JOURNAL OF BACTERIOLOGY, Mar. 1993, p. 1221–1234
0021-9193/93/051221-14$02.00/0

Vol. 175, No. 5

Nucleotide Sequence of the *adi* Gene, Which Encodes the Biodegradative Acid-Induced Arginine Decarboxylase of *Escherichia coli*

KATHLEEN P. STIM AND GEORGE N. BENNETT*

Department of Biochemistry and Cell Biology, Rice University, P.O. Box 1892, Houston, Texas 77251

Received 4 August 1992/Accepted 23 December 1992

Arginine decarboxylase (encoded by *adi*) is induced under conditions of acidic pH, anaerobiosis, and rich medium. The DNA sequence of a 3-kb fragment of the *Escherichia coli* chromosome encoding biodegradative arginine decarboxylase was determined. This sequence encodes a protein of 755 amino acids with a molecular size of 84,420 daltons. The molecular weight and predicted Adi amino acid composition agree with those found in earlier work. The amino acid sequence of arginine decarboxylase showed homology to those of three other decarboxylases of *E. coli*: (i) CadA, encoding lysine decarboxylase; (ii) SpeC, encoding biosynthetic ornithine decarboxylase; and (iii) SpeF, encoding biodegradative ornithine decarboxylase and the lysine decarboxylase of *Hafnia alvei*. Unlike SpeC and SpeF, Adi is not similar to the biosynthetic arginine decarboxylase, SpeA. *adi* is also dissimilar to *cadA* and *speF* in that it does not appear to be part of an operon containing a metabolically related transport protein, indicating that it represents a new type of biodegradative decarboxylase regulation. Transcriptional fusions between fragments upstream of *adi* and *lacZ*, primer extension, and site-directed mutagenesis experiments defined the pH-regulated promoter. Deletion analysis of the upstream region and cloning of fragments to make *adi::lacZ* protein fusions implicated a region beyond an upstream *Ssp*I site in pH regulation. Induction of *adi* in the presence of sublethal concentrations of novobiocin or coumermycin A1, inhibitors of DNA gyrase, was dramatically decreased, indicating that DNA supercoiling is involved in *adi* expression. These results and those of promoter structure studies indicated that acid regulation of *adi* may involve a mechanism different from that of acid regulation of *cad*.

Escherichia coli contains two types of amino acid decarboxylases. The biosynthetic decarboxylases are constitutively expressed regardless of variations in pH and are involved in the synthesis of polyamines (57). The biodegradative decarboxylases, such as arginine and lysine decarboxylases, are strongly induced in rich medium at a low pH in the presence of excess substrate (4, 5, 21, 39, 44, 52, 57) and appear to play a role in pH homeostasis by consuming protons and neutralizing the acidic by-products produced during carbohydrate fermentation (22, 49). Biodegradative arginine decarboxylase acts on arginine to produce agmatine and has been thoroughly characterized (5–8, 52). The native protein is a decamer of 820,000 daltons and is composed of 10 identical subunits (6, 8).

The biodegradative arginine (encoded by *adi*) and lysine (encoded by *cadA*) decarboxylases have several properties in common. Both are induced under anaerobic, acidic conditions (although CadA is also induced under aerobic conditions) and are major cellular proteins, representing over 2% of the total cell protein under maximal induction. Arginine and lysine decarboxylases are capable of increasing the surrounding pH by removing acidic carboxyl groups and releasing CO_2 from their substrates (arginine and lysine, respectively), and both contain pyridoxal 5'-phosphate as a cofactor (5). However, Adi and CadA exhibit differences in induction, as shown by studies involving the Mu d*lac* fusion strains GNB7145K (*adi*) and GNB8385K (*cadA*) (4). Genes regulated by alterations in external pH (*hyd* and *aniG*) have also been identified in *Salmonella* spp. (1, 20). The *hyd* gene only responds to changes in pH under anaerobic conditions, as does *adi*, while the *aniG* gene is similar to *cadA*, being inducible under aerobic and anaerobic conditions (1). At this time, no unique regulatory genes have been identified as interacting with *adi*, whereas *cadR* was identified by the absence of a lysine requirement for the induction of *cadA* (47). *cadA* is part of an operon including *cadB*, the gene for a proposed lysine-cadaverine membrane-bound transport protein (40). Immediately adjacent to the *cadBA* operon is *cadC*, a gene encoding a protein with homology to other prokaryotic transcriptional activators involved in environmental sensing (58). If homologous genes exist in the arginine decarboxylase system, they are arranged differently with respect to the decarboxylase gene on the chromosome.

In this report, we present the nucleotide sequence of the *adi* gene and identify the promoter responsible for pH-regulated expression. An investigation of specific regions of the promoter involved in pH control was initiated; a possible role of DNA gyrase is indicated. Finally, we present a comparison of the deduced *adi* amino acid sequence with the amino acid sequences of four other amino acid decarboxylases.

MATERIALS AND METHODS

Materials. Restriction enzymes were purchased from Promega Corp., New England Biolabs, Inc., and Bethesda Research Laboratories, Inc. T4 DNA ligase and its buffer were purchased from United States Biochemical Corp. and used in accordance with the manufacturer's recommendations. Subclones were sequenced by use of the Sequenase version 2.0 sequencing kit from United States Biochemical Corp. and [^{35}S]dATP from NEN Research Products. The −40 primer was provided by the manufacturer; other oligo-

* Corresponding author.

TABLE 1. Bacterial strains

E. coli strain	Relevant genotype	Reference
71-18	*SupE thi* Δ(*lac-proAB*) F′ (*proAB lacI*^{q} *lacZ* ΔM15)	12
BW313	*dut ung thi-1 relA spoT1*/F′ *lysA*	34
GNB7145	MC4100 *adi*::Mu dX (Ap^r *lac*)	4
GNB7145K	MC4100 *adi*::Mu dI 1734 (Km^r *lac*)	4
GNB2039	MC4100 *adi*::Mu dX (Ap^r *lac*)	4
GNB3112	MC4100 *adi*::Mu dX (Ap^r *lac*)	4
GNB5948	MC4100 *adi*::Mu dX (Ap^r *lac*)	4
MC4100	*araD139* Δ(*argF-lac*)U169 *rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	10

mers (18-mers) for DNA sequencing (or other procedures) were synthesized on a Biosearch 8600 DNA synthesizer by K. Muthukrishnan in the Department of Biochemistry and Cell Biology, Rice University. Southern hybridizations were performed by use of [α-^{32}P]dATP from ICN Biomedicals, Inc., and the Random Primers DNA labeling system from Bethesda Research Laboratories, with either nitrocellulose membranes from Schleicher & Schuell or Immobilon-N transfer membranes from Millipore. The 1.1-kb polymerase chain reaction (PCR) fragment was purified by use of the Millipore Ultrafree-MC filter unit. Primer extension was performed by use of a primer labeled with [γ-^{32}P]dATP from ICN Biomedicals. Deoxyribonucleoside triphosphates, avian myeloblastosis virus reverse transcriptase, and placental RNase inhibitor were from Promega. Arginine decarboxylase assays were performed with L-[U-^{14}C]arginine from ICN Biomedicals. Site-directed mutagenesis was performed by use of the Muta-Gene M13 in vitro mutagenesis kit, version 2, from Bio-Rad. Plasmids were prepared by use of the QIAGEN plasmid kit in accordance with the manufacturer's instructions. All other chemicals were purchased from Sigma or Research Organics.

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used are listed in Tables 1 and 2. Falkow decarboxylase medium was described previously (18). Modified Falkow arginine decarboxylase medium contained 5 g of Bacto Peptone, 3 g of yeast extract, and 5 g of L-arginine (monohydrochloride) per liter and was buffered with 20 g of morpholineethanesulfonic acid (MES) for pH 5.5 or 23 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) for pH 8. Cultures were grown on Luria broth (LB) plates (10 g of NaCl, 10 g of tryptone, 5 g of yeast extract, and 30 mg of cysteine per liter; for solid media, 15 g of agar was added to the medium before autoclaving) or MacConkey lactose plates (40 g of MacConkey agar base and 10 g of lactose per liter). The presence of arginine decarboxylase activity was tested by use of Bacto decarboxylase medium base (9 g/liter) plus 5 g of L-arginine monohydrochloride. Five milliliters of medium was placed in screw-cap test tubes, inoculated, and overlaid with 1 ml of sterile mineral oil. The tubes were incubated for 4 days at 37°C, and the activity was determined on the basis of the color of the pH indicator dye (bromcresol purple): purple was positive and yellow was negative. Antibiotics were included in the media at all times to retain plasmids during growth.

Identification of fragments used for cloning by Southern hybridization. The construction of mini-Mu-derived plasmid pKER110 (*cad mel*$^+$ *adi*$^+$) was described previously (4). Thirteen restriction enzymes (*Eco*RI, *Bam*HI, *Bgl*II, *Pst*I, *Pvu*II, *Hin*dIII, *Eco*RV, *Xho*I, *Bgl*I, *Hpa*I, *Sal*I, *Sma*I, and *Sac*I) were used to digest pKER110 singly or in pairs. After digestion, the fragments were separated on 0.8% agarose gels and transferred to nitrocellulose membranes. For preparation of the probe, pSTY3 (*mel*$^+$) (25) was digested with *Eco*RI and *Sal*I. After polyacrylamide gel electrophoresis, the 1.8-kb *Eco*RI-*Sal*I fragment was eluted overnight in buffer X (0.5 M ammonium acetate, 0.01 M magnesium acetate, 1% sodium dodecyl sulfate [SDS], 0.1 mM EDTA) and precipitated with 2 volumes of ethanol. The probe was labeled with [α-^{32}P]dATP and purified by use of a Sephadex G-50 chromatography column as described by Maniatis et al.

TABLE 2. Bacterial plasmids

Plasmid	Relevant marker	Reference
pEMBL8$^+$	Ap^r	12
pEMBL9$^+$	Ap^r	12
pKER110	Mu d5005 *cad mel*$^+$ *adi*$^+$ Km^r	4
pSTY3	*adi* N terminus; Ap^r	25
pXA	St^r Sp^r	11
pRS551	Km^r Ap^r	54
pRS552	Km^r Ap^r	54
pKS4	4.3-kb *Sal*I fragment from pKER110 cloned in pEMBL8$^+$	This work
pKS29	4.3-kb *Bgl*II fragment from pKER110 cloned in pEMBL8$^+$	This work
pKS31	Same as pKS29 but cloned in the opposite orientation	This work
pKS10	4.3-kb *Bgl*II fragment from pKER110 cloned in pEMBL9$^+$ (same orientation as pKS31)	This work
pKS53	pKS10 with a deletion of a 2-kb *Eco*RI fragment	This work
pKS103	pKS29 with a deletion of a 1.9-kb *Hin*dIII fragment	This work
pX39	418-bp *Eco*RV-*Sau*3A fragment from pKS29 cloned in pXA	This work
pX26	218-bp *Eco*RV-*Bam*HI PCR fragment cloned in pXA	This work
pRS551-1	226-bp *Eco*RI-*Bam*HI PCR fragment from pX26 cloned in pRS551	This work
pRS552-1	226-bp *Eco*RI-*Bam*HI PCR fragment from pX26 cloned in pRS552	This work
pX-B/S-1	124-bp *Bam*HI-*Ssp*I PCR fragment cloned in pXA	This work
p8-5	226-bp *Eco*RI-*Bam*HI PCR fragment from pX26 cloned in pEMBL8$^+$	This work
p8$^+$-S/RV-1	94-bp *Ssp*I-*Eco*RV PCR fragment cloned in pEMBL8$^+$	This work
pSDM1	pX26 with a −35 mutation	This work
pSDM2	pX26 with a −10 mutation	This work
pSDM2-16	pX26 with a 1-base deletion between the −10 and −35 regions	This work
pSDM3	pX26 with two mutations in the −10 region	This work

(37). Hybridization and washing were performed in accordance with the nitrocellulose membrane manufacturer's protocol. Fragments containing the *adi* gene region were identified by autoradiography.

Recombinant DNA techniques. Cloning experiments were conducted in accordance with standard procedures (37). DNA fragments from pKER110 were shotgun cloned into phagemid pEMBL8$^+$, pEMBL9$^+$, or pXA to prepare subclones for sequencing (pKS4, pKS29, pKS31, pKS53, pKS103, and pX39). For creation of operon (pRS551) or protein (pRS552) fusions, the promoter region of *adi* was amplified by PCR with 19-mer primer X (11) and primer PCR1. PCR1 incorporates a *Bam*HI restriction site (5′ TT GATGGAGAggaTCcCTTTCAACAA 3′ [lowercase letters indicate changes to create the *Bam*HI restriction site]) for cloning purposes. After amplification, the PCR fragment was digested with *Eco*RV and *Bam*HI and inserted into *Sma*I- and *Bam*HI-cleaved pXA to yield pX26. The resulting insert was cleaved with *Eco*RI and *Bam*HI and subcloned into pRS551, pRS552, and pEMBL8$^+$ at the *Eco*RI and *Bam*HI sites to yield pRS551-1, pRS552-1, and p8-5, respectively. The original PCR-amplified product was digested with *Bam*HI and *Ssp*I or *Eco*RV and *Ssp*I and inserted into either pXA (digested with *Bam*HI and *Sma*I) or pEMBL8$^+$ (digested with *Sma*I) to yield pX-B/S-1 and p8$^+$-S/RV-1, respectively. Inserts were subjected to DNA sequence analysis for confirmation of proper cloning.

DNA sequence analysis. Single-stranded DNA was induced by infecting host strain 71-18 containing the recombinant phagemid with phage IR1 (17). Clones used for sequencing in pEMBL8$^+$ or pEMBL9$^+$ (pKS4, pKS29, pKS31, pKS53, and pKS103) were first sequenced in accordance with the manufacturer's instructions with the −40 primer provided in the Sequenase version 2.0 sequencing kit. Clone pX39 was initially sequenced with primer X (11). Subsequent sequencing was done by creating primers based on a previously determined sequence to traverse the DNA. Both strands of the DNA were sequenced by this method. After manual reading of the autoradiograms, the DNA sequence was analyzed by use of MacVector release 3.5 (International Biotechnologies, Inc.). Homology searches and multiple alignments were performed by use of the EuGene software package developed by the Molecular Biology Information Resource at Baylor College of Medicine (36).

Primer extension analysis. For maximal induction of the production of *adi* mRNA, 0.5 ml of an overnight LB culture of 71-18 cells containing pKS29 (*adi*$^+$) or pKS31 (*adi*$^+$) was used to inoculate 10 ml of modified Falkow arginine decarboxylase medium at pH 5.5. As a negative control, cells were also grown in modified Falkow arginine decarboxylase medium at pH 8. All tubes were incubated anaerobically until reaching an optical density at 600 nm of 0.6 and RNA was isolated by the procedure of Gilman (23). For determination of the 5′ end of the *adi* mRNA, 18-mer primer T (CGGTAACATTTTGCTGGC) complementary to nucleotides 277 to 294 was labeled with [γ-^{32}P]dATP as described by Kingston (32). After heat inactivation of the T4 polynucleotide kinase, labeled primer T was purified by use of a Sephadex G25-150 chromatography column as described by Maniatis et al. (37). The primer extension procedure of Kingston (32) was used, except that hybridization was carried out at 25°C. The products were separated on a sequencing gel by use of the sequence generated from pKS53 and primer T as a reference.

Site-directed mutagenesis of the *adi* promoter region. For further investigation of the promoter region, three primers that would alter bases in the proposed −10 and −35 regions were designed. Primer SDM1 (ACGCGCTTgACAGCCCG [the changes are represented by lowercase letters]) corresponds to positions 117 to 133 of the *adi* sequence. Primer SDM2 (GGCCGGAAtATACTTGC) corresponds to positions 138 to 154, and primer SDM3 (AAGGCCGGAAGAc ACcTGCCCGCAACG) corresponds to positions 136 to 162. pX26 was transformed into BW313 and grown in 2×YT medium (16 g of Bacto tryptone, 10 g of Bacto yeast extract, and 5 g of NaCl per liter [a 2× version of the YT medium used by Kunkel {34}, except that the NaCl concentration remained the same]) containing 1.25 mg of uridine per liter. After growth to an optical density at 660 nm of 0.2, uracil-containing single-stranded DNA was isolated by infection with phage IR1. The phosphorylated primers were annealed to the uracil-containing single-stranded DNA, and the synthesis of the second strand of DNA with T4 DNA polymerase and T4 DNA ligase was performed in accordance with the instructions in the Muta-Gene M13 in vitro mutagenesis kit, version 2. The product was transformed into 71-18 cells. Single colonies were isolated, and single-stranded DNA was prepared. The DNA was then sequenced with primer X to verify the correct mutations. QIAGEN plasmid preparations were made from isolates containing the desired mutations. These plasmids were transformed into MC4100, and β-galactosidase values were determined.

Identification of the chromosomal location of the Mu d*lac* insert by Southern hybridization. Chromosomal DNAs from MC4100 and the *adi*::*lacZ* strains GNB7145, GNB2039, GNB5948, and GNB3112 were isolated and purified as described by Rodriguez and Tait (50). The DNA samples, along with pKER110, were digested with *Bgl*II or *Sal*I, separated on 0.8% agarose gels, and transferred to Immobilon-N transfer membranes. The DNA was hybridized to a DNA probe prepared by labeling pKER110 with [α-^{32}P]dATP by use of the Random Primers DNA labeling system and purified by use of a Sephadex G-50 column as described by Maniatis et al. (37). For further definition of the Mu d*lac* insert, chromosomal DNAs from GNB5948, GNB7145, and MC4100 were digested with *Dra*I, electrophoresed, and transferred as stated previously. The DNA was hybridized to a DNA probe prepared by labeling a 1.1-kb PCR fragment (see Fig. 1) (purified by use of the Millipore Ultrafree-MC filter unit) created by use of primers PCR6 (TGAAGGCGGAATTCTTTAAATAACA) and PCR7 (GTTGAATTCTTATCGACCG) with [α-^{32}P]dATP by use of the Random Primers DNA labeling system and purified as stated previously. This PCR product contains a segment of 1.1 kb beginning 13 bases downstream of *adi*.

β-Galactosidase assays. Cultures to be analyzed for β-galactosidase activity were grown anaerobically in pH-buffered modified Falkow arginine decarboxylase medium as described by Auger et al. (4). The cells were grown to an A_{600} of 0.4 to 0.7 and harvested by centrifugation. β-Galactosidase assays were performed, and the units were calculated as described by Miller (42) by the SDS-chloroform permeabilization method. In experiments done to measure the promoter activity of operon or protein fusions, plasmids were transformed into *E. coli* MC4100. In experiments done to test the effect of novobiocin or coumermycin A1, β-galactosidase assays were performed as stated, except that 0, 25, 50, or 100 μg of novobiocin per ml (final concentration) or 50 μg of coumermycin A1 per ml was added to modified Falkow arginine decarboxylase medium.

Arginine decarboxylase assays. *E. coli* 71-18 with either pEMBL8$^+$, pKS29, or pKS31 or without a plasmid was

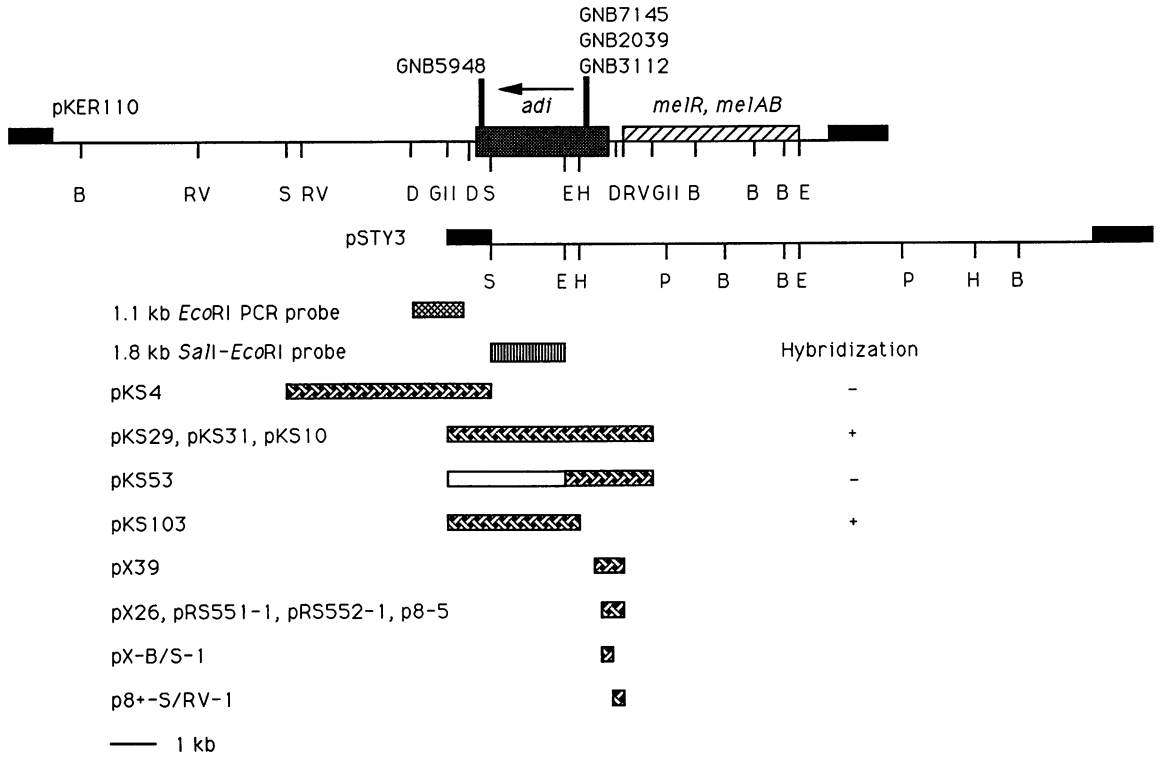

FIG. 1. Chromosomal regions cloned in pKER110 and pSTY3 and chromosomal location of the Mu d*lac* insertion in GNB2039, GNB3112, GNB5948, and GNB7145, as deduced from Southern hybridizations with radiolabeled pKER110 or a 1.1-kb *Eco*RI PCR fragment as a probe. The location of the Mu d*lac* insertion is denoted by heavy vertical bars. The coding region for the *mel* operon is denoted by a hatched rectangle. The coding region for *adi* is denoted by a stippled rectangle, and the direction of its transcription is indicated by an arrow. Solid rectangles represent vector sequences. DNAs subcloned from pKER110 to produce pKS4, pKS29, pKS31, pKS10, pKS53, pKS103, pX39, pX26, pRS551-1, pRS552-1, p8-5, pX-B/S-1, and p8$^+$-S/RV-1 are denoted by cross-hatched rectangles to indicate the regions cloned (see Table 2). The 2-kb *Eco*RI fragment removed from pKS10 to create pKS53 is denoted by an open rectangle. Fragments displaying positive hybridization to the 1.8-kb *SalI-Eco*RI fragment from pSTY3, used to probe the cloned regions, are denoted by a +; those that did not hybridize are denoted by a −. Relevant restriction sites are as follows: B, *Bam*HI; D, *Dra*I; E, *Eco*RI; GII, *Bgl*II; H, *Hin*dIII; P, *Pst*I; RV, *Eco*RV; S, *Sal*I.

assayed for arginine decarboxylase activity by the method of Morris and Boeker (44).

Nucleotide sequence accession number. The sequence of *adi* has been deposited in the GenBank data base under accession number M93362.

RESULTS

Identification of DNA fragments used for sequencing. The location of *adi* had been previously mapped to 93.4 min on the *E. coli* chromosome (4), upstream of the melibiose operon (Fig. 1). pKER110 (*cad mel*$^+$ *adi*$^+$) containing this region of the chromosome was used to investigate the exact location of *adi*. Restriction enzymes were used to digest pKER110, and the resulting fragments were transferred to nitrocellulose and probed with the 1.8-kb *SalI-Eco*RI fragment from pSTY3 (*mel*$^+$) (25). This fragment was used as a probe because it is upstream of the melibiose operon, in the region in which *adi* had been previously localized. Figure 1 shows the restriction maps of pKER110 and pSTY3. The hybridization experiment revealed that a 4.3-kb *Bgl*II fragment hybridized to this probe and possibly contained *adi*. This 4.3-kb *Bgl*II fragment and the overlapping 4.3-kb *Sal*I fragment of pKER110 were then cloned into pEMBL8$^+$ or pEMBL9$^+$ to create pKS4, pKS29, pKS31, and pKS10. By use of the *Eco*RI site in the polylinker of pEMBL9$^+$ and the *Eco*RI site in *adi*, pKS10 was digested with *Eco*RI to remove the 2-kb *Eco*RI fragment to create pKS53. For creation of pKS103, pKS29 was digested with *Hin*dIII (by use of the pEMBL8$^+$ polylinker *Hin*dIII site and the *Hin*dIII site in *adi*) to remove the 1.9-kb *Hin*dIII fragment containing the *melR* gene. pX39 was created by cloning a 420-bp *Eco*RV-*Sau*3A fragment from the end of *melR* through the N terminus of *adi* into pXA. All inserts were confirmed by restriction mapping, and these plasmids were used to sequence this region.

For testing of these plasmids for the functional expression of *adi*, they were each transformed into GNB7145K (*adi*), as was pSTY3, and inoculated into arginine decarboxylase medium as previously described (4). GNB7145K bearing pKS29 and pKS31 was positive, indicating that only these plasmids contained the entire *adi* gene. GNB7145K bearing pKS4, pKS53, pKS103, and pSTY3 was negative, indicating that these plasmids contained only portions of *adi*.

Identification of the chromosomal location of the Mu d*lac* insert. Southern hybridization analysis was performed with *Bgl*II- or *Sal*I-digested chromosomal DNA from MC4100, GNB2039, GNB3112, GNB7145, and GNB5948 and plasmid DNA from pKER110. DNA was probed with radiolabeled pKER110. Fragments showing positive hybridization in MC4100 but not in the other strains indicated that the insertion of Mu d*lac* had occurred within those fragments.

Figure 1 displays a map derived from the results of these experiments. In all cases, the 4.3-kb *Bgl*II fragment was altered and a new, larger fragment showed positive hybridization. This result indicates that the insertion of Mu d*lac* had occurred in the 4.3-kb *Bgl*II fragment. An analysis done with *Sal*I-digested fragments revealed that one strain (GNB5948) had an insertion in the 4.3-kb *Sal*I fragment. The remaining strains (GNB2039, GNB3112, and GNB7145) showed no changes in the 4.3-kb *Sal*I fragment. These results indicate that GNB2039, GNB3112, and GNB7145 had insertions in the regions between the *Sal*I site and the N terminus of *adi*, whereas GNB5948 had an insertion in the *Sal*I-*Bgl*II fragment, which contains the C terminus of *adi*. For determination of whether the Mu d*lac* insertion was in *adi* or downstream of *adi*, chromosomal DNA from MC4100, GNB5948, and GNB7145 was digested with *Bgl*II, *Dra*I, and *Sal*I and probed with a radiolabeled 1.1-kb PCR fragment distal to *adi*. The results showed that GNB5948 and GNB7145 had insertions in the 4.3-kb *Bgl*II fragment. GNB5948 had an insertion in the 4.3-kb *Sal*I fragment, while GNB7145 did not, as found previously. None of these strains had an insertion in the 1.1-kb *Dra*I fragment immediately downstream of *adi*, indicating that the insertion in GNB5948 was in the C terminus of *adi* and that the orientation of Mu d*lac* was backwards relative to the direction of transcription of *adi*. These results are consistent with the β-galactosidase activities reported for these strains by Auger et al. (4). In this study, GNB2039 and GNB7145 exhibited significant induction under anaerobic conditions at pH 5.5, while GNB5948 did not show any significant induction.

DNA sequence analysis. DNA was sequenced in both directions by use of the above-mentioned plasmids, and the sequence was analyzed by use of MacVector release 3.5. The DNA sequence of 3 kb from the *Eco*RV site at the end of *melR* to the *Bgl*II site beyond the C terminus of *adi* (shown in Fig. 1) was determined and is presented in Fig. 2. The amino acid sequences of the N terminus (MKVLIVE; 52), the pyridoxal 5′-phosphate binding site (ATHSTHKLL NALSQASY; 7), and the C terminus (A; 7) of *adi* had been previously determined by protein analysis. This information was used to scan the six reading frames of the DNA sequence to precisely locate *adi*. This information allowed the precise localization of *adi* on the map of Kohara et al. (33) and indicated that it is adjacent to *melR*.

The sequence of a DNA segment adjacent to *melR* and a region now identified as encoding the N terminus of *adi* had been previously published (59). The sequence that we have determined is identical to the previously determined sequence in the overlapped region, except for four base changes. At position 236 in Fig. 2, Webster et al. (59) reported a T, and we found a C. This base is in the third position of a threonine codon and would cause no amino acid change. At positions 414 and 415, they reported two C's, and we found two T's. These bases are in the first and second positions of the codon, and our sequence would encode a leucine rather than a proline. In the amino acid alignment with other decarboxylases (see Fig. 4), this residue is a leucine in two of the other decarboxylases; in contrast to the high degree of alignment of most prolines, a proline was not present in any of the other decarboxylases at this position. At position 422, they reported a C, and we found a T; because this base is in the third position of a glycine codon, it would not alter the amino acid encoded.

The promoter region has been identified, and a possible Shine-Dalgarno ribosome binding site is also indicated by underlining in Fig. 2. This ribosome binding site does not seem to be as optimal as some other highly expressed genes. The C-terminal amino acid of *adi* is followed by a region containing several possible stem-loop structures, indicating a probable rho-independent terminator. The first begins 17 bases after the *adi* stop codon and has an 8-base stem with a 12-base loop (bases 2476 to 2503; Fig. 2). Two more stem-loop structures are located 56 bases downstream of the first stem-loop structure, and these two are adjacent to each other. The second structure has a 5-base stem with an 8-base loop, and the third has a 6-base stem and an 8-base loop (bases 2560 to 2596; Fig. 2) followed by a poly-T region.

Promoter identification. Enzyme studies indicated that the plasmids conferring an *adi*$^+$ phenotype produced a high level of *adi* in an acid pH-inducible fashion. Arginine decarboxylase assays were performed at pH 5.5 and pH 8 with strain 71-18 containing no plasmid or plasmid pEMBL8$^+$, pKS29 (*adi*$^+$), or pKS31 (*adi*$^+$). Strains 71-18 and 71-18(pEMBL8$^+$) showed low levels of arginine decarboxylase when grown at pH 8 (0 and 0.2 nmol/min/mg of protein, respectively) and levels of 8.5 and 7.0 nmol/min/mg of protein when grown at pH 5.5. Strains containing pKS29 and pKS31 showed levels of only 0.3 and 0.4 nmol/min/mg of protein, respectively, when grown at pH 8 and levels of 35 and 42 nmol/min/mg of protein, respectively, when grown at pH 5.5. To readily detect and analyze the 5′ end of the mRNA transcript, we performed primer extension analysis with RNA isolated from 71-18(pKS29) grown anaerobically under inducing (pH 5.5) or noninducing (pH 8) conditions. Primer T, corresponding to nucleotides 277 to 294 (Fig. 2), was used in the extension. Figure 3 presents the results of this analysis. Under maximal induction at pH 5.5, an extension product was present and ended at position 157 of the *adi* sequence shown in Fig. 2 (indicated by +1). At pH 8, a faint extension product was also present at this position. A second band was present at position 166 of the *adi* sequence.

To investigate which of these bands represents the correct 5′ end of the mRNA, we performed site-directed mutagenesis of the *adi* promoter. To begin this analysis of the promoter region of *adi*, we cloned fragments into protein or operon fusion vectors as indicated in Fig. 1. For creation of in-phase fusions to *lacZ*, a *Bam*HI restriction site was constructed in the *adi* coding region by use of primer PCR1. Primer PCR1 and primer X were then used to amplify the *adi* promoter region by PCR, and this region (from the *Eco*RV site at the end of *mel*R to the *Bam*HI site at codon eight of *adi*) was fused to *lacZ* in pXA to create pX26. Site-directed mutations within the promoter were constructed to investigate the proposed −10 and −35 regions of the *adi* promoter. Three primers (SDM1, SDM2, and SDM3) that would alter one or two bases in the promoter region were synthesized. These primers were used with pX26, which contains the *adi* promoter and a protein fusion of the eighth codon of *adi* to *lacZ* (bases 1 to 215 in Fig. 2). Thus, changes in β-galactosidase activity could be used to monitor the effect of the mutations. Primer SDM1 changed the −35 region from 5′ TTTACA to 5′ TTGACA, and primer SDM2 changed the −10 region from 5′ GATACT to 5′ TATACT. Both of these changes resulted in promoter sequences that are more similar to the *E. coli* σ^{70}-dependent promoter sequences (26), and plasmid constructs (pSDM1 and pSDM2) containing these changes would be expected to show increased expression if the proposed −10 and −35 regions represented the correct promoter. Primer SDM3 introduced two mutations in the −10 region, changing it from 5′ GATACT to 5′ GAC ACC. This change resulted in a sequence less similar to the *E. coli* σ^{70}-dependent promoter sequences (26), and the

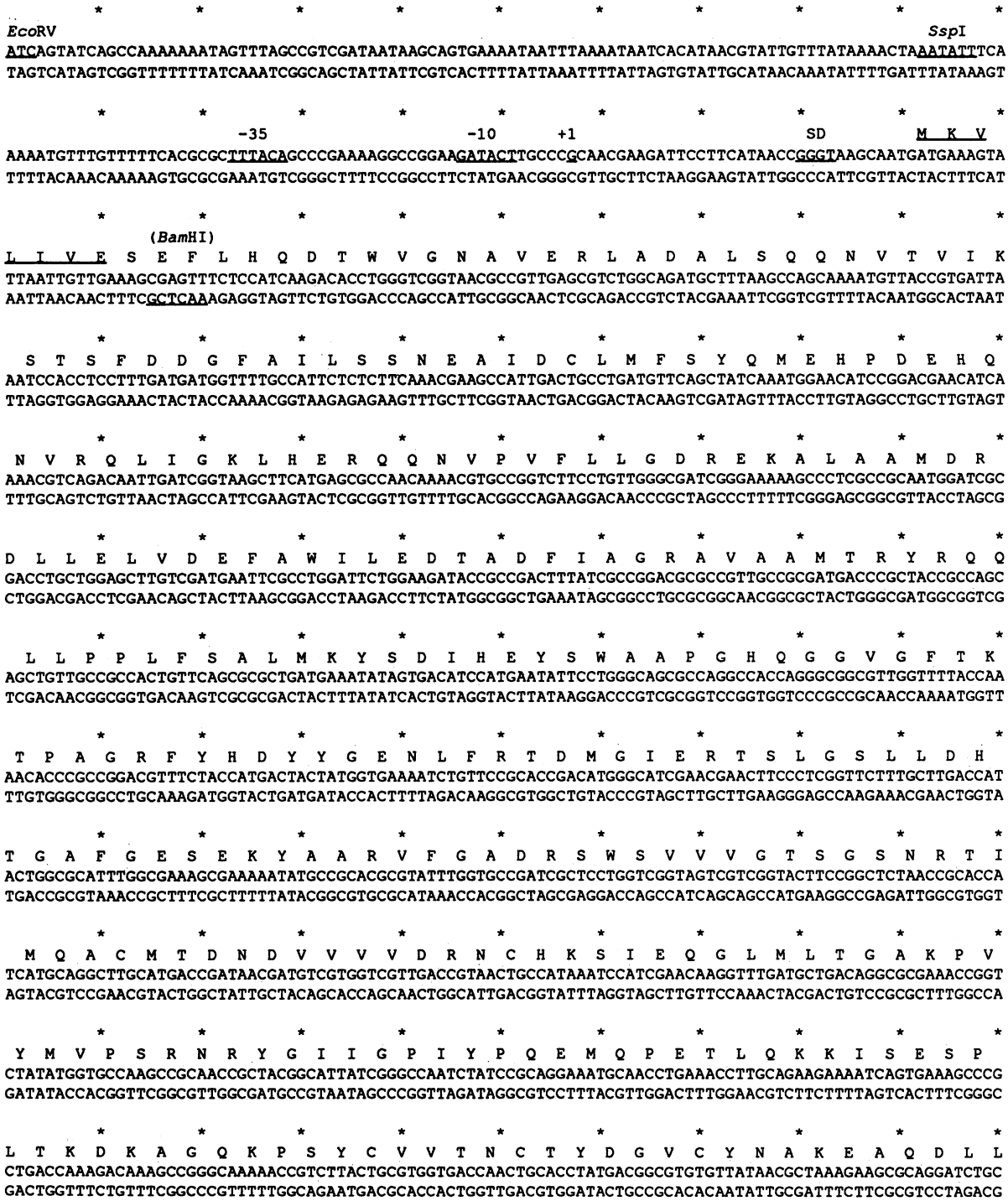

FIG. 2. Nucleotide and deduced amino acid sequences of *adi*. The total sequence shown starts from an *Eco*RV site and ends with a *Bgl*II site. Selected restriction sites used in this work are shown above the sequence: the *Ssp*I site at bp 92 was used to cleave the promoter region to create pX-B/S-1 or p8$^+$-S/RV-1; the *Bam*HI site in parentheses was created by use of primer PCR-1 to clone the promoter region (bp 1 to 215) (by use of this site and the *Eco*RV site); and the *Sal*I site at bp 2208 indicates where the overlap between pKS4 and pKS31 occurred. The numbers on the left indicate the first base pair at the beginning of each row of the double-stranded DNA sequence; a sequence from the region of bp 1 to 424 was reported previously (59) and corresponds to this sequence, except for four base changes noted in the text. The transcription start site (+1), the −10 and −35 sequences, and the Shine-Dalgarno sequence (SD) are underlined. The Adi amino acid sequence is translated from the top strand and is positioned above each codon. The N-terminal and pyridoxal 5′-phosphate sequences of arginine decarboxylase used to identify *adi* are underlined. Possible stem-loop structures and an adjacent poly-T region located just beyond the translational stop codon for *adi* are underlined.

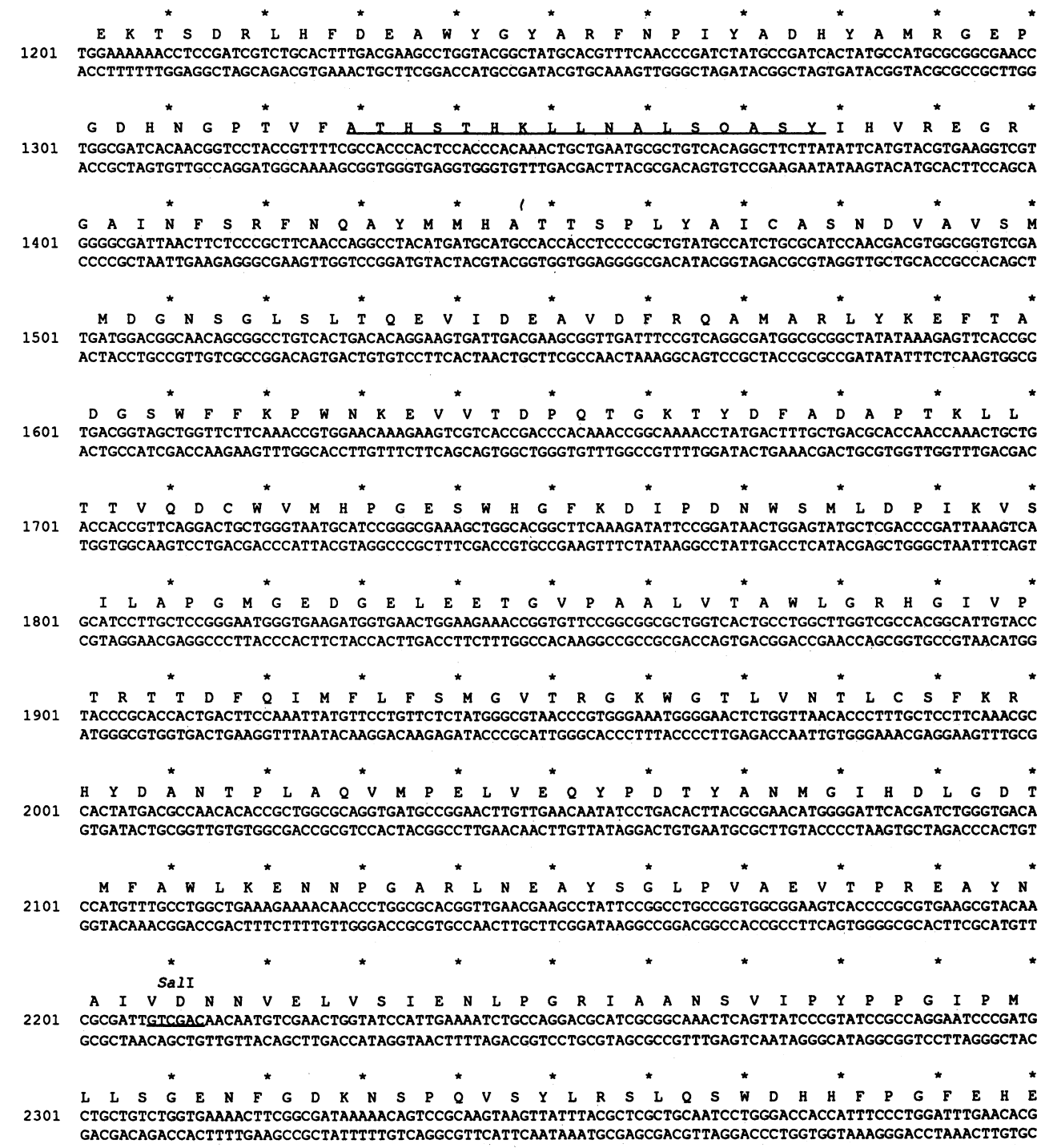

FIG. 2—*Continued.*

plasmid construct (pSDM3) containing these mutations would be expected to show decreased β-galactosidase activity. During the site-directed mutagenesis experiments, a spacing mutant that had a deletion of one base between the proposed −10 and −35 regions (the A at position 137 in Fig. 2) was identified (pSDM2-16) by DNA sequence analysis. This deletion changed the spacing between the −10 and −35 regions from 17 bases (the most common spacing arrangement; 26) to 16 bases but did not alter the −10 and −35 regions.

Table 3 shows that the mutations introduced by primers SDM1 and SDM2 increased the expression of β-galactosidase from the *adi*::*lacZ* protein fusions, as expected. Compared with the wild-type *adi* promoter fragment on pX26, pSDM1 showed a 6-fold increase in pH 5.5 β-Galactosidase expression and a 33-fold increase in pH 8 β-galactosidase

```
                *         *         *         *         *         *         *         *         *         *
          T  E  G  T  E  I  I  D  G  I  Y  H  V  M  C  V  K  A  ***
2401  AAACTGAAGGGACTGAAATTATTGACGGTATTTACCACGTTATGTGCGTGAAAGCGTAACCACTATTCCGCTGAAGGCGTAATTGTTTAAATAACATTAC
      TTTGACTTCCCTGACTTTAATAACTGCCATAAATGGTGCAATACACGCACTTTCGCATTGGTGATAAGGCGACTTCCGCATTAACAAATTTATTGTAATG

                *         *         *         *         *         *         *         *         *         *
2501  GCCGCCTGGCCTTAGGCCTTTTGAGTATGGCAACGTTTTCATAAAAATTGCTGCAAACAAAAATGTCATACTTTTTGCGCGGCCCCACCCCGCGCTTTTT
      CGGCGGACCGGAATCCGGAAAACTCATACCGTTGCAAAAGTATTTTTAACGACGTTTGTTTTTACAGTATGAAAAACGCGCCGGGGTGGGGCGCGAAAAA

                *         *         *         *         *         *         *         *         *         *
2601  TTTGCCTGTTATTTATCCTGTAAAAAAATATGTACATGAGAAATTACTATAAAAATTTGTACTATTAGTAAAACTCGTTATTTTTATGCATGTTTATATT
      AAACGGACAATAAATAGGACATTTTTTTATACATGTACTCTTTAATGATATTTTTAAACATGATAATCATTTTGAGCAATAAAAATACGTACAAATATAA

                *         *         *         *         *         *         *         *         *         *
2701  CATCATACAATTATATAACCATTTCCCGGTATCGCTTTGCTTTAGCGAGAACTGGTCTTTTATTCGCACTCAGGAGTACATGTATGAGGATTTGCAGCGA
      GTAGTATGTTAATATATTGGTAAAGGGCCATAGCGAAACGAAATCGCTCTTGACCAGAAAATAAGCGTGAGTCCTCATGTACATACTCCTAAACGTCGCT

                *         *         *         *         *         *         *         *         *         *
2801  CCAACCTTGTATTGTTTTATTGACTGAAAAAGATGTCTGGATAAGGGTGAATGGGAAAGAACCTATTAGCCTTAAAGCTAACCATATGGCGTTATTAAAT
      GGTTGGAACATAACAAAATAACTGACTTTTTCTACAGACCTATTCCCACTTACCCTTTCTTGGATAATCGGAATTTCGATTGGTATACCGCAATAATTTA

                *         *         *         *         *         *         *         *         *         *
2901  TGTGAAAATAATATTATCGACGTCTCCTCTCTTAACAACACTTTGGTTGCTCATATTAGTCACGACATCATCAAAGATTACCTCCGGTTTCTGAATAAAG
      ACACTTTTATTATAATAGCTGCAGAGGAGAGAATTGTTGTGAAACCAACGAGTATAATCAGTGCTGTAGTAGTTTCTAATGGAGGCCAAAGACTTATTTC

      BglII
3001  ATCT
      TAGA
```

FIG. 2—*Continued.*

expression. On the other hand, pSDM2 showed a 7-fold increase in pH 5.5 β-galactosidase expression and a 130-fold increase in pH 8 β-galactosidase expression. pSDM3 exhibited β-galactosidase activity <1% the wild-type β-galactosidase activity at pH 5.5 and no detectable activity at pH 8. pSDM2-16 showed that the one-base deletion caused a decrease in β-galactosidase expression to 4% of the pH 5.5 wild-type value and 7% of the pH 8 wild-type value.

Taken together, the results of primer extension and site-directed mutagenesis led us to conclude that the indicated −10 and −35 regions (Fig. 2) were indeed the site of a σ^{70}-type promoter of *adi* and that the second band detected in the primer extension experiment was due to partial degradation or incomplete extension of the mRNA.

Amino acid sequence of biodegradative arginine decarboxylase and comparison of this decarboxylase with other decarboxylases. The amino acid sequence of arginine decarboxylase is presented in Fig. 2. The open reading frame encoding *adi* extends from a methionine codon at nucleotide 192 to nucleotide 2459 and encodes a 755-amino-acid protein of 84.4 kDa. The amino acid composition and molecular weight of *adi* are quite similar to those reported previously. Table 4 presents a comparison of the amino acid composition of arginine decarboxylase determined by chemical degradation (6) with the amino acid sequence deduced on the basis of the nucleotide sequence. The molecular mass is within the range of 77 to 89 kDa estimated by Boeker and Snell (8), as determined by ultracentrifuge studies of the reduced and carboxymethylated enzyme in denaturing solvents, and 74 to 86 kDa estimated by Boeker et al. (6). Kyte-Doolittle (35) hydrophilicity analysis done with MacVector software is consistent with the fact that the inducible arginine decarboxylase is a cytosolic protein.

A search of the GenBank and Swiss-Prot data bases by use of the BLAST network service (2) of EuGene (36) revealed four decarboxylases with protein sequences similar to that of the inducible arginine decarboxylase. These proteins include

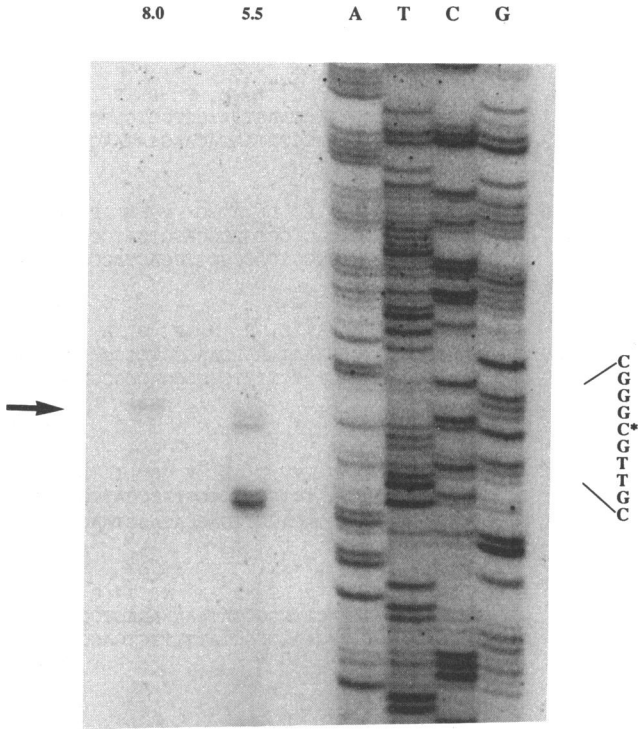

FIG. 3. Primer extension analysis. The two left lanes contain the product of a primer extension experiment with RNA isolated from 71-18(pKS29) grown in modified Falkow arginine decarboxylase medium at pH 5.5 or pH 8. The extension was performed with an antisense primer corresponding to nucleotides 277 to 294 of the *adi* sequence (Fig. 2). The right lanes contain a DNA sequencing ladder generated by use of the same antisense primer and plasmid pKS53. The asterisk to the right of the gel indicates the endpoint of the extended product. The arrow indicates the location of the extended product.

TABLE 3. Effects of promoter mutations on β-galactosidase expression from *adi*::*lacZ* protein fusion constructs

Plasmid[a]	β-Galactosidase activity[b] at pH:	
	5.5	8
pX26 (wild-type fusion)	700	15
pSDM1 (−35 mutation)	4,200	500
pSDM2 (−10 mutation)	5,200	2,000
pSDM2-16 (one-base deletion)	30	1
pSDM3 (−10 mutations)	6	0
None (MC4100)	0	0

[a] All plasmids were transformed into MC4100.
[b] Cells were grown and assayed as described in Materials and Methods. Values are given in Miller units (42) and represent the averages of six assays.

three from *E. coli*: CadA—the inducible lysine decarboxylase (40; GenBank accession no. M76411), SpeC—the biosynthetic ornithine decarboxylase (8a; GenBank accession no. M33766), and SpeF—the inducible ornithine decarboxylase (31; GenBank accession no. M64495). The fourth protein is lysine decarboxylase from *Hafnia alvei* (19; GenBank accession no. X03774). An alignment of these five proteins by use of the pattern-induced multialignment program (56) of EuGene (36) is presented in Fig. 4. There are 119 completely conserved sites among the five proteins, and these sites occur throughout the entire protein sequences. Clusters of conserved residues occur throughout the protein sequences, most notably around the region of the pyridoxal 5′-phosphate binding sites of the inducible arginine and lysine decarboxylases of *E. coli*. In the comparison, the alignment of a number of proline and glycine residues (18 and 23, respectively) suggests a conservation of parts of the secondary structures (most probably turns) of the proteins. Secondary structure prediction programs for the aligned sequences also indicate some correlation of structural features. Arginine decarboxylase shares 35, 31, 30, and 35% homology (number of identical amino acid residues/number of residues of the shortest of the two proteins compared) with the inducible lysine decarboxylase, the ornithine decarboxylase, the inducible ornithine decarboxylase, and the *H. alvei* lysine decarboxylase, respectively.

TABLE 4. Amino acid composition of the *E. coli* inducible arginine decarboxylase

Amino acid	Amino acid composition determined by:			
	Chemical degration[a]		DNA sequencing	
	No.	%	No.	%
Ala	59	8.3	62	8.2
Arg	32	4.5	33	4.4
Asx	84	11.8	83	11.0
Asn			34	4.5
Asp			49	6.5
Cys	7	1.0	10	1.3
Glx	75	10.5	76	10.1
Gln			29	3.8
Glu			47	6.2
Gly	51	7.2	56	7.4
His	23	3.2	25	3.3
Ile	32	4.5	34	4.5
Leu	56	7.9	59	7.8
Lys	28	3.9	29	3.8
Met	25	3.5	28	3.7
Phe	30	4.2	32	4.2
Pro	35	4.9	39	5.2
Ser	40	5.6	46	6.1
Thr	42	5.9	46	6.1
Trp	13	1.8	14	1.8
Tyr	28	3.9	31	4.1
Val	51	7.2	52	6.9
Total	711		755	

[a] From Boeker et al. (6).

Regulation of *adi* expression. Experiments designed to define the region required for acid pH induction and to determine whether selected promoter fragments could titrate the effect of an activator, as was observed for *cad* (41), were undertaken. For evaluation of the effect of various upstream regions of the promoter, several constructs were made for expression studies or in vivo protein titration experiments. The *Bam*HI-*Eco*RI fragment of pX26 was moved into pRS551, pRS552, and pEMBL8$^+$ to create pRS551-1, pRS552-1, and p8-5, respectively. The PCR fragment was then cleaved into fragments of 124 and 94 bases by *Bam*HI-*Ssp*I digestion and *Ssp*I-*Eco*RV digestion, respectively, and the fragments were cloned into pXA (*Bam*HI-*Ssp*I) and pEMBL8$^+$ (*Ssp*I-*Eco*RV), respectively. These manipulations yielded pX-B/S-1 and p8$^+$-S/RV-1, respectively. For expression studies, plasmids were transformed into MC4100, and for investigation of the possibility of activator or repressor titration effects, pEMBL8$^+$, p8$^+$-S/RV-1, and p8-5 were transformed into GNB7145K.

Assays of expression constructs are shown in Table 5. The results of β-galactosidase assays with pX26, pRS551-1, and pRS552-1 showed that acid pH induction was still observed with a plasmid fragment bearing a 218-base promoter region. Operon fusion pRS551-1 showed elevated expression at both pHs, but this increased basal expression has sometimes been observed in other situations (41) and was probably due to nonspecific promoter expression. Protein fusions generally yield a more tightly controlled β-galactosidase level and are more widely used. The β-galactosidase levels of the protein fusion constructs indicated acid pH induction. The β-galactosidase levels obtained with pX-B/S-1 indicated that cutting at the *Ssp*I site and removal of the 94-base fragment closest to *melR* removed the pH control of this promoter. Alternatively, a nonregulated promoter was inadvertently created by the construction. Thus, it appears that sequences beyond the *Ssp*I site may be involved in acid pH induction.

Transformation of GNB7145K with p8$^+$-S/RV-1 or p8-5 allowed us to determine whether this region has an effect on the expression of β-galactosidase from the *adi*::*lacZ* Mu dI1734 insertion in the chromosome. If a regulatory protein that binds to this region is present, then the presence of multiple copies of the 93-base *Ssp*I-*Eco*RV region or the entire promoter region would create competition for the regulatory protein. This competition would, in effect, titrate this regulator and prevent its interaction with the chromosomal *adi*::*lacZ* fusion, resulting in a decrease in β-galactosidase expression in GNB7145K if an activator mechanism were operating on *adi*. Table 5 shows no significant change at either pH, indicating no titration of a regulatory protein for *adi*. Thus, perhaps some other type of activation occurs in this system, sequences outside this region are required for most effective binding, or the activator is not present in low abundance. These results differ from those found for *cad* (41).

Effect of novobiocin and coumermycin A1 on *adi* expression. Table 6 shows the effect of the DNA gyrase inhibitors novobiocin and coumermycin A1 on the expression of

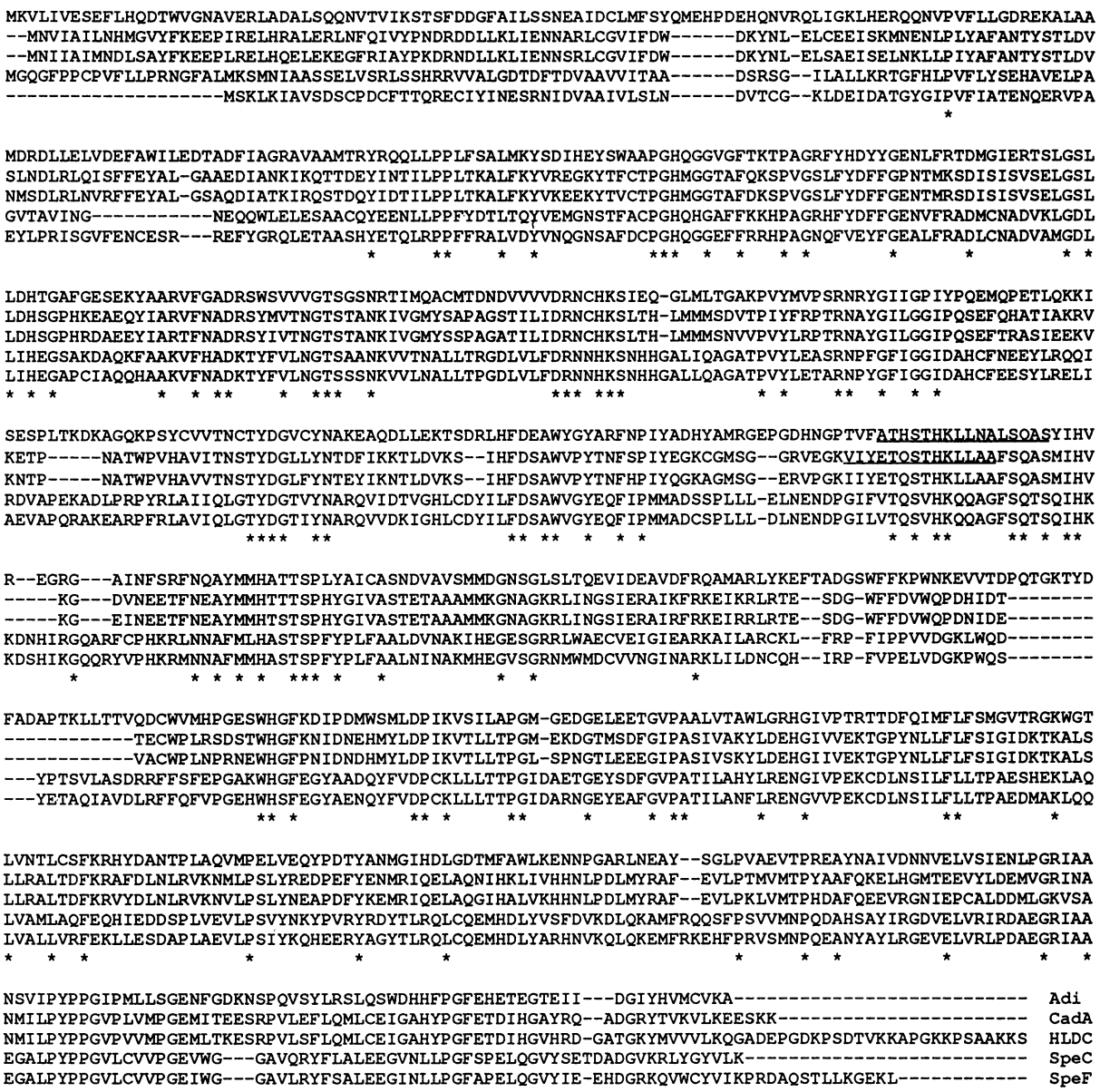

FIG. 4. Sequence alignment of the biodegradative lysine decarboxylase (CadA), the biosynthetic ornithine decarboxylase (SpeC), and the inducible ornithine decarboxylase (SpeF) of *E. coli*, the lysine decarboxylase of *H. alvei* (HLDC), and the biodegradative arginine decarboxylase (Adi) of *E. coli*. Conserved amino acid residues are noted by an asterisk below the conserved site. Gaps in the alignment are indicated by dashes. The pyridoxal 5′-phosphate binding sites of the biodegradative arginine and lysine decarboxylases are underlined.

adi::*lacZ* in GNB7145K and *cadA*::*lacZ* in GNB8385K. Cells grown without novobiocin or coumermycin A1 showed normal acid pH induction of the fusion. The inclusion of novobiocin or coumermycin A1 dramatically decreased the acid pH expression of *adi*::*lacZ* under anaerobic conditions (by 70 to 80%), indicating a role of the DNA supercoiling state in *adi* expression. The addition of either of these antibiotics to GNB8385K cells resulted in only a small decrease; neither antibiotic significantly altered acid pH induction. Measurements of pH during growth showed no significant change from the starting pH. The modest decrease in growth rate under conditions in which a significant reduction in the expression of *adi*::*lacZ* was observed, coupled with the lack of a significant effect on the acid pH induction of the *cadA*::*lacZ* fusion, and the fact that the strains were derived from the same parent (MC4100) led to the conclusion that the effects of the DNA gyrase inhibitors are specific for *adi* and are not side effects due to altered cell growth.

DISCUSSION

Several decarboxylase genes induced by an acidic pH have now been sequenced and characterized. These genes include *adi*, for the inducible arginine decarboxylase, *cadA*, for the inducible lysine decarboxylase (40, 41, 58), and *speF*, for the inducible ornithine decarboxylase (31). A group of constitutively produced decarboxylases has also been char-

TABLE 5. Effect of promoter region length on β-galactosidase expression

Plasmid[a]	Type of fusion	Upstream region length[b]	β-Galactosidase activity[c] at pH:	
			5.5	8
MC4100(pX26)	Protein	218	380	20
MC4100(pX-B/S-1)	Protein	124	470	250
MC4100(pXA)	Vector		0.2	0.4
MC4100(pRS551-1)	Operon	226	740	200
MC4100(pRS551)	Vector		0.3	0
MC4100(pRS552-1)	Protein	226	120	12
MC4100(pRS552)	Vector		0.2	0
GNB7145K(p8-5)		226	770	7
GNB7145K(p8$^+$-S/RV-1)		94	770	6
GNB7145K(pEMBL8$^+$)	Vector		640	6
None (GNB7145K)			500	5

[a] Plasmids were present in MC4100 or in GNB7145K as indicated.
[b] Length (in base pairs) of the region upstream from the *adi* transcription initiation site remaining intact in the construction.
[c] Values are given in Miller units (42) and represent the averages of six tubes for plasmids in MC4100 and nine tubes for plasmids in GNB7145K.

acterized. This group includes the biosynthetic ornithine (*speC* gene; 8a) and arginine (*speA* gene; 43) decarboxylases of *E. coli* and the lysine decarboxylase (19) of *H. alvei*. All of these decarboxylases show at least 30% amino acid sequence homology to Adi, except for SpeA. SpeA and Adi are only 10% homologous, and SpeA is 9% similar to the *H. alvei* lysine decarboxylase, SpeC, and SpeF and 8% similar to CadA. A search of the GenBank and Swiss-Prot data bases for proteins similar to Adi revealed CadA, SpeC, SpeF, and the *H. alvei* lysine decarboxylase but did not list SpeA among the possible candidates. A comparison of the amino acid alignments of the five similar decarboxylases (Adi, CadA, SpeC, SpeF, and *H. alvei* lysine decarboxylase) to the alignments of these five decarboxylases plus SpeA shows that the number of completely conserved amino acid residues drops from 119 to 7. A phylogeny constructed by the Hein program (27) of EuGene (36) placed SpeA in a separate, distant branch from the other five decarboxylases.

TABLE 6. Effect of novobiocin or coumermycin A1 on β-galactosidase expression in GNB7145K and GNB8385K

Strain	Treatment[a]	β-Galactosidase activity[b] at pH:	
		5.5	8
GNB7145	None	390	2
	Novobiocin (25 μg/ml)	120	2
	Novobiocin (50 μg/ml)	90	2
	Novobiocin (100 μg/ml)	60	3
	Coumermycin A1 (50 μg/ml)	150	2
	DMSO (50 μl)	470	1
GNB8385K	None	2,700	210
	Novobiocin (25 μg/ml)	2,000	260
	Novobiocin (50 μg/ml)	1,700	280
	Novobiocin (100 μg/ml)	2,200	330
	Coumermycin A1 (50 μg/ml)	2,500	290
	DMSO (50 μl)	2,900	250

[a] DMSO, dimethyl sulfoxide.
[b] Cells were grown and assayed as described in Materials and Methods. Values are given in Miller units (42) and represent the averages for six tubes not treated or with novobiocin added and for three tubes with coumermycin A1 or DMSO added.

The finding that Adi and SpeA are only 10% similar is quite different from the results of comparisons of the biosynthetic and biodegradative decarboxylases. CadA and the lysine decarboxylase of *H. alvei* show 80% amino acid sequence homology, while the two ornithine decarboxylases (SpeC and SpeF) show 67% homology. The lack of similarity between Adi and SpeA could be due to the different cellular locations of these two proteins. Adi is a soluble cytosolic protein, whereas SpeA has been localized within the inner periplasmic space (9). SpeA monomers are synthesized as 74-kDa precursor polypeptides that are posttranslationally processed to a 70-kDa mature protein (9). The presence of a signal peptide typical of translocated proteins is supported by the accumulation of the 74-kDa species but not the 70-kDa species in an *E. coli* strain incapable of processing signal sequences (9). An analysis of the predicted N terminus of SpeA, however, did not reveal a typical prokaryotic signal sequence (43). On the other hand, Adi is synthesized in its mature form as a 755-amino-acid protein of 84.4 kDa. The two lysine decarboxylases as well as the two ornithine decarboxylases are all soluble cytosolic proteins, and they differ in length from Adi by less than 6%. The lengths of the Adi and SpeA amino acid sequences differ by more than 13%.

Applebaum et al. (3) proposed that the biodegradative ornithine, lysine, and arginine decarboxylases plus the biosynthetic ornithine decarboxylase shared a common evolutionary ancestor on the basis of structural similarities. An analysis of the amino acid sequence data supported this conclusion. A phylogeny of the five similar decarboxylases was determined by use of the Hein program (27). It revealed that the two lysine decarboxylases (CadA and *H. alvei* lysine decarboxylase) were most closely related to each other and were grouped with the biodegradative arginine decarboxylase. The two ornithine decarboxylases (SpeC and SpeF) were most closely related to each other and were distantly related to the other three decarboxylases. These groupings supported the hypothesis proposed by Applebaum et al. (3) that the biodegradative ornithine decarboxylase was derived from an ancestral biosynthetic ornithine decarboxylase. If the biodegradative decarboxylases share a common ancestor, Adi, CadA, *H. alvei* lysine decarboxylase, and SpeF should cluster together, while the biosynthetic ornithine decarboxylase should form a separate branch.

Although the genes for both arginine and lysine decarboxylases are located on a similar region of the *E. coli* chromosome and are induced under the same conditions of low pH, anaerobiosis, and excess substrate, they are clearly different in several ways. *cadA* is part of the *cad* operon, which also includes *cadB*, the gene for a proposed lysine-cadaverine membrane-bound transport protein (40). Immediately upstream from the *cad* operon is *cadC*, a gene that encodes a protein with homology to other prokaryotic transcriptional activators involved in environmental sensing (58). A model of how this system is regulated has been proposed (40, 58). Basically, CadC is proposed to act as an environmental sensor detecting changes in extracellular pH. Under low-pH conditions, it undergoes an unspecified alteration and functions as an activator of the *cad* operon, either directly or through another regulatory factor. CadB is proposed to exchange lysine and cadaverine, bringing in lysine and excreting cadaverine (the end product of lysine decarboxylation) produced through the activity of CadA. A similar arrangement of transport and decarboxylase genes has been found for ornithine decarboxylase. The genes encoding the biodegradative ornithine decarboxylase (*speF*) and a membrane-bound putrescine-ornithine antiporter (*potE*) have

been sequenced and the transport function has been demonstrated (30, 31). These two genes also are part of an operon that is induced at a low pH.

adi does not appear to be part of an operon involving upstream regions, as the distance between the *melR* C terminus and the *adi* N terminus is 198 bp. The possibility exists that a gene downstream of *adi* is involved in this system. However, the stem-loop structures deduced after the C terminus of *adi* indicate that it is probably not part of an operon. Preliminary experiments indicated that a downstream fragment exhibits acid pH induction of β-galactosidase when cloned into fusion vectors. If the *adi* system proves similar to the *cad* and *speF* operons, this downstream region may contain a transport-related gene or a gene that has a regulatory role.

The differences found in the regulation of *adi* and *cadBA* were not surprising, as previous experiments had determined that both *adi* and *cadBA* are induced by external acidic pH conditions but that *cadBA* is induced in the presence or absence of oxygen, while *adi* is induced only under anaerobic conditions (4, 55). Genes responsive to external acidic pH conditions (*hyd* and *aniG*) have also been found in *Salmonella* spp. (1, 20). *adi* is more similar to *hyd* in that both genes are induced anaerobically, while *cadBA* is more similar to *aniG*, both of which are induced aerobically and anaerobically.

To investigate the differences between *adi* and *cad*, we undertook an analysis of promoter structure. Site-directed mutagenesis of the *adi* promoter to change conserved regions to sequences unlike the *E. coli* σ^{70} promoter sequence resulted in a dramatic decrease in β-galactosidase activity at both pH 5.5 and pH 8. Site-directed mutagenesis of the *adi* promoter to enhance the similarity to the *E. coli* σ^{70}-dependent promoter consensus sequence resulted in increased expression from the mutant promoters. The wild-type *adi* promoter has a higher induction ratio (47-fold) than the altered promoter sequences do when the pH 8 and pH 5.5 β-galactosidase activities are compared. These results indicate that the inducing mechanism has less of an effect on an already strong promoter. This suggestion would be consistent with a mechanism in which the relative levels of RNA polymerase binding to the promoter are altered by induction. These constructs may be useful in further studies of the mechanism of activation. Sequences of a repeating type or palindromes, like those found in the *cad* promoter (41), were not so apparent upstream of the *adi* promoter.

The bacterial chromosome is folded and maintained by negative superhelical tension (supercoiling) (for reviews of agents that alter supercoiling, see references 15 and 28) and a set of DNA-binding proteins (48; for reviews, see references 16 and 46). The interaction of topoisomerases I and III and DNA gyrase functions to maintain supercoiling at the proper level. Transcription can contribute to the level of supercoiling and lead to local variations in supercoiling. It has been shown that DNA supercoiling changes occur in response to environmental stresses, such as changes in osmolarity and oxygen conditions, and that other control mechanisms specifically involved in certain responses are superimposed on this process (13, 29, 45). Several genes have been found to be affected by inhibitors of DNA gyrase. DNA gyrase inhibition can either increase the expression of genes such as *tonB* (13) or decrease gene expression (for a review, see reference 14). The experiments reported here support the inclusion of *adi* among those genes subject to regulation by supercoiling. The greater effect of supercoiling inhibitors on *adi* would correlate with its greater dependence on anaerobic conditions (compared with *cad*), under which increased negative supercoiling occurs. Whether this effect occurs directly on the *adi* promoter through some other mechanism is not known. A study of the *cadA*::*lacZ* fusion strain showed little effect on β-galactosidase activity when the DNA gyrase inhibitor novobiocin or coumermycin A1 was added to the culture, indicating that supercoiling is not a major factor in the regulation of the *cad* operon. Some mutations in *hns* (*osmZ*), a locus that can adjust supercoiling levels, affect the expression of both lysine and arginine decarboxylases (53). Other agents that alter supercoiling, such as novobiocin or coumermycin A1, significantly affect only *adi*. Such results may suggest that *hns* mutations, although yielding increased expression at pH 8 in both the lysine and arginine decarboxylase systems, do so by different mechanisms. Thus, H-NS, the product of the *hns* gene, may have a role more in competition with the activator in the case of *cad* and a role more related to effects on DNA topology in the case of *adi* (53).

One hypothesis for the mechanism of *adi* induction would involve an interaction of the RNA polymerase with an environmentally responsive activator, causing the induction of *adi* at a low pH. Preliminary experiments with *adi*::*lacZ* fusions that also possess mutations in the α subunit of RNA polymerase (*rpoA*; 38, 51) suggest that a possible interaction of this type is important (52a). The induction could be particularly sensitive to the local supercoiling environment. A possible integration host factor consensus binding sequence in the *adi* promoter has been identified by use of the MacTargsearch program (24). It is interesting to note that both integration host factor and H-NS, the product of the *hns* gene, seems to be involved in interactions with curves or bends in DNA, and the role and regulation of this aspect of DNA structure are areas of broad current interest. Localization and characterization of the *adi* activator and analysis of specific protein sites within the *adi* promoter region should further assist our understanding of the acid regulation of the *adi* gene and its relation to cell physiology.

ACKNOWLEDGMENTS

We thank Kamali Muthukrishnan for oligonucleotide synthesis and Kevin Redding for mini-Mu *adi* constructs.

This work was supported by NIH grant GM 039420 and by the Texas Advanced Technology Program 003604-035.

REFERENCES

1. **Aliabadi, Z., Y. K. Park, J. L. Slonczewski, and J. W. Foster.** 1988. Novel regulatory loci controlling oxygen- and pH-regulated gene expression in *Salmonella typhimurium*. J. Bacteriol. **170**:842–851.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
3. **Applebaum, D. M., J. C. Dunlap, and D. R. Morris.** 1977. Comparison of the biosynthetic and biodegradative ornithine decarboxylases of *Escherichia coli*. Biochemistry **16**:1580–1584.
4. **Auger, E. A., K. E. Redding, T. Plumb, L. C. Childs, S.-Y. Meng, and G. N. Bennett.** 1989. Construction of *lac* fusions to the inducible arginine- and lysine decarboxylase genes of *Escherichia coli* K12. Mol. Microbiol. **3**:609–620.
5. **Blethen, S. L., E. A. Boeker, and E. E. Snell.** 1968. Arginine decarboxylase from *Escherichia coli*. I. Purification and specificity for substrates and coenzyme. J. Biol. Chem. **243**:1671–1677.
6. **Boeker, E. A., E. H. Fischer, and E. E. Snell.** 1969. Arginine decarboxylase from *Escherichia coli*. III. Subunit structure. J. Biol. Chem. **244**:5239–5245.
7. **Boeker, E. A., E. H. Fischer, and E. E. Snell.** 1971. Arginine

decarboxylase from *Escherichia coli*. IV. Structure of the pyridoxal phosphate binding site. J. Biol. Chem. **246**:6776–6781.
8. **Boeker, E. A., and E. E. Snell.** 1968. Arginine decarboxylase from *Escherichia coli*. II. Dissociation and reassociation of subunits. J. Biol. Chem. **243**:1678–1684.
8a. **Boyle, S. M.** Unpublished data.
9. **Buch, J. K., and S. M. Boyle.** 1985. Biosynthetic arginine decarboxylase in *Escherichia coli* is synthesized as a precursor and located in the cell envelope. J. Bacteriol. **163**:522–527.
10. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
11. **De Lorenzo, V., M. Herrero, and J. B. Neilands.** 1988. pCON4 and pCON5: improved plasmid vectors to study bacterial promoters. FEMS Microbiol. Lett. **50**:17–23.
12. **Dente, L., G. Cesareni, and R. Cortese.** 1983. pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11**:1645–1655.
13. **Dorman, C. J., G. C. Barr, N. Ni Bhriain, and C. F. Higgins.** 1988. DNA supercoiling and the anaerobic and growth phase regulation of *tonB* gene expression. J. Bacteriol. **170**:2816–2826.
14. **Drlica, K.** 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. **48**:273–289.
15. **Drlica, K.** 1992. Control of bacterial DNA supercoiling. Mol. Microbiol. **6**:425–433.
16. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histonelike proteins of bacteria. Microbiol. Rev. **51**:301–319.
17. **Enea, V., and N. D. Zinder.** 1982. Interference resistant mutants of phage f1. Virology **122**:222–226.
18. **Falkow, S. N.** 1958. Activity of lysine decarboxylase as an aid in the identification of *Salmonellae* and *Shigellae*. Am. J. Clin. Pathol. **29**:598–600.
19. **Fecker, L. F., H. Beier, and J. Berlin.** 1986. Cloning and characterization of a lysine decarboxylase gene from *Hafnia alvei*. Mol. Gen. Genet. **203**:177–184.
20. **Foster, J. W., and Z. Aliabadi.** 1989. pH-regulated gene expression in *Salmonella*: genetic analysis of *aniG* and cloning of the *earA* regulator. Mol. Microbiol. **3**:1605–1615.
21. **Gale, E. F.** 1940. The production of amines by bacteria. 1. The decarboxylation of amino acids by a strain of *Bacterium coli*. Biochem. J. **34**:392–413.
22. **Gale, E. F.** 1946. The bacterial amino acid decarboxylases. Adv. Enzymol. **6**:1–32.
23. **Gilman, M.** 1987. Preparation of bacterial RNA, p. 4.4.2.–4.4.4. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 1. John Wiley & Sons, Inc., New York.
24. **Goodrich, J. A., M. L. Schwartz, and W. R. McClure.** 1990. Searching for and predicting the activity of sites for DNA binding proteins: compilation and analysis of the binding sites for *Escherichia coli* integration host factor (IHF). Nucleic Acids Res. **18**:4993–5000.
25. **Hanatani, M., H. Yazyu, S. Shiota-Niiya, Y. Moriyama, H. Kanazawa, M. Futai, and T. Tsuchiya.** 1984. Physical and genetic characterization of the melibiose operon and identification of the gene products in *Escherichia coli*. J. Biol. Chem. **259**:1807–1812.
26. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15**:2343–2361.
27. **Hein, J.** 1990. Unified approach to alignment and phylogenies. Methods Enzymol. **183**:626–644.
28. **Higgins, C. F., C. J. Dorman, and N. Ni Bhriain.** 1990. Environmental influences on DNA supercoiling: a novel mechanism for the regulation of gene expression, p. 421–432. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
29. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer.** 1988. A physiological role for DNA supercoiling in the osmotic control of gene expression in *S. typhimurium* and *E. coli*. Cell **52**:569–584.
30. **Kashiwagi, K., S. Miyamoto, F. Suzuki, H. Kobayashi, and K. Igarashi.** 1992. Excretion of putrescine by the putrescine-ornithine antiporter encoded by the *potE* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89**:4529–4533.
31. **Kashiwagi, K., T. Suzuki, F. Suzuki, T. Furuchi, H. Kobayashi, and K. Igarashi.** 1991. Coexistence of the genes for putrescine transport protein and ornithine decarboxylase at 16 min on *Escherichia coli* chromosome. J. Biol. Chem. **266**:20922–20927.
32. **Kingston, R. E.** 1987. Primer extension, p. 4.8.1.–4.8.3. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 1. John Wiley & Sons, Inc., New York.
33. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for the rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
34. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
35. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
36. **Lawrence, C. B., T. Y. Shalom, and S. Honda.** 1989. EuGene: a software package for nucleotide and protein sequence analysis for UNIX systems. Molecular Biology Information Resource, Department of Cell Biology, Baylor College of Medicine, Houston, Tex.
37. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Matsuyama, S., and S. Mizushima.** 1987. Novel *rpoA* mutation that interferes with the function of OmpR and EnvZ, positive regulators of the *ompF* and *ompC* genes that code for outer-membrane proteins in *Escherichia coli* K-12. J. Mol. Biol. **195**:847–853.
39. **Melnykovych, G., and E. E. Snell.** 1958. Nutritional requirements for the formation of arginine decarboxylase in *Escherichia coli*. J. Bacteriol. **76**:518–523.
40. **Meng, S.-Y., and G. N. Bennett.** 1992. Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low extracellular pH. J. Bacteriol. **174**:2659–2669.
41. **Meng, S.-Y., and G. N. Bennett.** 1992. Regulation of the *Escherichia coli cad* operon: location of a site required for acid induction. J. Bacteriol. **174**:2670–2678.
42. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Moore, R. C., and S. M. Boyle.** 1990. Nucleotide sequence and analysis of the *speA* gene encoding biosynthetic arginine decarboxylase in *Escherichia coli*. J. Bacteriol. **172**:4631–4640.
44. **Morris, D. R., and E. A. Boeker.** 1983. Biosynthetic and biodegradative ornithine and arginine decarboxylases from *Escherichia coli*. Methods Enzymol. **94**:125–134.
45. **Ni Bhriain, N., C. J. Dorman, and C. F. Higgins.** 1989. An overlap between osmotic and anaerobic stress responses: a potential role for DNA supercoiling in the coordinate regulation of gene expression. Mol. Microbiol. **3**:933–942.
46. **Pettijohn, D. E.** 1988. Histone-like proteins and bacterial chromosome structure. J. Biol. Chem. **263**:12793–12796.
47. **Popkin, P. S., and W. K. Maas.** 1980. *Escherichia coli* regulatory mutation affecting lysine transport and lysine decarboxylase. J. Bacteriol. **141**:485–492.
48. **Pruss, G. J., and K. Drlica.** 1989. DNA supercoiling and prokaryotic transcription. Cell **56**:521–523.
49. **Rescei, P. A., and E. E. Snell.** 1972. Histidine decarboxylaseless mutants of *Lactobacillus* 30a: isolation and growth properties. J. Bacteriol. **112**:624–626.
50. **Rodriguez, R. L., and R. C. Tait.** 1983. Recombinant DNA techniques: an introduction, p. 45–46. Addison-Wesley Publishing Co., Reading, Mass.
51. **Rowland, G. C., P. M. Giffard, and I. R. Booth.** 1985. *phs* locus of *Escherichia coli*, a mutation causing pleiotropic lesions in metabolism, is an *rpoA* allele. J. Bacteriol. **164**:972–975.
52. **Sabo, D. L., and E. H. Fischer.** 1974. Chemical properties of *Escherichia coli* lysine decarboxylase including a segment of its pyridoxal 5′-phosphate binding site. Biochemistry **13**:670–676.

52a. **Shi, X.** Personal communication.
53. **Shi, X., B. C. Waasdorp, and G. N. Bennett.** 1993. Modulation of acid-induced amino acid decarboxylase gene expression by *hns* in *Escherichia coli*. J. Bacteriol. **175**:1182–1186.
54. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53**:85–96.
55. **Slonczewski, J. L., T. N. Gonzalez, F. M. Bartholomew, and N. J. Holt.** 1987. Mu d-directed *lacZ* fusions regulated by low pH in *Escherichia coli*. J. Bacteriol. **169**:3001–3006.
56. **Smith, R. F., and T. F. Smith.** 1990. Automatic generation of primary sequence patterns from sets of related protein sequences. Proc. Natl. Acad. Sci. USA **87**:118–122.
57. **Tabor, C. W., and H. Tabor.** 1985. Polyamines in microorganisms. Microbiol. Rev. **49**:81–99.
58. **Waston, N., D. S. Dunyak, E. L. Rosey, J. L. Slonczewski, and E. R. Olson.** 1992. Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. J. Bacteriol. **174**:530–540.
59. **Webster, C., K. Kempsell, I. Booth, and S. Busby.** 1987. Organization of the regulatory region of the *Escherichia coli* melibiose operon. Gene **59**:253–263.