Hydrolysis of organophosphorus compounds by an esterase isozyme from insecticide resistant pest Helicoverpa armigera

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Esterase activity of resistant and susceptible H. armigera were compared in gels with different substrate such as naphthyl acetate, naphthyl phosphate, paraoxon and monocrotophos. Whole body extract of resistant H. armigera hydrolyzed paraoxon, monocrotophos and naphthyl phosphate in gels. Resistant H. armigera showed high esterase, phosphatase and paraoxon hydrolase activity compared to susceptible ones.

Keywords: Esterase isozyme, Insecticide resistance, Helicoverpa armigera, Organophosphorus compounds.

Helicoverpa armigera (Hübner) is an economical pest of cotton, pulses and vegetable crops. In Asia and Australia H. armigera has developed resistance virtually to all insecticides that have been applied against it in any quantity. Several mechanisms of resistance have been identified in H. armigera population in various parts of the world.

The most usual resistance mechanism consists of an increased capacity for detoxification. Variety of enzymes and enzyme systems are available in most insects, reflecting the need to cope with many noxious chemicals in their environment. Esterases, oxidases, tranferases and other enzymes have been found to have an augmented capacity in some resistant strains due to increased efficiency or amount of enzyme.

Model substrates such as naphthyl esters are commonly used in preference to insecticidal esters, which are normally more difficult to use as assaying agents. Insecticide breakdown by metabolism is the common mechanism that has evolved to protect insects. Role of increased 1-naphthyl esterase activity in resistance was confirmed by enhanced hydrolysis of several insecticides by the resistant insects such as Myzus persicae, Culex quinquefasciatus, and Musca domestica. In the paper, an attempt has been made to study the hydrolysis of some of the organophosphorus i.e. paraoxon and monocrotophos compounds in the polyacrylamide gels by esterase isozyme from resistant H. armigera.

A laboratory susceptible strain of Helicoverpa armigera, obtained from Project Directorate of Biological Control, ICAR, Bangalore was reared for several generations in the lab and used as a susceptible strain for comparative studies of enzymes. The resistant pests were provided by Dr S S Hudikeri, Agriculture Research Station, Gulbarga. These pests have developed broad resistance to various insecticides.

Fifth instar larvae of the resistant strains were dissected to remove the gut contents and homogenized in Tris-HCl buffer (0.1 M; pH 7.6) containing phenyl thiourea (0.1 mM) and Triton X-100 (0.01%). The homogenate was filtered through a four layered cheese cloth and then centrifugated at 10,000 g for 30 min at 4°C and the supernatant was used as an enzyme source. Protein concentration was determined by Lowry et al.

Esterases were separated by discontinuous, non-denaturing polyacrylamide gel (12%) electrophorosis by the method of Hughes and Raftos. The gels were stained for esterase activity at 37°C for 10 min using a solution containing 0.02% w/v α-naphthyl acetate dissolved in acetone and 0.05% fast blue RR salts in phosphate buffer (0.1 M; pH 6.8).

For insecticide hydrolysis activity, primarily the gels were incubated with citrate buffer (0.1 M; pH 4.5) for about 10 min and the gels were transferred to the 50 ml of solution containing calcium chloride (0.2%) and 20 mg of substrate (monocrotophos and α-naphthyl phosphate in 0.1 ml of acetone) in 50 mM of barbitur buffer (pH 9.0) for 10-60 min at room temperature. Gels were transferred to cobaltous acetate (2%) solution and incubated for 5 min, and then the gels were washed in distilled water. Further the gels were incubated with dilute solution of ammonium sulfide. The phosphate ions liberated were precipitated in the form of calcium phosphate. The latter was transformed first into cobalt phosphate and then, into black cobalt sulfide. Paraoxon hydrolase activity was detected in the gel by liberation of p-nitrophenol at the site of activity.

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Fig. 1.—Polyacrylamide gel electrophoresis of general esterases stained with α-naphthyl acetate and fast blue RR salt. Lane R, 100 μg of enzyme extract of resistant pest. Lane S, 100 μg of enzyme extract of susceptible pest. Experimental details are given in text.

Esterase activities were assayed by the method of Meghji et al. 22 Reaction was carried out in a final volume of 3 ml containing 50 mM of phosphate buffer (pH 7.4), 100 μM of p-nitrophenyl acetate as substrate and 50 μl of enzyme extract. The tubes were incubated at 37°C for exactly 10 min. The reaction was terminated by addition of 200 μl of sodium carbonate (0.3 M).

Paraoxon hydrolase activity was measured spectrophotometrically. The mixture contained an aliquot of enzyme, methyl paraoxon (1 mg) in 10 μl of acetone in a total volume of 1 ml of Tris-HCl buffer (0.1 M; pH 8.3). The reaction was recorded continuously at 405 nm at 30°C. Activity of the enzyme was calculated by the rate of formation of p-nitrophenol. Non-enzymatic degradation of methyl paraoxon was corrected in the reference cell.

Polyacrylamide gel electrophoresis showed that there were differences in esterase activity between resistant and susceptible *H. armigera*. While most bands were common to both strains, susceptible *H. armigera* lacked E1, E7 and E8 esterases when naphthyl acetate was a substrate (Fig. 1), whereas susceptible strain did not show any activity with the above insecticides (data not shown). The resistant pest extract exhibited esterase activity in gels with all the substrates tested such as naphthyl phosphate, paraoxon and monocrotophos (Fig. 2) and identified as an E6. The resistant pest showed the paraoxon hydrolase activity (0.195 ± 0.12 nmole min⁻¹ mg⁻¹) whereas susceptible pest whole body extract did not show paraoxon hydrolase activity, and showed low esterase activity (0.027 nmole min⁻¹ mg⁻¹) in vitro.

These results indicated that quantitative differences in individual esterases between both resistant and susceptible insects as well as novel esterase found only in resistant pests. The resistance related esterase activities were monitored in the gels by using different OP compounds.

Increased levels of esterase activity have been reported as a mechanism of resistance in many insects and is particularly well documented in the pea aphid, *Myzus persicae* and species of *Culex* sp. *mosquito*. Increased enzyme activity can be either due to a more catalytically efficient enzyme able to hydrolyze insecticides or to higher amounts of enzyme which protect acetylcholinesterase (AChE) by offering a large number of alternative sites of phosphorylation and therefore reduce the amount of OP available to bind AChE. This is particularly well illustrated by comparison of a malathion carboxylesterase in the sheep blowfly 23 and esterase E4 in *Myzus persicae* 24.

In insects, increased production of esterase is a result of an amplification of structural gene encoding for these enzymes 25. For *H. armigera*, the genetic basis of the increased esterase production is not yet understood. However, enzyme activity data has been shown that resistant *H. armigera* are grouped in a number of discrete populations which may be the result of a series of gene amplifications.

References


