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# Purification, crystallization and X-ray crystallographic analysis of *meso*-diaminopimelic acid decarboxylase from *Corynebacterium glutamicum*

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*meso*-Diaminopimelic acid decarboxylase from *Corynebacterium glutamicum* (CgDAPDC) is the key enzyme for the production of  $\epsilon$ -lysine and it catalyzes *meso*-DAP to produce the final product,  $\epsilon$ -lysine. The CgDAPDC was overexpressed and purified to homogeneity by Ni-NTA affinity and size-exclusion chromatography. The CgDAPDC protein was crystallized using sitting-drop vapor-diffusion method in the presence of 0.8 M sodium citrate tribasic and 0.1 M sodium cacodylate, pH 6.5 at 293 K. X-ray diffraction data were collected to a maximum resolution of 2.4 Å. The crystal belonged to space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with unit cell parameters  $a = 114.54$  Å,  $b = 91.702$  Å,  $c = 95.161$ ,  $\alpha = \beta = \gamma = 90^\circ$ . With one molecules per asymmetric unit, the crystal volume per unit protein mass was  $2.64$  Å<sup>3</sup> Da<sup>-1</sup>, which correspond to a solvent content of approximately 53.37%.

## INTRODUCTION

*Corynebacterium glutamicum* was first described in 1896, and the whole genome sequence analysis of *C. glutamicum* ATCC 13032 was completed in 2003 (Ikeda and Nakagawa, 2003; Kalinowski et al., 2003; Lehmann, 1896). *C. glutamicum* is generally recognized as a safe (GRAS) organism used in industrial biotechnology, and especially, *C. glutamicum* has been widely used to produce amino acids such as  $\epsilon$ -glutamate and  $\epsilon$ -lysine, and other chemicals such as cadaverine, ethylene glycol, and isobutanol (Becker and Wittmann, 2012; Blombach et al., 2011; Buschke et al., 2011; Chen et al., 2016; Huang et al., 2017; Wendisch et al., 2016).

$\epsilon$ -lysine is an essential amino acid that has been used as a feed additive with  $\epsilon$ -methionine,  $\epsilon$ -threonine, and  $\epsilon$ -tryptophan, and can be converted into other high value bio-molecules such as diaminopentane and poly- $\epsilon$ -lysine (Mimitsuka et al., 2007; Sagong and Kim, 2017; Sagong et al., 2016). The de novo  $\epsilon$ -lysine biosynthetic pathway, starting from aspartate, is followed by a four-step enzyme reaction to produce tetrahydropicolinate (THDP). THDP is then converted to *meso*-diaminopimelic acid (*meso*-DAP) through four different pathways such as succinyl-, acetyl-, dehydrogenases-, and aminotransferase pathway, and in *C. glutamicum*, succinyl- and dehydrogenase pathways are used to produce *meso*-DAP. As a final step in the  $\epsilon$ -lysine biosynthesis, *meso*-DAP decarboxylase (DAPDC) catalyzes *meso*-DAP to produce the final product,  $\epsilon$ -lysine (Figure 1).

The DAPDC enzyme reacts with pyridoxal 5'-phosphate (PLP) as a cofactor. PLP is one of the active forms of vitamin

B6, and enzymes using PLP as cofactor can be classified into five types based on protein structures (Kumar, 2016; Percudani and Peracchi, 2003). Each type of PLP dependent enzymes has aminotransferase and decarboxylase (DC) (type I),  $\beta$ -elimination (type II), alanine racemase (type III), D-amino acid

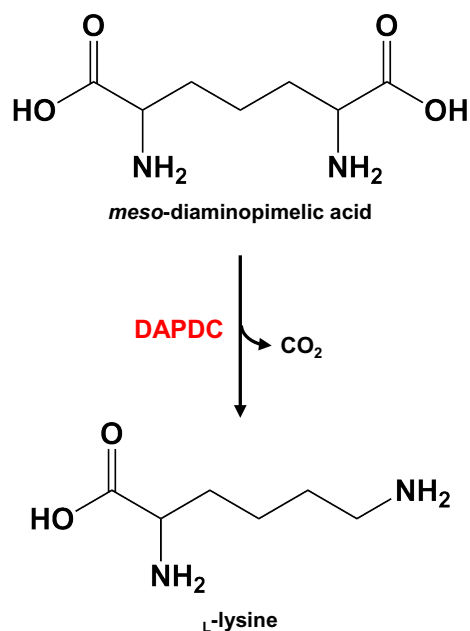


FIGURE 1 | Enzyme reaction of CgDAPDC.

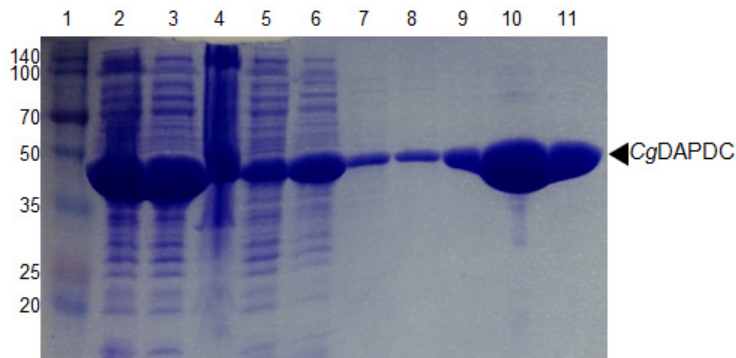
aminotransferase (type IV), and glycogen and starch phosphorylases reactions (type V), respectively. Furthermore, PLP-dependent decarboxylases (PLP-DCs) are divided into four groups according to amino acid sequence and evolutionary origin (Sandmeier et al., 1994). Group I PLP-DC contains glycine DC, and aromatic amino acid DCs are belong to group II PLP-DC. Group III and IV PLP-DCs include DAPDC, lysine DC, ornithine DC, and arginine DC; prokaryotic and eukaryotic enzymes belong to group III and IV PLP-DC, respectively (Sandmeier et al., 1994).

*meso*-Diaminopimelic acid decarboxylase from *C. glutamicum* (CgDAPDC) is an attractive target for increasing L-lysine

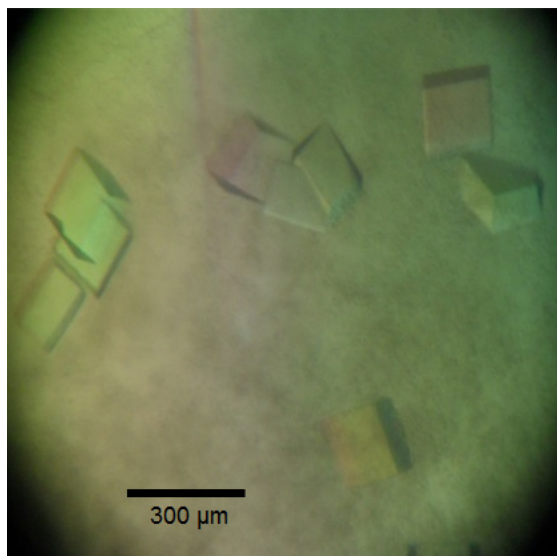
productivity through structure based protein engineering. DAPDC utilizes pyridoxal PLP as a cofactor and belong to the group III PLP dependent decarboxylases. Despite the importance of *C. glutamicum* as a producer of L-lysine, structural studies had not been reported on CgDAPDC prior to this study. In this study, we describe the cloning, expression, purification, crystallization and X-ray crystallographic analysis of the CgDAPDC protein.

## RESULTS AND DISCUSSION

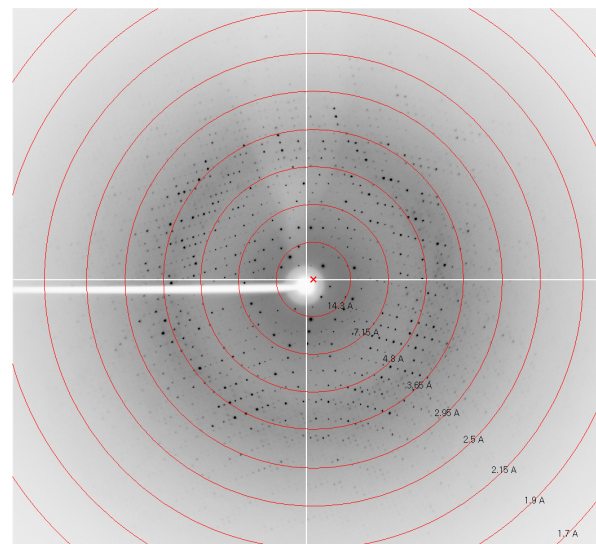
CgDAPDC was purified to apparent homogeneity by Ni-NTA affinity chromatography followed by size-exclusion chromatography. The eluted protein from gel-filtration column, calibrated with Gel Filtration Calibration Kits (GE Healthcare Life Sciences), had a molecular weight of ~ 95 kDa, which corresponds to a dimeric form of CgDAPDC (data not shown). SDS-PAGE of the purified protein showed a single band with a monomeric molecular weight of 47.4 kDa (Figure 2). The CgDAPDC protein was crystallized in 0.8 M sodium citrate tribasic and 0.1 M sodium cacodylate, pH 6.5 (Figure 3). The crystals were transferred to cryo-protectant solution containing 0.8 M sodium citrate tribasic, 0.1 M sodium cacodylate, pH 6.5, and 30% (v/v) glycerol, and the crystals were fished out with a loop larger than the crystals, and flash-frozen by immersion in liquid nitrogen. The crystals of the CgDAPDC diffracted to resolution of 2.4 Å (Figure 4). The CgDAPDC crystals belonged to the space group P2<sub>1</sub>2<sub>1</sub>2 with unit cell parameters  $a = 114.54$  Å,  $b = 91.702$  Å,  $c = 95.161$ ,  $\alpha = \beta = \gamma = 90^\circ$ . Assuming two molecules of CgDAPDC per asymmetric unit, the crystal volume per unit of protein mass was 2.64



**FIGURE 2 | Purification of CgDAPDC.** SDS-PAGE of purification of recombinant CgDAPDC protein. Lane 1 shows molecular-weight markers (labelled in kDa). Lane 2-11 show the purification procedure of CgDAPDC using Ni-NTA chromatography. Lane 2, whole cell extract; lanes 3 and 4, supernatant and pellet fraction after centrifugation of whole cell extract, respectively; lane 5, flow-through from Ni-NTA column; lane 6 to 9, wash with 0, 10, 20, and 30 mM imidazole, respectively; lane 10 and 11, elution with 300 mM imidazole. Purified CgDAPDC is indicated with an arrow and labeled.



**FIGURE 3 | Crystals of CgDAPDC.** Crystals of the best quality were produced from the condition 0.8 M sodium citrate tribasic and 0.1 M sodium cacodylate pH 6.5 and grew to maximum dimensions of 0.2 x 0.2 x 0.05 mm within 3 days.



**FIGURE 4 | Diffraction pattern of the CgDAPDC crystal.** The crystal diffracted to a maximum resolution of 2.4 Å.

**TABLE 1** | Macromolecule-production information

Source organism	<i>Corynebacterium glutamicum</i> ATCC 13032
DNA source	Chromosomal DNA
Forward primer	GCGCGCATATGGCTACAGTTGAAAATTTCAATGAAC
Reverse primer	GCGCGCTCGAGTGCCTCTAGTGAGAGGATGTCGTC
Cloning vector	pET-30a
Expression vector	pET-30a
Expression host	<i>E. coli</i> BL21(DE3)-T1 <sup>R</sup>
Complete amino-acid sequence of the construct produced	MATVENFNEPAAHVWPRNAVRQEDGVVTVAGVPLPLDAEEY GTPLFVWDEDDFRSRCRDMATAFGGPGNVHYASKAFLTKTIA RWWDEEGLALDIASINELGIALAAGFPASRITAHGNKGVFL RALVQNGVGHVVLDSAQELELLDYVAAGEGKIQDVLIRVKP GIEAHTHEFIATSHEDQKFGFSLASGSAFEAANAENLNL VGLHCHVGSQVFDAGFKLAAERVLGLYSQIHSELGVALPEL DLGGGYGIAYTAAEELNVAEASDLLTAVGKMAAELGIDAP TVLVEPGRAIAGPSTVTIYEVGTTKDVHVDDDKTRRYIAVDG GMSDNIRPALYGSEYDARVVSRFAEGDPVSTRIVGSHCESGDI LINDEIYPSDITSGDFLALAATGAYCYAMSSRYNAFTRPVVSV RAGSSRLMLRRETLDDI LSLEALEHHHHHHH

The underlined characters in the primers indicate restriction-enzyme sites.

$\text{\AA}^3 \text{ Da}^{-1}$ , which means the solvent content was approximately 53.37% (Matthews, 1968). The structure of CgDAPDC was determined by molecular replacement with the CCP4 version of MOLREP (Vagin and Teplyakov, 2010) using the structure of DAPDC from *Mycobacterium tuberculosis* (MtDAPDC, PDB code 1HKV) as a search model (Gokulan et al., 2003). CgDAPDC has 58% amino acid sequence identity to MtDAPDC. Model building was performed manually using the program WinCoot (Emsley and Cowtan, 2004), and refinement was performed with CCP4 remlac5 (Murshudov et al., 1997). The initial electron density map, which was good quality with backbone atoms well defined by electron density, allowed us to build a three-dimensional CgDAPDC structure. Crystallographic model building and

**TABLE 2** | Crystallization

Method	Sitting-drop vapor diffusion
Plate type	MRC Crystallization plate (96-well)
Temperature (K)	293
Protein concentration (mg ml <sup>-1</sup> )	60
Buffer composition of protein solution	40 mM Tris-HCl pH 8.0, 150 mM NaCl
Composition of reservoir solution	0.8 M sodium citrate tribasic and 0.1 M sodium cacodylate pH 6.5
Volume and ratio of drop	2 $\mu$ l; 1:1 ratio of protein and reservoir solutions
Volume of reservoir ( $\mu$ l)	50

refinement of the structure to 2.4  $\text{\AA}$  resolution are in progress.

## METHODS

### Production of CgDAPDC

The forward and reverse primers were designed as 5'-GCGCGCATATGGCTACAGTTGAAAATTTCAATGAAC-3' and 5'-GCGCGCTCGAGTGCCTCTAGTGAGAGGATGTCGTC-3' to introduced NdeI and XhoI restriction sites, respectively (underlined). The CgDAPDC coding gene was amplified from *C. glutamicum* strain ATCC 13032 chromosomal DNA by polymerase chain reaction (PCR). The PCR products were then subcloned into pET30a (Novagen) with a six-His tag at the C-terminus. The resulting expression vector pET30a:CgDAPDC was transformed into the *E. coli* BL21(DE3)-T1<sup>R</sup> strain, which was grown in LB medium containing 50 mg L<sup>-1</sup> of kanamycin at 310 K. At an OD<sub>600</sub> of 0.6, CgDAPDC protein expression was induced by the addition of 1 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) and the culture medium was maintained for a further 20 h at 290 K. The culture was then harvested by centrifugation at 4,000  $\times g$  for 20 min at 277 K. The cell pellet was resuspended in buffer A (40 mM Tris-HCl, pH 8.0 and 150 mM NaCl) and then disrupted by ultrasonication. The cell debris was removed by centrifugation at 13,500  $\times g$  for 40 min and the lysate was applied to a Ni-NTA agarose column (QIAGEN). After washing with buffer B (40 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 30 mM imidazole), the bound proteins

**TABLE 3** | Data collection and processing

Diffraction source	Beamline 7A, PAL
Wavelength ( $\text{\AA}$ )	0.97933
Temperature (K)	100
Detector	Quantum 270 CCD
Crystal-to-detector distance (mm)	180
Rotation range per image ( $^{\circ}$ )	1
Total rotation range ( $^{\circ}$ )	250
Exposure time per image (s)	1
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2
<i>a</i> , <i>b</i> , <i>c</i> ( $\text{\AA}$ )	114.65, 92.013, 94.753
$\alpha$ , $\beta$ , $\gamma$ ( $^{\circ}$ )	90.0, 90.0, 90.0
Resolution range ( $\text{\AA}$ )	50.00-2.40 (2.44-2.40) <sup>a</sup>
Total No. of reflections	36222
Completeness (%)	96.0 (92.3) <sup>a</sup>
<i>R</i> <sub>sym</sub> or <i>R</i> <sub>merge</sub> (%)	10.0 (30.7) <sup>a</sup>
<i>I</i> / $\sigma$	26.88 (4.07) <sup>a</sup>
Redundancy	4.7 (3.2) <sup>a</sup>

<sup>a</sup>The numbers in parentheses are statistics from the highest resolution shell.

were eluted with buffer C (40 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 300 mM imidazole). Finally, the trace amount of contaminants was removed by size-exclusion chromatography by using a Sephacryl S-300 column (320 mL, GE Healthcare Life Sciences) equilibrated with buffer A. All purification experiments were performed at 277 K and SDS-polyacrylamide gel electrophoresis analysis of the purified proteins shows a single polypeptide of 47.4 kDa corresponding to the estimated molecular weight of the CgDAPDC monomer. The purified protein was concentrated to 60 mg/mL in buffer A. Macromolecule-production information is given in Table 1.

### Crystallization of CgDAPDC

Crystallization of the purified CgDAPDC protein was initially performed with commercially available sparse-matrix screens from Hampton Research, Rigaku Reagents, and Molecular Dimensions by using the sitting-drop vapor-diffusion method on the MRC Crystallization plate (Molecular Dimensions) at 293 K. Each experiment consisted of mixing 1.0  $\mu$ L protein solution (60 mg/ml in 40 mM Tris-HCl, pH 8.0, 150 mM NaCl) with 1.0  $\mu$ L reservoir solution and then equilibrating this against 50  $\mu$ L reservoir solution. CgDAPDC crystals were observed from several crystallization screening conditions. After several rounds of crystal improvement, the best quality crystal appeared in 0.8 M sodium citrate tribasic and 0.1 M sodium cacodylate, pH 6.5. Crystallization information is summarized in Table 2.

### X-ray diffraction analysis of CgDAPDC

Diffraction data were collected from CgDAPDC crystals on 7A beamline of the Pohang Accelerator Laboratory (PAL, Pohang, Korea), using a Quantum 270 CCD detector (ADSC, USA). The CgDAPDC crystals diffracted to a resolution of 2.4 Å. The data were indexed, integrated, and scaled together using the HKL-2000 software package (Otwinowski and Minor, 1997). The data statistics are summarized in Table 3.

### CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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