

## Novel targets for control of the sheep blowfly and other insect pests.

Vern Bowles and Norm Mancuso

Centre for Animal Biotechnology, University of Melbourne, 3010, Victoria.

Email: [v.bowles@vet.unimelb.edu.au](mailto:v.bowles@vet.unimelb.edu.au)

### Summary

*The identification of novel targets for controlling the sheep blowfly is an important process for discovering new generation compounds that are safer and more environmentally friendly. Experiments in our laboratory have indicated a role for serine proteases in influencing egg hatching in the sheep blowfly. Isolation of the proteases involved using benzamidine affinity chromatography identified a group of molecules with homology to both trypsin and chymotrypsin-like proteases. These proteases represent potential new targets for controlling egg hatching in the sheep blowfly.*

### Keywords

Sheep blowfly, enzymes, egg hatch, inhibitors

### Introduction

The identification of critical biochemical pathways within a parasite's lifecycle is a key stage in the development of improved parasite control. Furthermore, the ability to produce highly specific inhibitors against crucial enzymatic targets represents an equally important step in the production of safer and more environmentally friendly anti-parasite compounds. Recent research at the Centre for Animal Biotechnology (CAB), The University of Melbourne, has focussed on understanding developmental processes within the sheep blowfly *Lucilia cuprina* with the aim of discovering novel targets for the development of highly specific therapeutics to control this important ectoparasite.

Flystrike infections are initiated when eggs are laid in the wool or on the skin of sheep. The eggs then mature over several hours as the embryo develops until the first stage larvae emerge. During this period the eggs are accessible to agents that may be able to disrupt the normal physiological processes that are responsible for egg hatch. Determining the important biological processes occurring during this period may identify new targets for control not only for *L. cuprina*, but also for other ectoparasites including other flies and other insect pests.

Previous studies have indicated a key role for enzymes/proteases in the hatching of a number of endoparasites (Young *et al.*, 1999). While there are reports demonstrating a role for enzymes in the egg hatching of non-parasitic insects, there is very little information concerning the importance of enzymes in the hatching of ectoparasites with the exception of the sucking lice, *Pediculus sp.* (Baudisch, 1958), and the mosquito, *Aedes aegypti* (Thomas, 1944). Enzymes have been implicated in important aspects of the lifecycle of the sheep blowfly through their involvement in wound initiation and larval nutrition (Young *et al.*, 1996). In addition our studies identified developmentally regulated enzymes released from the egg during egg hatch, indicating a potential role for these proteases in this process (Young *et al.*, 1997). This paper describes the progress on characterising the enzymes present in the hatching fluids, termed egg shell washings (ESW) of the sheep blowfly and outlines strategies for the use of these molecules as targets for high throughput screens to identify novel inhibitors.

### Methods

The studies described in this paper involve the use of a highly sensitive egg hatching *in vitro* bioassay as previously outlined (Young *et al.*, 1997; Young *et al.*, 2000). Earlier studies in our laboratory had indicated that the ESW were composed predominantly of serine proteases. A

benzamidinium affinity column was therefore used in order to isolate serine proteases within the ESW and evaluate the effect of the bound molecules compared to the unbound on the rate of egg hatch. The bound molecules from the affinity column were isolated and characterised on the basis of their N-terminal amino acids and the sequences obtained compared to a number of databases using the Blast logarithm.

## Results

Benzamidinium affinity chromatography resulted in an enrichment of a group of eight molecules that specifically bound to the column ranging in size from 20-55kDa. This semipurified bound fraction was tested in the egg hatch assay and found to significantly enhance the rate of egg hatch compared to the unbound material. N-terminal amino acid sequence data was obtained for each of the eight components in the Benzamidinium Affinity Purified Fraction (BAPM) and significant homologies to a number of serine proteases were identified. It was found that BAPM 1-6 shared significant similarity with a chymotrypsinogen of *L. cuprina* (Casu *et al.*, 1994) in a 20 amino acid overlap. A similar degree of homology was also identified to a serine protease from *Drosophila heteroneura*, a trypsin precursor from *Aedes aegypti* and a serine protease precursor from *Drosophila melanogaster*.

## Discussion

The development of novel approaches to identify potentially useful targets within specific stages of a parasite's lifecycle represents a significant advancement in the field of parasite control. We have chosen to focus on the critical process of egg hatch in the sheep blowfly, as it is one of the earliest developmental stages of this fly. A number of enzymes have been identified that represent potential targets for intervention. The development of highly specific inhibitors capable of effecting the hatching process offers the potential for controlling this fly before any damage to the host has occurred. Proteases have been used as targets for the development of therapeutics against other parasitic infections. For example, specific inhibitors have been designed to block the activity of an elastase protease important in skin penetration by *Schistosoma* sp. (Ring *et al.*, 1993). More recently, the effectiveness of this approach has been demonstrated (Engel *et al.*, 1998), through the use of synthetic protease inhibitors to cure an experimental *Trypanosoma cruzi* infection in mice.

The development of highly specific protease inhibitors that specifically target egg hatch in the sheep blowfly leaving non-target species unaffected would provide an alternative control strategy that is potentially more environmentally responsible compared to the current use of more broad spectrum chemicals. This would provide a significant benefit to Australian sheep producers. In addition, the generic approach being used to identify novel targets provides future opportunities to identify both key targets involved in egg hatch and specific inhibitors of other pests of economic importance.

## Conclusion

The results from these studies indicate that benzamidinium-binding molecules are able to significantly influence egg hatch in *L. cuprina*. Partial characterisation of these molecules by N-terminal sequencing indicates a predominance of chymotrypsin-like molecules confirming previous studies using serine protease inhibitors. Future studies will require the expression of active forms of these enzymes and their use in high throughput screens against natural and synthetic chemical libraries to identify inhibitory molecules. These compounds will then need to be further evaluated as novel targets for control of egg hatching in blowfly.

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