Isolation of Ovine Hemoglobin, its Apoglobins and Peptides, for the Determination of Antimicrobial Activities

A thesis presented in partial fulfilment of the requirements for the degree of

Masterate of Engineering

in

Biotechnology

at Massey University, Palmerston North,

New Zealand.

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2012

Abstract

The objective of the research presented here was to investigate the properties of ovine hemoglobin, its subunits and its peptides as potential antimicrobial therapeutics or biopreservatives. This objective addresses two issues. The first is the growing lack of novel and effective antimicrobials against drug resistant microorganisms (superbugs); ovine hemoglobin and its components may provide an effective alternative. The second is the large volume of ovine blood generated from sheep slaughter in New Zealand, from which, currently only low value products such as blood meal are made; it is proposed that this blood be used as a source of antimicrobial peptides - high value products.

The research was divided into three parts. First, ovine hemoglobin was isolated from whole blood using isotonic ammonium chloride lysis of erythrocytes and the subunits were separated and de-hemed by acid acetone precipitation. Two conditions for pepsin digestion of hemoglobin into short random coiled peptides were also identified - hemoglobin as a starting substrate in its native and denatured conformations.

Secondly, the alpha and beta apoglobins were separated into their respective fractions by semi-preparative RP-HPLC. The kinetics of the two pepsin digestion conditions were also compared by RP-HPLC and it was found that denatured hemoglobin is digested into peptides significantly more rapidly than native hemoglobin, and a different set of peptides resulted. However, observation of RP-HPLC profiles showed that ovine hemoglobin, unlike bovine hemoglobin (mentioned in the literature), was not fully denatured by 5.3M urea.

Thirdly, native ovine hemoglobin, its apoglobins, and its peptides from pepsin digestion were tested for antimicrobial activity using the radial diffusion assay. The native hemoglobin tetramer displayed no activity at the highest concentration of 30mg/ml, but the separation of subunits at 0.5 to 2.0mg/ml provided moderate activity against *E.coli* and *S.aureus*. A greater proportion of the RP-HPLC fractions from the denatured hemoglobin pepsin digest were active towards *E.coli* and many were also more potent in comparison to those from the native digest. After further testing the denatured digest fractions against *S.aureus* and *C.albicans*, six candidates were selected for mass spectroscopy and MIC (Minimum Inhibitory

Concentration) testing based on their potency and reproducibility in RP-HPLC. Most of the peptides within these complex fractions were largely small random coils as desired. However, none of these fractions were highly antimicrobial, in fact, they had poor MICs ranging from 12mg/ml to 44mg/ml against the three test organisms.

It is recommended that further research be carried out focussing on the antimicrobial activity of a wider range of peptides with various secondary structures and peptide lengths. This would involve optimising digestion conditions and analysis of peptides from different degrees of hydrolysis. Synthetic peptides based on this information can be tested for their activities also. Then the feasibility of ovine hemoglobin peptides as components of antimicrobial treatments and products can be further investigated.

Acknowledgements

First and foremost I would like to thank my project supervisor, Associate Professor Pak-Lam Yu, for all the support, knowledge and enthusiasm you have brought to my research and my undergraduate years as a student.

I would like to thank Professor Kim Brogden from the University of Iowa for providing me with further insight into his group's research on ovine antimicrobial peptides, and relevant documents. I must also thank Ms Diana Carne from the Centre for Protein Research at the University of Otago. Diana, your efforts to go above and beyond with the mass spectrometry and identification of my peptide samples are greatly appreciated.

Next I would like to thank the technical and administration staff of the School of Engineering and Advanced Technology. In particular, I owe a big thanks to the staff in the Microbiology Suite laboratory - Ann-Marie Jackson, Judy Collins, Julia Fordyce Stevenson, John Sykes, and John Edwards. These people were always more than willing to help with any technical difficulties, or simply locating equipment.

Thanks to all the other post-graduates and honours students for your positivity, especially when things weren't going to plan. It is comforting to know there is always someone else who has been in the same position as you.

Finally, I would like to thank my family and friends for their love and support. Even though most of you live far away I appreciate that I am in your thoughts. Most of all I am thankful to be blessed with such an amazing and loving family. I dedicate this thesis to Mum, Dad, Darren and Cynthia. Thank you for your constant faith in me. When times are tough I think of you and then nothing seems unattainable.

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