

HIGH-RESOLUTION GRADIENT POLYACRYLAMIDE ELECTROPHORESIS OF ISOZYMES OF THE MOSQUITO, *Aedes aegypti*

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ABSTRACT. A procedure for high resolution electrophoresis of isozymes of the mosquito *Aedes aegypti* using nonlinear (2.5%–20%) polyacrylamide gradient gel slabs is described. Crude mosquito homogenates were electrophoresed on gradient and on homogeneous (7%) polyacrylamide gels and stained for esterase (EST), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH). The resulting zymograms were compared to demonstrate the high resolving power of the polyacrylamide gradient gel system.

INTRODUCTION

Several investigators have employed electrophoretic techniques for genetic analysis and characterization of mosquitoes, especially species of medical importance (Saul et al. 1976, Saul et al. 1978, Kreutzer 1979, Mathews and Craig 1980, Powell and Tachnick 1980, Steiner and Joselyn 1979). These investigators employed homogeneous supported media such as starch, cellulose acetate or polyacrylamide in their studies. Electrophoretic techniques that use homogeneous supported media separate proteins by ionic charge. Although these methods are adequate in some cases, the resolution and separation of electrophoretic variants in homogeneous supported media in many instances are ambiguous (Johnson 1977a). For example, Johnson (1976a) examining α -glycerophosphate dehydrogenase in the butterfly *Colias meadii*, found two variants using a 5% polyacrylamide gel, compared with 5 variants when the pore size of the gel varied. Additional results due to variation of gel pore size and protein separation have been reported by Johnson (1976b, 1977b, 1977c, 1977d).

The purposes of this investigation were to compare the quality of several enzyme assays from the mosquito *Aedes aegypti* (Linn.) using gradient and homogeneous polyacrylamide gel electrophoresis (PAGE) and to describe a simple and cost-effective method for using this procedure for genetic and biochemical studies.

MATERIALS AND METHODS

Two buffer systems were employed for both homogeneous and gradient PAGE systems

(Munstermann 1979)³. The Tri-Borate-EDTA (pH 8.9) buffer consisted of 0.081 M tris (hydroxymethyl) aminomethane, 0.020 M boric oxide and 0.002 methylenediaminetetraacetic acid. The Tris-Citrate buffer (pH 7.1) was composed of 0.034 M Tris and 0.010 M citric acid. The gel buffer for the Tris-Citrate system was diluted 50% with distilled water and 25% for the tank buffer. The Tri-Borate-EDTA buffer system was used undiluted.

Homogeneous and gradient gel densities were controlled by adjusting the % T and % C as described by Hjerten (1962). The % T is defined as the number of grams of monomer, acrylamide (A) plus N,N'-methylenebisacrylamide (Bis) added to a volume of buffer. The formula used for this calculation is: $T = \frac{A(g) + Bis(g)}{Volume (ml)} \times 100\%$. The % C or the cross-linking concentration is defined as the weight of Bis expressed as a percent of the total weight of the monomer such that $C = \frac{Bis(g)}{A(g) + Bis(g)} \times 100\%$.

All gels were cast in lots of 16 using 82 × 82 × 5 mm glass cassettes and a gradient maker and gel-casting tower from Isolabs, Inc., Akron, OH⁴. Homogeneous gels with a % T of 7.0 and a % C of 4.5 were made using a monomer solution composed of 8.17 g of acrylamide, 0.38 g of Bis and 122.2 ml of buffer. To obtain an optimal polymerization time of 20 min, 17.3 ml of a 0.75% ammonium persulfate initiator solution was added to the monomer solution. The amount of catalyst, a 10% TEMED solution, used was 0.04 ml for Tris-Borate-EDTA based gels and 0.01 ml for Tris-Citrate based gels.

Gradient gels were made using a modification of the method described by Margolis and

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³ Munstermann, L. E. 1979. Isozymes of *Aedes aegypti*. Phenotypes, linkage and use in the genetical analysis of sympatric subspecies populations in East Africa. Ph.D. dissertation, University of Notre Dame, Notre Dame, IN.

⁴ Use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health and Human Services.

Kendrick (1968). Using this method, low and high concentration monomer solutions are added separately to each of two chambers of the gradient maker apparatus. As the contents of these two chambers mix, a solution with a low monomer concentration is delivered into the gel cassettes first with a linear increase in monomer concentration over time. To obtain 2.5–20.0% gradient gels a heavy solution contained 75.0 g of acrylamide, 3.75 g of Bis, 2.5 ml 10% ammonium persulfate (w/v) solution and 250.0 ml of buffer. The light solution contained 2.25 ml 10% ammonium persulfate solution and 0.07 ml of a 20% TEMED solution (v/v) and 225.0 ml of buffer. In order to obtain an optimal polymerization time for Tris-Citrate based gels it was necessary to decrease the volume of TEMED to 0.04 ml. Both gradient and homogeneous gels were allowed to stand for at least 8 hr in order to insure complete polymerization. The gels were then removed from the casting tower, covered with paper towels saturated with a 0.02% sodium azide solution to inhibit bacterial growth and stored at 4°C.

Pupae from a colony of *Ae. aegypti* originally derived from Vero Beach, Florida, were used for all isozyme assays. Mosquitoes were reared using the procedures as outlined in Saul et al. (1980). Pupae were homogenized individually in a 30 μ l solution containing either Tris-

Borate-EDTA or Tris-Citrate buffers with 17.8% sucrose, 0.36% 2-mercaptoethanol and 0.02 mM bromphenol blue. The homogenates were then centrifuged at 1000 \times g for 30 min at 4°C.

Electrophoresis was performed using a Pharmacia Vertical Gel System. All gels were pre-electrophoresed without samples for 30 min at 150 v constant voltage. Electrophoretic conditions for each isozyme assay are given in figures 1, 2 and 3. Staining solutions were prepared according to the methods outlined in Munstermann (1979)³ The reagents used in this study were obtained from Sigma Chemical Co., St. Louis, Mo.⁴

Homogeneous and gradient gels were compared for clarity, compactness and number of electrophoretic bands per individual mosquito. In all three isozyme assays, the same mosquito was used in corresponding sample slots for both homogeneous and gradient gels.

RESULTS

The ten mosquitoes assayed for esterases (EST) on gradient and homogeneous gels are shown in Fig. 1. Although greater separation of the EST bands occurred on the homogeneous gel, distinct compact bands are apparent on the gradient gel. The homogeneous gel had ad-

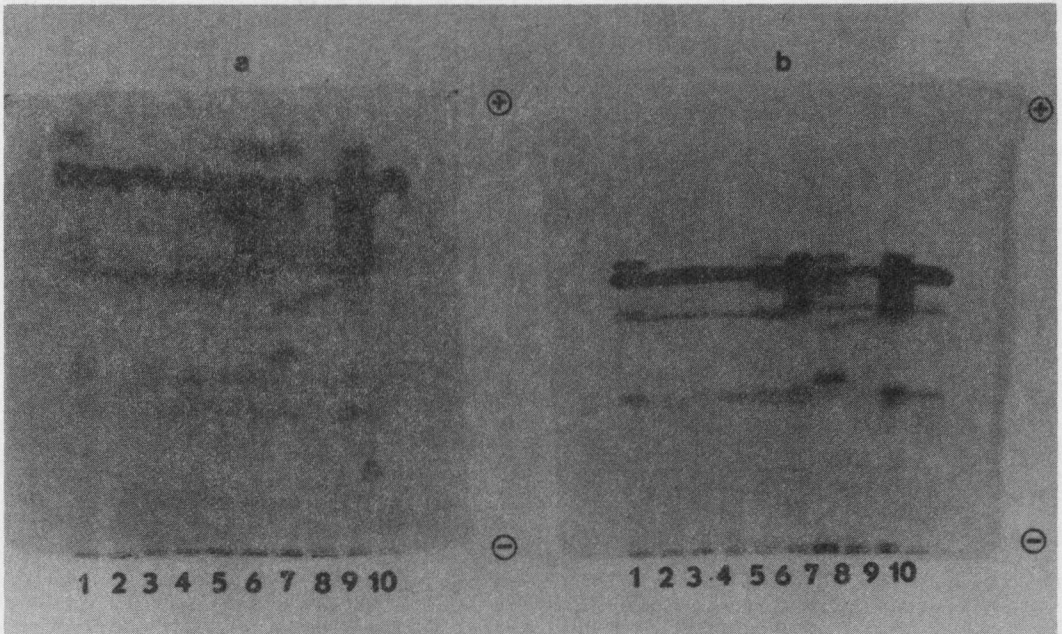


Fig. 1. Stained homogeneous gel (a) and gradient gel (b) zymograms for EST. Each position, 1 to 10, was loaded with 4 μ l of homogenate on each gel in the same sequence. Electrophoresis was toward the anode (+) at 500 V in system I Tris-Borate-EDTA buffer for 0.5 hr. and 1.0 hr on gels (a) and (b), respectively.

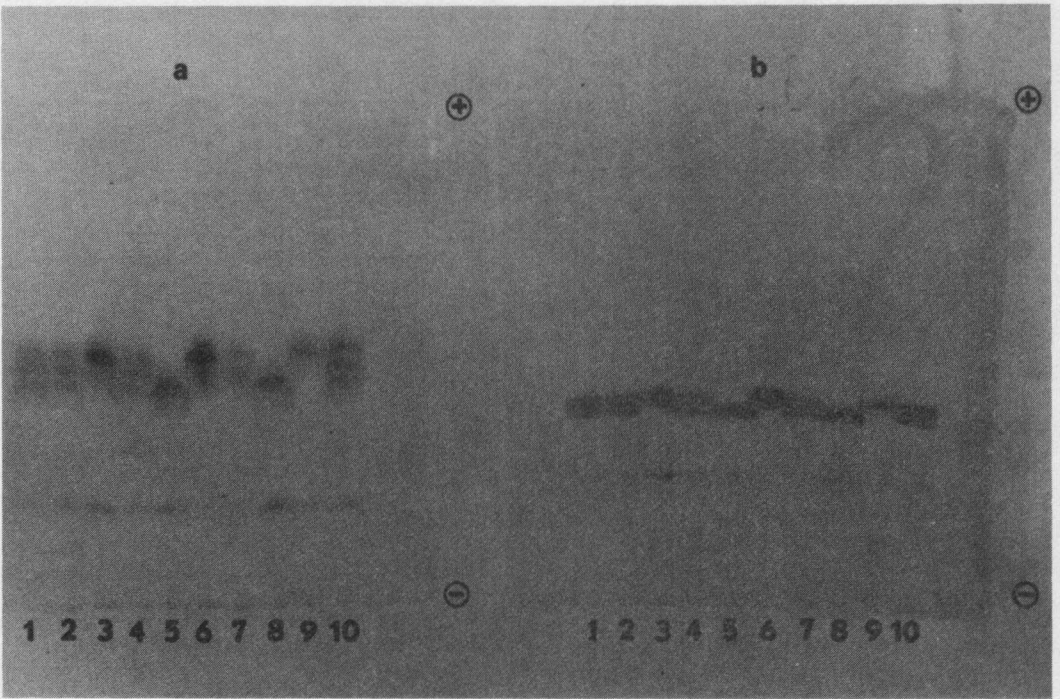


Fig. 2. Stained homogeneous gel (a) and gradient gel (b) zymograms for IDH. Each position, 1 to 10, was loaded with $3\ \mu\text{l}$ of homogenate on each gel in the same sequence. Electrophoresis was toward the anode (+) at 375 V in system II Tris-Citrate buffer for 1.5 hr and 3.0 hr on gels (a) and (b), respectively.

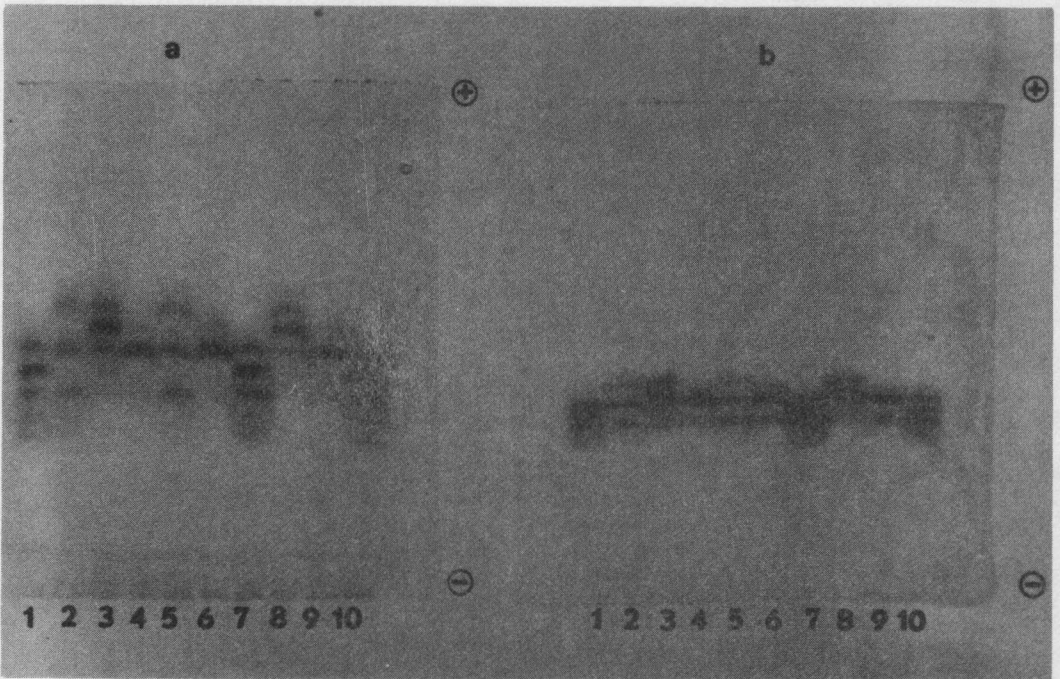


Fig. 3. Stained homogeneous gel (a) and gradient gel (b) zymograms for MDH. Each position, 1 to 10, was loaded with $3\ \mu\text{l}$ of homogenate on each gel in the same sequence. Electrophoresis was toward the anode (+) at 375 V in system II Tris-Citrate buffer for 1.5 hr and 3.0 hr on gels (a) and (b), respectively.

ditional bands in samples 1, 4, 5, 8 and 9 which were absent on the gradient gel. Distinct bands are present in samples 5, 6, 7, 9 and 10 on the gradient gel. These EST bands were either missing or obscured on the corresponding homogeneous gel.

Figures 2 and 3 show the homogeneous and gradient gels for isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) isozyme assays respectively. Although both zymograms show similar banding phenotypes, distinct compact bands were observed using the gradient gel system.

DISCUSSION

The procedure described here provides a simple and cost-effective method for the characterization of mosquito isozymes. The gradient gel system produces electrophoretic bands that are visually distinct and compact.

With the gradient PAGE system, the resolution is improved through a continuous increase in retardation of migrating proteins caused by a progressive decrease in gel pore size (Margolis and Kenrick 1968). The problem of "hidden" heterogeneity (Johnson, 1976a, 1977a) and determining epigenic bands are minimized by using the gradient PAGE technique.

One of the major advantages of separation by ionic charge alone is that step charge reflects substitutions of a discrete class of amino acids. Substitution of these amino acids may or may not translate changes in conformation of the enzyme. We have not seen this as a problem since the average heterozygosity we have established in populations of *Ae. aegypti* are statistically similar to those using homogeneous gel systems (San Juan Laboratories, unpublished data).

The advantage of the gradient system is that additional alleles provide a broader range of characters for taxonomic and population genetic studies. Also migration distances, R_f values and densitometric scans can be used to accurately measure the gradient PAGE gels, primarily because of background clarity and compact banding properties. The advantages described here make this versatile gradient PAGE system very useful for taxonomic and

genetic characterization of mosquito isozymes compatible with polyacrylamide.

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