characterized primarily by a single extremely hydrophobic cluster.

The conservation of cysteine position and regions of hydrophobicity in the small subunits can be seen in Fig. 4. This figure displays hydrophobic regions (24) and cysteine positions for all four small subunits. Nine cysteine residues (at *B. japonicum* positions 63, 66, 134, 195, 261, 267, 276, 295, and 298) aligned well in all subunits (Fig. 3). The cysteine residues between 260 and 299 were contained in the consensus sequence $GXCX_4GCKGPXTYXXCX_{16-17}CIXC$ identified above. In addition, *B. japonicum, R. capsulatus*, and *D. baculatus* had hydrophobic regions at 158–170 and 190–205, which were well conserved. These regions were not present in *D. gigas*. Interestingly, *B. japonicum* and *R. capsulatus* small subunits contained at their C termini very hydrophobic regions that may contribute to their location in the bacterial membrane. The sequences were absent from *D. baculatus* and *D. gigas* enzymes, which are located in the periplasmic space.

DISCUSSION

The arrangement of the structural genes in *B. japonicum* is similar to that in *R. capsulatus, D. baculatus*, and *D. gigas*. The small-subunit gene is followed by that encoding the large subunit. The structural gene for the small subunit encodes not only the mature monomer but also a leader peptide of 46 amino acids. The same is true in *R. capsulatus, D. baculatus*, and *D. gigas*. The opposite situation prevails for the hydrogenase of *D. vulgaris*, where the subunit gene order is reversed and the leader peptide is associated with the small subunit. The two genes of *B. japonicum* are separated by a small intergenic region, as are the corresponding genes in *R. capsulatus, D. baculatus*, and *D. gigas*. Since the transcription start site is located upstream from the small-subunit gene, since there are no obvious promoter elements in the

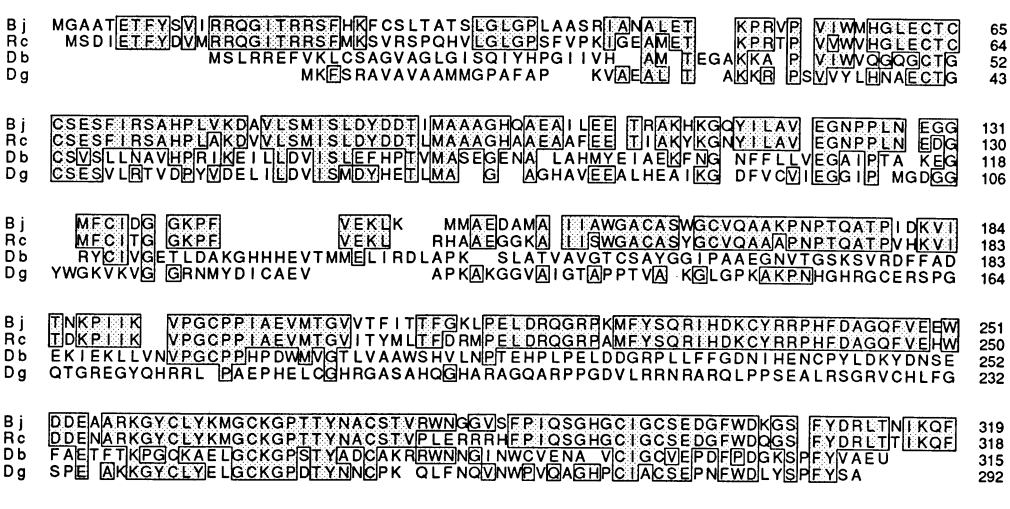

FIG. 3. Sequence comparison of *B. japonicum* (Bj) uptake hydrogenase small subunit and leader peptide with comparable sequences from *R. capsulatus* (Rc), *D. baculatus* (Db), and *D. gigas* (Dg). Identical residues are boxed. P. Vignais (5) has informed us that the corrected initial sequence (upper left) of the *R. capsulatus* leader peptide is QLSDI instead of MSDI.

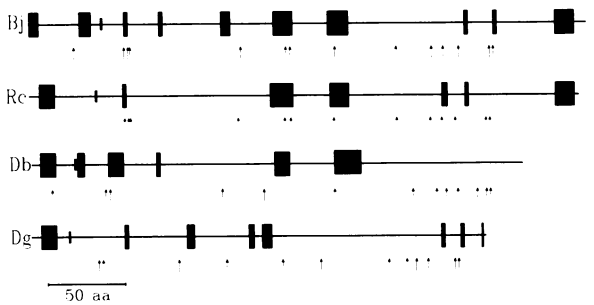

FIG. 4. Cysteine positions (arrows) and hydrophobic domains (black boxes) in hydrogenase small subunits. Mature subunit N termini are shown by shortened bars. Bj, *B. japonicum*; Rc, *R. capsulatus*; Db, *D. baculatus*; Dg, *D. gigas*. aa, Amino acids.

intergenic region, and since the only putative transcription terminator is at the end of the large-subunit structural gene, it is likely that the two genes constitute a single operon.

The striking conservation of key amino acid sequences in the small subunits from this rather diverse group of bacteria, with the exception of *D. vulgaris*, suggests an important structural or functional role. Stabilization of tertiary structure and participation in metal binding sites are obvious possibilities. Evidence has been presented (8) that the consensus iron binding site of ferredoxin (CIXCACXXECPVX$_{26}$C) may have a homolog in the large subunit of *D. vulgaris* hydrogenase. Our data indicate that a significant feature of these primary sequences is the retention of a set of widely distributed cysteine residues and the presence in all small subunits of the sequence GXCX$_4$GCKGPXTYXXCX$_{16-17}$CIXC. This sequence corresponds to that identified by Li *et al.* (6) as a potential iron-binding sequence but is not similar to the iron binding site of ferredoxin. Indeed the cysteine content of the large subunits of these bacterial hydrogenases is variable, and in *B. japonicum* there is no cysteine-containing binding site resembling that described by Voordouw and Brenner (8). Other metal-binding domains may be present. For example, the hemerythrin ion-binding domain HXXH (25) has a close homolog, HFYHXHALD, in the large subunit of *B. japonicum*. This last sequence is conserved in all large subunits and may well play a role in metal binding. The data regarding the content of Fe, Ni, and Se in *B. japonicum* hydrogenase are incomplete (1).

The hydrophobic domains and conserved cysteine positions of the *B. japonicum* small subunit coincide closely with those from *R. capsulatus*, *D. baculatus*, and *D. gigas* (Fig. 4). The extremely hydrophobic domains that are present at the C termini of the small subunits of *B. japonicum* and *R. capsulatus* but absent from the termini of the *D. baculatus* and *D. gigas* hydrogenases may well provide extra anchorage for these enzymes in the bacterial membrane. This would explain the need for detergents in the purification of hydrogenases from *B. japonicum* and *R. capsulatus* (26) but not in preparations from *D. gigas* and *D. baculatus*.

Selenium increases the activity of the H_2-uptake system of *B. japonicum*,[||] and the UGA codon has been reported to encode selenocysteine in mammalian glutathione peroxidase and *E. coli* formate dehydrogenase (27). No evidence for a role of a UGA codon in selenocysteine incorporation in *B. japonicum* hydrogenase has been found.

[||]Hanus, F. J., Papen, H., Becker, M., Boursier, P., Russell, S. A. & Evans, H. J. (1988) Proceedings of the VII International Congress on Nitrogen Fixation, Mar. 1988, Cologne, F.R.G. (abstr. 12-44).

The sequence similarity of the hydrogenase structural genes from *B. japonicum* and *R. capsulatus* explains their similar enzymatic characteristics and crossreactivity toward antibodies raised against the *B. japonicum* hydrogenase (26). Li *et al.* (6) discussed the different immunological properties of the hydrogenases from *D. baculatus* and *D. gigas* and suggested that their differences are in their cellular origin and metal content. It would be interesting to test the affinity of these two hydrogenases for the antibodies against *B. japonicum* hydrogenase.

We thank Dr. D. Arp and Dr. L. Seefeldt for information on the N-terminal amino acid sequences of *B. japonicum* hydrogenase and Dr. Herbert Paaren for the oligonucleotide probe for the large subunit. The expert assistance of Mr. J. Kramer of the Gene Research Center of Oregon State University is gratefully acknowledged. Also, we appreciate sequence data on *hup* genes in *Azotobacter* from Dr. Geoffrey Yates and Dr. Chris Ford. This research was supported by the Agrigenetics Co., the U.S. Department of Agriculture (Competitive Research Grants Office Grant 87-CRCR-1-2472), and The Oregon Agricultural Experiment Station, from which this is Technical Paper 8575.

1. Evans, H. J., Harker, A. R., Papen, H., Russell, S. A., Hanus, F. J. & Zuber, M. (1987) *Annu. Rev. Microbiol.* **41,** 335–361.
2. Lambert, G. R., Cantrell, M. A., Hanus, F. J., Russell, S. A., Haddad, K. R. & Evans, H. J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3232–3236.
3. Cantrell, M. A., Haugland, R. A. & Evans, H. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 181–185.
4. Zuber, M., Harker, A. R., Sultana, M. A. & Evans, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7668–7672, and correction (1988) **85,** 1835.
5. Leclerc, M., Colbeau, B., Cauvin, B. & Vignais, P. M. (1988) *Mol. Gen. Genet.*, in press.
6. Li, C., Peck, H. D., LeGall, J. & Przybyla, A. E. (1987) *DNA* **6,** 539–551.
7. Menon, N. K., Peck, H. D., Jr., LeGall, J. & Przybyla, A. E. (1987) *J. Bacteriol.* **169,** 5401–5407.
8. Voordouw, G. & Brenner, S. (1985) *FEBS Lett.* **148,** 515–520.
9. Ruiz-Argueso, T., Maier, R. J. & Evans, H. J. (1979) *Appl. Environ. Microbiol.* **37,** 582–587.
10. United States Biochemical Co. (1988) Technical literature accompanying Sequenase kit (United States Biochemical Co., Cleveland).
11. Boyer, H. W. & Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41,** 459–472.
12. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
13. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
14. Henikoff, S. (1984) *Gene* **28,** 351–359.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Hicks, D. A. & Vecoli, C. (1987) *Biotechniques* **5,** 206–208.
17. Wood, W. I., Gitschier, J., Lasky, L. A. & Lawn, R. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1585–1588.
18. Davis, G. L., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York).
19. Corbin, D., Barran, L. & Ditta, G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3005–3009.
20. Arp, D. J. (1985) *Arch. Biochem. Biophys.* **237,** 504–512.
21. Stormo, D. G., Schneider, T. D. & Gold, L. M. (1982) *Nucleic Acids Res.* **10,** 2971–2986.
22. Rostas, K., Kondorosi, E., Horvath, B., Simoncsits, A. & Kondorosi, A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1757–1761.
23. Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48,** 443–453.
24. Engleman, D. M., Steitz, T. A. & Goldman, A. (1986) *Ann. Biophys. Chem.* **15,** 321–353.
25. Berg, J. M. (1986) *Science* **232,** 485–486.
26. Seefeldt, L. C., MacCollum, L. C., Doyle, C. M. & Arp, D. J. (1987) *Biochim. Biophys. Acta* **914,** 299–303.
27. Chambers, I. & Harrison, P. R. (1987) *Trends Biochem. Sci.* **12,** 255–256.