

Global exploration of functional biomolecules from amphibian skin

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Twelve novel peptides (Pxt-1 - Pxt-12) were isolated from the skin of *Xenopus tropicalis* using topological mass spectrometry analysis. Among them, Pxt-8, Pxt-9, and Pxt-10 were N-terminus of Pxt-1, N-terminus of Pxt-3, and C-terminus of Pxt-11, respectively. The Pxt-3 and Pxt-11 peptides shared significant sequence homologies with magainins 1, 2 and levetide, respectively that were all isolated from *X. laevis*. Pxt-12 was identical to the *X. tropicalis* XT-6-like precursor previously isolated by ESI-MS/MS. None of the Pxt peptides contained any Cys, Asp, Tyr or Trp, while Ile, Leu and Lys were frequently found as typical frog-skin peptides. RT-PCR analysis confirmed the gene expressions of Pxt-2, Pxt-3, Pxt-4, Pxt-5, Pxt-7 and Pxt-11 in *X. tropicalis* skin. Several ion peaks corresponding to all identified Pxt peptides, were observed with MALDI-MS analysis of *X. tropicalis* secretory fluids, collected after *in vivo* stimulation which suggested that Pxt peptides were definitely secretory molecules. Circular dichroism studies and the Schiffer-Edmundson helical wheel projections suggested that Pxt-5, as well as mastoparan, at least, could form a typical amphiphilic α -helix without a phospholipid or a membrane-mimetic solvent. Moreover, Pxt-2 showed growth inhibitory effects on both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive), at MIC values of 50 and 9.7 ($\mu\text{g/ml}$), respectively. Measurements of dynamic light scattering and the surface tensions of Pxt peptides solutions suggested that both Pxt-2 and Pxt-5 could form associations as micelles and behave like a general surfactant, affording critical association concentrations (CAC) of 38.1 and 57.3 μM , with γ_{CAC} of 46.9 and 38.3 mN/m.