Title: Antimicrobial activity of the venom of the Peruvian snake *Bothrops oligolepis:* involvement of *a* metalloprotease, a type-C lectin and a serine-protease

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Abstract:

Snake venoms contain many biologically active proteins with high therapeutic potential. Among them are antimicrobial proteins, so these venoms have acquired importance as sources for the development of new antimicrobial agents. In this work, we studied the venom of the Peruvian snake *Bothrops oligolepis*. The antimicrobial activity was measured by liquid growth inhibition assay. The venom was active against Gram-positive (*S. aureus*, *M. luteus*) and Gram-negative (*E. coli*, *P. aeruginosa*, *S. choleraesius*) bacteria, but not against *Candida albicans*, *C. parapsilosis* and *C. krusei*. Fractionation by anion-exchange chromatography (on a DEAE-cellulose column) gave eight fractions (P1-P8), being P2, P4 and P8 active against *S. aureus*. P2 was then subjected to gel filtration (on a Superose 12 10/300 column) to provide seven other fractions (P2-I – P2-VII), but only P2-I showed antimicrobial activity against *S. aureus*. Fraction P8 was re-chromatographed on a Superose 12 10/300 column to give three new fractions (P8-I – P8-III), but only P8-II was active against *S. aureus*. Fraction P4 was subjected to fractionation by RP-HPLC (on a C_{18} column) to give fractions P4-I – P4-II, being the P4-II active against *S. aureus*. Biochemical characterization by SDS-PAGE of all fractions showed that P2-I and P8-II contained components with molecular masses of 73 and 14-28 kDa, respectively, and that fraction P4-II presented a homogeneous band corresponding to 27 kDa. Thus, the bands of P2-I, P8-II and the homogeneous P4-II were cut off from the gels and trypsinized to furnish peptides, which were sequenced by MALDI-TOF/TOF mass spectrometry. Some peptide amino acid sequences led to three types of proteins: metalloproteinase (P2-I), C-type lectin (P8-II) and serine proteinase (P4-II). These enzyme activities were confirmed by the measurement of gelatinolytic activity (by zymography to evaluate protease activity) and hemagglutinating activity and carbohydrate specificity (to evaluate C-type Lectin activity). In conclusion, we first report the partial purification of a metalloproteinase and a C-type lectin with antibacterial activity as well as the complete purification of a serine-proteinase with antibacterial activity from *Bothrops oligolepis* venom.

Key words: Antimicrobial activity, snake venom, metalloproteinase, type-C Lectin, serine protease.

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