Preparative Nondenaturing Gel Electrophoresis Used in the Purification of an Esterase Involved in Insecticide Resistance

Dr Albert J Ketterman and SHP Parakrama Karunaratne, Dept. of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT UK

Introduction

In attempts to control *Culex quinquefasciatus* as a worldwide vector of diseases such and filariasis and encephalitis, mosquito field populations are sprayed with organophosphate insecticides. However, mosquitoes develop tolerance to organophosphate.

One of the mechanisms of organophosphate resistance involves either a different form and/or increased amounts in a carboxyolesterase (EC 3.1.1.1). Carboxylesterase A_2 is one of two enzymes found elevated together in *Culex* mosquitoes. The putative mechanism involving the resistance-conferring carboxylesterase A_2 is an increased expression in this enzyme for the sequestration of, and cross-resistance to, various organophosphate and carbamate insecticides (Raymond et al. 1991).

The question of a qualitative change in the carboxylesterase has not been fully explored with a purified enzyme. Here we report the use of Bio-Rad's Model 491 prep cell for preparative, nondenaturing gel electrophoresis as a final step in purifying the native carboxylesterase A_2.

Methods

Carboxylesterase A_2 was isolated from the supernatant of a mosquito homogenate by sequential ion exchange, hydrophobic interaction, and hydroxyapatite chromatography, followed by a final purification step using the Model 491 prep cell (Ketterman et al. 1992).

Preparative Native PAGE

As suggested in the Model 491 prep cell instruction manual, the gel composition (monomer concentration) for maximum resolution was determined empirically by analytical, nondenaturing PAGE using the Mini-PROTEAN® II cell (Figure 1). Native PAGE separates proteins both by charge and size and we had protein bands above and below our A_2 protein band of interest. At each gel concentration used, the distance was measured between the band of interest and the nearest bands above and below it. The gel concentration was

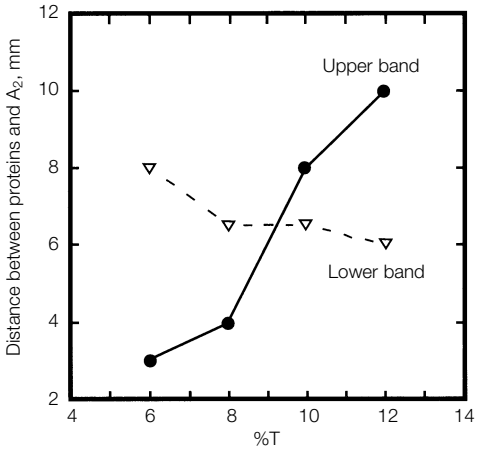

Fig. 1. Determining the optimal gel composition for the Model 491 prep cell. Preliminary experiments were performed on analytical Mini-PROTEAN II gels, using the sample to be purified with the Model 491 prep cell. After protein detection, the distance was measured between the band of interest and the nearest bands above and below it for each gel concentration used.

9%T/3%C where the two curves intersect (Figure 1). The electrode running buffer, in both the upper and the lower buffer chambers, and the buffer used to prepare the gel, was 0.1 M Tris-borate (pH 8.0) containing 2.0 mM EDTA. The sample, after elution from hydroxyapatite, was concentrated using a Centriprep 10 concentrator (Amicon) to approximately 0.5 ml. The sample remained in the elution buffer from the hydroxyapatite column, that is, approximately 60 mM phosphate buffer (pH 6.8). The concentrated sample was mixed with a 5x solution of glycerol and marker dye Xylene Cyanole FF to give a final concentration of 10% glycerol and 0.02% dye marker. The sample, approximately 1.6 mg of protein, was loaded on a 9%T/3%C, 6 cm gel in the Model 491 prep cell (28 mm ID). The Model 491 prep cell had previously been equilibrated overnight in a 4ºC coldroom. Electrophoresis was performed at 15 W constant power (350–400 V). Elution (1 ml/min) and fraction collection (5 ml fractions) began when the marker dye eluted from the gel, after about 1.5 hr run time (Figure 2). The enzyme activity peak was completely eluted about 3.75 hr later. The total run time was 5.5 hr. Electrode buffer was also used as elution buffer because the enzyme is stable in this buffer.

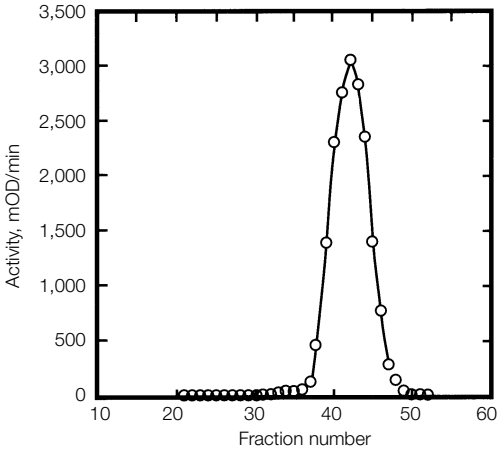

Fig. 2. Elution of enzyme activity from the Model 491 prep cell. The protein concentration in the fractions was below the limits of online ultraviolet detection.

Results

As shown in Table 1, there was an 87.0% recovery of enzyme activity applied to the Model 491 prep cell. The protein appeared to be homogeneous as determined by SDS-PAGE (Figure 3). In addition, several other esterases that appear as electrophoretic allozymes on native PAGE also have been separated by the Model 491 prep cell under the conditions described above. The purified enzymes have been characterized (by kinetics) with various insecticides to determine that qualitative differences exist in mosquito populations from different geographic locations.

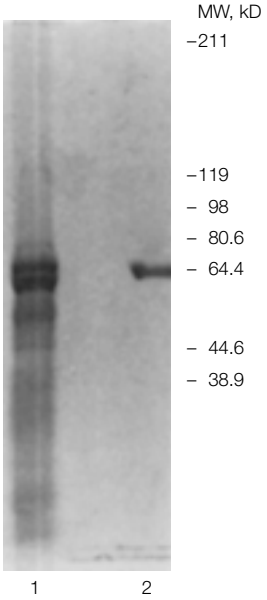

Fig. 2. SDS-PAGE of esterase protein before and after Model 491 prep cell purification. Lane 1 contains 15 µg of the sample applied to the Model 491 prep cell; lane 2 contains 4 µg of the esterase A_2 concentrate after elution from the Model 491 prep cell. The gel used is a Mini-PROTEAN® II Ready Gel® 4–20% gradient gel.

References

Ketterman AJ et al., Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus,* Biochem J 287, 355–360 (1992)

Raymond M et al., Worldwide migration of amplified insecticide resistance genes in mosquitoes, Nature 350, 151–153 (1991)

Table 1. Esterase activity was followed by the hydrolysis of *p*-nitrophenyl acetate at 405 nm.

Step	Specific Activity (U/mg)	Protein (mg)	Total Activity (U)	Purification Factor	% Total Recovery
10,000 x g supernatant	1.03	692.5	713.3	1	
Ion exchange chromatography	13.15	63.9	840.3	12.8	117.8
Hydrophobic interaction chromatography	33.22	12.67	420.9	32.2	58.9
Hydrophobic chromatography	204.78	1.63	333.8	198.8	46.8
Model 491 prep cell	363.15	0.8	290.5	352.6	40.7

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