Enzymes implicated in disease processes attack one another instead of harming body proteins

August 13 2012

Research led by Manu Platt, an assistant professor in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, has shown for the first time that members of the cathepsin family of proteases can attack one another -- instead of the protein substrates they normally degrade. Credit: Georgia Tech Photo: Gary Meek

Researchers for the first time have shown that members of a family of enzymes known as cathepsins – which are implicated in many disease processes – may attack one another instead of the bodily proteins they

normally degrade. Dubbed "cathepsin cannibalism," the phenomenon may help explain problems with drugs that have been developed to inhibit the effects of these powerful proteases.

Cathepsins are involved in disease processes as varied as cancer metastasis, atherosclerosis, cardiovascular disease, osteoporosis and arthritis. Because cathepsins have harmful effects on critical proteins such as collagen and elastin, pharmaceutical companies have been developing drugs to inhibit activity of the enzymes, but so far these compounds have had too many side effects to be useful and have failed clinical trials.

Using a combination of modeling and experiments, researchers from the Georgia Institute of Technology and Emory University have shown that one type of cathepsin preferentially attacks another, reducing the enzyme's degradation of collagen. The work could affect not only the development of drugs to inhibit cathepsin activity, but could also lead to a better understanding of how the enzymes work together.

"These findings provide a new way of thinking about how these proteases are working with and against each other to remodel tissue – or fight against each other," said Manu Platt, an assistant professor in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University. "There has been an assumption that these cathepsins have been inert in relationship to one another, when in actuality they have been attacking one another. We think this may have broader implications for other classes of proteases."

The research was supported by the National Institutes of Health, the National Science Foundation and the Georgia Cancer Coalition. Details of the study were reported August 10 in the *Journal of Biological Chemistry*.

Platt and student Zachary Barry made their discovery accidentally while investigating the effects of cathepsin K and cathepsin S – two of the 11-member cathepsin family. Cathepsin K degrades both collagen and elastin, and is one of the most powerful proteases. Cathepsin S degrades elastin, and does not strongly attack collagen.

When the researchers combined the two cathepsins and allowed them to attack samples of elastin, they expected to see increased degradation of the protein. What they saw, however, was not much more damage than cathepsin K did by itself.

Platt at first believed the experiment was flawed, and asked Barry – an undergraduate student in his lab who specializes in modeling – to examine what possible conditions could account for the experimental result. Barry's modeling suggested that effects observed could occur if cathepsin S were degrading cathepsin K instead of attacking the elastin – a protein essential in arteries and the cardiovascular system.

That theoretical result led to additional experiments in which the researchers measured a direct correlation between an increase in the amount of cathepsin S added to the experiment and a reduction in the degradation of collagen. By increasing the amount of cathepsin S ten-fold over the amount used in the original experiment, Platt and Barry were able to completely block the activity of cathepsin K, preventing damage to the collagen sample.

"We saw that the cathepsin K was going away much faster when there was cathepsin S present than when it was by itself," said Platt, who is also a Georgia Cancer Coalition Distinguished Scholar and a Fellow of the Keystone Symposia on Molecular and Cellular Biology. "We kept increasing the amount of cathepsin S until the collagen was not affected at all because all of the cathepsin K was eaten by the cathepsin S."

The researchers used a variety of tests to determine the amount of each enzyme, including fluorogenic substrate analysis, Western blotting and multiplex cathepsin zymography – a sensitive technique developed in the Platt laboratory.

Beyond demonstrating for the first time that cathepsins can attack one another, the research also shows the complexity of the body's enzyme system – and may suggest why drugs designed to inhibit cathepsins haven't worked as intended.

"The effect of the cathepsins on one another complicates the system," said Platt. "If you are targeting this system pharmaceutically, you may not have the types or quantities of cathepsins that you expect, which could cause off-target binding and side effects that were not anticipated."

Platt's long-term research has focused on cathepsins, including the development of sensitive tools and assays to quantify their activity in cells and tissue, as well as potential diagnostic applications for breast, lung and cervical cancer. Cathepsins normally operate within cells to carry out housekeeping tasks such as breaking down proteins that are no longer needed.

"These enzymes are very powerful, but they have been overlooked because they are difficult to study," said Platt. "We are changing the way that people view them."

For the future, Platt plans to study interactions of additional cathepsins – as many as three or four are released during certain disease processes – and to develop a comprehensive model of how these proteases interact while they degrade collagen and elastin. That model could be useful to the designers of future drugs.

"As we build toward a comprehensive model of how these enzymes work, we can begin to understand how they behave in the extracellular matrix around these cells," said Platt. "That will help us be smarter about how we go about treating diseases and designing new drugs."

Provided by Georgia Institute of Technology