ISOLATION AND CHARACTERISATION OF HOST DEFENCE PEPTIDES OSTRICACINS FROM OSTRICH HETEROPHILS

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ABSTRACT

Host defence peptides are ubiquitous components of innate immunity within all living organisms. These peptides are small, positively charged and amphiphilic molecules. The biological roles of these peptides are direct antimicrobial activity against pathogens and to induce the innate and adaptive immune response within the host.

The research presented in this thesis was focused on isolating host defence peptides from ostrich blood and characterising their antimicrobial properties. Four ostrich β-defensins, named ostricacins-1-4 (Osp-1-4), were successfully purified from ostrich blood. These peptides contained 36-42 amino acid residues, with the main residues including: arginines, lysines, glycines and cysteines. The molecular weight of these four ostricacins ranged between 4-5 kDa. They displayed antimicrobial activity against Gram-negative bacteria and Gram-positive bacteria with minimum inhibitory concentration (MIC), ranging between 1-12 μg/ml. In addition, Osp-2 displayed antimicrobial activity against yeast, with MIC of 6.2 μg/ml. Osp-1 and Osp-2 were further characterised with the investigation of the effects of cationic ions and temperature changes on their antimicrobial activity against Gram-negative bacteria and Gram-positive bacteria. The antimicrobial activity of both peptides significantly declined with the presence of cationic ions. Both peptides were relatively stable when heated to temperatures between 30-70°C. Finally, an investigation of the mode of action of Osp-1 and Osp-2 against Gram-negative bacteria was carried out. Both peptides were compared with a sheep cathelicidin, SMAP-29, and a human α-defensin, HNP-1. SMAP-29 showed the strongest affinity to LPS and it was the most potent peptide to cause disruption of the outer and cytoplasmic membrane. The two ostricacins showed stronger affinity than HNP-1 and they also indicated partial permeabilisation of the outer membrane and a slight depolarisation of the cytoplasmic membrane. HNP-1 did not indicate disruption of the outer membranes or depolarisation of the cytoplasmic membrane. Further investigation indicated that the partial disruption allowed the ostricacins to pass through the membranes and interact with the intracellular components. However, these peptides could not inhibit the bacterial colony forming potential, and therefore, they were considered bacteriostatic. It is recommended that further research be carried out to investigate the feasibility of ostricacins in adding value to existing topical products.

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