

**ANTIBACTERIAL PROTEINS IN SKIN MUCUS
FROM RAINBOW TROUT**

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EXTENDED ABSTRACT ONLY -DO NOT CITE

Teleost fish, in common with higher vertebrates, have the ability to mount adaptive 'memory' type immunity against invasive micro-organisms. Unlike mammals, however, adaptive immunity in fish is dependent upon temperature, fish age or level of development. Thus, most teleosts spend a large part of their life without the protection conferred by antibodies and so rely on the non-specific responses of the blood or mucosal surfaces to deal with opportunistic infection. A key part of innate immune defences in animals is the expression of a repertoire of antimicrobial peptides. These are low molecular weight (<10 kDa) proteins that display broad spectrum microbicidal activity (Boman, 1995). Whilst they have been extensively studied in many vertebrate and invertebrate animals, their presence in teleosts has received only scant attention.

To investigate the presence of such proteins in skin secretions of rainbow trout, *Oncorhynchus mykiss*, surface mucus was collected from instimulated adult trout and subjected to ethanol precipitation, 2.5 % trifluoroacetic acid extraction, batch cation exchange on CM-Sepharose and reverse phase HPLC on 20 % acetonitrile gradients and a C₁₈ column (Smith et al., 2000). Fractions were assayed for antibacterial activity by the double layer radial diffusion procedure of Lehrer et al. (1991) using mainly the Gram positive bacterium, *Planococcus citreus*, and lyophilised cell walls of *Micrococcus luteus*, as test agents. Fractions of interest

were run on Tris-tricine 16 % SDS polyacrylamide gels and silver stained by standard procedures.

These experiments revealed that *O. mykiss* mucus contains at least five low molecular weight antimicrobial proteins. One is a conventional lysozyme with a molecular mass of ca 14 kDa and a pI 9.0. Another is an unusual muramidase with a molecular mass of ca 14 kDa but a pI of ca 6.0. Three other non-muramidase proteins are also present. One is a peptide of ca 3 kDa, active against Gram positive bacteria. Its partial amino acid sequence, obtained by Edman degradation, indicates that it has an amphipathic α -helical secondary structure; a feature shown by many other animal antimicrobial peptides. However, it does not appear to be expressed consistently in trout mucus. By contrast, two other antimicrobial proteins in *O. mykiss* mucus, which have molecular masses of ca 6.6 kDa and 7.9 kDa, respectively, occur more constantly throughout the year. One of these (the 6.6 kDa peptide) has been isolated by reverse phase HPLC and subjected to mass spectrometry and partial N-terminus amino acid sequence analyses. It has a molecular mass of 6,621 Da and the first 10 residues are identical to a member of the High Mobility Group (HMG) family of proteins from mammals (Landsman et al, 1986). Broth micro-dilution antibacterial assays (Giacometti et al., 2000) have revealed that this protein is active against both Gram positive and Gram negative bacteria, is inhibited by high salt concentrations and is non-lytic. The fifth protein fractionated by reverse phase-HPLC is a peptide of 7,959 Da, as determined by mass spectrometry. Internal sequence data, obtained after trypsin digestion, show that it contains repeats of a GGHD motif, known to be present in holotricin, an antibacterial peptide previously purified from coelopteran beetles by Lee et al. (1995).

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