Renewable resource: To produce vital lipoic acid, sulfur is used, then replenished

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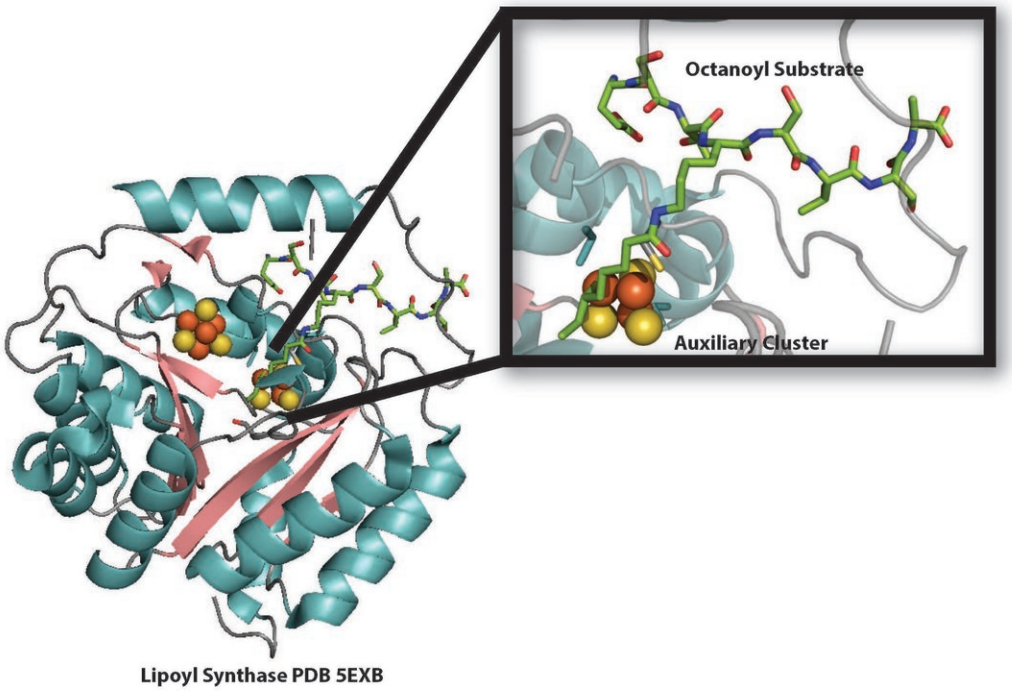

Model of the crystal structure of the lipoyl synthase enzyme (LipA) from the bacteria Mycobacterium tuberculosis revealing the destruction of one of its iron-sulfur clusters (orange and yellow balls) to use as a sulfur source for the production of lipoic acid. New research demonstrates that the iron-sulfur cluster that is destroyed during the production of lipoic acid is replaced by an iron-sulfur carrier protein, NfuA, so that LipA can continue to produce lipoic acid. Credit: Booker laboratory, Penn State University

New research shows how a protein is consumed and then reconstituted during the production of lipoic acid, a compound required by our bodies to convert energy from food into a form that can be used by our cells. The lipoyl synthase enzyme (LipA) removes two hydrogen atoms from an inert carbon chain and replaces them with sulfur atoms from one of its own iron-sulfur clusters to create lipoic acid, rendering itself inactive in the process. The new research from Penn State University, which will be published in the journal *Science* on October 20, 2017, shows that another protein, an iron-sulfur cluster carrier called NfuA, replaces the destroyed iron-sulfur cluster in LipA, allowing it to continue producing lipoic acid. The results also could help scientists to understand why humans with defects in the iron-sulfur carrier gene—a fatal condition—have deficiencies of lipoic acid.

"LipA cannibalizes itself to provide the sulfur atoms needed for the production of lipoic acid," said Squire Booker, professor of chemistry and of biochemistry and molecular biology at Penn State University, an investigator of the Howard Hughes Medical Institute, and the corresponding author of the research paper. "When we demonstrated this in 2011, it was perplexing because if LipA is destroyed, how could the cell make enough lipoic acid?"

LipA is a member of the radical SAM (S-adenosylmethionine) family of enzymes. Like most radical SAM enzymes, it contains a cluster of four iron and four sulfur atoms, which it uses to convert SAM into a high energy radical. In turn, that radical can remove hydrogen atoms from other molecules, a step required to activate many important cellular metabolic reactions. The hydrogen atoms are replaced with sulfur to complete the process.

Where the sulfur atoms that LipA uses to produce lipoic acid come from and how they are attached have been major questions in the field. How other enzymes attach oxygen atoms to inert carbon centers is fairly well

understood. In those instances, oxygen, which is ubiquitously available in the atmosphere, is used to create high-energy radicals and is also the source of the appended oxygen atom. Sulfur, on the other hand, is not similarly available, but unlike most other radical SAM enzymes, LipA has an additional iron-sulfur cluster.

"We knew from earlier work that LipA used its second iron-sulfur cluster as the source of sulfur atoms to create lipoic acid," said Erin L. McCarthy, a graduate student in Booker's laboratory and the first author of the paper. "But this created a problem. If LipA stole sulfur atoms from its own iron-sulfur cluster, the enzyme would be destroyed and therefore could not create any more lipoic acid. When we learned that humans with defective NFU1 genes, the human equivalent of the bacterial NfuA gene used in our experiments, had deficiencies in lipoic acid, we thought that this iron-sulfur carrier could be replacing the consumed iron-sulfur cluster in LipA, allowing it to continue making lipoic acid."

To test this hypothesis, the researchers performed two key experiments. First, they tested whether LipA and NfuA associate with each other by assessing how fast the molecules migrate through a gel by a technique called gel-filtration chromatography. In this technique, large molecules migrate faster than smaller molecules. When LipA and NfuA were combined and then analyzed by this technique, they migrated faster than either molecule does on its own, suggesting that the two molecules were bound together to form a larger, faster migrating molecule. The researchers then created a version of NfuA that contained a slightly different form of sulfur (34S rather than 32S) atoms, a form that they could trace if it was incorporated into LipA and then into lipoic acid. In this second experiment, the researchers showed that after the 32S originally present in LipA was consumed in the chemical reaction to produce lipoic acid, lipoic acid was produced containing 34S, which could only have come from their engineered NfuA.

"We've been interested for quite some time in both the process that adds sulfur to an inert carbon compound to make lipoic acid and the source of the added sulfur," said Booker. "Lipoic acid is a vital component of the basic metabolic processes that keep our cells alive. Understanding the reaction that creates it not only allows us to understand this process better, it also gives us insight into human diseases like the one caused by mutations in NFU1 that result in lipoic acid deficiency and death."

More information: "Destruction and reformation of an iron-sulfur cluster during catalysis by lipoyl synthase" *Science* (2017). science.sciencemag.org/cgi/doi … 1126/science.aan4574

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