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Characterization of Tryptophanase from *Vibrio cholerae* O1

Taiyeebah Nuidate[a], Natta Tansila[b], Kanda Panthong[c], Varaporn Vuddhakul[a]*

[a]*Food Safety and Health Research Unit, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand*
[b]*Faculty of Medical Technology, Prince of Songkla University, Hat Yai 90112, Thailand*
[c]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand*

Abstract

Tryptophanase (Trpase) encoded by the *tnaA* gene catalyzes the conversion of tryptophan to indole, which is an extracellular signaling molecule detected in various bacteria including *Vibrio cholerae*. Indole has been demonstrated to regulate biofilm formation, drug resistance, plasmid maintenance and spore formation of bacteria. In the present study, the *tnaA* gene from *V. cholerae* O1 (VcTrpase) was cloned and expressed in *E. coli* BL21(DE3) *tn5:tnaA* (a Trpase-deficient competent). VcTrpase was purified by Ni^{2+}-NTA chromatography. The obtained VcTrpase had a molecular mass of approximately 49 kDa, a specific activity of 3 U/mg protein, and absorption peaks at 330 and 435 nm. Using a site-directed mutagenesis technique, replacement of Arg419 by Val resulted in a VcTrpase completely devoid of activity. Thus, this site can be a target for drug design for controlling *V. cholerae.*

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Keywords: V. cholerae; tryptophanase; indole

* Corresponding author. Tel.: +66-7428-8328; fax: +66-7444-6661
E-mail address: varaporn.v@psu.ac.th

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1. Introduction

Tryptophanase (Trpase) is detected in a wide variety of bacteria. *V. cholerae* and many other Gram-negative bacteria use this enzyme to catalyze the conversion of L-tryptophan into indole, ammonium and pyruvate (Fig. 1)[1]. This enzyme is of particular interest because indole has been demonstrated to act as an extracellular signaling molecule to control virulence and biofilm formation[2]. Thus, tryptophanase is a useful target for novel potential inhibitors to inhibit *V. cholerae* biofilm formation. The aim of this work was to investigate amino acid residues in tryptophanase that could abolish its activity. Arg419 of tryptophanase has been demonstrated to bind the tryptophan substrate. Thus, in this study, arginine 419 was replaced with valine using site directed mutagenesis and the R419V mutant was evaluated.

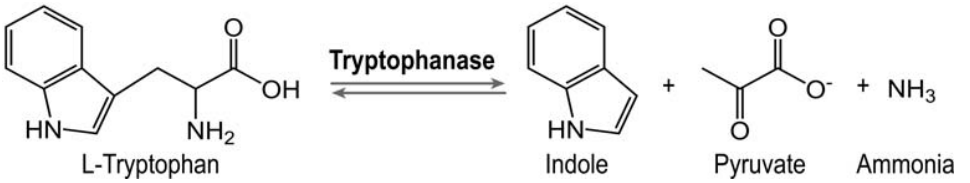

Fig. 1. Reaction catalyzed by Trpase.

2. Methods

2.1. Strains and plasmid

V. cholerae PSU966 serogroup O1 was used for PCR amplification of the *tnaA* gene. *E. coli* BL21 (DE3) *tn5:tnaA* was used as Trpase-deficient competent cells for expression of recombinant VcTrpase[3]. Plasmid pET20b (+) vector containing a hexa-histidine tag (Novagen, Germany) was used for cloning and expression of the *tnaA* gene.

2.2. Cloning of tnaA gene

An overnight culture of *V. cholerae* PSU966 in Luria Bertani (LB) broth was harvested by centrifugation at 12,000x*g* for 5 min and chromosomal DNA was extracted by the phenol-chloroform method[4]. The *tnaA* gene was amplified from the total DNA of *V. cholerae* by the polymerase chain reaction (PCR) with primers tnaA-VC-F 5'-TACATATGGAAAATTTTAAACACTTACCAGAACC-3' and tnaA-VC-R 5'-TTGTCGACGGCTTTTTCTT TTAAGCG-3'. The amplification cycles were 35 cycles of denaturation at 95°C for 45 sec, annealing at 53°C for 30 sec, and extension at 72°C for 2 min. The PCR product was digested with *Nde*I and *Sal*I enzymes and purified from agarose gel with the QIAquick Gel Extraction Kit (Qiagen, Germany). Then it was ligated to the pET20b(+) vector. The recombinant plasmid pET20b(+)-VcTrpase was transformed into *E. coli* BL21(DE3) *tn5*:*tnaA* host cells by the $CaCl_2$ technique[5]. The transformants were selected on LB agar plates containing kanamycin (50 μg/ml) and ampicillin (100 μg/ml), and screened for indole production. Then the recombinant plasmid (pET20b(+)-VcTrpase) was extracted and confirmed for the presence of the *tnaA* gene by PCR and DNA sequencing (Macrogen, Korea).

2.3. Site-directed mutagenesis

For amplification of the *tnaA* gene, site-directed mutagenesis was performed using the pET20b(+)-VcTrpase as a template. To replace arginine 419 with valine, the primer pair R419V_F 5′-CCAGCCGAATTGCT CGCGTTAACCATTCCACGC-3' and R419V_R-5'-CGCTGGAATGGTTAACGCGAGCAATTCGGCTGG-3' with mutagenized codon (underlined) was used. Then the template plasmid was removed by *Dpn*I restriction enzyme digestion. After that the R419V plasmid was transformed into *E. coli* BL21 (DE3) *tn5:tnaA*. The plasmid was extracted and the presence of the correct codon substitution in the *tnaA* gene was confirmed by DNA sequencing (Macrogen, Korea).