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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TABLE OF CONTENTS

Abstract iii

Acknowledgement v

Table of Contents vii

List of Figures xi

List of Table xiii

Chapter 1 Literature Review 1

1.1 Introduction 1

1.2 Animal and Human Immune Systems 2

1.2.1 Innate Immunity 3

1.2.2 Adaptive Immunity 6

1.3 Antimicrobial Peptides 9

1.3.1 Classes of Host Defence Peptides 10

1.3.2 Host Defence Peptides Roles in Immunity 12

1.3.2.1 Roles in Innate Immunity 12

1.3.2.2 Roles in Adaptive Immunity 15

1.4 Mode of Actions 17

1.4.1 Membrane Disruptive Mechanisms 18

1.4.2 Non-Membrane Disruptive Mechanisms 22

1.5 Defensins 23

1.5.1 Animal and Human Defensins 24

1.5.2 Biological roles 27

1.6 Purification and Characterisation of Avian β-Defensins 29

1.6.1 Avian Heterophil β-Defensins 30

1.6.2 Avian Non-Heterophil β-Defensins 32

1.6.3 Antimicrobial Activity of Avian β-Defensins 34

1.6.4 Purification and Characterisation Techniques of Avian Heterophil β-Defensins 36

1.7 Evolutionary of β-defensins 38

1.8 Applications of Host Defence Peptides 41

1.8.1 Current Developments of Host Defence Peptides into Therapeutic Applications 42

1.8.2 Potential Applications for Ostrich Host Defence Peptides 44

1.9 Conclusion 45

1.10 Objectives of Research 47

Chapter 2 Materials and Methods 49

2.1 Materials, Chemicals and Media 49

2.2 Peptide Extraction and Purification 51

2.2.1 Crude Extraction 51

2.2.2 Cation-exchange Chromatography 52

2.2.3 RP-HPLC (Analytical Column) 52

2.2.4 Mass Spectrometry 53

2.2.5 N-Terminal Sequencing 53

2.2.6 Radial Diffusion Plate Assay 54

2.2.7 Protein Concentration Determination 54

2.2.8 Minimum Inhibitory Concentration (MIC) 55

2.3 Assessment of Peptide Activity in Variety of Conditions 55

2.3.1 RP-HPLC (Semi-prep column) 55

2.3.2 MIC using Micro Broth Dilution 56

2.3.3 Monovalent cation Effects 57

2.3.4 Divalent Cation Effects 57

2.3.5 Temperature Effects 58

2.3.6 Analysis of Variance (ANOVA) 58

2.4 Study of Antimicrobial Peptide Mechanisms of Action 59

2.4.1 LPS Binding Assay 59

2.4.2 Outer Membrane Permeabilisation Assay 60

2.4.3 Cytoplasmic Membrane Depolarisation Assay 61

2.4.4 Optical Density and Colony Forming Unit Measurements 62

2.4.5 DNA Gel Electrophoresis 62

Chapter 3 Extraction and Purification of Host Defence Peptides from Ostrich Heterophils 65

3.1 Introduction 65

3.2 Results and Discussion 67

3.2.1 Peptide Extraction 67

3.2.2 Peptide Purification with Cationic Exchange 69

3.2.3 Peptide Purification with RP-HPLC (Analytical Column) 70

3.2.4 Mass Spectrometry and N-terminal sequence Analysis 72

3.2.5 Antimicrobial activity of OSP-1-4 74

3.2.6 Amino Acid Composition Analysis 75

3.2.7 Evolutionary Analysis of the Ostricacins 77

3.3 Conclusion 81

Chapter 4 Investigation of Antagonist Factors Affecting Antimicrobial Activity of Ostricacins-1 and 2 on *E. coli* O157:H7 and *S. aureus* 1056MRSA 83

4.1 Introduction 83

4.2 Results and Discussion 85

4.2.1 Effect of Monovalent Cation on E. coli O157:H7 85

4.2.2 Effect of Monovalent Cation on S. aureus 1056MRSA 86

4.2.3 Effect of Divalent Cation on E. coli O157:H7 89

4.2.4 Effect of Divalent Cation on S. aureus 1056MRSA 90

4.2.5 Effect of Temperature 92

4.3 Conclusion 96

Chapter 5 Investigation of Mechanisms of Action of Ostricacins against Gram-Negative Bacteria 99

5.1 Introduction 99

5.2 Results and Discussion 101

5.2.1 LPS Binding Assay 101

5.2.2 Outer Membrane Permeabilisation Assay 102

5.2.3 Cytoplasmic Membrane Depolarisation Assay 105

5.2.4 Kill curve 108

5.2.5 DNA Gel Electrophoresis 109

5.3 Conclusion 113

Chapter 6 Conclusions and Recommendations 115

6.1 Overall Conclusions 115

6.2 Recommendations for Future Work 118

References 121

Appendix 1 Raw Data and Calculations from Investigation of Antagonist Factors Affecting Osp-1 and 2 133

1.1 Example Calculations of MIC 133

1.2 Calculation of the Average and Confidence Intervals of MIC from the Raw Data 137

1.3 Analysis of Variance (ANOVA) of MIC Data from Different Conditions ... 139

Appendix 2 Replicates Results of the Investigation of Mechanisms of Actions of Ostricacins 141

2.1 Raw Data of LPS Binding Assay and Example Calculations of Fraction Fluorescence Inhibited 141

2.2 Results of LPS Binding Assay 142

2.3 Results of Outer Membrane Permeabilisation Assay 144

2.4 Results of Cytoplasmic Membrane Depolarisation Assay 146

2.5 Results of Kill Curves on *E. coli* O111 148

Appendix 3 List of Publications 151

LIST OF FIGURES

Figure 1.1 Molecular Components of Innate Immune System..... 4

Figure 1.2 The Phagocytosis Mechanisms of a Phagocyte on a Microbe or a Foreign Particle..... 5

Figure 1.3 Molecular Components of Adaptive Immune System. 7

Figure 1.4 Adaptive Immune Responses Upon Recognition of Antigens. 8

Figure 1.5 Structural Representations of The Four-Classes Host Defence Peptides..... 11

Figure 1.6 Host Defence Peptides Roles in Innate and Adaptive Immunity..... 14

Figure 1.7 The three models of host defence peptides membrane disruptive mechanisms on Gram-negative membrane. 20

Figure 1.8 Micellar-aggregate on Gram-negative bacteria. 21

Figure 1.9 Intracellular Targets of Host Defence Peptides in *E. coli*. 22

Figure 1.10 Schematic processing of two nine-amino-acid segments of mutated α-defensins precursor into θ-defensins. 26

Figure 1.11 Sequence and Structure of Human Neutrophil Peptide-3 (HNP-3) and Human β-Defensins 2 (HBD-2). 27

Figure 1.12 Interaction of Defensins with Immune System..... 28

Figure 1.13 Evolutionary tree of existing β-defensins..... 40

Figure 3.1 Microscopic Examination (400x) of Blood Cells Stained with Diff-Quick.. 68

Figure 3.2 Ion-Exchange Chromatograph of the Ostrich Crude Extract. 69

Figure 3.3 RP-HPLC chromatograph of Fraction 2 of the Cationic Exchange Column. 71

Figure 3.4 Phylogenetic tree illustrating the homology of Osp-1-4 with other avian and mammalian β-defensins. 78

Figure 4.1 The effect of monovalent cations, Na^+ (A) and K^+(B), on the MIC of Osp-1 and 2 against *E. coli* O157:H7..... 86

Figure 4.2 The effect of monovalent cations, Na^+ (A) and K^+ (B), on the MIC of Osp-1 and 2 against *S. aureus* 1056MRSA. 87

Figure 4.3 The effect of divalent cations, Mg^{2+} (A) and Ca^{2+} (B), on the MIC of Osp-1 and 2 against *E. coli* O157:H7..... 90

Figure 4.4 The effect of divalent cations, Mg^{2+} (A) and Ca^{2+} (B), on the MIC of Osp-1 and 2 against *S. aureus* 1056MRSA. 91

Figure 4.5 The effect of temperatures on the MIC of Osp-1 and 2 against *E. coli* O157:H7 (A) and *S. aureus* 1056MRSA (B). 93

Figure 5.1 DPX/LPS binding assay of SMAP-29, HNP-1, OSP-1 and 2 indicating the affinity of the peptides to LPS. 101

Figure 5.2 Kinetics of NPN uptake assay using *E. coli* UB 1005 that was treated with SMAP-29, HNP-1, OSP-1, OSP-2 and Polymyxin B (PXB). 103

Figure 5.3 Cytoplasmic membrane depolarisation that induced by SMAP-29, HNP-1, OSP-1, OSP-2 and Gramicidin. 106

Figure 5.4 Measurements of OD_{600nm} taken in parallel with the colonies counting of OSP-1 and 2 against *E. coli* O111. 108

Figure 5.5 Interactions between different ratios of bacterial λDNA and the host defence peptide using DNA Gel Electrophoresis. 110

Figure 5.6 Model illustrating the mechanism of ostricacins on Gram-negative bacteria 112

LIST OF TABLE

Table 1.1 Comparison of Innate and Adaptive Immune Response. 3

Table 1.2 Comparison of Vertebrate Defensins. 25

Table 1.3 Amino acid sequences of existing avian and mammalian β-defensins. 30

Table 1.4 Antimicrobial activity of avian β-defensins. 35

Table 2.1 Sequence and Molecular Mass of The Ostrich Antimicrobial Peptide, Osp-1 and 2. 56

Table 2.2 Ingredients for Peptide-DNA Binding Gel Electrophoresis. 63

Table 3.1 Plate Assay of Ion Exchange Column Fractions and Crude Extract. 70

Table 3.2 Plate Assay of RP-HPLC Peaks. 71

Table 3.3 Mass Spectrometry and N-terminal Sequencing of Osp-1-4. Using FASTA, these Ostricacins were aligned with chicken gallinacins and turkey THPs. 73

Table 3.4 Minimum Inhibitory Concentrations (μg/ml) of Ostricacins-1-4. 75

Table 3.5 Amino Acid Composition of Ostricacins-1-4 (mole percent) 76