Isolation and Characterization of a Novel Organophosphorus Acid Hydrolase of *Pseudomonas cepacia* BY21

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Organophosphorous acid hydrolase (OPH; EC 3.1.8.1) catalyzes the hydrolysis of many highly toxic, acetylcholinesterase-inhibiting compounds, including chemical warfare nerve agents (sarin, soman, and VX) and pesticides [1, 2]. The OPH genes (*opd*) from *Pseudomonas diminuta* MG and *Flavobacterium* sp. Strain had been cloned [3, 4]. The open reading frame of the known *opd*gene contains 975 bases, which encode a polypeptide of 325 amino acids with a molecular mass of 35 kDa. During screening microorganisms in environments, a new soil strain, *Pseudomonas cepacia* BY21, was found to produce a very effective OPH.

To purify the OPH protein, microbial cells were cultured in LB media, harvested by centrifugation, and suspended in 50mM phosphate buffer (pH7.5). Cell walls were disrupted by ultra-sonication for 30 min. An equal volume of 1% protamin sulfate solution was added to the supernatant and centrifuged. The supernatant was treated with ammonium sulfate (70% saturation). After centrifugation for 20 min at 10,000g, the protein precipitates were suspended in 50mM phosphate buffer (pH7.5) and loaded to a Sephadex G-100 gel chromatography (3 x 20 cm). The activity of OPH was measured by monitoring the appearance of *p*-nitrophenol from paraoxon at 400 nm. Enzymatic reactions were performed with various concentrations of substrates in 1 ml cuvette at 25 C for 1 min. The kinetic parameters (Vmax and Km) were determined using a SIGMA PLOT curve fitting program. Partially purified OPH was immobilized on Eupergit C and then optimal conditions for the immobilized enzyme were determined.

Keywords: Chemical Warfare, Detoxification, Nerve Agent, Organophosphorous Compound, Paraoxon.

References

[1] Cheng, T-C., S. P. Harvey, and G. L. Chen. (1996) Cloning and expression of a gene encodinga bacterial enzyme for decontamination of organophosphorus nerve agents and nucleotide sequence of the enzyme. *Appl. Environ. Microb.*62: 1636-1641.

[2] Raushel, F. M. (2002) Bacterial detoxification of organophosphate nerve agents. *Curr. Opin. Microbiol.* 5: 288-295.

[3] Mulbry, W., J. S. Karns, P. C. Kearney, J. O. Nelson, C. S. McDaniel, and J. R. Wild (1986) Identification of a plasmid-borne parachion hydrolase gene from *Flavobacterium* sp. by southern hybridization with *opd* from *Pseudomonas diminuta*. *Appl. Environ. Microbiol.* 51: 926-930.

[4] Harper, I. I., C. S. McDaniel, C. E. Miller, and J. R. Wild (1988) Dissimilar plasmids isolated from *Pseudomoans diminuta* MG and a *Flavobacterium*sp. (ATCC 27551) contain identical *opd* genes. *Appl. Environ. Microbiol.* 44: 246-249.