CATCHING AN ENZYME IN THE ACT OF CATALYSIS

Previously invisible enzyme structure revealed with protein engineering and NMR.

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PHOTO by **JOHAN GUNSÉUS**

ENZYMES ARE DYNAMIC proteins. They shift, bend and flex as they catalyze a reaction. X-ray crystallography shows their stable forms but not their fleeting, intermediate conformations. To design enzymes for applications such as retrieving high-value molecules from cellulose, we need to understand their transitional states.

An enzyme has now been captured, mid-catalysis, in an unstable, previously unseen form, by an Umeå University group.

Dr. Magnus Wolf-Watz, senior author on the report in Nature Communications, says, "Other studies suggested that this transient, high-energy state should exist, but this is the first direct handle on it. We can not only observe the structure but quantify the enzyme's properties and see why they are important for catalysis."

MULTIPLE METHODS

The breakthrough required multiple methods. The research group used targeted protein engineering to make a version of the model enzyme adenylate kinase (AdK) that was slightly slower than normal. They used standard biochemical assays and x-ray crystallography to confirm that the engineered enzyme had the same catalytic traits and overall structure as the nonengineered version. Then, they used NMR (nuclear magnetic resonance spectroscopy) to visualize the engineered AdK as it went through catalysis.

"NMR was initially promoted in protein chemistry as a way to determine structure," says Wolf-Watz, "but its biggest contribution has been in measuring protein dynamics."

ADDING DYNAMICS TO DE NOVO DESIGN

Knowledge of enzyme dynamics, says Wolf-Watz, boosts our capacity for de novo enzyme design, creating protein catalysts from scratch. The Umeå group, for example, is interested in designing enzymes to break down cellulose into more valuable molecules. It's still basic research, says Wolf-Watz, but to design enzymes we must understand their dynamics. Right now, computer modeling can create structures for new proteins but lacks the dimension of dynamics.

"Our research," says Wolf-Watz, "is aimed at finding general rules about how to modulate large-scale conformational changes that enzymes go through in catalysis. We'd like to contribute to building dynamics into the generation of new enzymes."

THE GROUP CONTINUES to work with AdK as a model for how protein conformational changes lead to function and interactions. They are also studying how enzymes work in living organisms. The researchers are doing in vivo studies with AdK in yeast to understand the amount of enzyme a cell needs to grow normally or under stress and why enzymes have certain catalytic properties in living systems. Wolf-Watz sees basic and applied directions for research on enzyme dynamics and he radiates enthusiasm about the work. "Right now," he says, "NMR is really taking off for protein studies." NLS

Reference: Kovermann M, Ådén J, Grundström C, Sauer-Eriksson AE, Sauer UH, Wolf-Watz M. Structural basis for catalytically restrictive dynamics of a high-energy enzyme state. Nature Communications. 2015; 6:7644.

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