CLONING AND EXPRESSION OF *E. COLI* ORNITHINE TRANSCARBAMYLASE GENE,argI

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Abstract

E. coli ornithine transcarbamylase is the enzyme which catalyzes the citrullin biosynthesis from ornithine and carbamyl phosphate. To facilitate the purification of enzyme which will be used for many purposes, cloning and expression of E. coli argI gene, ornithine transcarbamylase gene, have been conducted. argI has been amplified from genomic DNA of E. coli DH5a strain by PCR method, and cloned in prokaryotic expression vector, pKK223-3. The enzyme expressed has been purified by salt fractionation, heat denaturation and affinity chromatography. The result of SDS PAGE shows a single band of the purified enzyme. Kinetic data for expressed enzyme give almost same results as those of the wild type enzyme. Vmax of the enzyme $1.0x10^5$ min^{-1}, and K_Ms of ornithine and carbamyl phosphate are 0.35 mM and 0.06 mM, respectively. On the other hand, TB2 cell has been obtained by plasmid curing. This strain does not have argI gene and resulting ornithine transcarbamylase activity. The strain has been prepared for site-directed mutagenesis work.

INTRODUCTION

Ornithine transcarbamylase (EC 2.1.3.3) catalyzes the synthesis of citrulline from carbamyl phosphate and ornithine. The enzyme is located in the arginine biosynthetic patway of E. coli. Transcarbamylation of ornithine in mammals is a step in the urea cycle. The function of the enzyme is highly conserved in evolution. Ornithine transcarbamylases obtained from most of the sources studied are trimers composed of identical subunits. Its molucular weight is about 110,000 daltons. Ornithine transcarbamylase normally observes Michaelis-Menten kinetics for both substrates, ornithine and carbamyl phosphate. The enzyme is not known to be an allosteric enzyme until zinc ion mediates the positive cooperativity (Lee et al., 1990). Even though several amino acids have been proposed to play roles on substrate bindings, the environment of active site is not well understood. The protein over-production system is needed to have enough enzymes to study structure and function relationships of ornithine transcarbamylase.

MATERIALS AND METHODS

Oligodeoxynucleotides and Taq DNA polymerase for PCR (Polymerase Chain Reaction) were obtained from Korea Biotech. Restriction enzymes and deoxynucleotides were purchased from BM Korea, and prokaryotic expression vector pKK223-3 was obtained from Pharmacia. Other chemicals for DNA work were purchased from SIGMA.

E. coli genomic DNA was purified from E. coli strain DH5a. The single colony of bacterial cell was obtained from plate streak, and was inoculated in LB medium for overnight culture. The procedures for the DNA purification were followed the protocols in Molecular Cloning (Maniatis et al.,1989). E. coli TB2 cell was obtained by successive culturing of TB2 cell containing other plasmid on LB plate of the minimal media and ampicilline.

RESULTS AND DISCUSSION

To clone the argI gene of ornithine transcarbamylase, argI gene was amplified from wild type E. coli genomic DNA by PCR. Oligodeoxynucleotides for the amplification were 5'-CA GGT GAA TTC ATG TCC GGG TTT CAT AAG CAT-3' and 5'-CA TGT AAG CTT TTA TTT ACT GAG CGT CGC GAC CAT-3' for N-terminus and C-terinus, respectively. These primers have restriction enzyme sites, EcoR1 and Hind III, to facilitate the cloning in the expression vector and the M13 mutagenic vector. To check the amplification of arg I gene, agarose gel electrophoresis was performed. The results showed clearly single band at size of 1.0kb in Figure 1. For a control experiment, the

genomic DNA purified from TB 2 cell cured was tested at the same condition. no DNA band was appeared on the gel as expected.

PCR product of argI gene was digested with EcoR1 and Hind III, and the expression vector pKK233-3 was also double digested with same enzymes. The small flanking fragments of argI gene amplified were filtered off with the Centricon (Amicon Co.). Then, argI gene was subcloned to the pKK233-3, and transformed to the TB 2 cell. Cloned plasmids were purified and digested with EcoR1 and Hind III to confirm the sucess of subcloning. The results are shown in Figure 2. Figure 2 shows two bands, 4.5 kb of pKK233-3 vector and 1.0kb of arg I gene.

To check whether the cloned argI gene is functionally expressed, TB 2 cell and TB 2 cell transformed with arg I gene were grown at various growth conditions. The results are shown in Table 1. TB 2 cell lacking functional genes for ornithine transcarbamylase are not able to grow in the M9 minimal medium without arginine. while TB 2 cell containing argI gene can grow well at the same condition. Since arginine is the penultimate product of biosynthetic pathway ornithine transcarbamylase is involved, the results support the cloned argI gene is gene for ornithine transcarbamylase.

Table 1. The viabilities of TB 2 strains at various growth conditions.

Strains	LB	LB/AMP	M9/ Uracil Arg	M9/ Uracil	M9/ Uracil Arg/Amp
TB2	o	x	o	x	x
TB2(argI)	o	o	o	o	o

Ornithine transcarbamylase expressed in the TB 2 cell was purifed by ammonium sulfate fractionation, heat denaturation, and Amicon's Matrex Blue Gel A affinity chromatography. The molecular weight of the purified enzyme was tested on the SDS PAGE (Laemmli, 1970). The result is shown in Figure 3. The estimated size of argI gene product is about 38KD. While the size of the enzyme deduced from the wild-type DNA coding sequences is 36.6KD. Since there is only 5% difference between them, the size of the expressed enzyme is assumed to be same as that of the wild type.

The kinetic parameters of expressed were obtained and compared to the those of wild type ornithine transcarbamylase. The results are shown in Table 2.

Table 2. Kinetic parameters of ornithine transcarbamylases.

Source	Vmax min^{-1}	Ornithine K_M, mM	Carbamyl Phosphate K_M, mM
E. coli K-12	$1.4x10^5$	0.32	0.05
TB 2 (arg I)	$1.0x10^5$	0.35	0.06

The data show kinetic parameters obtained are very much same between wild-type and cloned enzymes, even though there are slight differences.

In conclusion, along with the growth control test and SDS-PAGE, combined results directly suggest that argI gene of E. coli ornithine transcarbamylase is correctly cloned and expressed. However, further experiments such as DNA sequencing and other kinetic parameters of the expressed enzyme are needed to confirm the degree of genetic variance of argI gene and the correct behaviors of the cloned enzyme.

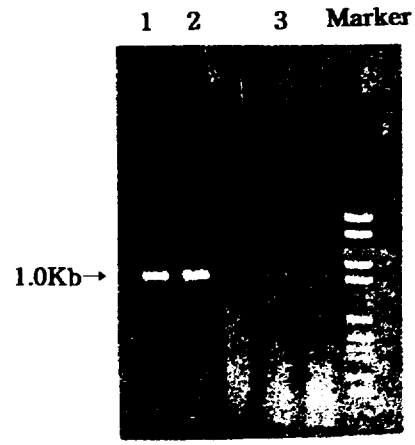

Figure 1. The results of the agarose gel electrophoresis for the argI PCR product. Genomic DNA used as PCR templates (Lanes 1 and 2; DH5α. Lane 3;TB2)

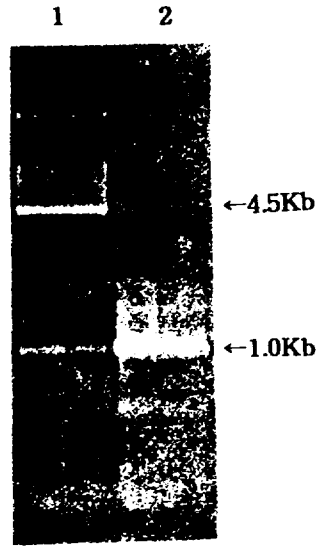

Figure 2. EcoRI/Hind III digestion of subcloned argI on expression vector pKK223-3.
Lane 1; pKK223-3(argI), Lane 2; PCR product of genomic DNA of DH5α.

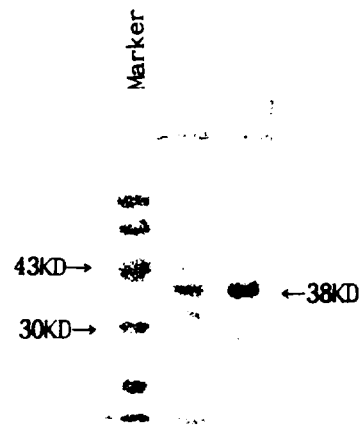

Figure 3. The results of SDS PAGE of purified enzyme which is expressed from pKK223-3(argI). Lane 1; Marker, Lane 2 and 3; purified enzyme.

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