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Purification of the Integration Host Factor Homolog of *Rhodobacter capsulatus*: Cloning and Sequencing of the *hip* Gene, Which Encodes the β Subunit

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We describe a method for rapid purification of the integration host factor (IHF) homolog of *Rhodobacter capsulatus* that has allowed us to obtain microgram quantities of highly purified protein. *R. capsulatus* IHF is an αβ heterodimer similar to IHF of *Escherichia coli*. We have cloned and sequenced the *hip* gene, which encodes the β subunit. The deduced amino acid sequence (10.7 kDa) has 46% identity with the β subunit of IHF from *E. coli*. In gel electrophoretic mobility shift DNA binding assays, *R. capsulatus* IHF was able to form a stable complex in a site-specific manner with a DNA fragment isolated from the promoter of the structural *hupSL* operon, which contains the IHF-binding site. The mutated IHF protein isolated from the Hup⁻ mutant IR4, which is mutated in the *himA* gene (coding for the α subunit), gave a shifted band of greater mobility, and DNase I footprinting analysis has shown that the mutated IHF interacts with the DNA fragment from the *hupSL* promoter region differently from the way that the wild-type IHF does.

The purple nonsulfur photosynthetic bacterium *Rhodobacter capsulatus* possesses a membrane-bound hydrogenase that functions in H_2 uptake under physiological conditions (31). This enzyme enables *R. capsulatus* cells to grow autotrophically with H_2 as the source of electrons (17). It is also synthesized under heterotrophic growth conditions, in particular in H_2-evolving cultures in which hydrogenase synthesis is stimulated by H_2 (9).

The level of hydrogenase expression is closely coupled to environmental and growth conditions (9). Regulatory genes necessary for hydrogenase synthesis in *R. capsulatus* have recently been identified in the cluster of *hup* and *hyp* genes (8). However, the environmental stimuli to which they respond have not yet been identified. The identification of another gene, *himA*, isolated by complementation of the hydrogenase-deficient (Hup⁻) mutant IR4 and capable of encoding a protein similar to the α subunit of the integration host factor (IHF) of *Escherichia coli*, indicated that *R. capsulatus* contains an IHF-like protein (30). The mutation in the *himA* gene (30), which lowers hydrogenase synthesis in IR4 (33), suggested that IHF is involved in hydrogenase gene expression. Indeed, the promoter region of the structural hydrogenase operon *hupSL* contains an IHF-binding consensus sequence, and we have shown by gel retardation assays that IHF from *E. coli* specifically binds to the *R. capsulatus* DNA fragment of 274 bp isolated from the *hupS* promoters, which contains the IHF-binding site (30).

In *E. coli*, IHF is an αβ heterodimer. We report here on the isolation and purification of the *R. capsulatus* IHF-like protein, which is also an αβ heterodimer. The *hip* gene, which encodes the β subunit, has been cloned from *R. capsulatus*, and its sequence is presented.

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MATERIALS AND METHODS

Bacterial strains and cultures. *E. coli* TB1 and competent cells of *E. coli* DH5α (hosts for pUC plasmids) were purchased from and used as suggested by Bethesda Research Laboratories, Inc.; they were grown aerobically at 37°C in liquid Luria-Bertani medium (25). *R. capsulatus* B10 (wild type) (20) and IR4 (*himA* mutant) (30, 33) were grown anaerobically in the light in minimal salts (RCV) medium (15, 32) supplemented with DL-malate (30 mM) and L-glutamate (7 mM) as C and N sources, respectively, as previously described (7).

DNA manipulations. Standard recombinant DNA techniques were performed as described previously (25). Plasmids pIβ2 and pIβ3 were constructed by inserting into the polylinker of pUC18 (34) the 5-kbp *Pst*I-*Pst*I and 2.2-kbp *Hin*dIII-*Hin*dIII fragments, respectively, of genomic *R. capsulatus* DNA that hybridized with the IHFβ probe. DNA sequencing on both strands was performed by the dideoxy chain termination method (26). DNA sequence analysis and homology searching were performed with LASERGENE programs (DNASTAR, Madison, Wis.). Gel retardation assays were performed as previously described (30), using the 274-bp *Eco*RI-*Eco*RI DNA fragment containing the IHF-binding site isolated from the *hupS* promoter.

Purification of IHF. *R. capsulatus* strains were grown anaerobically in 40-liter cultures until the A_{660} reached 1.2, typically yielding 120 g of wet cells. The cells were harvested by centrifugation and resuspended in 100 mM Tris HCl–20 mM EDTA, and 0.2 mg of lysosyme per ml was added. The cell extract (150 ml), obtained after sonication and ultracentrifugation (150,000 × *g*, 2 h) and containing 3 g of protein, was applied to a 5-ml Econo-Pac heparin cartridge (Bio-Rad) equilibrated with buffer A (20 mM Tris HCl, 0.5 mM EDTA, 10% glycerol, pH 8), and the column was developed with a gradient of 0 to 1 M NaCl. DNA was eluted from the heparin column at a low salt concentration (between 0 and 0.25 M NaCl). The fraction that eluted with 0.6 to 0.65 M NaCl in buffer A (0.5 mg of protein in a volume of 10 ml) contained the

IHF protein in an amount sufficient to be detectable by gel shift assays with the 274-bp DNA fragment from the *R. capsulatus hupS* promoter. The IHF-containing fraction (20 ml) was dialyzed for 2 h against buffer P (10 mM phosphate, 0.5 mM EDTA, pH 7) and then loaded onto a 5-ml Econo-Pac-S cartridge (Bio-Rad) equilibrated in buffer P. The column was developed with a gradient of 0 to 1 M NaCl in buffer P. The fraction that eluted at between 0.42 and 0.45 M NaCl (4 ml) contained 100 μg of pure IHF protein.

Cloning of the *hip* gene. The N-terminal sequence of the 10-kDa polypeptide was determined and found to be MIRSELIA. The polypeptide was then cleaved by Asp endoprotease digestion. After purification of the cleaved fragments by high-performance liquid chromatography (HPLC), the sequence DEKHVPFF was obtained for the N terminus of the major proteolytic peptide. Both amino acid sequences could be aligned to that of the β subunit of *E. coli* IHF. On the basis of these two last amino acid sequences and by using the *R. capsulatus* codon usage, the following two oligonucleotides were synthesized: IA922, 5′ATGATCCG(C/G)(T/G)C(C/G)GA(A/G)CT(C/G)ATC; and IA924, 5′GAAGAA(G/C)GG(G/C)AC(G/T)TGCTT(T/G)TC(G/T)TC. They were used for amplification by polymerase chain reaction on total genomic DNA. A 200-bp DNA fragment was thus obtained and was then used for hybridization with *Pst*I-*Pst*I genomic DNA fragments. A 5-kb *Pst*I-*Pst*I DNA fragment was isolated, cloned into pUC18, and sequenced, using first IA922 and IA924 as primers and then synthetic oligonucleotides to progress on both DNA strands.

Protein analytical methods. The protein concentration was determined by the method of Bradford (3). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a small vertical slab gel unit (Mighty Small SE200; Hoefer Scientific Instruments, San Francisco, Calif.) by a method adapted from that of Laemmli (18), with a 5% (wt/vol) stacking gel and a 15% (wt/vol) resolving gel. For N-terminal sequence analysis, proteins were transferred to Problott membranes (Applied Biosystems) in a TE22 Mini Transphor unit (Hoefer) by the protocol recommended by the manufacturer. Following SDS-PAGE, the gel was slightly stained for protein with 0.1% Coomassie blue R-250 in 20% methanol–0.5% acetic acid. The stained protein band was excised, destained with 30% ethanol for 1 h at room temperature, washed twice with 20 ml of 50% acetonitrile for 15 min at 30°C, and partially dehydrated in a Speed Vac concentrator (Savant). The gel slice was then rehydrated in an Eppendorf tube with 20 μl of 50 mM phosphate buffer (pH 8.0) containing 10% acetonitrile and was treated with Asp endoprotease (Boehringer, sequencing grade) added at an enzyme-to-protein ratio of about 1/50 (wt/wt). The digestion was carried out for 15 h at 37°C. The cleaved peptides were extracted from the gel with 60% acetonitrile and 0.1% trifluoracetic acid as described previously (24), separated by reverse-phase HPLC using a VYDAC TP C4 column (150 by 2.1 mm) in 0.1% trifluoroacetic acid, and eluted with a gradient of 0 to 80% acetonitrile supplemented with 0.1% trifluoroacetic acid as described previously (2). The peptide-containing fractions were spotted onto a glass fiber disk coated with Polybrene, and amino acid sequence analysis was performed on an automated Applied Biosystems sequencer (477A) equipped with an online phenylthiohydantoin-amino acid analyzer (model 120A).

DNase I footprinting. An *Eco*RI-*Hin*dIII fragment of 180 bp isolated from the *hupS* promoter and containing the IHF consensus sequence was cloned into the polylinker of pUC18. The resulting plasmid, pRSA1, was digested by the *Eco*RI and *Pvu*II or the *Hin*dIII and *Pvu*II restriction enzymes, yielding fragments of 274 and 354 bp, respectively, in order to obtain double-stranded fragments for labeling of either of the two strands. These fragments were end labeled by 3′ end filling with the Klenow fragment of DNA polymerase, ^{35}S-dATP, and ^{35}S-dCTP. The binding reaction mixture (80 μl) contained 2 μl (5 ng) of ^{35}S-labeled DNA probe (10,000 cpm/ng), various amounts of IHF protein, 5 μg of poly(dI)-poly(dC), and 0.3 μg of bovine serum albumin in 10% glycerol–25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.2)–1 mM EDTA–1 mM dithiothreitol. After 15 min of preincubation at room temperature, $MgCl_2$ (final concentration, 10 mM) and 80 ng of DNase I were added and allowed to react for 1 min at room temperature. The reaction mixture was added in a 2:1 ratio to the stop mixture (95 mM EDTA, 0.8% SDS, 1.6 M ammonium acetate, 0.3 mg of sonicated calf thymus DNA per ml). Nucleic acids were precipitated with ethanol, dissolved in sequencing loading buffer, and loaded onto a 6% denaturing polyacrylamide gel containing 7 M urea. After electrophoresis (1.5 h at 1,600 V), the gel was exposed to a Storage Phosphor Screen which was then introduced in a Phosphorimager apparatus (Molecular Dynamics) for image treatment.

Nucleotide sequence accession number. The sequence shown in Fig. 3 has been deposited in GenBank (accession no. L13998).

RESULTS

Purification of IHF-like protein from *R. capsulatus*. The purification scheme presented here is a two-step procedure which allows rapid recovery of microgram quantities of highly purified IHF. It does not include the polymin P fractionation steps of the procedure used earlier to purify IHF from *E. coli* (22), it makes use of commercial heparin and cation-exchange columns without prior elimination of DNA to avoid loss of IHF, and it allows the direct isolation of the protein from cells containing small amounts of IHF. The IHF protein present in the collected fractions was detected by gel mobility shift assays using the 274-bp DNA fragment from the *R. capsulatus hupS* promoter, which has been shown earlier to contain an IHF-binding site (30). As shown in Fig. 1, 1 μg of the fraction eluted from the heparin column (lane 3) was as efficient as 400 μg of the crude extract for complexing 1 ng of DNA isolated from the *hupS* promoter. Fig. 1, lane 4, shows that 0.5 μg of the fraction eluted at 0.4 to 0.5 M NaCl from the Econo-Pac S column was able to complex the same amount of DNA as 1 μg of the fraction eluted from the heparin column. With a recovery of approximately one-third of the initial IHF content in the crude extract, the IHF protein was obtained with an enrichment factor of 3,200 (Table 1). We calculate that the total amount of IHF is approximately 0.3 mg/g of total soluble proteins.

SDS-PAGE analysis of the protein fractions at each purification step showed that most of the contaminants present in the eluate of the heparin column were removed by ion-exchange chromatography. On the electrophoretograms stained either by Coomassie blue or by silver nitrate, the IHF protein appeared clearly as a heterodimeric protein with subunits with apparent molecular masses of ca. 11 and 10 kDa (Fig. 2). Similarly, the IHF-like protein from the *himA* mutant IR4 could be purified by the same two-step procedure.

Cloning and sequencing of the *hip* gene, which encodes the IHF β subunit. The N-terminal amino acid sequence of each of the bands was determined. The sequence of the N terminus of the 11 kDa polypeptide, SEKTLTRMDLSEAV, coincided with the predicted sequence of the *himA* gene product from

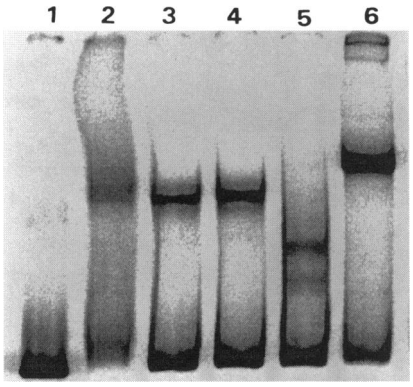

FIG. 1. Binding of IHF to the 274-bp DNA fragment from the *hupSL* promoter studied by gel retardation assays. The amounts of proteins (micrograms) indicated in parentheses were added to the 274-bp DNA fragment: Lane 1, free DNA; lane 2, soluble extract from *R. capsulatus* B10 (400); lane 3, Econo-Pac heparin eluate from B10 (1); lane 4: Econo-Pac S eluate from B10 (0.5); lane 5: Econo-Pac heparin eluate from IR4 (1); lane 6, soluble extract from *E. coli* TB1 (50).

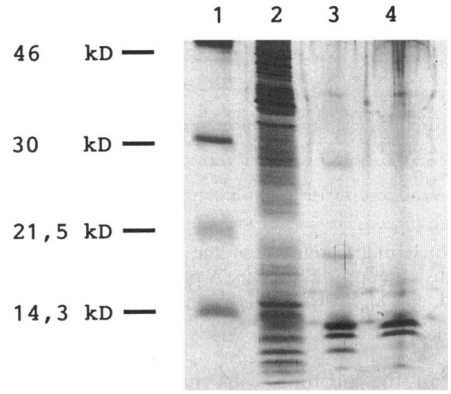

FIG. 2. SDS-PAGE analysis of the protein fractions obtained at each step of the purification procedure. An SDS–15% polyacrylamide gel stained with silver nitrate is shown. Lane 1, molecular weight markers, lane 2, soluble bacterial extract (5 μg of protein); lane 3, eluate from Econo-Pac heparin column (1 μg of protein); lane 4, Econo-Pac S column eluate (0.5 μg of protein).

amino acids 2 to 14 (30). The N-terminal amino acid sequences of the whole 10-kDa polypeptide and of an internal proteolytic fragment derived from it could be aligned with the corresponding amino acid sequence of the β subunit of *E. coli* IHF. By polymerase chain reaction amplification from genomic DNA with the two oligonucleotides IA922 and IA924, we isolated the DNA fragment containing an open reading frame capable of encoding a protein of 94 amino acids (10.7 kDa) (Fig. 3), the deduced product of which shared 43 identical amino acids with a 93-amino-acid overlap (i.e. 46% identity) with the β subunit of *E. coli* IHF. The *R. capsulatus* and *E. coli* proteins had an overall similarity of 71%. Crucial amino acid sequences were either identical or homologous in particular in the region from position 58 to 92 (Fig. 4). The Glu-44 residue, which alters DNA-binding specificity in *E. coli* IHF (19), is also conserved in the IHF β subunit of *R. capsulatus*. Similarly to the *E. coli* gene encoding the β subunit (11), the *R. capsulatus* gene has been called *hip*.

The 5′ upstream region of the *R. capsulatus hip* gene contains an IHF consensus sequence (ttcCAAGCGATTG GTG), as is also the case for the promoter region of the *himA* gene, which encodes the α subunit (30). In the promoter region of the *hupS* gene, which encodes the small hydrogenase subunit, two IHF consensus sequences are present (8). The first one, CACACCATTGA, is located 144 bp upstream from the TTG start codon of the putative signal peptide preceding the *hupS* gene. The second one, CAAGGCATTCT, is located 297 bp upstream from the same TTG start codon. Gel mobility shift assays with the IHF protein from *R. capsulatus* showed that the protein binds to the DNA fragment containing the IHF consensus sequence which is closer to the TTG start codon (data not shown).

DNA binding and promoter recognition. The *R. capsulatus* IHF proteins isolated from B10 (IHF_{B10}) and from the *himA* mutant IR4 (IHF_{IR4}) were used in gel retardation experiments. Figure 1 shows that while the wild-type protein formed a single stable complex with the 274-bp DNA fragment (lanes 3 and 4), IHF_{IR4} formed more than one complex (lane 5), an indication that IHF_{IR4} may have lost part of its binding specificity. Furthermore, the protein isolated from the wild-type strain B10 produced a complex of lower mobility (Fig. 1, lanes 3 and 4) relative to free DNA (lane 1) than did the mutated protein isolated from the *himA* mutant, IR4 (lane 5). The interactions of the IHF_{B10} and IHF_{IR4} proteins and DNA were studied further by DNase I protection experiments using IHF site-containing DNA fragments labeled at the 3′ end of either of the two DNA strands. The protection patterns are shown in Fig. 5. In the control experiment done with the antisense strand and no protein added (lane 3), a region naturally DNase I protected was apparent from positions −128 to −120 relative to the TTG translation start codon, which interfered with the determination of the beginning of the region interacting with IHF on that strand. In contrast, the sense strand allowed us to see that the IHF_{B10} or IHF_{IR4} protein covers 40 bases located between positions −167 and −127 (compare lanes 4, 5, and 6 of Fig. 5). This region encompasses the CACACCATTGA IHF consensus sequence. Thus, this sequence is the binding site of *R. capsulatus* IHF, the first one to be determined for that bacterium. Although the mutated IHF_{IR4} protein, in which Arg-8 is replaced by Cys-8 in the α subunit, still binds to the same site as IHF_{B10}, some

TABLE 1. Purification of the IHF protein of *R. capsulatus*[a]

Fraction	Vol (ml)	Protein (mg/ml)	DNA-binding activity (U/μl)	DNA-binding sp act (U/mg of protein)	Enrichment factor	Total activity (U)	Yield (%)
Crude extract	50	20	1	50		50,000	100
Heparin column	10	0.05	2	40,000	800	20,000	40
Cation-exchange column	4	0.025	4	160,000	3,200	16,000	32

[a] The activity, estimated from gel shift analyses, was arbitrarily given the value of 1 U for 1 μl of crude extract.

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1    CATGCGATAATTTCCCGCCTTTCCAAGCCATTGCTGTTTGTGTCGCGGAAAATTCCTCCTATAGTCGG
                                        Hip →  M  I  R  S  E  L  I  A
69   CGCAAGAACCAGTCCGGGTCGAGCCGGGTGACGGGGGAGATACGCATGATCCGCTCTGAATTGATCGCC
     K  I  A  E  E  N  P  H  L  F  Q  R  D  V  E  K  I  V  N  T  I  F  E
138  AAGATCGCCGAAGAGAATCCGCATCTGTTTCAGCGCGACGTGGAGAAGATCGTGAACACGATCTTCGAA
     E  I  I  E  A  M  A  R  G  D  R  V  E  L  R  G  F  G  A  F  S  V  K
207  GAGATCATCGAGGCGATGGCCCGCGGTGATCGCGTCGAGCTGCGCGGCTTTGGCGCCTTTTCGGTGAAG
     K  R  D  A  R  T  G  R  N  P  R  T  G  T  S  V  A  V  D  E  K  H  V
276  AAACGGGACGCTAGAACCGGGCGCAATCCGCGCACCGGCACATCGGTCGCGGTGGATGAAAAACACGTG
     P  F  F  K  T  G  K  L  L  R  D  R  L  N  G  G  E  E
345  CCCTTCTTCAAGACCGGCAAGCTTTTGCGTGACCGGCTGAACGGGGGAGAGGAGTAAGACCCGATGTTC
414  CGCGCGATCCGCTACCTGTCCTGGCTTGCCTGATCCTTGTCTGGTGACGTTGGCGATGTCCAACCGC..
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FIG. 3. Nucleotide sequence of the fragment containing the *hip* gene and translation product of *hip*. A potential ribosome binding site is underlined, and an upstream IHF consensus sequence is overlined.

differences in the protein-DNA interaction were observed, namely, an alteration of the protection in the DNA region from −145 to −165 on the antisense strand (compare lanes 1 and 2 of Fig. 5). Although DNase I protection is not the best way to probe DNA-protein contacts at the level of one nucleotide, densitograms of protection patterns of the antisense strand obtained by the Image Quant software (Molecular Dynamics) allowed us to obtain more precise data. With IHF_{IR4}, the band corresponding to the C at −161 is lighter, indicating a lower accessibility to DNase I, whereas the bases positioned at −159, −150, and −148 appear to be less protected.

DISCUSSION

The occurrence of IHF-like proteins in gram-negative bacteria other than *E. coli* is a very recent discovery (14, 30). It is now well established that, together with HU (1), these small heterodimeric DNA-binding proteins have an important role to play in the formation of high-order DNA structures and, consequently, in gene regulation.

The method presented here can be applied to purify and microsequence IHF homolog proteins from other organisms and then to probe and clone the corresponding *himA* and *hip* genes. The internal sequence GRNPKTG, which is highly conserved in the known sequences of both the α and β subunits of IHF and in HU proteins (14, 21), may be used to design the antisense nucleotide sequence necessary for the synthesis of the probe by PCR. This internal motif, which is part of a larger motif encompassing amino acids 55 to 80 of IHF and HU subunits, would belong to the β-sheet secondary structure of IHF and of HU, which is thought to interact with the minor groove of DNA (19). This domain is homologous to the DNA-binding domain of the TATA box-binding protein (TFIID) of eucaryotes at the levels of both the primary and secondary structures (21, 23). The DNA consensus sequence, 5′ TATAAA, binding TFIID, is reminiscent of the 5′ ATCAA sequence found in typical IHF-binding sites (13) and also in FNR-binding sites (29).

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Hip-R.c.  MIRSELIAKIAEENPHLFQRDVEKIVNTIFEEIIEAMARGDRVELRG
          M :SELI ::A :::H:  : VE  V: ::E::  ::A:G:R:E:RG
Hip-E.c.  MTKSELIERLATQQSHIPAKTVEDAVKEMLEHMASTLAQGERIEIRG

Hip-R.c.  FGAFSVKKRDARTGRNPRTGTSVAVDEKHVPFFKTGKLLRDRLNGGEE
          FG:FS:  R :RTGRNP:TG  V :: K VP FK GK LRDR N
Hip-E.c.  FGSFSLHYRAPRTGRNPKTGDKVELEGKYVPHFKPGKELRDRANIYG
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FIG. 4. Amino acid sequence alignment of the IHF β subunits from *R. capsulatus* and from *E. coli*. A comparison of the deduced amino acid sequences of the *hip* gene products from *E. coli* (E.c.) and *R. capsulatus* (R.c.) is shown. Colons indicate conservative substitutions.

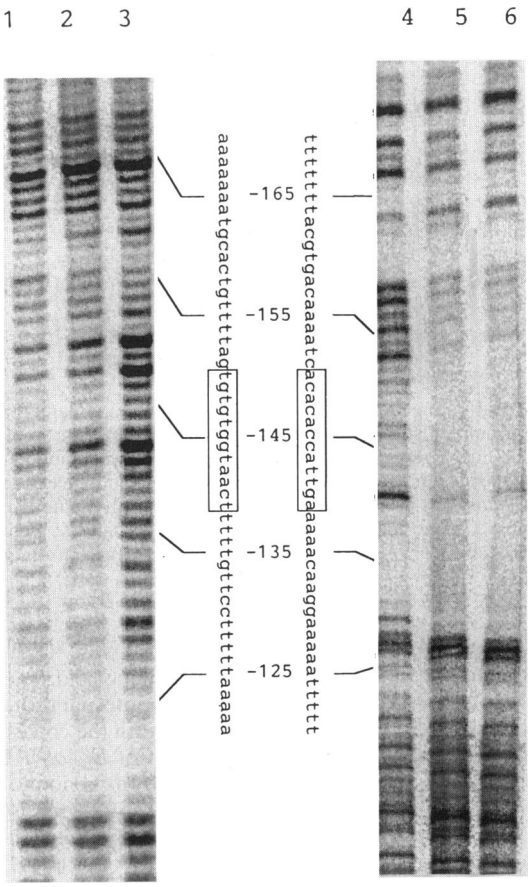

FIG. 5. DNase I footprinting of the IHF-binding site of the *R. capsulatus hupS* promoter complexed with either IHF_{B10} or IHF_{IR4}. Experiments were carried out with 5 μg of the double-stranded DNA fragments from the *hupS* promoter (see Materials and Methods) labeled by ^{35}S at the 3′ end of either the antisense strand (lanes 1, 2, and 3) or the sense strand (lanes 4, 5, and 6). The amounts of proteins used in the incubation mixtures were as follows: IHF_{B10}, 0 μg (lane 3) and 5 μg (lanes 1 and 6); IHF_{IR4}, 0 μg (lane 4) and 5 μg (lanes 2 and 5). The nucleotide sequence of each strand is shown. Numbers indicate positions on the DNA sequence with respect to the TTG translation start codon of the *hupS* gene. The IHF consensus sequence is boxed.

By inducing a strong DNA bending, the IHF protein can have a positive or a negative effect on the regulation of transcription from a distant site (6). Recent studies have shown that IHF binds just upstream from the promoters of some genes whose expression requires an alternative form of RNA polymerase which uses the *rpoN* gene product, the σ^{54} factor (6, 27). Transcription depends on an activator protein which catalyzes the isomerization of closed complexes between RNA polymerase holoenzyme and a promoter to produce transcriptionally productive open complexes. This is the case for the nitrogen fixation genes *nifH* and *nifU* of *Klebsiella pneumoniae* (4, 16, 27), for *glnH* (glutamine permease) of *E. coli* (5), for the *flbG* operon (flagellar proteins) of *Caulobacter crescentus* (12), and for *xylCAB* (xylene and toluene catabolic enzymes) of *Pseudomonas putida* (10).

For *R. capsulatus*, it has been shown earlier (30) that the impairment of in vivo hydrogenase expression in the IR4 mutant (33) is the result of a point mutation in the α subunit of IHF (in IHF_{IR4}, Arg-8 has been replaced by Cys-8). Gel retardation assays (Fig. 1) show that the mutated IHF protein

isolated from the IR4 strain does bind to the *hupS* promoter but less specifically than the wild-type IHF protein. In the computer model for IHF and DNA interaction assembled by David and Toussaint (9a), it appears that, depending on the level of DNA bending, Arg-8 can interact with the DNA. The relatively higher mobility of the main IHF_{IR4}-DNA complexes suggests that in these complexes the degree of DNA bending is probably less than in the IHF_{B10}-DNA complex (28). The footprinting data have permitted us to pinpoint the slight differences in the interaction between the DNA chain and the IHF_{B10} and IHF_{IR4} proteins. The footprinting data indicated that the interactions between IHF and the region from −165 to −145 are weaker with IHF_{IR4} than with IHF_{B10}. It is known that the specific capacity of IHF binding to a particular site depends not only on the presence of the IHF consensus sequence but also on the flanking nucleotide sequences which contribute to the DNA conformation around the site (13). The IHF_{B10} DNA contact region covers 40 bases which contain 70% A+T with a series of four to six consecutive A or T residues, whereas the average A+T content of *R. capsulatus* DNA is 33 to 35%. The DNA conformation in complexes with the mutated IHF_{IR4} maybe different from that of complexes with IHF_{B10} because of an altered interaction of nucleotides flanking the binding site with amino acid residues of the body of the protein. These subtle differences in binding are apparently sufficient to alter expression of hydrogenase in the IR4 mutant (33). Indeed, transcription of the structural hydrogenase genes in the IR4 mutant, monitored by the use of a plasmid-borne *hupS::lacZ* fusion, has been found to be 20% of that in the wild-type B10 (9), confirming that the mutation in *himA* altered the capacity of IHF to activate hydrogenase gene transcription.

It is not well understood how the synthesis of IHF is regulated in vivo. The presence of an IHF consensus sequence in the promoter regions of the *himA* and the *hip* genes suggests that IHF synthesis may be autoregulated in vivo.

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