Inducible tolerance to *Bacillus thuringiensis* (Bt) endotoxins based on cell-free immune reactions

by

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Abstract

The use of *Bacillus thuringiensis* (Bt) endotoxins to control insect vectors of human diseases and agricultural pests is threatened by the possible evolution of resistance in major pest species. Despite the use of Bt-endotoxins in transgenic crops covering about 80 million hectares, the precise details of how endotoxins bind to gut cells to kill insects are poorly understood. This limitation impedes our understanding of potential mechanisms of insect resistance to Bt-endotoxins other than the loss or modification of receptors. We explored a novel mechanism, where tolerance to Bt-endotoxins is correlated with an elevated immune status involving cell-free immune reactions in the gut lumen. The thesis project is based on investigations of a laboratory culture of the flour moth *Ephestia kuehniella*, which showed induction of hemolymph melanization a sign of immune induction, after feeding sub-lethal concentrations of a Bt-formulation. Since the elevated immune status was transmitted to subsequent generations by a maternal effect, an increase of toxin in the food by increments was possible every generation. Investigations of strains exposed to various toxin levels revealed a correlation between systemic immune induction and Bt-tolerance. Molecular analysis revealed a possible mechanism of immune mediated inactivation of Bt-endotoxins in the gut lumen.

To gain more specific information about the effector pathways involved in the protection against the toxin, we studied the effects of Bt-toxin formulations in susceptible (non-induced) and tolerant (immune-induced) larvae after natural (parasitism-mediated) and chemical (tropolone-mediated) suppression of defence reactions. Although melanization in hemolymph was significantly reduced, there was no significant effect on susceptibility to the toxin in parasitised or tropolone-treated larvae. This suggests that melanization of hemolymph is correlated with an elevated immune status but not responsible for the observed tolerance to Bt-toxin. This leaves coagulation as a likely mechanism for Bt-tolerance in the gut lumen. To examine whether hemolymph proteins exist in the gut lumen were they could function as pro-coagulants to

inactivate the toxin; we compared gut and plasma proteins of immune-induced larvae with those of non-induced larvae. This analysis revealed that the lipid carrier lipophorin represents a major component in the gut lumen and interacts with mature Bt-toxin like an oligomeric lectin that may inactivate the toxin in a cell free coagulation reaction in the gut lumen before it can reach the brush border membrane.

Further analysis showed that lipophorin particles are the regulatory and effector components in innate immune defence reactions, which are involved in the recognition and inactivation of lipopolysaccharides (LPS) and bacteria even in the absence of hemocytes. Examination of proteins from lipophorin particles separated by low-density gradient centrifugation have shown that in immune-induced insects sub-populations of lipophorin particles are associated with pattern recognition proteins, phenoloxidase and regulatory proteins that activate prophenoloxidase. Moreover, interactions with lectins resulted in the assembly of lipophorin particles into cage-like coagulation products, effectively protecting the surrounding tissues and cells from the potentially damaging effects of pathogens and phenoloxidase products. This cell-free immune reaction mediated by lipophorin particles may potentially involve in detoxification of pore-forming toxins (Bt-endotoxins) in the gut lumen.

This thesis is based on the following publications and manuscripts:

Rahman, M.M., Ma, G., Roberts, H.L.S. and Schmidt, O. (2006). Cell-free immune reactions in insects. *Journal of Insect Physiology* **52**: 754-762

Schmidt, O., **Rahman, M.M**., Ma, G., Theopold, U., Sun, Y., Sarjan, M., Fabbri, M. and Roberts, H.L.S. (2005). Mode of action of antimicrobial proteins, pore-forming toxins and biologically active peptides (Hypothesis). *Invertebrate Survival Journal*. **2**: 82-90.

Rahman, M.M., Roberts, H.L.S., Sarjan, M., Asgari, S. and Schmidt, O. (2004). Induction and transmission of *Bacillus thuringiensis* tolerance in the flour moth *Ephestia kuehniella*. *Proceedings of the National Academy of Sciences of the United States of America* **101(9)**: 2696-2699.

Rahman, M.M., Roberts, H.L.S. and Schmidt, O. (2004). The development of the endoparasitoid *Venturia canescens* in Bt-tolerant, immune induced larvae of the flour moth *Ephestia kuehniella*. *Journal of Invertebrate Pathology* **87 (2-3)**: 129-131

Rahman, M.M., Roberts, H.L.S. and Schmidt, O. Tolerance to *Bacillus thuringiensis* endotoxin in immune-suppressed larvae of the flour moth *Ephestia kuehniella*. *Applied and Environmental Microbiology* (under review).

Rahman, M.M. and Schmidt, O. Cell-free sequestration of mature *Bacillus thuringiensis* endotoxin by lipophorin particles. *Applied and Environmental Microbiology* (under review).