PC-6

Characterization of the recombinant metalloprotease from *Vibro mimicus* and its hemagglutinating activity

In-Soo Kong · Seung-Yeol Shin · Jong-Hee Lee · Jin-Man Kim* · Young-Seo Park**

Pukyung Natl. Univ. · *Yosu Natl. Univ. · **Kyungwon Univ.

Introduction

Metalloprotease produced in *Vibrio mimicus* , in which zinc is an essential meta ion for catalytic activity, degrades a variety of biologically important substances including human collagen, several complement components, and immunoglobulin. For gene overexpression and convenient purification, VMC gene was constructed in pET22b(+) expression vector by using of PCR. VMC was purified by His-bin affinity column chromatography from inclusion body in *E.coli* BL21(DE3). To determine VMC activity, insoluble collagen, acid-soluble collagen, gelatin, synthetic peptide and azocasein were used as substrate. Effect of the protease inhibitors, pH and temperature were observed. We have previously isolated the metalloprotease gene (*vmc*) from *V. mimicus* ATCC 33653 and demonstrated that the *vmc* gen contained 1884 nucleotides with a conserved zinc-binding motif (His-Glu-Tyr-Thr-His). However, molecular weight of the VMC protein (71kD predicted from the primary amino acid sequences was much larger than that of the HA/protease (31kDa) purified from an environmental *V. mimicus* E33. This finding suggest that the VMC protein may act as another virulence factor in vivo system Although the VMC protein is expected to participate in pathogenesis and physiological function to host cell, biochemical characters have not been defined. To investigate of these properties, we present the purification of a His-tagged recombinant protein and biochemical properties.

Materials and Methods

1. Constructuion of overexpression plasmid.
2. Purification of recombinant VMC.
3. Protease assay.

Results and Summary

1. The metalloprotease gene (*vmc*) was amplified by PCR method and subcloned to pET22b(+) and overexpressed in *E. coli* BL21(DE3)

2. SDS-PAGE of the purified metalloprotease showed a predicted band with a molecular weight of 71kDa.

3. The optimum temperature and pH of the VMC were 37℃ and pH 7.5, respectively.

4. The VMC was showed proteolytic activity about various substrates, excep casein and hemagglutianting activity against human erythrocytes.

5. The VMC was strongly inhibited 고 by EDTA and other metal-chelating agent but other protese inhibitors were not affected.

References

1.Kim, G. T., Lee, J. H. and Kong, I. S. ; Nucleotide sequence of the *vmh*A gene encoding hemolysin from *Vibrio mimicus*, *Biocim.Biophys.Acta*, 1360, 102-104(19

2.Lee,J.H., Kim,G.T. and Kong,I.S. ; Isolation and sequence analysis o metalloprotease gene from *Vibrio mimicus*, *Biochim.Biophys.Acta*, 1384, 1-6(1998