

## PROTEINACEOUS INHIBITORS FROM LEGUME SEEDS: STRATEGIES TO CONTROL OF INSECT PESTS

Maurício Pereira de Sales

Departamento de Bioquímica, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN, cep 59072-970.

Enzyme proteinaceous inhibitors have been purified and characterized from a variety of plant sources. The role these inhibitors as defensive compounds against predators was studied as early as 1947 when Mickel and Standish observed that larvae of certain insects were unable to develop on soybean products. Protein inhibitors of proteinases from the Kunitz soybean inhibitor superfamily (SKTI) form one of major groups of inhibitors from plants. Inhibitors from this superfamily with molecular weights 20-34 kDa and two disulfide bonds were previously found mainly in legumes and cereals. These inhibitors attract particular interest because of their likely participation in the protection of plants from damage caused by insects and phytopathogenic microorganisms. In this study two inhibitors from wild legume seeds were purified, characterized, determined their N-terminal amino acid sequences and *in vitro* and *in vivo* potential deleterious effects against insect pests were examined. CpaTI (*Crotalaria pallida* Trypsin inhibitor) and TTI (*Tamarindus indica* Trypsin inhibitor) are protein with molecular weights around 20-32 kDa, with subunit linked by disulfide bridges. N-terminal 25-26 amino-acid sequence of the inhibitors were determined by Edman degradation and showed that they belong to the Kunitz type superfamily. The specificities of the inhibitors were different, CpaTI showed be bi-functional, inhibiting cysteine and serine proteinases, and TTI inhibited only serine proteinases like trypsin. TTI and CpaTI were noncompetitive inhibitors with  $K_i$  values of  $1.7 \times 10^{-9}$  M and  $8.8 \times 10^{-7}$  M, respectively. Biological effects of both inhibitors were evaluated. CpaTI and TTI inhibited, in different degrees, digestive enzymes from Lepidoptera (*Spodoptera frugiperda*, *Alabama argillacea*, *Plodia interpunctella*) Coleoptera (*Anthonomus grandis*, *Callosobruchus maculatus* and *Zabrotes subfasciatus*) and Diptera (*Ceratitis capitata*) guts. *In vivo* susceptibility of *Callosobruchus maculatus* and *Ceratitis capitata* to CpaTI was evaluated. Due bi-functionality of CpaTI, it was added to artificial diets and offered to *C. capitata* and *C. maculatus* larvae. Results showed that *C. maculatus* was more susceptible to CpaTI with an  $LD_{50}$  of 3.0 and  $ED_{50}$  of 2.17%. *C. capitata* larvae were more resistant to CpaTI, in disagreement with the *in vitro* effects. The larvae were more affected at lower concentrations, causing 27% mortality and 44.4% mass decrease. The action was constant at 2-4% (w/w) with 15% mortality and 38% mass decrease. *In vivo* bioinsecticidal assays toward *C. capitata* was developed with TTI too. The concentration of TTI (w/w) in the artificial seed necessary to cause 50% mortality ( $LD_{50}$ ) of larvae was 3.6%, and that to reduce mass larvae by 50% ( $ED_{50}$ ) was 3.2%. Furthermore, the mass *C. capitata* larvae were affected at 53.2% and produced 34% mortality at a level of 4.0% (w/w) of TTI incorporated in artificial diets. The weak *in vivo* effect of CpaTI and TTI to *C. capitata* can be due two reasons: first, instability of the enzyme-inhibitor complex, promoting slight activity *in vivo*, as observed in Zymogram using SBTI that showed interactions between enzyme and inhibitor, with complex retardation during electrophoresis; second, due the complex digestive system of *C. capitata* larvae, which is based on serine proteinases, such as chymotrypsin and trypsin, with predominance of chymotrypsin-like enzymes. As result, strategies to pest control using proteinases inhibitors require the isolation of inhibitors that are also active towards these insensitive proteinases.