Protein Spotlight *Issue 7; February 2001*

WHEN A FROG SWALLOWS A FLY

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When you punch a hole in a tyre, it deflates. This seemingly simple strategy is used by plants and animals as a defence system. For this, they use antimicrobial peptides which can alter enemy cellular membranes in such a way that the inside leaks out, or the outside leaks in, thus causing cell death. Antimicrobial peptides have been discovered in all kinds of plants and animals, from fruit flies to horseshoe crabs, and honeybees to humans. It is a rapid immune response to microbes that surround us daily. When a frog swallows a fly, it also ingurgitates an army of microbes, which have to be eliminated or their growth rate at least controlled. Magainins, from the Hebrew "magain" meaning shield, do just this. They are the first antimicrobial peptides to have been described.

Magainins were discovered in 1987 in *Xenopus laevis*. Michael Zasloff had been working for some time on the frog's oocyte system. Though his discovery is a very fortunate one, associations for the prevention of cruelty to animals may wince. He was accustomed to practising non-sterile surgery on female frogs; the incisions were repaired with sutures and the animals were tossed back into microbially contaminated tanks… Zasloff, himself, was amazed at the absence of infection in such conditions and predicted a "sterilizing" activity in the frog's skin. Indeed, soon after, he discovered a number of small peptides secreted by the frog's skin, which could kill a host of bacteria and virus, as well as some fungi and protozoa. A favorite trick of Zasloff's is to rub some of the hormone adrenalin onto the frog's back. Within a few seconds, a horde of tiny white spots appear and soon merge into a milky film on the creature's skin. What Zasloff calls "a beautiful bandage". And that is just what it is.

Though magainins were first extracted from *Xenopus*' skin, they have also been found, understandably, in the frog's gastrointestinal tract. They are synthesized both in the skin and the stomach, and stored in the granular dermal glands of the skin and the granular multinucleated cells in the gastric mucosa, respectively. What is more, and fortunately, they attack non-eurkaryotic cells, exclusively. How is it that these peptides can recognize a non-friendly membrane from a friendly one? And how do they work? This is what is quite remarkable in this novel defence system. It all has to do with the physicochemical properties of the lipid bilayers, and of course those of the magainins.

Figure 1
A female African clawed frog awaiting a fly

Magainins are particulary short (20 to 26 amino acids) and specifically attracted to lipid bilayers. Not all lipid bilayers, however. Eukaryotic cell membranes consist, mainly, of zwitterionic phospholipids and cholesterol, and only carry a very low electric charge. Bacterial membranes are packed with negatively charged phospholipids and sugars, and thus carry a strong negative charge. In the presence of lipid bilayers, magainins curl up into amphipathic alpha helices and are immediately attracted to the charged microbial membranes. Eukaryotic membranes are ignored because of their low charge. The cholesterol also acts as a barrier to the antimicrobial peptides.

Magainins are just long enough to span lipid bilayers. Indeed, they have been observed to do just that. However, over the years, it became apparent that magainins also coat the membranes. In fact, it is now known that it is all a question of peptide to lipid ratio. When there are not too many peptides, the latter curl into their alpha-helix conformation and recline on the bacterial membrane. Once more of their

pals join the party, they turn and adopt a transmembrane orientation. Here they get together with a few phospholipids and form a peptide-lipid supramolecular complex pore.

There is not much the bacteria can do with a punched membrane: resistance mechanisms such as enzymatic degradation or export processes are hopeless. When the peptide ratio diminishes, the supramolecular pore is disrupted and the magainins rest on the inner phospholipid bilayer. The peptide waltz from the outside phospholipid monolayer to the inner phospholipid monolayer spells death.

Naturally, magainins fast became a pole of therapeutic interest. Their broad anti-viral, anti-bacterial, anti-fungal and even spermicidal activities have therapeutic potential in the treatment of infections in man. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two of the "superbugs" that infest hospitals and are fast becoming antibiotic-resistant. Antimicrobial peptides could take over thanks to their pore-forming mode of action. One particularly interesting development is the use of these peptides as a potent vaginal contraceptive. In a world where there are 250 million new cases per year of sexually transmitted diseases, besides the AIDS virus, a contraceptive with anti-viral, anti-bacterial and anti-fungal activity would be a godsend. There are obstacles, however. The antimicrobial peptides can be digested by the host or can even be toxic for the host. Tests have been carried out with more resistant synthetic peptides, which seem to be less prone to enzymatic digestion in their host. However, there is still a long way to go before such drugs are deemed safe for humans.

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Protein Spotlight (ISSN 1424-472) is published by the SWISS-PROT group at the Swiss Institute of Bioinformatics (SIB).