# Cellular processing of proteins found in Congolese child birthing tea now revealed

December 12 2011

Many plants produce compounds that serve as a defense against predators or pathogens. Some are also used by humans for a variety of beneficial purposes, such as in medicines. As recently as the early 1990s, a unique class of proteins previously unknown to science, the cyclotides, was discovered. First noted through African tribal use as a tea given to speed up delivery during childbirth, cyclotides have since been determined to serve as a powerful insecticidal and nematocidal defense in the plants that produce them, and they also have anti-HIV and antimicrobial properties, with obvious benefits for humans. However, scientists are still working on unlocking much of the basic science of these fascinating proteins, including how they work and where in the plant cell they are produced.

Among the scientists interested in cyclotides, as well as other immune proteins, is Marilyn Anderson of LaTrobe University, Australia.

"Cyclotides are small cyclic peptides of only 28-37 residues that most plant biologists may not have heard of, yet they form the largest family of cyclic proteins described to date in any organism," explains Anderson. "Cyclic cyclotides are widespread in members of the *Rubiaceae*, *Violaceae*, *Cucurbitaceae* and *Fabaceae* families, yet linear cyclotides are also produced by major monocots such as rice, corn and barley."

"The big question is what do they do?" she continues. "We have discovered that some are potent insecticidal and nematocidal molecules but it is likely that some have other functions as yet undescribed."

Indeed, cycolotides have a unique shape resulting from three disulfide bonds and a peptide backbone that twists in such as way as to produce a cystine knot. This cyclic configuration provides the protein with a very stable structure that is hard to break down—which is how it maintains its bioactivity despite, for example, the high temperatures used to brew the tea used to aid childbirth in the Congo.

"The tea, called kalata kalata, was prepared by boiling the leaves so the active constituent had to be stable to boiling, as well as passage through the human intestinal tract where sufficient amounts were absorbed into the bloodstream to stimulate the uterus," comments Anderson. "Some years after its use was noted, in 1995, the structure of kalata B1, the active constituent from the tea, was solved and its cyclic structure was discovered."

Since then, Anderson, in collaboration with other researchers, has discovered that cyclotides are gene encoded—and in fact are encoded by a single gene, which was at the time unique for a cyclic protein from a eukaryote—and continues to investigate how plants make these cyclic peptides. Her most recent discovery is published in the December issue of the *American Journal of Botany*. She and her colleagues successfully determined where in the plant cell the cyclotide kalata B1 is produced and how its precursor protein, Oak1, is directed to the appropriate processing location (http://www.amjbot.org/content/98/12/2018.full.pdf+html).

Kalata B1 is found in the leaves of *Oldenlandia affinis* (*Rubiaceae*) and, as with all proteins, is made up of building blocks of amino acids put together in a genetically determined sequence. The precursor protein to kalata B1, called Oak1, is linear and is made up of a series of domains, the centerpiece of which contains a cyclotide domain sandwiched between the N- and C-terminal segments. Other cyclotide precursors may contain up to three cyclotide domains.

Anderson and co-authors used a novel approach to determine where in the plant cell the precursor protein was sent and which segment contained the signal sequence responsible for directing it there. They split the precursor protein Oak1 up into its component parts and tagged each segment with the green fluorescent protein (GFP). They then transferred the different constructs into *Agrobacterium*, injected the *Agrobacterium* into living leaves of *Nicotiana benthamiana*, and two days later injected a dye to visually highlight the plasma membrane and the intracellular membranes, such as the tonoplast, which surrounds the vacuole.

Their first finding was that the precursor protein Oak1 was sent to the vacuole to be processed. When viewed under a microscope, cells that were injected with the Oak1-GFP construct had vacuoles that were entirely filled with florescent green dye.

This was a very exciting finding because enzymes hypothesized to play a key role in the ring formation of cyclotides are naturally found in the vacuole. These two pieces of information led the authors to conclude that the vacuole must be the location where Oak1 is converted into kalata B1.

## But how is the precursor protein directed to the vacuole?

When the authors looked at cells injected with the different constructs, they found that the only cells that had green florescent vacuoles were those containing constructs with segments of the Oak1 precursor protein that contained propeptides from the N-terminal region. Thus, while the C-terminal segment of the precursor protein is critical for the formation of the ring structure, the N-terminal segments get it to the appropriate cyclization processing location.

Knowing precisely how the precursor protein is directed to its target location (the vacuole) where the cyclotide domain is excised and the ligation of the N- and C- termini occurs is a critical step in understanding the biology of these proteins.

"After conducting the research described in this paper," Anderson concludes, "we now know that the cyclization reactions occur in the vacuole and this provides more insight into the pH conditions required for cyclization and supports our hypothesis that the vacuolar enzyme asparaginyl endoproteinase is the crucial enzyme involved."

**More information:** Conlan, Brendon F., Amanda D. Gillon, Barbara L. Barbeta, and Marilyn A. Anderson. (2011). Subcellular targeting and biosynthesis of cyclotides in plant cells. *American Journal of Botany* 98(12): 2018-2026. DOI: 10.3732/ajb.1100154

Provided by American Journal of Botany