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Researchers create artificial enzyme that mimics the body's internal engine

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The protein cytochrome c oxidase (CcO) is the ultimate enzyme responsible for all aerobic life on Earth, from bacteria to people. It is also a crucial component of the cellular machinery that generates energy in our body. With such impressive credentials, you might expect that scientists would have a clear understanding of how CcO works. But they don't, according to James P. Collman, professor emeritus of chemistry at Stanford University.

To help scientists achieve a better understanding of how CcO works, Collman and his colleagues have built a new model of the enzyme's active site-a region on the protein's surface where chemical reactions occur. According to Collman, this new model might eventually help researchers gain insights into the causes of cancer and other major diseases, and might even prove useful in the development of new forms of alternative energy. The team's findings appear in the March 16 issue of the journal *Science*.

Energy source

Many organisms, including humans, derive their energy from tiny organelles in cells known as mitochondria. Embedded in the membrane of each mitochondrion is a structure called the electron transport chain, which produces adenosine triphosphate (ATP), a molecule that is the source of the cell's energy. The transport chain is made up of a series of proteins known as electron carriers. Each carrier receives electrons from the preceding one, then transfers them down the chain. The final receptor of the electrons is a molecule of oxygen that is transformed into water and, in the process, generates energy in the form of ATP and heat.

CcO is the last electron carrier in the transport chain. It receives four electrons from the other carriers and transfers the electrons to the molecule of oxygen, converting it into two molecules of water.

"CcO has to behave perfectly," Collman said. "If it adds less than four electrons, it can produce partially reduced oxygen molecules, and these are known to be very toxic." The two most deleterious forms of reduced oxygen are superoxide and hydrogen peroxide, which have been implicated in cancer, heart failure, Alzheimer's disease and other illnesses, he added.

The good news is that CcO rarely fails, said Stanford postdoctoral fellow Neal K. Devaraj, whose doctoral dissertation was the basis of the Science paper. According to Devaraj, CcO has more than 99 percent efficiency in transforming oxygen into water.

To understand why CcO is so efficient, Collman's group, led by Stanford research associate Richard Decreau, created an artificial version of the enzyme active site using organic compounds as building materials. The imitation site, which involves an elaborate sequence of 32 chemical steps, was built from scratch and took several years to develop.

The site contains the three active centers found in the naturally occurring enzyme: an organic molecule called phenol, an iron atom and a copper atom. Working together, these three centers provide the four electrons necessary to transform oxygen into water. "How all four electrons are added to oxygen has always been mysterious," Collman said. "Very few people study it. It's quite complex, and it's been broadly ignored."

Each electron is brought to the enzyme one at a time, Collman said: "It's like a bucket brigade in a Western movie." But the electrons are consumed too fast to study individually, he noted. Therefore, the researchers had to invent a technique that supplied electrons to their enzyme model in a slow and continuous way. They solved the problem by attaching the model to a liquid crystalline film on a gold electrode, which provided a nonstop supply of electrons to the model as it transformed oxygen molecules into water-a process called steady turnover.

"The biochemists that study the enzyme typically study single turnover," Collman said. "They let the enzyme have only one oxygen molecule and watch what happens." He said that single turnover is like taking a single photograph of an event, while steady turnover is like shooting a movie.

Damaged enzymes

Once Collman's group had solved the continuous electron supply problem, the scientists systematically removed each of the three active centers-phenol, iron and copper-one at a time, as if the enzyme had been damaged and a specific active center was missing. "We found that great damage occurred, and that partially reduced oxygen species were produced in large amounts," Collman said. This finding led the researchers to conclude that all three active sites are essential for the proper functioning of the enzyme.

According to Devaraj, the new laboratory techniques developed in this experiment may have applications for research involving other enzymes. Understanding what makes CcO so efficient in reducing oxygen to water may even be useful to the study of fuel cells-very efficient power sources that convert chemical energy to electricity. "If we can develop better catalysts to do that reduction, we can get better fuel cells," Devaraj explained.

Source: Stanford University