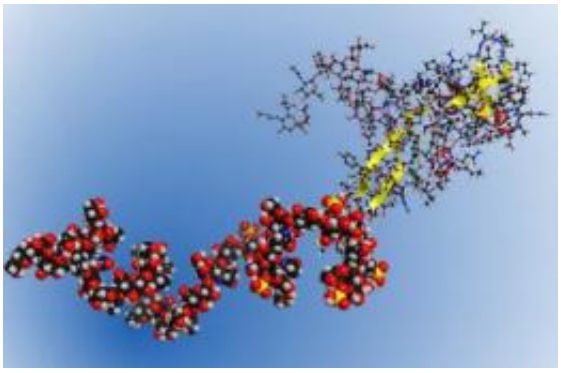
Uncharted territory: Scientists sequence the first carbohydrate biopolymer

October 11 2011, by Gabrielle DeMarco



The portion on the left corresponds to the sugar part of the molecule, the sequence of which was determined in the current study. The portion on the right corresponds to the protein part of bikunin. Credit: Rensselaer Polytechnic Institute

(PhysOrg.com) -- DNA and protein sequencing have forever transformed science, medicine, and society. Understanding the structure of these complex biomolecules has revolutionized drug development, medical diagnostics, forensic science, and our understanding of evolution and development. But, one major molecule in the biological triumvirate has remained largely uncharted: carbohydrate biopolymers.

Today, for the first time ever, a team of researchers led by Robert Linhardt of Rensselaer Polytechnic Institute has announced in the October 9 Advanced Online Publication edition of the journal *Nature*

Chemical Biology the sequence of a complete complex carbohydrate biopolymer. The surprising discovery provides the scientific and medical communities with an important and fundamental new view of these vital biomolecules, which play a role in everything from cell structure and development to disease pathology and blood clotting.

The paper is titled "The proteoglycan bikunin has a defined sequence."

"Carbohydrate biopolymers, known as glycosaminoglycans, appear to be really important in how cells interact in higher organisms and could explain evolutionary differences and how development is driven. We also know that carbohydrate chains respond to disease, injury, and changes in the environment," said Linhardt, who is the Ann and John H. Broadbent Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering at Rensselaer. "In order to understand how and why this all happens, we first need to know their structure. And today, at least for the simplest glycosaminoglycan structure, we can now do this."

The first glycosaminoglycan sequenced was obtained from bikunin. Bikunin is a proteoglycan, a protein to which a single glycosaminoglycan chain is attached. Unlike less sophisticated carbohydrate biopolymers, such as starch and cellulose, the proteoglycans are decorated with structurally complex carbohydrates that enable them to perform more sophisticated and defined roles in the body. Bikunin, for example, is a natural anti-inflammatory that is used as a drug for the treatment of acute pancreatitis in Japan. It has the simplest chemical structure of any proteoglycan. Linhardt views the discovery of the structure of bikuin as the first step on the ladder to the discovery of the structure of more complex proteoglycans.

"The first genome sequences of DNA were on the simplest organisms such as bacteria. Once the technology was developed it ultimately led to the sequencing of the human genome," he said. "In our efforts to

sequence carbohydrate biopolymers we don't yet know if the defined structure we observe for this simple protoglycan will hold for much more complex proteoglycans."

But, looking for structure in more complex proteoglycans will be among the next steps in the research for Linhardt and his team. The search for structure could help put to rest a long-running debate in the scientific community as to whether complex carbohydrate biopolymers require a defined structure to function.

"Despite all that is known about glycan formation, our understanding has not yet been deep enough to infer sequence or even determine if sequence occurs," Linhardt said. "These findings represent a new way of looking at these complex biomolecules as ordered structures."

Linhardt's research into carbohydrate sequencing began 30 years ago. In his previous work, he determined that some order existed in at least a portion of some carbohydrate biopolymers, but it did not represent the entire finished puzzle.

"Previously, we could see a pattern, but we could not see if all the chains were playing the same music. The tools did not yet exist. Now we can recognize it as a symphony."

To uncover the entire structure, Linhardt and his team, which was led by his doctoral student Mellisa Ly, borrowed a technique from the field of protein research called the proteomics top-down approach. As opposed to the bottom-up approach that first breaks apart a complex biopolymer into pieces and then rebuilds it piece by piece like a jigsaw puzzle, the top-down approach used by Linhardt and colleagues allows the researcher to picture the whole intact puzzle. This can only be accomplished with some of the most sophisticated technology available to the scientific community today, including very high-powered mass

spectrometers.

Linhardt used a mass spectrometer located in the Rensselaer Center for Biotechnology and Interdisciplinary Studies (CBIS) to make his initial discoveries, and had these results independently confirmed on a separate and higher-level spectrometer at the University of Georgia. Mass spectrometers break down a molecule into separate charged particles or ions. These ions can then be categorized and analyzed based on their mass-to-charge ratio. These ratios then allow for sequencing of the entire molecule.

"This was truly the convergence of really sophisticated spectroscopy and its application to biology," Linhardt said. "We were fortunate to have a lot of time to play with the instrument at CBIS to understand its capabilities."

Beyond the technology it also took faith and determination. According to Linhardt, "It takes a student that is willing to try something even when the odds are pretty low. If it doesn't work, you make incremental progress. If it does work, you can make a great discovery. But, from the beginning you need to be a believer that it is worth taking the chance because it takes a lot of hard work in the lab."

And the odds weren't in Linhardt's favor. Despite being the most simple of proteoglycans, there were still 290 billion different possible sequences for the molecule.

"The first sample we looked at, we got the structure," Linhardt said. "In the end we did 15 chains and they all came back playing the same exact symphony."

Provided by Rensselaer Polytechnic Institute