Nitrogenase Structure Revealed

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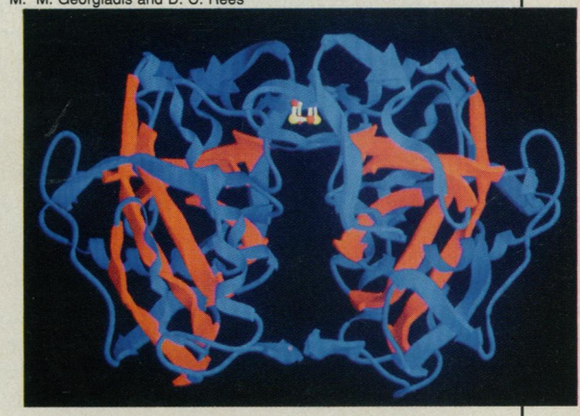

Float like a butterfly. *The two subunits of the nitrogenase iron protein form the butterfly's wings with the iron sulfur cluster at the head.*

Industrial chemists have long wished they could mimic the action of a bacterial enzyme known as nitrogenase. The active component of nitrogen-fixing bacteria, nitrogenase somehow manages to convert atmospheric nitrogen to ammonia at normal temperatures and soil pressures. Such biological nitrogen fixation provides an estimated 200 million tons of nitrogen fertilizer every year. But chemists are far less adept at synthesizing ammonia. They have to use extremely high temperatures and pressures, requiring a great deal of expensive energy, as well as a catalyst, to get the reaction to go.

Despite years of effort, however, researchers have been at a loss to explain how nitrogenase performs its job so efficiently. That situation may now be changing as researchers have recently obtained critical clues that may help solve the mystery. The hope is that this research may lead to easier and cheaper industrial ways to manufacture nitrogen fertilizers.

The new results have come from the efforts of the x-ray crystallographers, who have finally begun to unravel the structure of nitrogenase, which is actually a complex containing two different proteins. Caltech workers Doug Rees, Millie Georgiadis, and Pinak Chakrabarti have recently determined the three-dimensional structure of the smaller of these, the iron protein, which is so named because it contains a cluster of iron and sulfur atoms. Moreover, the structure of the larger component may soon follow, as another group has started its crystallographic analysis of that protein.

One of the major problems that has hindered researchers' efforts to understand how nitrogenase works is the extreme rapidity with which it is inactivated by oxygen; it is irreversibly poisoned within seconds of exposure. "The nitrogenase complex is one of the most sensitive proteins on Earth," says James Howard, a nitrogenase biochemist at the University of Minnesota.

Nevertheless, Rees took on the challenge of determining the structure of the iron protein, which has a molecular weight of 65,000, about 9 years ago while he was still a postdoc at Harvard University. At the time, he says, he did not fully appreciate the logistical problems he would encounter. "My original grant proposal to support the project suggested it would take 3 years to solve the structure," Rees says. "If I had known then how long it would take I might not have taken on the project."

Among other things, Rees and his colleagues had to protect the iron protein from oxygen during crystallization and the subsequent x-ray analysis. But that was not the biggest difficulty they had to overcome. Doing x-ray crystallography requires making "isomorphous" derivatives of the crystals by inserting metal atoms into the protein to be examined without distorting its shape. The metal atoms provide points of reference that are needed to calculate a three-dimensional protein structure from x-ray crystallographic data. Rees and his colleagues had to try several metals before they hit on one—gold—that would give them good crystals. That took until 1988, and the researchers spent the remaining $2^1/_2$ years collecting and analyzing data.

Georgiadis unveiled the iron protein's structure for the first time at the 8th International Congress on Nitrogen Fixation, which was held in May in Knoxville, Tennessee. So far the group has achieved a resolution of 3.0 Å, good enough to see how the protein folds, although they cannot yet locate the position of every amino acid.

The iron protein has an unusual structure, Rees says, with features that help to explain its extreme sensitivity to oxygen, as well as its function. The protein exists as a butterfly-shaped dimer of two protein subunits, with the metallic iron-sulfur cluster located between them at the butterfly's "head." The immediate environment of the cluster may be described as a "gem setting," Rees says, with segments of the protein acting as prongs to hold the cluster in place like a diamond in a ring.

As a result, he notes, the metal cluster is highly exposed, and that may be why the iron protein is so readily inactivated by oxygen, which attacks the iron atoms. Rees says that other iron-sulfur proteins have their metallic clusters buried in a web of amino acids. Such enzymes are not as oxygen sensitive as nitrogenase.

The iron protein also binds ATP (adenosine triphosphate), which is thought to provide the energy needed for the reduction of nitrogen to ammonia. The ATP attachment site is apparently near the bottom of the cleft between the two wings of the butterfly.

In addition, there is a possible binding site for the second protein component of the complex, the other star performer in the nitrogenase act. This is a molybdenum-iron protein that sits at the top of the iron protein near the iron-sulfur cluster. During nitrogen fixation, the role of the iron protein is to transfer electrons from a high-energy donor protein such as ferredoxin to the iron-molybdenum protein, which in turn passes them on to nitrogen during its reduction to ammonia. "The surface accessibility of the iron metal cluster is presumably important for electron transfer interactions with the molybdenum-iron protein," says Rees.

Nitrogen fixation researchers are now waiting to see what the iron-molybdenum protein, a veritable giant with a molecular weight of 240,000, looks like. The heroic task of determining that structure is under way at Purdue University in the laboratory of Jeffrey T. Bolin, who is making progress but is not yet ready to discuss his results.

Analyzing the structures of the two proteins separately may still not be enough to give a complete understanding of the nitrogenase system, however. It may also be necessary to solve the structure of the complex as a whole. What is already known about the interplay of the iron and the molybdenum-iron proteins suggests that nitrogen fixation is not a solo turn, but is a more complex *pas de deux* of two partner proteins.

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