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Characterization of a Second Lysine Decarboxylase Isolated from *Escherichia coli*

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We report here on the existence of a new gene for lysine decarboxylase in *Escherichia coli* K-12. The hybridization experiments with a *cadA* probe at low stringency showed that the homologous region of *cadA* was located in λ Kohara phage clone 6F5 at 4.7 min on the *E. coli* chromosome. We cloned the 5.0-kb *Hin*dIII fragment of this phage clone and sequenced the homologous region of *cadA*. This region contained a 2,139-nucleotide open reading frame encoding a 713-amino-acid protein with a calculated molecular weight of 80,589. Overexpression of the protein and determination of its N-terminal amino acid sequence defined the translational start site of this gene. The deduced amino acid sequence showed 69.4% identity to that of lysine decarboxylase encoded by *cadA* at 93.7 min on the *E. coli* chromosome. In addition, the level of lysine decarboxylase activity increased in strains carrying multiple copies of the gene. Therefore, the gene encoding this lysine decarboxylase was designated *ldc*. Analysis of the lysine decarboxylase activity of strains containing *cadA*, *ldc*, or *cadA ldc* mutations indicated that *ldc* was weakly expressed under various conditions but is a functional gene in *E. coli*.

There are two types of bacterial amino acid decarboxylase, constitutive and inducible. The former type includes decarboxylases for L-ornithine, L-arginine, *S*-adenosyl-L-methionine, and diaminopimelic acid (27). In *Escherichia coli*, a previously characterized lysine decarboxylase (EC 4.1.1.18) is encoded by *cadA* at 93.7 min and participates in the synthesis of cadaverine from lysine. This enzyme is inducible under anaerobic conditions at pH 5.5 and by adding lysine to the culture medium (7, 19, 23, 27). Some evidence has been presented for the existence of a second, much less active, constitutive lysine decarboxylase in *E. coli* (6, 30), but the data are still too incomplete to permit any definitive conclusions on the presence of this second enzyme. Igarashi et al. made several observations concurring with the suggestion that cadaverine is actually formed by ornithine decarboxylase, which would then account for the alleged constitutive lysine decarboxylase (8). The presence of constitutive lysine decarboxylase has not been detected in organisms, except for *Selenomonas ruminantium*, a strictly anaerobic gram-negative bacterium (12).

We previously reported the overproduction of lysine by strain WC196, a lysine analog (*S*-aminoethyl-L-cysteine)-resistant strain of W3110 formed by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis. During the construction of strains derived from this strain, we found that a *cadA* deletion mutant (WC196C) could still degrade lysine to cadaverine (15a). This finding suggested the existence of another lysine decarboxylase besides *cadA* in *E. coli*.

Here, we report the existence of a new lysine decarboxylase gene, designated *ldc*, at 4.7 min on the *E. coli* chromosome, and we describe the lysine decarboxylase activities of strains containing *cadA*, *ldc*, or *cadA ldc* mutations.

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MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The *E. coli* K-12 strains, bacteriophages, and plasmids used are listed in Table 1. The Kohara-ordered λ *E. coli* chromosomal DNA library was kindly provided by Y. Kohara, National Institute of Genetics (Mishima, Japan).

Southern blot hybridization. Southern blot hybridization was performed at 55°C for 16 h as specified by the manufacturer (Du Pont-New England Nuclear). After hybridization, the membrane was washed at 55°C in 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]) containing 0.5% sodium dodecyl sulfate (SDS).

Construction of a *cadA* deletion mutant of *E. coli*. To construct an ampicillin-sensitive *cadA* deletion mutant, the DNA of the λ Kohara phage clone 21H11 was isolated from a culture of *E. coli* W3110 that had been infected with λ phage 21H11 and digested with *Eco*RV. The 5.4-kb *Eco*RV fragment containing the *cadBA* operon region was cloned into the *Eco*RI site of a temperature-sensitive plasmid vector (pTS1) after blunting the ends with T4 DNA polymerase, and plasmid pTCAD was obtained. The *cadA* coding sequence between the *Eco*RI sites of pTCAD was removed, and plasmid pTCADΔE was obtained. Then W3110 was transformed with pTCADΔE and grown at 30°C for 2 h. It was further incubated overnight at 42°C on Luria-Bertani medium (LB) plates containing ampicillin. Since pTCADΔE has a temperature-sensitive replicon, the transformants should appear as ampicillin-resistant colonies at 42°C only after incorporation of the plasmid into the chromosome by homologous recombination. About 1% of the population of ampicillin-resistant colonies grown at 42°C was ampicillin sensitive. In ampicillin-sensitive colonies, *cadA* deletion mutants were screened for lysine decarboxylase activity by inoculating the wells of sterile microtiter dishes containing 150 μl of lysine decarboxylase broth (Difco) per well from the master plates (7). Each well was overlaid with 50 μl of sterile mineral oil and incubated at 37°C for 1 day. The *cadA* deletion mutants appeared yellow, while wells containing wild-type strains were purple; strain WYK011, which is *cadA* deletion mutant, was obtained. The *cadA* deletion in this strain was confirmed by PCR using primers 5′-TGGATAACCACACCGCGTCT-3′ and 5′-GGAAGGATCATATTGGCGTT-3′, which were complementary to the upstream and downstream regions of *cadA* (19), respectively.

Construction of an *ldc* deletion mutant of *E. coli*. To construct an *ldc* deletion mutant, we first removed the *ldc* coding sequence between the *Eco*RV sites of pU6F5H. A chloramphenicol-resistant gene cassette was excised as a 1.0-kb *Acc*II fragment from the cloning vector pHSG399 and inserted into the truncated *ldc* DNA coding region to produce plasmid pU6F5HCm. The *Hin*dIII-digested fragment of pU6F5HCm, containing the *ldc* gene disrupted with the chloramphenicol-resistant gene cassette, was cloned into temperature-sensitive plasmid vector pTS1 at the *Hin*dIII site, yielding plasmid pT6F5HCm. W3110 was then transformed with pT6F5HCm, and homologous recombination was performed. The resulting ampicillin-sensitive and chloramphenicol-resistant *ldc* deletion mutant was designated WYK020.

WYK031, a *cadA ldc* double deletion mutant, was constructed from WYK011.

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TABLE 1. Bacterial strains, bacteriophages, and plasmids used in this study

Strain, phage, or plasmid	Genotype or characteristics[a]	Reference or source
E. coli K-12		
W3110	F^- IN(*rrnD-rrnE*)	2
GNB10181	Δ*cadA* Ap^r Cm^r	7
WC196	W3110 NTG mutant (*S*-aminoethyl-L-cysteine resistant mutant)	Our laboratory stock
WC196C	WC196 Δ*cadA* Ap^r Cm^r [WC196 × P1(GNB10181)]	Our laboratory stock
WYK010	W3110 Δ*cadA* Ap^r Cm^r [W3110 × P1(GNB10181)]	This work
WYK011	W3110 Δ*cadA*	This work
WYK020	W3110 Δ*ldc* Cm^r	This work
WYK031	WYK011 Δ*ldc* Cm^r [WYK011 × P1(WYK020)]	This work
WYK040	WC196 Δ*ldc* Cm^r [WC196 × P1(WYK020)]	This work
WYK050	WYK040 Δ*cadA* Ap^r Cm^r [WYK040 × P1(GNB10181)]	This work
Bacteriophages		
P1*vir*	Used for generalized transduction	Our laboratory stock
21H11	λ Kohara phage clone 647 in the miniset library	16
6F5	λ Kohara phage clone 122 in the miniset library	16
Plasmids		
pUC19	Cloning vector, Ap^r	31
pU6F5H	pUC19 carrying *ldc* on a 5.0-kb *Hin*dIII fragment from 6F5	This work
pHSG399	Cloning vector, Cm^r	28
pU6F5HCm	pU6F5H derivative carrying Δ*ldc* with in-frame deletion	This work
pTS1	Temperature-sensitive cloning vector, Ap^r	32
pT6F5HCm	pTS1 carrying Δ*ldc* with in-frame deletion on a 4.9-kb *Hin*dIII fragment from pU6F5HCm	This work
pTCAD	pTS1 carrying Δ*cadA*	This work
pTCADΔE	pTS1 carrying Δ*cadA* with 900 bases of *Eco*RI fragment deletion	This work
pHSG299	Cloning vector, Km^r	28
pHLDC	pHSG299 carrying *ldc* on a 2.8-kb *Sma*I-*Hin*dIII fragment from 6F5	This work
pTrc99A	Expression vector, Ap^r	1
pTrcLDC	pTrc99A carrying *ldc*	This work

[a] Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin.

Assay for lysine decarboxylase. LB was buffered to pH 5.5 with 100 mM 2-(*N*-morpholino)ethanesulfonic acid or buffered to pH 7.6 with 100 mM HEPES as described previously (25). In certain experiments, 0.5% L-lysine hydrochloride was also included in the medium.

Strains were grown to an optical density of 0.5 to 0.7 (at 600 nm) in a 500-ml Sakaguchi flask containing 50 ml of modified LB with appropriate antibiotics at 37°C with reciprocal agitation (120 rpm). Cells were harvested by centrifugation, washed once with saline, and suspended in 4 ml of 0.5 M sodium acetate buffer (pH 6.0) containing 0.1 mM pyridoxal-5′-phosphate. The suspension was cooled in an ice-salt bath and sonicated for 5 min at 150 W. The sonicated samples were centrifuged at 150,000 × *g* for 1 h to remove unbroken cells, and the supernatant was used as the crude cell extract. Lysine decarboxylase activity was measured as reported previously (12, 22), using L-lysine as a substrate.

Construction of a plasmid for Ldc expression in a *trc*-promoter expression system. To achieve expression of Ldc, the DNA fragment containing *ldc* and its Shine-Dalgarno sequence was amplified from pU6F5H by PCR using primers 5′-GGGATTTTCCAGGAGGAACAC-3′ and 5′-GCCAAGCTTTGCTCACC GCATAA-3′, which were complementary to the upstream and downstream regions of *ldc*, respectively, and was digested with *Hin*dIII after blunting the ends with T4 DNA polymerase. The purified 2,286-bp fragment was cloned into the *Sma*I-*Hin*dIII site of the *trc* promoter expression vector pTrc99A, yielding plasmid pTrcLDC. The *ldc* region of pTrcLDC was sequenced, and it was confirmed that there was no point mutation in the plasmid. Then pTrcLDC was introduced into strain WYK031.

Isolation and purification of Ldc and CadA from *E. coli* K-12. *E. coli* WYK031(pTrcLDC) was grown in TY medium at 37°C for 20 h under aerobic conditions, and the cells were collected by centrifugation at 4°C. The cells (12 g) were suspended in 50 mM sodium phosphate buffer (pH 6.5, buffer A) and disrupted in a French pressure cell. After centrifugation at 200,000 × *g* for 2 h, the supernatant was collected. Solid ammonium sulfate was added with stirring to the enzyme fraction at a final concentration of 1 M with stirring. After 3 h, the supernatant was collected by centrifugation and was applied to a TSK gel phenyl-5PW column (21.5 by 150 mm; Tosoh, Tokyo, Japan). The column was washed with 500 ml of buffer A containing 1 M ammonium sulfate. The enzyme was then eluted with a linear gradient created by mixing 125 ml of 1 M ammonium sulfate in buffer A with 125 ml of buffer A. The fractions which eluted from 0.4 to 0.5 M ammonium sulfate were pooled. After being dialyzed against buffer A, the enzyme preparation was located onto a prepacked Q-Sepharose column (HiLoad 16/10; Pharmacia) equilibrated with buffer A. The column was washed with 0.1 M NaCl in buffer A and then eluted with a linear gradient of NaCl in buffer A (0.1 to 0.8 M). The fractions eluted from 0.45 to 0.55 M NaCl were pooled. Final purification was achieved by fast protein liquid chromatography (FPLC) with a Mono Q column (Pharmacia). The column was washed with 0.2 M NaCl in buffer A and then eluted with a linear gradient of NaCl in buffer A (0.2 to 0.8 M). The fractions eluted from 0.55 to 0.60 M NaCl were pooled.

For the purification of CadA, *E. coli* WYK020 with deletion of the *ldc* gene was grown in chemically defined medium containing 0.8% lysine at 37°C at pH 5.5 under anaerobic conditions (23). CadA was purified by the method of Sabo et al. (23).

One unit of lysine decarboxylase activity was defined as the amount forming 1 μmol of cadaverine from L-lysine at 37°C in 1 min. Specific activity is reported as units per milligram of protein.

N-terminal amino acid sequences of intact Ldc and CadA and the peptide fragments obtained by cyanogen bromide cleavage. Purified Ldc was cleaved with CNBr by the method of Matsudaira (18). Ldc (500 μg) was dissolved in 1 ml of 70% formic acid containing 500 μg of CNBr and was kept in the dark under argon gas at 24°C for 24 h. After lyophilization, the sample was dissolved in 5 M guanidine-HCl and subjected to high-performance liquid chromatography using an ODS 120T column (4.6 by 250 mm; Tosoh) at 40°C. The CNBr fragments were eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (10 to 60%) and were directly analyzed for the N-terminal amino acid sequence. Fifty-one fragments were obtained. Fragment 38 (eluted at 40% acetonitrile) was used for N-terminal amino acid sequence analysis. The N-terminal amino acid sequences of intact Ldc and CadA were also analyzed. The N-terminal sequence was determined as described previously (3), using a gas-phase protein sequencer (model PSQ; Shimadzu, Kyoto, Japan) equipped with an on-line amino acid analyzer (model RF-550; Shimadzu).

Other analytical procedures. SDS-polyacrylamide gel electrophoresis (PAGE) was done as described previously (9). Nucleotide sequences were determined by the dideoxy-chain termination method (24), using [α-^{35}S]dCTP (Amersham).

Nucleotide sequence accession number. The sequence of *ldc* has been deposited in the GenBank/EMBL/DDBJ database under accession no. D87518.

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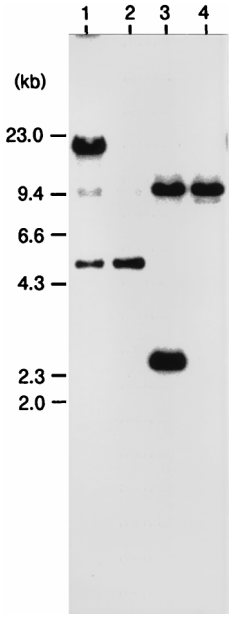

FIG. 1. Southern blot hybridization of the chromosomal DNA from strains W3110 and WYK010 with the *cadA* probe. Purified chromosomal DNA of *E. coli* W3110 and WYK010 was digested with restriction enzymes and subjected to agarose gel electrophoresis. After Southern blotting, the DNA was hybridized with a ^{32}P-labeled 2,063-bp DNA fragment which was the DNA of the whole *cadA* gene obtained by PCR using primers 5′-GGGATTTTCCAGGAGGAAC AC-3′ and 5′-GCCAAGCTTTGCTCACCGCATAA-3′. Lane 1, W3110, *Hin*dIII-digested; lane 2, WYK010, *Hin*dIII-digested; lane 3, W3110, *Pst*I-digested; lane 4, WYK010, *Pst*I-digested.

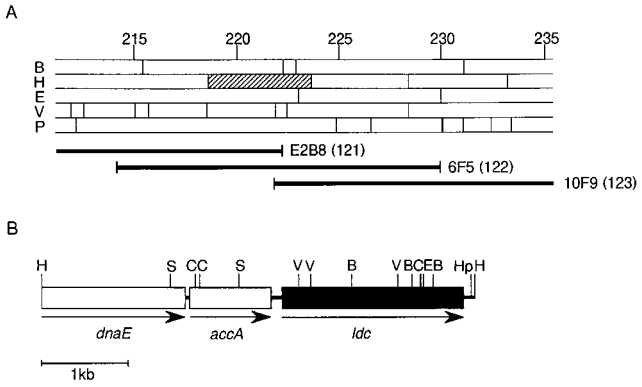

FIG. 2. Physical mapping of the *ldc* gene. (A) Scale restriction map of the *E. coli* chromosome around position 220 kbp. Thick lines show the extent of the chromosomal DNA carried on the λ Kohara phage clones E2B8, 6F5, and 10F9. Numbers in parentheses at the right of the clone names are the serial numbers of the Kohara miniset genomic library. The hybridizable 5.0-kb *Hin*dIII fragment with the *cadA* probe is denoted by a hatched rectangle. (B) Restriction map of the fragment denoted by a hatched rectangle in panel A. The coding regions for *dnaE* and *accA* are denoted by open rectangles, and the coding region of *ldc* is denoted by a solid rectangle. The arrows below the genes indicate the direction of transcription. Relevant restriction sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; S, *Sma*I; V, *Eco*RV.

RESULTS AND DISCUSSION

Identification of a *cadA* homologous region on chromosomal DNA from *E. coli* K-12 W3110 and WYK010. While attempting to construct lysine-overproducing *E. coli*, we noticed that a *cadA*-deleted mutant of *E. coli* still degraded lysine to cadaverine (15a). We therefore digested chromosomal DNA from W3110 and WYK010 with the restriction enzymes *Hin*dIII and *Pst*I. Southern blot hybridization was performed with a 2,063-bp *cadA* DNA fragment containing most of the *cadA* coding region (Fig. 1). The 27.0-kb *Hin*dIII and the 2.5-kb *Pst*I fragments corresponding to the *cadA* region hybridized strongly to this probe, while 5.0-kb *Hin*dIII and 12.6-kb *Pst*I fragments from strain W3110 also hybridized weakly (Fig. 1, lanes 1 and 3). The 5.0-kb *Hin*dIII and 12.6-kb *Pst*I fragments were similarly detected in WYK010, in which *cadA* was deleted (Fig. 1, lanes 2 and 4). This result indicates that there is a region homologous to *cadA* in *E. coli*, which was designated the *cadA*-like region. Meng and Bennett previously reported that preliminary Southern hybridization using *cadA* to probe *E. coli* chromosomal DNA failed to identify a second region homologous to *cadA* under standard conditions (19); in this study, we detected the *cadA*-like region only under low-stringency conditions. To map its position in the *E. coli* chromosome, we hybridized DNA from the Kohara miniset genomic library with the *cadA* probe under the latter conditions. The λ Kohara phage clones 21H11 and 5G7, corresponding to the *cadA* region at 93.7 min on the *E. coli* chromosome, gave a strong hybridization signal, and clones E2B8, 6F5, and 10F9 at 4.7 min on the *E. coli* chromosome also gave hybridization signals (data not shown). This result clearly indicated that clone 6F5 contained the 5.0-kb *Hin*dIII fragment bearing the *cadA*-like region (Fig. 2A). Accordingly, the 5.0-kb *Hin*dIII fragment was cloned into the *Hin*dIII site of plasmid vector pUC19 to obtain plasmid pU6F5H. Then restriction mapping of the 5.0-kb *Hin*dIII fragment of this plasmid was done (Fig. 2B).

DNA sequence analysis of the *cadA*-like region in the 5.0-kb fragment. The nucleotide sequence of the 2.8-kb *Sma*I-*Hin*dIII fragment in pU6F5H was determined (Fig. 3). The *cadA*-like region was located downstream of the gene for a subunit of acetyl coenzyme A carboxylase (*accA*) (17). In this region, we detected one open reading frame (ORF), which is designated *ldc*. The ORF for *ldc* extended from a methionine codon at nucleotide 571 to nucleotide 2709 and encoded a protein of 713 amino acid residues with a calculated molecular mass of 80,589 Da. A GenBank/EMBL/DDBJ database record, D49445, exists which has the *ldc* gene sequence; the Ldc sequence in this entry has 712 amino acids, rather than the 713 amino acids that we have determined.

The nucleotide sequences of the ORFs for *ldc* and *cadA* had 68.2% identity. However, there was no similarity of the 5′-flanking regions between *ldc* and *cadA*. A sequence resembling a strong transcription terminator (ΔG of −23.9 kcal, calculated with the model of Tinoco et al. [29]) was found immediately downstream of *accA* (horizontal arrows in Fig. 3).

Comparison of the predicted amino acid sequence of Ldc with those of other lysine decarboxylases, including CadA of *E. coli* (19), lysine decarboxylase of *Hafnia alvei*, (5), and lysine decarboxylase of *Salmonella typhimurium* (21) (Fig. 4), showed homologies of 69.4, 68.6, and 68.9% respectively, of the entire amino acid sequence. On the other hand, the entire amino acid sequence of Ldc showed 34.2, 20.0, 30.8, and 34.0% identity with those of inducible arginine decarboxylase (26), constitutive arginine decarboxylase (20), inducible ornithine decarboxylase (14), and constitutive ornithine decarboxylase (15), respectively. Bacterial amino acid decarboxylases contain a pyridoxal 5′-phosphate-binding consensus sequence, S-X-H-K (20), which was also found in the predicted peptide sequence of Ldc (Fig. 4).

Lysine decarboxylase activity. Table 2 shows the effects of different growth conditions known to affect lysine decarboxylase activity. A basal activity of lysine decarboxylase was ob-

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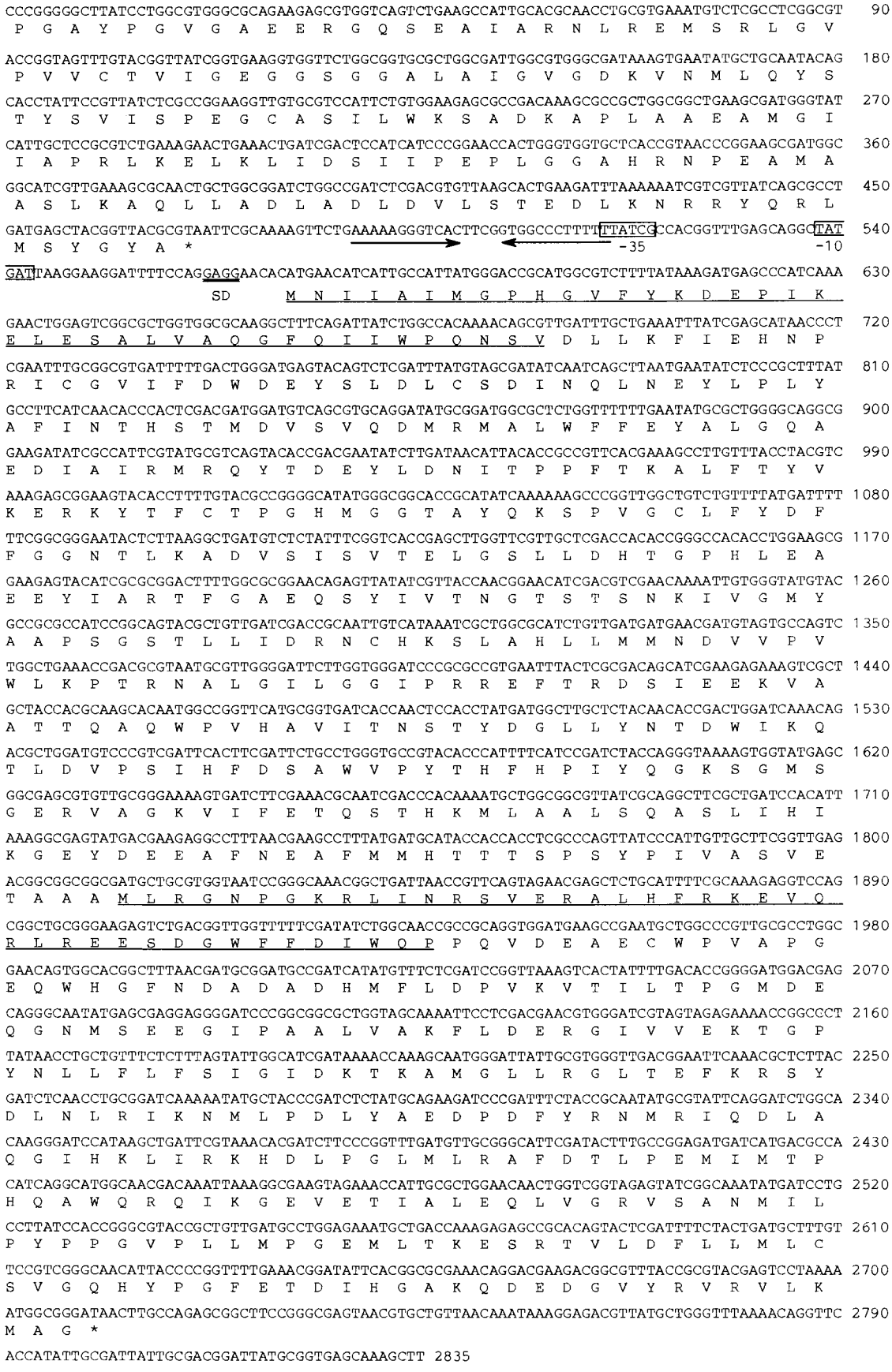

FIG. 3. Nucleotide sequence containing *ldc* from *E. coli* and the deduced amino acid sequence. The strand is shown in the 5′-to-3′ direction. The deduced amino acid sequence is shown under the nucleotide sequence. Amino acid sequences analyzed chemically, the putative −10 and −35 regions of the promoter site and the Shine-Dalgarno sequence of *ldc* are indicated by single underlines, boxes, and a double underline, respectively. The putative termination signal of *accA* is indicated by horizontal arrows.

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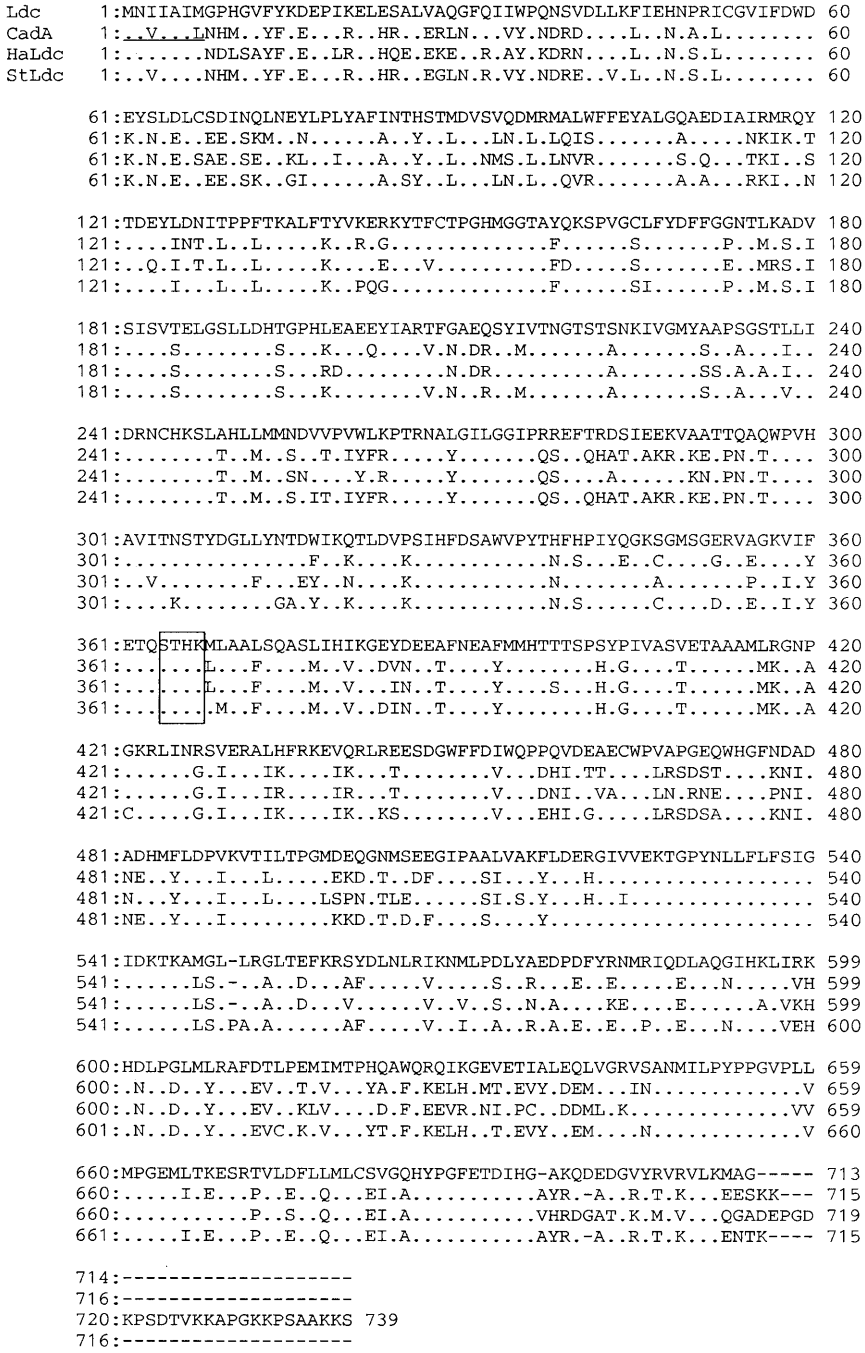

FIG. 4. Comparison of the deduced amino acid sequences of Ldc, CadA of *E. coli*, lysine decarboxylase of *H. alvei* (HaLdc), and lysine decarboxylase of *S. typhimurium* (StLdc). Numbers indicate amino acid positions. Gaps in the alignment are indicated by dashes. The amino acid residues of CadA, HaLdc, and StLdc are replaced by dots if they are identical with those of Ldc. The amino acid sequence of the pyridoxal-5′-phosphate binding site is boxed. The amino acid sequence of CadA purified in this study and analyzed chemically is indicated by a single underline.

served in WYK031 (*cadA ldc*) which may be due to ornithine decarboxylase (8). The same low level of lysine decarboxylase activity was found in WYK011 (*cadA*), and it was not significantly increased by low pH or the presence of extra lysine in the LB broth. Both strains W3110 and WYK020 (*ldc*) showed the high lysine decarboxylase activity encoded by *cadA*$^+$ and expressed in medium of low pH. The basal activities observed under noninducing conditions were similar in W3110 and WYK020. In lysine decarboxylase broth (see Materials and Methods), WYK011 (*cadA*) appeared yellow, indicating low if any activity of lysine decarboxylase encoded by *ldc*$^+$ under these conditions. However, in strain WYK011 transformed with plasmid pHLDC (*ldc*$^+$), in which the orientation of the *ldc* gene was opposite that of the *lac* promoter, we observed that lysine decarboxylase activity was high at both pHs (Table 2). Thus, although *ldc* is little expressed in single copy in the

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TABLE 2. Levels of lysine decarboxylase activity in W3110 derivatives and WYK011 carrying pHLDC

Strain	Lysine decarboxylase activity (nmol/min/mg of protein)[a]			
	pH 5.5		pH 7.6	
	−Lys	+Lys	−Lys	+Lys
W3110	82 ± 6	90 ± 6	0.16 ± 0.05	0.17 ± 0.05
WYK011 (*cadA*)	0.14 ± 0.04	0.20 ± 0.06	0.11 ± 0.02	0.07 ± 0.02
WYK020 (*ldc*)	81 ± 4	89 ± 6	0.07 ± 0.02	0.05 ± 0.01
WYK031 (*cadA ldc*)	0.12 ± 0.03	0.21 ± 0.06	0.10 ± 0.03	0.06 ± 0.01
WYK011(pHLDC [*ldc*$^+$])	140 ± 10	NT[b]	161 ± 10	NT
WC196C (*cadA*)	15 ± 2	13 ± 1	14 ± 1	14 ± 1
WYK050 (*cadA ldc*)	0.12 ± 0.03	0.12 ± 0.04	0.10 ± 0.03	0.07 ± 0.02

[a] Values are averages of three independent lysine decarboxylase assays ± standard deviations.
[b] NT, not tested.

normal context under the conditions tested, including anaerobic conditions, it appears to be functional in high copy number and not a pseudogene.

During the construction of lysine-producing strains, we found that WC196C, a lysine analog (*S*-aminoethyl-L-cysteine)-resistant and *cadA* deletion mutant, could still degrade lysine to cadaverine. The activity of lysine decarboxylase in WC196C was significantly increased, and the level of activity was almost steady under the conditions tested (Table 2). The level of lysine decarboxylase activity in WYK050, a *cadA ldc* double deletion mutant derived from WC196, decreased to the basal level. It is clear that the increased activity of lysine decarboxylase in WC196C is due to lysine decarboxylase encoded by *ldc*. When we cloned the promoter region of *ldc* in WC196C and determined the nucleotide sequence of this region, no base change was detected in comparison with the wild type. It is possible that the significantly increased lysine decarboxylase activity encoded by *ldc* in WC196C occurs because an *ldc* repressor is not functional due to NTG mutagenesis and that the marked increase seen in WYK011(pHLDC) occurs because the *ldc* repressor is being titrated out by the presence of a binding site on the cloned *ldc* fragment.

Expression, purification, and characterization of Ldc in *E. coli*. *E. coli* WYK031(pTrcLDC) cells expressed *ldc*, and Ldc accumulated in the cells at a final concentration of 9.4% of total protein. Ldc was purified to homogeneity (using FPLC), as demonstrated by the presence of a single band on gel electrophoresis (Fig. 5). The enzyme was purified about 100-fold, and the specific activity of the purified enzyme was 1,125 U/mg of protein (Table 3). The molecular mass of the native enzyme was about 800 kDa, as determined by Superdex 75 gel (HR 10/30; Pharmacia) filtration with buffer A containing 0.15 M NaCl. The molecular mass of its subunit was determined by SDS-PAGE. A single band was found in the gel, and the size of the protein was estimated to be 80 kDa, indicating that the native enzyme contained 10 subunits of identical molecular mass. Intact CadA was also found to be composed of 10 subunits, each with a molecular mass of 78 kDa (23). The molecular mass of the subunit of Ldc was the same as that calculated from the deduced amino acid residues of the nucleotide sequence of *ldc*. The N-terminal 40 amino acid residues of intact Ldc and the N-terminal 42 residues of its CNBr fragment were identical to the deduced amino acid residues 1 to 40 from the N terminus and residues 416 to 457, respectively (Fig. 3). The purified enzyme preparation decarboxylated L-lysine, while neither L-ornithine nor L-arginine was a suitable substrate. The optimum pH for enzyme activity was 6.2 to 8.0. At pH 5.2 and 8.8, the activity was decreased to 40 and 30%, respectively. In contrast, purified CadA had an optimum pH of 5.7. At pH 5.2 and 8.0, its activity was decreased to 70 and 20%, respectively. Goldemberg had reported that two kinds of lysine decarboxylase exist: an inducible form, which is thermostable, and a postulated constitutive form, which is thermosensitive (6). It was also described that the postulated constitutive form showed about 30% decrease in activity after heating for 4 min at 60°C (6). In our experiments, purified CadA was very stable after heating for 15 min at 60°C, but purified Ldc showed about 45% decrease in activity after heating for 5 min at 60°C, followed by a plateau. It is obvious that our result agrees with that of Goldemberg.

Until now, it has been believed that *E. coli* possesses only CadA, which is strongly induced in rich medium at a low pH in the presence of excess substrate and appears to play a role in pH homeostasis by consuming protons and neutralizing the acidic by-products of carbohydrate fermentation (19). In the present study, we demonstrated the existence of a second lysine decarboxylase in *E. coli* encoded by *ldc*. Since *ldc* is little expressed, it is possible that an *ldc* repressor exists. Conditions leading to higher expression of *ldc* may exist, but these have not yet been elucidated. Although the physiological role of this second lysine decarboxylase is not clear, it is interesting that cadaverine but not putrescine participates in closing the porin channels in the outer membrane of *E. coli* growing in lysine-

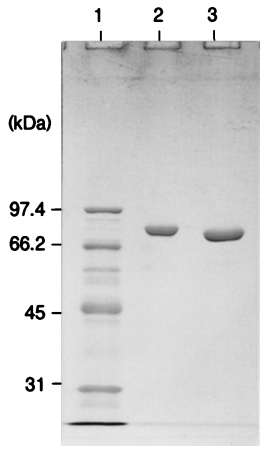

FIG. 5. SDS-PAGE of purified Ldc and CadA preparations from *E. coli*. The gel was stained with Coomassie brilliant blue R-250. Molecular mass standards were phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), and bovine carbonic anhydrase (31 kDa). Lane 1, molecular mass standards; lane 2, Ldc from *E. coli* WYK031(pTrc99A); lane 3, CadA from *E. coli* WYK020.

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TABLE 3. Purification of the lysine decarboxylase encoded by *ldc* from *E. coli*

Step no., process	Total vol (ml)	Total activity[a] (U)	Total protein (mg)	Sp act (U/mg)	Yield (%)
1, Supernatant from crude extract	85	16,000	1,500	10.7	100
2, TSK gel phenyl-5PW chromatography	20	7,500	12.0	625	46.9
3, Q-Sepharose chromatography	7.0	5,000	7.7	649	31.3
4, Mono Q chromatography	6.5	2,700	2.4	1,125	16.9

[a] 1 U = 1 μmol/min.

free medium at a neutral pH (4) and that an essential component of *S. ruminantium* peptidoglycan is cadaverine, which is constitutively synthesized from lysine (10–13). Thus, it seems reasonable to suppose that constitutively synthesized cadaverine plays an important role in bacteria.

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