Understanding enzyme cascades key to understanding metabolism

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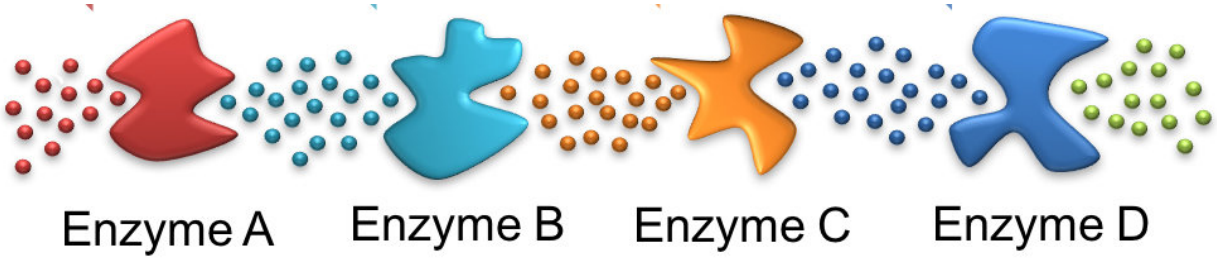

Like ants, one enzyme follows the trail left behind by the previous one. In this case, the initial substrate is acted upon by enzyme A, leaving a substrate suitable for enzyme B and on down the line. Credit: Ayusman Sen, Penn State

A spoonful of sugar may make the medicine go down, but understanding what happens to that sugar in the cell is far more complicated than simple digestion, according to researchers. For sugars to metabolize and provide energy to the cells, a series of enzymes - biological catalysts—must each, in turn, break down a reactant. In this case, the researchers used glucose, the sugar found in corn syrup and one of the two sugars that result when table sugar—sucrose—is broken down in the body.

In this cascade, the first enzyme acts on the glucose supplied to the cell and the subsequent enzymes work on successive products. In the process, two adenosine triphosphate molecules—ATP—are consumed but four

are produced. The hydrolysis of ATP powers many cellular processes to maintain the cell's viability. Similar enzyme cascades are responsible for many metabolic processes in the body.

Enzymes that participate in such reaction pathways have in some cases been shown to form intracellular, reversible complexes termed metabolons by Paul Srere (deceased), University of Texas Southwestern Medical School. Having the enzymes in proximity to one another facilitates the series of reactions they catalyze. One such example is the purinosome discovered in Evan Pugh University Professor and Eberly Chair in Chemistry Stephan J. Benkovic's Lab at Penn State that consists of six enzymes involved in the biosynthesis of purines.

The researchers asked whether one of the factors contributing to metabolon formation could be a gradient of chemicals generated by the participating enzymes. They report their results in today's (Dec. 18) issue of *Nature Chemistry*.

"We discovered some time ago that simple catalyst molecules such as enzymes will also chemotax up the gradient of a reactant," said Ayusman Sen, distinguished professor of chemistry, Penn State. "They move toward higher and higher concentrations of reactant."

The movement is termed chemotaxis, where individual molecules migrate along a concentration gradient of other molecules.

"All living things chemotax," said Sen. "If you are hungry and suddenly smell French fries, you will try to walk toward the fries. If the smell decreases, you will randomly turn to try to find the higher concentration of French-fry odor molecules until you are at the French-fry counter."

In their study, the researchers used only the first four enzymes of the glycolytic pathway—hexokinase, phosphoglucose isomerase,

phosphofructokinase and aldolase. These four steps actually consume ATP. To study the movement of the enzymes, the researchers used fluorescent tagging of hexokinase and aldolase, the first and fourth enzymes in the pathway. Each was tagged with a different fluorescent dye so the movement of both enzymes could be followed.

They looked at three cases—the normal reaction where hexokinase phosphorylates glucose; the reaction of hexokinase with mannose, a sugar that binds more strongly but has a slower reaction rate; and finally with L-glucose, a form of glucose that is not used by hexokinase. The phosphorylation requires ATP. In the presence of phosphoglucose isomerase—the second enzyme—and phosphofructokinase—the third enzyme—the reactant for aldolase is produced.

The researchers observed that the aldolase moves towards the hexokinase in their flow experiment, revealing that aldolase was chemotaxing up the reactant gradient produced by the functioning of the first three enzymes in the pathway. The chemotaxis was greatest with D-glucose, less with mannose and not observed with L-glucose.

Theoretical modeling of the enzyme movement qualitatively predicted the extent of enzyme movement.

The researchers also looked at whether chemotaxis of enzymes would occur in a model of the exceptionally crowded intracellular environment. They added a large molecular weight substance to simulate such crowding. Chemotaxis still occurred, but at a slower rate.

"Chemotaxis along a chemical gradient could be a factor in assembly of enzyme clusters such as metabolons," said Benkovic. "Other factors, such as noncovalent interactions would still be expected to contribute."

The resolution of the research instrument, however, was insufficient to

demonstrate in this case that the four enzymes were assembling into a metabolon. The researchers observed the formation of large aggregates of enzymes, but could not demonstrate they were functioning metabolons.

More information: Substrate-driven chemotactic assembly in an enzyme cascade, *Nature Chemistry* (2017). nature.com/articles/doi:10.1038/nchem.2905

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