Point Mutations in the Regulatory Region of the ilvGMEDA Operon of Escherichia coli K-12

ROBERT P. LAWThER

Department of Biology, University of South Carolina, Columbia, South Carolina 29208

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The ilvGMEDA operon of Escherichia coli K-12 is preceded by a regulatory region containing a promoter, a leader, and an attenuator. This region has been extensively characterized biochemically. In this note point mutations of the regulatory region are reported. The effect of these mutations on expression from the ilv regulatory region supports the previous biochemical analysis.

The genes for biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine are divided into several transcriptional units (26). The largest is the ilvGMEDA operon, which is multivalently regulated by all three amino acids (26). Limitation of the growth of either Escherichia coli K-12 or Salmonella typhimurium by deficiencies in any of the three amino acids results in derepression of the operon (6). Alternatively, the addition of all three amino acids to minimal medium results in repression of the operon (26). Eidlic and Neidhardt (5) made the first observation that ilv gene regulation is tRNA mediated by using an E. coli strain that contained a temperature-sensitive valyl-tRNA synthetase. Subsequent studies of mutant strains of S. typhimurium (4) and E. coli K-12 (13) with altered tRNA modification supported this conclusion. DNA sequence analysis suggested that the ilvGMEDA operon is preceded by a leader-attenuator. In both E. coli K-12 (14, 20) and S. typhimurium (25), the leader region encodes a putative 32-amino-acid polypeptide that contains 15 branched-chain amino acid residues (Fig. 1).

The isolation and characterization of mutant or variant strains assisted in elucidating the mechanisms of the regulation of gene expression. Such mutants provided much insight into the regulation by attenuation of the trp (24, 28), his (10), thr (16, 17), and leu (3) operons. Investigation of the regulation of the ilvGMEDA operon has been limited by the absence of mutations that alter the leader-attenuator. Comparison of the regulatory region of S. typhimurium with that of E. coli K-12 (25) indicated only seven nucleotide (nt) differences over more than 200 base pairs (bp). None of the observed nucleotide differences would be expected to affect leader-attenuator function. Harms et al. (7) also determined the DNA sequences of the regulatory regions of the ilvGMEDA operons of E. coli B, Klebsiella aerogenes, Edwardsiella tarda, and Serratia marcescens. The nucleotide sequences for E. coli B and K. aerogenes were found to be similar to those reported for E. coli K-12 and S. typhimurium; i.e., no nucleotide changes that should alter either the secondary structure of the leader RNA or the amino acid composition of the leader peptide were observed. The nucleotide sequences determined for both Edwardsiella tarda and Serratia marcescens were found to differ substantially from those for E. coli K-12. As a result, the RNA secondary structure should change and the deduced amino acid sequence of the leader peptide should also vary. Thus, regulation of the attenuation of the ilvGMEDA operon by leucine for Edwardsiella tarda and Serratia marcescens requires response to a single leucine codon (9). By altering the leucine codon with oligonucleotide site-directed mutagenesis, Harms and Umbarger (8) demonstrated that the single leucine codon could account for leucine-specific regulation of the ilvGMEDA operon. Bennett and Umbarger (1) isolated a pair of deletions that extended through the ilv attenuator of E. coli K-12 by using a lambda bacteriophage containing an ilvD-lacZ fusion. They observed a 30-fold derepression of β-galactosidase relative to levels in the parental bacteriophage. Because of the size of the deletions, the observed extent of derepression must in part reflect deletion of the nonsense mutation (at bp 1,252) in the wild-type allele of ilvG, which results in reduced expression of the distal portion of the operon (12).

This note reports the first isolation of organisms carrying the point mutations that alter gene expression from the ilvGMEDA regulatory region of E. coli K-12. These mutants were isolated on the basis of observations of E. coli K-12 M152 (galK2 recA3 epsL200 1N[rrnD-rrnE]) transformed with plasmid pRL137. This plasmid, constructed by using BamHI and HindIII linkers to insert a 700-bp HarHI-AluI restriction fragment into the galK expression vector pKO6 (21), extends from 50 to 350 bp beyond the sequence presented in Fig. 1 (i.e., to bp 645). The plasmid is constructed so that expression of galactokinase is dependent on the ilv promoter, leader, and attenuator. Transformants of strain M152 with pRL137 yielded white colonies on galactose McConkey agar (Table 1) and showed growth after 24 h on M63 minimal agar (19) with galactose as the sole carbon source (Table 1). Therefore, organisms with mutations in the ilv regulatory region that decreased galactokinase formation would fail to grow on minimal galactose, while organisms with mutations that increased expression from the ilv region would yield red colonies on galactose McConkey agar.

Our initial mutagenesis of plasmid pRL137 was done by the hydroxylamine hydrochloride procedure described by Busby et al. (2). pRL137 (50 μg) was treated with 1 M hydroxylamine hydrochloride for 2 h at 75°C. Plasmid DNA was recovered and digested with the restriction endonucleases BamHI and HindIII. The 700-bp ilv insert was purified by polyacrylamide gel electrophoresis as described by Maxam and Gilbert (18). The ilv fragment was inserted into unmutagenized parental vector pKO6 and then transformed into strain M152. Transformants were selected on galactose McConkey agar containing ampicillin (100 μg/ml) and tested for growth on minimal galactose agar.

When this procedure is used, colonies produced from the initial transformation should represent unique events because, after mutagenesis, the hydroxylamine-treated DNA is
not replicated until it is inserted into unmutagenized pKO6 and transformed into M152. Selection and screening of several hundred transformants yielded approximately 60 colonies with the selected phenotypes. The plasmids from these colonies were isolated by miniscreen or rapid-screen isolation, and the plasmid structure was analyzed by restriction endonuclease digestion. After repeated retransformation and resoliation (a total of three times), 25 plasmids which yielded transformants with either of the two phenotypes described were isolated. Plasmid DNA was prepared, and the DNA sequence (18) of the ilv promoter-leader-attenuator region was determined. Subsequently, the remainder of the ilv insert was sequenced for the plasmids found to contain mutations within the regulatory region. This stringent analysis yielded seven plasmids belonging to either of two classes. The small number (both in type and quantity) of mutants recovered probably reflect several factors: (i) mutagenesis with hydroxylamine yields only G-C-to-A-T transitions, (ii) ilvGp2 is already a weak or poor promoter, and (iii) the selection and screening criteria used by us were highly stringent.

All the plasmids uniquely altered in the ilv regulatory region belonged to two classes, represented by the mutations ilvGp2950 and ilvGp951. The first group (four isolates) yielded white colonies on galactose McConkey agar and failed to grow on minimal galactose agar (Table 1, PRL201). Galactokinase expression was assayed as described previously (21). These mutant plasmids yielded an approximately 10-fold-lower expression of galactokinase (0.69 nmol/mg of protein per fmol of PRL201 versus 8.0 nmol/mg of protein per fmol of PRL137). DNA sequence determination indicated that there is a G-to-A transition at −15 bp (Fig. 1) relative to the transcription initiation site (defined by analysis both in vivo and in vitro; 15). This mutation is consistent with similar mutations previously identified as down mutations in the promoters of bacteriophage lambda (23) and of the arg operon (22). As such, they confirm that the sequence TAACTCT (−13 to −7) corresponds to the −10 region of other bacterial promoters (14).

The second group of mutants (three isolates) yielded red colonies on galactose McConkey agar and grew on minimal

### TABLE 1. Characteristics of ilv wild-type and mutant plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Mutation</th>
<th>Galactose McConkey agar&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Minimal galactose agar&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Galactokinase&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKO6</td>
<td>Parental vector</td>
<td>W</td>
<td>−</td>
<td>1.0</td>
</tr>
<tr>
<td>pRL137</td>
<td>Wild type</td>
<td>W</td>
<td>+</td>
<td>8.0</td>
</tr>
<tr>
<td>pRL201</td>
<td>ilvGp2950 (bp −15, G→A)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>W</td>
<td>+</td>
<td>0.69</td>
</tr>
<tr>
<td>pDM287</td>
<td>ilvGp951 (bp 177, G→A)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>R</td>
<td>+</td>
<td>24</td>
</tr>
<tr>
<td>pMO167</td>
<td>ΔilvGa</td>
<td>R</td>
<td>+</td>
<td>25</td>
</tr>
<tr>
<td>pMW333</td>
<td>ilvGp952 (bp 34, T→C)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>W</td>
<td>−</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Galactokinase assays utilized extracts of M152 transformed with the indicated plasmid. Each variant was grown on M63 glucose medium and assayed as described elsewhere (21). Galactokinase units are nanomoles of galactose-1-phosphate formed per minute per milligram of protein per femtomole of plasmid.

<sup>b</sup> W, White colony at 15 to 18 h; R, red colony.

<sup>c</sup> Growth (+) after or lack of growth (−) after 24 h on M63 (19) with galactose as the sole carbon source.

<sup>1</sup> Nucleotide change indicated in Fig. 1.
galactose agar. This phenotype is represented by pDM287 (Table 1), in which there has been a G-to-A transition at bp177 (Fig. 1, ilvGa951). The change of this base pair would reduce the stability of the RNA stem-loop structure formed from bp 151 to 180, which is required for transcription termination (15). Calculation of the free energy of formation of the RNA stem-loop structure for the wild-type ilv operon yields a value of \(-14.0\) kcal (ca. \(-5.9 \times 10^{-4}\)) J, while that for the ilvGa951 mutation is \(-7.4\) kcal (ca. \(-3.1 \times 10^{-4}\)).

Consistent with this mutation resulting in reduced transcription termination at the attenuator is the finding that the level of galactokinase observed for pDM287 (24 U; Table 1) is similar to that observed for plasmid pMO167 (25 U; Table 1), in which the attenuator is deleted by insertion of the restriction fragment from \(-50\) (HaeIII) to +133 (Sau3A) bp. This group of mutations corresponds to similar mutations in the attenuator observed in other amino acid biosynthetic systems regulated by a leader-attenuator (reviewed by Landick and Yanofsky [11]).

As described above, the several hundred transformants isolated after hydroxylamine mutagenesis yielded some hundred mutations within the ilv regulatory region. These mutations were either down promoter mutations at \(-15\) bp or attenuator mutations at 177 bp. It had been expected that mutations would also be obtained at the initiating AUG for translation of the leader peptide, mutations similar to those described for the trp operon by Zurawski et al. (28). To construct such a mutation, an oligonucleotide (RL36; GGGCTGTCGTTTTGTCT) was synthesized to change bp 34 from T \cdot A to C \cdot G, thus changing the codon from AUG (Met) to ACG (Thr). To accomplish this, the 700-bp ilv fragment from pRL137 was transferred to the single-stranded phage MP8 and the virus was mutagenized with RL36 as described by Zoller and Smith (27). Phage lysates were screened by differential hybridization of the mutagenic oligonucleotide and by DNA sequence analysis (27). The mutagenized ilv fragment was inserted into pKO6, yielding pMW333. A five- to sixfold decrease in the expression of galactokinase resulted from altering this base pair (1.4 versus 8.0 U; Table 1).

The phenotypes of these three mutants are consistent with the results of previous analyses (15, 26). Transcription in vitro was performed to further analyze these mutants (Fig. 2). Transcription in vitro of pRL137 yields two short products (21), the 108-nt RNA from the replication region of the plasmid and the 186-nt RNA from ilvGp2 into the ilv attenuator (Fig. 2, lane 1). Lanes 2, 3, and 4 in Fig. 2 show the products of transcription in vitro of pRL201, pDM287, and pMW333, respectively. Altering either the promoter or the attenuator resulted in reduced formation of the 186-nt transcript. However, alteration of the translation initiation triplet appeared to have no effect on formation of the 186-nt RNA (pMW333; Fig. 2, lane 4).

Quantitation of the transcription products by densitometric analysis further supported the effect of these mutations on transcription. The molar ratios of the 186- to 108-nt RNAs for pRL137 and pMW133 are 1.54 and 1.57, respectively, while for pRL201 and pDM287 the ratios are 0.210 and 0.303, respectively. These data are again consistent with the ilv-950 mutation affecting expression from ilvGp2 and with the ilv-951 mutation affecting termination. Furthermore, analysis of the transcription of purified restriction fragments is consistent with the transcription of the plasmids (data not shown).

The point mutations described in this note are the first described for the regulatory region of the ilvGmeda operon of E. coli K-12. They confirm previous conclusions about the structure of the ilv regulatory region that were based on biochemical analysis. The mutation ilvGp2950 reduces both the expression in vivo and the transcription in vitro of galactokinase, in agreement with the proposed location of ilvGp2 (14, 15). Our analysis of mutation ilvGe952 is consistent with the site of translation initiation of the proposed ilv leader peptide. The characteristics of mutation ilvGa951 confirm the site for formation of the stem-loop required for transcription termination. Together, these mutations minimize any uncertainty in our previous analysis (15).

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LITERATURE CITED


