

The Perfect Pill?

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A protein found in sunflower seeds could be the key to developing plants as pharmaceutical factories.

A tiny protein ring derived from sunflower seeds has inspired drug designers for more than a decade, but the unusual way that sunflowers make it has only recently been revealed in a discovery that could transform plants into drug production factories and turn seeds into pills.

Our recent research has shown that this protein ring is surprisingly buried within much a larger protein that makes a completely different protein called albumin. Even more unusual is that the ring and the albumin emerge from the larger protein simultaneously, using the machinery that normally only matures the albumin. This “hijacking” of the sunflower’s processing machinery to make a protein ring can be transferred to other plants and offers a means of synthesising drug-like rings on a large scale.

Interest in the Sunflower Ring by Drug Designers

This whole story began in the late 1990s when Russian scientist Alexander Konarev studied proteins that blocked protein-digesting enzymes. He was interested in these proteinases because plants often use proteinase blockers to protect themselves from insects.

Konarev discovered one proteinase blocker that was of extraordinary potency. It was also one of the smallest he’d ever found. This particular proteinase blocker came from extracts made from

sunflower seeds, and he purified it during a collaborative visit to the Rothamsted Research Station in the United Kingdom.

The extract was handed to PhD student Sue Luckett who, with scientists from Bristol, characterised its sequence and structure. She did so by incubating the extract with the proteinase Konarev used to discover it – a much-used proteinase called trypsin.

Trypsin formed a complex with its blocker, and Luckett was able to simultaneously solve the structures for both. They named the blocker sunflower trypsin inhibitor 1 (SFTI-1), and when they looked at the structure they could see that it was a protein ring.

This was unusual as almost all proteins have a start and an end, known as the amino and carboxyl termini. The lacks of ends, as well as its structure, make SFTI-1 extremely stable and an excellent blocker of trypsin. It is these properties that later inspired drug designers to develop drugs using the SFTI-1 framework.

Pharmaceutical interest in proteinase inhibitors comes mainly from the importance of proteinases in cancer. Tumours often switch on otherwise silent proteinases in order to chew into the surrounding flesh, giving the tumour space to grow into.

Which proteinase is activated depends on the type of cancer, so the proteinases themselves are often used diagnostically as an

indicator of existing cancer. In some cases researchers have shown that the switching-on of a proteinase is all that is required to trigger some forms of cancer. A common component of many cancer treatments is a proteinase inhibitor that blocks the proteinase employed by the specific form of cancer.

What grabbed the attention of drug designers was the finding in 2001 that SFTI-1 in its natural form could very efficiently block a proteinase involved in breast cancer called matriptase. A series of patents were filed for the use of SFTI-1 in treating breast cancer but it has not reached the market, possibly because SFTI-1 blocks other proteinases and is not specific enough, so it could cause side-effects.

More promising work by scientists at the Queensland University of Technology showed that by making three changes to the amino acid sequence of SFTI-1 they could make a specific inhibitor that blocked a proteinase involved in prostate cancer but did not affect closely related proteinases.

What Makes This Sunflower Ring Drug-Like?

What makes SFTI-1 so good at inhibiting proteinases is its structure. Recently an important observation was made while looking at the structures of proteinases bound to either substrates or inhibitors: proteinases preferred a “flat” area of protein surface in front of the amino acid that it targets and binds. This explained why one inhibitor could be better than another, but also explained why proteinases don’t always cut at every target amino acid.

The structure of SFTI-1 is perfect for inhibition. Trypsin targets the amino acid lysine, which juts out from SFT-I and is preceded by a perfectly flat surface. This ideal shape is what drug designers make use of, and by changing the target from lysine to other amino acids they can make different versions of SFT-I that block different proteinases. Further small changes around the target amino acid can improve the specificity of the modified proteinase blocker.

A protein is the best drug for blocking proteinases, but protein drugs face two challenges when they leave the test tube.

The first challenge is their instability in biological fluids. When they enter the body, protein drugs are treated no differently to any other protein. If taken orally, an expensive protein drug will

be digested just like proteins in a meat pie would be as they passed through the digestive system. As a result, protein drugs on the market are delivered directly to the site of action by hypodermic needle or directly into the spinal column in the case of the nerve pain drug Prialt®. The cyclic and rigid structure of SFTI-1 is likely to be much less digestible than normal proteins so it may already be able to meet this challenge.

The second challenge that protein drugs face is that they are very expensive to manufacture compared with small chemical drugs. To develop a suitable drug, chemical synthesis is the best approach but kilograms, not picograms, are needed when it comes to making that drug. Creating protein drugs like this synthetically requires a lot of harsh chemicals, expensive amino acid precursors and a lot of wastage purifying the correct forms away from incorrectly formed products. For SFTI-1 based drugs in particular, the ring closure reaction has not been transferrable to simple biological systems, like bacteria, that are traditionally used for synthesising drugs, but in this case plants are obviously good at it.

How Do Sunflowers Make Protein Rings?

It was this international interest in SFTI-1 and an opportunity to meet the challenge of large-scale production that prompted me to understand how sunflowers went about making SFTI-1. I did not know it at the time that it would be 3 years before I would fully appreciate the unusual manner of its production.

The first discovery came relatively quickly: the isolation of the gene that encoded SFTI-1. This was unusual in itself, as although SFTI-1 was only 14 amino acids in length, its precursor was 151 amino acids in length. Furthermore, far from being dedicated to making SFTI-1, this 151 amino acid protein looked a lot more like a protein that made albumin.

Albumin is a seed storage protein. Its role in plants, along with another protein called globulin, is to act as a store of nitrogen and sulfur that is broken down and built into new proteins that the seedling needs to make as it germinates. A seedling needs to establish a root and a shoot before it can start taking nutrients from the soil and photosynthesising, and to construct these tissues it relies on the proteins, oil and starch from its seeds. Seed proteins may constitute up to 50% of the kernel of a seed.



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Being buried inside a precursor for albumin seemed a strange place for SFTI-1 to begin. Over the next few years my colleagues and I at the Institute for Molecular Bioscience, as well as a talented mass spectrometrists from CSIRO, would unpick the way that SFTI-1 emerges from within this albumin precursor.

After isolating it, I named this unusual precursor gene PawS1, a catchy abbreviation for the more cumbersome “Preproalbumin with SFTI-1”. Almost immediately I found a related gene called PawS2, and it encoded an even smaller protein ring that we purified and called SFT-L1 for “SFTI-Like 1”.

Looking at all the known albumin precursors, it was clear that having an extra piece of protein inside them was unique to PawS proteins. Despite being used to make these rings we showed that both PawS1 and PawS2 still go on to make normal mature albumins, so they have not lost their original function of making a seed storage protein.

Seed storage proteins are “matured” from a precursor (in our case PawS1 and PawS2) into their final form, which is a little smaller and much more knotted and stable. Much of this seed protein maturation is performed by a proteinase called AEP, which targets the amino acids asparagine and aspartic acid. What was interesting about PawS1 was that SFTI-1 was preceded immediately by asparagine and ended itself with aspartic acid. This suggested that the enzyme AEP, long known for its important role in maturing albumin, might cut SFTI-1 out of PawS1 as well.

To understand the processing of SFTI-1 any further I had to turn to the model plant *Arabidopsis thaliana*. I have worked with this “lab rat of the plant kingdom” since 1997 when I started my PhD, and currently some 16,000 labs worldwide use *Arabidopsis* to study plant processes.

We made a transgenic *Arabidopsis* plant that made PawS1 protein in its seeds, and

were delighted to find that *Arabidopsis* could process SFTI-1 from it. This meant we had a system to further study SFTI-1 processing, but it also meant that PawS1 could be moved around. I will come to the significance of this application later.

Using *Arabidopsis* we found which parts of PawS1 were critical for making SFTI-1, but we also used an *Arabidopsis* mutant lacking AEP to prove that AEP was indeed needed to cut SFTI-1 out of PawS1. In a paper published this year in *Nature Chemical Biology* we described how SFTI-1 and SFT-L1 emerge from within albumin precursors by “hijacking” the albumin-maturing machinery.

A seed could eventually be the ultimate delivery system for a drug.

So we knew now that AEP released SFTI-1 out of PawS1, but most interesting of all was trying to understand how the ends got joined together to make the ring. As we discovered more and more about what was happening, the work done in *Arabidopsis* made it clear that during the final cut at the end of SFTI-1, the conditions were ideal for the front part of SFTI-1 to chemically react with the back part of SFTI-1 as it still sat in the “jaws” of the AEP proteinase.

Proteinases cut in two steps. First, they bind to the target amino acid and then water reacts with this intermediate, breaking the protein and releasing the proteinase. But proteinases are like any enzyme – if the conditions are right they can push a reaction in both directions, which in this case means making instead of breaking a bond.

What we think happens is that instead of water it is the front of SFTI-1 that reacts with the AEP intermediate and makes a bond between the front and end of SFTI-1, effectively turning it into a ring.

Although we’re certain that AEP cuts

SFTI-1 at both ends, this notion that it also joins the ring is still a hypothesis we’re trying to prove. It’s not going to be easy to separate these cutting and joining reactions if they are happening simultaneously on the same site, but we’ve got a few ideas about how it can be done.

Make Drugs Cheaply

For most scientists the unusual dual processing and the implications it has for protein evolution are the most interesting aspects of this discovery, but the original purpose for understanding how plants make SFTI-1 can be applied. Successfully making SFTI-1 in *Arabidopsis* and understanding how it is made has opened the door for plants to be production systems for SFTI-like drugs, thereby overcoming the cost-of-production challenge that protein drugs typically face. Although *Arabidopsis* has exceptionally small seeds, our success with it suggests that we could transfer modified versions of PawS1 into species with larger seeds like canola, safflower or, once the transformation technology becomes available, sunflower itself.

Seeds are an attractive system for making protein drugs. Agricultural practices are already in place for large-scale production, harvest, storage, transport and extraction of seeds. Seeds are self-perpetuating units, they are stable at room temperature and the inside of their seed coat is sterile. A seed could eventually be the ultimate delivery system for a drug – the “perfect pill” if you will. Seed-based drugs could be especially helpful for Third World nations lacking the financial strength or the infrastructure to import, distribute and administer drugs.

This is all a long way off, and what we are working on now is trying to find out what changes PawS1 will accept and still go on to make the protein ring. So far things look promising, and we can change the target amino acid lysine as well as the amino acids around it without disrupting its processing within plants.

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