Tailor-made recombinant proteins in mammals

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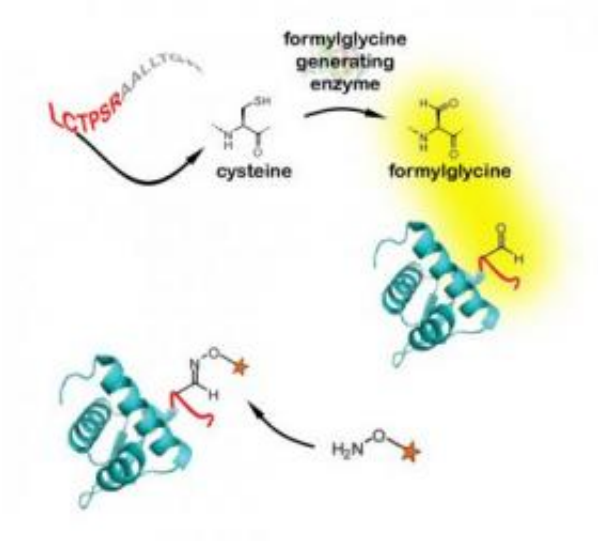

DNA for the core sequence of six amino acids -- leucine, cysteine, threonine, proline, serine, and arginine (red letters) -- is cloned into the gene for the recombinant protein at the locus to be chemically modified. The cell's own FGE converts the cysteine in the sequence to formylglycine, outfitting the protein with an aldehyde group. Synthetic molecules (starred) that are specially equipped to react with the aldehyde group modify the protein at that site and no other. Many kinds of proteins can be tagged in this way; "generic" proteins are pictured here. Credit: Carolyn Bertozzi laboratory, UC Berkeley, Lawrence Berkeley National Laboratory

A new way to direct chemical modifications to specific sites on recombinant proteins - including the monoclonal antibodies so important

in the pharmaceutical industry - has been developed by Carolyn Bertozzi and her colleagues at the U.S. Department of Energy's Lawrence Berkeley National Laboratory and the University of California at Berkeley.

Many therapeutic proteins, including insulin for diabetes, can be made in bacterial systems like *Escherichia coli*, but most protein pharmaceuticals must be expressed in cultured mammalian cells. The researchers have now found a way to extend the use of "aldehyde tags," which they previous developed for recombinant proteins expressed in bacteria, to label proteins that can only be expressed by mammalian systems.

"Proteins made with recombinant DNA are a major weapon in the armory against disease," says Bertozzi, who is a member of Berkeley Lab's Materials Sciences and Physical Biosciences Divisions and director of the Molecular Foundry, a Department of Energy (DOE) nanoscience user research facility. "But protein therapeutics are far from perfect. Many have short half-lives, so the patient must inject them repeatedly. And it is often difficult to introduce novel features into a recombinant protein."

While a specific chemical change can extend a protein's lifetime or turn it into a target for diagnostic imaging, for example, or convert an antibody into a drug that seeks out and attacks cancer cells, directing a chemical modification to the right place in the protein can be a challenge.

"Some protein modification methods have been around for a long time; typically they make use of the amino acid residue lysine. But there are lots of kinds of proteins in a cell, and any of them may include dozens or even hundreds of lysines, so it's hard to modify just one and not all of them," says Bertozzi, who is also a professor in the Departments of Chemistry and Molecular and Cell Biology and a Howard Hughes

Medical Institute Investigator at UC Berkeley.

Says Bertozzi, "Ways to get around nonspecificity have been found in the laboratory, but these lab methods usually aren't very practical for drug manufacture because they can't be generalized or scaled up."

Enter Bertozzi's aldehyde tag: the tag is an aldehyde group displayed as part of an amino acid side chain with chemical reactivity completely unlike the 20 standard amino acids that normally make up a protein. When the aldehyde tag appears at a specific site on a given protein, a chemical equipped to react with an aldehyde group - which is not an ordinary event, but instead is what Bertozzi calls "orthogonal to nature" - will target that location and none other.

"To do this we actually borrowed a machinery from nature, which is out there in pretty much all organisms and was just waiting for us to take advantage of," Bertozzi says. "It's called the formylglycine generating enzyme, or FGE, and it resides in the endoplasmic reticulum, a part of the eukaryotic cell that participates in protein secretion."

In nature, FGE is part of the cell's machinery for regulating the catalytic functions of enzymes called sulfatases. FGE does this by converting a cysteine residue, one in a sequence of six core amino acids in a particular sulfatase, into the nonstandard amino acid residue formylglycine. "Formyl" is another name for a simple aldehyde group.

"So all we had to do was clone the nucleotides encoding those six residues from the sulfatase sequence into the DNA of the target protein we want to produce, at the site corresponding to where we wanted the chemical modification to occur." Bertozzi calls that six-residue sequence the aldehyde tag. "You just sit back and allow the cell to express the protein. When it does, the FGE system is already in place to convert the cysteine to formylglycine" - the nonstandard amino acid bears the

aldehyde.

Having developed this technique for pinpointing chemical reactions to sites on recombinant proteins from *E. coli*, Bertozzi and her colleagues showed that it works equally well in the most common mammalian system, CHO cells (Chinese hamster ovary cells), as well as in the HEK cell system (human embryonic kidney cells).

"You can put anything you want on the tagged protein," she says, "You attach small molecule drugs, or polymers that change the pharmacokinetics such as polyethylene glycol polymers, or another protein molecule, or anything you're interested in attaching to a protein."

Because of their obvious therapeutic importance, the researchers used antibodies in their first tests. Immunoglobulin G (IgG) is the most widely used, clinically important type of antibody. For example, it can be rendered anti-inflammatory by chemically modifying part of its structure known as the Fc fragment. Conventionally this is done by targeting lysines, but there are many of these, and some lie near sites that, if modified, would cripple the antibody instead of making it therapeutic.

Bertozzi's group proved they could precisely tag the Fc fragment of the IgG molecule so that it would selectively bind with chemicals engineered to react with the aldehyde. The Fc fragments were the only proteins in the mix with which the experimental labels reacted.

"From the biotech perspective, secreted antibody-like proteins like these are the most interesting," Bertozzi says, "but the aldehyde tag technique is versatile and easy to apply, with a wide range of applications in the biotech industry." Her team showed that aldehyde tags could successfully label mammalian membrane-associated proteins and proteins occurring in the intracellular fluid as well.

"What's so appealing about the method is that it's really simple," Bertozzi says. She's sufficiently confident of the value of the aldehyde tag method for pharmaceutical manufacture to have licensed the technology through the University of California and, with former graduate student David Rabuka, cofounded a startup named Redwood Biosciences. "You don't need any high-tech genetic engineering or any fancy chemistry - it's really as simple as cloning a little six-residue tag into the gene of your target protein. That's why we call it 'low-tech, high-concept.'"

More information: "Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag," by Peng Wu, Wenqing Shui, Brian L. Carlson, Nancy Hu, David Rabuka, Julia Lee, and Carolyn R. Bertozzi, appears in Proceedings of the National Academy of Sciences and is available in the PNAS Early Edition online at www.pnas.org/content/early/200 … /0807820106.abstract .

Source: Lawrence Berkeley National Laboratory