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The protein with a topological twist

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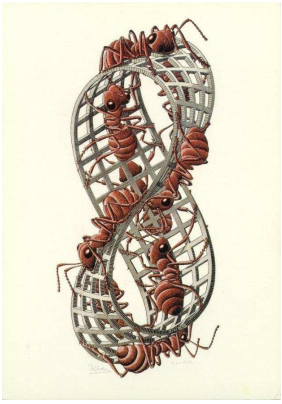
Scientists discovered the use of *Oldenlandia affinis* as an oxitocic agent in Africa, in the 1960s. *O. affinis* is a perennial weed with a woody root and blue-violet flowers, and is found in the tropical zones of Africa and western Asia. There are 196 different species of *Oldenlandia* and their use in traditional medicine is as widespread as their geography. India uses many of the different species in as many different drugs. However, it was the uteroactive activity of a green brew, Kalata-Kalata, given to African women during labour that first triggered an interest. The decoction was made from a handful of dried *O. affinis* boiled in about a litre of water. Women about to give birth were either given the tea to sip or it was directly applied *per vaginum*; contractions became stronger and delivery was shortened. What was the nature of the uteroactive agent? The green potion was whipped to a laboratory and the main uteractive agent turned out to be a small protein named after the traditional medicine from which it was extracted: kalata B1.



MC Escher's Moebius Strip II

It took a further 25 years to determine the protein's sequence and structure, which proved to be quite particular. Kalata B1 is not only a cyclic protein but it also sports a biological knot…with a twist. Small cyclic proteins of microbial origin are quite common. However macrocyclic proteins, such as kalata B1, are not so frequent and seem to be favoured only by higher plants. Kalata B1 is 29 amino-acids long and begins its life as a linear sequence. As it evolves through the plant's secretory pathway, it three disulfide bridges are created, two of which form a ring. The third plunges through the ring creating a knot. Once the protein sequence is cyclised it cannot be undone without causing damage to the chain. Mathematicians may not qualify this as a true knot, but for the layman a good definition of a knot is something you cannot undo.

In addition to its three cystine bridges and cyclization, kalata B1 also has a twist. The biological significance of this twist is unknown but from a structural point of view it is fascinating. As David Craik from the Centre for Drug Design and Development in Queensland explains, imagine a thin strip of paper. There are two ways of attaching the ends. You can attach them as you would a bracelet, i.e. without a twist. Or you can add one twist and then attach the ends.

What's the deal? The 'bracelet' type has an inside and an outside; the 'twisted' type has neither. To grasp the point, take a pen and a strip of paper. Start from any point on the 'bracelet' type; you have to lift the pen to mark the other side. Now do the same on the 'twisted' type. You will find that you do not have to lift the pen to mark every part of the paper strip. This means that the 'twisted' type has neither an inside nor an outside! Such a strip is termed a Moebius strip. And plant cyclotides are now grouped into two types: bracelet cyclotides and

Moebius cyclotides. Kalata B1 is a Moebius cyclotide.

It is hardly surprising that a cyclic protein bearing a cystine knot and a twist is particularly stable. Kalata B1 survives boiling temperatures... It is also a complex structure for enzymes to get their teeth into. And as a consequence of its discovery, small peptides have been cyclised in the pharmaceutical industry for a number of years now for greater stability.

Despite the knowledge of such an intricate knotting system, scientists still do not know what the precise role of kalata is in the plant. There is proof of insecticidal and antimicrobial properties. Plants have quite an arsenal against insect predators, fungal and bacterial pathogens as well as grazing animals. Plant toxins known to date are – like kalata B1 – cysteine rich, expressed in each plant tissue and trafficked through the secretory pathway.

Besides its oxitocic powers, kalata is also hemolytic. Though how, no one knows. What is known is that kalata B1 does not act the way other plant defence molecules do. It has a major effect on *Helicoverpa punctigera* larvae, for example, by stunting their growth and development. However, it does not inhibit protein and starch degradation, hinting that it does not function as a proteinase or alpha-amylase inhibitor.

Evidently, much remains to be learned from this contorted protein. Besides its oxitocic activity which could be used to produce an oxitocic agent for clinical purposes, its particularly strong framework – and hence its chemical and biological stability – will most certainly be used for agricultural and pharmaceutical applications. It is not too far-fetched to imagine inserting specific amino-acid sequences onto kalata's scaffolding, which in turn would confer specific functions to a stable chimeric protein. And is it not a refreshing thought to be told that knots and twists can be a bonus?

Cross-references to Swiss-Prot

Kalata B1, *Oldenlandia affinis* : P56254

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