Scientists find E. coli enzyme must move to function

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Slight oscillations lasting just milliseconds have a huge impact on an enzyme's function, according to a new study by Scripps Research Institute scientists. Blocking these movements, without changing the enzyme's overall structure or any of its other properties, renders the enzyme defective in carrying out chemical reactions.

The study, published in April 8, 2011 issue of the journal *Science*, adds to a growing body of evidence pointing to the importance of movement in the ability of enzymes and other types of proteins to do their job. The findings may also help scientists design more specific and effective drugs targeting enzymes.

"Ever since the first X-ray structures of proteins emerged, scientists have been talking about proteins as though their structures were fixed in space," said Peter Wright, chair of the Department of Molecular Biology and member of the Skaggs Institute for Chemical Biology at Scripps Research who was senior author of the study, "but that is not how proteins work. They are like the machines we build. They have moving parts and they need motion to work."

A Model Enzyme

The new study examined the enzyme dihydrofolate reductase (DHFR) from the common bacterium *Escherichia coli*, which the Wright group has been using as a model for understanding how enzymes catalyze

(cause or accelerate) chemical reactions. Most strains of *E. coli* are harmless, but some can cause serious food poisoning.

Bacterial cells cannot live without DHFR, thus this enzyme is the target for many antibiotics. Human cells, and in particular rapidly dividing cells, also use DHFR; drugs that target human DHFR, such as methotrexate, are often used in cancer chemotherapy.

DHFR spurs the conversion of a compound called dihydrofolate (DHF) to a different form, tetrahydrofolate (THF), which is needed by cells for synthesis of DNA. In its chemical reaction, DHFR uses a helper or co-factor, called NADPH. It catalyzes the transfer of a hydride (a negative hydrogen ion) from NADPH to DHF to produce THF. Previous studies by Wright and others have shown that the loops surrounding the active site are flexible, and that one of the loops in particular, called the Met20 loop can adopt two different conformations during the catalytic cycle.

Until now, however, the significance of these motions remained obscure.

Linking Motion to Function

Wright, graduate student Gira Bhabha, and colleagues from both Scripps Research and Pennsylvania State University decided to investigate.

For the new study, the scientists turned to an imaging technique known as nuclear magnetic resonance (NMR) spectroscopy, in combination with X-ray crystallography. Unlike X-ray crystallography, a technique used to determine the structure of proteins in crystals, recently developed NMR methods allow scientists to visualize the motions of proteins in solution. The technique can capture protein motions "in a time scale that is relevant to biology, from microseconds to milliseconds to seconds," said Wright.

To determine the importance of the oscillations, the team set out to make a mutation in the DHFR enzyme that prevented the flexible Met20 loop from moving. To know which amino acids to change, the scientists compared the bacterial DHFR protein sequence to that of the human enzyme, since in the human enzyme the Met20 loop is more rigid.

Using this approach, the scientists successfully produced a rigidified mutatant *E. coli* DHFR. When the scientists examined it using X-ray crystallography, they could see the mutant enzyme's structure was almost identical to the wild type enzyme. However, NMR analysis revealed that the Met20 loop and other parts of the active site were no longer flexible in the mutant.

Significantly, the mutated *E. coli* enzyme transferred hydride at a rate that was 16 fold slower than that of the wild type enzyme—a substantial loss in enzyme function.

"We demonstrated that locking down the motion in the active site prevents catalysis," said Wright.

While previous work had indicated that enzymes can exist in different shapes and forms and that changes in enzyme shape enable enzymes to bind to their substrates and co-factors or release the products, "this is the first demonstration that motions play a role in the actual chemistry of a reaction," said Wright.

Clamping Down on the Active Site

The scientists reason that, when the *E. coli* DHFR carries out its chemical reaction, motions in the active site assist in pushing NADPH and DHF closer to one another. This proximity makes the transfer of the hydride from NAPDH to DHF more efficient. If the active site can't move, the molecules are not sufficiently close to one another for the

chemical reaction to occur. "We think that the mutations prevent the enzyme from clamping down on the hydride donor and acceptor, so they can no longer get as close to each other as is necessary for efficient catalysis," explained Bhabha.

Taking motion into account when designing drugs to either inhibit or increase enzyme function could result in more effective or more specific drugs. For example, because the motions in the bacterial DHFR differ from those in the human enzyme, this difference might be exploited to design drugs that are specific for the bacterial enzyme. "It might help reduce the serious side effects of drugs that target DHFR," said Wright.

"The idea is to harness these motions in drug design," added Bhabha. "It's a difficult and challenging problem, but it could have huge impact."

More information: "A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis," *Science*.

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