Biochemists discover how a ‘molecular slingshot’ disrupts key proteins

January 26 2011

(PhysOrg.com) -- An important basic science discovery reported by University of Massachusetts Amherst biochemists this week describes how certain proteins use an unusual, spring-loaded loop mechanism to cripple their target enzymes as part of normal function. But the work also reveals how a misfire, with no target present, causes the proteins to polymerize and form damaging clumps, which lead to misfolding diseases such as familial emphysema and liver cirrhosis.

"It’s a remarkable mechanism," says Lila Gierasch, professor of biochemistry and molecular biology at UMass Amherst. "One just has to sit back and once again reflect that nature is amazing." Gierasch and Beena Krishnan, a senior postdoctoral fellow, show how members of a large family of serine protease inhibitors, or serpins, dangle a spring-loaded loop structure to deactivate enzymes involved in processes as wide-ranging as blood coagulation and lung function. Once touched, the serpin acts like a catapult, instantly flinging the enzyme like an arrow deep into a slatted structure in the serpin. There, it becomes tangled in a chemical dead-end.

The researchers found that this mechanism comes with a risk, however. When not acting on their targets, serpins are prone to form cell-damaging polymer clumps. The findings appear in the current issue of *Nature Structural and Molecular Biology*.

Serpins make up the largest family of proteins that inhibit proteases, the proteins that control many step-by-step or cascade reactions in cells. For

example, people deficient in an elastase-inhibiting serpin called antitrypsin in the lung are genetically prone to emphysema. Other serpins control blood clotting, while a deficiency in still others causes an inherited form of cirrhosis of the liver.

This week's publication is the first to describe in detail how the serpin's structure is triggered to change. Protein-folding researchers like Gierasch and Krishnan are fascinated with this molecular catapult because it's so rare. It's one of very few proteins that can exist in two distinct and meta-stable states, one of which must be triggered to snap or fold into the other. The hemagglutinin molecule, critical to infection by the influenza virus, is another. The consequence of the serpins' unusual mechanism is that a certain number will accidentally fire with no target in place, aggregate into clumps and damage normal cells.

With the catapult mechanism known, Gierasch says, medical researchers may be able to use the new information to design therapies for treating protein-misfolding diseases and to devise genetic tests for early identification of family risk factors. "This is a class of molecule that works very well most of the time, but occasionally it goes wrong and causes some very difficult-to-treat diseases," says Gierasch.

To unravel secrets of the serpin's baited-loop and catapult action, Krishnan constructed several variants of the serpin alpha-one antitrypsin and marked parts of it with cysteine probes. These would not react in the "trigger cocked" state but when chemically tickled, some would and some would not be reactive, thus showing her where the structure had changed.

Krishnan selected probe positions "to interrogate different regular structural elements of the protein" and reveal how the catapult-and-arrow penetrates and disrupts the serpin's structure of parallel slats known as the "sheet."

As Gierasch explains, "We wanted to know how the arrow gets shot in and how it opens the sheet or persuades it to breathe open. Beena placed the cysteine probes throughout the sheet so she could track them and watch the partial unfolding of events. It's quite a clever way of getting a picture of a molecular state that's not normally observable. What emerged is that part of the molecule changes shape while much of it remains the same."

Genetic mapping clearly shows that disease-causing mutations in alpha-one antitrypsin affect vulnerable regions of the molecule where structural changes are known to occur, she adds.

Gierasch's work is supported by a Pioneer Award from the National Institutes of Health, designed to support scientists of exceptional creativity who propose pioneering and possibly transforming approaches to major challenges in biomedical research. She says the experiments not only advance basic knowledge about molecular processes, they afford scientists a peak into evolutionary forces and how nature balances risks and benefits.

"In the evolutionary selection for this serpin machine, nature accepts the risk of disease," she says. "By leaving these trigger-cocked molecules lying around in the cell in a vulnerable state, they can spring open on their own and cause damage through polymerization. But apparently the advantage of having these powerful serine protease inhibitors around overshadows the risk."

Provided by University of Massachusetts Amherst