MAGAININ-LIKE ANTI-BACTERIAL PROPERTIES OF TISSUE EXTRACTS FROM SQUALUS ACANTHIAS AND MYXINE GLUTINOSA

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Magainins are a family of peptides with which are responsible for broad-spectrum anti-microbial activity in the skin of the African clawed frog, Xenopus laevis (Zasloff, M., Proc. Natl. Acad. Sci., USA, 84:5449-5453, 1987; Westerhoff, H.V., S. Juretic, R.W. Hendler and M. Zasloff, Proc. Natl. Acad. Sci., USA, 86:6597-6601, 1989). They represent a new class of peptides of a type excreted by prokaryotes as well as lower eukaryotes as a defense against pathogenic or predatory organisms (Cramer, W. A., J.A. Dankert and Y. Uratani, Biochim. Biophys. Acta 737:175-193, 1983; Ando, K. and Natori, S. J. Biochem. 103:735-739, 1988) through a mechanism involving disruption of membrane-linked free-energy transduction (Westerhoff et al., op. cit.). The general extent of phylogenetic expression of the anti-bacterial activity of these peptides among higher eukaryotes is a matter of considerable interest.

In July of 1989, we prepared at the MDIBL extracts of a variety of tissues from two Squalus acanthias including stomach, skin, rectal gland, and oviduct, as well as the non-adherent skin of two Myxine glutinosa for subsequent assay for Magainin-like properties. Squalus were sacrificed by spinal transection, while the Myxine were anaesthetized in MS 222 for about 20 minutes and subsequently sacrificed. Tissues were cut into small pieces, placed in 4 volumes (based on initial weight) of a 1:10 dilution of glacial acetic acid, brought to a boil in a small microwave oven, and subsequently homogenized using a Polytron blender. The foamy gray homogenates were centrifuged at 15,000 x g and the supernatants removed and returned for assays at the Children's Hospital of Philadelphia. The extracts were absorbed to a C18 reverse phase resin (Sep-Pak, Waters) and eluted with 60% acetonitrile, 0.1% trifluoracetic acid. Samples were lyophilized to dryness. Extracts were resuspended with water to a volume corresponding to 50 microliters/gm starting tissue, and two microliters of each extract were assayed for antibacterial activity. Two microliters of a 0.05 micrograms/ml solution of a member of the magainin family (Westerhoff et al., op. cit.), PGLa, a 21-amino acid peptide was used as a positive control.

The standard anti-bacterial assay used suppression of growth of Escherichia coli D31 to which supernatant fractions from various tissue extracts were applied to the top agar surface (Figure 1; for details of procedure see Zasloff, op. cit.). The results were quantified by measuring the diameter of the circular zone of anti-microbial activity of the various preparations and are shown in Table 1.

Table 1: Diameters (mm) of zones of anti-microbial activity of two samples of extracts from Squalus and Myxine and a single PGLa control. PGLa is a synthetic Xenopus antibiotic peptide, provided as a positive control. Stomach, rectal gland and oviduct were from Squalus only.

Squalus Skin	Stomach	Rectal Gland	Oviduct	Myxine Skin	PGLa
17	23	15	20	20	24
18	24	18	20	20	

All of the experimental extracts showed significant anti-microbial activity when com-

pared with an expected zone of activity of zero mm for a distilled water control (Kruskal-Wallis Analysis of Variance by Ranks; $T = 11.22$, $p < 0.05$) Further analysis revealed that the activity of five groups of experimental extracts did not differ significantly from that resulting from the application of PGLa, a magainin ($T = 7.47$, $p < 0.20$). There was some question about whether contaminating blood might not be partially responsible for some of the activity observed in rectal gland and skin.

These results suggest that epithelial oligopeptide anti-microbial defenses may be widespread among at least the lower vertebrates. Particularly interesting is the activity of the oviduct extract. Female Squalus harbor their young in an oviduct which maintains communication with seawater for an 18 month fetal period, during which metabolic wastes from active and developing young might provide an effective medium for bacterial growth and subsequent infection of either the pups or mother. The extent to which magainin-like anti-bacterial defenses are significant in elasmobranchs and agnathans is an intriguing matter worth pursuing.

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Figure 1: Extracts were assayed for anti-microbial activity as described in Zasloff (1987). Two microliters of each preparation were assayed and the plate photographed 12 hours after incubation. Duplicate assays are presented.