JOURNAL OF BACTERIOLOGY, July 1989, p. 3667–3672
0021-9193/89/073667-06$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 171, No. 7

Genetic Characterization of *Bacillus subtilis odhA* and *odhB*, Encoding 2-Oxoglutarate Dehydrogenase and Dihydrolipoamide Transsuccinylase, Respectively

PETER CARLSSON* AND LARS HEDERSTEDT

Department of Microbiology, University of Lund, Sölvegatan 21, S-223 62 Lund, Sweden

Received 19 December 1988/Accepted 10 April 1989

The 2-oxoglutarate dehydrogenase complex consists of three different subenzymes, the E1o (2-oxoglutarate dehydrogenase) component, the E2o (dihydrolipoyl transsuccinylase) component, and the E3 (dihydrolipoamide dehydrogenase) component. In *Bacillus subtilis*, the E1o and E2o subenzymes are encoded by *odhA* and *odhB*, respectively. A plasmid with a 6.8-kilobase-pair DNA fragment containing *odhA* and *odhB* was isolated. Functional E1o and E2o are expressed from this plasmid in *Escherichia coli*. Antisera generated against *B. subtilis* E1o and E2o expressed in *E. coli* reacted with antigens of the same size from *B. subtilis*. The nucleotide sequence of *odhB* and the terminal part of *odhA* was determined. The deduced primary sequence of *B. subtilis* E2o shows striking similarity to the corresponding *E. coli* protein, which made it possible to identify the lipoyl-binding lysine residue as well as catalytic histidine and aspartic acid residues. An mRNA of 4.5 kilobases hybridizing to both *odhA* and *odhB* probes was detected, indicating that *odhA* and *odhB* form an operon.

The 2-oxoglutarate dehydrogenase multienzyme complex (ODHC) catalyzes the oxidative decarboxylation of 2-oxoglutarate to succinyl coenzyme A, a reaction which is part of the citric acid cycle. In eucaryotes and eubacteria, this enzyme complex is composed of three different enzymes, present in nonequivalent stoichiometry. In *Escherichia coli*, each ODHC contains 12 E1o (2-oxoglutarate dehydrogenase; EC 1.2.4.2), 24 E2o (dihydrolipoamide transsuccinylase; EC 2.3.1.61) and 12 E3 (dihydrolipoamide dehydrogenase; EC 1.8.1.4) subenzymes (32). E1o and E2o are unique for ODHC, whereas E3 is shared between ODHC and the analogous pyruvate dehydrogenase and the branched-chain 2-oxo-acid dehydrogenase complexes.

E. coli E2o polypeptides form a cubic core with octahedral symmetry to which the E1o and E3 components are bound (9). The substrate is transferred between the different active sites by a lipoyl moiety, bound to a lysine residue in the E2o polypeptide.

In *E. coli*, the genes encoding E1o and E2o are situated adjacent to each other as part of an operon which also contains the genes encoding the α and β subunits of succinyl coenzyme A synthetase. The gene encoding E3, however, is part of another operon also encoding the pyruvate dehydrogenase (E1p) and the dihydrolipoamide transacetylase (E2p) subenzymes of the pyruvate dehydrogenase multienzyme complex (35). The organization of the corresponding genes in the gram-positive, endospore-forming bacterium *Bacillus subtilis* appears similar (6, 7, 13, 19), but has not been analyzed in detail. In *B. subtilis*, E1o and E2o are encoded by the *odhA* (formerly *citK*) and *odhB* (formerly *citM*) genes, respectively (the genes have been renamed to let the designations more directly refer to the gene products). The aim of this work was to analyze the primary structure of *B. subtilis* E2o and to determine the genetic organization of *odhA* and *odhB*.

The nucleotide sequence of the previously cloned *odhB* (7) is presented, and the deduced primary structure of *B. subtilis* E2o is compared with that of other lipoyl-containing enzymes. The complete *odhA* has been cloned and is shown to express functional E1o in *E. coli*. Transcript and nucleotide sequence analyses show that *odhA* and *odhB* constitute an operon.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used are listed in Table 1.

Media and growth of bacteria. *B. subtilis* and *E. coli* strains were kept on Penassay agar plates (17.5 g of Antibiotic Medium no. 3 [Difco Laboratories, Detroit, Mich.] and 20 g of Bacto-Agar [Difco] per liter). The Cit phenotype was checked on purification agar plates (5). Antibiotics were added to the following final concentrations: ampicillin, 35 μg/ml; chloramphenicol, 5 μg/ml (*B. subtilis*) or 12.5 μg/ml (*E. coli*). Minimal glucose medium was prepared as described by Spizizen (36) with the addition of $MnCl_2$ to a final concentration of 10 μM.

In vitro DNA and RNA techniques. Agarose gel electrophoresis was performed by the method of Maniatis et al. (25). *B. subtilis* chromosomal DNA was prepared by using the plasmid preparation procedure of Canosi et al. (4). Plasmid DNA was prepared as described by Ish-Horowicz and Burke (21). Restriction endonucleases and T4 DNA ligase were purchased from Boehringer Mannheim (Mannheim, Federal Republic of Germany) and used as recommended by the manufacturer. Preparation of RNA and Northern (RNA) blot analysis were performed as described by Melin et al. (26).

Transformation. *E. coli* cells were made competent by the $CaCl_2$ method of Mandel and Higa (24). *B. subtilis* was grown to competence as described by Arwert and Venema (1).

DNA sequence analysis. Nucleotide sequence determination was performed using the dideoxy chain termination method of Sanger et al. (33). DNA fragments were cloned into M13mp18 and mp19 (29) and sequenced using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio), [α-^{35}S]dATP, universal M13 primers, and *odhB*-specific oligonucleotide primers (see Fig. 2). Computer analysis of the

TABLE 1. Bacterial strains and plasmids used

Bacterial strain or plasmid	Genotype or phenotype[a]	Source	Reference
B. subtilis			
3G18	*trpC2 ade met*	G. Venema	
CU1693	*trpC2* Δ(SPβ *kauA odhA odhB gltB gltA*)	T. P. Iismaa	20
E. coli			
5K	*hsdM hsdR rpsL thr thiA*	L. O. Hedén	23
JRG72 (W1485 *sucl*)	*sucA1 supE42 iclR*	M. E. Spencer	15, 18
JM83	*ara* Δ(*lac-proAB*) *rpsL* φ80 *lacZ* M15		40
Plasmids and phages			
pHV14	Ap^r Cm^r	S. D. Ehrlich	12
pHV32	Ap^r Tc^r Cm^r	S. D. Ehrlich	28
pUC18	Ap^r		29
M13	mp18		29
M13	mp19		29
pLUC3	Ap^r Cm^r *odhB*		7
pLUC5	Ap^r Cm^r *odhA odhB*	This work	
pLUC51	Ap^r *odhA*	This work	

[a] Ap, Ampicillin; Cm, chloramphenicol; Tc, tetracycline.

nucleotide sequences obtained was performed with the GCG Sequence Software Package, version 5.3 (10).

Preparation of cell extracts. *B. subtilis* cell extracts were prepared as described by Ohné et al. (30) from bacteria grown in Penassay broth (17.5 g of Antibiotic Medium no. 3 per liter) at 37°C. *E. coli* cell extracts were prepared from spheroplasts (22), which were lysed in 50 mM potassium phosphate buffer (pH 7.4). Cell membranes were removed by centrifugation at 35,000 × *g*, at 4°C, for 30 min.

Generation of antisera. To generate an E1o-specific antiserum, a cytoplasmic extract of *E. coli* JRG72(pLUC5) was fractionated at 4°C on a Sephacryl S-300 (Pharmacia, Uppsala, Sweden) gel filtration column equilibrated and eluted with 50 mM NaCl–30 mM Tris hydrochloride (pH 8.0). Fractions containing E1o were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and pooled. The pooled fractions were dialyzed against water, lyophilized, suspended in phosphate-buffered saline, and used for immunization.

E2o-specific antiserum was obtained as follows. An *E. coli* JRG72(pLUC3) cytoplasmic extract was subjected to preparative SDS-PAGE. The E2o polypeptide was localized in the gel and isolated according to a procedure described in detail previously (17).

Immunization of rabbits with the partially purified E1o (0.6 mg per immunization) and with isolated E2o (2.5 μg per immunization) was done as described previously (17). The immunoglobulins were purified and stored as described by Harboe and Ingild (16) except that the DEAE-Sephadex step was omitted.

SDS-PAGE and immunoblotting. SDS-PAGE was performed as described by Neville (27). The proteins, separated in a 10% (wt/vol) acrylamide–0.27% (wt/vol) bisacrylamide gel, were transferred to nitrocellulose filters (BA85; Schleicher & Schüll, Dassel, Federal Republic of Germany) by the method of Towbin et al. (38). The filters were blocked, probed with primary and secondary antibodies, and developed as described by Blake et al. (2). The partially purified immunoglobulin fraction of rabbit antisera was used as primary antibody. The secondary antibody, affinity-purified alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.), was used at a 1:1,000 dilution.

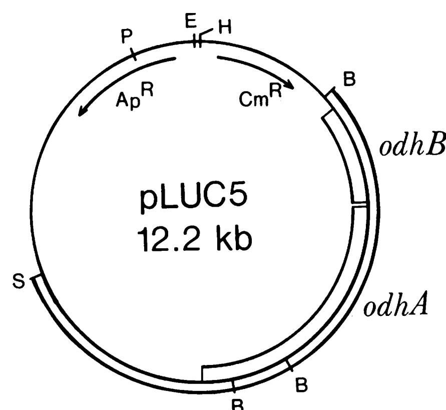

FIG. 1. Schematic map of pLUC5. The boxed region corresponds to cloned *B. subtilis* DNA, and *odhA* and *odhB* are indicated. The plasmid is a pHV32 derivative. Restriction sites: P, *Pst*I; E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sal*I. P, E, and H sites in the cloned fragment are shown in Fig. 2. Ap^R, Ampicillin resistance gene; Cm^R, chloramphenicol resistance gene.

RESULTS AND DISCUSSION

Cloning of *odhA*. The previously described plasmid pLUC3 is a derivative of pHV32 that contains *odhB* and the terminal part of *odhA* on a 3.4-kilobase-pair (kbp) *Bam*HI fragment (7). It cannot replicate in *B. subtilis*, and in order to express the vector-coded chloramphenicol resistance, the plasmid has to integrate in the *B. subtilis* chromosome by homologous recombination (Campbell-like mechanism). To clone the complete *odhA*, *B. subtilis* 3G18 was transformed to chloramphenicol resistance with pLUC3. Chromosomal DNA from one such transformant was isolated and digested with *Sal*I, which cuts only once in the vector part. Transformation of *E. coli* 5K to ampicillin resistance with the digested and then ligated chromosomal DNA was predicted to result in clones containing both *odhA* and *odhB*. Plasmid pLUC5 was isolated from one ampicillin-resistant *E. coli* transformant (Fig. 1).

The positions of the *Sal*I and *Bam*HI restriction sites in the 6.8-kbp cloned fragment in pLUC5 are in good accordance with the restriction map of this part of the chromosome established by Weiss and Wake (39). Subcloning of the 5.7-kbp *Sal*I-*Kpn*I DNA fragment (Fig. 2) of pLUC5 into pUC18 resulted in pLUC51, which lacks *odhB*.

Expression of *odhA* in *E. coli*. *B. subtilis* E2o expressed in an E2o-deficient *E. coli* mutant can complement the Odh$^-$ phenotype of the mutant (7). Expression of functional *odhA* gene product (E1o) from pLUC5 and pLUC51 was similarly determined. An *E. coli* E1o-deficient mutant, JRG72, was transformed with the two plasmids selecting for the wild-type ODHC phenotype (ability to grow on minimal glucose plates) as described previously (7). The wild-type phenotype was restored in both *E. coli* JRG72(pLUC5) and *E. coli* JRG72(pLUC51), which shows that functional *B. subtilis* E1o can be expressed from both plasmids.

A 60- and a 110-kilodalton (kDa) polypeptide were the most abundant proteins in cytoplasmic extracts of *E. coli* 5K(pLUC5). These polypeptides were not found in *E. coli*

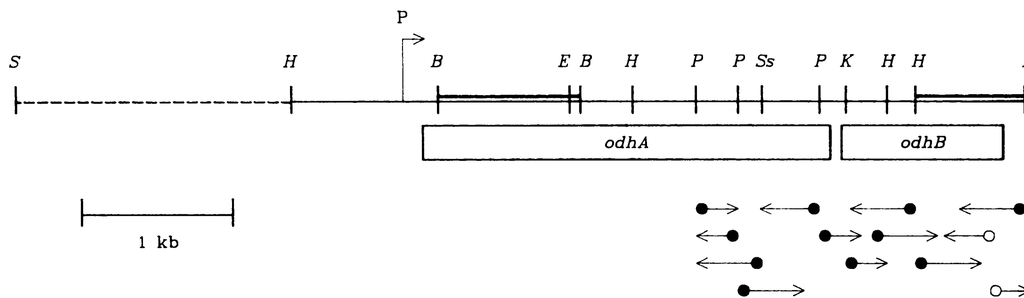

FIG. 2. Map of the cloned 6.8-kbp *B. subtilis* DNA fragment containing *odhA* and *odhB*. The boxes indicate the open reading frames of *odhA* and *odhB* as determined from nucleotide sequences and the size of E1o (this work and unpublished results). Restriction sites: *S*, *Sal*I; *H*, *Hin*dIII; *B*, *Bam*HI; *Ss*, *Sst*I; *E*, *Eco*RI; *P*, *Pst*I; *K*, *Kpn*I. *H* and *P* sites in the dashed *S-H* fragment are not presented. P indicates the *odhAB* promoter (Melin et al., in preparation). The strategy and extent of nucleotide sequencing are shown by arrows. Open circles indicate that specific oligonucleotides were used as sequencing primers. Double lines show DNA fragments used as probes in DNA-RNA hybridization (Fig. 5). Note that the 0.2-kbp *Hin*dIII fragment in *odhB* is misplaced in the maps in reference 7.

5K(pHV32) (Fig. 3). *E. coli* JM83(pLUC51) extracts contained only the 110-kDa polypeptide. *E. coli* E1o has a molecular mass of 105 kDa (8). The 60-kDa polypeptide has previously been identified as *B. subtilis* E2o (7). Thus, the 110-kDa polypeptide was concluded to be *B. subtilis* E1o. This conclusion was confirmed by immunoblotting analyses. Antisera against the 60- and 110-kDa polypeptides expressed in *E. coli* reacted with *B. subtilis* wild-type cytoplasmic antigens of the same size (Fig. 4). These antigens were not present in *B. subtilis* CU1693, which has a deletion spanning *odhA* and *odhB* (20). The results show that pLUC5 and pLUC51 contain the complete *odhA* and that they encode functional E1o in *E. coli*.

Nucleotide sequence of *odhB*. The nucleotide sequence of the *odhB* region, including part of *odhA*, was determined using subcloned DNA fragments from pLUC3. The sequencing strategy is shown in Fig. 2. The *odhB* sequence contains an open reading frame consisting of 1,251 base pairs (bp) (including ATG) (Fig. 5). This open reading frame is preceded by a putative ribosome-binding site, d(AAGGGGG), 10 bp from the ATG translational start codon. The nucleotide sequence of the open reading frame shows 56% identity to *E. coli sucB*, which encodes E2o (34). The deduced amino acid sequence shows 66% similarity to *E. coli* E2o, including conserved exchanges.

Genetic mapping experiments (6, 7, 13, 19) and complementation studies (e.g., with pLUC51) demonstrate that *odhA* is located adjacent to and upstream of *odhB*. The open reading frame of *odhA* was identified by comparing the deduced C-terminal amino acid sequence with that of *E. coli* E1o (8). Only 15 bp separates the *odhA* translational stop codon from the *odhB* translational start codon (Fig. 5).

Transcription analysis of *odhA* and *odhB*. To determine whether the *odhA* and *odhB* genes are members of one operon as are their counterparts in *E. coli*, RNA-DNA hybridization experiments (Northern blots) were performed (Fig. 6). Internal *odhA* and *odhB* DNA fragments were used as DNA probes (Fig. 2). Both probes hybridized to an mRNA species of approximately 4.5 kilobases (kb) (Fig. 6), suggesting that *odhA* and *odhB* are transcribed from the

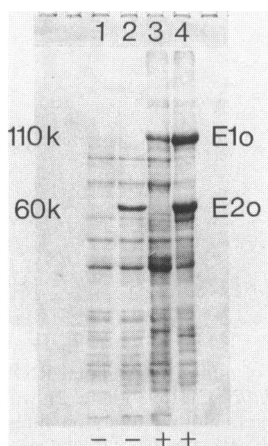

FIG. 3. SDS-PAGE of *E. coli* extracts. Total cytoplasmic extracts were fractionated on an SDS–10% (wt/vol) acrylamide gel and stained for protein with Coomassie blue R250. Lanes: 1, 5K(pHV32); 2, 5K(pLUC3) (pLUC3 contains only the 3.4-kbp *Bam*HI fragment, i.e., only *odhB* [Table 1; 7]); 3, JM83(pLUC51); 4, 5K(pLUC5). Approximately 80 μg of protein was loaded on each lane. k, Kilodaltons. The ability of the respective plasmid to transform *E. coli* JRG72 (E1o deficient) to the Odh$^+$ phenotype is indicated by + or −.

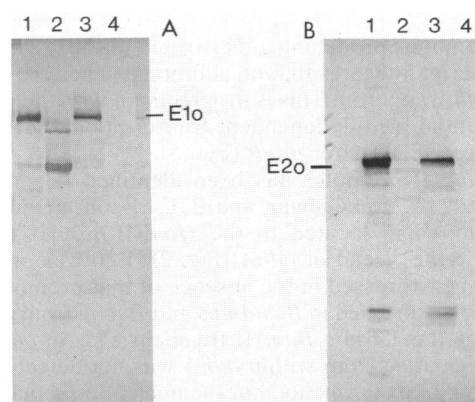

FIG. 4. Immunoblotting analyses of cell extracts from *B. subtilis* strains and *E. coli* plasmid-containing cells. Cytoplasmic extracts of *E. coli* 5K(pLUC5) (lane 1), *E. coli* 5K(pHV32) (lane 2), *B. subtilis* 3G18 (lane 3), and *B. subtilis* CU1693 (which lacks *odhA* and *odhB*) (lane 4) were fractionated on an SDS–10% (wt/vol) acrylamide gel, blotted onto nitrocellulose filters, and probed with antibodies against *B. subtilis* E1o (panel A) and *B. subtilis* E2o (panel B). About 100 μg of protein was loaded on each lane except for lane 1, where only 2 μg of protein was loaded. No cross-reactivity between *E. coli* and *B. subtilis* E2o antigens was detected despite their high similarity in primary structure.

```
   2 TGCAGCACATCATTTGCTGTTCATAACAGCCCGCTGTCTGAAGGATCGGTTCTCGGTTTT 61
     CysSerThrSerPheAlaValHisAsnSerProLeuSerGluGlySerValLeuGlyPhe

  62 GAATACGGCTATAACGTGCATTCACCGGAAACGCTTGTTTTGTGGGAAGCACAGTACGGA 121
     GluTyrGlyTyrAsnValHisSerProGluThrLeuValLeuTrpGluAlaGlnTyrGly

 122 GATTTTGCCAACGCTGCTCAAGTGTATTTTGACCAATTTATTTCTGCCGGACGCGCGAAA 181
     AspPheAlaAsnAlaAlaGlnValTyrPheAspGlnPheIleSerAlaGlyArgAlaLys

 182 TGGGGCCAAAAATCCGGATTAGTCATGCTGCTCCCGCACGGTTACGAGGGACAGGGGCCT 241
     TrpGlyGlnLysSerGlyLeuValMetLeuLeuProHisGlyTyrGluGlyGlnGlyPro

 242 GAGCACTCAAGCGGAAGAATTGAGCGATTCCTGCAGCTTGCAGCGGAAAACAACTGGACG 301
     GluHisSerSerGlyArgIleGluArgPheLeuGlnLeuAlaAlaGluAsnAsnTrpThr

 302 GTTGCCAACCTGACAAGCGCGGCACAGTATTTCCATATTTTAAGAAGACAGGCTAAAATG 361
     ValAlaAsnLeuThrSerAlaAlaGlnTyrPheHisIleLeuArgArgGlnAlaLysMet

 362 CTGCTTCGTGAAGAAATCAGACCGCTCGTCATTATGACGCCGAAGAGCCTTCTTCGCAAC 421
     LeuLeuArgGluGluIleArgProLeuValIleMetThrProLysSerLeuLeuArgAsn

 422 CCAAATACGGTGTCGGAAGTGCAGGAGCTCAGCGAAAGCCGCTTCCAGCCTGTTTATGAA 481
     ProAsnThrValSerGluValGlnGluLeuSerGluSerArgPheGlnProValTyrGlu

 482 CAGTCGGGACTTTCTCATGACTATGAAAAAGTAACCAGACTTGTATTATCCAGCGGTAAA 541
     GlnSerGlyLeuSerHisAspTyrGluLysValThrArgLeuValLeuSerSerGlyLys

 542 GTGTCTATAGACATTGACGTACATTTTAATAAGTTAGAAGACGGTAAAGAGTGGCTTCAC 601
     ValSerIleAspIleAspValHisPheAsnLysLeuGluAspGlyLysGluTrpLeuHis

 602 ATTGCGAGAATCGAACAGCTGTATCCATTCCCGGCAAAAGGAGTCAAAGAATTATTTGCA 661
     IleAlaArgIleGluGlnLeuTyrProPheProAlaLysGlyValLysGluLeuPheAla

 662 AAACTTCCGAACCTGAAAGAAATCGTTTGGGTGCAGGAAGAGCCGCAGAACATGGGGGCT 721
     LysLeuProAsnLeuLysGluIleValTrpValGlnGluGluProGlnAsnMetGlyAla
                                     -35                     -10
 722 TGGGGTTATATCAGCCCGTATTTGACAGAGATTGCACCAGAGGGAGTAAGCGTTCAATAT 781
     TrpGlyTyrIleSerProTyrLeuThrGluIleAlaProGluGlyValSerValGlnTyr

 782 ATTGGACGAAGAAGACGATCCAGCCCTGCAGAGGGAGATCCGACGGTTCATAAAAAAGAA 841
     IleGlyArgArgArgArgSerSerProAlaGluGlyAspProThrValHisLysLysGlu
                                       stop E1o RBS     start E2o
 842 CAGGAACGTATTGTATCTGATAGCTTGACTCGCAAAAACTAAGGGGGAAATGAAAAAATG 901
     GlnGluArgIleValSerAspSerLeuThrArgLysAsn***                Met   1

 902 GCGGAAATTAAGGTACCTGAATTAGCAGAATCAATCTCAGAAGGAACAATAGCCCAATGG 961
   2 AlaGluIleLysValProGluLeuAlaGluSerIleSerGluGlyThrIleAlaGlnTrp  21

 962 TTAAAGCAGCCTGGTGACTATGTAGAACAGGGTGAATATCTGCTTGAACTAGAAACGGAT 1021
  22 LeuLysGlnProGlyAspTyrValGluGlnGlyGluTyrLeuLeuGluLeuGluThrAsp  41

1022 AAAGTGAATGTTGAATTGACAGCAGAAGAATCGGGTGTACTTCAAGAGGTATTGAAAGAT 1081
  42 LysValAsnValGluLeuThrAlaGluGluSerGlyValLeuGlnGluValLeuLysAsp  61

1082 TCGGGTGATACCGTCCAGGTCGGAGAAATTATCGGTACGATTTCAGAAGGCGCGGGTGAA 1141
  62 SerGlyAspThrValGlnValGlyGluIleIleGlyThrIleSerGluGlyAlaGlyGlu  81

1142 AGTTCTGCCCCTGCTCCTACAGAGAAAACAGAAAGCAAGGAAAGCGTAAAAGAAGAGAAA 1201
  82 SerSerAlaProAlaProThrGluLysThrGluSerLysGluSerValLysGluGluLys 101

1202 CAGGCTGAACCAGCTGCACAAGAGGTGAGCGAGGAAGCACAATCTGAAGCAAAATCAAGA 1261
 102 GlnAlaGluProAlaAlaGlnGluValSerGluGluAlaGlnSerGluAlaLysSerArg 121

1262 ACGATCGCTTCTCCGTCGGCCCGTAAGCTTGCGAGAGAAAAAGGAATTGACCTGTCTCAA 1321
 122 ThrIleAlaSerProSerAlaArgLysLeuAlaArgGluLysGlyIleAspLeuSerGln 141

1322 GTTCCAACTGGAGATCCGCTTGGAAGAGTGCGCAAGCAGGATGTCGAAGCGTACGAAAAA 1381
 142 ValProThrGlyAspProLeuGlyArgValArgLysGlnAspValGluAlaTyrGluLys 161

1382 CCGGCATCAAAACCGGCTCCTCAGCAAAAGCAGCAGCCTCAGGCTCAAAAAGCACAGCAA 1441
 162 ProAlaSerLysProAlaProGlnGlnLysGlnGlnProGlnAlaGlnLysAlaGlnGln 181

1442 AGCTTTGACAAACCTGTTGAAGTGCAAAAAATGTCACGCCGCAGACAAACGATTGCAAAA 1501
 182 SerPheAspLysProValGluValGlnLysMetSerArgArgArgGlnThrIleAlaLys 201

1502 CGCCTTGTAGAGGTACAGCAAACATCAGCGATGCTGACTACATTTAATGAAGTGGACATG 1561
 202 ArgLeuValGluValGlnGlnThrSerAlaMetLeuThrThrPheAsnGluValAspMet 221

1562 ACGGCTGTCATGAATCTCAGAAAACGCCGCAAAGATCAATTTTTTGAGCAAAATGAAGTG 1621
 222 ThrAlaValMetAsnLeuArgLysArgArgLysAspGlnPhePheGluGlnAsnGluVal 241

1622 AAGCTCGGCTTTATGTCTTTCTTCACGAAAGCGGTCGTGGCTGCATTGAAAAAATATCCG 1681
 242 LysLeuGlyPheMetSerPhePheThrLysAlaValValAlaAlaLeuLysLysTyrPro 261

1682 CTGTTGAATGCAGAAATTCAAGGCGATGAGTTGATCGTTAAAAAATTCTACGATATCGGA 1741
 262 LeuLeuAsnAlaGluIleGlnGlyAspGluLeuIleValLysLysPheTyrAspIleGly 281

1742 ATCGCTGTTGCTGCTGTAGAAGGTCTTGTCGTTCCGGTTGTACGGGATGCGGATCGCCTG 1801
 282 IleAlaValAlaAlaValGluGlyLeuValValProValValArgAspAlaAspArgLeu 301

1802 ACATTTGCAGGAATCGAAAAAGAGATCGGCGAGCTTGCGAAAAAAGCAAGAAACAATAAA 1861
 302 ThrPheAlaGlyIleGluLysGluIleGlyGluLeuAlaLysLysAlaArgAsnAsnLys 321

1862 TTAACCCTTAGCGAGCTTGAGGGAGGCTCCTTCACGATTACAAACGGAGGGACTTTCGGT 1921
 322 LeuThrLeuSerGluLeuGluGlyGlySerPheThrIleThrAsnGlyGlyThrPheGly 341

1922 TCATTGATGTCAACTCCAATTTTAAACAGCCCGCAAGTCGGTATACTGGGCATGCATAAG 1981
 342 SerLeuMetSerThrProIleLeuAsnSerProGlnValGlyIleLeuGlyMetHisLys 361

1982 ATTCAGCTGCGCCCTGTAGCCATTGATGAAGAGCGTTTCGAAAACCGTCCGATGATGTAT 2041
 362 IleGlnLeuArgProValAlaIleAspGluGluArgPheGluAsnArgProMetMetTyr 381

2042 ATCGCTTTATCTTATGATCACCGAATTGTAGACGGTAAAGAAGCGGTTGGTTTCCTCGTG 2101
 382 IleAlaLeuSerTyrAspHisArgIleValAspGlyLysGluAlaValGlyPheLeuVal 401
                                                   stop E2o
2101 ACAATCAAAAATTTACTGGAAGATCCTGAACAGCTTTTATTAGAAGGATAATAAAAAAGG 2161
 402 ThrIleLysAsnLeuLeuGluAspProGluGlnLeuLeuLeuGluGly***  ------- 417
          terminator
2162 GTACATCACGATAAAGTGATGTACCCTTTTTGATGCAATATTTAAAGTGATAGATTGCTG 2221
     -------->      <---------------

2222 CTTTTGGATCC                                                  2232
```

FIG. 5. Nucleotide sequence of *B. subtilis odhB*. The 2.2-kbp sequence contains the end of *odhA* and the complete *odhB*. The deduced amino acid sequences are shown below the nucleotide sequence. Potential promoters, transcription terminators, and ribosome binding sequences (RBS) are indicated. The nucleotide at position 1 is not shown and corresponds to the first nucleotide in the *Pst*I recognition sequence.

same promoter, producing a dicistronic mRNA. The size of the transcript indicates that no additional genes are included in this *odhAB* operon. This is in agreement with the presence of a potential [*rho*]-independent transcriptional termination signal located just after *odhB* (Fig. 5).

The *odhAB* promoter has been identified (L. Melin, L. Hederstedt, A. von Gabain, and P. Carlsson, manuscript in preparation) and located to the *Hin*dIII-*Bam*HI fragment containing the 5′ end of *odhA* (Fig. 2). E2o was previously found to be expressed in the absence of that promoter; e.g., E2o can be expressed in *B. subtilis* and *E. coli* from plasmids containing the 3.4-kbp *Bam*HI fragment (7). An *odhB* transcript originating from within *odhA* was not detected in the Northern blot experiments, but the nucleotide sequence just upstream of *odhB* contains a possible σ^A promoter (11) (Fig. 5).

Structure of *B. subtilis* E2o. The E2o polypeptide, as deduced from the nucleotide sequence of *odhB*, consists of 417 amino acid residues including the N-terminal methionine, is acidic with a net charge of −13, and has a calculated molecular weight of 45,988. A consensus sequence for the attachment site of lipoate in various lipoylated proteins has been found (3), and one such sequence is found in the N-terminal part of *B. subtilis* E2o. This suggests that the E2o

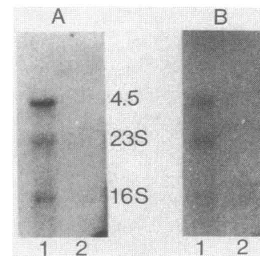

FIG. 6. Analysis of *odh* mRNA. Total RNA of *B. subtilis* 3G18 (lane 1) and *B. subtilis* CU1693 (lane 2) was fractionated on a denaturing agarose gel, blotted to nylon filters, and probed with *odhA* (A)- and *odhB* (B)-specific ^{32}P-labeled DNA probes. DNA fragments used as probes are indicated in Fig. 2. Autoradiographs of hybridized filters are shown. 23S and 16S indicate 23S and 16S rRNAs, respectively. The *odhAB* mRNA is indicated as 4.5, the size given in kilobases. 23S and 16S rRNAs of strain 3G18, but not that of CU1693, which lacks *odhAB*, seemingly hybridized to both DNA probes. The same phenomenon, which probably is a result of trapping of mRNA degradation products, was observed when *B. subtilis sdhCAB* mRNA was analyzed in a wild-type strain and a mutant with a *sdhCAB* deletion (26).

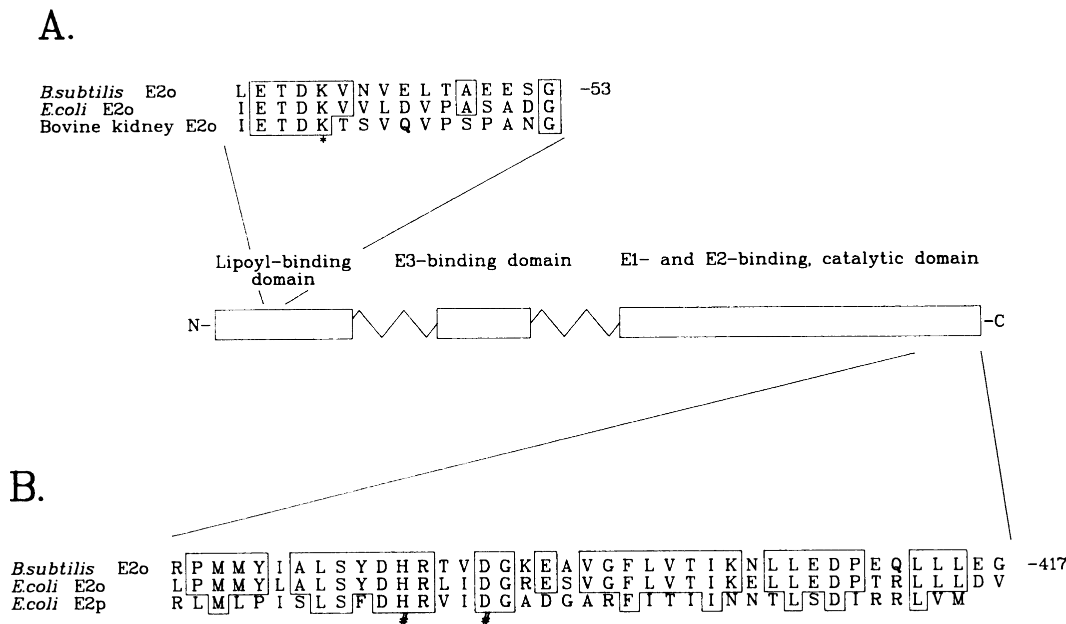

FIG. 7. Drawing of the general domain structure of E2o and amino acid sequence comparisons. The relative size of the respective domains in the drawing is not according to scale. Zig-zag lines indicate potential flexible regions. Lipoate is most probably covalently bound to the lysine residue indicated in panel A (*). Panel B shows the C terminus with potential active site histidine and aspartic acid residues indicated (#). The sequence data for *E. coli* E2o and E2p and bovine kidney E2o are from references 34, 37, and 3, respectively.

subenzyme contains one lipoyl residue covalently bound to the lysine at position 42 (Fig. 7A).

E2o in assembled ODHC functionally binds E1o and E3 as well as other E2o polypeptides. From studies of dihydrolipoamide acyltransferases from *E. coli* and other organisms, different functions of E2o can be attributed to protein domains identifiable in the primary structure (31). These proteins have an N-terminal lipoyl domain consisting of about 100 residues, followed by an E3-binding domain flanked by flexible segments and a C-terminal E1o- and E2o-binding domain which also contains active site residues (Fig. 7). *B. subtilis* E1o and E2o can form a functional ODHC together with *E. coli* E2o-E3 and E1o-E3, respectively, as shown by complementation analyses of mutants (6, 7; this work). This means that *B. subtilis* E1o can be bound to *E. coli* E2o and probably vice versa. However, from these complementation data we cannot determine whether E3 can be bound to heterologous E2o or not. The interfunctionality of *E. coli* and *B. subtilis* E2o is also supported by the primary sequence comparisons. The respective lipoyl and C-terminal domains show a high degree of similarity (65 and 74%, including conserved exchanges), whereas the E3-binding domains with flanking putative flexible regions are less similar (47%). A difference between these flexible segments in *E. coli* and *B. subtilis* E2o is that the first segment is shorter in *B. subtilis* E2o. Furthermore, the second segment, which in *E. coli* E2o contains mainly proline and alanine, in *B. subtilis* E2o has glutamine as the predominant amino acid. The considerable difference between the molecular mass of 60 kDa estimated from SDS-PAGE (7) and the molecular mass of 46 kDa deduced from the nucleotide sequence may be explained by the structure of the flexible segments (34).

B. subtilis and *E. coli* E2o polypeptides are very similar at the C terminus (Fig. 7B), which most likely contains active site residues as discussed by Guest (14). This similarity indicates that histidine 388 and aspartate 392 in *B. subtilis* E2o are active site residues. Short regions in the C-terminal domain of *E. coli* E2o which show poor homology to *E. coli* E2p can be proposed to prohibit the respective E2 component to bind inappropriate E1 components. These sequences in *E. coli* E2o also show poor homology to those in *B. subtilis* E2o. Since *B. subtilis* and *E. coli* E2o are functionally exchangeable, these differences may not reflect sorting of E2 polypeptides to different multienzyme complexes.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council and Emil and Wera Cornells Stiftelse.

We are grateful to Marie Mannerlöf for expert technical assistance.

LITERATURE CITED

1. **Arwert, F., and G. Venema.** 1973. Transformation in *Bacillus subtilis*. Fate of newly introduced transforming DNA. Mol. Gen. Genet. **123:**185–198.
2. **Blake, M. S., K. H. Johnston, G. J. Russel-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline-phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136:**175–179.
3. **Bradford, A. P., A. Aitken, F. Beg, K. G. Cook, and S. J. Yeaman.** 1987. Amino acid sequence surrounding the lipoic acid cofactor of bovine kidney 2-oxoglutarate dehydrogenase complex. FEBS Lett. **222:**211–214.
4. **Canosi, U., G. Morelli, and T. Trautner.** 1978. The relationship between molecular structure and transformation efficiency of some *S. aureus* plasmids isolated from *B. subtilis*. Mol. Gen. Genet. **166:**259–267.
5. **Carls, R. A., and R. S. Hanson.** 1971. Isolation and characterization of tricarboxylic acid cycle mutants of *Bacillus subtilis*. J. Bacteriol. **106:**848–855.
6. **Carlsson, P., and L. Hederstedt.** 1986. In vitro complementation of *Bacillus subtilis* and *Escherichia coli* 2-oxoglutarate dehydrogenase complex mutants and genetic mapping of *B. subtilis citK* and *citM* mutations. FEMS Microbiol. Lett. **37:**373–378.
7. **Carlsson, P., and L. Hederstedt.** 1987. *Bacillus subtilis citM*, the structural gene for dihydrolipoamide transsuccinylase: cloning

and expression in *Escherichia coli*. Gene **61**:217–224.

8. **Darlison, M. G., M. E. Spencer, and J. R. Guest.** 1984. Nucleotide sequence of the *sucA* gene encoding the 2-oxoglutarate dehydrogenase of *Escherichia coli* K12. Eur. J. Biochem. **141**:351–359.
9. **DeRosier, D. J., R. M. Oliver, and L. J. Reed.** 1971. Crystallization and preliminary structural analysis of dihydrolipoamide transsuccinylase, the core of the 2-oxoglutarate dehydrogenase complex. Proc. Natl. Acad. Sci. USA **68**:1135–1137.
10. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
11. **Doi, R. H., and L.-F. Wang.** 1986. Multiple procaryotic ribonucleic acid polymerase sigma factors. Microbiol. Rev. **50**:227–243.
12. **Ehrlich, S. D.** 1978. DNA cloning in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **75**:1433–1436.
13. **Fisher, S. H., and B. Magasanik.** 1984. Synthesis of oxaloacetate in *Bacillus subtilis* mutants lacking the 2-ketoglutarate dehydrogenase enzymatic complex. J. Bacteriol. **158**:55–62.
14. **Guest, J. R.** 1987. Functional implications of structural homologies between chloramphenicol acetyltransferase and dihydrolipoamide acetyltransferase. FEMS Microbiol. Lett. **44**:417–422.
15. **Guest, J. R., and I. T. Creaghan.** 1973. Gene-protein relationships of the α-keto acid dehydrogenase complexes of *Escherichia coli* K-12. Isolation and characterization of lipoamide dehydrogenase mutants. J. Gen. Microbiol. **75**:297–310.
16. **Harboe, N., and A. Ingild.** 1973. Immunization, isolation of immunoglobulins, estimation of antibody titre, p. 161–164. *In* N. H. Axelsen, J. Kröll, and B. Weeke (ed.), A manual of quantitative immunoelectrophoresis. Universitetsförlaget, Oslo.
17. **Hederstedt, L., and L. Rutberg.** 1980. Biosynthesis and membrane binding of succinate dehydrogenase in *Bacillus subtilis*. J. Bacteriol. **144**:941–951.
18. **Herbert, A. A., and J. R. Guest.** 1969. Biochemical and genetic studies with lysine + methionine mutants of *Escherichia coli*. Mol. Gen. Genet. **105**:182–190.
19. **Hoch, J. A., and H. J. Coukoulis.** 1978. Genetics of the α-ketoglutarate dehydrogenase complex of *Bacillus subtilis*. J. Bacteriol. **133**:265–269.
20. **Iismaa, T. P., and R. G. Wake.** 1987. The normal replication terminus of the *Bacillus subtilis* chromosome, *terC*, is dispensable for vegetative growth and sporulation. J. Mol. Biol. **195**:299–310.
21. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
22. **Kaback, H. R.** 1971. Bacterial membranes. Methods Enzymol. **22**:99–120.
23. **Kreft, J., H. Berger, M. Härtlein, B. Müller, G. Weidinger, and W. Goebel.** 1983. Cloning and expression in *Escherichia coli* and *Bacillus subtilis* of the hemolysin (cereolysin) determinant from *Bacillus cereus*. J. Bacteriol. **155**:681–689.
24. **Mandel, M., and A. Higa.** 1979. Calcium-dependent bacteriophage DNA infections. J. Mol. Biol. **53**:159–162.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Melin, L., K. Magnusson, and L. Rutberg.** 1987. Identification of the promoter of the *Bacillus subtilis sdh* operon. J. Bacteriol. **169**:3232–3236.
27. **Neville, D. M., Jr.** 1971. Molecular weight determination of protein-dodecyl sulphate complexes by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. **246**:6328–6334.
28. **Niaudet, B., A. Goze, and S. D. Ehrlich.** 1982. Insertional mutagenesis in *Bacillus subtilis*: mechanism and use in gene cloning. Gene **19**:277–284.
29. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
30. **Ohné, M., B. Rutberg, and J. A. Hoch.** 1973. Genetic and biochemical characterization of mutants of *Bacillus subtilis* defective in succinate dehydrogenase. J. Bacteriol. **115**:738–745.
31. **Packham, L. C., and R. N. Perham.** 1986. Chain folding in the dihydrolipoyl acyltransferase components of the 2-oxo-acid dehydrogenase complexes from *Escherichia coli*. FEBS Lett. **206**:193–198.
32. **Pettit, F. H., L. Hamilton, P. Munk, G. Namihira, M. H. Eley, C. R. Willms, and L. J. Reed.** 1973. α-Keto acid dehydrogenase complexes. XIX. Subunit structure of the *Escherichia coli* α-ketoglutarate dehydrogenase complex. J. Biol. Chem. **248**:5282–5290.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
34. **Spencer, M. E., M. G. Darlison, P. E. Stephens, I. K. Duckenfield, and J. R. Guest.** 1984. Nucleotide sequence of the *sucB* gene encoding the dihydrolipoamide succinyltransferase of *Escherichia coli* K-12 and homology with the corresponding acetyltransferase. Eur. J. Biochem. **141**:361–374.
35. **Spencer, M. E., and J. R. Guest.** 1985. Transcription analysis of the *sucAB*, *aceEF* and *lpd* genes of *Escherichia coli*. Mol. Gen. Genet. **200**:145–154.
36. **Spizizen, J.** 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Natl. Acad. Sci. USA **78**:167–171.
37. **Stephens, P. E., M. G. Darlison, H. M. Lewis, and J. R. Guest.** 1983. The pyruvate dehydrogenase complex of *Escherichia coli* K12. Nucleotide sequence encoding the dihydrolipoyl acetyltransferase component. Eur. J. Biochem. **133**:481–489.
38. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
39. **Weiss, A., and R. G. Wake.** 1983. Restriction map of DNA spanning the replication terminus of the *Bacillus subtilis* chromosome. J. Mol. Biol. **171**:119–137.
40. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.