Engineers convert a natural plant protein into drug-delivery vehicles

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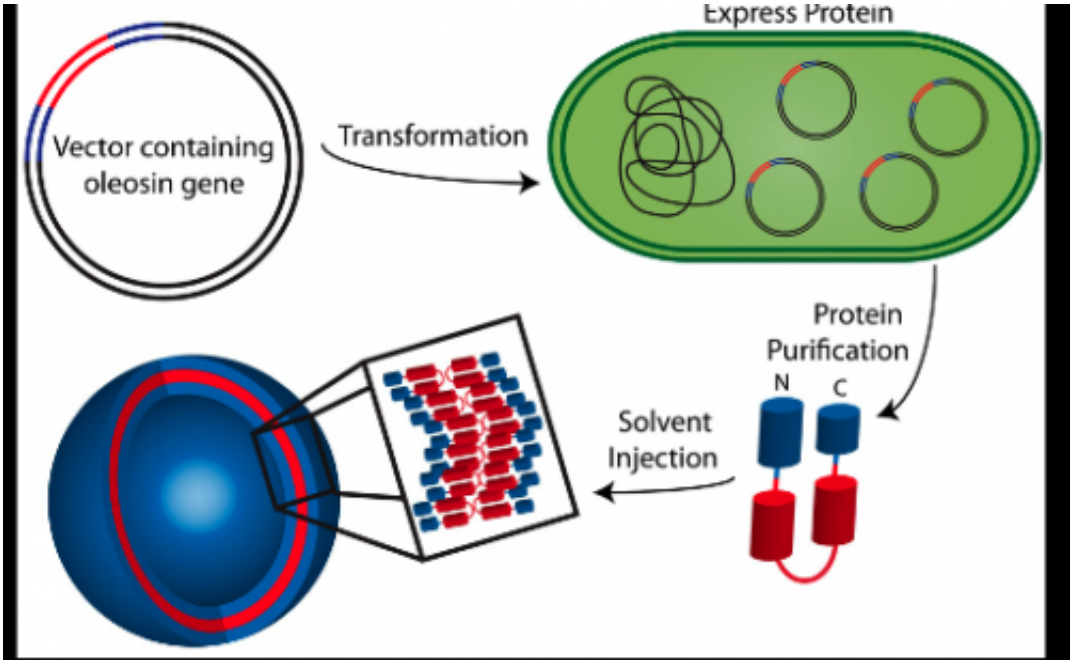

The gene for oleosin, a protein from a plant, was inserted into a vector and expressed in bacteria. Many different variants were made by recombinant methods. The purified proteins were assembled in structures, including vesicles, a capsule that can carry a large payload of drugs. This is the first demonstration of vesicles being made from a recombinant protein. Credit: University of Pennsylvania

(Phys.org) -- Finding biocompatible carriers that can get drugs to their targets in the body involves significant challenges. Beyond practical concerns of manufacturing and loading these vehicles, the carriers must

work effectively with the drug and be safe to consume. Vesicles, hollow capsules shaped like double-walled bubbles, are ideal candidates, as the body naturally produces similar structures to move chemicals from one place to another. Finding the right molecules to assemble into capsules, however, remains difficult.

Researchers from the University of Pennsylvania have now shown a new approach for making vesicles and fine-tuning their shapes. By starting with a protein that is found in sunflower seeds, they used genetic engineering to make a variety of protein molecules that assemble into vesicles and other useful structures.

Daniel A. Hammer, Alfred G. and Meta A. Ennis Professor of Bioengineering, graduate student Kevin Vargo and research scientist Ranganath Parthasarathy of the Department of Chemical and Biomolecular Engineering in Penn's School of Engineering and Applied Science conducted the research.

Their work was published in the *Proceedings of the National Academy of Sciences*.

"To our knowledge, this is the first time a vesicle has been made from a recombinant protein," Hammer said.

Recombinant proteins are the products of a well-established technique that involves introducing a designed gene sequence into a host organism — in most cases, the bacterium E. coli — in order to get that organism to make a protein it would not normally produce.

Hammer's group worked for nearly a decade to find a protein that was biocompatible, could be produced through recombinant methods and, most important, could be induced to form vesicles.

“The molecule we identified is called oleosin,” Hammer said. “It’s a surfactant protein found in sunflower and sesame seeds.”

Surfactants are soap-like chemicals that have two distinct sides; one side is attracted to water and the other is repelled by it. They can make many structures in solution but making vesicles is rare. Most often, surfactants make micelles, in which a single layer of molecules aggregates with the water-loving part on the outside and the water-hating part on the inside. Micelles have a limited ability to carry drugs. Vesicles, in contrast, have two walls aligned so the two water-hating sides face each other. The water-loving interior cavity allows the transport of a large payload of water-soluble molecules that are suspended in water. Since many drugs are water soluble, vesicles offer significant advantages for drug delivery.

The team systematically modified oleosin to find variants of the molecule that could form vesicles. Getting oleosin to take this complex shape meant selectively removing and changing parts of oleosin’s gene sequence so that the corresponding protein would fold the way the researchers wanted after it was produced by the E.coli.

“We started by truncating the sequence that codes for the hydrophobic part, shortening the protein itself,” Hammer said. “We did more complex truncations at the ends for separation and to control the shape of the assembly.”

“There are simple ways to correlate the gene sequence to the geometry you get in the protein,” Vargo said. “For example, getting the right amount of curvature to make a spherical vesicle means the chains should be sufficiently large that they do not pack tightly.”

In the process of finding the right protein for this task, the researchers came up with several other useful protein variants that form different shapes, including sheets and fibers, when grown in the appropriate salt

solutions.

Materials made by recombinant methods offer an additional advantage in that the precise sequence of amino acids can be controlled for targeting to specific receptors and other biological targets. For proteins of this size, this level of control is not attainable by any other method.

“Other groups have synthesized polypeptide vesicles, but they have a hard time controlling the sequences in individual sections of their molecules,” Vargo said. “We can go in a change a single amino acid in the protein by modifying the corresponding part of the gene.”

“Recombinant methods mean we can make polymers that are all of a defined length and dictate the chemical composition at each location along that length,” Hammer said. “You get the exact length and sequence every time.”

According to Hammer’s team, the hardest part of the research was confirming that these sequences did indeed fold into vesicles. This was only possible with specialized equipment available to the researchers through their association with Penn’s Materials Research Science and Engineering Center and made possible by a grant written by professor Karen Winey from Materials Science and Engineering.

“The vast majority of our time in this project was doing the imaging; making the protein was relatively easy,” Hammer said.

The imaging technique used is known as cyro-transmission electron microscopy, or cryoTEM

“With cryoTEM,” Vargo said, “we create a thin layer of solution, then plunge it into ethane, freezing it fast enough that the water doesn’t crystallize. Ice crystals would also destroy the vesicles, so this technique

leaves you with your particles and structures intact."

As their protein is already routinely eaten, the researchers are confident that their oleosin vesicles will be of great interest in drug-delivery applications, particularly oral-drug delivery. Future work will entail adding genes for functional groups to allow the vesicles to target certain tissues, as well as determining whether the proteins can be induced to change shape once they reach their targets.

"This research opens up the possibility of using switchable motifs to allow us to release high concentrations of drugs on different cues, such as a change in acidity," Hammer said. "Tumor microenvironments and the interior of tumors are known to be acidic, so a vesicle that falls apart in acidic environments would be extremely valuable."

Provided by University of Pennsylvania

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