Scientists used accelerated evolution to develop enzymes that provide protection against nerve gas

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Protection against nerve gas attack is a significant component of the defense system of many countries around the world. Nerve gases are used by armies and terrorist organizations, and constitute a threat to both the military and civilian populations, but existing drug solutions against them have limited efficiency.

A multidisciplinary team of scientists at the Weizmann Institute of Science succeeded in developing an enzyme that breaks down such organophosphorus nerve agents efficiently before damage to nerves and muscles is caused. Their results have recently been published in the journal Nature Chemical Biology. Recent experiments performed in a U.S. military laboratory (USAMRICD) have shown that injecting a relatively small amount of this enzyme into animals provides protection against certain types of nerve agents, for which current treatments show limited efficacy.

Nerve agents disrupt the chemical messages sent between nerve and muscle cells, causing loss of muscle control, and ultimately leading to death by suffocation. Nerve agents interfere with the activity of acetylcholinesterase, the enzyme responsible for the breakdown of the chemical messenger – acetylcholine. As a result, acetylcholine continues to exert its effect, resulting in constant muscle contraction throughout the body.

Several drugs exist that are used to treat cases of nerve agent poisoning. Although these drugs are somewhat effective when exposed to small doses of the nerve agent, they do not provide protection against high-dose exposure; they are not effective against all types of nerve agents; or they cause serious side effects. Neither are they able to prevent nor repair cerebral and motor nerve damage caused by the nerve agent.

An ideal solution to the problem is to use enzymes – proteins that speed up chemical reactions – to capture and break down the nerve agent before it gets the chance to bind to the acetylcholinesterase, thereby preventing damage. The main obstacle facing the realization of this idea, however, is that nerve agents are man-made materials and therefore, evolution has not developed natural enzymes that are able to carry out this task.

Scientists worldwide have previously succeeded in identifying enzymes that are able to break down similar materials, but these enzymes were characterized by low efficiency. Large amounts of the enzyme were therefore required in order to break down the nerve agent, rendering their use impractical.

This is where Prof. Dan Tawfik of the Weizmann Institute's Biological Chemistry Department enters the picture. Tawfik's group developed a special method to artificially induce "natural selection" of enzymes in a test tube, enabling them to engineer "tailor-made" enzymes.

The method is based on introducing many mutations to an enzyme, and scanning the variety of mutated versions that were created in order to identify those that exhibit improved efficiency. These improved enzymes then repeatedly undergo further rounds of mutations and selection for higher efficiency. In previous studies, Tawfik showed that this method can improve the efficiency of enzymes by factors of hundreds and even thousands.

For the current task, Tawfik selected an enzyme that has been extensively studied in his laboratory, known as PON1. The main role of this enzyme, found naturally in the human body, is to break down the products of oxidized fats that accumulate on blood vessel walls, thus preventing atherosclerosis. But PON1 seems to be a bit of a "moonlighter" as it has also been found to degrade compounds belonging to the family of nerve agents.

However, because this activity has not fully evolved and developed through natural selection, its efficiency in carrying out the task remains very low. But by using the directed evolution method, scientists hope that they will be able to evolve this random "moonlighting" activity into PON1's main "day job," which would be carried out more quickly and efficiently than before.

In the first phase, Tawfik and his team, including research fellow Dr. Moshe Goldsmith and postdoctoral student Dr. Rinkoo Devi Gupta, induced a number of mutations in PON1 – some random and others directed at key sites on the enzyme. To identify the most effective PON1 mutants, the scientists joined forces with Yacov Ashani of the Structural Biology Department.

The method that the scientists developed closely mimics what happens in the body upon exposure to nerve agents: They put the acetylcholinesterase in a test tube together with a specific mutant PON1 enzyme that they wanted to test, and added a small amount of nerve agent to it. In cases where the acetylcholinesterase continued to function properly, it could be concluded that PON1 rapidly degraded the nerve agent before it was able to cause damage to the acetylcholinesterase.

After several rounds of scanning, the scientists succeeded in indentifying active mutant enzymes, which are able to break down the nerve agents soman and cyclosarin effectively before any damage is caused to the

acetylcholinesterase. These mutant enzymes have been structurally analyzed by a team of scientists from the Structural Biology Department, which included Profs. Joel Sussman and Israel Silman, and research student Moshe Ben-David. Further experiments have shown that when these enzymes were given as a preventative treatment before exposure, they afforded animals near-complete protection against these two types of nerve agents, even when exposed to relatively high levels.

The scientists plan to further expand the scope and develop preventive treatment that provides protection against all types of existing nerve agents. They are also trying to develop enzymes with high enough efficiency to be able to very rapidly break down the nerve agent so they could be used to prevent the lethal effects of nerve agents by injection immediately after exposure.

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