JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2659–2669
0021-9193/92/082659-11$02.00/0

Vol. 174, No. 8

Nucleotide Sequence of the *Escherichia coli cad* Operon: a System for Neutralization of Low Extracellular pH

SHI-YUAN MENG AND GEORGE N. BENNETT*

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

Received 30 September 1991/Accepted 8 February 1992

Lysine decarboxylase of *Escherichia coli* has been the subject of enzymological studies, and the gene encoding lysine decarboxylase (*cadA*) and a regulatory gene (*cadR*) have been mapped. This enzyme is induced at low pH in the presence of lysine and achieves maximal level under anaerobic conditions. The induction of lysine decarboxylase increases the pH of the extracellular medium and provides a distinctive marker in tests of clinical strains. We report the sequence of the *cad* operon encoding lysine decarboxylase, a protein of 715 amino acids, and another protein, CadB, of 444 amino acids. The amino acid sequence of lysine decarboxylase showed high homology to that of the lysine decarboxylase of *Hafnia alvei* with less homology to the sequence of *speC*, which encodes the biosynthetic ornithine decarboxylase of *E. coli*. The *cadA* and *cadB* genes were separately cloned and placed under the control of *lac* and *tac* promoters, respectively, to facilitate independent study of their physiological effects. The *cadB* gene product had a mobility characteristic of a smaller protein on protein gels, analogous to that found for some other membrane proteins. The CadB sequence showed homology to that of ArcD of *Pseudomonas aeruginosa*, encoding an arginine/ornithine antiporter. Excretion studies of various strains, the coinduction of *cadB* and *cadA*, and the attractive physiological role for an antiport system led to a model for the coupled action of *cadA* and *cadB* in uptake of lysine, the reduction of H^+ concentration, and excretion of cadaverine.

Two types of amino acid decarboxylases exist in *Escherichia coli*. Biosynthetic enzymes are involved in the synthesis of polyamines and are expressed at low levels regardless of pH variation (56). Biodegradative enzymes such as lysine decarboxylase are strongly induced at acidic pH (5, 49, 56) and hence have a possible role in maintaining pH homeostasis or extending the growth period by detoxification of the extracellular medium (20). Although it has been stated elsewhere that the cadaverine generated from decarboxylation of L-lysine is not further metabolized for energy production but is excreted out of the cell as part of a countermeasure to acidic environment (49), no direct detailed analysis of this aspect has been reported. Unlike arginine and ornithine decarboxylases, two types of enzymes that have been well characterized previously (56), only the inducible form of lysine decarboxylase has been analyzed previously (49, 50), although some evidence has indicated the presence of a pH-constitutive form of lysine decarboxylase in *E. coli* (24, 64).

Mutants of *cadA* have been isolated, and the gene was first mapped to around 92 min on the *E. coli* genome (57). Subsequent detailed mapping of in vivo Mu dX operon fusion strains deficient in lysine decarboxylase activity and mini-Mu clones revealed that *cadA* was at 93.7 min (5). In studies of protein expression from the plasmid pLC4-5, it was found that *cadA* and *lysU* were both expressed (59); however, the orientation and order relative to the surrounding genes were not established. We report here the complete sequence of the *cad* operon, including *cadA*, the gene encoding the inducible lysine decarboxylase, and *cadB*, an upstream gene whose gene product appears to be homologous to the ArcD protein of *Pseudomonas aeruginosa* (34). The gene *arcD* is in an operon which encodes enzymes for using arginine as an energy source for motility and slow growth under anaerobic, nitrate-free conditions (60). ArcD has been proposed as an arginine/ornithine antiporter (22). From the similarities between the *cadBA* and *arcDABC* operons, and on the basis of our experimental results, we propose that a similar lysine/cadaverine antiporter also exists in *E. coli* and that CadB plays an essential role in it.

* Corresponding author.

MATERIALS AND METHODS

Materials. Restriction enzymes and T4 DNA ligase were purchased from Promega Corp., New England Biolabs, Inc., and Bethesda Research Laboratories, Inc., and were used according to the recommendations of the manufacturers. Subclones were sequenced with the Sequenase Version 2.0 sequencing kit from United States Biochemical Corp. with ^{35}S-dATP from NEN Research Products. Universal primer and −40 primer were provided by the manufacturer; other oligoprimers (18- to 20-mers) for DNA sequencing and polymerase chain reactions (PCRs) were synthesized with a Biosearch model synthesizer by K. Muthukrishnan in the Department of Biochemistry and Cell Biology, Rice University. *trans*-^{35}S protein label was purchased from ICN Biomedicals, Inc. The GeneAmp DNA amplification reagent kit was purchased from Perkin-Elmer Cetus. PCR amplification conditions were as suggested by the manufacturer.

Bacterial strains, plasmids, and media. Bacterial strains and plasmids are listed in Table 1. Modified Falkow lysine decarboxylase medium was described elsewhere (37).

Recombinant DNA techniques. All cloning experiments were conducted according to standard procedures (36). DNA fragments prepared from pLC4-5 and pKER65 were subcloned into phagemid pEMBL8$^+$, pEMBL9$^+$, or pBGS18$^+$. Shotgun cloning as well as cloning of specific fragments eluted from polyacrylamide gels with buffer X (37) was used to prepare suitable subclones for sequencing.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Reference
E. coli strains		
71-18	(*lac-proAB*) (F′ *proAB lacI*^{q} *Z* Δ*M15*)	15
CSR603	F$^-$ *phr-1 recA1 uvrA6 supE44*	52
CSR603F′	CSR603 (F′ Tn*10 proAB lacI*^{q} *Z* Δ*M15*)	This work
GNB581	MC4100 *cadA*::Mu dX (Ap^r *lac*)	5
GNB8385	MC4100 *cadB*::Mu dX (Ap^r *lac*)	5
GNB8385K	MC4100 *cadB*::Mu dI *1734* (Km^r *lac*)	5
MC4100	(*arg-lac*)U169 *rps150 relA1*	11
XL1-blue	*lac* (F′ Tn*10 proAB lacI*^{q} *Z* Δ*M15*)	10
Plasmids		
pBGS18$^+$	Km^r	54
pCADA	*cadA* Km^r	This work
pCADB	*cadB* Ap^r	This work
pEMBL8$^+$	Ap^r	15
pEMBL9$^+$	Ap^r	15
pKER65	Mu *d5005 cad*$^+$ *mel*$^+$ *adi*$^+$	5
pLC4-5	*lysU*$^+$ *cadA*$^+$	14

Plasmids bearing inserts were identified as white colonies on plates containing Luria broth, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μg/ml), and IPTG (isopropyl-β-D-thiogalactopyranoside) (40 μg/ml) plus ampicillin (30 μg/ml) or kanamycin (50 μg/ml). Plasmid DNA was isolated and subjected to restriction enzyme analysis (36).

DNA sequence analysis. Synthesis of single-stranded DNA was induced by infecting the host strain 71-18 bearing the recombinant phagemid with phage IR1 (16). Sequencing was carried out by the modified dideoxy chain termination method as described by U.S. Biochemicals. Synthesized oligonucleotide primers based on the known sequences were used to bridge the gap regions. DNA sequences from autoradiograms were read manually and were analyzed with MacVector sequence analysis software (International Biotechnology, Inc.). The Eugene data bank system provided by the Molecular Biology Information Resources at the Baylor College of Medicine was used for homology searches and prediction of secondary structure.

Protein expression of *cadA*. An overnight Luria broth culture of strain MC4100 harboring pBGS18$^+$ or pCADA was grown to an optical density at 600 nm of 0.3 in modified Falkow lysine decarboxylase medium at pH 8 with agitation and in the presence of IPTG and kanamycin, a noninducing condition for the pH-regulated chromosomal *cadA* gene. Total-cell protein extracts were separated on a 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and stained by Coomassie brilliant blue.

Cloning of the *cadB* gene by PCR. The total sequence upstream from *cadA* present in the insert pSM10 (37) was determined. An open reading frame (ORF) (1,332 bp long) was found and designated *cadB*. A forward primer aggagaagaCCATGGgttctgcc corresponding to the N terminus with two mismatches (underlined) and a backward primer cgCTCGAGatgaaaggaggagcctcg corresponding to a region beyond the C terminus of the *cadB* gene were designed to contain an *Nco*I site (capital letters) and an *Xho*I site (capital letters), respectively, for insertion of the PCR fragment into pPV9$^+$700 (61) such that *cadB* is under the control of the *tac* promoter without any interference from the *cad* upstream regulatory sequence. This manipulation introduced only one base pair change in the second codon of *cadB*: a change from AGT (Ser) to GGT (Gly).

Expression of *cadB* gene product in maxicells. The attempt to express the *cadB* gene in strain CSR603 was hampered by extremely slow growth. Therefore, a *lacI*^{q} gene on an F factor was introduced from strain XL1 to CSR603 by mating. Successful conjugants (CSR603F′) were selected on Luria broth plates with streptomycin (25 μg/ml) and tetracycline (15 μg/ml). Transformed CSR603F′ cells were grown in special medium and were UV irradiated as described by Sancar et al. (52). The *tac* promoter was induced with IPTG 30 min prior to labeling with *trans*-^{35}S. Samples were analyzed by SDS-PAGE and autoradiography.

Cadaverine excretion assay. Strains were grown in modified Falkow lysine decarboxylase media buffered with 100 mM MES (morpholineethanesulfonic acid) (pH 5.5) or with 100 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 8) as described elsewhere (37). Anaerobic cultures were grown in screwcap test tubes (16 by 125 mm) containing 10 ml of modified Falkow lysine decarboxylase medium and were incubated at 37°C without shaking. When cell density reached an optical density at 600 nm of 0.3, IPTG was added to induce the expression of *cadA* or *cadB* carried by plasmid pCADA or pCADB, respectively. The cells were pelleted after the absorbance reached 0.65 to 0.7, and 1 ml of each supernatant was tested for cadaverine content. All reagents and procedures used for the cadaverine assay were as described by Phan et al. (41), except that the reaction temperature was changed from 40 to 43°C. A 1.02×10^{-2} M concentration of TNBS (2′,4′,6′-trinitrobenzylsulfonic acid) was added to react with cadaverine, lysine, and trace amounts of other amines and amino acids at 43°C for 5 min. The colored product, N,N′-bistrinitrophenylcadaverine (TNP-cadaverine), was separated from N,N′-bistrinitrophenyllysine by extraction with 2 ml of toluene. The A_{340} of the extract was read. In order to verify that other trace amounts of amines and amino acids present in the supernatant did not affect the validity of cadaverine excretion assay by the spectrophotometric method, appropriate medium blanks were used. Also, in a separate specificity experiment, [U-^{14}C]lysine (1.5 μCi/ml) was added to the culture 10 min before the cells were pelleted. Subsequent reaction of the supernatant with TNBS and extraction with toluene were conducted as described above. An aliquot of the extracts was mixed with scintillation fluid and counted.

Nucleotide sequence accession number. The nucleotide sequence of the *cad* operon has been deposited in GenBank, accession no. M76411.

RESULTS

Localization of *cadA* gene. Preliminary data indicated that *cadA* was close to the right end of the insert in pLC4-5, as shown in Fig. 1. Therefore, fragments covering a region of about 3 kb of this end were subcloned and sequenced. Previous Southern hybridization mapping experiments with GNB581 and GNB8385 (5), two Mu dX fusion strains deficient in lysine decarboxylase activity, had shown that the insertion positions of Mu dX were in the 0.6-kb *Pst*I-*Xho*I fragment and in the 0.9-kb *Eco*RI fragment, respectively (Fig. 1). The sequence revealed a 715-amino-acid polypeptide whose N terminus matched the known peptide sequence of the N-terminus (Fig. 2). The active-site peptide (50) also was found (corresponding to nucleotide positions 2984 to 3028). Only one glutamine residue at the active site did not match the originally reported glutamic acid residue, which

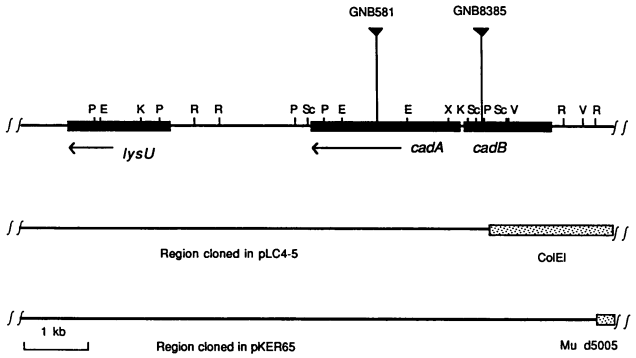


FIG. 1. Chromosomal regions cloned in pLC4-5 and pKER65. The coding regions of *cadB*, *cadA*, and *lysU* are covered by filled rectangles, and the directions of transcription are indicated by arrows. The insertion positions of Mu dX in Mu *lac* fusion strains GNB581 and GNB8385 are indicated by filled triangles. Dotted boxes are vector sequences of ColE1 and Mu d5005. Regions beyond each side of ∫∫ are not shown in this figure. Relevant restriction sites are as follows: B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; R, *Eco*RV; Sc, *Sca*I; V, *Pvu*II; X, *Xho*I.

can be explained by loss of the amino group during chemical degradation of the polypeptide (5, 50). Because the start codon of *cadA* was close to the end of the chromosomal insert in pLC4-5, the plasmid did not contain a complete upstream regulatory region (37); therefore, pKER65 (5), an in vivo mini-Mu constructed plasmid bearing *cadA* with a longer upstream region, was used for sequencing studies. There was no variation in the overlapping sequenced region because of strain differences between pLC4-5, originally constructed from *E. coli* CS520 (14), and pKER65, constructed from *E. coli* MC1040-2 (13). The sequence also agrees in the overlapped region with that found by Watson et al. (62), who have analyzed the N-terminal amino acid coding region of *cadA* and a large upstream sequence containing *cadB* and *cadC*. The single termination codon TAA is followed by a hairpin structure typical of a rho-independent transcription terminator (43). A Shine-Dalgarno sequence (GGAGA) is 7 bp upstream of the translation start codon.

The plasmid pCADA containing the entire *cadA* gene together with its ribosome binding site was made by cloning a 2.6-kb *Sca*I fragment from pLC4-5 into the *Sma*I fragment on the plasmid pBGS18$^+$ (54), placing *cadA* under the control of the *lacUV5* promoter. Upon induction by IPTG, this plasmid made a protein the size of inducible lysine decarboxylase (81 kDa) (Fig. 3a), which was also seen in a maxicell system (Fig. 3b). Plasmid pCADA also expressed a functional lysine decarboxylase upon induction by IPTG, as determined by lysine decarboxylase indicator medium (17) and cadaverine excretion assay.

Amino acid sequence of biodegradative lysine decarboxylase. The amino acid composition and molecular weight of inducible lysine decarboxylase deduced from the DNA sequence are quite similar to the results reported from chemical degradation (50). Kyte and Doolittle (32) hydrophobicity analysis is consistent with the fact that the inducible lysine decarboxylase is a soluble cytosolic protein. The predicted profile of the secondary structure as proposed by the method of Garnier et al. (23) indicated some similarities among decarboxylases. Two sequences in the GenBank were found to be significantly similar to those of the biodegradative lysine decarboxylase of *E. coli*: biosynthetic ornithine decarboxylase (*speC*) of *E. coli* (9a; GenBank accession no. M33766) and lysine decarboxylase of *Hafnia alvei* (18; GenBank accession no. X03774).

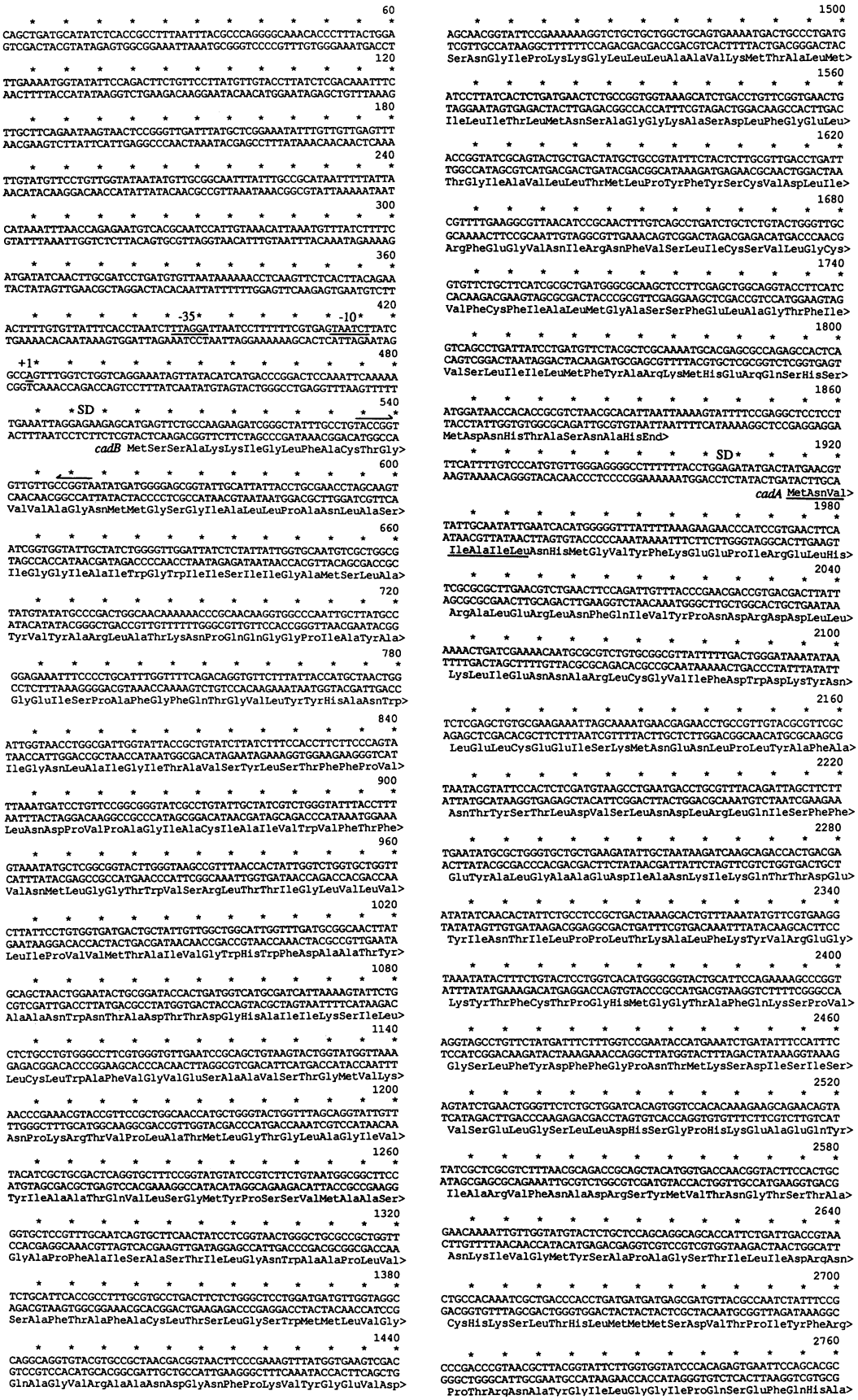
Although *H. alvei* and *E. coli* are distantly related among *Enterobacter* species (55), their lysine decarboxylase nucleotide sequences have 73% homology (number of the same nucleotide residues/total number of nucleotide residues of the *E. coli* lysine decarboxylase gene) and the amino acid sequences have an 80% homology (number of identical amino acid residues/total number of amino acid residues of *E. coli* lysine decarboxylase) (Fig. 4); however, only 41% of the amino acids use the same codon. The amino acids and spacing exhibit strong regions of homology except for a small portion at the carboxyl end. The two enzymes catalyze the same decarboxylation reaction of lysine to cadaverine. The difference is that, in *E. coli*, the basal level of lysine decarboxylase is very low, but upon induction at acidic pH and under anaerobic conditions the expression rises sharply (20); on the other hand, in *H. alvei*, the production of the enzyme is independent of pH changes (51). This may suggest an explanation for the observation that although these two lysine decarboxylase genes are very similar, no similarity was found in the flanking regions.

Biosynthetic ornithine decarboxylase catalyzes the decarboxylation of ornithine to putrescine in the polyamine biosynthesis pathway. Although the overall amino acid sequences of biodegradative lysine decarboxylase and biosynthetic ornithine decarboxylase have only 29% homology, there are several more conserved domains, including the pyridoxal 5′-phosphate binding site. The conserved domains are aligned to biodegradative lysine decarboxylase throughout the sequence, except for the first 120 N-terminal amino acid residues (Fig. 4).

Identification of the *cadB* gene and its protein product. That the pH-regulated promoter of *cadA* could not be detected within 600 bp upstream from *cadA* by earlier primer extension and by promoter cloning experiments led us to suspect that *cadA* might reside in a multigene operon. Indeed, sequencing of the upstream region revealed another ORF, which we have designated *cadB*. This ORF would encode a polypeptide of 444 amino acids (Fig. 2). To identify its gene product and understand its physiological role, a PCR fragment containing the *cadB* gene was amplified and cloned into multicopy plasmid pPV9$^+$700 (61); the resulting plasmid was designated pCADB. The higher mobility (Fig. 3b) relative to the deduced molecular mass of CadB (~47 kDa) suggested that this protein may have an atypical structure, and this might correlate with the proposed membrane role of the protein, since certain membrane proteins have been observed to migrate as significantly smaller proteins in similar gel systems (34).

In order to check for proper retention of the coding triplet phase of the *cadB* sequence, several in phase protein fusions were made at various points within *cadB* from very close to the beginning of the gene to one fusion in the third-to-last codon. These *cadB*::*lac* protein fusions were assayed for their pH responses, and similar levels of induction were observed among the *cadB*::*lac* protein fusion constructs (37). This supported the idea that the entire ORF was being translated in the phase shown and that the true size of the protein is ~47 kDa. Its highly hydrophobic profile (Fig. 5) suggested that CadB was a membrane-associated protein.

Homology between CadB of *E. coli* and ArcD of *P. aeruginosa*. In addition to some similarities between short stretches of the CadB sequence and the sequences of several integral membrane proteins from a GenBank search, the


-35*
-10*
+1*
SD
cadB MetSerSerAlaLysLysIleGlyLeuPheAlaCysThrGly>
cadA MetAsnVal>
IleAlaIleLeu

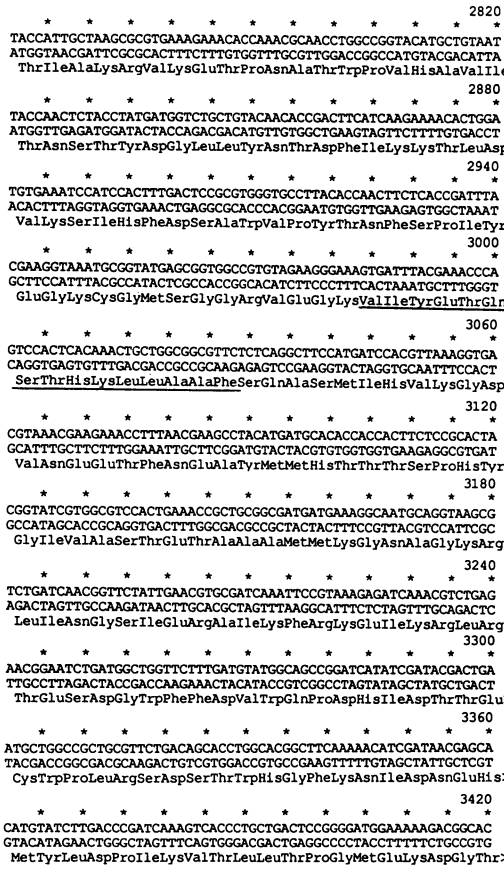


FIG. 2. Nucleotide and deduced amino acid sequences of the *cadBA* operon. The total sequence shown starts from a *Pvu*II site and ends with a *Pst*I site. The numbers on the right side indicate the last base pair at the end of each row of the double-stranded DNA sequence; bp 1 to 131 are part of the C-terminal coding region of *cadC* (62). The transcriptional start point (+1) and the −10 and −35 sequences of the *cad* operon were identified by Watson et al. (62). The CadB and CadA amino acid sequences are translated from the top strand and are positioned below each codon. The Shine-Dalgarno sequence (SD) of each ORF is indicated. The N-terminal and active site sequences of lysine decarboxylase used to identify the *cadA* gene are underlined. The stem-loop structure 35 bp downstream from the *cadB* start codon is indicated by a pair of arrows. Also indicated is the GC-rich hairpin structure downstream from the *cadA* coding sequence, and this is followed by a T region as shown.

most significant finding was the homology with ArcD of *P. aeruginosa*. Not only do they have comparable sizes (444 versus 482 amino acids), but the similarity is observed throughout their sequences. Although the homology was not reflected by using identical amino acid residues, a very close relatedness between CadB and ArcD was apparent. The arrangement of CadB and ArcD membrane spanning domains as revealed by Kyte and Doolittle hydropathy graphs (32) strongly suggested a similar natural folding pattern for both CadB and ArcD (Fig. 5), and therefore they might perform a similar function or at least adopt a similar structure in their physiological roles.

Cadaverine excretion. Upon full induction of the *cad* operon, large quantities of lysine decarboxylase are produced, and if high amounts of lysine are present in the medium, one would expect significant amounts of cadaverine to be formed under the induced condition, since no other pathways to further metabolize cadaverine in *E. coli* are known and excessive levels of some polyamines can be toxic (56). Cadaverine was speculated to be excreted from the cell on the basis of some indirect evidence (27), and cadaverine was isolated from the medium (19). The discovery of the *cadB* gene ignited our interest to test for release of cadaverine using a colorimetric assay originally designed for assaying lysine decarboxylase activity assay (41). Two Mu *lac* fusion strains, GNB581 (*cadA*::*lac*Ap^r) and GNB8385 (*cadB*::*lac*Ap^r), have phenotypes of CadB$^+$ CadA$^-$ and CadB$^-$ CadA$^-$, respectively. Together with MC4100 (CadB$^+$ CadA$^+$) and in combination with plasmids pCADA and pCADB, various phenotypes of CadB and CadA could be manipulated. For the convenience of drug selection, GNB8385K (*cadB*::*lac* Km^r) was used to harbor plasmid pCADB. The strategy to overcome growth inhibition by induced pCADB on CSR603 in maxicell experiments was implemented again by introducing the *lacI*^{q} gene on an F factor into respective strains through mating. The presence

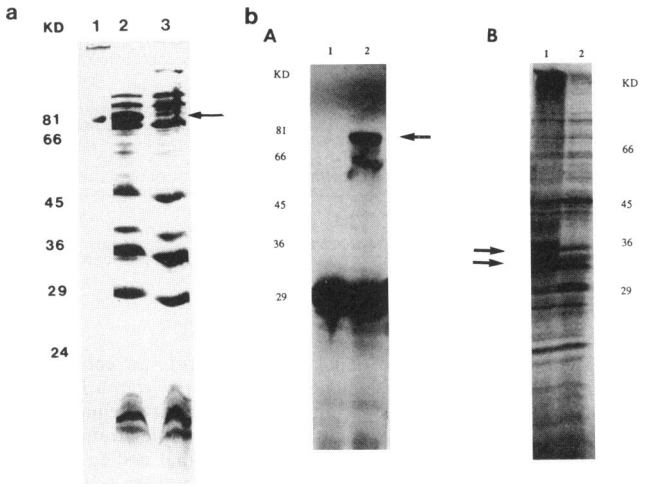


FIG. 3. (a) Protein expression of lysine decarboxylase from pCADA. Strain MC4100 harboring plasmid pCADA (lane 2) or vector pBGS18$^+$ (lane 3) was grown aerobically in modified Falkow lysine decarboxylase medium at pH 8 in the presence of IPTG. The IPTG-induced expression of *cadA* from pCADA (lane 2) is indicated by an arrow. This band comigrated with the purified *E. coli* lysine decarboxylase purchased from Sigma Chemical (lane 1). No expression of *cadA* from pBGS18$^+$ was observed (lane 3). (b) Protein expression of *cadA* and *cadB* from pCADA and pCADB by maxicells. ^{35}S-labeled protein extracted from UV-irradiated maxicell CSR603 bearing pBGS18$^+$ (gel A, lane 1) or pCADA (gel A, lane 2) was separated on an SDS-polyacrylamide gel as described in Materials and Methods. The same experiment was done with CSR603F′ harboring pCADB (gel B, lane 1) or pPV9$^+$700 (gel B, lane 2). The gene products of *cadA* and *cadB* are indicated by arrows, and the positions of protein markers are shown. Two polypeptides expressed from pCADB (gel B, lane 1) but not present in pPV9$^+$700 (gel B, lane 2) migrated significantly faster than would a protein with the predicted molecular mass of CadB (~47 kDa). The protein of the faster-migrating band may be a processed form of the more slowly migrating protein.

```
: MNVIAILNHMGVYFKEE.PIRELHRALERLNFQIVYPNDRDDLLKLIENNARLCGVIFDW
: --I---M-DLSA-----.-L----QE--KEG-R-A--K--N---------S---------
: -GQGFPPCPVFLLPRNGfALMKSMNIAASSELVSRLSSH-RVVALGDTDFTDVAA-VITA

: DKYNLELCEEISKMNENLPLYAFANTYSTLDVSLNDLRLQISFFEYALGAAEDIANKIKQ
: -------SA---EL-KL--I-----------NMS----NVR-------S-Q---T--R-
: ADSRSGILALLKRTGFH--VFLYSEHAVE-PAGVTAV.........INGN-QQWLELES

: TTDEYINTILPPLTKALFKYVREGKYTFCTPGHMGGTAFQKSPVGSLFYDFFGPNTMKSD
: S--Q--D--------------K-E---V----------D-------------E---R--
: AACQ-EENL---FYDT-TQ--EM-NS--AC---QH-AF-K-H-A-RH------E-VFRA-

: ISISVSELGSLLDHSGPHKEAEQYIARVFNADRSYMVTNGTSTANKIVGMYSAPAGSTIL
: ------------------RD--E----T-------I----------------S---A---
: MCNADVK--D--I-E-SA-D-QKFA-K--H--KT-F-L----A---V-TNALLTR-DLV-

: IDRNCHKSLTH.LMMMSDVTPIYFRPTRNAYGILGGIPQSEFQHATIAKRVKETPNATWP
: ----------.-----N-V-V-L-----------------TR-S-EEK--N-------
: F---N---NH-gALIQAGA--V-LEAS--PF-FI---DAHC-NEEYLRQQIRDVAPEKAD

: VH.....AVITNSTYDGLLYNTDFIKKTLDVKS..IHFDSAWVPYTNFSPIYEGKCGMSG
: --.....-V--------F---EY--N------..-----------H---Q--A----
: LPrpyrl-I-QLG----TV--ARQVID-VGHLCdy-L------G-EQ-I-MMADSSPLLL
                    ↓
: GRVEGKV.IYETQSTHKLLAAFSQASMIHVKGDVNEET........FNEAYMMHTTTSPH
: E--P--I.----------------------EI----.........---------S----
: ELN-NDPg-FV---V--QQ-G---T-Q--K-DNHIRGQarfcphkrL-N-F-L-AS---F

: YGIVASTETAAAMMKGNAGKRLINGSIERAIKFRKEIKRLRTESDGWFFDVWQPDHIDTT
: ------------------------------R-----R----------------N--EV
: -PLF-ALDVN-KIHE-ES-R--WAECV-IG-EA--A-LARCKLFRPFIPP-VDGKLWQDY

: ECWPLRSD.........STWHGFKNIDNEHMYLDPIKVTLLTPGMEKD.GTMSDFGIPASI
: A----NPR.........NE----P----D---------------LSPN.--LEEE------
: PTSV-A--xrffsfepgAK----EGYAADQYFV--C-LL-T---IDAEt-EY----V--T-

: VAKYLDEHGIVVEKTGPYNLLFLFSIGIDKTKALSLLRALTDFKRAFDLNLRVKNMLPSL
: -S---------I-----------------------------------VY---------V----
: L-H--R-N---P--CDLNSI---LTPAESHE-LAQ-VAM-AQ-EQHIEDDSPLVEV---V

: YREDPEFYENMRIQELAQNIHKLIVHHNLPDLMYRAF..EVLPTMVMTPYAAFQKELHGM
: -N-A-D--KE--------G--A-VK-------------.----KL-----HD---E-VR-N
: -NKY-VR-RDYTLRQ-C-EM-D-Y-SFDVK--QKAM-rqQSF-SV--N-QD-HSAYIR-D

: TEEVYLDEMVGRINANMILPYPPGVPLVMPGEMITEESRPVLEFLQMLCEIGAHYPGFET
: I-PCA--D-L-KVS-----------V------L-K------S-----------------
: V-L-RIRDAE---A-EGA-------LC-V---VWGG...A-QRYFLA-E-GVNLL---SP

: DIHGAYRQ...ADGRYTVKVLKE.....................ESKK  E. coli  LDC
: ----VH-Dg..-T-K-M-V---QgadepgdkpsdtvkkapgkkpsaAK-S  H. alvei LDC
: ELQ-V-SE.tp---VKRLYGY.....................VL-   E. coli  ODC
```

FIG. 4. Sequence alignment of lysine decarboxylase of *H. alvei* and biosynthetic ornithine decarboxylase of *E. coli* to the biodegradative lysine decarboxylase of *E. coli*. The sequence alignment of three amino acid decarboxylases is shown. Amino acid residues of the lysine decarboxylase (LDC) of *H. alvei* (middle sequence) and the biosynthetic ornithine decarboxylase (ODC) of *E. coli* (bottom sequence) are replaced by dashes if they are the same as those of the biodegradative lysine decarboxylase of *E. coli* (top sequence). Gaps between sequences are filled with dots. Extra residues appearing in the gaps are indicated by lower-case letters. The lysine residue for binding of pyridoxal 5′-phosphate is indicated by an arrow.

of the large amount of *lac* repressor from the overproducing *lacI*^{q} allele would act to more fully repress expression of the *cad* gene under *lac* control on the high-copy-number plasmid. A summary of the results of cadaverine measurements is presented in Table 2. A large amount of cadaverine in the medium was detected at pH 5.5 but not at pH 8 and not in the absence of lysine decarboxylase. The IPTG-induced expression gave levels of cadaverine comparable to those found with pH induction (GNB581F′/pCADA versus MC4100F′). The expression of *cadA* was a prerequisite for the source of cadaverine. However, in the absence of CadB (GNB8385F′/pCADA versus MC4100F′ or GNB581F′/pCADA), the amount of cadaverine detected in the medium was significantly reduced. To ensure the specificity of the experiment, [U-C^{14}]lysine was used as the substrate and the amount of radioactive cadaverine was measured after a 10-min incubation. The results also showed a higher excretion of labeled cadaverine in the presence of CadB (data not shown). CadB appeared to facilitate the excretion of cadaverine at pH 5.5, but the effect of CadB was less conspicuous at pH 8.

Chromosomal location of the *cad* operon and its neighboring genes. Genes that have been localized in the 93.2- to 94-min region are *adi*, *melR*, *melAB*, *fumB*, *lysU*, *cadBA*, *cadC*, and *pheR* (5, 6, 13, 25, 42, 48, 62, 63). Among them, nine genes (underlined) have been sequenced. Figure 6 shows the relative positions of these genes by comparing their restriction enzyme maps with Kohara's *E. coli* genomic map (31, 48). The gene *lysU*, also present on pLC4-5, has recently been sequenced (13, 33). It is in the same orientation as *cadA*, on the basis of comparison of restriction maps. According to the restriction map, there is a space of about 2 kb between *cadA* and *lysU*. Immediately upstream from *cadB*, another ORF, *cadC*, was recently identified as a positive regulator of the *cad* operon (62).

DISCUSSION

In the study of amino acid decarboxylases of *E. coli*, one should distinguish biodegradative amino acid decarboxylases from those biosynthetic enzymes involved in polyamine biosynthesis: arginine decarboxylase (*speA*), ornithine decarboxylase (*speC*), *S*-adenosyl-L-methionine decarboxylase (*speD*), and diaminopimelic acid decarboxylase (*lysA*). The biosynthetic decarboxylases are produced in low amounts and are unaffected by variation in pH (56). In contrast, the expressions of the biodegradative decarboxylases—arginine decarboxylase (*adi*), glutamic acid decarboxylase (*gadS*), histidine decarboxylase, lysine decarboxylase

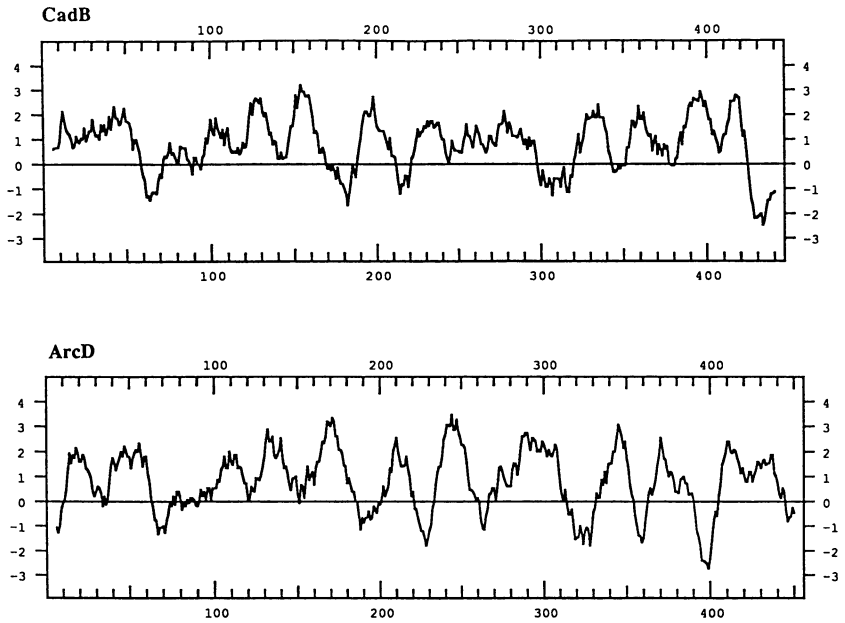


FIG. 5. Hydrophobicity profiles of CadB of *E. coli* and ArcD of *P. aeruginosa*. The hydrophobicity of the CadB and ArcD sequences is displayed as Kyte and Doolittle plots (32). Higher positive values indicate the more hydrophobic regions.

(*cadA*), and ornithine decarboxylase—are all induced at low pH and have special nutritional requirements (20). Among them, the inducible arginine, lysine, and ornithine decarboxylases exhibit similarities in overall structure, although they display individual characteristics (56). Although inducible arginine and lysine decarboxylases exist in almost all *E. coli* strains, inducible ornithine decarboxylase is absent in *E. coli* K-12 (2). The evolutionary relationship of these three amino acid decarboxylases has been discussed elsewhere on the basis of similarities and differences in physical and chemical properties in distribution (3) and on the basis of available sequence information (38).

Biosynthetic arginine decarboxylase (38) has no homology with biosynthetic ornithine decarboxylase (9a; GenBank accession no. M33766) or biodegradative lysine decarboxylase, and sufficient physical differences even between the two forms of arginine decarboxylases are known. On the other hand, the amino acid sequence of biosynthetic ornithine decarboxylase bears a strong resemblance to that of biodegradative lysine decarboxylase and provides further evidence that biosynthetic ornithine decarboxylase and biodegradative arginine, lysine, and ornithine decarboxylases share a common origin (3). Intriguingly, biodegradative arginine decarboxylase has a subunit structure more similar to that of biodegradative lysine decarboxylase than to that of biosyn-

TABLE 2. Cadaverine excretion assay[a]

Strain and pH condition	Amt of cadaverine excreted[b]	% of cadaverine content[c]	Phenotype[d]
pH 5.5			
MC4100F′	10.0	100	CadB$^+$ CadA$^+$
GNB581F′(pCADA)	8.2	82	CadB$^+$ <u>CadA</u>$^+$
GNB581F′	0.1	1	CadB$^+$ CadA$^-$
GNB8385KF′(pCADB)	0.3	3	<u>CadB</u>$^+$ CadA$^-$
GNB8385F′(pCADA)	2.0	20	<u>CadB</u>$^-$ <u>CadA</u>$^+$
GNB8385F′	0.04	0.4	CadB$^-$ CadA$^-$
pH 8.0			
MC4100F′(pCADB, pCADA)	1.3	13	<u>CadB</u>$^+$ <u>CadA</u>$^+$
MC4100F′(pCADB)	0.1	1	<u>CadB</u>$^+$ CadA$^-$
MC4100F′(pCADA)	0.8	8	<u>CadB</u>$^-$ <u>CadA</u>$^+$
MC4100F′	0.08	0.8	CadB$^-$ CadA$^-$

[a] The growth of cells and the cadaverine excretion assay are described in Materials and Methods. Cells with or without plasmids were grown anaerobically in modified Falkow lysine decarboxylase medium at pH 5.5 or 8.

[b] Expression of the plasmid-borne *cadB* or *cadA* gene was induced by IPTG, and the amount of cadaverine excreted into the medium was determined by measuring the optical density at 340 nm of TNP-cadaverine.

[c] The amount of cadaverine excreted into the medium is presented as the percentage of the cadaverine content in the growth medium (the CadB$^+$ CadA$^+$ strain value is 100%).

[d] The underlined phenotype indicates induction by IPTG as compared with the pH-induced phenotype.

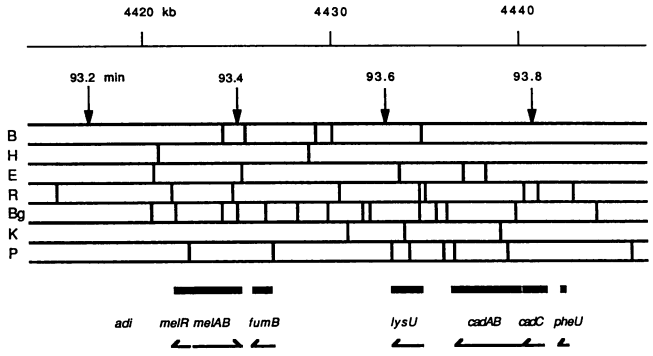


FIG. 6. Chromosomal localization of *cad* operon and its neighboring genes. The coordinates on the top line are positioned according to Kohara's map (31). The minute calibration unit follows that of Bachmann (6). The restriction map is that provided by Kohara (31). Restriction enzyme site designations are B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; and R, *Eco*RV.

thetic arginine decarboxylase. The high degree of similarity between the pH-inducible lysine decarboxylase of *E. coli* and the non-pH-inducible lysine decarboxylase of *H. alvei* might be explained by a loss of specific controls in the *H. alvei* case. The noninducible lysine decarboxylase may substitute for arginine decarboxylase as a component in polyamine synthesis in *H. alvei*, since biosynthetic arginine decarboxylase is absent in *H. alvei* (51). The existence of a second lysine decarboxylase has been observed in *E. coli*. Mutants with reduced lysine decarboxylase activity isolated from an *E. coli* polyamine auxotroph (24) and the inhibitory effect on putrescine argued against the possibility that the low level of activity might represent a basal level of the inducible lysine decarboxylase (64). The locus of a second lysine decarboxylase gene has not been mapped, and the purified protein has not been reported. Our preliminary Southern hybridization experiment using *cadA* to probe *E. coli* chromosomal DNA failed to identify a second region homologous to *cadA* under the conditions used.

The genes *lysU*, which encodes the thermoinducible lysyl-tRNA synthetase, and *cadA* are both present on pLC4-5 and are transcribed in the same orientation. That *lysU* can also be induced under the same conditions as *cadA* (28) has prompted consideration of the hypothesis that these genes may belong to the same operon (13). However, according to available information, these two genes are about 2 kb apart, and there is a sequence resembling a strong transcription terminator (ΔG of -26.6 kcal [1 cal = 4.184 J] calculated with the model of Tinoco et al. [58]) immediately downstream of *cadA*. The free energies of most hairpin loop transcription terminators are in the range of 20 to 30 kcal (43); therefore, it is unlikely that there is significant expression of genes downstream of this terminator. However, because of the magnitude of expression of *cadA*, the possibility of some carryover transcription to the *lysU* region from the *cadA* promoter cannot be completely excluded.

The control of intracellular pH in bacteria is complex and involves coordination of the activities of a number of ion transport systems (9). Although *E. coli* can grow with little difference in generation time over a pH range of 5.5 to 8.5 (21), the intracellular pHs of vigorously growing *E. coli* cells remain constant at pH 7.4 to 7.8 (53, 65). A rapid shift to low pH transiently lowers the internal pH, but it quickly recovers. Major factors required in maintaining pH homeostasis in response to acid challenge appear to include having a source of energy, the proton translocating ATPase, and the ability to transport potassium ion, but the complicated interrelationships among transport processes should not be underestimated (9). After the discovery of pH induction of amino acid decarboxylases, their role in a pH response was considered. In support of their importance were the observations of reduced ability of arginine decarboxylase mutants to grow at low pH (7) and the decrease in growth of a histidine decarboxylase *Lactobacillus* mutant at low pH (46). Proposed models of how the action of amino acid decarboxylases can allow further growth under acidic growth conditions have considered the production of CO_2 by the reaction, in light of the requirement of CO_2 for growth and as a compensatory mechanism for the loss of CO_2 at low pH by chemical equilibrium (8). Also considered has been the effect of the decarboxylase reaction on the intracellular pH in preventing overacidification (20). At low pH, the increase in the permeability of H^+, especially in the presence of the weak organic acids produced during fermentation, presents a problem in maintaining the appropriate electrochemical gradients. This conjecture for the role of decarboxylases is based on the fact that during the decarboxylase reaction, one H^+ is removed per molecule of lysine processed. Thus, the reaction could eliminate a large quantity of H^+ ions from the cell. Another aspect of the action of decarboxylases is the increase in the extracellular pH value due to the large-scale removal of lysine and H^+ with the concomitant production of a polyamine. Indeed, specific acid-base-indicating media which allow the screening of bacterial strains for the presence or absence of inducible arginine, lysine, or ornithine decarboxylases have been developed elsewhere (17). The rationale of the method is based precisely on the fact that decarboxylase-positive strains could prevent the color change caused by excessive acidification from glucose fermentation.

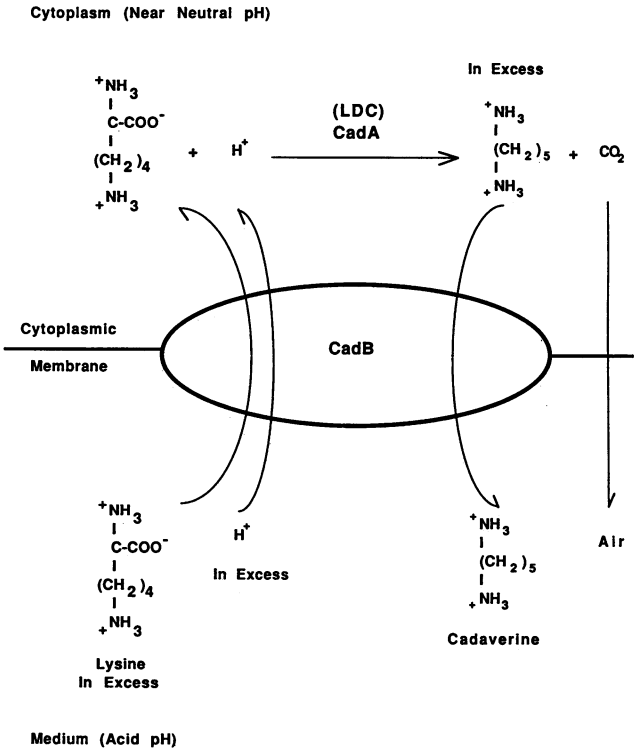


FIG. 7. Model for detoxification of extracellular high H^+ concentration by the CadB-CadA system. Upon encountering a low pH environment in a rich medium, the *cadBA* operon is highly induced in *E. coli* cells. The membrane protein CadB functions as a lysine/cadaverine antiporter, bringing in lysine and excreting cadaverine, the end product of the CadA (lysine decarboxylase [LDC])-catalyzed enzymatic reaction. In order to balance the electrochemical charge inside the cell, CadB may take in H^+ from the medium at the same time that it imports lysine into the cell, leaving other mechanisms to cope with the strain on intracellular pH. Alternatively, the action of lysine decarboxylase could remove H^+ from the intracellular environment that enters by other routes or by leakage under conditions of low pH. This model would propose that CadB functions best when the medium contains a high concentration of both lysine and H^+; this condition, together with the enzymatic reaction of CadA, would create an inward concentration gradient for lysine and H^+ as well as an outward concentration gradient for cadaverine and CO_2. The driving force for the lysine/cadaverine antiporter can solely depend on the concentration gradients described above without other energy expense. In this way, the extracellular H^+ entering the cell is neutralized inside the cell with minimum interference to cell metabolism.

In the proposed model (Fig. 7), lysine is taken in vigor-

ously and turned over quickly to cadaverine and CO_2. The driving force of this movement would be further aided if the end products can be rapidly removed from the system. Carbon dioxide can freely escape through the cell membrane to the atmosphere, and this property was used in identification of *cadR* and *cadA* mutants (57), but how does the cell deal with the excess amount of cadaverine? There are no enzymes in *E. coli* that normally catabolize cadaverine; logically, if the cell does not possess some kind of secretion device(s), the accumulation of cadaverine will not only disrupt the electrochemical equilibrium and eventually become toxic but will also slow the lysine decarboxylase reaction and therefore prevent further processing of H^+ and lysine. The existence of membrane protein CadB helps solve this basic problem. Although the excretion of polyamines has previously been studied with respect to K^+ and osmolarity (40) and polyamine transport systems have been investigated elsewhere (29, 30, 39), the relation of these studies to the pH response has not been thoroughly considered. An ability of CadB not only to facilitate the excretion of cadaverine but to act in such a way as to coordinate cadaverine excretion with lysine uptake makes an attractive model. In order to resolve this role for *cadB*, more detailed transport assays need to be undertaken. However, because of the similarity between CadB and ArcD of *P. aeruginosa*, it is tempting to speculate that CadB might constitute a lysine/cadaverine antiporter or at least part of it.

A hypothetical working model for maintaining pH homeostasis by the CadBA system is presented in Fig. 7. In this case, amino acid decarboxylases would play an ideal role in the efficient reduction of extracellular H^+. The source of the H^+ used in the decarboxylation could come from several routes: H^+ accumulated inside the cell by proton permeability or brought in by weak acids or via other transporters. An alternative possibility is that H^+ is brought in at the same time as lysine by the CadB system. Examples of concomitant uptake of H^+ together with a specific amino acid are known (1). The plausible explanation is that symport systems allow the accumulation of some amino acids and carbohydrates from the medium beyond the limitation of electrochemical equilibrium in uniport systems (35). The same strategy can also be adopted to raise the external pH, if the significance of H^+ uptake is considered. It may seem fruitless to bring in extra H^+ ions while the cell is suffering from acid stress, but if the external low pH can be slightly raised, the culture will benefit by extended growth. The presence of an antiporter which couples uptake of amino acid with release of the polyamine product would constitute a complete system for feeding substrate to the decarboxylase while removing product.

P. aeruginosa uses the arginine deiminase pathway to convert arginine to ornithine and carbamoylphosphate, which serves to generate ATP under anaerobic, nitrate-free conditions (60). A similar pathway also exists in *Streptococcus* spp., in which the arginine/ornithine exchange has been well studied previously (44). The speculation that CadB is a lysine/cadaverine antiporter is based on the observation that a $CadB^-$ strain showed significant decrease in cadaverine excretion even in the presence of CadA (lysine decarboxylase). The energy cost-free arginine/ornithine antiporter has been shown to exist in bacteria, and the driving force solely depends on the opposite concentration gradients of arginine and ornithine across the membrane (44). Similarly, the high level of lysine decarboxylase activity can also create a concentration gradient that would assist in providing the driving force to favor the antiport model. The attempt to detect cadaverine secretion from cells grown in minimal medium supplemented with 10 μM lysine (as opposed to >43 mM in the modified Falkow medium) was unsuccessful even with IPTG-induced expression of both *cadA* and *cadB* (data now shown). This might be explained as the necessity of a high concentration gradient at low pH for proper functioning of CadB. If the transporter behaves such that high external H^+ concentration increases the uptake of lysine and concomitant excretion of cadaverine, this would be very supportive of the coordinate role of the CadA CadB system in neutralizing H^+ from the medium.

Is there a specific lysine transport system that functions only for the purpose of raising the external pH, or does the expression of *cadB* modify the usual transport mechanism to a more efficient and energy cost-free antiport? The relation of *cadB* to previously studied lysine transport systems of *E. coli* (1, 45, 47) is unclear. The only known locus affecting the expression of *cadA* (and *cadB*) is the *cadR* gene, which has been mapped to 46 min (57). The spontaneous CadR mutant strains were selected by resistance to SAEC (*S*-aminoethyl cysteine), and they showed a lowered level of lysine transport and relieved a requirement for the presence of exogenous lysine for the maximal induction of *cadA* (45). SAEC-resistant strains have also been shown to excrete a large amount of lysine into the medium (26). However, *cadR* is not responsible for the pH and air regulation of *cadA* (4). One possible explanation is that *cadR* is the pH-constitutive lysine transport system or regulates these systems, which mainly provide lysine for protein synthesis, whereas *cadB* dominates the massive lysine uptake at low pH for the purpose of neutralizing H^+. This model is supported by the observation that normal lysine transport systems are repressible by lysine (12), where as *cadB* is further induced by lysine. The validity of this hypothesis awaits detailed analysis of the transport behavior of *cadB* and the elucidation of the sequence and function of the *cadR* locus.

Recent studies of the regulation of the *cad* operon by extracellular pH have located the site of pH regulation of the promoter (37) and shown the requirement for an upstream gene *cadC* in the pH response (62). Analysis of the mechanism of pH sensing and signal transduction can now be approached. Preliminary experiments with the inducible arginine decarboxylase indicate that *cadC* is not required for pH regulation of this gene although some mutations (i.e., *osmZ*) that affect both the lysine and the arginine decarboxylase systems have been isolated (data not shown).

ACKNOWLEDGMENTS

We thank Kevin Redding for mini-Mu *cad* constructs and K. Muthukrishnan for oligonucleotide synthesis.

This work was supported by NIH grant GM 039420.

REFERENCES

1. **Anraku, Y.** 1978. Active transport of amino acids, p. 171–219. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
2. **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.
3. **Applebaum, D. M., D. L. Sabo, E. H. Fischer, and D. Morris.** 1975. Biodegradative ornithine decarboxylase of *Escherichia coli*. Purification, properties, and pyridoxal 5′-phosphate binding site. Biochemistry **14**:3675–3681.
4. **Auger, E. A., and G. N. Bennett.** 1989. Regulation of lysine decarboxylase activity in *Escherichia coli* K12. Arch. Microbiol. **151**:466–468.
5. **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.
6. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
7. **Becker, E.** 1967. Mutants of *E. coli* B in enzyme of putrescine synthetic pathways. Fed. Proc. **26**:812.
8. **Boeker, E. A., and E. E. Snell.** 1972. Amino acid decarboxylases, p. 217–253. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 6. Academic Press, New York.
9. **Booth, I. R.** 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. **49**:359–378.
9a. **Boyle, S. M.** Unpublished data.
10. **Bullock, W. O.** 1987. XL1-blue: a high effeciency plasmid transforming *recA Escherichia coli* strain with beta-galactosidase selection. BioTechniques **5**:376–378.
11. **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.
12. **Celis, T. F. R., H. J. Rosenfeld, and W. K. Maas.** 1973. Mutant of *Escherichia coli* K-12 defective in the transport of basic amino acids. J. Bacteriol. **116**:619–626.
13. **Clark, R. L., and F. C. Neidhardt.** 1990. Roles of the two lysyl-tRNA synthetases of *Escherichia coli*: analysis of nucleotide sequences and mutant behavior. J. Bacteriol. **172**:3237–3243.
14. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *E. coli* genome. Cell **9**:91–99.
15. **Dente, L., G. Cesareni, and R. Cortes.** 1983. pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11**:1645–1655.
16. **Enea, V., and N. D. Zinder.** 1982. Interference resistant mutants of phage f1. Virology **122**:222–226.
17. **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.
18. **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.
19. **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.
20. **Gale, E. F.** 1946. The bacterial amino acid decarboxylases. Adv. Enzymol. **6**:1–32.
21. **Gale, E. F., and H. M. R. Epps.** 1942. The effect of the pH of the medium during growth on the enzymic activities of bacteria (*Escherichia coli* and *Micrococcus lysodiekticus*) and the biological significance of the changes produced. Biochem. J. **36**: 600–619.
22. **Gamper, M., A. Zimmermann, and D. Haas.** 1991. Anaerobic regulation of transcription initiation in *arcDABC* operon of *Pseudomonas aeruginosa*. J. Bacteriol. **173**:4742–4750.
23. **Garnier, J., D. J. Osguthorp, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120**:97–120.
24. **Goldemberg, S. H.** 1980. Lysine decarboxylase mutants of *Escherichia coli*: evidence for two enzyme forms. J. Bacteriol. **141**:1428–1431.
25. **Guest, J. R., J. S. Miles, R. Roberts, and S. A. Woods.** 1985. The fumarase genes of *Escherichia coli*: location of the *fumB* gene and discovery of a new gene (*fumC*). J. Gen. Microbiol. **131**:2971–2984.
26. **Halsall, D. M.** 1974. Overproduction of lysine by mutant strains of *Escherichia coli* with defective lysine transport systems. Biochem. Genet. **13**:109–124.
27. **Hanka, M. E., and K. K. Koessler.** 1924. Studies on proteinogenous amines. J. Biol. Chem. **59**:835–855.
28. **Hickey, E. W., and I. N. Hirshfield.** 1990. Low-pH-induced effects on patterns of protein synthesis and on internal pH in *Escherichia coli* and *Salmonella typhimurium*. Appl. Environ. Microbiol. **56**:1038–1045.
29. **Kashiwagi, K., and K. Igarashi.** 1988. Adjustment of polyamine contents in *Escherichia coli*. J. Bacteriol. **170**:3131–3135.
30. **Kashiwagi, K., H. Kobayashi, and K. Igarashi.** 1986. Apparently unidirectional polyamine transport by proton motive force in polyamine-deficient *Escherichia coli*. J. Bacteriol. **165**:972–977.
31. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large library. Cell **50**:495–508.
32. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
33. **Leveque, F., P. Plateau, P. Dessen, and S. Blanquet.** 1990. Homology of *lysS* and *lysU*, the two *Escherichia coli* genes encoding distinct lysyl-tRNA synthetase species. Nucleic Acids Res. **18**:305–312.
34. **Lüthi, E., H. Baur, M. Gamper, F. Brunner, D. Villeval, A. Mercenier, and D. Hass.** 1990. *arc* operon for anaerobic arginine catabolism in *Pseudomonas aeruginosa* contains an additional gene, *arcD*, encoding a membrane protein. Gene **87**:37–43.
35. **Maloney, P. C.** 1987. Coupling to an energized membrane: role of ion-motive gradients in the transduction of metabolic energy, p. 222–243. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
36. **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.
37. **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.
38. **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.
39. **Munro, G. F., C. A. Bell, and M. Lederman.** 1974. Multiple transport components for putrescine in *Escherichia coli*. J. Bacteriol. **118**:952–963.
40. **Munro, G. F., and W. Sauerbrier.** 1973. Osmotically induced excretion of putrescine by mutants of *Escherichia coli* defective in potassium transport. J. Bacteriol. **116**:488–490.
41. **Phan, A. P. H., T. T. Ngo, and H. M. Lenhoff.** 1982. Spectrophotometric assay for lysine decarboxylase. Anal. Biochem. **120**:193–197.
42. **Pittard, J., J. Praszkier, A. Certoma, G. Eggertsson, J. Gowrishankar, G. Narasaiah, and M. J. Whipp.** 1990. Evidence that there are only two tRNA Phe genes in *Escherichia coli*. J. Bacteriol. **172**:6077–6083.
43. **Platt, T.** 1986. Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. **55**:339–372.
44. **Poolman, B., A. J. M. Driesen, and W. N. Konings.** 1987. Regulation of arginine-ornithine exchange and the arginine deiminase pathway in *Streptococcus lactis*. J. Bacteriol. **169**: 5597–5604.
45. **Popkin, P. S., and W. K. Maas.** 1980. *Escherichia coli* regulatory mutation affecting lysine transport and lysine decarboxylase. J. Bacteriol. **141**:485–492.
46. **Rescei, P. A., and E. E. Snell.** 1972. Histidine decarboxylaseless mutants of *Lactobacilla* 30a: isolation and growth properties. J. Bacteriol. **112**:624–626.
47. **Rosen, B. P.** 1971. Basic amino acid transport in *Escherichia coli*. J. Biol. Chem. **146**:3653–3663.
48. **Rudd, K. E., W. Miller, J. Ostill, and D. A. Benson.** 1990. Alignment of *Escherichia coli* K12 DNA sequences to a genomic restriction map. Nucleic Acids Res. **18**:313–321.
49. **Sabo, D. L., E. A. Boeker, B. Byers, H. Waron, and E. H. Fischer.** 1974. Purification and physical properties of inducible *Escherichia coli* lysine decarboxylase. Biochemistry **13**:662–670.
50. **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.
51. **Sakazaki, R.** 1981. The genus *Hafnia*, p. 1181–1186. *In* M. P.

Starr, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag, Berlin.

52. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. B. Clark, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148:**45–62.
53. **Slonczewski, J. L., B. P. Rosen, J. R. Alger, and R. M. Macnab.** 1981. pH homeostasis in *Escherichia coli*: measurement by ^{31}P nuclear magnetic resonance of methylphosphonate and phosphate. Proc. Natl. Acad. Sci. USA **78:**6271–6275.
54. **Spratt, B. G., P. J. Hedges, S. te Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene **4:**337–342.
55. **Steigerwalt, A. G., G. R. Fanning, M. A. Fife-Asbury, and D. J. Brenner.** 1976. DNA relatedness among species of *Enterobacter* and *Serratia*. Can. J. Microbiol. **22:**121–137.
56. **Tabor, C. W., and H. Tabor.** 1985. Polyamines in microorganisms. Microbiol. Rev. **49:**81–99.
57. **Tabor, H., E. W. Hafner, and C. W. Tabor.** 1980. Construction of an *Escherichia coli* strain unable to synthesize putrescine, spermidine, or cadaverine: characterization of two genes controlling lysine decarboxylase. J. Bacteriol. **144:**952–956.
58. **Tinoco, I., P. N. Borer, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature New Biol. **246:**40–41.
59. **VanBogelen, R. A., V. Vaughn, and F. C. Neidhardt.** 1983. Gene for heat-inducible lysyl-tRNA synthetase (*lysU*) maps near *cadA* in *Escherichia coli*. J. Bacteriol. **153:**1066–1068.
60. **Vander Wauven, C., A. Pierard, M. Kley-Raymann, and D. Haas.** 1984. *Pseudomonas aeruginosa* mutants affected in anaerobic growth on arginine: evidence for a four-gene cluster encoding the arginine deiminase pathway. J. Bacteriol. **160:**928–934.
61. **Vermersch, P. S.** 1988. Genetic strategies for analyzing proteins: applications utilizing the R388 type II dihydrofolate reductase. Ph.D. thesis. Rice University, Houston.
62. **Watson, 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.
63. **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.
64. **Wertheimer, S. J., and Z. Leife.** 1983. Putrescine and spermidine sensitivity of lysine decarboxylase in *Escherichia coli*: evidence for a constitutive enzyme and its mode of regulation. Biochem. Biophys. Res. Commun. **114:**882–888.
65. **Zilberstein, D., V. Agmon, S. Schuldiner, and E. Padan.** 1984. *Escherichia coli* intracellular pH, membrane potential, and cell growth. J. Bacteriol. **158:**246–252.