Crystal structure for "biological transistor" revealed by team of scientists at UCSD and SDSC

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CRYSTAL STRUCTURE FOR "BIOLOGICAL TRANSISTOR" REVEALED

The structure of an essential regulatory protein, considered by some to be a type of biological transistor, has been revealed in atomic detail by a team of scientists at the University of California, San Diego and the San Diego Supercomputer Center.

The finding offers three-dimensional insights into an essential family of enzymes. These enzymes are turned on and off in the body to regulate everything from the release of glucose for quick energy, to gene expression and cell motility, to the triggering of brain chemicals for thought and memory.

It also opens a potential new avenue for drug development, and provides researchers with a template for the rational design of inhibitors to block the activity of regulatory mechanisms gone awry, as in cancer.

In two articles appearing in today's issue of Science, the journal of the American Association for the Advancement of Science, a team of UCSD researchers describe the first atomic structure of one member of a family of proteins, known as protein kinases, and the structure of a naturally occurring molecule that inhibits its activity.

The UCSD scientists participating in the study include: Daniel R. Knighton and Jianhua Zheng, graduate students in chemistry; Susan S. Taylor, professor of chemistry; Lynn F. TenEyck, a computational crystallographer with SDSC and codirector with Taylor of the Computational Center for Macromolecular Structure; Victor A. Ashford, a biologist; Nguyenhuu Xuong, with the departments of biology, chemistry and physics. Janusz M. Sowadski, a crystallographer with the departments of medicine and biology, directed the project. The work constitued Knighton's doctoral dissertation and won this year's UCSD Martin Kamen Prize for the best dissertation in the areas of biology and chemistry.

Protein kinases, like transistors used in electrical circuits, can serve as simple on/off switches or they can amplify certain signals to increase or decrease specific activities that take place in the cell. The kinase family includes the insulin receptor, the epidermal growth factor (EGF) receptor and many other important signal-transmitting proteins. A small but vitally important subgroup are produced by oncogenes (cancer genes) and are thus implicated in tumor initiation and growth. All of these kinases retain a homologous catalytic core that has been conserved throughout evolution.

The exact sequences of amino acids (basic protein units) for the more than 200 kinases are known, but until now, no one has known just how they fold up into active proteins. It is the three-dimensional structure that is essential for understanding how they act as enzymes to catalyze reactions in the cell.

In the first Science article, the researchers describe the structure of cAMP-dependent-protein kinase, so-called because it is activated by a chemical messenger known as cyclic-adenosine monophosphate (cAMP). This protein is one of the first members of the kinase family to be discovered, and it also is one of the best understood chemically.

Here's how this one works:

Normally, the protein kinase lies dormant in the body until it is called upon by cAMP. In this inactive state, the kinase exists as a complex composed of four pieces -- two regulatory subunits and two catalytic subunits.

This period of dormancy is disrupted only when the specific chemical messenger approaches, CAMP, approaches. The production of cAMP inside the cell is triggered by extra-cellular hormones such as adrenalin that initiates a cascade of reactions. By binding to the regulatory subunit, CAMP sets in motion of chain of events that breaks up the kinase into three parts -- two free catalytic subunits and one piece containing the regulatory subunits.

On their own, the catalytic subunits are free to bind to and modify specific proteins through a chemical process called phosphorylation. Here, a phosphate is transferred from adenosine triphosphate (ATP) onto an amino acid group belonging to another protein. During the process, both the ATP and the other protein are temporarily bound to the kinase. These substrate proteins are typically key regulatory enzymes that catalyze the rate-limiting steps in essential pathways. Addition of a phosphate can either activate or inactivate the enzyme.

Once activated, the protein kinases can perform a variety of functions, depending on where they are located in the body.

"Every cell has many protein kinases, including cAMP-dependent protein kinase," said Taylor. "They have different effects depending on the cell type. In the liver, cAMP causes glucose to be released into the blood; in the fat cell, triglycerides are released for energy; in the muscle, CAMP mobilizes stored glycogen for energy; in the brain, it may potentiate memory. You have a tremendous diversity."

Certain kinases also can set in motion a cascade of chemical events that result in tumor formation.

"Being able to design specific inhibitors that would target these oncogenic (cancer-forming) kinases is a goal that has been sought after for a long time," said Taylor.

The researchers took the first step toward such a goal by finding the three-dimensional structure of cAMP-dependent protein kinase. The work brought together a team of experts that included crystallographers, protein chemists, biochemists, and molecular biologists.

It also relied heavily on the CRAY Y-MP8/864 supercomputer at SDSC, the graphics workstations and expertise available at the Advanced Scientific Visualization Laboratory (VisLab) at SDSC, and the National Research Resource of X-ray Crystallography at UCSD for data collection, operated by Nguyen-huu Xuong.

In its final form, the image of the catalytic subunit featured two lobes, with a cleft between them. This cleft provides the docking sites for ATP and the proteins which must come together at the active site to allow for the transfer of the phosphate.

"The ATP binding site is on the smaller lobe and the protein that is acted upon -- the substrate -- is bound at sites in the cleft and on the surface of the larger lobe," said Taylor.

In the second Science article, the researchers described the structure of a natural inhibitor bound to the catalytic subunit. It is the structure of this peptide and its interaction with the protein that provides the real basis for the rational design of kinase-specific inhibitors.

Essential chemical and structural features of the catalytic subunit were found to be remarkably similar to previously studied kinases found in eukaryotic cells, including simple organisms, plants and other animals. To biologists, this means the structure has evolved from a common origin indicating that the general folding has been conserved as well as the mechanism for phosphate transfer.

"You can use this simple kinase as a kind of a template for much more complex enzymes," said Taylor.

Among other things, the revelation of the kinase structure is expected to prompt new studies to map members of the family associated with with tumor formation.

Once these structures have been mapped, researchers can begin the painstaking task of trying to design specific inhibitor molecules.

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