Scientists develop powerful new methodology for stabilizing proteins

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A team of scientists at The Scripps Research Institute has discovered a new way to stabilize proteins — the workhorse biological macromolecules found in all organisms. Proteins serve as the functional basis of many types of biologic drugs used to treat everything from arthritis, anemia, and diabetes to cancer.

As described in the February 4, 2011 edition of the journal *Science*, when the team attached a specific oligomeric array of sugars called a "glycan" to proteins having a defined structure, the proteins were up to 200 times more stable in the test tube. In the body, this stability may translate into longer half-lives for therapies, possibly lowering the overall cost of treatment for certain protein-based drugs and requiring patients to have fewer injections during a course of treatment.

The work may have major implications for the drug industry because there are a large number of protein-based drugs on the market, more in clinical trials, and many more under development worldwide. Nearly all of these protein-based drugs have glycans attached to them and are therefore called "glycoproteins". Glycoprotein-based drugs can be quite expensive to produce and usually need to be administered intravenously.

One of the challenges in producing these drugs has been increasing their stability, which generally extends their half-life in the bloodstream — issues that the new discovery appears to address directly.

"We've now provided engineering guidelines for glycoprotein stability,"

said Scripps Research Professor Jeffery W. Kelly, who is chair of the Department of Molecular and Experimental Medicine, Lita Annenberg Hazen Professor of Chemistry, and member of The Skaggs Institute for Chemical Biology at Scripps Research. Kelly led the study with Scripps Research Associate Professor Evan Powers and Staff Scientist Sarah R. Hanson, in collaboration with Research Associates Elizabeth K. Culyba, Joshua Price, and colleagues.

In Search of Stability

Making therapeutic proteins more stable by attaching glycans to them is nothing new. Scientists have known for many years that the human body widely modifies proteins in this way after they are made inside cells. By some estimates, as many as a third of all types of proteins in the human body are "glycosylated," the scientific name for the process whereby glycans are attached to proteins. Scientists also know that these modifications can be directly linked to protein stability.

Attaching a glycan to one part of a protein can have a dramatic stabilizing effect, accounting for the difference between it lasting in the bloodstream for a few minutes or a few days. But attaching the same glycan to another part of the same protein can have a distinctly different destabilizing effect, turning it into the microscopic equivalent of a cooked egg — unfolded and worthless as a medicine.

Scientists who work on these sorts of drugs often try to stabilize their therapeutic proteins with glycans, but until now nobody understood the rules that govern the process — nobody even knew for sure if there were general rules governing it. Researchers have always made such modifications through trial-and-error — more of a time-consuming art than an exact science.

But now, predicts Powers, "Having a rational design approach will

streamline protein drug optimization quite a bit."

Simple Engineering Rules

The new research shows simple engineering rules do exist for achieving stability of glycoproteins in the test tube. In the new paper, the Scripps Research team showed that scientists could dramatically stabilize proteins by integrating the standard N-glycan into a particular part of the protein — a structure known as a "reverse turn" containing a certain combination of amino acids. Reverse turns are found in the vast majority of proteins, making this methodology broadly applicable.

The scientists tested their ability to increase the stability of proteins by creating glycoproteins from proteins that are not normally glycosylated — leading to increased stabilization in the test tube. These scientists have not yet looked at how long the proteins survive in the bloodstream — that work is currently under way. But the team is confident that the principles they discovered will now give scientists a new way to predictably stabilize proteins by design.

Kelly added that this portable stabilizing structural module called the "enhanced aromatic sequon" also leads to more efficient production of glycoproteins by cells, a result that is potentially very important, since glycoproteins remain difficult to produce and purify.

More information: In addition to Kelly, Powers, Hanson, Culyba, and Price, the article, "Protein Native-State Stabilization by Placing Aromatic Side Chains in N-Glycosylated Reverse Turns" is authored by Apratim Dhar, Chi-Huey Wong, and Martin Gruebele.

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