Molecular Design Gets Into a Hole

A bacterial toxin that self-assembles into cell-penetrating pores might give rise to new molecular technologies

BACTERIAL BRUTALITY KNOWS NO LIMITS. Take *Staphylococcus aureus*. This common lowlife secretes toxin molecules that riddle the membranes of susceptible cells with tiny pores. Water gushes in and, like overfilled balloons, the besieged cells burst.

It's not a pretty picture, but to molecular biologist Hagan Bayley of the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, it has powerful attraction. He and his colleagues have been dissecting the pore-forming proteins by genetic engineering to learn how they work. Armed with that structural knowledge, they hope to transform the proteins from bacterial weapons to biotechnological plowshares. They are betting that tailored pores could open the way to new medicines, chemical sieves, and high-tech microporous materials.

The *Staphylococcus* toxin that has been on Bayley's mind for the better part of a decade is alpha-hemolysin—alpha-toxin for short. The 293 amino acid protein gets its name from its penchant for lysing, or rupturing, red blood cells. Nobody knows just what the bacteria gain by bursting cells—though it may be a rough-and-ready way to break them down into food fragments—but investigators in Germany, Sweden, England, the United States, Italy, and elsewhere have gathered enough clues to build a general picture of pore formation. After binding to cell membranes, the protein monomers gather in groups, which then self-assemble into pores big enough to admit ions and small molecules but too small for other proteins.

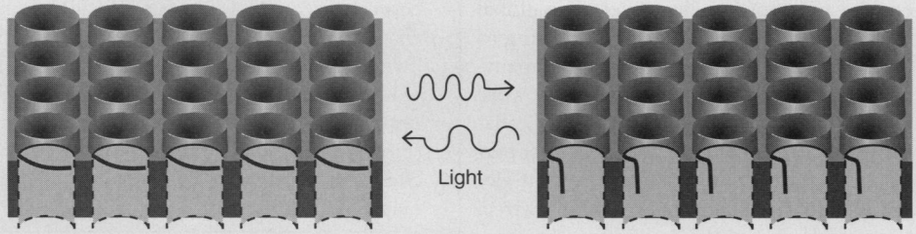
Bayley and his colleagues, though, wanted a deeper understanding of how the protein does its dirty work. That meant developing the ability to genetically alter specific parts of the protein and study the effects of each change. The first step—cloning and sequencing the gene—was taken in the mid-1980s by other researchers. Building on that work, Bayley and his co-workers developed cell-based methods for making relatively large amounts—milligrams, that is—of the protein in biotech's favorite laboratory organism, *Escherichia coli*.

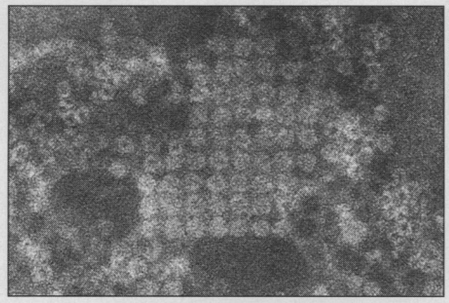
By mutating the gene and producing new versions of the protein that lacked various parts of the amino acid chain, Bayley and colleagues were able to pinpoint domains that are crucial to its function. Combining these clues with evidence for a hinge-like domain in the protein, previously reported by researchers at Karolinska Institute in Stockholm, Bayley's team came up with a more detailed model of pore formation. First, specific segments of the protein monomers, which are folded in two at the hinge, probably bind to still-unidentified receptors in cell membranes. Next, hydrophilic (water-loving) regions of each protein find each other and huddle like football players to minimize their contact with the hydrophobic, or oily, membrane around them. When six of the molecules have joined the huddle, physical interactions cause each folded protein to spring open and plunge through the membrane. The result is a pore surrounded by six proteins arranged like the staves of a barrel.

That aptitude for piercing membranes makes the unaltered protein attractive to biologists who need to get foreign substances into cells. "It's better than detergents" for making cells permeable, says Sidney Harshman, a microbiologist and microbe toxin aficionado at Vanderbilt University, referring to the standard—but less discriminating—lab technique for disrupting cell membranes. When cells are exposed to low concentrations of the protein, their membranes become temporarily porous.

Now that Bayley and his colleagues have a means of making lots of mutant protein monomers and a schematic picture of how the molecule works, they think they can take it much further. By tweaking the protein's chemical personality, says Bayley, they're finding that "this is a blank slate for making pores of many kinds."

Last September, at a biology conference in Virginia, the researchers reported equipping the protein with what could serve as a chemical hook for attaching other molecular accessories. They did so by enlisting genetic engineering techniques to replace a serine and a threonine residue—the 3rd and 292nd amino acids in the protein—with cysteine residues. The substitute amino acids contain relatively reactive sulfur atoms, which should open the way to adorning the basic protein with other chemicals. Add-ons that respond to light, other substances, or voltage, for example, might form the basis of molecular gates that could be opened and closed with external signals. Such gated pores might in turn be cross-linked into sheets that could yield chemical sensors and even pore-based memory devices, speculates John Kasianowicz, a biophysicist at the National Institutes of Health who is collaborating with Bayley.





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Pore prospects. *Protein pores array in a membrane* (left)*; bioengineered, they might open and close in response to light* (above).

Other chemical modifications might transform pore proteins from weapons of destruction into medicinal agents, Kasianowicz and Bayley think. Appropriately modified, pore proteins might seek out and puncture disease-causing cells while ignoring healthy cells. Or they might shield organ transplants from rejection by the recipient's immune system, suggests Harshman. His strategy would exploit the molecules' gregarious nature rather than their ability to form pores. Adorned with antibodies targeted to the cell-surface antigens that trigger rejection, Harshman suggests, the proteins might aggregate into clusters on the transport cells. In a normal cellular response, the cells would engulf the protein-antigen clusters, thereby deceiving the host immune system.

Bayley and company admit that none of these scenarios is a sure thing. But if they can somehow weave holes into the design repertoire of molecular engineers, the brutality of bacterial pores will have yielded unexpected peace dividends. ■ IVAN AMATO