STRUCTURAL BIOLOGY

Biologists Catch Their First Detailed Look at NO Enzyme

Nitric oxide is a Jekyll-and-Hyde substance. In tiny puffs inside the body, it helps cement memories, control blood pressure, and battle disease. But it has also been implicated in ailments as varied as Alzheimer's disease, diabetes, and impotence. The key to this character switch is an enzyme called a nitric oxide synthase (NOS), which makes NO inside the body. If it does its job correctly, NO is the benign Dr. Jekyll; if it produces too much or too little, NO becomes the perfidious Mr. Hyde.

Now researchers have gotten their first detailed look at this crucial enzyme's key section: the catalytic site at which NO is produced. On page 425 of this issue, a team led by John Tainer at The Scripps Research Institute in La Jolla, California, and Dennis Stuehr of the Cleveland Clinic in Ohio reports determining the three-dimensional structure of NOS's active site, bound to substances known to inhibit its catalytic action. This information could help researchers design drugs to dampen NO production in diseases linked to excess NO.

"This is a magnificent achievement," says Carl Nathan, an immunologist at Cornell University Medical College in New York City who has studied NOS for a decade. "For drug development, a picture of the active site is indispensable." Drugs that inhibit NOS, he notes, could be useful in treating septic shock, Alzheimer's disease, multiple sclerosis, stroke, inflammatory bowel disease, rheumatoid arthritis, and many forms of inflammation. "Probably every major drug company is looking for drugs to inhibit this enzyme," adds Solomon Snyder, a neuroscientist at Johns Hopkins University whose team was the first to clone and sequence one form of NOS.

The shape of the catalytic part of NOS—which turned out to be quite different from what experts expected—should also provide insights into a class of chemical reactions that produce steroids and break down toxins. Both processes, like the production of NO, entail adding single oxygen atoms to molecules. "It is a very exciting structure," says Douglas Rees, a crystallographer at the California Institute of Technology in Pasadena.

Long studied as an ingredient of air pollution, NO first achieved biological notoriety in the mid- to late 1980s, as hints emerged of its myriad physiological roles. In 1987, for example, John Hibbs's team at the University of Utah in Salt Lake City found that immune cells called macrophages released it as a toxic defense against tumor cells. But in smaller amounts and other contexts, NO seemed to function as a signaling molecule important to regulating blood pressure or transmitting messages between nerve cells.

In the early 1990s, research teams isolated and cloned three variant forms, or isozymes, of NO synthase. Two of them, extracted from the brain or the lining of the blood vessels, produced NO for signaling purposes. But the enzyme isolated from macrophages, dubbed iNOS for inducible NOS, churned out NO in much larger amounts, to cope with threats from cancer or invading microbes. It's this isozyme that is largely responsible for NO's dark side: In the large amounts made by the enzyme, NO can also trigger deadly blood pressure drops, causing toxic shock, or destroy healthy tissue in the brain, bowel, or joints, leading to neurodegenerative or inflammatory diseases.

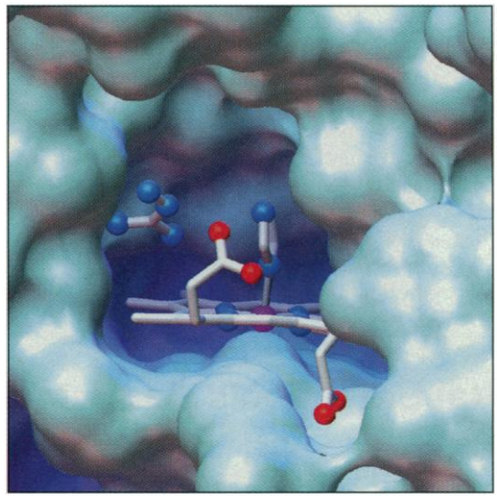

ILLUSTRATION: M. E. PIQUE

In hand. Heme group (horizontal structure) sits in baseball mitt–shaped pocket of NO synthase. Small blue molecules above the heme are inhibitors.

In 1993, the Cleveland Clinic's Stuehr, a pioneer in NO biology, co-authored an article on the many properties of this gas in *Chemical & Engineering News*, and the article piqued the interest of Scripps's Tainer, a crystallographer. "I was fascinated," Tainer recalls. "Whatever it took, I wanted to solve the structure of this enzyme."

He wrote to Stuehr in hopes of collaborating with him. But at the time, neither Stuehr nor anyone else had enough of any NOS isozyme to conduct structural studies. So Stuehr left Tainer's letter unanswered for 18 months, until he had coaxed bacteria to mass-produce iNOS, or rather, the part of it called the oxygenase domain. He sent Tainer a solution of the enzyme fragment in 1995.

Tainer, along with colleagues Brian Crane, Andrew Arvai, and Elizabeth Getzoff, then began the painstaking process of crystallizing the fragment and determining its structure by measuring the diffraction of x-rays beamed through the crystal. They crystallized it in a complex with inhibitors to show where the substrate and drugs would bind. They were startled by what they found.

Tainer and his colleagues had expected NOS's active site to look much like that of similar enzymes such as cytochrome p-450, which shares NOS's penchant for splitting and shuttling oxygen molecules, and also contains a "heme," or iron-containing group. In those enzymes, the active site is embedded in a protective thicket of protein coils. In NOS, however, the researchers found an exposed pocket made from overlapping protein sheets shaped like a catcher's mitt, with the heme clasped inside the pocket like a ball in a glove.

The shape, says Crane, "was something no one had seen before." It provides critical clues to how the enzyme works. For example, the position of the heme in the pocket beside a binding site for the amino acid arginine strongly suggests that the heme produces NO by catalyzing a reaction between oxygen and arginine. Such an understanding may someday enable chemists to mimic the enzyme's activity to make complex compounds such as steroids, which require similar chemistry. In addition, the pocket's exposed position indicates that NOS may be more vulnerable to inhibitors than was previously thought.

Another surprise was that two inhibitor molecules, instead of one, bind in the palm of this enzymatic glove. This suggests, says Tainer, that the most effective and least harmful drug inhibitors would be dumbbell-shaped molecules that could attach to both sites. Ideally, such inhibitors would specifically interact with iNOS and not the other NOS isozymes, so that they don't compromise signaling in the nervous and vascular systems.

But more pieces of this enzyme may have to be crystallized before researchers can craft superspecific iNOS inhibitors. In the complete molecule, the oxygenase Tainer's group crystallized is linked to other enzymatic machinery, including a reductase, which shunts electrons to it, and another oxygenase-reductase pair. "What they have is a piece of a huge enzyme," Nathan cautions. "We need to know how all the pieces fit together." Even so, this piece should open many eyes. "For me this is a watershed," says Stuehr. "Seeing this structure is like seeing somebody's face after knowing him for years as just a pen pal."

–Ingrid Wickelgren