SUMMARY

The floral organs and wounded leaves of *Nicotiana alata*, the ornamental tobacco, produce a serine protease precursor molecule (NaPI) which is cleaved into six individual inhibitors of 6 kDa. Two of these inhibitors have chymotrypsin activity and four have trypsin activity. These proteinase inhibitors inhibit trypsins and chymotrypsins in the gut of Lepidopteran pests, and when incorporated into artificial diets or transgenic plants they have a detrimental effect on growth and development of *Helicoverpa punctigera* larvae, but some larvae are unaffected. This study describes the characterisation of one of the major targets for NaPI, the chymotrypsins and their potential role in the tolerance of larvae to ingestion of NaPI. Chymotrypsin cDNAs were isolated from a cDNA library made from the gut *H. punctigera* larvae. Phylogenetic analysis of the encoded protein sequences indicated there were six major families of chymotrypsin genes in Lepidoptera.

In vitro inhibition assays were used to screen a number of proteinase inhibitors against chymotrypsin activity from gut of *H. punctigera* larvae. These assays revealed that NaPI was a poor inhibitor when compared to potato inhibitor I (Pot I), which was a strong inhibitor of chymotrypsin activity. A purification protocol was designed to isolate the C1-insensitive chymotrypsins in order to characterise their PI-resistant properties. The cDNAs encoding a C1-sensitive and insensitive chymotrypsin were isolated and used to construct molecular models. Comparison of the models indicated that a single amino acid change from a glutamine in the C1-sensitive chymotrypsin to an arginine in the C1-insensitive chymotrypsin may prevent binding of the NaPIs.

A number of important biochemical and molecular tools have been developed in this study. Specific antibodies were produced against both the C1-sensitive and insensitive chymotrypsins, which will allow monitoring of the levels of both these enzymes in response to PIs. The potential to engineer NaPI to target NaPI-insensitive enzymes was investigated using site-directed mutagenesis and phage display. A library of C1-variants was produced and has the potential to be screened against insects to identify new inhibitors.