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A Protein That Binds to the Regulatory Region of the *Escherichia coli ilvIH* Operon

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The *ilvIH* operon of *Escherichia coli* encodes acetohydroxyacid synthase III, an isoenzyme involved in branched-chain amino acid biosynthesis. Transcription of the *ilvIH* operon is repressed by growing cells in the presence of leucine (C. H. Squires, M. DeFelice, S. R. Wessler, and J. M. Calvo, J. Bacteriol. 147:797–804, 1981). A protein in crude extracts of *E. coli*, termed the *ilvIH*-binding (IHB) protein, bound specifically in vitro to DNA upstream of the *ilvIH* operon. The binding protein, partially purified by Polymin precipitation, gel filtration, and phosphocellulose chromatography, has a native molecular weight of 43,000 and is composed of two subunits of identical size. As determined by protection against lambda exonuclease and DNase I, the protein binds within a region −190 to −260 relative to the start point of transcription. In addition, the IHB protein binds to a site between positions −100 and −40. The following evidence suggests that binding of this protein to the region upstream of *ilvIH* is related to the regulation of this operon by leucine. Binding of the IHB protein to the *ilvIH* regulatory region in vitro was reduced by leucine but not by isoleucine, valine, or threonine. In a mutant strain isolated by M. V. Ursini, P. Arcari, and M. DeFelice (Mol. Gen. Genet. 181:491–496, 1981), transcription was not repressed by leucine. A protein in extracts of this mutant strain bound to the *ilvIH* regulatory region, but the complex migrated through agarose gels with a mobility different from that of the complex formed by wild-type protein. Furthermore, a concentration of leucine that substantially reduced binding of the wild-type protein to DNA did not affect binding of the protein from the mutant strain. A simple model consistent with these findings is that transcription from the *ilvIH* promoter is stimulated by binding of the IHB protein to one or more sites upstream of the promoter and that leucine interferes with this binding.

The *ilvIH* operon codes for one of two acetohydroxyacid synthase (AHAS) isoenzymes in *Escherichia coli* K-12 catalyzing the first step unique to branched-chain amino acid biosynthesis (3). *ilvI* encodes the catalytic subunit of AHAS III, and *ilvH* encodes a subunit that confers sensitivity to inhibition by valine. In addition, the *ilvH* subunit is required for efficient catalysis (15). The DNA of the entire *ilvIH* operon, including the regulatory region, has been sequenced (8, 14).

Transcription of the *ilvIH* operon is repressed 5- to 10-fold in cells grown in the presence of leucine (15). This repression mediated by leucine is sufficiently severe that an *E. coli* K-12 strain lacking the other AHAS isoenzyme (AHAS I) because of a mutation in *ilvB* does not grow in the presence of leucine. Among *ilvB* mutants that grew in the presence of leucine, Ursini et al. (16) characterized one strain, LR16, in which *ilvIH* expression was not repressed by leucine. The mutation causing this phenotype, *lrs-1*, is not within the *ilvIH* operon (16; J. Platko and J. Calvo, unpublished data).

Both the *ilvIH* promoter and sequences necessary for leucine-mediated repression are within a 361-base-pair region located immediately upstream from the ATG start codon of *ilvI* (8). A deletion analysis suggested that the promoter and regulatory region of this operon may be unusual in that sequences several hundred base pairs upstream of the presumed site of transcription initiation are required for efficient transcription initiation (8). In an attempt to further characterize the *ilvIH* promoter and regulatory region, we searched crude extracts of *E. coli* for proteins that bind specifically to this region. We report here the preliminary characterization of such a protein from wild-type and *lrs*-containing strains. Our results suggest that this binding protein, termed the *ilvIH*-binding (IHB) protein, is involved in leucine-mediated regulation of *ilvIH* operon expression. A model consistent with our results is that the IHB protein stimulates transcription of the *ilvIH* operon upon binding to one or more sites upstream of the promoter and that leucine represses expression of the operon by interfering with the binding of the IHB protein to these sites.

MATERIALS AND METHODS

Strains and growth conditions. *E. coli* K-12 W3102 (F^- *galK2*) was employed as a source of wild-type binding protein. Strains PS1035 (HfrH *thi-1 glyA bglR20 ilvB619*) and LR16 (PS1035 containing the *lrs-1* mutation) were a gift from M. DeFelice. Minimal medium contained SSA salts (8) supplemented with 0.2% glucose, micronutrients (12), and 5 μg of thiamine per ml. Cells were grown at 37°C (except for PS1035 and LR16, which were grown at 32°C) with shaking.

Preparation of extracts and partial purification of binding protein. Bacteria were harvested by centrifugation, suspended in cold TGED buffer (10 mM Tris hydrochloride [pH 8], 10% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol) containing 0.2 M NaCl (3.5 ml/g [wet weight] of cells), and disrupted either by sonication or by passage through a French pressure cell (1,000 kg/cm^2). Cell debris was removed by centrifugation at 7,000 × *g* for 20 min. The resulting supernatant was used for experiments with crude extract.

All steps in the following purification scheme were carried out at 4°C. A 5% solution of Polymin P (ICN Biomedicals) was added slowly to crude extract (15 mg of protein per ml) to bring the Polymin P concentration to 0.2%. Following incubation for 5 min and centrifugation for 15 min at 5,000 ×

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g, the pellet (containing about 50% of the binding activity) was suspended in one-fifth volume of TGED containing 0.5 M NaCl and incubated for 5 min. Following centrifugation for 15 min at 5,000 × *g*, 433 g of solid ammonium sulfate per liter of supernatant (60% saturation) was added slowly. After standing for 20 min and being centrifuged for 45 min at 6,000 × *g*, the pellet was suspended in one-half volume of TGED buffer containing 0.2 M NaCl and the sample was dialyzed against the same buffer for at least 5 h with one change of buffer. A 5-ml portion of the sample containing 40 mg of protein was applied to a Sephadex G-100 Superfine (Pharmacia) column (2.5 by 46 cm) equilibrated with TGED buffer containing 0.2 M NaCl. The column was eluted with the same buffer at a flow rate of 7.5 ml/h, and 1.1-ml fractions were collected. Fractions containing binding activity (see below) were pooled and applied to a 0.75-ml P11 phosphocellulose column (Whatman, Inc.) equilibrated with TGED containing 0.2 M NaCl. The column was washed with 5 column volumes of the same buffer and eluted with TGED containing 0.5 M NaCl at a flow rate of 4 ml/h. Material with binding activity eluted at about 0.3 M NaCl as determined by conductivity measurements. At this stage of purification, the IHB protein was estimated to be about 15% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Gel electrophoresis binding assay. Plasmid pCV112 was constructed by inserting a 361-base-pair *Hae*III fragment containing the *ilvIH* promoter and regulatory region (8) into the *Hin*cII site in plasmid pUC13. Plasmid pCV112 was cut with *Eco*RI, *Hin*dIII, and *Bgl*I and those fragments that contained protruding 5′ ends were end labeled with ^{32}P by using reverse transcriptase (13). This procedure yields labeled fragments of 1,370, 406 (contains the *ilvIH* promoter and regulatory region), and 150 base pairs. For the binding assay, samples contained the following in a total volume of 20 μl: ^{32}P-labeled DNA fragments, 10^5 cpm (10^7 cpm/μg); crude or partially purified extract, up to 8 μg of protein; calf thymus DNA, 3 μg; 20 mM Tris-acetate buffer [pH 7.9] containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 4 mM magnesium acetate, 50 mM NaCl, and 12.5% glycerol. After incubation at room temperature for 10 min, samples were fractionated by electrophoresis through 1.5% agarose gels (200 V, 2 to 3 h) cast and run in Tris-borate buffer (90 mM Tris, 90 mM boric acid, 3 mM EDTA [pH 8.3]). Gels were dried at 50°C under vacuum and subjected to radioautography.

For quantitation of binding, lanes were scanned with a model 620 densitometer (Bio-Rad Laboratories). The extent of binding was calculated from $a/(a + b)$, where a is the absorbance (arbitrary units) of the shifted *Eco-Hin*d_{406} band and b is the absorbance of the unshifted band. Normalization of a and b to the 1,370-base-pair band did not change the results significantly.

Renaturation of IHB protein after SDS-PAGE. Samples of crude extract (5 μg of protein) or partially purified IHB protein (5 μg; purified by Polymin P precipitation, gel filtration and phosphocellulose chromatography) were heated to 100°C in a buffer containing 1% SDS and then fractionated by electrophoresis (4 h at 25 mA of constant current) on a 1.2-mm-thick SDS-PAGE gel containing 13% acrylamide, 0.4% bisacrylamide, and 0.1% SDS as described by Laemmli (9). Size standards included bovine serum albumin (68 kilodaltons [kDa]), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 kDa), and lysozyme (14 kDa). Proteins were visualized by staining with silver. An unstained adjacent lane (1.25 μg of partially purified protein) was sliced, and protein was eluted and renatured by the procedure of Hager and Burgess (7). Samples were concentrated 20-fold with a Centricon 10 centrifuge filter (Amicon Corp.), and a portion was tested for binding activity by the gel retardation assay.

Subfragments derived from the promoter-regulatory region. Fragments B, C, D, and E (see Fig. 6B) were prepared as follows. A *Hin*fI$_{187}$ fragment cut from fragment *Eco-Hin*d_{406} (fragment A in Fig. 6B) was labeled at the 3′ ends with reverse transcriptase, and fragments B, C, D, and E were prepared from it by cleavage with the indicated restriction enzymes and isolation of the individual fragments following electrophoresis through 5% acrylamide. Similarly, a *Hin*fI-*Hin*dIII$_{192}$ fragment cut from fragment *Eco-Hin*d_{406} was labeled at the 3′ ends, and fragments F, G, H, and I were prepared from it.

Protection against lambda exonuclease. Fragment *Eco-Hin*d_{406} labeled with ^{32}P at the *Hin*dIII end (label on the noncoding strand) was prepared from plasmid pCV112 by cutting with *Hin*dIII, labeling with reverse transcriptase, cutting with *Eco*RI, and then isolating the labeled *Eco-Hin*d_{406} fragment by acrylamide gel electrophoresis. Fragment *Eco-Hin*d_{406} labeled at the *Eco*RI end (label on the coding strand, i.e., the strand of the operon that is transcribed) was prepared in a similar way. Labeled fragment (10^5 cpm; 10^7 cpm/μg) was incubated with partially purified IHB protein (purified by Polymin P precipitation and phosphocellulose chromatography) for 10 min at room temperature and then further incubated without or with 1 U of lambda exonuclease for 5 min at 37°C. Samples were ethanol precipitated and analyzed by electrophoresis through a 40-cm 5% acrylamide–50% urea gel, and bands were visualized by radioautography.

Protection against DNase I. Fragment *Eco-Hph*I$_{260}$, derived from fragment *Eco-Hin*d_{406} (see Fig. 6B), was labeled at the *Eco*RI end with ^{32}P (5×10^5 cpm; 10^7 cpm/μg) and was

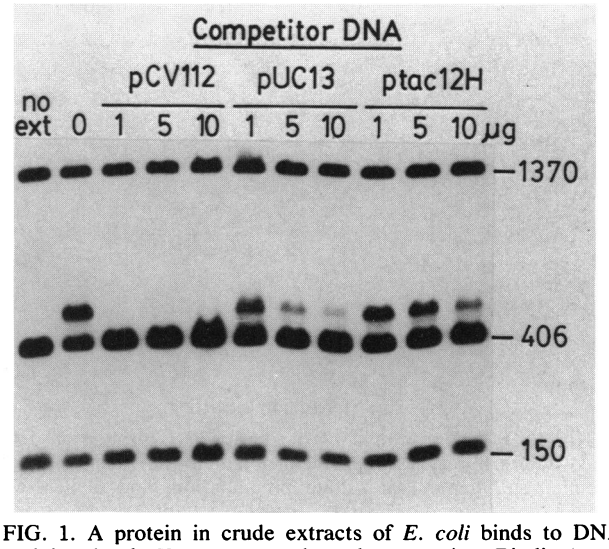

FIG. 1. A protein in crude extracts of *E. coli* binds to DNA containing the *ilvIH* promoter and regulatory region. Binding reactions contained ^{32}P-labeled fragments from plasmid pCV112 (sizes in base pairs are indicated on right), 6 μg of protein from a crude extract of *E. coli* W3102 (ext), and the indicated amounts (in micrograms) of competitor DNA, added in addition to the calf thymus DNA that is normally present in the assay. Samples were treated as described in the text for the gel electrophoresis binding assay.

incubated without or with 50 ng of partially purified IHB protein (purified by Polymin P precipitation, Sephadex and phosphocellulose chromatography) for 10 min at room temperature. Samples were treated with 2.5 ng of DNase I for 2 min at room temperature, phenol extracted, ethanol precipitated, and then fractionated by electrophoresis through a 40-cm 8% acrylamide–50% urea gel.

RESULTS

A protein that binds to the *ilvIH* regulatory region. Plasmid pCV112 contains the *ilvIH* promoter and regulatory region cloned into the polylinker of plasmid pUC13. Three labeled fragments are produced from plasmid pCV112 after digestion with endonucleases *Bgl*I, *Eco*RI, and *Hin*dIII and end labeling with reverse transcriptase. Upon incubation of these fragments with a crude extract from *E. coli* followed by agarose gel electrophoresis, a fraction of the 406-base-pair *Eco*RI-*Hin*dIII fragment (*Eco-Hind*$_{406}$, containing the *ilvIH* promoter and regulatory region) migrated more slowly than did the same fragment analyzed in the absence of protein (Fig. 1, lane 0). We used this "gel retardation" assay (4, 6) to characterize the binding activity.

To investigate the specificity of the binding, unlabeled competitor DNA was added in addition to the calf thymus DNA that is normally included to reduce nonspecific binding and nuclease action. One microgram (100-fold molar excess) of either plasmid pUC13 DNA or plasmid ptac12H DNA (contains a strong promoter) (1) diminished binding less than 2-fold, whereas a 100-fold molar excess of plasmid pCV112 decreased binding by more than 20-fold (Fig. 1). The binding activity was destroyed by treatment with proteinase K, SDS, or phenol and by heating 2 min at 100°C, suggesting that it is at least in part proteinaceous (data not shown). For the presentation that follows, we assume that the binding factor is just a single protein and we call it the IHB protein.

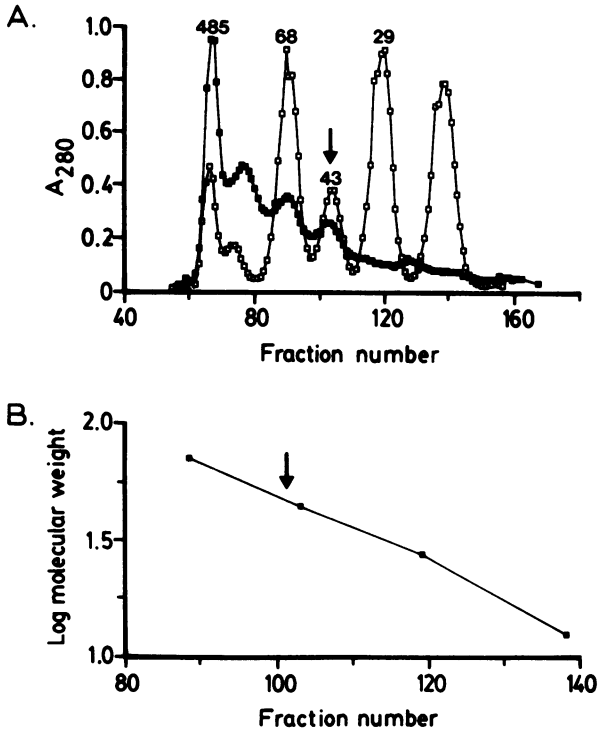

FIG. 2. Molecular weight of the native IHB protein as determined by gel filtration. (A) Protein obtained following Polymin P precipitation, ammonium sulfate precipitation, and dialysis was applied to a Sephadex G-100 Superfine column. Fractions were assayed for A_{280} (■) and binding activity, measured by the gel retardation assay. Binding activity was limited to fractions 95 to 114, with peak activity occurring at fraction 102 (arrow). The following size standards, analyzed in a separate experiment using the same column, are (from left to right) fatty acid synthetase (485 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), and cytochrome *c* (12.5 kDa) (5 mg each) (□). (B) Estimation of molecular weight. The peak of binding activity (arrow) corresponds to a molecular weight of 43,000.

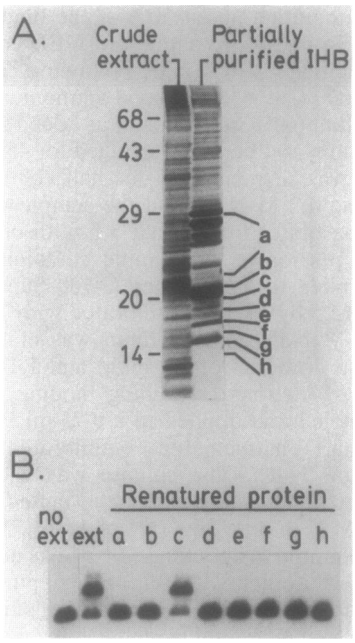

FIG. 3. Renaturation of IHB protein after SDS-PAGE. (A) Samples of crude or partially purified IHB protein fractionated by SDS-PAGE. (B) An unstained adjacent lane containing partially purified protein was sliced (a, b, c, etc., in panel A), and protein from each slice was eluted, renatured, and tested for binding activity by the gel retardation assay. ext, Partially purified extract that was not subjected to SDS-PAGE.

Partial purification and size determination of the IHB protein. The IHB protein was partially purified by a combination of Polymin P precipitation, gel filtration, and chromatography on phosphocellulose. The native molecular weight, as estimated by sizing on a Sephadex G-100 column, is 43,000 (Fig. 2).

To estimate subunit sizes, samples of partially purified protein were fractionated by electrophoresis through a polyacrylamide gel containing SDS. The gel was cut into slices, and from each slice, protein was eluted, renatured, and tested for binding activity by the gel retardation assay. Binding activity was associated with a fraction containing polypeptides of molecular weights 20,000 to 21,500 (Fig. 3). The mobility of the complex formed from renatured protein was identical to that formed from native protein, indicating that the native and renatured proteins have the same size. Taken together, the results indicate that the IHB protein is composed of two polypeptides, each of molecular weight about 21,000.

Effect of leucine on binding. The binding of the partially purified IHB protein to the *Eco-Hind*$_{406}$ fragment in vitro was reduced by 20 mM leucine but not by equivalent

concentrations of valine, isoleucine, or threonine (Fig. 4A). The relationship between leucine concentration and binding activity is shown in Fig. 4B. Inhibition of binding caused by leucine was not complete, even at higher concentrations of leucine. In different experiments, the inhibition varied from 50 to 70%. Thus, leucine, which represses expression of the *ilvIH* operon in vivo, reduces but does not eliminate binding of the IHB protein to the *ilvIH* regulatory region in vitro.

A mutation that affects *ilvIH* operon expression affects binding. Ursini et al. (16) described a mutation in *E. coli* located outside the *ilvIH* operon, *lrs-1*, that caused slightly elevated expression of the *ilvIH* operon. Furthermore, *ilvIH* operon expression in a strain containing this mutation (LR16) was not markedly inhibited by leucine. In a gel retardation assay, the mobility shift caused by a crude extract prepared from a wild-type strain was greater than that caused by an extract prepared similarly from the mutant strain (Fig. 5A). Note that an extract of PS1035 (isogenic to LR16 except for *lrs-1* mutation) gave the same mobility shift as an extract from W3102 (strain used throughout this study; wild-type *lrs*). This experiment was repeated with partially purified preparations of protein under conditions of higher resolution (Fig. 5B). With protein from a wild-type strain, three bands of altered mobility were observed, and the

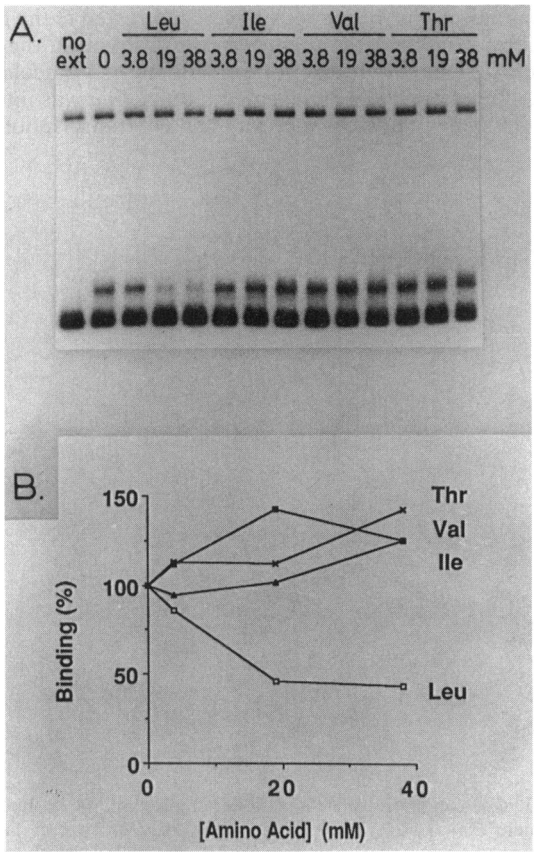

FIG. 4. Effect of leucine, isoleucine, valine, and threonine on binding of IHB to the *ilvIH* regulatory region. (A) Binding reactions, containing end-labeled fragments prepared from plasmid pCV112, 50 ng of partially purified protein (purified by Polymin P precipitation and phosphocellulose chromatography), and the indicated concentrations of each amino acid, were analyzed by the gel retardation assay. (B) Quantitation of binding in panel A by densitometry. Each value is expressed as a percentage of the value from a sample that had no added amino acid.

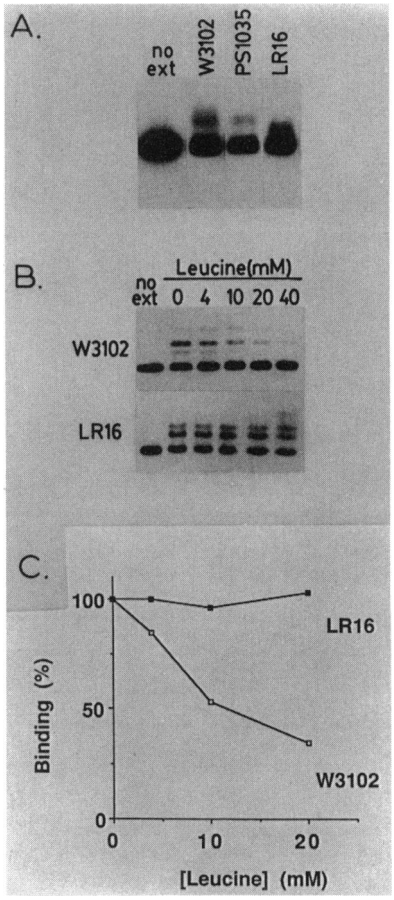

FIG. 5. Gel retardation assays employing crude extract and partially purified IHB protein from strain LR16. (A) Binding reactions contained end-labeled fragments derived from plasmid pCV112 and no extract or crude extract containing 6 μg of protein from each of the indicated strains. (B) Binding reactions contained end-labeled fragments derived from plasmid pCV112, 50 ng of partially purified extract from strain W3102 or LR16 (purified by Polymin P and phosphocellulose chromatography), and the indicated amount of leucine. (C) Densitometric scans of the lanes shown in panel B.

middle prominent band corresponded to the shifted band in panel A of the figure. High concentrations of IHB protein favored formation of the slowest moving of the three bands (data not shown). Under our assay conditions, the fastest moving of the three was never more than a minor component. By comparison, the major band observed with partially purified protein from the mutant apparently corresponds to the fastest moving complex.

The effect of leucine upon DNA binding is shown in Fig. 5B and C. Leucine at a concentration of 20 mM had no effect upon DNA-binding activity of a partially purified protein from strain LR16, but it reduced binding to less than 40% for the samples containing wild-type protein.

In summary, in a mutant strain that lacks leucine-mediated repression of *ilvIH* expression, the IHB protein is altered in some way and the binding of this protein to the *ilvIH* regulatory region is not affected by leucine.

IHB protein binds to at least two sites upstream of *ilvIH*. A variety of shorter fragments, prepared by treating fragment *Eco-Hind*$_{406}$ with restriction endonucleases, were isolated following electrophoresis and tested for binding to the IHB protein by using the gel retardation assay. The results

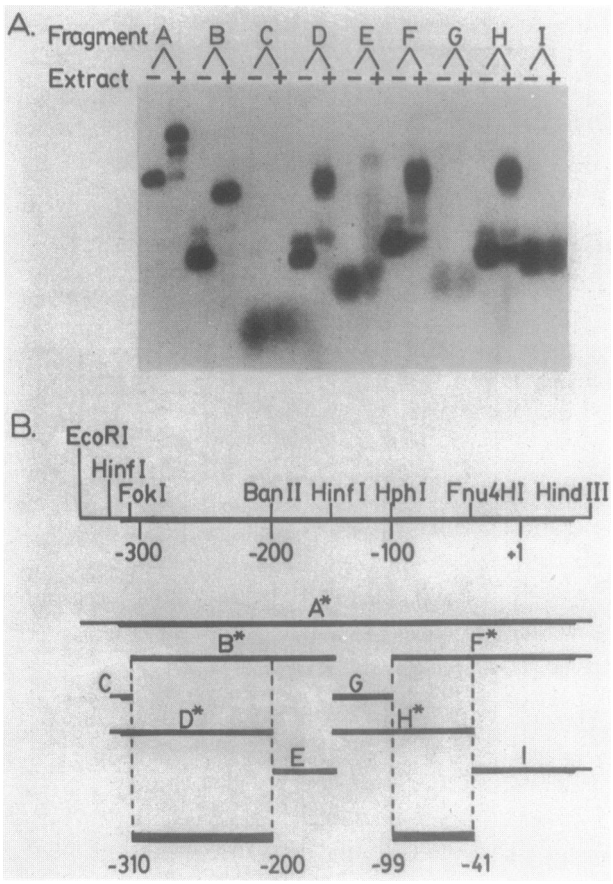

FIG. 6. Binding of IHB protein to subfragments derived from the promoter and regulatory region of *ilvIH*. (A) End-labeled fragments identified in panel B were incubated without (−) or with (+) partially purified IHB protein (50 ng, purified by Polymin P precipitation and Sephadex and phosphocellulose chromatography) and analyzed by the gel retardation assay. (B) The topmost line represents a map of fragment *Eco-Hind*$_{406}$, with the thick line corresponding to bacterial DNA and the thin line corresponding to polylinker in plasmid pCV112. +1, Position at which transcription begins; *, fragments that bind IHB protein, as shown in panel A. The thick bars at the bottom represent regions within which binding occurs, as deduced from the results of this experiment.

demonstrate that the IHB protein binds to at least two different regions, one located between −310 and −200 and the other between −99 and −41 relative to the point at which transcription begins (Fig. 6). A shifted band of weak intensity was also observed with fragment E, suggesting a weak binding site within the region −200 to −150. It may be noted that, as opposed to the full-length fragment, the subfragments give rise to only a single band of shifted mobility (for example, compare fragments A and D in Fig. 6). A simple possibility consistent with this finding is that for the full-length fragment, the faster moving complex contains IHB protein bound at one site and the slower moving complex has protein bound at two sites.

To further define sites at which the IHB protein binds, nuclease protection experiments (17) were performed. Figure 7 shows the results of treating fragment *Eco-Hind*$_{406}$, labeled separately at the *Eco* end or the *Hind* end, with lambda exonuclease (degrades in a 5′-to-3′ direction). From the sizes of fragments protected by the IHB protein, we deduce that at least one binding site lies between −205 and −260. At the concentration of IHB protein used, this was the only binding site observed. IHB protein protects the *Eco-Hind*$_{406}$ fragment from DNase I action (2, 5) in the region −190 to −260 (Fig. 8). Other regions of the fragment, including the region −60 to −80, were protected to some extent, but more highly purified preparations of binding protein will be required to define these regions.

Taken together, these results indicate that the IHB protein binds to fragment *Eco-Hind*$_{406}$ between positions −190 and −260. In addition, the binding experiments with shorter DNA fragments indicate that there is at least one other binding site, which is within the region −99 to −41. The fact that this latter site was not protected against lambda exonuclease suggests that the IHB protein binds to this site with lower affinity than it does to the more upstream site.

DISCUSSION

The experiments reported here were prompted by several considerations. First, optimal in vivo expression of the *ilvIH* operon of *E. coli* requires sequences several hundred base pairs upstream of the presumed site of transcription initiation (8). Second, the *ilvIH* promoter is extremely weak in in vitro transcription experiments with purified RNA polymerase (D. Aker, G. Haughn, and J. Calvo, unpublished data), suggesting that some factor may be necessary for transcription initiation. Furthermore, we were interested in the mechanism by which leucine represses transcription of the *ilvIH* operon. Since a transcription attenuation mechanism seemed unlikely (8), we suspected that leucine was interacting with some regulatory factor to control transcription. For

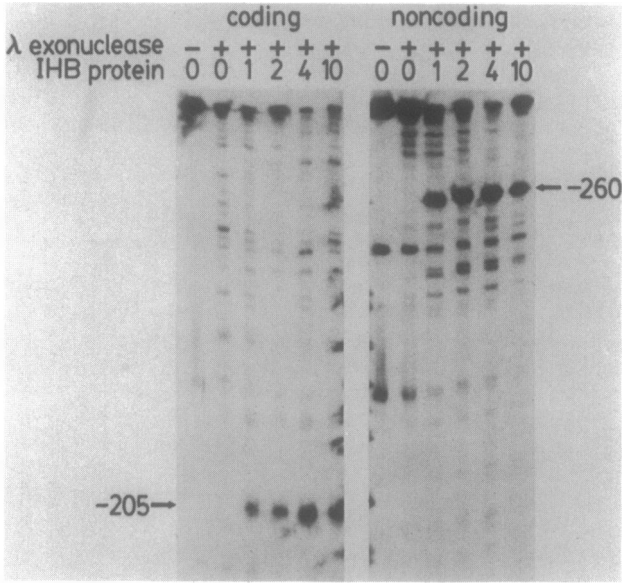

FIG. 7. Protection by IHB protein against lambda exonuclease. Fragment *Eco-Hind*$_{406}$ labeled with ^{32}P at the *Hind*III end (label on the noncoding strand) or at the *Eco*RI end (label on the coding strand, i.e., the strand of the operon that is transcribed) was incubated with partially purified IHB protein (2, 4, and 10 are amounts of protein relative to what was added in lane 3) and then further incubated without (−) or with (+) lambda exonuclease. Samples were analyzed by electrophoresis through an acrylamide-urea gel. Arrows identify the major fragments protected by the IHB protein. Note that for the noncoding strand (DNA alone), there are two extra bands in addition to the band representing the full-length fragment. We do not know the origin of these extra bands.

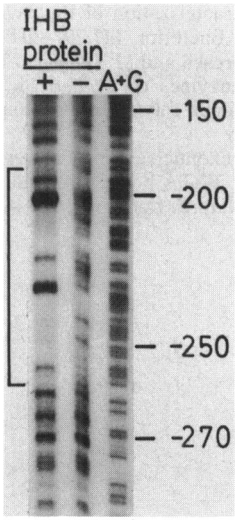

FIG. 8. Protection by IHB protein against DNase I. Fragment *Eco-Hph*I_{260} (Fig. 3B), labeled at the *Eco*RI end with ^{32}P, was incubated without (−) or with (+) partially purified IHB protein, treated with DNase I, and then fractionated by electrophoresis through an acrylamide-urea gel. A sample of labeled fragment *Eco-Hind*$_{406}$, subjected to the A+G sequencing reaction (10), served as size standards.

these three reasons, we searched extracts of *E. coli* for a protein that bound specifically to the *ilvIH* promoter and regulatory region.

Using a gel retardation assay, we identified a protein of molecular weight 43,000, called the IHB protein, that binds specifically to the *ilvIH* promoter and regulatory region. Renaturation experiments following SDS-PAGE indicate that the IHB protein is composed of two subunits of molecular weight about 21,000 each. The results of exonuclease and DNase I protection experiments identify one region of binding between −190 and −260. In vivo, more than 50% of the expression from the *ilvIH* promoter is lost as a consequence of a deletion that removes sequences upstream of position −207 (8). Other experiments suggest that the IHB protein (or another protein that exists in the same partially purified preparation) also binds to the region −99 to −41. A deletion extending to position −60 lowers expression from the *ilvIH* promoter by more than 80% (8). Thus, binding of the IHB protein may be important for normal expression of the *ilvIH* operon.

In strain LR16 containing the *lrs* mutation, *ilvIH* operon expression is not repressed by leucine (16). Partially purified IHB protein from strain LR16 has binding activity in the gel retardation assay, but the protein-DNA complex moves more rapidly than does the complex formed with protein from the parent strain. The results of recent experiments indicate that in strain LR16, the lack of leucine-mediated repression and the altered mobility of the IHB protein-DNA complex are caused either by a single mutation or by several closely-linked mutations (J. Platko and J. Calvo, unpublished data). In addition, binding of the IHB protein from strain LR16 is not affected by 20 mM leucine, whereas binding of the protein from the wild-type strain is reduced by more than 60% by 20 mM leucine. For cells grown in a medium containing 50 μg of leucine per ml, the intracellular leucine pool can approach 20 mM (11). Finally, we note that deletion of the upstream binding site results in an operon that is less sensitive to repression by leucine (E. Ricca and J. Calvo, unpublished data). Taken together, these results suggest that regulation of the *ilvIH* operon by leucine is mediated, at least in part, by the IHB protein.

How might leucine repress expression of the *ilvIH* operon? A mechanism involving leucine-dependent binding of a repressor (i.e., the IHB protein) to an operator site seems unlikely for the following reasons. First, deletion of the region containing the IHB binding sites lowers rather than raises expression of the operon. Second, leucine interferes with rather than promotes binding of the IHB protein to DNA upstream of the promoter. A simple model consistent with our results is that the IHB protein stimulates transcription from the *ilvIH* promoter upon binding to one or more sites upstream of the promoter and that leucine decreases transcription by interfering with binding. The *lrs* locus may code for the IHB protein or for some factor that modifies the IHB protein. Should this model prove correct, then the regulation of the *ilvIH* operon is different from that of other operons involved in branched-chain amino acid biosynthesis or indeed of other amino acid operons in general.

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LITERATURE CITED

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