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13. ABSTRACT (Maximum 200 words)

The major aim is to determine the three-dimensional atomic structure of an organophosphorus acid anhydrolase (OPAA) by x-ray crystallography. This structure is a prerequisite for remodeling the active site, in collaboration with scientists at ECBC and Geo-Centers, Inc. in order to enhance catalytic activity towards fluorinated G-type and other extremely toxic chemical warfare (CW) nerve agents. A decontamination system based on the remodeled OPAA not only provides rapid removal of CW agents, but is also environmentally safe and non-corrosive in nature. After overcoming the major difficulty in crystallizing OPAA, we have now determined by MAD and SAD techniques and refined the crystal structure of OPAA to 2.5 Å resolution. The structure analysis of OPAA with the bound enzyme inhibitor MIPAFOX (N,N′-di-isopropylphosphorodiamidic fluoride) is in progress.

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B. Final Progress Report

B.1. Statement of the problem

The major objective of the research project is to determine the three-dimensional atomic structure of a 60 kilodalton organophosphorus acid anhydrolase (OPAA) by x-ray crystallographic technique. The structure will serve as the basis for improving the catalytic activity of the enzyme, which is the long goal of the collaboration between the structural laboratory at Baylor College of Medicine and the laboratories Edgewood Chemical and Biological Center (ECBC) and Geo-Centers, Inc. Organophosphorus (OP) compounds, including fluorinated G-type nerve agents, are among the most toxic chemicals known, inhibiting acetylcholinesterase (AChE) and thereby disrupting nerve function. A number of bacterial enzymes (organophosphorus acid anhydrolases, OPAA) isolated and characterized at ECBC have been found to contain high levels of activity for the detoxifying the G-type chemical nerve agents such as soman (GD), sarin (GB), GF, tabun (GA) and others. Recently, the genes encoding such enzymes have been cloned and sequenced at ECBC from two Alteromonas sp, A. spJD6.5 and A. haloplanktis. Sequencing and biochemical analysis of the cloned enzymes have established OPAA to be a prolidase (E.C. 3.4.13.9), a type of dipeptidase cleaving dipeptide bonds with a prolyl residue at the carboxyl terminus (X-Pro). An enzyme-based decontamination system not only provides rapid removal of nerve agents, but is also environmentally safe and non-corrosive in nature. The range of substrates catalyzed by OPAA and OPH far exceeds any known chemical catalyst for hydrolysis of CW agents. Recently, these enzymes were also demonstrated to possess catalytic activity against GV and other extremely toxic CW agents. However, the enzymatic activities are low relative to those seen with the G-agent substrates. The major goal of the collaboration is to improve the catalytic efficiency of OPAA for detoxifying GV and other threat agents. This goal will be accomplished by a structure-based redesign of OPAA. This redesign requires the three-dimensional atomic structure of OPAA, the long range goal of the collaboration. The structure will be used for molecular modeling and mutagenesis approach to create and characterize the OPAA mutants with enhanced activity.

B.2. Summary of the most important results

Although we obtained four different crystal forms of OPAA in the initial phase of our investigation, they diffracted poorly. In the ensuing period, our work focused in obtaining better diffracting crystals, which proved to be challenging. We did finally succeed; the crystals belong to space group I222, with unit cell dimensions of a = 124.4 Å, b = 143.9 Å and c =219.2 Å and 3 molecules in the asymmetric unit. This breakthrough has led to the structure determination using MAD and SAD techniques. The structure has been refined at resolution 2.5 Å to R-cryst value of 0.23, R free of 0.30 and good geometry (RMSD of bonds and angles of 0.025 Å and 2.80°, respectively. A total of 58,310 reflections (85.6% of theoretical total number) was used in the refinement, of which 10% of random reflections was used for the R-free calculation.

The OPAA crystal structure is depicted in Fig. 1. The structure is composed of a trimer. Since there is no evidence in solution that the enzyme exits as a trimer, the trimeric structure may be an artifact of crystallization. The monomer structure is made up of two domains, an amino- or N-terminal domain and a carboxy- or C-termianl domain. Interestingly, sequence comparison indicates that the sequence of residues 155 to 445 in the C-terminal domain is very similar to that of aminopeptidase or aminohydrolase. With the exception of one helix at the beginning, the N-terminal domain is nearly entirely composed of one sheet. In contrast, the C-terminal domain shows an α/β fold, with five helices flanking one side of the β sheet.

Search of the 3D protein structure data base indicates that the OPAA structure is similar to that of an aminopeptidase (e.g., aminopeptidase P from E. coli, PDB accession #1A29) especially, as expected, the C-terminal domain. Aminopeptidase P consists of similar number (440) of residues as OPAA and also has two

domains. The N-terminal domains of the aminopeptidase P and OPAA are not as structurally similar as the C-terminal domains, which is consistent with essentially no sequence identity.

Based on the structural similarity and the fact that aminopeptidases use manganese as cofactor, the active site of OPAA was initially tentatively located to a site that contains two nearest Mn^{2+} atoms on the exposed side of the β sheet of the C-terminal domain (Fig. 1). The active site location has been confirmed by the structure analysis of OPAA crystals with the bound MIPAFOX inhibitor.

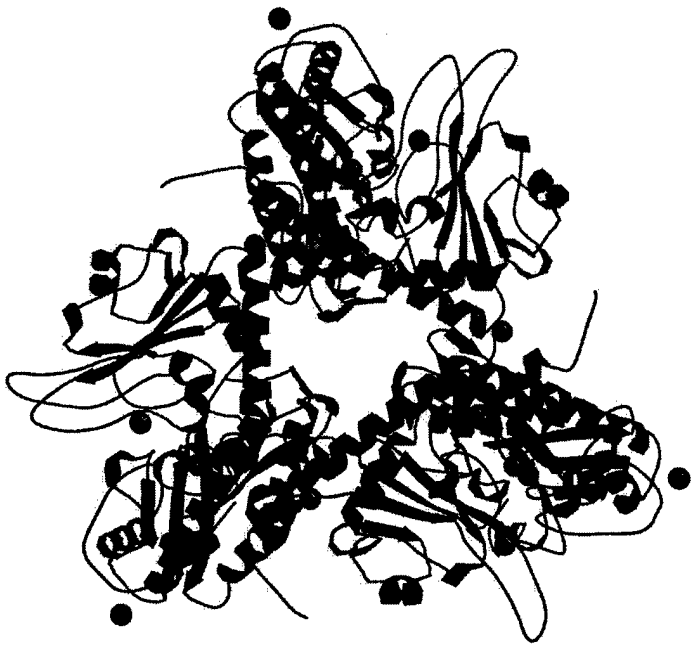

Figure 1. Ribbon diagram of OPAA trimer with manganese ions in gray spheres.

Important note: We are on the verge of obtaining the requisite atomic structures to initiate the structure-based redesign of the OPAA active site in order to enhance the catalytic activity towards detoxifying fluorinated G-type and other extremely toxic chemical warfare (CW) nerve agents, which is the long range goal of the collaboration between the different laboratories. This structure-based redesign will require several rounds of iteration of the following major steps:

1) Redesign of the active site by site-directed mutagenesis based on the OPAA crystal structures.
2) Identify those enzyme mutants with enhanced activities.
3) Determine the crystal structures of the enzymes identified in #2 for further leads for iteration of steps 1 to 2.

Considering the importance of the goal from a scientific standpoint and from a practical consideration in detoxifying chemical nerve agents, further funding or support for the structure analysis component is imperative.

B.3. Publications: None, although one paper is being written.

B.4. List of participating personnel: Dr. Alexei Nickitenko and Mrs. Abha Choudhary