New antibiotics for anthrax?

Stephani Sutherland, freelance writer

A US patent was granted early this year for a new class of compounds that could eventually represent a family of drugs with antibiotic properties against *Bacillus anthracis*. These drugs would have the important benefit of being active against other pathogenic bacteria while leaving the endogenous microflora of the human intestinal system intact.

Although the drugs are still in the earliest stages of development, officials at both VDDI Pharmaceuticals (http://www.virtualdrugdevelopment.com) and the University of Alabama at Birmingham's Center for Biophysical Sciences and Engineering (UAB-CBSE; http://www.cbse.uab.edu), who jointly filed the patent, are optimistic about the future use of the compounds, the first of which appears to discriminate between Gram-positive and Gram-negative bacteria.

NAD synthetase blocker

As director of the UAB-CBSE, Larry DeLucas chose to first develop compounds that were designed to block NAD synthetase, an enzyme critical to the bacteria's transition from its sporulated state to its potentially lethal vegetative form (Fig. 1). The enzyme is one of several that could provide future drug targets for the anthrax bacterium [1], and was a natural first choice because the crystal structure had already been published [2].

The research is partially funded by the US Department of Defense, in hopes that the compounds could be used to protect potential victims of a biological attack with anthrax. Normally, when a spore is inhaled, DeLucas explains, it is engulfed by a macrophage. Once the spore encounters the right environment – 'water, a few crucial amino acids, like alanine,' – it begins to germinate. 'The spore is harmless… it has to lose its outside core… but then it becomes a vegetative cell. It looks black; it begins to divide and multiple, you get so many in the lungs, and then they begin to release toxin.' He sums up the consequences simply: 'Then you have big problems.'

Bioterrorism

The new compounds were designed to shut down the bacteria's emergence from its protective coating. DeLucas explains that the spore tries to become the bug and it eventually loses its shell; however, when NAD synthetase is blocked, the walls break apart and it looks like 'a bomb went off, and you get no bug.' DeLucas stresses that the compounds have only just begun down the long road of drug design but, if successful, could be a powerful prophylactic against a biological attack. Even with a large number of spores in the air, they would just fall apart and infection would be prevented. The currently used antibiotic Cipro, in contrast, is only effective when anthrax exists in its vegetative state, which means that the victim is already becoming infected. Compounds that inactivate NAD synthetase would also be useful against the vegetative cell after the spore has germinated, adds DeLucas, because the enzyme is still vitally important to the organism.

Indeed, the enzyme is vital to any bacterium, so DeLucas was somewhat surprised when the new inhibitors showed 'an absolute predilection for Gram-positive bacteria,' while leaving Gram-negative bacteria, such as *E. coli*, intact. The researchers are hoping that this means that it is not going to kill all internal microflora, 'but it is difficult to predict whether a compound will act with such specificity,' says DeLucas; 'it is likely to depend on subtle structural differences in the proteins'.

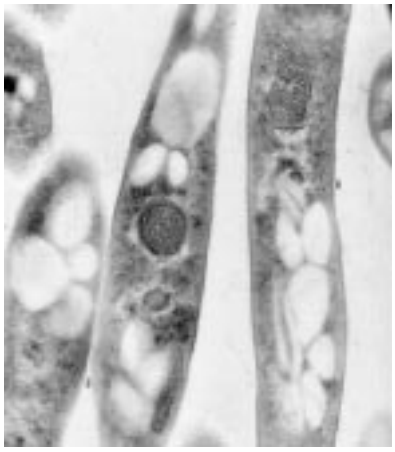

Figure 1. Transmission electron micrograph of the vegetative (rod) state of *Bacillus anthracis* (from http://phil.cdc.gov/phil/search.asp).

Crystal structures

Although researchers at the UAB-CBSE have not yet co-crystallized NAD synthetase with the bound inhibitor, they have compared the crystal structures of the enzyme from *Bacillus subtilis* [3] and from *E. coli* [4], and they hypothesize that a small amino acid loop near the active site in the Gram-negative bacterial enzyme might prevent the compound from disabling NAD synthetase.

DeLucas and his group aimed to design compounds that would specifically disable their target by binding to the enzyme's active site [5]. Structure-based drug design has many advantages, says DeLucas, primarily

1359-6446/03/$ – see front matter PII: S1359-6446(03)02665-5

that such a drug is effective: 'the enzyme just can't work.' In addition, a compound that binds at a protein's active site is less likely to lead an organism to develop resistance. Explains DeLucas, amino acids on the surface of a protein could be easily mutated without affecting function but the active site is different. 'If you look at any protein…there will be certain amino acids that are critical for it to function.' By making a drug that binds to those specific residues, you use an area of the protein that is less likely to naturally mutate and thereby confer resistance.

New antimicrobial drugs

J. Todd Weber, of the National Center for Infectious Disease at the US Center for Disease Control (http://www.cdc.gov), says that it is 'clear that we do need new classes of antimicrobial drugs,' because the 'rates of resistance are increasing' all the time. Although Weber is not familiar with the unpublished details of the current work, he noted, 'any advance to create new drugs that get around the mechanisms of developing antibiotic resistance would certainly be valuable'.

Of course, today we must also consider a non-natural source of mutations that might lead to drug resistance: those engineered by potential bioterrorists. DeLucas suggests that by making the active site the drug target, you could protect the drug's efficacy. Vaccines, too, are subject to lose their effectiveness through natural protein mutations. 'Does this mean we should not use vaccines' to protect people against a biological attack, DeLucas asks. 'No. They present another hurdle for terrorists,' and fighting biological warfare, he says, 'should use a dual-pronged approach'. DeLucas is betting that one day the newly developed compounds will be used in that fight.

References

1 Jedrzejas, M.J. (2002) The structure and function of novel proteins of *Bacillus anthracis* and other spore-forming bacteria: development of novel prophylactic and therapeutic agents. *Crit. Rev. Biochem. Mol. Biol.* 37, 339–373

2 Rizzi, M. *et al.* (1996) Crystal structure of NH3-dependent NAD^+ synthetase from *Bacillus subtilis*. *EMBO J.* 15, 5125–5134

3 Symersky, J. *et al.* (2002) NH3-dependent NAD+ synthetase from *Bacillus subtilis* at 1Å resolution. *Acta Crystallog.* D58, 1138–1146

4 Ozment, C. (1999) Structural study of *Escherichia coli* NAD synthetase: overexpression, purification, crystallization, and preliminary crystallographic analysis. *J. Struct. Biol.* 127, 279–282

5 Devedjiev, Y. *et al.* (2001) Stabilization of active-site loops in NH3-dependent NAD+ synthetase from *Bacillus subtilis*. *Acta Crystallogr.* 57, 806–812

News in brief

Targets and mechanisms

The worm has turned

Scientists at Massachusetts General Hospital (http://www.mgh.harvard.edu/) and their colleagues have scoured thousands of genes in the *Caenorhabditis elegans* worm and have identified hundreds of promising candidates that could determine how fat is stored and used in a variety of animals [1]. Their findings represent the first survey of an entire genome for all genes that regulate fat storage.

The research team, led by Gary Ruvkun, used RNA-mediated interference (RNAi) to disrupt the expression of each of the 16,757 genes of *C. elegans* in a systematic screen of the genome for genes that are necessary for normal fat storage. In this way they identified ~300 genes that, when inactivated, cause reduced body fat and ~100 genes that cause increased fat storage when turned off. The identified genes were diverse and included both the expected genes involved in fat and cholesterol metabolism, as well as new unexpected candidates. Many of the fat regulatory genes identified in this study have counterparts in humans and other mammals.

'This study is a major step in pinpointing fat regulators in the human genome,' says Ruvkun. 'Of the estimated 30,000 human genes, our study highlights about 100 genes as likely to play key roles in regulation of fat levels,' he continued. Most of these human genes had not previously been predicted to regulate fat storage and could pave the way for designing drugs to treat obesity and its associated diseases such as diabetes.

1 Ashrafi, K. *et al.* (2003) Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421, 268–272

Piecing together the HIV virus

New information has been revealed on the interactions that mediate virus assembly. A team led by Peter Prevelige of the University of Alabama at Birmingham (UAB; http://main.uab.edu/) used MS to probe the assembly of HIV-1 capsid protein [2], a target with obvious therapeutic significance.

Much data has been collected over the past few decades on the structures of the protein constituents of viruses. However, the means by which the numerous capsid subunits unite to form the virus particle are less-well understood, especially in retroviruses. New research on the nature of such interactions has obvious implications for drug research. Advanced structural knowledge would be an important foundation for developing anti-viral drugs that could target interactions between capsid particles and disrupt the virus.

The team from UAB used high-resolution MS to probe the interactions between individual HIV-1 capsid proteins. The HIV-1 virus, like other retroviruses, has frustrated attempts at detailed structural

 1359-6446/03/$ – see front matter PII: S1359-6446(03)02666-7