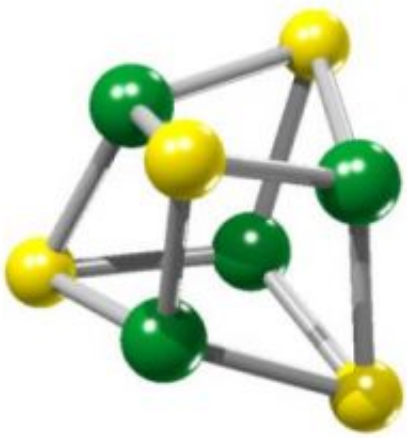
Researchers witness assembly of molecules critical to protein function

June 16 2008



Depicted is a molecular representation of a four iron- four sulfur cluster in which the sulfur is shown in yellow and iron in green. Credit: Callie Raulfs

A Virginia Tech research group lead by two biochemistry graduate students has isolated proteins responsible for the iron-sulfur cluster assembly process and witnessed the necessary protein interactions in vivo – within a cell. They have captured pathway intermediates and observed protein interactions between the two major players in iron-sulfur cluster assembly.

Iron-sulfur clusters are critical to life on earth. They are necessary for

protein function in cellular processes, such as respiration in humans and other organisms and photosynthesis by plants. "But we do not understand how Fe-S molecules are made or the specifics of how they bond," said Callie Raulfs of Christiansburg, Va. "It does not happen spontaneously. It has to be regulated."

Diseases such as Friedrich's ataxia and several types of anemia are a result of iron-sulfur cluster (ISC) assembly malfunctions.

Using genetic and biochemical techniques, Ph.D. students Raulfs and Ina P. O'Carroll, of Tirana, Albania, have isolated components of the ISC machinery in the process of making iron-sulfur clusters. "This work provides insight into the sequential steps of the iron-sulfur cluster assembly process, helping to explain how molecules of iron and sulfur are synthesized and distributed in cells," said O'Carroll.

The work, "In vivo iron-sulfur cluster formation," by Raulfs, O'Carroll, Virginia Tech post-doctoral associates Patricia C. Dos Santos of Brazil and Mihaela-Carmen Unciuleac of Romania, and Dennis R. Dean of Blacksburg, professor of biochemistry and director of the Fralin Biotechnology Center at Virginia Tech, has been published in the *Proceedings of the National Academy of Science* (*PNAS*) Online Early Edition the week of June 16-20, 2008.

Previous studies by Dean and others have demonstrated that proteins can assemble clusters from components in vitro systems – that is, outside of an organism. Ten years ago, working with nitrogen-fixation systems, Dean's lab was the first to discover ISC proteins. Now Dean's students, Raulfs and O'Carroll, are the first to witness the assembly process in vivo – within a cell.

"The cool thing is we've come up with a way to observe ISC proteins from their native host with a cluster attached," said O'Carroll. "The

system also allows us to capture different phases of the process."

The students have isolated three different intermediates of the ISC proteins involved in intercellular biosynthesis – or the cluster assembly process.

Rather than multiplying the proteins by placing them in E. coli, the Virginia Tech team used Azotobacter vinelandii, an aerobic, soil microbe that fixes nitrogen from the atmosphere, to obtain natural levels of the ISC proteins. "A vinelandii grows quickly and keeps the interior of the cell free of oxygen, which is important, since oxygen can destroy Fe-S clusters," said Raulfs, who first isolated a protein complex with a cluster attached, providing in vivo evidence that the two proteins get together and form a cluster.

"Because we are isolating proteins from the cell, we are also able to observe interactions between different Fe-S cluster asembly proteins," said O'Carroll. "We have been able to isolate a complex between the two major players in iron-sulfur assembly, the cluster assembly scaffold (IscU) and the sulfur-delivery protein (IscS)."

The methodology is to add a histidine amino acid tag to the ISC proteins "so we can fish the proteins out of the cell," said O'Carroll.

"Because we are fishing the cluster-containing protein out of the cell that has all of the other assembly proteins present at physiological levels, we are able to observe what else comes with the protein. What was really exciting in this case was that we saw large amounts of one of the other iron sulfur cluster assembly protein, IscS." said O'Carroll.

The work marks the first time researchers have been able to observe ISC proteins from the balanced environment of the native cell.

Next, they plan to determine the role of the individual genes in the set that produces ISC proteins in order to determine the effect of each gene on the assembly process. "The goal is to determine the events and the order in the ISC assembly process so we can figure out how cells make clusters and deliver them to specific target proteins," said O'Carroll.

The researchers are now developing a system that others can use to study proteins.

Source: Virginia Tech