Researchers crack code of 3-D structure in key metabolic protein

March 10 2008

Using X-ray crystallography, researchers at the University of Pittsburgh School of Medicine led by structural biologist Joanne I. Yeh, Ph.D., have become the first to decipher the three-dimensional structure of a membrane-bound enzyme that plays a crucial role in glycerol metabolism – a discovery that could lead to important advances against obesity, diabetes and a potential host of other diseases. Their findings are reported in the March 4 issue of the *Proceedings of the National Academy of Sciences*.

The sugar-alcohol glycerol is an essential source of energy that is required to help drive cellular respiration. In addition to powering some of the most central reactions of the body, glycerol also provides key precursors needed to regulate fatty acid and sugar metabolism. Figuring out the complex ways that cells break down or produce glycerol and use this vital chemical could be critical to combating obesity, diabetes and other chronic disorders. Recent findings also have linked glycerol metabolism to cellular processes related to aging, infectivity in certain organisms such as Mycobacterium tuberculosis, and in other energy-related illnesses.

“Everybody wants a golden bullet for obesity, and certainly we need better ways of controlling diabetes,” said Dr. Yeh, the study’s senior author and associate professor of structural biology at Pitt. “I think that glycerol metabolism will be on the forefront of developing treatments for these diseases, and so many others, since it is a pivotal yet underappreciated link among some very important metabolic pathways.”

The protein structure Dr. Yeh's team solved is a large enzyme called Sn-glycerol-3-phosphate dehydrogenase – known simply as GlpD – found in abundance in the cell membranes of almost all organisms, including humans. GlpD is a monotopic membrane protein, which means that although it is embedded partially into the cell membrane, the protein does not span the entire membrane to the interior of the cell. As a result, it is technically challenging to produce enough highly purified and active protein to obtain clear, relevant information about the enzyme's atomic structure. This study marks the highest resolution structure of a monotopic membrane protein that scientists have solved to date, and is one of only a handful of structures of this important class of membrane proteins that have been determined.

"These findings and data help to fill an important scientific and technical gap in the structural field and present new information and ideas about how the enzyme works and the importance of the cell membrane in stabilizing the enzyme and in processes related to energy production," said Dr. Yeh, who published the paper along with postdoctoral research associate Unmesh N. Chinte, Ph.D., and research assistant professor, Shoucheng Du, Ph.D., both in Pitt's Department of Structural Biology.

Studying the proteins and enzymes involved in oxidative and glycerol metabolism, as well as characterizing their structures, functions and regulatory relationships, has been a major research interest of Dr. Yeh's lab. It took Dr. Yeh and her colleagues only three months – an unusually short time – to decipher the set of 3-D structures of GlpD isolated from E. coli bacteria, thanks to other methodologies they developed in earlier studies.

Rather than make conclusions based on a single structure, the team additionally determined the structures of GlpD bound with its metabolic product and several substrate analogues to evaluate the enzyme in its native and combined forms. By careful unraveling of this collection of

structures, researchers could gain a more complete understanding of how the enzyme functions, details about how GlpD interacts with the membrane, works to catalyze the enzymatic reaction, and links to cellular-energy production.

As part of these challenging studies, the Pitt researchers used novel peptide-based detergents called “peptergents” that they developed in their lab to carefully separate GlpD from the cell membrane and keep it in an active form to ensure that their studies revealed a physiologically relevant enzyme structure. The team then used detergents to crystallize the enzyme and screened the protein crystals in Pitt’s new state-of-the-art X-ray crystallography facility, directed by Dr. Yeh.

Next, they applied beams of high intensity parallel X-rays to the protein crystals in order to collect the diffraction data necessary to determine the protein’s atomic configuration. These experiments were performed using cyclic particle accelerators at the Argonne National Laboratory in Illinois and the Paul Scherrer Institute in Switzerland. Called synchrotrons, these accelerators are the size of a football field and produce X-ray beams millions of times more intense than those generated by conventional X-ray machines. Highly advanced computational techniques were then used to analyze the diffraction data and to uncover, through complex mathematical approaches, the atomic matter in the crystals responsible for the diffraction. Ultimately, the unique 3-D topology of GlpD was deciphered, atom by atom.

The main role of GlpD in the cell is to remove hydrogen from a form of glycerol called glycerol-3-phosphate (G3P) to generate dihydroxyacetone phosphate (DHAP), a biochemical compound vital to the process of metabolizing the sugar-alcohol. In the process, electrons are produced and shuttled to a molecule called ubiquinone that works to power cellular respiration. Based on the structural information acquired in their study, Dr. Yeh’s team proposed mechanisms by which the enzyme carries out

this fundamental metabolic reaction.

Their data revealed that GlpD is a dimer, or a protein with two subunits, that is embedded into and interacts substantially with the lipids that make up the cell membrane. This interaction with the membrane is required to keep the enzyme energetically and functionally stable so that it doesn't collapse on itself, the PNAS study reports.

Dr. Yeh's team also found that the enzyme is made up of two major domains: a soluble extracellular "cap" and a FAD-binding region, whose base is rooted in the membrane. The location of the enzyme's active site – where the chemical reaction actually occurs – is at this FAD-binding region. G3P fastens tightly here, much like a key fitting into a lock, and is then transformed into DHAP. The researchers also proposed a docking site for where ubiquinone binds to the enzyme to accept electrons produced in the reaction. Eventually, ubiquinone feeds these electrons into respiration to produce the crucial energy to fuel cellular processes.

In addition, Dr. Yeh's team discovered a never-before-seen type of protein fold consisting of about 100 amino acids in the "cap" domain of GlpD. They also identified areas where other proteins might bind to regulate the enzyme's activity and transmit chemical signals.

With the GlpD structure in hand, Dr. Yeh's team is already examining how mutating, or changing, certain amino acids in the enzyme affects its function and fold. These studies target the roles that these specific amino acids play in enzymatic function and regulation of activity. These questions are important because glycerol metabolism is a key link between sugar and fatty acid metabolism. The Pitt group also has determined the atomic resolution structures of other enzymes involved in mediating glycerol and oxidative metabolism. In all, these structural results provide some of the first three-dimensional views of these highly

important proteins and enzymes.

Source: University of Pittsburgh