ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Molecular Catalysis B: Enzymatic 26 (2003) 193–200

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Catalytic domain of *Salmonella typhimurium* 2-oxoglutarate dehydrogenase is localized in N-terminal region☆

Cheorl-Ho Kim*

National Research Laboratory for Glycobiology, Department of Biochemistry and Molecular Biology, Dongguk University COM, Kyungju, Kyungbuk 780-714, South Korea

Received 18 March 2003; received in revised form 11 June 2003; accepted 19 June 2003

This paper is dedicated to Dr. J.-S. Lee, KAIST with regard to his retirement

Abstract

2-Oxoglutarate dehydrogenase (lipoamide) [OGDH or E1o: 2-oxoglutarate: lipoamide 2-oxidoreductase (decarboxylating and acceptor-succinating); EC 1.2.4.2] is a component enzyme of the 2-oxoglutarate dehydrogenase complex. *Salmonella typhimurium* gene encoding OGDH (*ogdh*) has been cloned in *Escherichia coli*. The libraries were screened for the expression of OGDH by complementing the gene in *E. coli* E1o-deficient mutant. Three positive clones (named Odh-3, Odh-5 and Odh-7) contained the identical 2.9 kb *Sau*3AI fragment as determined by restriction mapping and Southern hybridization, and expressed OGDH efficiently and constitutively using its own promoter in the heterologous host. This gene spans 2878 bases and contains an open reading frame of 2802 nucleotides encoding a mature protein of 927 amino acid residues ($M_r = 110{,}000$). The comparison of the deduced amino acid sequence of the cloned OGDH with *E. coli* OGDH shows 91% sequence identity. To localize the catalytic domain responsible for *E. coli* E1o-complementation, several deletion mutants lacking each portion of the *ogdh* gene were constructed using restriction enzymes. From the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, a polypeptide which showed a complementation activity with an M_r of 30,000 was detected. The catalytic domain was localized in N-terminal region of the gene. Therefore, this is a first identification of the catalytic domain in bacterial *ogdh* gene.
© 2003 Elsevier B.V. All rights reserved.

Keywords: 2-Oxoglutarate dehydrogenase; Functional domain; Molecular cloning; *Salmonella typhimurium*

1. Introduction

The 2-oxoglutarate dehydrogenase (lipoamide) [OGDH or E1o: 2-oxoglutarate: lipoamide 2-oxidoreductase (decarboxylating and acceptor-succinating); EC 1.2.4.2] multienzyme complex (ODHC) catalyzes the oxidative decarboxylation of 2-oxoglutarate to succinyl CoA and CO_2, a reaction which is part of the citric acid cycle [19,8]. Of them, OGDH or E1o catalyzes the decarboxylation of a 2-oxoglutarate and use thiamine pyrophosphate (TPP) as a prosthetic group. It is located in mitochondria within the inner membrane/matrix compartment. In eucaryotes and eubacteria, this enzyme complex is composed of three different enzymes, present in nonequivalent stoichiometry [8]. Porcine OGDH ($M_r = 216{,}000$) is

Abbreviations: OGDH, 2-oxoglutarate dehydrogenase (lipoamide) (2-oxoglutarate:lopoamide 2-oxidoreductase, EC 1.2.4.2); ODHC, 2-oxoglutarate dehydrogenase (lipoamide) multienzyme complex

☆ The nucleotide sequence of the *ogdh* gene has been deposited in the GenBank data base with an accession no. AF093783.

* Tel.: +82-54-770-2663; fax: +82-54-770-2281.
E-mail address: chkimbio@dongguk.ac.kr (C.-H. Kim).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2003.06.002

a dimer of identical subunits ($M_r = 97{,}000$–$113{,}000$) [8]. The sequence of yeast OGDH gene was found to have an open reading frame of 3042 nucleotides capable of coding for a protein of $M_r = 114{,}470$ [15]. *Escherichia coli* OGDH ($M_r = 190{,}000$) is similarly a dimer of identical subunits ($M_r = 104{,}905$) from the deduced amino acid sequence [5,16].

In *E. coli*, 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 [14]. 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 [5]. The substrate is transferred between the different active sites by a lipoyl moiety, bound to a lysine residue in the E2o polypeptide. 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 CoA synthetases. The gene encoding E3, however, is part of another operon also encoding the pyruvate dehydrogenase (E1p) and dihydrolipoamide transacetylase (E2p) subenzymes of the pyruvate dehydrogenase multienzyme complex [18]. These gene organizations in gram positive *Bacillus subtilis* appear similar [4].

However, *Salmonella typhimurium* OGDH has not been reported yet. In this paper, the OGDH gene has been cloned by complemention of the gene in *E. coli* E1o-deficient mutant. Furthermore, the functional OGDH domain was identified using complementation method of E1o-deficient *E. coli*.

2. Materials and methods

2.1. Bacterial strains and culture

Restriction enzymes and modification enzymes were purchased from BMS Korea, and were used as recommended by the suppliers. Molecular marker kits for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad Laboratories Korea (Seoul, Korea). Nylon membrane filter (Hybond-C) and ECL DNA hybridization kit were purchased from Amersham Korea (Seoul, Korea). Other chemicals used were of the purest grade commercially available.

S. typhimurium ATCC 14028 was used on the source of the gene that codes for the ODGH. E1o-deficient *E. coli* JRG72 (*suc*A1 *sup*E42 *icl*R) mutant, *E. coli* JM109 and plasmid pUC19 were used from our deposit in Bacterial Collection Laboratory (BCL) of Department of Biochemistry and Molecular Biology, Dongguk University COM, Kyungju, Kyungbuk, Korea. *Vibrio parahaemolyticus* ATCC 27519, *Vibrio vulnificus* ATCC 29307, *Enterobacter cloacae* ATCC 13047, *E. coli* O157:H7, *Pseudomonas aeruginosa* ATCC 27582 and *Proteus* sp. N 13838 were also our deposit in BCL of Dongguk University College of Oriental Medicine, Kyungju City, Kyungpook, Korea. *S. typhimurium*, *E. coli* JRG72 and *E. coli* JM109 were maintained in Luria broth (1% peptone, 0.5% yeast extract and 0.5% NaCl, pH 7.2), while JM109, JRG72/plasmid was maintained in Luria broth containing 40 μg/ml ampicillin. Cultures were preserved in 25% glycerol at −70 °C. For selection of the cloned gene in *E. coli* JRG72, minimal glucose medium was also used.

2.2. DNA manipulation and cloning of ogdh genem

Chromosomal DNA was extracted from *S. typhimurium* according to the method of Canosi et al. [2]. Large scale preparation of plasmid DNA was carried out by the method as described [6]. Restriction endonuclease (BRL, Gaithersberg, MD, USA) were used under the assay conditions described by the manufacturers. Agarose gel electrophoresis of DNA fragments was carried out in Tris–acetate buffer, pH 7.8, containing EDTA. DNA from the gel was transferred onto Hybond Q membrane (Amersham) and used for hybridization. The DNA probe was nick translated with [α-^{32}P]dCTP and hybridized as described by Maniatis et al. [11].

E. coli cell extracts were prepared from spheroplasts [7], 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.

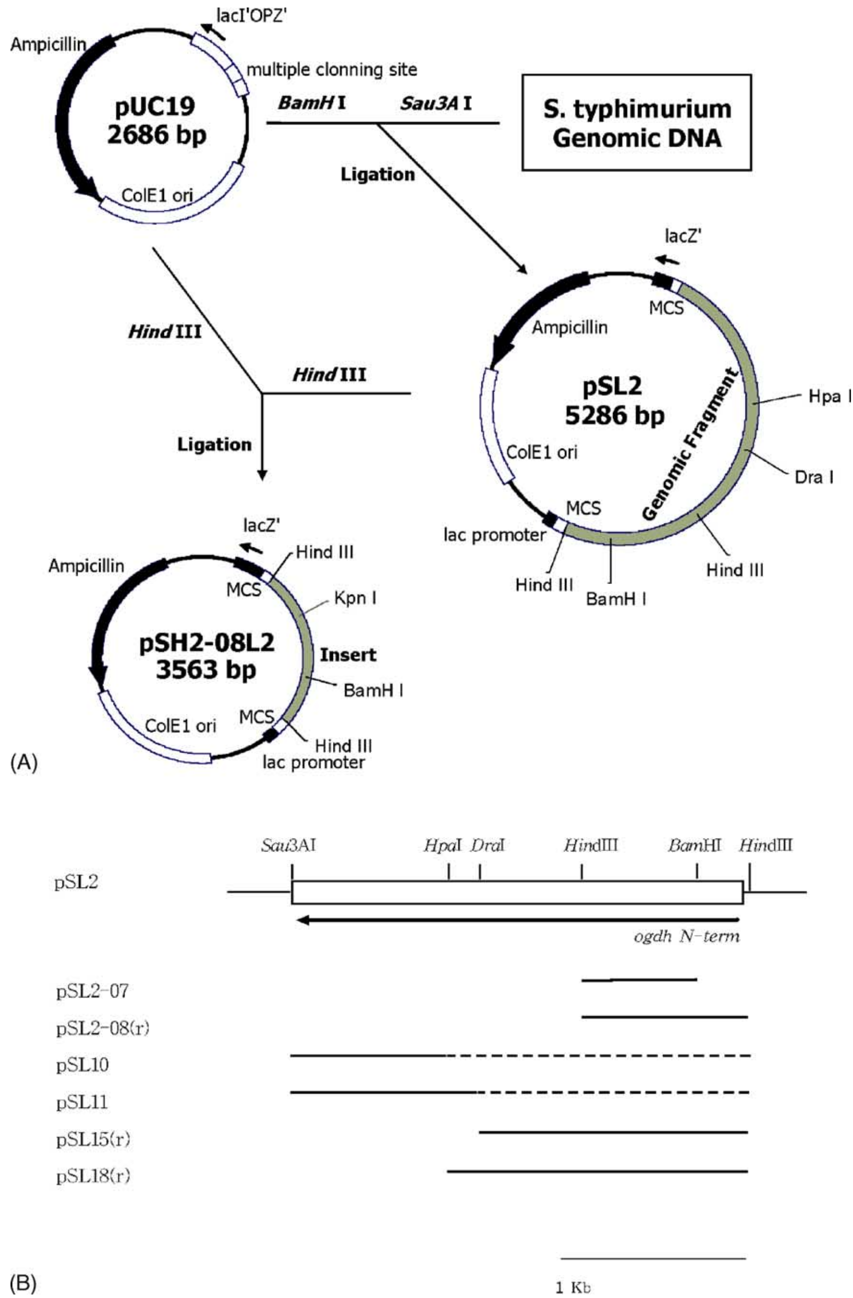

Fig. 1. Cloning and map of the *ogdh* gene from *S. typhimurium.* (A) Cloning scheme of the *ogdh* gene. The closed box regions correspond to cloned *S. typhimurium* DNA containing *ogdh* gene. (B) Map of pSL2 carrying the *ogdh* gene and its derivative plasmids. Open box and arrow indicate the cloned DNA fragment carrying plasmid pSL2 and ORF direction of *ogdh* gene, respectively. Bar and dashed lines indicate the cloned DNA fragment and deletion region, respectively.

Chromosomal DNA from *S. typhimurium* was partially digested with *Sau*3AI. After removal of proteins, the resulting fragments were ligated to *Bam*HI-digested pUC19 DNA using T4 DNA ligase. *E. coli* JRG72 was transformed using these recombinant plasmids according to the method described by Mandel and Higa [10]. The transformants were selected for growth in minimum glucose medium.

S. typhimurium OGDH expressed in an E1o-deficient *E. coli* mutant was screened by complementing the E1o-negative phenotype [3,12]. *E. coli* E1o-deficient mutant, JRG72, was transformed with the plasmid library of the chromosomal inserts, selecting for the wild-type E1o phenotype (ability to grow on minimal glucose plates).

2.3. DNA sequence analysis and Southern hybridization

DNA sequence was determined by the dideoxy chain reaction termination method [17] with T7 DNA polymerase to the manufacturer's instructions as described [6]. The nucleotide sequence of the *ogdh* gene has been deposited in the GenBank data base with an accession no. AF093783. Southern hybridization was also carried out as described [11] to the supplier's instructions. DNA sequence information was analyzed through the National Center for Biotechnology Information, using the BLAST network service to search the GenBank database [1], and with MacVector sequence analysis software (version 6).

2.4. SDS-PAGE and analytical methods

Sodium dodecyl sulfate-polyacrylamide gel electrohporesis was performed in 17% (w/v) gels with SDS by the method of Nevillei [13]. *E. coli* JRG72 strains carrying plasmids were cultured with or without 1 mM isopropyl β-D-thiodgalactoside (IPTG) for induction. The sample buffer was 0.01 M Tris–HCl (pH 8.0) containing 2.5% SDS and in some cases 5% (v/v) β-mercaptoethanol. Protein content was determined by the method of Lowry et al. [9] with bovine serum albumin as standard. Protein in the column eluates was routinely followed by the absorbance at 280 nm.

3. Results and discussion

3.1. Cloning of 2-oxoglutarate dehydrogenase (OGDH) gene in E. coli

S. typhimurium OGDH expressed in an E1o-deficient *E. coli* mutant can complement the E1o-negative phenotype of the mutant [3]. Expression of functional *ogdh* gene product from recombinant plasmids was similarly determined. *Sau*3AI-digests of genomic DNA was ligated into the *Bam*HI site of plasmid pUC19 and *E. coli* E1o-deficient mutant, JRG72, was transformed with the plasmid library of the chromosomal inserts, selecting for the wild-type E1o phenotype (ability to grow on minimal glucose plates) as described previously [3]. The libraries were screened for the expression of OGDH by complementing the gene in *E. coli* E1o-deficient mutant. Three positive clones (named Odh-3, Odh-5 and Odh-7) contained the identical 2.9 kb *Sau*3AI fragment as determined by restriction mapping and Southern hybridization, and expressed OGDH efficiently and constitutively. Plasmids isolated from three Odh clones were named pSL2.

The plasmid pSL2 carrying *ogdh* gene, which complement the E1o-negative phenotype of the mutant *E. coli* JRG72, on a 2.9 kb *Sau*3AI fragment was isolated (Fig. 1). Subcloning of the 0.9 kb *Hin*dIII–*Hin*dIII DNA fragment of pSL2 into pUC19 also resulted in pSH2-08, which also complement E1o-negative mutant. The wild type phenotype was restored in *E. coli* JRG72 (pSL2) and *E. coli* JRG72 (pSH2-08), which shows that functional *S. typhimurium* can be expressed from both plasmids (Fig. 1). When Southern hybridization of cloned *ogdh* gene with *S.*

Table 1
Complementation activity in OGDH-negative *E. coli* mutant carrying pSL2 and its derivative plasmids

Plasmid	Complementation (+/−)
pSL2	+
pSL18	+
pSL15	+
pSL18r	+
pSL15r	+
pSL10	−
pSL11	−
pSH2-08	+
pSH2-08r	+

typhimurium chromosomal DNA was carried out, strong bands in only *S. typhimurium* and *E. coli* were detected, not in *V. parahaemolyticus*, *V. vulnificus*, *E. cloacae*, *P. aeruginosa* and *Proteus* sp. N 13838 (data not shown). This indicates that the *S. typhimurium* *ogdh* gene is structurally similar to that of *E. coli.*

3.2. Localization of the gene encoding OGDH

To localize the structural gene in a 2.9 kb insert fragment of pSL2 (5.3 kb), *Hpa*I–*Hin*dIII-digested DNA fragment (1.8 kb DNA) or *Dra*I–*Hin*dIII-digested DNA fragment (1.5 kb DNA) were religated into

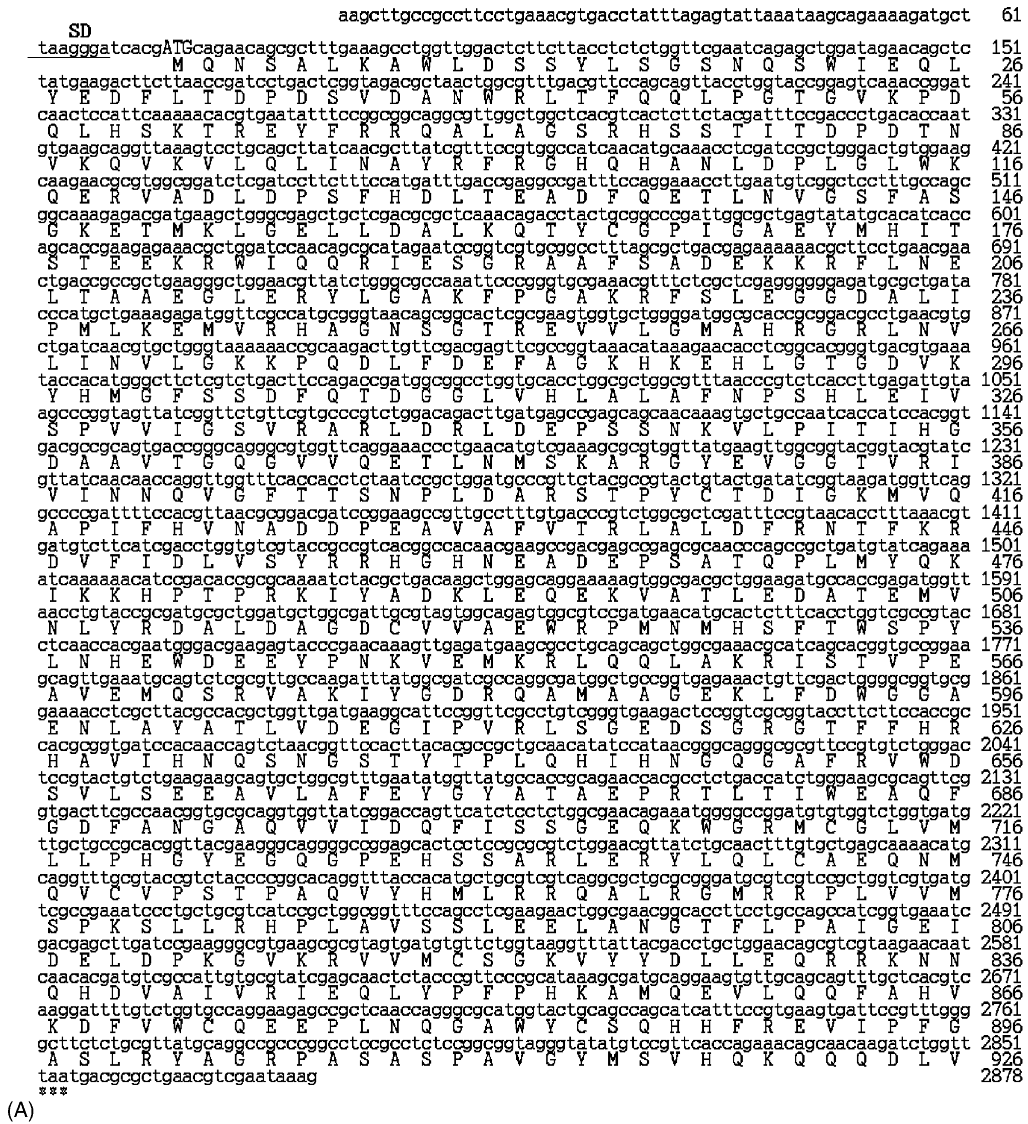

Fig. 2. Nucleotide sequence of *S. typhimurium ogdh* gene. (A) The 2.9 kb sequence contains the 5′-starting region of *ogdh* gene. Ribosome binding sequence is indicated as SD. The nucleotide at position 1 corresponds to the first nucleotide in the *Hin*dIII recognition sequence. (B) Comparison of DNA sequence corresponding to the catalytic domain of *S. typhimurium ogdh* with that of *E. coli*. Mismatched nucleotides are indicated as stars. (C) Comparison of the deduced amino acid sequence corresponding to the catalytic domain of *S. typhimurium* OGDH with that of *E. coli*. Mismatched amino acids are indicated as stars.

```
                            aagcttgccgccttcctgaaacgtgacctatttagagtattaaataagcag
S. typhymurium     aaaagatgcttaagggatcacgatgcagaacagcgctttgaaagcctggttggactcttc
E. coli            aaaagatgcttaagggatcacgatgcagaacagcgctttgaaagcctggttggactcttc

S. typhymurium     ttacctctctggttcgaatcagagctggatagaacagctctatgaagacttcttaaccga
E. coli            ttacctctctggcgcaaaccagagctggatagaacagctctatgaagacttcttaaccga
                               ** *  *
S. typhymurium     tcctgactcggtagacgctaactggcgtttgacgttccagcagttacctggtaccggagt
E. coli            tcctgactcggttgacgctaactggcgttcgacgttccagcagttacctggtacgggagt
                               *                *                        *
S. typhymurium     caaaccggatcaactccattcaaaaacacgtgaatatttccggcggcaggcgttggctgg
E. coli            caaaccggatcaattccactctcaaacgcgtgaatatttccgccgcctggcgaaagacgc
                                *    *  **    *              *  * *    *** ** *
S. typhymurium     ctcacgtcactcttctacgatttccgaccctgacaccaatgtgaagcaggttaaagtcct
E. coli            ttcacgttactcttcaacgatctccgaccctgacaccaatgtgaagcaggttaaagtcct
                   *      *       *     *
S. typhymurium     gcagcttatcaacgcttatcgtttccgtggccatcaacatgcaaacctcgatccgctggg
E. coli            gcagctcattaacgcataccgcttccgtggtcaccagcatgcgaatctcgatccgctggg
                         *  *     *  *  *        *  *  *        *
S. typhymurium     actgtggaagcaagaagccgtggcggatctcgatccttctttccatgatttgaccgaggc
E. coli            actgtggcagcaagataaagtggccgatctggatccgtctttccacgatctgaccgaagc
                          *       ****     *     *     *        *   *       *
S. typhymurium     cgatttccaggaaaccttgaatgtcggctcctttgccagcggcaaagagacgatgaagct
E. coli            agacttccaggagaccttcaacgtcggttcatttgccagcggcaaagaaaccatgaaact
                   *  *        *     *  *     *  *                 *  *     *
S. typhymurium     gggcgagctgctcgacgcgctcaaacagacctactgcggcccgattggcgctgagtatat
E. coli            cggcgagctgctggaagccctcaagcaaacctactgcggcccgattggtgccgagtatat
                   *           *  *  *     *  *                    *  *
S. typhymurium     gcacatcaccagcaccgaagagaaacgctggatccaacagcgcatagaatccggtcgtgc
E. coli            gcacattaccagcaccgaagaaaaacgctggatccaacagcgtatcgagtctggtcgcgc
                          *             *                    *  *  *  *     *
S. typhymurium     ggcctttagcgctgacgagaaaaaacgcttcctgaacgaactgaccgccgctgaagggct
E. coli            gactttcaatagcgaagagaaaaaacgcttcttaagcgaactgaccgccgctgaaggtct
                      *  * *****  *                 * *                     *
S. typhymurium     ggaacgttatctgggcgccaaattcccgggtgcgaaacgtttctcgctcgagggggaga
E. coli            tgaacgttacctcggcgcaaaattccctggcgcaaaacgcttctcgctggaaggcggtga
                   *        *  *     *        *  *  *     *        *  *  *  *
S. typhymurium     tgcgctgatacccatgctgaaagagatggttcgccatgcgggtaacagcggcactcgcga
E. coli            cgcgttaatcccgatgctcaaagagatgatccgccacgctggcaacagcggcacccgcga
                   *   * *  *  *     *         * *     *  *  *           *
S. typhymurium     agtggtgctggggatggcgcaccgcggacgcctgaacgtgctg
E. coli            agtggttctcgggatggcgcaccgtggtcgtctgaacgtgctg
                         *  *              *  *  *
```

(B)

```
S. typhymurium     MQNSALKAWLDSSYLSGSNQSWIEQLYEDFLTDPDSVDANWRLTFQQLPGTGVKPDQLHS  60
E. coli            MQNSALKAWLDSSYLSGANQSWIEQLYEDFLTDPDSVDANWRSTFQQLPGTGVKPDQFHS  60
                                    *                        *              *

S. typhymurium     KTREYFRRQALAGSRHSSTITDPDTNVKQVKVLQLINAYRFRGHQHANLDPLGLWKQERV 120
E. coli            QTREYFRRLAKDASRYSSTISDPDTNVKQVKVLQLINAYRFRGHQHANLDPLGLWQQDKV 120
                   *       * ***  *    *                                  * **

S. typhymurium     ADLDPSFHDLTEADFQETLNVGSFASGKETMKLGELLDALKQTYCGPIGAEYMHITSTEE 180
E. coli            ADLDPSFHDLTEADFQETFNVGSFASGKETMKLGELLEALKQTYCGPIGAEYMHITSTEE 180
                                     *                  *

S. typhymurium     KRWIQQRIESGRAAFSADEKKRFLNELTAAEGLERYLGAKFPGAKRFSLEGGDALIPMLK 240
E. coli            KRWIQQRIESGRATFNSEEKKRFLSELTAAEGLERYLGAKFPGAKRFSLEGGDALIPMLK 240
                                * ***      *

S. typhymurium     EMVRHAGNSGTREVVLGMAHRGRLNVLI                                 268
E. coli            EMIRHAGNSGTREVVLGMAHRGRLNVLV                                 268
                   *                     *
```

(C)

Fig. 2. (*Continued*).

*Hpa*I- and *Dra*I-digested plasmid pUC19. Plasmids obtained in forward direction were named as pSL18 and pSL15, and reverse-directed plasmids were named as pSL18r and pSL15r, respectively. They showed complementation activity screened in the E1o-deficient mutant (Table 1). However, plasmids pSL10 and pSL11, which were constructed by deleting *Hpa*I-digested DNA fragment (1.8 kb) and *Dra*I-digested DNA fragment (1.5 kb), had not any complementation activity. Also, *E. coli* carrying plasmid pSH2-07, which *Hin*dIII–*Bam*HI-digested DNA fragment (0.7 kb) was cloned in pUC19, did not result in any complementation activity. These results clearly indicated that plasmid pSL2 carried the DNA fragment responsible for the complementation gene with its own promoter and a 0.9 kb DNA fragment of *Hin*dIII–*Hin*dIII site might be its own 5′-promoter region for the gene transcription and structure gene for the OGDH. Although the pUC9 as a plasmid vector has lac transcriptional promoter, there was not any significant difference between the complementing activities by the forward or reverse-directed plasmids (Table 1).

The newly designated plasmids pSL18r and pSL15r conferred the same complementation activities as those produced by pSL18, pSL15 and pSH2-08 to the mutant *E. coli*, indicating that transcription of the gene arose in part from a *S. typhimurium* promoter. For further characterization of the gene and of the promoter localization for complementation, a *Hin*dIII-digested 0.9 kb DNA fragment of pSH2-08 was subcloned to reverse direction of pUC19. Two derivative plasmids, pSH2-08 and pSH2-08r in which the pSH2-08 insert was in direct and reverse orientation, revealing that a promoter was present in this fragment.

3.3. Nucleotide sequence and deduced amino acid sequence of S. typhimurium ogdh gene

The nucleotide sequence of the *ogdh* gene was determined using subcloned DNA fragment from pSL2. The *ogdh* gene sequence contains an open reading frame consisting 2802 bp (including ATG) with a protein having 927 amino acids (Fig. 2A). This open reading frame is preceded by a putative ribosome-binding site, d(TAAGGG), 12 bp from the ATG translational stard codon. The nucleotide sequence of the open reading frame shows 86% identity to *E. coli sucA*, which encodes E1o [5] (Fig. 2B). The deduced amino acid sequence of catalytic domain shows 91% similarity to *E. coli* E1o (Fig. 2C).

The amino acid sequence of E1o, as deduced from the nucleotide sequence, is presently known from *B. subtilis* [3], *E. coli* [5], *Sacharomyces cerevisiae* [14] and human [8]. When these sequences were compared with the cloned *S. typhimurium* OGDH region, they showed significant sequence homology; the overall identity between the *S. typhimurium* OGDH sequence and that of *E. coli* is 91% (Fig. 2C), indicating that the *S. typhimurium* OGDH is highly similar to the *E. coli* E1o. *S. typhimurium* OGDH region can complement an *E. coli* mutant defective in E1o, showing that the E1o components of these two bacteria are also functionally similar. However, the overall identity between the *S. typhimurium* OGDH sequence and those of *B. subtilis*, *S. cerevisiae* and human was 41, 37 and 37%, respectively (data not shown).

3.4. Functional expression of ogdh gene carrying a catalytic region of S. typhimurium OGDH in E. coli

As the smallest protein expresses the complementation activity, 30 kDa polypeptide was the most abundant protein in cytoplasmic extracts of both *E. coli* JRG72 (pSH2-08) and *E. coli* JRG72 (pSL2). This polypeptide was not found in *E. coli* JRG72 (Fig. 3). It was reported that yeast, porcine and human E1os were 114 kDa [14], 113 kDa [8] and 108 kDa [8], respectively. In procaryotes, it was also known that *E. coli* E1o has a molecular mass of 105 kDa [5] and *B. subtilis* E1o has a molecular mass of 110 kDa [4], respectively. Thus, the 30 kDa polypeptide was concluded to be too small in its molecular weights than those reported, even though the cloned 30 kDa protein functions as an active enzyme. Furthermore, it was suggested that the 30 kDa would be a N-terminal part of *S. typhimurium* OGDH having the functional activity, indicating that the catalytic domain of OGDH is localized on the N-terminal region. Therefore, this is of interest in analysis of relationship between molecular structure and biological activity of OGDH. We are in progress to characterize the molecular structure for complementation activity.

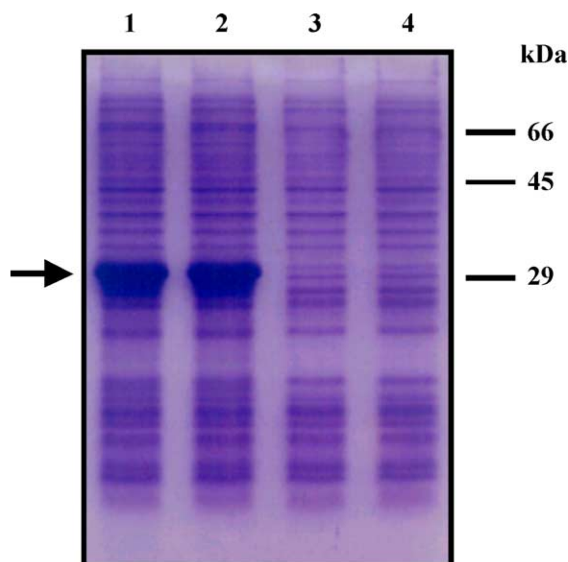

Fig. 3. SDS-PAGE of the *E. coli* extracts carrying the functional N-terminal region having complementation activity. Total cell extracts were fractionated on an SDS, 10% (w/v) acrylamide gel and stained for protein with Coomassie blue R250. Lanes: (1) *E. coli* JRG72 (pSl2); (2) *E. coli* JRG72 (pSH2-08); (3) *E. coli* JRG72 (pUC19) without IPTG; (4) *E. coli* JRG72 (pUC19) with IPTG induction. Approximately 20 μg of protein was loaded on each lane. The left indicates molecular weight positions as kDa dimension.

Acknowledgements

This work was supported by Dongguk University Research Fund.

References

[1] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, J. Mol. Biol. 215 (1990) 403.
[2] U. Canosi, G. Morelli, T. Trautner, Mol. Gen. Genet. 72 (1978) 619.
[3] P. Carlsson, L. Hederstedt, Gene 61 (1987) 217.
[4] P. Carlsson, L. Hederstedt, J. Bacteriol. 171 (1989) 3667.
[5] M.G. Darlison, M.E. Spencer, J.R. Guest, Eur. J. Biochem. 141 (1984) 351.
[6] D. Ish-Horowicz, J.F. Burke, Nucleic Acids Res. 9 (1981) 2989–2998.
[7] H.R. Kaback, Methods Enzymol. 22 (1971) 99.
[8] K. Koike, Y. Urata, S. Goto, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 1963.
[9] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[10] M. Mandel, A. Higa, J. Mol. Biol. 53 (1979) 159–162.
[11] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning, A Laboratory Manual. Cold Spring Harbour Laboratory, New York, 1982, p. 68, 150.
[12] U.H. Jin, T.W. Chung, Y. C Lee, S.D. Ha, C.H. Kim, Glycoconj. J. 18 (2001) 779.
[13] D.M. Neville Jr., J. Biol. Chem. 246 (1971) 6328.
[14] F.H. Pettit, L. Hamilton, P. Munk, G. Namihira, M.H. Eley, C.R. Willms, L.J. Reed, J. Biol. Chem. 248 (1973) 5283.
[15] B. Repetto, K. Tzagoloff, Mol. Cell. Biol. 9 (1989) 2695.
[16] P.M. Ricaud, M.J. Howard, E.L. Roberts, R.W. Broadhurst, R.N. Perham, J. Mol. Biol. 264 (1996) 179.
[17] F. Sanger, S. Nichlen, A.R. Coulson, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 5463.
[18] M.E. Spencer, J.R. Guest, Mol. Gen. Genet. 200 (1985) 145.
[19] S.J. Yeaman, Biochem. J. 257 (1989) 625.