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Structural basis for substrate specificity of *meso*-diaminopimelic acid decarboxylase from *Corynebacterium glutamicum*

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ABSTRACT

L-lysine is an essential amino acid that is widely used as a food supplement for humans and animals. *meso*-Diaminopimelic acid decarboxylase (DAPDC) catalyzes the final step in the *de novo* L-lysine biosynthetic pathway by converting *meso*-diaminopimelic acid (*meso*-DAP) into L-lysine by decarboxylation reaction. To elucidate its molecular mechanisms, we determined the crystal structure of DAPDC from *Corynebacterium glutamicum* (*Cg*DAPDC). The PLP cofactor is bound at the center of the barrel domain and forms a Schiff base with the catalytic Lys75 residue. We also determined the *Cg*DAPDC structure in complex with both pyridoxal 5′-phosphate (PLP) and the L-lysine product and revealed that the protein has an optimal substrate binding pocket to accommodate *meso*-DAP as a substrate. Structural comparison of *Cg*DAPDC with other amino acid decarboxylases with different substrate specificities revealed that the position of the α15 helix in *Cg*DAPDC and the residues located on the helix are crucial for determining the substrate specificities of the amino acid decarboxylases.

1. Introduction

Corynebacterium glutamicum is a gram-positive, aerobic, and rod-shaped bacterium. It has been traditionally used in biotechnological research, because it is easy to handle, safe, and capable of large-scale fermentation [1–4]. *C. glutamicum* has been widely used for producing amino acids such as L-glutamate and L-lysine; recently, the use of *C. glutamicum* has been extended to the production of other chemicals such as cadaverine, ethylene glycol, and isobutanol [5–8].

L-Lysine is an essential amino acid that has been widely used as a feed additive with L-threonine, L-tryptophan, and L-methionine. Because of its potential to be converted into other high value biomolecules such as diaminopentane and poly-ε-lysine [9–11], L-lysine is the fastest growing amino acid in global market [4,12]. The *de novo* L-lysine biosynthetic pathway is classified into four pathways, namely, succinylase, acetylase, dehydrogenase, and aminotransferase pathways [13–15]. In bacteria, the succinylase pathway is commonly used, and *C. glutamicum* synthesizes L-lysine through the succinylase and dehydrogenase pathways [16]. All four *de novo* L-lysine biosynthetic pathways lead to production of *meso*-diaminopimelic acid (*meso*-DAP), which is converted into L-lysine by *meso*-DAP decarboxylase (DAPDC) through a decarboxylation reaction (Fig. 1A).

Here, we report the crystal structure of DAPDC from *C. glutamicum* (*Cg*DAPDC) in complex with the PLP cofactor and the L-lysine product. Using the identified crystal structures, the binding mode of the PLP cofactor and substrate was elucidated. Biochemical studies using the DAPDC mutants were used to confirm the results. These results could be used in protein engineering to increase the activity of *Cg*DAPDC to improve L-lysine productivity.

2. Materials and methods

2.1. Cloning, expression and purification of CgDAPDC

The *Cg*DAPDC coding gene (Gene accession code: X07563) was amplified from *Corynebacterium glutamicum* chromosomal DNA by a polymerase chain reaction (PCR). The PCR products were then sub-cloned into pET30a (Novagen) with 6x-His tag at the C-terminus. The resulting expression vector pET30a:*Cg*DAPDC was transformed into *E. coli* BL21(DE3)-T1^{R} strain, which was grown to an OD_{600} of 0.6 in LB medium containing 50 mg L^{-1} of kanamycin at

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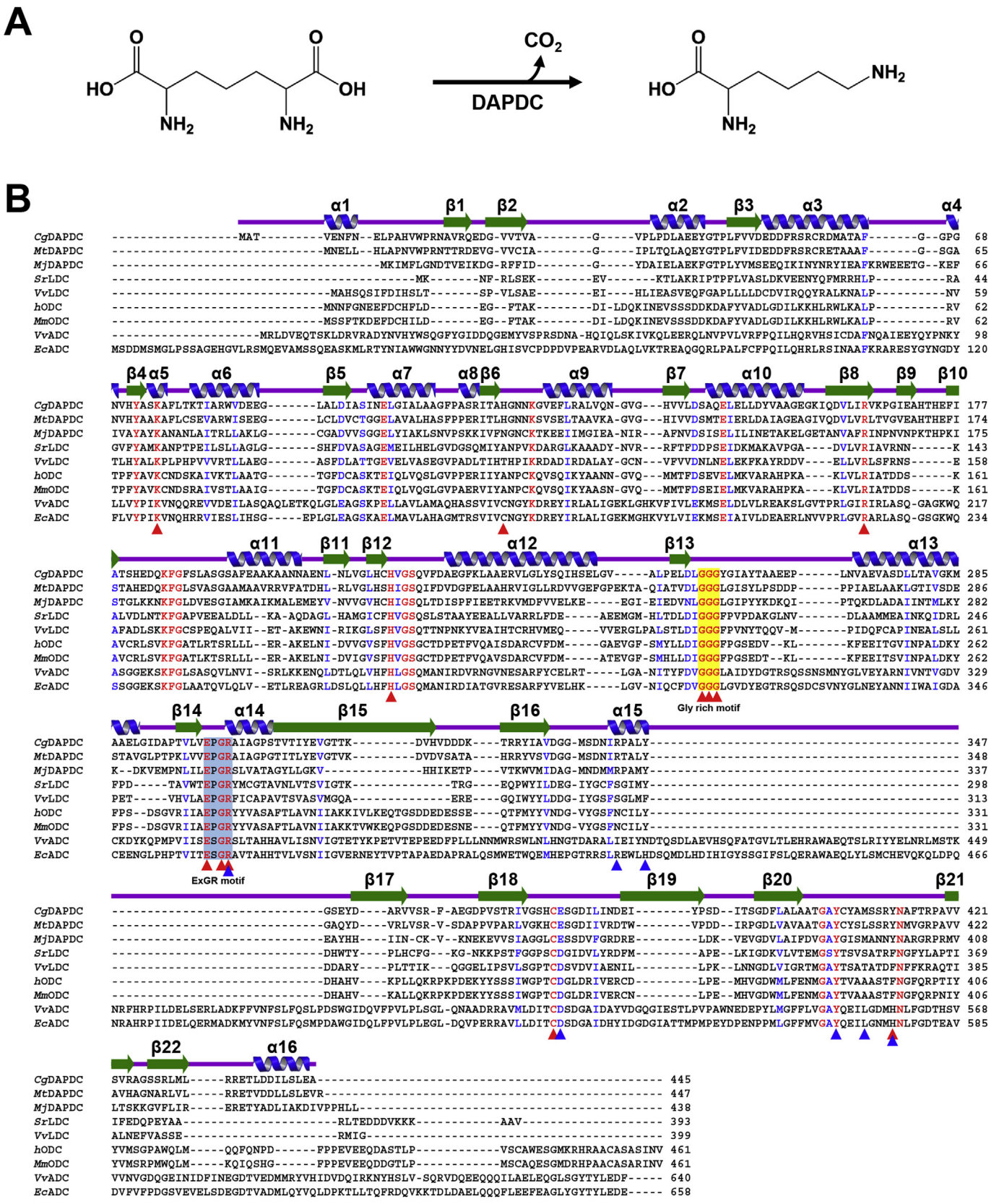

Fig. 1. Enzyme reaction and amino acid sequence alignment of amino acid decarboxylases.
(A) Enzyme reaction of *Cg*DAPDC. (B) Amino acids sequence alignment of *Cg*DAPDC with other reported amino acid decarboxylases. The secondary structure elements are drawn based on the structure of *Cg*DAPDC. The residues involved in the binding of PLP cofactor and in the formation of substrate binding pocket are indicated by red colored and blue colored triangles, respectively. The glycine rich motif and the ExGR motif are shown in boxes with yellow and light-blue colors, respectively. *Cg*DAPDC, *Mt*DAPDC, *Mj*DAPDC, *Sr*LDC, *Vv*LDC, *h*ODC, *Mm*ODC, *Vv*ADC, and *Ec*ADC are representatives of *meso*-diaminopimelic acid decarboxylase (DAPDC) from *Corynebacterium glutamicum*, DAPDC from *Mycobacterium*

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