Exogenous leucine affects the expression of a number of different operons in *Escherichia coli*. For at least some of these operons, the leucine-related effect is mediated by a protein called Lrp (Leucine-responsive regulatory protein). The purification of Lrp to near homogeneity is described. Lrp is a moderately abundant, basic protein composed of two subunits of molecular mass 18.8 kDa each. In addition, the corresponding protein was purified from a strain having a mutation within the gene that encodes Lrp (lrp). This mutation (lrp-1) causes high constitutive expression of ilvIH, one of the operons controlled by Lrp (Platko, J. V., Willis, D. A., and Calvo, J. M. (1990) *J. Bacteriol.* 172, 4563–4570). The Lrp-1 and Lrp proteins have similar physical properties, but they show some differences in the characteristics with which they bind DNA upstream of the ilvIH promoter. The nucleotide sequences of the *lrp* and *lrp-1* genes differ by only a single nucleotide, a C to G change that would substitute a Glu for an Asp at amino acid 114. Lrp has some amino acid sequence similarity to AsnC, a protein that regulates *asnA* expression (Kolling, R., and Lother, H. (1985) *J. Bacteriol.* 164, 310–315).

The *ilvIH* operon of *Escherichia coli* encodes acetohydroxy-acid synthase III, one of three isoenzymes catalyzing the first step common to the biosynthesis of isoleucine, leucine, and valine (1). Transcription of the *ilvIH* operon is repressed when cells are grown in the presence of leucine (2). This leucine effect is mediated by a positively-acting regulator of *ilvIH* transcription, termed Lrp (3) (referred to as IHB in Ref. 4). A Tn10 insertion within the *lrp* gene that abolishes Lrp activity reduces transcription of the *ilvIH* operon more than 30-fold (3). Lrp binds to at least two sites within the *ilvIH* promoter/regulatory region, at positions -40 to -100 and -190 to -260, relative to the startpoint of transcription (4) and mutations within these binding sites reduce expression of the *ilvIH* operon.1 In *vitro*, leucine reduces the extent of binding of Lrp to these binding sites (4). Taken together, these results indicate that Lrp stimulates transcription of *ilvIH* by binding to the promoter/regulatory region and that leucine represses expression of the *ilvIH* operon by preventing the binding of Lrp.

Ursini *et al.* (5) isolated a mutant in which *ilvIH* expression was not repressed by leucine (mutation designated *lrp-1*). Lrp from this strain binds in *vitro* to the *ilvIH* promoter/regulatory region, but in a leucine-independent manner (4). Furthermore, the nature of the resulting DNA-protein complex is different from the corresponding DNA-wild-type Lrp complex, as evidenced by its altered mobility in a gel retardation assay (4). The *lrp-1* mutation was recently shown to be an allele of the *lrp* gene and was renamed *lrp-1* to reflect that fact (3).

Leucine regulates the expression of a number of operons in *E. coli*, including *serA* (6), *iliJ/K* (7), *ilvIH* (2), *oppABCDF* (8), *sdA* (6), *tdh* (6), *lysU* (9), and an operon involved in serine transport (10). *serA* encodes phosphoglycerate dehydrogenase and *iliJ/K* (proteins involved in transport of branched-chain amino acids), as well as *ilvIH*, are repressed by leucine. A transposon insertion that inactivates *lrp* reduces expression of *serA* and *ilvIH* and the remaining expression is not affected by leucine (3, 6). By contrast, expression of the following operons is stimulated by leucine: *oppABCDF* (encodes proteins involved in oligopeptide transport) (8), *sdA* (encodes l-serine deaminase) (11, 12), *tdh* (encodes l-threonine dehydrogenase, which catalyzes the first step in a pathway of glycine synthesis from threonine) (13), and *lysU* (encodes a lysyl-tRNA synthetase) (9). Transposon insertions that inactivate *lrp* increase expression of these genes and expression is no longer affected by leucine (6, 43).1,2

For the *ilvIH* operon, it has been demonstrated that a plasmid containing *lrp* as the only bacterial DNA complemented transposon insertion mutations (3). Also, in the case of *ilvIH* and *iliJ/K*, it has been established that *lrp-1*, a single base pair change, affects the expression of the relevant operon (3).1 For these cases, at least, it seems likely that the observed phenotypes are due to effects upon *lrp*, rather than upon some other gene that is in the same operon whose expression was affected by polarity. In summary, the available evidence suggests that Lrp is a positive regulator of some operons (*serA* and *ilvIH*) and a negative regulator of others (*oppABCDF, sdA, tdh, and lysU*). Thus, Lrp can be viewed as a global regulatory protein mediating the action of leucine on these operons, and possibly others.

The work described here details the purification of Lrp from wild-type and *lrp-1* mutant strains and the characterization of the purified proteins. Lrp is a small, moderately abundant, basic protein composed of two subunits of molec-
ular mass 18.8 kDa each. The nucleotide sequences of Lrp and Lrp-1 were also determined and are consistent with the properties of the proteins.

MATERIALS AND METHODS

Strains and Growth Conditions—The following strains were used: CSH26, F' ara thi (lac-pro); CV975, F' ara thi (lac-pro) thi-2:His6::Mu d1734 (5); CV1154, F' ara thi (lac-pro) thi-2:His6::Mu d1734 lrp-1 (5); CV1008, F' ara thi (lac-pro) thi-2:His6::Mu d1734 lrp-1 (3); CV1009, F' ara thi (lac-pro) iioI2Hy:Mu d1734 lrp-1 (pCV168, lrp) (3); and CV1056, F' ara thi (lac-pro) Mu cts ze:n:10 lrp-l(pCV170). Plasmids pCV168 and pCV170 were described by Platko et al. (3). Strains were grown at 37 °C (except that CV1056, F' ara thi (lac-pro) Mu cts ze:n:10 lrp-l(pCV170) was grown at 30 °C) in a minimal medium containing M9 salts (14) and 0.2% glucose supplemented with 5 μg/ml thiamine, 50 μg/ml proline, 25 μg/ml isoleucine, 50 μg/ml valine, and micronutrients (15). LB was used as a rich medium (14).

Purification of Lrp—Cells (24 g wet weight) were harvested from strain CV1009 grown to late-log phase in 10 liters of M9 minimal medium containing 100 μg/ml ampicillin. All remaining steps were done at 4 °C, except that fractions were stored at -20 °C prior to analysis. Cells were resuspended in 30 ml of a solution containing 10 mM Tris-HCl, pH 8.0, 10% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 0.2 M NaCl. Cells were lysed by passage through a French pressure cell at 920 kg/cm², and cell debris was removed by centrifugation at 8,000 × g for 20 min. The resulting crude extract (37 ml) was passed over a 30-ml phosphocellulose column (Whatman P11) that had been equilibrated with TGloED with 0.2 M NaCl. After washing with 7.5 ml of the starting buffer, the column was eluted with 4 ml of a linear gradient of NaCl from 0.2 to 1.5 M. When analyzed by the gel retardation assay, fractions adjacent to the peak of activity caused DNA to be shifted to a diffuse band of low mobility. These fractions did not contain a 20-21.5-kDa species as seen by SDS-PAGE and were discarded. Appropriate fractions were pooled, desalted by dialysis against TGE6D with 0.2 M NaCl, and fractionated on a 7.5 ml heparin-agarose column (Sigma H6508) that had been equilibrated with TGE6D with 0.2 M NaCl. After washing with 21 ml of starting buffer, the column was eluted with 70 ml of TGE6D containing a linear gradient of NaCl from 0.2 to 1.5 M. When analyzed by the gel retardation assay, some fractions adjacent to the peak of activity caused DNA to be shifted to a diffuse band of low mobility. These fractions did not contain a 20-21.5-kDa species as seen by SDS-PAGE and were discarded. Appropriate fractions were pooled, desalted by dialysis against TGE6D with 0.2 M NaCl, and fractionated on a 7.5 ml heparin-agarose column (Sigma H6508) that had been equilibrated with TGE6D with 0.2 M NaCl. After washing with 7.5 ml of starting buffer, the column was eluted with 25 ml of TGE6D containing a linear gradient of NaCl from 0.2 to 1.0 M. Appropriate fractions were pooled and desalted against TGE6D with 0.1 M NaCl (Gdn-HCl increases to 50% glycerol)

Subcloning the lrp-1 Gene—A polymerase chain reaction (PCR) product containing only the coding region of the lrp-1 gene (no promoter) was prepared from chromosomal DNA of strain CV976 using primers N1 (5'GGAAA AGATTCAGAAGGACATAAS') and C2 (5'TGACCGGATCGTGGGTTAGG') (3). The resulting 552 base pair fragment was digested with restriction endonucleases EcoRI and BamHI, purified from an agarose gel, and ligated to EcoRI and BamHI-digested plasmid pHB retract II SK (Stratagene). Transformation of strain CV1008 with the ligation mixture (selection for resistance to ampicillin) yielded strain CV1040. The plasmid in CV1040, pCV183, carries the lrp-1 gene downstream of a T7 RNA polymerase promoter.

Purification of Lrp—CV1040 cells were grown to an Abs of 1 in 10 liters of LB medium supplemented with 50 μg/ml of ampicillin. MgSO₄ was added to a final concentration of 10 mM and 2 × 10⁷ pfu of λ-phage C6 (carries the gene for T7 RNA polymerase) (16) were added and growth continued for 2.5 h. 50 g (wet weight) of cells were harvested. Purification and assay procedures were essentially the same as for the Lrp purification, except that volumes were scaled up in proportion to the amount of protein present.

Quantitation of DNA-Binding Activity— Gel retardation assays were performed as described previously (4). The extent of Lrp binding to [3H]labeled phage DNA was estimated after assaying a dilution of Lrp that would give 25-75% conversion of the DNA to the major fast mobility DNA-protein complex. Quantitation was carried out with a Betascope model 603 Biot Analyzer (Betagen Corporation). The extent of binding was linear with the amount of Lrp under these conditions. One unit of DNA-binding activity is the amount of Lrp that causes conversion of 50% of the DNA to this complex. Protein concentration was determined by the Bradford method (17) using the Coomassie Protein Assay Reagent (Pierce Chemical Co.). For the protein assay, Lrp samples were diluted to a final buffer concentration of one-fifth that of TGE6D with 0.2 M NaCl. Lysozyme diluted in the same buffer was used as the standard.

Gel Filtration Chromatography—A 0.2 ml sample containing 350 μg of TGE6D with 0.1 M NaCl was applied to a 0.7 × 24 cm column of Sephadex G-100, Superfine grade (Pharmacia LKB Bio- technology Inc.). The column was equilibrated and eluted with TGE6D containing 0.1 M NaCl (same as TGE6D with 0.1 M NaCl except EDTA was omitted and 10 mM sodium phosphate buffer, pH 8.0, was substituted for Tris-HCl). The latter buffer was chosen for its transparency, as column fractions were monitored for protein by their absorbance at 215 nm in a quartz microcuvette. The flow rate was 1.5 ml/h. Fractions of 0.22 ml were collected and assayed for protein and DNA-binding activity.

Electrophoresis Methods—SDS-PAGE was performed as described previously (4). For isoelectric focusing, Servalyt Precotes, pH range 3-10 (Serva Biochemicals) were employed. Gels were prefocused with the voltage increasing from 200 to 350 V over a 30-min period. Samples of Lrp, Lrp-1, and standards were loaded separately toward the cathode side of the gel. Electrophoresis was carried out at 4 watts for 1 hour and 200 volts for 2 hours. When the power had dropped to 1.5 watts, gels were fixed in 20% trichloroacetic acid and stained with Coomassie Brilliant Blue.

Amino Acid Composition—Samples of pure Lrp and Lrp-1 (about 2 μg) were spotted on Immobilon-P Transfer Membrane (Millipore). Hydrolysis of the protein and analysis of the resulting amino acids was performed by the Synthetic and Analytic Laboratory of the Cornell University Biotechnology Program using the Pico-Tag Amino Acid Analysis System (Waters) (18).

Antibody Methods—Rabbit antibodies were raised by Cocalico Biologicals, Inc. (Reamstown, PA). A rabbit was inoculated with 200 μg of Lrp, and was boosted with 275 μg of Lrp on day 14 days and again 21 days after the initial injection. Serum obtained 14 days after the final injection was preabsorbed by incubating 0.12 ml of serum with 0.3 ml of crude extract from strain CV1008 and 3.6 ml of Tris-buffered saline (10 mM Tris-HCl, pH 8.0, 100 μM NaCl) for 1 h at 37 °C and removing the debris by centrifugation.

For Western blotting experiments, proteins fractionated by SDS-PAGE were electrophoretically transferred to Immobilon P Transfer Membrane (Millipore). The membrane was incubated for 0.5 h with a blocking solution containing 0.05% Tween 20 and 1% bovine serum albumin in Tris-buffered saline and for 0.5 h with a solution containing 0.05% Tween 20, 1% bovine serum albumin, and 0.1% sodium dodecyl sulfate. The membrane was washed three times, 5 min each, with 200 ml of Tris-buffered saline containing 0.05% Tween 20 (TBST) and incubated with a 1:7500 dilution of goat-anti-rabbit antibodies conjugated to alkaline phosphatase. After washing with TBST as above, bound antibody was visualized upon incubation with substrates nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Protoblot kit from Promega).

Measurement of Abundance of Lrp in E. coli—Strains CSH26 and CV1009 were grown in minimal medium at 37 °C to an OD₆₀₀ of 0.9 and 0.6, respectively. The number of cells/ml was estimated by measuring cell turbidity (standard curve prepared by measuring OD₆₀₀ of dilutions of a culture whose cell number was determined by plate counts). Cells were harvested by centrifugation and lysed by passage through a French pressure cell. Dilutions of the resulting cell extracts were analyzed by Western blotting next to dilutions of pure Lrp. Dilutions of cell extracts and pure Lrp were made in TGE6D containing 0.2 M NaCl and 1.7 ng/ml of protein from a crude extract of strain CV1008. In the absence of carrier protein in the diluent, the signal was markedly reduced. The concentration of solutions of pure Lrp was determined by the Bradford (17) and Lowry (19) assays using both bovine serum albumin and lysosome as standards. In addition, the concentration of Lrp was estimated by absorbance at 278 nm, assuming a molar extinction coefficient of 1340 for tyrosine (20) and δ tyrosines and 0 tryptophans/monomer of Lrp (predicted from nucleotide sequence; confirmed by amino acid composition measurement). We used in all calculations the protein content determined by spectrophotometry; this value (1.4 mg/ml) did not differ appreciably from those obtained by the Lowry (1.0 mg/ml) or
RESULTS

Purification of Lrp and Lrp-1—Purified Lrp was prepared from strain CV1009 which has a Tn10 insertion within the chromosomal lrp gene and a wild type lrp gene on a multicopy plasmid (3). This strain produces 20-25-fold more Lrp than does a strain having lrp in single copy. The progress of purification was followed using SDS-PAGE and a gel retardation assay which measures binding of Lrp to DNA upstream of the iluZH promoter (4). Fig. 1A shows that fractionation through phosphocellulose, heparin-agarose, and DNA-cellulose yielded protein of greater than 98% purity. The yield of pure protein was about 2.2 mg from 24 g of cells (wet weight). Table I summarizes data from one such purification of Lrp. Lrp was also purified from a strain carrying lrp-1, a mutation that prevents repression of the iluIH operon by leucine (3). Lrp isolated from such strains (hereafter referred to as Lrp-1) binds to the iluIH promoter/regulatory region in vitro but the pattern of binding and the effect of leucine upon binding are different than for wild-type Lrp (4). Lrp-1 was isolated from strain CV1040 containing a Tn10 insertion in the chromosomal lrp gene and the lrp-1 gene cloned behind the T7 promoter on a multicopy plasmid. Transcription from the T7 promoter was induced after infection with a X-phage derivative carrying the T7 polymerase gene (16). Phage-injected cells overproduced Lrp-1 by a factor of about 100-fold as measured by a gel retardation assay, pure Lrp behaves like Lrp in crude extracts in these two respects (Fig. 2). Lrp-1 in crude extracts also binds to the iluIH promoter/regulatory region, but the mobility of the major DNA-protein complex formed is faster than that formed by Lrp and goat-anti-rabbit antibodies conjugated to alkaline phosphatase. As shown in Fig. 1C, Lrp and Lrp-1 are the major proteins in crude extracts detected with anti-Lrp antibody and they are the same size as the purified proteins.

In vitro, Lrp in crude cell extracts binds specifically to DNA containing the iluIH promoter/regulatory region, and leucine reduces the extent of binding (4). As measured by a gel retardation assay, pure Lrp behaves like Lrp in crude extracts in these two respects (Fig. 2). Lrp-1 in crude extracts also binds to the iluIH promoter/regulatory region, but the mobility of the major DNA-protein complex formed is faster than that formed by Lrp (4). Furthermore, the binding of Lrp-1 in crude extracts is not affected by leucine (4). As shown in Fig. 2, these characteristics are the same for pure Lrp-1 as for Lrp-1 in crude extracts.

Molecular Weight of Native Lrp—Purified Lrp was analyzed by Sephadex G-100 gel filtration chromatography. The DNA-binding activity of Lrp (closed squares) coeluted with the protein detected at 215 nm (closed circles) (Fig. 3). The molecular mass of native Lrp, estimated from a plot of log

FIG. 1. SDS-PAGE and Western blot analysis of Lrp and Lrp-1. Panels A and B, samples from purification steps of Lrp (A) and Lrp-1 (B) were fractionated on a 15% SDS-PAGE gel and stained with CBB Brilliant Blue. CE, crude extract (10 μg of protein); PC, phosphocellulose (10 μg); HA, heparin-agarose (10 μg); DC, DNA-cellulose (about 1 μg in the first lane and 10 μg in the second). The last lane in B contained 1.4 μg of purified Lrp. C, Western blot. Crude extract (CE) from a single copy lrp+ strain CSH26 (12.5 μg of protein) and single copy lrp-1 strain CV1006 (2 μg of protein), and purified Lrp (55 ng) and Lrp-1 (37 ng) were fractionated by SDS-PAGE as above, blotted, and incubated with antiserum raised against Lrp. Bound antibody was visualized by an alkaline phosphatase-based staining system. Dye-conjugated size standards used in all three panels were, respectively, phosphorylase b (92.5 kDa), bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and lysozyme (14.3 kDa) (Amersham Corp.).

CV1056, which carries a single copy of lrp-1. After fractionation of the crude extracts and pure proteins on SDS-polyacrylamide gels, protein was transferred to a membrane and antibody-reactive polypeptides were visualized after successive treatment with rabbit antibodies raised against Lrp and goat-anti-rabbit antibodies conjugated to alkaline phosphatase. As shown in Fig. 1C, Lrp and Lrp-1 are the major proteins in crude extracts detected with anti-Lrp antibody and they are the same size as the purified proteins.
**TABLE I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>10^3 × total activity (units)</th>
<th>Yield (%)</th>
<th>Specific activity (units/mg)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>37.0</td>
<td>1140.0</td>
<td>16,400</td>
<td>100</td>
<td>14,000</td>
<td>1</td>
</tr>
<tr>
<td>Phosphocellulose</td>
<td>16.0</td>
<td>19.2</td>
<td>6,800</td>
<td>42</td>
<td>359,000</td>
<td>26</td>
</tr>
<tr>
<td>Heparin-agarose</td>
<td>4.2</td>
<td>9.2</td>
<td>3,400</td>
<td>21</td>
<td>369,000</td>
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</tr>
<tr>
<td>DNA-cellulose</td>
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<td>2.2</td>
<td>1,000</td>
<td>6</td>
<td>454,000</td>
<td>32</td>
</tr>
</tbody>
</table>

* One unit of Lrp activity is the amount of Lrp that converts 50% of the labeled DNA fragment (IlelH promoter/regulatory region) to the major fast mobility DNA-protein complex under gel retardation assay conditions where the assay is linear.

**Fig. 2.** DNA-binding activity of purified Lrp and Lrp-1. A gel retardation assay was used to analyze binding to a 406-base pair DNA fragment containing the IlelH promoter/regulatory region. Binding reactions contained pure Lrp (3 ng), Lrp-1 (60 ng), or crude extract (CE) made as in Platko et al. (3) from strain CV975 (single copy lrp*, 2 µl 1:4 dilution) or strain CV976 (single copy lrp-1, 2 µl 1:3 dilution). Samples were fractionated by electrophoresis through 1.5% agarose (4). Leucine, when present, was added to the binding reaction at a final concentration of 30 mM.

**Fig. 3.** Sephadex G-100 gel filtration chromatography. Eluted Lrp was monitored by absorbance at 215 nm (closed circles, solid line) and DNA-binding activity (closed squares, solid line). A mixture of size standards (Sigma) in 0.2 ml was analyzed in a separate experiment on the same column under the same conditions (open circles, dotted line): 55 µg of alcohol dehydrogenase (150 kDa), 55 µg of bovine serum albumin (66 kDa), 29 µg of carboxylic anhydrase (29 kDa), 29 µg of cytochrome c (12.4 kDa). An analysis (not shown) of pure Lrp together with the 150- and 124-kDa size standards allowed alignment of the Lrp elution curve relative to that of the size standards.

**Fig. 4.** Isoelectric focusing of Lrp and Lrp-1. Purified Lrp and Lrp-1 (4.2 µg each) were loaded at a position corresponding to pH 7.5. Markers (M) were from Pharmacia, High pi Calibration Kit (9.3, 8.65, 8.45, 8.15, 7.35).

**Fig. 5.** Nucleotide sequence of the coding region of lrp. The sequence of lrp was submitted to Genbank and assigned accession number M35869. The sole difference between lrp and lrp-1 is the C to G change at position 342. Boxes designate the translation start and stop signals.

**Fig. 6.** Nucleotide sequence of the coding region of lrp. The sequence of lrp was submitted to Genbank and assigned accession number M35869. The sole difference between lrp and lrp-1 is the C to G change at position 342. Boxes designate the translation start and stop signals.

**Fig. 7.** Nucleotide sequence of the coding region of lrp. The sequence of lrp was submitted to Genbank and assigned accession number M35869. The sole difference between lrp and lrp-1 is the C to G change at position 342. Boxes designate the translation start and stop signals.

**Fig. 8.** Nucleotide sequence of the coding region of lrp. The sequence of lrp was submitted to Genbank and assigned accession number M35869. The sole difference between lrp and lrp-1 is the C to G change at position 342. Boxes designate the translation start and stop signals.

**Assign but are in the range of 9.2–9.4 for Lrp and 9.0–9.2 for Lrp-1. In any single experiment, Lrp-1 migrated with a pI of 0.2 units less than that of Lrp.**

**Nucleotide Sequence of lrp and lrp-1—**We previously reported cloning the lrp gene and localizing the gene to a 1.2 kilobase DNA fragment (3). The nucleotide sequence of lrp was determined by Austin et al. (43). We confirmed the nucleotide sequence of lrp using the dyeoxy procedure of Sanger (22), employing DNA from plasmid pCV180 as template (Fig. 5). The predicted amino acid sequence at the N terminus matches the experimentally determined sequence of 38 N-terminal amino acids found previously (3) (Fig. 8). A polypeptide of size 18.8 kDa and pl 9.24 is predicted from the sequence, which is in good agreement with the experimentally determined values.

The lrp-1 gene was cloned using chromosomal DNA from strain CV976 as template in a polymerase chain reaction. The oligonucleotides used as primers corresponded in sequence to the ends of the lrp gene. The PCR product was cloned into a Bluescript vector yielding plasmid pCV183 and the nucleotide sequence of lrp-1 was determined by the same procedure as for lrp, lrp and lrp-1 differ by a C to G change at position 342 (Fig. 5), which would change the Asp residue encoded there to Glu.

**Amino Acid Composition of Lrp and Lrp-1—**Samples of Lrp
and Lrp-1 were subjected to acid hydrolysis and their amino acid compositions determined. The correspondence between experimentally determined values and those predicted from the nucleotide sequence is good.

**Ultraviolet Absorption Spectrum of Purified Lrp**—The ultraviolet absorbance spectrum of purified Lrp is shown in Fig. 6. A typical peak for protein with a maximum absorbance around 280 was observed. The A_{278}/A_{260} ratio is 1.63. The peak at 278 nm is presumably due to the 5 tyrosines/mol of Lrp monomer, since Lrp is not predicted from the nucleotide sequence to contain tryptophan. There is no evidence for a bound nucleotide (the extinction coefficient for a nucleotide monomer, since Lrp is not predicted from the nucleotide sequence to contain tryptophan. There is no evidence for a bound nucleotide (the extinction coefficient for a nucleotide monomer, since Lrp is not predicted from the nucleotide sequence to contain tryptophan. There is no evidence for a bound nucleotide (the extinction coefficient for a nucleotide monomer, since Lrp is not predicted from the nucleotide sequence to contain tryptophan.

**Abundance of Lrp in E. coli**—The abundance of Lrp in crude extracts of E. coli strain CSH26 (single-copy lrp+ strain) was estimated by Western blot analysis, using as standards samples of pure Lrp analyzed on the same blot (Fig. 7). By this procedure, Lrp is about 0.1% of the total protein in strain CSH26 grown in minimal medium at 37 °C. Assuming a molecular mass of 38 kDa (the predicted size of the dimer by nucleotide sequence), E. coli contains about 3,000 Lrp dimers/cell. Using the same method, Lrp was estimated to be 2.5% of the total protein of overproducing strain CV1009 (75,000 dimers/cell) (data not shown).

Knowing the abundance of Lrp, we estimated the yield of Lrp after purification. 24 g wet weight of CV1009 cells should contain 60 mg of Lrp, assuming 1 g wet weight contains 0.1 g protein and an Lrp abundance of 2.5%. The yield of pure Lrp, 2.2 mg, was calculated to be 4% of the starting amount. This agrees well with the yield of Lrp determined from DNA-binding activity measurements (Table 1).

**DISCUSSION**

The nucleotide sequence of lrp is consistent with the known properties of Lrp. This comparison includes the size of the Lrp polypeptide (20–21.5 kDa by SDS-PAGE) versus 18.8 kDa predicted from the nucleic acid sequence), amino acid composition, and pl values (9.2–9.4 by isoelectric focusing versus 9.24 by calculation from the nucleotide sequence). Moreover, the sequence of 38 amino acids at the N terminus of Lrp (3) agrees perfectly with that predicted from the nucleotide sequence (Fig. 8). These results suggest that there are no errors in the nucleotide sequence that affect the reading frame because such errors would likely predict a polypeptide with properties different than those found. In addition, the nucleotide sequence reported here is the same as that reported by Austin et al. (43) for opp1, a gene that regulates the oppABCDEF operon.

In strains carrying the lrp-1 mutation, the iluIH operon is not repressed when leucine is added to the growth medium (5). The pattern of Lrp-1 binding to the iluIH promoter/regulatory region as visualized by a gel retardation assay is different than for wild-type Lrp (Fig. 2) (4). In addition, the site-specific binding of Lrp-1 is not reduced by the presence of leucine, as is the case for the wild-type protein (Fig. 2) (4). We show here that purified Lrp-1 is very similar to the wild-type protein in terms of subunit size (Fig. 1B) and amino acid composition. The pl values of the two proteins differ slightly, Lrp-1 being slightly less basic than Lrp (Fig. 4). Nucleotide sequencing shows that the difference between lrp and lrp-1 is a C to G change at position 342 (Fig. 5) which makes an Asp...
to Glu change at amino acid position 114.

A search of the NBRF and Swiss-Protein databases identified the asnC gene product (AsnC) as a protein that was possibly related to Lrp. asnC encodes a regulatory protein that regulates the expression positively (asnA codes for asparagine synthetase A, which synthesizes asparagine from aspartate and ammonia) and its own synthesis (25, 26). The amino acid sequences of Lrp and AsnC, shown in Fig. 6A, have 25% identities (three gaps were required for the best alignment). To determine the significance of the comparison, alignments were made between the Lrp polypeptide and 15 random amino acid sequences having the same length and composition as the AsnC polypeptide (27). Comparisons of Lrp to the random sequences gave an average of 16.7 ± 2.5% identity. The value for the AsnC/Lrp comparison, 25%, is more than 3 standard deviations above the average value. When the same analysis was performed using a quality score (includes comparisons of similar amino acids) instead of amino acid identities, then the AsnC/Lrp comparison was more than 12 standard deviations from the average. A comparison of Lrp and AsnC by the dot matrix procedure of Maizel and Lenk (24) shows that, with the exception of one small region near the center, the two polypeptides are related along their entire length (Fig. 8B). Taken together, these analyses strongly suggest that Lrp and AsnC are evolutionarily related.

The parallel between AsnC and Lrp extends beyond amino acid sequence similarity. Lrp stimulates expression from the ilvH promoter and that stimulation is reduced by leucine (3). Similarly, AsnC stimulates expression from the asnA promoter and that stimulation is reduced by asparagine (25, 26). Thus, for both cases, the regulatory protein stimulates operon expression and a pathway-related end product prevents this stimulatory action. In addition to acting positively, both Lrp and AsnC act negatively on other operons (Lrp represses oppA/B/C/D, tdh, and sda; AsnC represses asnC). Kolling and Lother (26) noted three 10-base pair sequences upstream of asnA (consensus TT(A/T)TT(T/G)(A/C)ATG) that could be binding sites for the AsnC protein, two that might account for activation of asnC, and one that might account for repression of asnC. Upstream of the ilvH promoter, in the region in which Lrp is known to bind (4), are two sequences that resemble these putative AsnC-binding sites (TTATGGATG and ATTCTGAATG centered at -234 and -217, respectively). It is conceivable that whereas the two proteins share only 25% sequence identity, that they have retained similar binding site specificities.

Kolling and Lother (26) pointed out that the N-terminal region of the AsnC polypeptide shares weak amino acid sequence similarity to regions of the Cro, cl, Crp, GaiR, and TrpR proteins. AsnC has a region centered at amino acid 35 that is similar to regions of other proteins known to have helix-turn-helix motifs (28). The corresponding region of Lrp, centered at amino acid 40, shows modest similarity to a set of 10 known helix-turn-helix sequences when analyzed by the procedure of Brennan and Matthews (29) (score for 10 known helix-turn-helix sequences, 0.69 ± 0.03; AsnC, 0.77; Lrp, 0.84; 40 random sequences, 0.95 ± 0.03).

E. coli genes that are expressed at a high level have a characteristic pattern of codon usage (30). The GCGG Codon Preference program was used to calculate "average codon preference," a parameter that compares the codon usage for a particular gene to the general pattern of codon usage for E. coli highly expressed genes. For the incorrect reading frames for genes rplA, rplK, rpsL (genes encoding ribosomal subunits), and crp (encodes cAMP receptor protein), average codon preferences were in the range 0.5 (0.48 ± 0.05). By contrast, the average codon preference values for the correct reading frames for these genes were 1.83, 1.49, 1.76, and 1.18, respectively. The corresponding value for Lrp, 0.75, suggests that Lrp is not an extremely abundant protein, but that it may be a moderately abundant protein. We used antibodies raised against Lrp to directly estimate the abundance of Lrp in E. coli. Lrp is about 0.1% of the total protein in a crude extract of wild-type E. coli, which corresponds to about 3,000 dimers/cell assuming a molecular mass of 38 kDa.

Another estimate of abundance can be made by analyzing E. coli proteins separated on two-dimensional gels (31). Purified Lrp migrated to position 48 × 33 on nonequilibrium two-dimensional gels and there was no spot at that position when strain CV1008, which lacks Lrp, was analyzed (analysis kindly performed by M. E. Hutton). There is a prominent, well-resolved spot in just that position for crude extracts prepared from 35S-labeled wild-type cells and analyzed on two-dimensional gels (31). We assumed that this latter spot is Lrp and that no other E. coli proteins migrate to position 48 × 33. Lrp was compared to other small, basic proteins having spots of comparable intensity. Crp (cAMP receptor protein) and Lrp, which have about the same number of sulfur-containing amino acids (9 versus 4/monomer, respectively) (32), have spots of similar intensity. The abundance of Crp in E. coli grown in a glucose-minimal medium is about 0.13% (33). Numerous ribosomal proteins having spots two to four times more intense than that of Lrp migrate to positions near 48 × 33, including L1 (rplA, 7 Cys + Met) (34), L11 (rplK, 6 Cys + Met) (34), and S9 (rpsL, 3 Cys + Met) (35). Ribosomal proteins as a group amount to 11% of the total protein in cells grown in a glucose-containing minimal medium, so that an average ribosomal protein amounts to about 0.21% of the total protein (36). The results of this analysis suggest that Lrp amounts to more than 0.1% and less than 0.2% of the total protein of E. coli.

The similarities and differences between Crp and Lrp are worth exploring further. They are both small, basic, relatively abundant proteins that form homodimers in solution (37). They both activate expression of some operons and inhibit expression of others (38). An important characteristic of Crp, which is shared by a number of other E. coli activator proteins, is that binding to specific sites on DNA requires a conformational change induced by a small molecule (cAMP in the case of Crp) (39). Whether the action of Lrp as an activator also requires a small molecule such as cAMP remains to be determined. In in vitro experiments employing purified RNA polymerase, Lrp stimulated transcription from the ilvH promoter about 15-fold in vivo, but only a few fold in vitro. If the activation function of Lrp in vivo requires a small molecule, then that small molecule is normally present in E. coli growing in a minimal medium because the ilvH promoter is maximally active under such conditions (40). What may be unusual about Lrp in comparison to other activator proteins is that the only known effector, leucine, reduces rather than increases the extent of activation.

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