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ABSTRACT

This research aimed to study the antimicrobial properties of leukocyte extracte of freshwater crocodiles (Crocodylus siamensis). Chromatography technique was used for the purification of the leukocyte extracts. Then, the mechanisms for destroying microorganism of peptides were studied using LC-MS-MS techniques. Results of the study indicated that the leukocyte extracts were able to destroy 8 bacteria; Staphylococcus. epidermidis, Bacillus. megaterium, B. licheniformis TISTR 1010, Pseudomonas. aeruginosa, Ps. aeruginosa ATCC 27853, Salmonella. typhi, S. typhi ATCC 5784, Vibrio. cholerae, and a kind of fungus; Candida. albicans, after testing with 30 species of microorganism. Further more, the leukocyte extracts were purified by using column chromatography. Four protein groups were obtained; P1, P2, P3 and P4. The study found that P1 and P3 proteins were able to inhibit S. epidermidis, S. typhi and C. albicans. Results from zymogram refolding gel and western immunoblotting indicated that the leukocyte extracts contained the proteins size of around 14 kDa that had weak interaction with anti hen egg white lysozyme antibody. Gel filtration was performed to separate the peptides, and Reverse phase high performance liquid column chromatography was done to purify the peptides. Results indicated that 10 peptides could be separated. Four peptides (Leucrocin I-IV) showed antibacterial activity against S. epidermidis, V. cholerae, S. typhi and C. albicans. Leucrocin I, II, III and IV exhibited different properties in antimicrobial activity, and different toxicity to human red blood cells. Electron microscopic study indicated that peptides leucrocin I and II destroyed inner membrane of bacterial cells, resulting in lysis or death of bacterial cells. Thus, the target site of peptide action is at the membrane of bacterial cells. Further, the permeabilization of outer membrane of S. typhi (Leucrocin I and II) and V. cholerae (Leucrocin III and IV) was studied by using detection of the increasing intensity of fluorescence of N-phenylnaphtylamine. Results showed that both peptide present the permeabilization of outer membrane of the bacterial cell tested, measured by the increasing of fluorescence intensity. Measurement of cell lyses performed by measuring DNA concentration at 260 nm showed that S. epidermidis could be lysed by the peptides tested. The study of primary structures of peptides using LC-MS-MS showed that amino acid sequence of Leucrocin I contain NGVQPQKY; and Leucrocin II contain NAGS_LSGWG. Sequence alignment analysis showed no homology to any proteins or polypeptides in data base.

This is the first study to report the antimicrobial peptides in the leukocyte of Siamese crocodiles of both the structure and antibacterial mechanism. These peptides are able to destroy bacteria by perturbing the permeabilization of bacterial cell membrane.