PERSPECTIVES: MICROBIOLOGY

Fighting Anthrax with a Mutant Toxin

Sjur Olsnes and Jørgen Wesche

Although anthrax is a rare disease, the microbe that causes it, *Bacillus anthracis*, has garnered more than its fair share of attention because of its potential use as a biological weapon in terrorist attacks as well as in wartime combat. In either scenario, aerosols of the bacterial spores are likely to be used because their inhalation induces the systemic form of the disease, which rapidly leads to death. Vaccination against anthrax is possible, but owing to the difficulty in predicting which populations are at risk, it is usually impractical. Antibiotics are effective if given at a very early stage of infection, but the bacteria multiply rapidly, quickly producing lethal amounts of the deadly anthrax toxin. Thus, there is a vital need for an effective treatment that can be administered after infection. On page 695 of this issue, Sellman *et al.* (*1*) describe a therapeutic approach that fits this bill.

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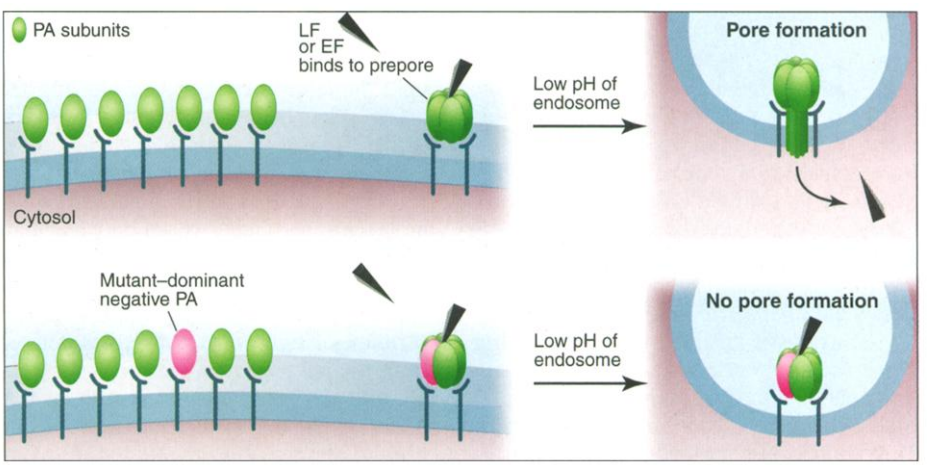
Anthrax is a multicomponent toxin that is assembled at the surface of host cells after infection (see the figure). The lethal action of the toxin, however, occurs in the host cell cytoplasm, where two of its components, edema factor (EF) and lethal factor (LF), interfere with cellular regulation. EF is an adenylate cyclase that is activated by calmodulin, and LF is a metalloprotease that cleaves several isoforms of mitogen-activated protein kinase kinase (MAPKK), a crucial signaling molecule. Both factors interfere with the ability of macrophages and other cells to fight the bacterial infection, and as a result the anthrax bacilli multiply rapidly in the blood (*2*).

Translocating EF and LF across cellular membranes so that they can reach their targets in the cytoplasm is not a trivial task. Thus, the anthrax toxin is equipped with a sophisticated translocation device. This structure is assembled on the host cell surface from seven identical subunits of protective antigen (PA)—so-called because it is a principal component of the anthrax vaccine. The doughnut-shaped heptamer binds EF and LF with high affinity (see the figure).

The assembled heptamer is taken into the cell by endocytosis. When exposed to the acidic pH of endosomes, a conformational change takes place in the structure and a loop in each subunit swings out and becomes inserted into the membrane, forming an ion-permeable pore. Concomitantly, the EF and LF attached to the outside of the cell are translocated into the cytoplasm. Some unfolding of EF and LF is required for their translocation (*3*), but it is not yet clear if they cross the membrane by passing through the pore or by another mechanism, as may be the case with diphtheria toxin (*4*).

Earlier observations from the Collier group (*5*) demonstrated that several mutations in PA can prevent translocation of EF and LF into host cells. The mutant forms of PA bind to the cell surface and assemble into heptamers, but the heptamers do not form pores, apparently because they cannot be inserted into the membrane. Sellman and colleagues (*1*) now demonstrate that if they mix normal and mutant PA molecules in a test tube, the mutant subunits behave in a dominant-negative manner, that is, they participate in the formation of the heptamer, but prevent pore formation and translocation of EF and LF into cultured cells. The Sellman *et al.* findings suggest that assembly of just one molecule of mutant (dominant-negative) PA into the heptamer can block translocation of EF and LF into the cytoplasm. They then tested their mutant PA in a rat model of anthrax intoxication. Rats injected with a lethal mixture of LF and PA became moribund within 90 minutes. If mutant dominant-negative PA was included in the lethal mixture, the rats showed no evidence of intoxication and survived happily. It will be interesting to see how long after infection with *B. anthracis* animals can still be protected by mutant PA. Administration of mutant PA to individuals infected with anthrax could potentially provide protection even at an advanced stage of disease. A major bonus for the Sellman *et al.* strategy is that large quantities of PA, which on its own is not toxic, are already produced to make the anthrax vaccine. Clearly, the Sellman *et al.* approach is a promising development in the treatment of human anthrax.



Blocking anthrax toxin. (Top) The bacterium that causes anthrax produces subunits of the protective antigen (PA, in green) that bind to receptors on mammalian host cells. After activation by the protease furin, the PA subunits oligomerize to form a prepore complex consisting of seven PA monomers, which binds lethal factor (LF) and edema factor (EF). The heptameric complex is then endocytosed by the host cell and the low pH of endosomes triggers a conformational change in the PA prepore, leading to pore formation. The toxic bacterial enzymes LF and EF (black) are translocated into the cytoplasm of the host cell. **(Bottom)** A mutant (dominant-negative) PA subunit (red) interferes with pore formation and prevents LF and EF from entering the host cell.

Anthrax toxin is only one of many multisubunit toxins that cause severe illness in humans. Some of these, such as cholera toxin and Shigella toxin, are assembled within the bacteria, and so interference by mutant dominant-negative subunits would not be feasible. However, a group of multi-

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subunit toxins—*Clostridium spiroforme* toxin, the ADP-ribosyltransferase from *Clostridium difficile*, the ι-toxin from *Clostridium perfringens*, and the C2 toxin from *Clostridium botulinum*—are assembled on the host cell surface like anthrax toxin (*6*), and their activity could be blocked with a dominant-negative strategy. Furthermore, important cytolysins, such as the α-hemolysin from *Staphylococcus aureus* and aerolysin from *Aeromonas hydrophila*, assemble into heptameric ring structures at the host cell surface, inserting themselves into the membrane and forming pores (*7*). These toxins could be inactivated by mutant dominant-negative subunits. The VacA toxin of *Helicobacter pylori*, the bacterium that causes gastric ulcers, forms hexa- and heptameric structures. A deletion mutant of the toxin interferes with the formation of active oligomers when tested on cells in culture (*8*). It may be difficult to exploit this finding therapeutically, however, because VacA attacks epithelial cells deep within the gastric crypts of the stomach. In the case of anthrax, the toxin is carried in the blood and can therefore be blocked by injecting mutant PA into the circulation.

A major concern when treating bacterial infections with antibiotics is the appearance of increasing numbers of antibiotic-resistant strains. Vaccination is a possible alternative for some diseases, but is often impractical either because it does not provide long-lasting protection or because the disease is uncommon. It is therefore important to be able to treat bacterial infections as soon as they occur with therapeutics other than antibiotics. The paper by Sellman *et al.* is an innovative example of how this can be achieved.

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PERSPECTIVES: LASER CHEMISTRY

Keeping Reactions Under Quantum Control

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Many chemical reactions can follow more than one reaction pathway, each leading to a different product. Usually, only one of these pathways is desired, and the side reactions decrease the yield of the desired product. Chemists therefore strive to devise methods for controlling chemical reactions and increasing the yield of specific products. Traditionally, they have relied on manipulating macroscopic parameters such as temperature and pressure; a catalyst may also alter the course of a reaction. Recently, femtosecond lasers have emerged as a powerful tool for quantum mechanical control of reactions. On page 709 of this issue, Levis *et al.* (*1*) show that strong-field laser pulses can be used to control a dissociative rearrangement reaction, in which bonds are not only selectively broken but also selectively formed.

Virtually since their creation, lasers have been suggested as a possible tool for controlling chemical reactions. A decade or more ago, schemes were developed that involved excitation to specific states, which were then supposed to decompose to specific products. In most cases, however, the laser energy was distributed rapidly into various bonds throughout the molecule, hindering the ability to select a specific pathway. All this changed with the development of femtochemistry, which opened up completely new ways of altering the course of a chemical reaction (*2*). Several scenarios for laser control of reactions have now been developed and implemented.

On a microscopic level, atoms, molecules, and light behave both as a particle and as a wave. It is the wave nature of the atoms and molecules that allows for quantum control to be accomplished. Absorption of light must obey rules derived from the laws of quantum mechanics, including the resonance condition, which dictates that the difference between the ground state and an excited state of an atom or molecule must match the energy of a single photon or the total energy of multiple photons. Short (femtosecond) laser pulses contain components of several different wavelengths; the frequency range depends on the bandwidth of the laser and hence its temporal characteristics. A single molecule can absorb photons of any of the component wavelengths as long as the absorption obeys the selection rules. When a molecule is coherently excited by the laser, a wave packet is created that accounts for the motion of the molecule in the superposition of excited states. Quantum mechanics predicts that if the molecule has a probability of residing in any one of the possible excited states, then it is in all of the possible excited states simultaneously.

The goal of a quantum control process is to alter this superposition and thus the wave packets, such that maximum constructive interference occurs in the desired reaction path while maximum destructive interference occurs in all other reaction pathways. This allows selective control beyond the trivial effects obtained through varying the intensity or simple temporal characteristics of laser pulses often used to alter the course of photochemical reactions.

Quantum control can be implemented in several ways. Two-pathway control, developed theoretically by Shapiro and Brumer (*3*) and experimentally by Gordon *et al.* (*4*) and others, uses laser light of sufficiently long pulses to excite molecules by distinct routes that reach the same final state. For example, absorption of one photon of 200 nm light and absorption of three photons of 600 nm are energetically equivalent and both could lead to the same final state. Varying the phase difference between the two laser fields changes the phase of the superposition wave packet and allows for quantum control. Some success has been achieved with this method (*4*).

A more general approach to quantum control, optimal control, uses an optimized laser field to create and control the excited molecular wave packets (*5*, *6*). Optimal control theory is used as a design technique to solve the equations of motion for the molecule and the exciting laser field to determine the optimal laser pulse that will give the maximum yield of the desired reaction products while minimizing side reaction channels (*7*). Solving the equations of motion can be computationally difficult, however, and the calculated laser field may be experimentally difficult to implement. The equations of motion depend on having an adequate description of the molecule including its interaction with the laser field. Reductions in the complexity of the calculations have been attempted, but in the case of the isomerization of HCN, the current levels of simplification were not up to the task of de-

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