Biological electron transfer route revealed in atomic detail by UCSD chemists

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Two chemists from the University of California, San Diego say they have caught a glimpse of an extremely rapid chemical reaction without which some of the fundamental processes of life would not occur.

According to an article published in the December 11 issue of Science, the researchers describe a molecular conduit through which electrons are shuffled very quickly (on the order of 1/50,000th of a second) between two key proteins in these reactions. Understanding how electrons are passed from protein to protein in so-called "electron transfer reactions" has been the subject of intense research for more than a decade, particularly among biochemists studying such fundamental processes as respiration, photosynthesis and drug metabolism.

As an indication of the field's importance, Rudolph A. Marcus at the California Institute of Technology was awarded the Nobel Prize in Chemistry earlier this year for developing the theory of electron transfer reactions in chemical systems.

"Our results represent a turning point in the study of electron transfer in living systems," said Huguette Pelletier, a UCSD postdoctoral research chemist and co-author of the study with Joseph Kraut, UCSD professor of chemistry. "There was a logjam in the field breaks things loose," she added.

Kraut cautioned that other work in his laboratory was needed to confirm the study's conclusions.

"We're going way out on a limb," he said. "We have this model and it is telling us something. And now we're doing the experiments to find out if we were wrong."

The centerpiece of the research described in the Science article is a small, highly mobile protein called cytochrome c, whose principal role in living organisms--from bacteria and yeast to horses and humans--is to shuttle electrons between larger, less mobile molecular partners.

Like its chemical cousin, hemoglobin (located in red blood cells), cytochrome c also contains a heme group, giving it a characteristic bright red color. However, instead of transferring oxygen, the heme in cytochrome c transfers electrons.

All the energy-generating processes of life hinge on reactions in which electrons are passed off from one molecule to another. Without cytochrome c, for example, oxygen could not be used by our bodies to burn fuel (food) and produce energy in the form of a chemical called adenosine triphosphate (ATP). During the final stage

of respiration, cytochrome c is responsible for the shuttling of electrons between two large enzymes--cytochrome reductase and cytochrome oxidase.

This reaction was first described by the late David Keilin more than a half century ago. Since then, researchers have been trying to understand how, precisely, electron transfer works.

"It's just one of those fundamental questions," said Pelletier. "We are asking how biology works at the molecular level."

Though several models were proposed for the electron transfer complex, based on experimental evidence and later computer simulations, none proved satisfactory.

"There was one camp that said that cytochrome c could bind almost anywhere and the electron would transfer," said Pelletier. "All cytochrome c needed to do was bump into a partner. The only problem with this idea is that there would be no control of the electron. In biology you need control. Specificity is very basic to all biology."

One way to resolve the question, several researchers recognized, was through a technique called x-ray crystallography. In this method, protein crystals are first grown from a highly concentrated protein solution. These crystals, when placed in an x-ray beam, diffract the x-rays in a unique way that allows X-ray crystallographers, such as Pelletier and Kraut, to deduce a high- resolution atomic picture of the protein molecule.

Although high-resolution crystal structures of cytochrome c from various species had been determined in the 1970s, a crystal of cytochrome c bound to an electron transfer partner remained elusive.

Pelletier took on this task as part of her Ph.D. dissertation research, working with cytochrome c bound to cytochrome c peroxidase--a complex that could serve as a simple model for other electron transfer complexes in the respiratory electron transport chain.

Even before she tried, some suggested the experiment couldn't be done because, they thought, the cytochrome c would not bind to one specific place, but would bind anywhere it landed.

After about three years of effort, however, Pelletier achieved what some thought was impossible. In fact, she was able to obtain crystals from two different complexes, one from yeast cytochrome c and the other from horse cytochrome c.

"This is the first time we have been able to visualize the cytochrome c molecule docking with an electron transfer partner," said Kraut. "We don't know if this is the docking required for electron transfer, although there is a lot of evidence that it probably is."

Although the static x-ray structure does not explicitly show an electron transfer reaction, the two researchers say the structure strongly suggests the pathway along which electron are transferred from one protein to another.

According to their interpretation of the model, an electron would travel along a straight pathway (about a billionth of a meter, which is quite long in the molecular world) between two hemes in each protein. This pathway is defined by a unique pattern of amino acid residues, the last of which is tryptophan 191--an amino acid which is in direct contact with the heme of cytochrome c peroxidase.

When tryptophan 191 is replaced with another amino acid, the electron transfer reaction is blocked. Kraut and Pelletier suggest this is one compelling piece of evidence supporting their proposals.

"This work may disappoint those who said that cytochrome c binds in many different places," said Pelletier. "This is a very specific pathway without which the reaction would probably not take place."

To further confirm their results, Mark Miller--another researcher in Kraut's lab--is working with other mutations of the pathway to determine if any other amino acid changes also inhibit electron transfer.

"That's the next big question," said Kraut. "We're hoping these mutations will really affect the electron transfer. If they do, then we are right. If not, then we are wrong. This comes as close to a definitive experiment as you can get in science."

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