

A New Defense against Nerve Agents

For many years now, a German team has been studying an obscure enzyme found in squid. Why? Because the enzyme could serve as a defense against nerve agents. Called DFPase because it catalyzes the destruction of the nerve agent DFP (di-isopropyl fluorophosphate), this enzyme is effective against a number of phosphorus-based nerve agents, including Sarin, the substance that killed 12 and sickened thousands in the Tokyo subway attacks of 1995.

DFPase, an enzyme that destroys nerve agents, was discovered accidentally in squid during early experiments on nerve conduction.

Two members of the team, Blum, who has been working with the medical branch of the German army, and Julian Chen, a California transplant and an assistant professor of biophysical chemistry at Goethe University in Frankfurt, recently came to the PCS to clinch their findings on the inner workings of DFPase. The new findings, published early this year in the Proceedings of the National Academy of Sciences (PNAS), not only have overthrown past ideas about how the enzyme works, but also have led to an engineered version of the enzyme, speeding its activity rate to the point that it can safely decontaminate exposed skin and sensitive surfaces on optics and electronics in less than 10 minutes. The engineered enzyme also works on another nerve agent, VX, which was immune to the action of DFPase. All these nerve agents act by quickly blocking the nervous system's "off switch" for muscles and glands, leaving them stuck in a semi-active state. As a result, they may tire, and in the case of large doses, the body can lose the ability to sustain breathing.

Marc-Michael Blum (left) and Julian Chen examining the PCS's sample holder. Photo by Dixon Wolf.

The original belief was that DFPase did its defensive work by catalyzing a reaction between water and the nerve agent. As shown in the diagram below, the standard picture was that oxygen from the water attacked by binding to the phosphorus in the nerve agent. That attack caused the fluorine that is bound to the phosphorus to be jettisoned, thereby detoxifying the nerve agent. (Without the fluorine, the agent no longer binds strongly to the critical off switch.) This detox reaction was presumably catalyzed in the area of the enzyme in which chemical reactions take place, DFPase's active site.

(a) DFP, Sarin, and other phosphorus-based nerve agents typically have a fluorine atom (blue) bound to a phosphorus atom. (b) It was thought that DFPase catalyzed a reaction in which the fluorine was jettisoned and an oxygen from a water molecule swooped in to replace it. The research reported here shows otherwise.

Before testing that theory, DFPase research was focused on just isolating the enzyme, that is, getting enough of it to work on. "At one point my Ph.D. advisor's group came back from the fish market with a couple hundred squid," says Blum. "We needed that many just to get micrograms of the enzyme." Fortunately, that amount was enough to determine the unique sequence of 314 amino acids that form the precious enzyme.

With the sequence in hand, the researchers could exploit gene technology to manufacture the enzyme in much larger quantities and pursue a slew of different techniques to probe its mode of operation.

X-ray crystallography revealed the structure of the enzyme (minus the hydrogen atoms, of course). The active site, where the enzyme presumably holds the nerve agent and the water in close proximity, turned out to be an indentation at the top where four of the enzyme's amino acids and three isolated oxygen atoms (red) were all loosely bound to a central calcium ion. The oxygen atoms were presumably water molecules (with their hydrogen atoms invisible) that had been frozen into position during the crystallization of the enzyme in

the presence of water.

Top view of the enzyme DFPase (white) shows six sections (the groupings of blue ribbons), arranged in a circle around a central tunnel containing two calcium ions (green). The central calcium holds the enzyme together; the upper calcium is in the enzyme's active site. Blowup: In the x-ray structure of DFPase's active site, four amino acids and three oxygen atoms (red) —presumably water molecules—surround and are bound to the calcium ion. Without this calcium ion, the enzyme is not active against nerve agents.

Blum says, "It was not clear which amino acid or water molecule would act to attack a nerve agent's phosphorus atom."

To uncover the active player, the team co-crystallized the enzyme with a nerve agent surrogate and again used x-rays to determine the combined structure.

To everyone's surprise, the nerve agent surrogate pushed the topmost water molecule out of the active site rather than interacting with it. Moreover, the surrogate had bound to the calcium in an unexpected and very exacting configuration. It looked as if one of the amino acids in the active site, rather than water, could easily provide the oxygen atom for detoxifying the nerve agent.

Blum and Chen began to suspect that, contrary to previous assumptions, a catalyzed reaction with water is not what destroys a real nerve agent. They proposed, instead, that a negatively charged oxygen from the enzyme's amino acid aspartate 229, is what binds to the nerve agent, causing the ejection of a bound fluorine atom. A water molecule later replaces the enzyme's oxygen.

Blum and Chen's newly discovered detox mechanism. An oxygen from aspartate 229 lines up with the fluorine-phosphorus chemical bond on the nerve agent Sarin. It then attaches to the phosphorus and jettisons the fluorine (not shown), destroying the nerve agent's toxicity.

To verify this radical proposal, the researchers did a series of tracer experiments in which the enzyme and the nerve agent DFP were surrounded by water in which oxygen-16, the common oxygen isotope, had been replaced by the heavier oxygen-18. By showing that the oxygen-18 atoms in the water showed up in the reaction not initially but only after a given DFPase molecule had acted multiple times, Blum and Chen were able to demonstrate that DFPase was indeed the source of oxygen that detoxified the nerve agent.

But the critics remained unconvinced, claiming that an invisible hydrogen ion was present on the aspartate 229's oxygen, neutralizing its negative charge and thereby preventing it from making a successful attack. These critics also claimed that the necessary oxygen came instead from a hydroxide ion bound to the calcium in the enzyme's active site.

To find out who was right, Blum and Chen abandoned x-ray crystallography and mounted a PCS neutron crystallography experiment to pinpoint the hydrogen atoms' positions in the DFPase structure.

Chen came to Los Alamos from Frankfurt to grow the needed crystals of DFPase and some weeks later returned for the delicate task of mounting a large, soft 2.4-mm-long crystal in a capillary tube. Enzyme crystals are typically soft and delicate because they are about 50 percent water.

Blum and Chen used this crystal to record 37 neutron diffraction images during one month. They then took advantage of new computer programs developed by Langan, Marat Mustyakimov (staff member at Los Alamos), and colleagues from Lawrence Berkeley Laboratory to compare x-ray diffraction data with the new neutron diffraction data and determine the most-likely structure for DFPase crystallized in the presence of water.

The neutron work unequivocally showed that the topmost oxygen atom in DFPase's active site had two hydrogen ions bound to it. It was indeed water, not a hydroxide ion. Also, there was no hydrogen ion on aspartate 229's active oxygen; it was negatively charged. All objections to the new theory about the detox mechanism had been knocked down.

"Our practical goal was to design better versions of DFPase," says Blum. "With proof for the new detox mechanism in hand, we redesigned the shape of the enzyme's active site so that the most-toxic versions of each nerve agent would naturally bind in the best orientation for an aspartate 229 attack. Our best new version of DFPase worked 2 to 10 times faster on all the known nerve agents than the original enzyme did, and it will likely become an important defense for first responders in the case of a nerve agent attack."