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Cloning, Nucleotide Sequence, and Expression of the *Escherichia coli* Gene Encoding Carnitine Dehydratase

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Carnitine dehydratase from *Escherichia coli* O44 K74 is an inducible enzyme detectable in cells grown anaerobically in the presence of L-(−)-carnitine or crotonobetaine. The purified enzyme catalyzes the dehydration of L-(−)-carnitine to crotonobetaine (H. Jung, K. Jung, and H.-P. Kleber, Biochim. Biophys. Acta 1003:270–276, 1989). The *caiB* gene, encoding carnitine dehydratase, was isolated by oligonucleotide screening from a genomic library of *E. coli* O44 K74. The *caiB* gene is 1,215 bp long, and it encodes a protein of 405 amino acids with a predicted M_r of 45,074. The identity of the gene product was first assessed by its comigration in sodium dodecyl sulfate-polyacrylamide gels with the purified enzyme after overexpression in the pT7 system and by its enzymatic activity. Moreover, the N-terminal amino acid sequence of the purified protein was found to be identical to that predicted from the gene sequence. Northern (RNA) analysis showed that *caiB* is likely to be cotranscribed with at least one other gene. This other gene could be the gene encoding a 47-kDa protein, which was overexpressed upstream of *caiB*.

L-(−)-carnitine [*R*-(−)-3-hydroxy-4-trimethylaminobutyrate] is a compound which is ubiquitous in nature. It can be found in animals, higher plants, and some procaryotes (6, 11). Within eucaryotes, L-(−)-carnitine serves as an essential factor in the transport of long-chain fatty acids through the inner mitochondrial membrane (3).

From the function of L-(−)-carnitine in cell metabolism cited above, a series of clinical and biotechnological applications follow. It is used, for example, in the treatment of patients with carnitine deficiency syndromes and of dialysis patients (1) and for the stimulation of production of monoclonal antibodies (26).

Some microorganisms, especially *Pseudomonas* species, assimilate L-(−)-carnitine as their sole source of carbon and nitrogen (9); others, for instance *Acinetobacter* species, degrade only the carbon backbone by formation of trimethylamine (16). Finally, different members of the family *Enterobacteriaceae*, such as *Escherichia coli*, *Salmonella typhimurium*, and *Proteus vulgaris*, are able to convert carnitine, via crotonobetaine, to γ-butyrobetaine in the presence of C and N sources and under anaerobic conditions (17, 20–22). The activities of L-(−)-carnitine dehydratase [L-(−)-CDHT] (8, 10), crotonobetaine reductase (7, 10), and carnitine racemase (9) were demonstrated in *E. coli*. Studies with whole cells of *E. coli* suggest that these enzymes are inducible in the presence of either L-(−)-carnitine or crotonobetaine in the growth medium during anaerobiosis (10). The biological significance of the pathway (Fig. 1) could lie in the transfer of redox equivalents to crotonobetaine in the absence of other electron acceptors. In support of this hypothesis is the suppression of this reaction by fumarate, nitrate, or glucose (17, 20–22).

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L-(−)-CDHT was purified and characterized by its kinetic and molecular properties (7, 8). During the purification process, a thermostable, low-molecular-weight factor which is essential for enzyme activity was lost. So far, it has been impossible to replace this component with any of the known cofactors which are involved in hydration (dehydration) reactions.

Further details concerning carnitine metabolism in members of the family *Enterobacteriaceae* are interesting in view of its possible application for stereoselective L-(−)-carnitine synthesis (18).

This study deals with the cloning, sequencing, and overexpression of CDHT from *E. coli* O44 K74. This is the first reported sequence of a gene belonging to the carnitine pathway in procaryotes.

MATERIALS AND METHODS

Strains, plasmids, and media. *E. coli* O44 K74 was used to isolate the gene encoding CDHT. *E. coli* Sure (Stratagene) and *E. coli* NM 522 (Stratagene) were used for gene library construction and for the propagation of plasmids. *E. coli* K38 (4) was used for overexpression of CDHT, and SK1592 (S. R. Kuschner, Athens, Georgia) was used for the integration of the *caiB*::*uidA* Km^r mutation. A *caiB* mutant was constructed by integration of an *uidA* Km^r operon fusion cassette (2) into the *Eco*RV-1 site (see Fig. 2) of a plasmid-borne *caiB* gene and subsequent transfer into the chromosome of strain SK1592 (*sbcB15 hsdR hsdM*) by the method described by Winans et al. (27). Plasmids pUC18 and pUC19 (Boehringer) were used as the cloning and sequencing vectors. Overexpression was carried out with the help of plasmids pT7-3 and pT7-4, described by Tabor and Richardson (25). Bacteria were grown aerobically in Luria broth (L broth) at 30, 37, and 42°C, as indicated, or on plates with L broth supplemented with 1.5% agar. Anaerobic growth took place in tightly stoppered 250-ml bottles filled almost to the top with L-broth medium supple-

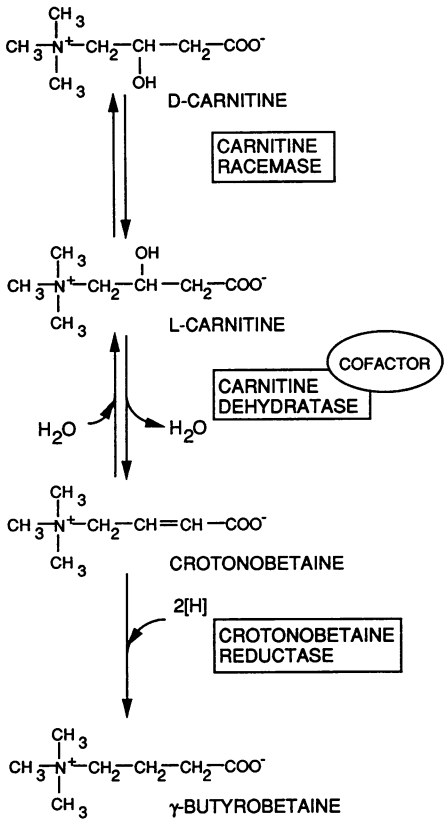

FIG. 1. Schematic representation of the biotransformation of trimethylammonium compounds in *E. coli*.

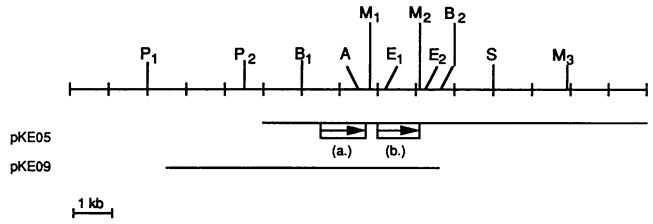

FIG. 2. Restriction map of two clones encoding CDHT isolated from a genomic library of *E. coli* O44 K74. The arrows indicate the direction of transcription of the gene encoding a 47-kDa protein (a.) and *caiB* encoding CDHT (b.). The 3.4-kb fragment *Bam*HI-1–*Bam*HI-2 (B_1-B_2) was subcloned for expression. The 1.5-kb fragment *Acc*I–*Eco*RV-2 (A-E_2) was subcloned for sequencing (see Fig. 3). Restriction enzyme abbreviations: A, *Acc*I; B, *Bam*HI; E, *Eco*RV; M, *Mlu*I; P, *Pst*I; S, *Sma*I.

mented with 2 μM ammonium molybdate as described previously (28). The minimal medium used was M9 (15). When required, antibiotics were added at the following final concentrations: ampicillin, 50 μg/ml; kanamycin, 20 μg/ml; and rifampin, 200 μg/ml.

DNA manipulation. All restriction enzymes were purchased from Boehringer Mannheim, and digestions were performed under the conditions recommended by the manufacturer. T4 DNA ligase and calf intestinal phosphatase were also obtained from Boehringer Mannheim. Chromosomal DNA was prepared from *E. coli* O44 K74 by the method of Smith (24). The following procedures were carried out by the standard methods described by Maniatis et al. (15): preparation of plasmid DNA, agarose gel electrophoresis, DNA ligation, bacterial transformation in the presence of calcium chloride, and Southern blotting.

N-terminal amino acid determination. The N-terminal amino acid sequence of the purified CDHT was determined by the Protein and Nucleic Acid Chemistry Facility in the Department of Chemistry, Zentralinstitut für Molekularbiologie, Berlin-Buch, Federal Republic of Germany.

Screening of the *E. coli* O44 K74 genome library. Chromosomal DNA from *E. coli* O44 K74 was partially digested with *Sau*3A and separated by sucrose density gradient centrifugation. Fragments between 9 and 11 kb in size were chosen and ligated into the dephosphorylated *Bam*HI site of pUC19. The ligation mixture was transformed into competent *E. coli* Sure, and the transformants were screened on L broth agar plates containing ampicillin (50 μg/ml), isopropyl-β-D-thiogalactopyranoside (IPTG) (120 μg/ml), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (50 μg/ml). Colonies were transferred onto a positively charged nylon membrane (Boehringer Mannheim) and screened with three different oligonucleotides directed against the N-terminal sequence of CDHT (see Fig. 3). Oligonucleotides were synthesized by the Protein and Nucleic Acid Chemistry Facility in the Department of Chemistry, Zentralinstitut für Molekularbiologie. Hybridization and detection were carried out with the digoxigenin (DIG) oligonucleotide 3′-end labeling kit and the DIG luminescence detection kit (Boehringer Mannheim).

DNA sequence analysis. All the components for DNA sequence analysis were purchased in the form of a kit from Pharmacia Biochemicals Inc.; the T7 sequencing kit was used for sequencing, and reactions were carried out by methods recommended by the manufacturer. [α-^{35}S]dATP (1,000 Ci/mmol) was purchased from NEN DuPont. DNA restriction fragments encompassing the entire region of interest were subcloned into pUC18 and pUC19, and both strands of the region were sequenced. Analysis of DNA and amino acid sequences was performed with Mac Molly Tetra from Soft Gene GmbH.

Northern (RNA) blot analysis. RNA was prepared from log-phase cultures harboring pKE05 or pKE09 which encode CDHT, or pUC19, as a standard, as previously described (23). Cells were grown under anaerobic conditions in the presence of 20 mM DL-carnitine (10). A Northern blot analysis was performed by standard molecular biological techniques (15). The probe used was an internal fragment of the gene encoding CDHT, excised from agarose gel and then labeled with the DIG-labeled dUTP by the random oligonucleotide method of Feinberg and Vogelstein (5). Detection was carried out with the DIG luminescence detection kit (Boehringer Mannheim).

Overexpression of CDHT. Plasmids pT7-3 and pT7-4 contain the T7 ϕ10 promoter upstream of different multiple cloning sites (25). These two plasmids were used for the construction of the hybrid plasmids pT7-3KE15 and pT7-4KE16 (which both contain the gene encoding CDHT and a gene encoding a 47-kDa protein) and plasmids pT7-3KE17 and pT7-4KE18 (which harbor only a DNA fragment encoding the 47-kDa protein). Cloning into pT7-3 and pT7-4 generates constructions with both orientations of the insert with respect to the promoter. These four plasmids were transformed into strain K38 harboring the compatible plasmid pGP1-2 containing the gene coding for T7 RNA polymerase under the control of the heat-inducible λ p_L promoter. The proteins specifically expressed from the genes cloned downstream of the ϕ10 promoter of pT7-derived plasmids were labeled with a mixture

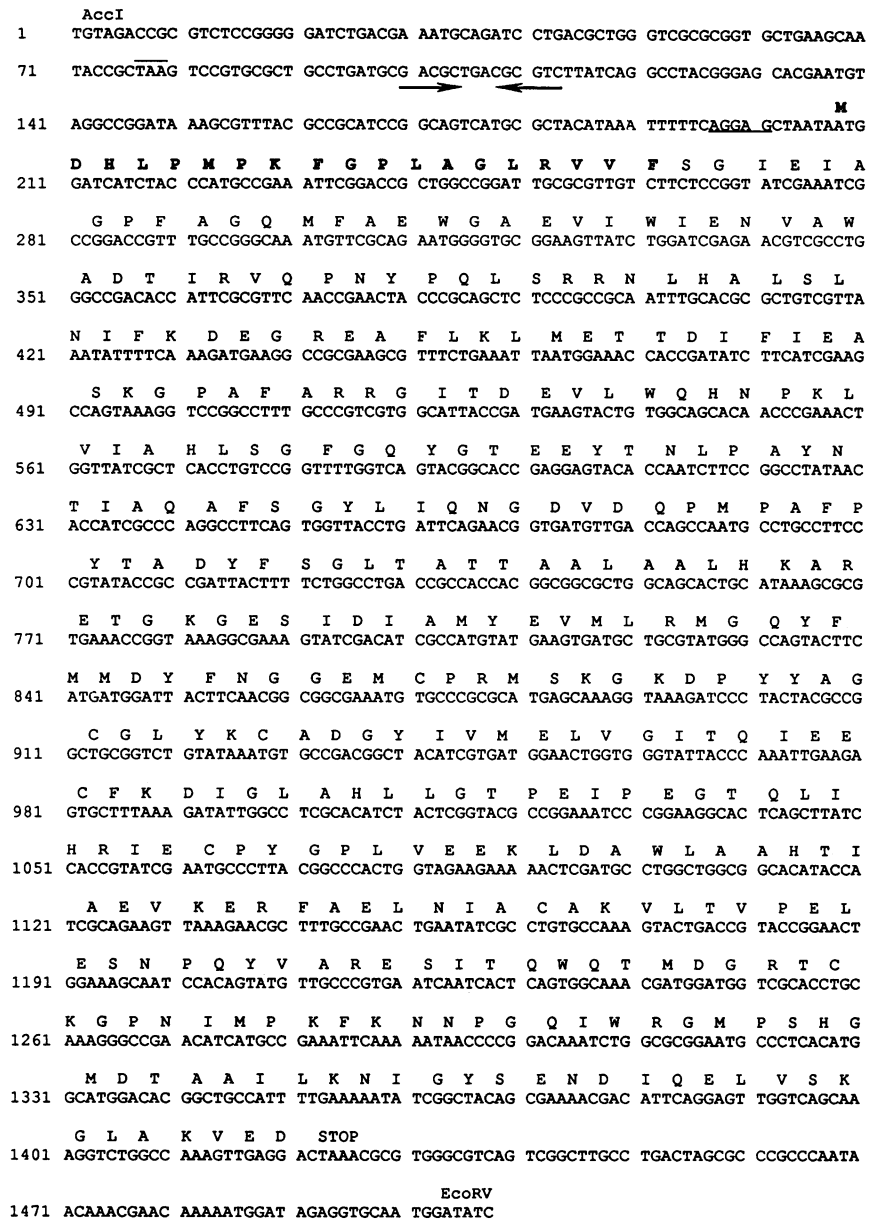

FIG. 3. Sequence of the 1.5-kb *Acc*I–*Eco*RV-2 fragment which gave a positive signal in Southern hybridization with oligonucleotide probes derived from the N-terminal sequence of CDHT. The deduced amino acid sequence of the *caiB* gene product is given over the coding sequence. The putative ribosome binding site is underlined. An inverted repeat located upstream is indicated by the two arrows. The putative termination codon of the gene encoding the 47-kDa protein is overlined. The N-terminal sequence of CDHT is presented in boldface type.

of [^{35}S]methionine and [^{35}S]cysteine (1,000 Ci/mmol; NEN DuPont) bythe protocol of Tabor and Richardson (25). Cells were then lysed in a solution containing 60 mM Tris-HCl (pH 6.8), 1% sodium dodecyl sulfate (SDS), and 1% 2-mercaptoethanol by heating the solution for 5 min at 95°C and finally, the cells were loaded onto an SDS–12.5% polyacrylamide gel as described by Laemmli (12).

Enzyme assays. CDHT was assayed by the method described by Jung et al. (8). Protein concentration was determined by the method of Lowry et al. (13), using bovine serum albumin as the standard.

Nucleotide sequence accession number. The nucleotide data reported in this article will appear in the EMBL data library under accession number X67748 ECCAIB.

RESULTS AND DISCUSSION

Cloning of CDHT gene from *E. coli* O44 K74. *E. coli* O44 K74 has a significantly higher CDHT activity than other *E. coli* strains (18). Therefore, we decided to construct a chromosomal library from this strain, which was isolated from the intestine of a rat which had been fed a carnitine-rich diet (19). Approximately 3,000 transformants of the Sure strain carrying fragments from the *E. coli* O44 K74 chromosome were obtained. This chromosomal library represents more than 99% of the *E. coli* O44 K74 genome, as calculated by the formula given by Maniatis et al. (15).

Five colonies harboring the recombinant plasmids pKE05,

pKE06, pKE07, pKE08, and pKE09 were isolated after screening with three different degenerate oligonucleotide probes derived from the N-terminal sequence of the purified CDHT (see Fig. 3).

Whole-cell preparations of these five colonies were assayed for CDHT activity after growth under anaerobic conditions in the presence of 20 mM DL-carnitine. For an unknown reason, two colonies harboring the recombinant plasmids pKE05 and pKE09 (Fig. 2) displayed a twofold increase of CDHT activity over that of *E. coli* Sure carrying the plasmid vector pUC19.

Restriction mapping of positive clones. Plasmids were isolated from the five positive colonies and digested with several restriction enzymes. Hybridization was confirmed by Southern analysis. The restriction map of pKE05 and pKE09 is shown in Fig. 2. Within each of the five clones (pKE05, pKE06, pKE07, pKE08, and pKE09), a hybridizing fragment *Mlu*I-1–*Eco*RV-1 0.35 kb long was located. A 3.4-kb *Bam*HI-1–*Bam*HI-2 fragment and a 2.0-kb *Bam*HI-1–*Eco*RV-1 fragment were subcloned into pT7-3 and pT7-4 for overexpression (discussed below), allowing us to determine the approximate location and transcription direction of the gene encoding CDHT. Thus, the 1.5-kb *Acc*I–*Eco*RV-2 fragment, assumed to contain the entire gene encoding CDHT, was chosen for further subcloning and sequenced in both directions.

Nucleotide sequence of the gene encoding CDHT. The nucleotide sequence of CDHT is shown in Fig. 3. Analysis of the sequence with the Mac Molly Tetra nucleotide and amino acid interpretation program revealed one open reading frame (ORF). All other reading frames had multiple stop codons. The sequenced ORF is 1,215 bp long and encodes a protein of 45,074 Da. This result is in perfect agreement with the estimated molecular mass of 45,000 Da proposed by Jung et al. (8) for the purified CDHT. In addition, the previously determined N-terminal sequence of purified CDHT was compared with the deduced amino acid sequence from the sequenced ORF. With the exception of a Leu or Arg residue at position 16, both sequences were identical. Since the nucleic acid sequence was determined five times and consistently revealed an arginine residue at position 16, we propose that arginine is the correct residue. Therefore, the mismatch might result from the technical limitations of the protein chemistry facility.

A putative Shine-Dalgarno sequence (AGGAG underlined in Fig. 3) is located 7 bp upstream of the ATG start codon. An inverted repeat from nucleotides 100 to 113 (GACGC----GCGTC, indicated by the arrows in Fig. 3) was found 94 nucleotides upstream of the initiating ATG codon. The inverted repeat may be a putative regulatory element for the expression of CDHT. Upstream of the gene encoding CDHT, another ORF was observed, at a distance of 129 nucleotides. Since this second ORF was expressed in the same transcription direction as that of the CDHT gene (see below) and the two were separated by a short distance that does not include a terminator sequence, they might belong to the same operon. The upstream ORF is currently being sequenced. Thus, we assigned the name *caiB* (carnitine) to the gene encoding CDHT.

A search of data bases (NBRL and Swiss-Prot) by use of the deduced amino acid sequence for *caiB* revealed that this protein sequence displayed a high degree of homology (FASTA 706, 38% identity in a 405-amino-acid overlap) with the sequence of the 47-kDa BaiF protein from *Eubacterium* sp. (14). The 47-kDa BaiF protein is involved in bile acid dehydration reactions in the human intestine. Since both proteins come from anaerobic intestinal bacteria and catalyze dehydration reactions, they may share a common evolutionary origin. Comparing the substrate specificity of CDHT for bile acids, as well as that of 47-kDa BaiF protein for carnitine, will require additional studies.

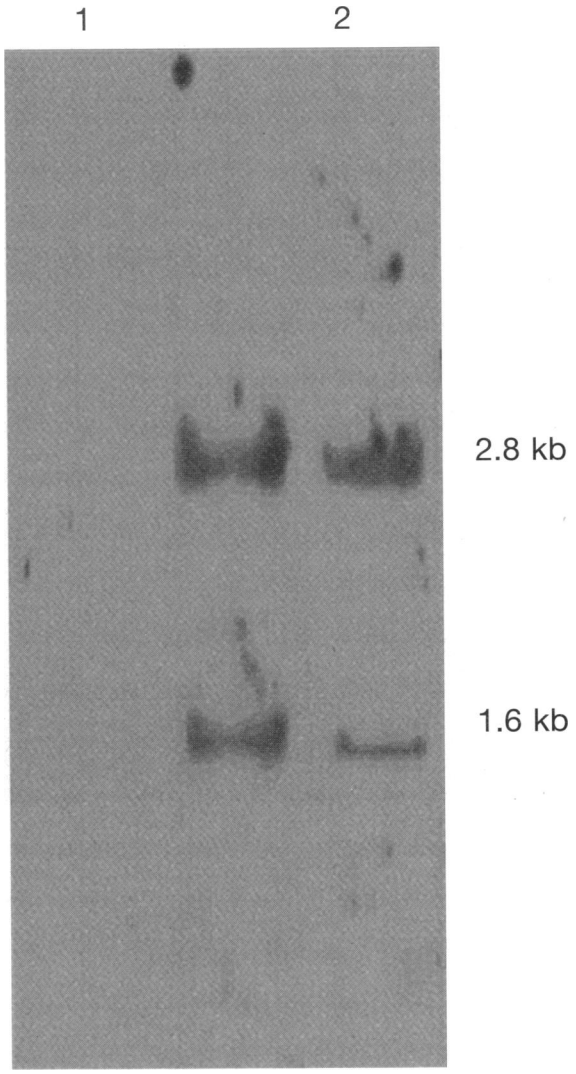

FIG. 4. Northern blot analysis of transcripts from the *caiB* gene and the upstream ORF. Luminograph of a Northern blot of RNAs extracted from *E. coli* Sure harboring pUC19 (control) (left lane), pKE09 (center lane), and pKE05 (right lane) probed with an internal fragment of *caiB* labeled with DIG dUTP and detected by the DIG luminescence detection kit (Boehringer Mannheim).

Northern analysis. In order to determine whether *caiB* is transcribed alone, Northern blot analysis was carried out. The mRNA species were extracted from log-phase anaerobic cultures of the Sure strain harboring either pKE05 or pKE09. Both plasmids encoded CDHT, and revealed an augmentation of CDHT activity in the enzymatic assay. A DNA probe which contained an internal fragment of *caiB* was used for hybridization. The probe hybridized to two mRNA species which appeared to be 2.8 and 1.6 kb long (Fig. 4). The 2.8-kb mRNA species therefore appears to have the coding capacity for an additional ORF(s), which indicates that the *caiB* gene is cotranscribed with at least one other gene. The upstream gene is a likely candidate. The 1.6-kb mRNA species might be a product of degradation or the result of the presence of a second promoter.

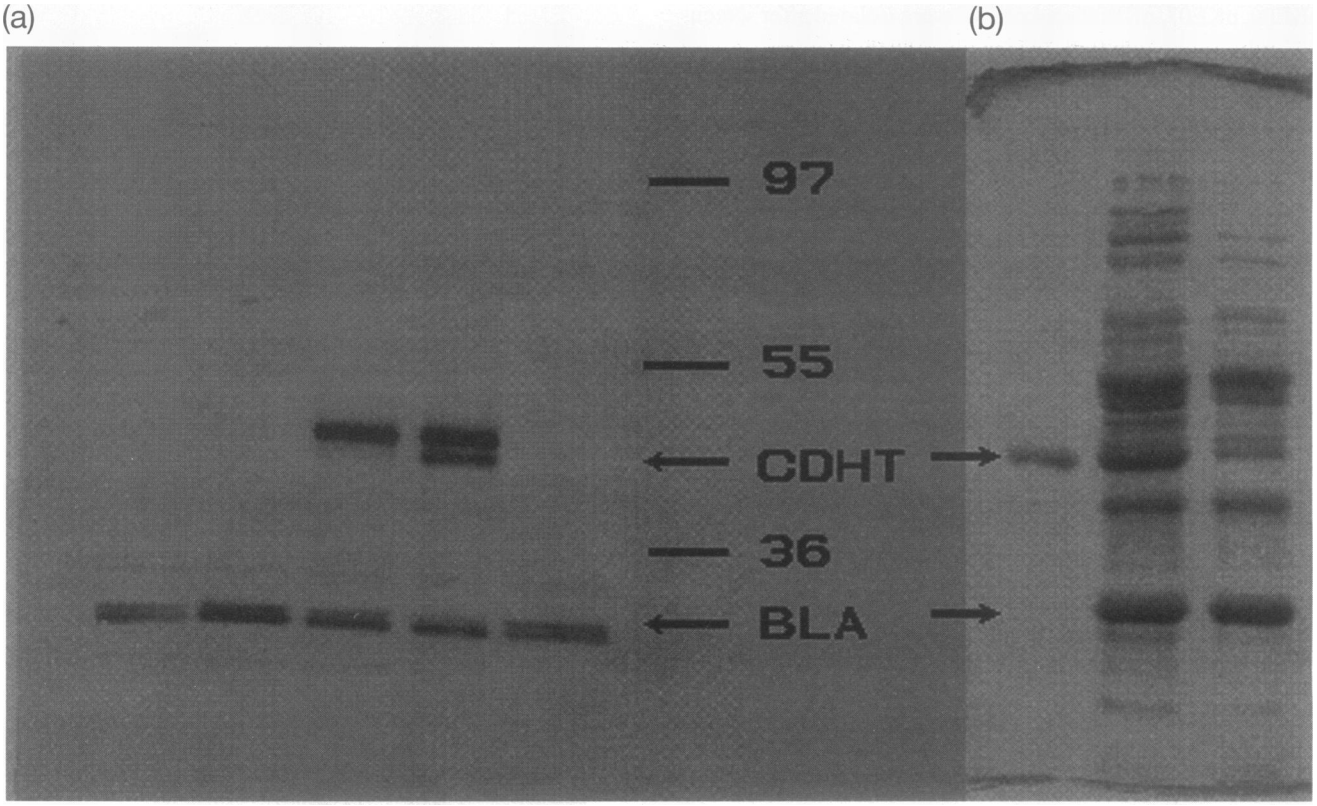

FIG. 5. Overexpression of the *caiB* gene product using the T7 RNA polymerase-promoter system of Tabor and Richardson (25). The ^{35}S-labeled polypeptides were separated on a 12.5% polyacrylamide gel containing 0.1% SDS. Visualization was done by autoradiography (a) and Coomassie blue staining (b). The radioactivity of each sample loaded onto the gel was approximately 10^6 cpm. The 31-, 29-, and 26-kDa polypeptides of β-lactamase (BLA) were expressed from pT7-3 and pT7-4 recombinant derivatives. The positions of molecular mass markers are shown in kilodaltons. From left to right, the five lanes in panel a contain pT7-3, pT7-4KE16, pT7-3KE15, pT7-3KE17, and pT7-4KE18, and the three lanes in panel b contain purified CDHT, pT7-3KE15, and pT7-3.

Overexpression of CDHT. Identification of the CaiB protein was performed with the in vivo T7 promoter-RNA polymerase system, in which the genes are expressed under the control of the φ10 promoter (25).

Derivatives of plasmids pT7-3 and pT7-4 gave rise to the synthesis of three protein bands of 31, 29, and 26 kDa, which are characteristic of β-lactamase (Fig. 5a). The middle band corresponding to the processed enzyme was expressed very strongly. Plasmid pT7-3KE15 (containing the 3.4-kb *Bam*HI-1–*Bam*HI-2 fragment) directed the synthesis of proteins with the estimated molecular masses of 47 and 45 kDa. Plasmid pT7-3KE17 (containing the 2.0-kb *Bam*HI-1–*Eco*RV-1 fragment) expressed only the 47-kDa protein. The expression of these proteins was not observed from pT7-4KE16 or from pT7-4KE18, where the encoding fragments are cloned in the opposite direction. The 45-kDa protein corresponded perfectly in size with the purified CDHT, as shown on a Coomassie blue-stained gel (Fig. 5b). The expression of *caiB* confirmed the estimated molecular mass of CDHT and showed that there is no posttranslational modification of the enzyme. Overexpression of the 47-kDa protein confirmed the presence of an ORF in the flanking DNA fragment upstream of *caiB*.

Enzymatic assay of overexpressed CDHT. In order to confirm the identity of the overexpressed product of *caiB* as CDHT, enzymatic activities of crude extracts from strains harboring the *caiB* recombinant plasmids pT7-3KE15 and pT7-3KE17 were measured in comparison with those detected in the strain carrying the vector pT7-3 alone. The results are shown in Table 1. The recombinant plasmid pT7-3KE15 showed levels of CDHT 21-fold higher than those of the wild-type O44 K74 after growth in the presence of 20 mM DL-carnitine (Table 1). It has been shown that CDHT requires an unknown cofactor which is necessary for activity. This

TABLE 1. Assay for CDHT activity in crude cell extracts of the wild-type *E. coli* O44 K74 and *E. coli* K38 harboring various plasmids

E. coli strain[a]	Sp act (μmol min^{-1} mg^{-1})		
	With 20 mM carnitine	Without carnitine	
		With cofactor[b]	Without cofactor
O44 K74	0.030	0.01	0
K38(pT7-3KE17)[c]	0.030	0.01	0
K38(pT7-3KE15)[c]	0.650	0.05	0
K38(pT7-3)[c]	0.030	0.01	0
SK1592	0.015		
KE3011[d]	0		

[a] Strains were grown anaerobically in L broth, in the presence of carnitine when required.
[b] Cofactor was prepared from *E. coli* O44 K74 by the method of Jung et al. (8).
[c] Overexpression took place under anaerobic conditions by using the protocol of Tabor and Richardson (25).
[d] This strain was a *caiB*::*uidA* Km^r mutant derived from strain SK1592.

cofactor can be found only in cells grown under anaerobic conditions in the presence of carnitine (7, 8). Moreover, it was possible to reconstitute the activity of CDHT after overexpression of the corresponding gene in the pT7 system, even in the absence of inducer, provided that a cofactor preparation necessary for the activity had been added (Table 1). In addition, an operon fusion cassette, which carries the promoterless gene of β-glucuronidase (*uidA*) and a kanamycin resistance gene flanked by a polylinker (2), was integrated into the *Eco*RV-1 site to disrupt the *caiB* gene. This construction was then transferred into the genome of *E. coli* SK1592. The obtained mutant, KE3011, was found to display no CDHT activity. These results strongly support the identity of the *caiB* gene product as CDHT.

In this study we report for the first time the isolation of a gene participating in the carnitine pathway in procaryotes. Sequencing, Northern blot analysis, and expression studies suggest that this gene belongs to a *cai* operon which also contains other genes. Current work in our laboratory is aimed at elucidating the genetic organization of the anaerobic carnitine pathway in *E. coli* and the functions of the proteins involved. It would be of particular interest to identify the control mechanism by which this system is regulated under anaerobiosis.

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REFERENCES

1. **Achenbach, H., H.-J. Kühn, J. Lössner, and H. Seim.** 1985. Klinische Anwendung des Carnitins unter besonderer Berücksichtigung der Carnitinmangelsyndrome. Wiss. Z. Karl Marx Univ. Leipz. Math. Naturwiss. Reihe **34:**259–272.
2. **Bardonnet, N., and C. Blanco.** 1992. *'uidA*-antibiotic-resistance cassettes for insertion mutagenesis, gene fusions and genetic constructions. FEMS Microbiol. Lett. **93:**243–248.
3. **Bremer, J.** 1983. Carnitine—metabolism and functions. Physiol. Rev. **63:**1420–1480.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:** 1141–1156.
5. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
6. **Fraenkel, G.** 1953. Studies on the distribution of vitamin B_T (carnitine). Biol. Bull. **104:**359–372.
7. **Jung, H., and K. Jung.** 1988. Zur Charakterisierung und Regulation der L(−)-Carnitin Metabolisierung in *Escherichia coli*. Ph.D. thesis. University of Leipzig, Leipzig, Federal Republic of Germany.
8. **Jung, H., K. Jung, and H.-P. Kleber.** 1989. Purification and properties of carnitine dehydratase from *Escherichia coli*—a new enzyme of carnitine metabolization. Biochim. Biophys. Acta **1003:** 270–276.
9. **Jung, H., and H.-P. Kleber.** 1991. Metabolism of D(+)carnitine by *Escherichia coli*. Appl. Microbiol. Biotechnol. **35:**393–395.
10. **Jung, K., H. Jung, and H.-P. Kleber.** 1987. Regulation of L-carnitine metabolism in *Escherichia coli*. J. Basic Microbiol. **27:** 131–137.
11. **Kleber, H.-P., and H. Aurich.** 1985. Stoffwechsel des Carnitins bei Mikroorganismen. Wiss. Z. Karl Marx Univ. Leipz. Math. Naturwiss. Reihe **34:**224–237.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
14. **Mallonee, D. H., W. B. White, and P. B. Hylemon.** 1990. Cloning and sequencing of a bile acid-inducible operon from *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **172:**7011–7019.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Miura-Fraboni, J., and S. Englard.** 1983. Quantitative aspects of γ-butyrobetaine and D- and L-carnitine utilization by growing cell cultures of *Acinetobacter calcoaceticus* and *Pseudomonas putida*. FEMS Microbiol. Lett. **18:**113–116.
17. **Seim, H., R. Ezold, H.-P. Kleber, and E. Strack.** 1980. Stoffwechsel des L-Carnitins bei Enterobakterien. Z. Allg. Mikrobiol. **20:**591–594.
18. **Seim, H., and H.-P. Kleber.** 1988. Synthesis of L(−)-carnitine by hydration of crotonobetaine by enterobacteria. Appl. Microbiol. Biotechnol. **27:**538–544.
19. **Seim, H., H.-P. Kleber, and E. Strack.** 1979. Reduktion von L-Carnitin zu γ-Butyrobetain durch *Escherichia coli*. Z. Allg. Mikrobiol. **19:**753–758.
20. **Seim, H., H. Löster, R. Claus, H.-P. Kleber, and E. Strack.** 1982. Stimulation of the anaerobic growth of *Salmonella typhimurium* by reduction of L-carnitine, carnitine derivates and structure-related trimethylammonium compounds. Arch. Microbiol. **132:**91–95.
21. **Seim, H., H. Löster, R. Claus, H.-P. Kleber, and E. Strack.** 1982. Formation of γ-butyrobetaine and trimethylamine from quaternary ammonium compounds structure-related to L-carnitine and choline by *Proteus vulgaris*. FEMS Microbiol. Lett. **13:**201–205.
22. **Seim, H., H. Löster, and H.-P. Kleber.** 1982. Reduktiver Stoffwechsel des L-Carnitins und strukturverwandter Trimethylammoniumverbindungen in *Escherichia coli*. Acta Biol. Med. Ger. **41:**1009–1019.
23. **Shimotsu, H., M. I. Kuroda, C. Yanofsky, and D. J. Henner.** 1986. Novel form of transcription attenuation regulates expression of the *Bacillus subtilis* tryptophan operon. J. Bacteriol. **166:**461–471.
24. **Smith, H. G.** 1967. Isolation of high molecular weight DNA from normal and phage infected *Escherichia coli*. Methods Enzymol. **12:**545–549.
25. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
26. **Typlt, H., R. Claus, and K. Nitzsche.** 1991. Influence of carnitine on the growth and productivity of murine hybridoma cells. J. Biotech. **18:**173–176.
27. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161:**1219–1221.
28. **Wu, L. F., and M. A. Mandrand-Berthelot.** 1986. Genetic and physiological characterization of new *Escherichia coli* mutants impaired in hydrogenase activity. Biochimie **68:**167–179.