Scientists find key to vitamin A metabolism

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Retinol or Vitamin A 3D space model (balls model). Credit: YassineMrabet, Wikipedia.

Researchers at Case Western Reserve University School of Medicine have uncovered the mechanism that enables the enzyme Lecithin: retinol acyltransferase (LRAT) to store vitamin A—a process that is indispensable for vision.

"Without this information, our knowledge was inadequate to understand the molecular mechanisms of blindness caused by mutations in the enzyme," said Marcin Golczak, assistant professor of pharmacology at Case Western Reserve and an author of the study.

The researchers hope the new information will be used to design small molecule therapies for degenerative eye diseases. The same enzymatic activity of LRAT that allows specific cells to absorb vitamin A can be used to transport small molecule drugs to the eye. These drugs would accumulate in eye tissue, lowering the effective dose and reducing risk of systemic side effects.

Their work is published in the journal *Nature Chemical Biology*.

Golczak, medical student Avery E. Sears, pharmacology instructor Philip D. Kiser and pharmacology chair Krzysztof Palczewski compared the function of LRAT and closely related enzymes that belong to N1pC/P60 family. They found that small variations in the protein sequences determine the substrate specificity—the substances on which the enzymes act—and thus govern physiological functions of these enzymes.

LRAT regulates cellular uptake of vitamin A by helping convert it to a usable form called retinyl ester. Retinyl ester is essential for our eyes to function. Consequently, lack of LRAT leads to vitamin A deficiency and blindness.

Unlike LRAT, a close relative, HRAS-like tumor suppressor 3, referred to as HRASLS3, does not process vitamin A, but is involved in regulation of triglycerides breakdown in white fat cells. Triglycerides provide an energy source for body tissues. But excess accumulation leads to obesity and related metabolic syndrome, increasing the risk of heart disease, diabetes and other health problems.

Mice lacking HRASLS3 gained no weight when fed high-calorie diets—even mice that were genetically engineered to be obese and lacking leptin, the hormone that signals mammals when they've eaten enough food.

The functions of these enzymes were known, but until now, there has been little understood about what enables the close relatives to go about their different jobs. The researchers looked at how LRAT is different from the rest of its protein family.

"Evolution of enzymatic activities via gene duplication, mutation and selection has led to the present diversity of metabolic capabilities," Golczak said. "Our studies explain what modification in the cellular enzymatic machinery enables vertebrates to efficiently take up and store excess vitamin A."

Overall, the two proteins have a common molecular structure. But, in the catalytic domain, LRAT has an insertion of 11 amino acids that HRASLS lacks, followed by a 19 amino acid stretch conserved in LRAT, but unseen in its HRASLS relatives, the researchers found.

To test the affects of the differences, the researchers created chimeric proteins in which they replaced the 30-amino-acid sequence in HRASLS3 and its two closest relatives, HRASLS2 and HRASLS4, with the sequence from LRAT.

Unmodified HRASLS proteins failed to catalyze the conversion of vitamin A into retinyl ester. But the modified HRASLS proteins robustly produced retinyl ester.

To understand the mechanical changes that resulted from the sequence replacement, the team determined the crystal structure of the HRASLS3/LRAT chimeric enzyme at 2.2 angstroms.

They found the replacement led to major structural rearrangements, including interactions between two protein molecules and domain swapping between neighboring subunits.

The rearrangements foster the new enzymatic activity by altering the active site architecture, protein/lipid membrane interactions, and promoting binding to a different substrate—in this case, to vitamin A.

More information: *Nature Chemical Biology*, www.nature.com/nchembio/journa … l/nchembio.1687.html

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