Malic and lactic dehydrogenases in the hemolymph and tissue extracts of cockroaches

Zakir Ahmad Warsi
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MALIC AND LACTIC DEHYDROGENASES IN THE HEMOLYMPH
AND TISSUE EXTRACTS OF
COCKROACHES

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
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FOR THE DEGREE OF MASTER OF SCIENCE

BY
ZAKIR AHMAD WARSI

DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA
MAY 1973
Master of Science Thesis

of

Zakir Ahmad Warsi

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ABSTRACT

Biology

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B.Sc. Aligarh Muslim University, 1968

Malic and Lactic Dehydrogenases in the Hemolymph and Tissue Extracts of Cockroaches

Advisor: Dr. Joseph P. Myers

Master of Science degree conferred
Thesis dated May, 1973

Malic dehydrogenase (MDH) and Lactic dehydrogenase (LDH) in tissue extracts and hemolymph of the adult cockroaches, Blatta orientalis (L), Periplaneta americana (L) and Blaberus craniifer (Brumeister) were identified and characterized. Identification of the above enzyme systems were carried out using polyacrylamide gel-disc electrophoresis. Characterization consisted primarily of spectrophotometric scanning of the tissue extracts.

The tissues selected for analyses were as follows: heart muscle, leg muscle, fat body along with perithoracic hemolymph.

The various tissues were placed in cold insect saline (pH 6.5) and homogenized by hand with twice their volume of Sigma alumina - 305 in a cooled mortar for 3-4 min. Crude extracts were then centrifuged at 8000 x g for 10 min. The clear supernatants were analyzed in two ways:
(a) Separation of negatively charged protein by electrophoresis using polyacrylamide gels and sites of dehydrogenase activity were noted by
immersing each gel in a specific reaction mixture. (b) Spectrophotometric determination of dehydrogenase activity was based on the rate of oxidation of NADH (decrease in optical density at 340 nm) in the presence of the enzyme and the substrate.

Multiple forms of MDH were detected in leg muscle and fat body tissue extracts but only a single form was demonstrated in the hemolymph and heart muscle extracts. The spectrophotometric results demonstrated high activity of MDH in all tissues examined. LDH was absent in hemolymph, heart muscle and fat body but was present in leg muscle extracts.

It was concluded that MDH is present in cockroaches in appreciable quantities. The results also indicated that the MDH in the various tissues examined had the same characteristics but varied in concentration.

LDH was identified only in the leg muscle extracts of all three species of cockroaches.
ACKNOWLEDGEMENTS

The author wishes to express appreciation to Dr. Joseph B. Myers for criticism, advice and guidance during the tenure of this investigation, and to his wife without whose help this work never could have been completed. I wish to also express gratitude to Dr. Lafayette Frederick for helpful suggestions.
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CHAPTER I

INTRODUCTION

Agrell and Kjellberg (1965) found two distinct forms of malate dehydrogenase (MDH) in mitochondrial fractions and in the cytoplasm of a variety of organisms. In relation to lactic dehydrogenase (LDH) five molecular forms or isozymes were found in vertebrate tissues (Markert and Massaro, 1966; Batellino and Blanco, 1970).

Only a few workers have studied LDH and MDH enzyme systems in insect tissues and our knowledge is accordingly very limited especially concerning LDH. Gilbert and Goldberg (1966) reported the first detailed study of metabolic enzymes in Leucophaea maderae. They found three metabolic enzymes, MDH, LDH and glyceraldehyde dehydrogenase (GDH) in muscle tissue, fat body, and sex-organs during the developmental stages. They successfully characterized MDH and GDH in all specimens and LDH in only muscle and fat body. Stephen and Cheldelin (1970) found extremely low activity of LDH and high activity of GDH in Periplaneta americana. Other reports regarding the existence of these enzymes in other insects were recorded by Muus (1968) in Glossina morsitans and Musca domestica; Aronson et al. (1968) in Glossina differentialis, and McReynolds and Kitto (1970), in Drosophila virilis. They all reported high activity of MDH and GDH and that the functions of LDH were replaced by GDH in contractile muscles of insects.

Gilmour (1965) stated that aerobic metabolism was the predominant mechanism for the oxidation of food stuffs in insects accompanied by
increased activity of GDH. He concluded that high activity of MDH was not surprising because MDH is an important oxidative enzyme of the Kreb's cycle. This cycle acts as a common pathway for the catabolism of the three main divisions of food stuffs (carbohydrates, fats and proteins).

There was a dearth of information concerning the presence of LDH in insect tissues. More specifically, no reports were found pertaining to the existence of LDH and MDH in *B. orientalis* and *B. crannifer*. This study was undertaken therefore to identify and characterize MDH and LDH in representative tissues (heart muscle, leg muscle, fat body) and hemolymph in the three species mentioned above.
CHAPTER II

REVIEW OF LITERATURE

Early interest in respiratory enzymes stemmed largely from the study of higher animals. Many of these enzymes have been reported to exist in more than one form. They were thoroughly studied by Cahn et al. (1962) in various organs and stages of development in chickens. Recently, LDH and MDH were found in multiple forms (isozymes) in several organisms. LDH isozymes are electrophoretic variants which are widespread in nature (Shaw, 1965). Vesell (1965) reported that LDH is common in higher vertebrate tissues whereas Zinkham et al. (1963) and Goldberg (1965) stated that sex-associated forms of LDH were present in mature testes and sperms of humans. Similarly, Blanco and Zinkham (1963) detected LDH in the sperms and testes of rabbits. Goldberg and Hawtry (1967) demonstrated five isozymes of LDH in mice (Mus musculus). Wong et al. (1971) reviewed the same work and detected a sixth-specific isozyme in Swiss mice. Large numbers of isozyme patterns of LDH have also been detected and discussed by Agrell and Kjellberg (1965) in the tissues of several invertebrates and vertebrates.

Appella and Markert (1961) and Cahn et al. (1962) proposed an explanation for the heterogeneity of enzymes, particularly LDH. They suggested that isozyme patterns of this dehydrogenase revealed five multiple forms and each represented a polymer of four subunits made up of two principal forms of enzymes, H and M monomers. This theoretically accounted for the five isozymes actually observed.
In recent years, considerable attention has been given to respiratory enzyme systems found in invertebrates. Goldberg (1958) reported the existence of the glycolytic cycle (anaerobic pathway) in Trichenella spiralis larvae and Bayne and Roberts (1969) demonstrated that the same cycle existed in Trypanosomes.

Several studies have shown that variable isozyme patterns and spectrophotometric activity of MDH can be demonstrated in organs of most animal systems. Comparative work by Agrell and Kjellberg (1965) showed that three MDH bands were active in pig kidneys and heart while in the same tissues of frogs and pigeons, only two forms were seen. Kulick and Barnes (1968) demonstrated three bands in tissue homogenates of pig heart.

Kaplan and Ciotti (1961) observed that erythrocytes contained high concentrations of MDH. They studied NAD-linked dehydrogenase activity in a variety of tissues and reported almost universal distribution of MDH. Shrago (1965) spectrophotometrically demonstrated very high activity of MDH in the mitochondria of erythrocytes in comparisons to that found in cytoplasm. Thus, it was suggested that most vertebrate tissues exhibit variable and multiple patterns of this enzyme.

In relation to insects, Agrell and Kjellberg (1965) found six bands of MDH in the blowfly Calliphora erythrocephala. Sims (1965) demonstrated only one to two bands in Drosophila species. Nuus (1968) was unable to find more than three bands in Tsetse flies and houseflies. Gilbert and Goldberg (1966) reported only two bands of MDH in all tissues examined in the cockroach, Leucophaea maderae.
Zebe and McShan (1957) detected LDH activity in muscles from insects belonging to various groups. In all cases, assays of flight muscle extracts showed extremely low LDH activity and leg muscles even lower activity. In the leg muscles of Belostoma (Hemiptera) and Melanoplus (Orthoptera), however, very active LDH was found. Zebe and McShan (1957) also reported LDH to be practically absent in gonad and fat bodies of the above species. Aronson et al. (1968) reported low activity of LDH in accessory tissues and essentially its absence in the muscle tissue of grasshoppers.

In relation to LDH, Gilbert and Goldberg (1966) reported low activity in leg muscles and midgut but very high activity in the ovary during developmental stages of L. maderae. A comparative study by Stephen and Cheldelin (1970), found low LDH and high GDH activity in the muscles of several species of cockroaches while Chiang (1971) observed the same relationship in P. americana.
CHAPTER III

MATERIALS AND METHODS

Adult male and female cockroaches representing two different families, Blattidae: *Periplaneta americana* (L) and *Blatta orientalis* (L) and Blaberidae: *Blaberus crannifer* (Bruneister) were used throughout this investigation. The animals were purchased from Carolina Biological Supply Company, Burlington, North Carolina. Each species was maintained in separate containers and fed a diet of Gerber's high protein baby cereal. Water was available at all times and at least once a week apple slices were added to supply additional nutrients.

Perithoracic hemolymph and three types of cockroach tissues were used: heart muscle, leg muscle and fat body. Tissue or hemolymph from three cockroaches of the same species was collected and pooled to obtain adequate samples. Four groups were established; Group I was the source of hemolymph; Group II supplied samples of heart muscle; Groups III and IV were designated for the collection of leg muscle and fat body respectively.

The insects were decapitated after which their wings were removed and discarded. The legs were removed and muscle tissue was obtained by removing the cuticle with the aid of fine scissors and forceps (Fig. 1). In most cases, muscle samples consisted of pooled segments of adductor coxae, flexor femoris and extensor tibialis (Fig. 2). Heart muscle was removed by making two parallel incisions on the right and left sides of the abdomen approximately 3.0 mm apart (Fig. 3). The abdominal portion
Fig. 1. A photograph showing the technique used for the removal of leg cuticle to obtain muscle tissue from B. crannifer Cuticle (Cl), Coxae (Cx), Coxae muscle (Cxm), Femur (Fr).
Fig. 2. A photograph of the Tarsus (Tr), Coxae (Cx), Femoris (Fr) and Tibialis (Tb) removed from *B. crannifer*. 
Fig. 3. A photograph demonstrating the procedure used for exposing the tubular heart in *B. crannifer* Incisions (Is) and Mid-dorsal portion (Md).
containing the tubular heart was placed in a watchglass and bathed with cold insect saline. A dissecting microscope and a pair of fine forceps was used to separate the heart from the mid-dorsal portion of the abdomen (Figs. 4 and 5). Fat body from the mid-abdominal region was teased out and pooled (Figs. 6 and 7). A Pasteur pipette was used to collect hemolymph by inserting the fine tip into the ventral side of the prothorax. In some cases, hemolymph was collected from the head region after decapitation (Fig. 8).

Each tissue sample was placed in chilled insect saline, rinsed and excess saline was removed with blotting paper. The tissues were then transferred to cooled mortars and thoroughly homogenized by hand with twice their volume of Sigma alumina-305 for 3 to 4 min. Five ml of cooled distilled water was added and mixed by stirring. The tissue homogenate was then placed in 50 ml polypropylene tubes and centrifuged for 10 min at 2 C in a Lourdes refrigerated centrifuge (8,000 x g). The supernatant was stored in the refrigerator until it was ready for use.

**Polyacrylamide Gel Electrophoresis**

The technique of electrophoresis on small columns of polyacrylamide gels was used for protein fractionation. The technique used was similar to the method used by Davis (1964). Complete gel preparation consisted of the following parts: (a) a large-pore anti-convection gel containing the protein sample, (b) a large-pore spacer gel in which electrophoretic concentration occurred, and (c) a small-pore gel in which electrophoretic separation was accomplished. A Buchler vertical column gel electrophoresis system and a regulated power supply, (Beckman Instruments, Incorporated, Atlanta, Georgia) were used for the separation.
Fig. 4. A photograph showing the tubular heart (H) of *B. crannifer*.
Anterior region (Ant), Posterior region (Pst).
Fig. 5. A photograph which illustrates the separation of the heart (H) from the mid-dorsal portion (Md) of the abdomen using the dissecting microscope.
Fig. 6. A photograph of fat body (F) in *B. crannifer* after removal of the mid-dorsal portions of the abdomen.
Fig. 7. A photograph illustrating the technique used to remove fatty tissue (F) from the abdomen of B. crannifer.
Fig. 8. A photograph demonstrating the method used to collect hemolymph from the cockroach. Prothrax (Pt), Pasteur pipette (P).
All special reagents for the gel system were purchased from Canalco Industrial Corporation, Rockville, Maryland. The following stock solutions were necessary to prepare the small-pore and large-pore solutions:

(A) 1N HCl
   2-Amino-2 Hydroxymethyl-1,3 Propanediol (Tris) 48 ml
   N,N,N',N'-Tetramethylethylene Diamine (TEMED) 36.3 gm
   Distilled water to make 100 ml

(B) 1M H₃PO₄
   2-Amino-2 Hydroxymethyl-1,3 Propanediol (Tris) 5.7 gm
   Distilled water to make 100 ml

(C) Acrylamide Monomer
   N,N,-Methylene Bisacrylamide (BIS) 0.8 gm
   Distilled water to make 100 ml

(D) Acrylamide Monomer
   N,N,-Methylene Bisacrylamide (BIS) 2.5 gm
   Distilled water to make 100 ml

(E) Riboflavin
   4.0 mg/100 ml of distilled water

The following working solutions were prepared from the stock solutions:

(1) Small-pore solution (Lower gel)
   Solution a
   1 part A
   2 parts C
   1 part distilled water

   Solution b
   Ammonium persulfate 0.14 gm/
   100 ml of distilled water
Solution b was prepared fresh and mixed with an equal volume of solution a for use.

(2) Large-pore solution (Upper gel; pH 6.8) was prepared as follows:
   1 part B
   2 parts D
   1 part E
   4 parts H₂O

The Tray buffer (10X) was prepared in the following manner: Tris 6.0 gm, Glycine 28.8 gm and distilled water to make 1,000 ml. One hundred ml of this buffer was then diluted to 1,000 ml after adding two drops of tracking dye (Bromophenol Blue). All solutions had a shelf life of several weeks when stored in brown glass bottles in the refrigerator.

The gel medium was placed in uniform glass tubes (7.0 cm x 0.5 cm I.D.) which were tightly closed at one end with flat rubber stoppers. While in a vertical position, each tube was filled with 0.85 ml of small-pore solution at room temperature. The gel was then carefully overlaid with 0.1 ml of distilled water and allowed to polymerize for 30 min. The water layer was removed and the inside of the tubes were washed once with large-pore gel. One-half ml of the large-pore gel was added, then carefully overlaid with 0.1 ml of distilled water. A day-light fluorescent lamp was placed approximately three inches behind the tubes to polymerize the large-pore gel layer which usually occurred within 15 to 20 min. After polymerization, the water layer was removed and 0.1 ml of tissue extract was added and the remaining space in each glass tube was filled with Tray buffer (10X).
The glass tubes were removed from the rubber stoppers and placed in vat holders of the cathodal compartment and again covered with Tray 10X buffer. A hanging drop of buffer was attached to the bottom of each tube before it was placed in the lower anodal compartment of the instrument. Care was taken to place equal volumes of Tray buffer in the upper and lower compartments of the instrument. Electrophoresis was carried out by applying a current of 42 mA to 12 gel columns for about 45 min. The average current therefore was approximately 3.5 mA for each gel column.

When electrophoresis was completed, the gels were removed by gently rimming the glass tubes with a 22-gauge needle through which a thin stream of water was allowed to pass. Each gel was then placed in a small test tube (7x1 cm OD) containing a reaction mixture.

Detection of LDH and MDH activity in the polyacrylamide gels was carried out by incubating the gels in a reaction mixture prepared in the following manner: 45 mg of Nitro-Blue-Tetrazolium (NBT) and 90 mg of Nicotinamide Adenine Dinucleotide (NAD) were mixed in 30 ml of Tris buffer (pH 7.4). This 30 ml reaction mixture was then divided into three parts: (1) the control, which contained no substrate, (2) a reaction mixture for MDH which contained DL malic acid (4 mg/ml), and phenazine methosulphate (0.1 mg/ml), and (3) a reaction mixture for LDH which contained DL lactic acid (0.05 mg/ml) and phenazine methosulphate (0.1 mg/ml). After incubating the gels for one hour at an average temperature of 25 C in the dark, the gels were removed from the reaction mixture and preserved in 7.5% acetic acid.
Spectrophotometry

MDH and LDH were assayed in a Beckman DB-G spectrophotometer connected to a ten-inch recorder. The activity of the enzyme systems was measured as a function of the rate of oxidation of NADH (decrease in optical density at 340 nm) in a total volume of 3 ml, using silica cells with 1.0 cm light paths.

MDH was assayed according to the method used by Ochoa (1955). All reagents were prepared separately in 0.25 M Tris buffer (pH 7.4). One-half mg of oxalacetic acid and 0.5 mg NADH were weighed and dissolved separately in 5 ml of Tris buffer; each equivalent to final concentrations of 0.0076M and 0.0015M respectively. The solutions were then stored in the dark until ready for use. Experimental assay systems contained 3 ml of reaction mixture, 2.7 ml of Tris buffer, 0.1 ml of oxalacetic acid, and 0.1 ml of NADH. The reaction was started by adding and mixing 0.1 ml of tissue extract. The controls contained 2.8 ml of water, 0.1 ml NADH and 0.1 ml tissue extract.

LDH assays were performed by the method of Kornberg (1955). The stock reagents were prepared separately as follows: 3.9 mg of sodium pyruvate (3.3 x 10^{-4}M) was dissolved in 100 ml of Tris buffer. NADH (6.7 x 10^{-5}M) was made daily by mixing 1.0 mg of NADH in 10 ml of Tris buffer. Three ml of this reaction mixture contained 2.8 ml of sodium pyruvate, 0.1 ml of NADH and the reaction was started by adding 0.1 ml of the tissue extract. The controls contained 2.8 ml of Tris buffer, 0.1 ml of NADH solution and 0.1 ml of tissue extract.

All samples were run in duplicate and the decrease in optical density at 340 nm between zero and 120 sec was used to determine the
enzyme activity. Units of activity were expressed as change in absorbance equivalent to 0.001 optical density units per minute.

The total protein was determined spectrophotometrically by measuring the absorption at wavelengths of 280 and 260 nm as described by Layne (1955). Protein concentrations were calculated utilizing the following equation:

\[ C = 1.55 \text{ O.D.}_{280} - 0.76 \text{ O.D.}_{260} \]

where \( C \) is the protein concentration in mg/ml and O.D., optical density at 280 and 260 nm, respectively.
CHAPTER IV

RESULTS

The following results are based on the separate analyses of MDH and LDH activity in the hemolymph and tissue extracts of the three species of cockroaches. Samples from each species were tested separately and all experiments were performed using the same procedure.

Protein concentrations applied to the gel columns for electrophoresis and that used for spectrophotometric assays are summarized in Table 1. The hemolymph and heart muscle extracts had much less protein content than leg muscle and fat body extracts. This was due to the difficulty in obtaining adequate amounts of hemolymph and heart tissue samples from B. orientalis and P. americana.

Disc gel electrophoretic analyses demonstrated several isozymes of MDH in most of the tissues examined. Fig. 9 (A, B and C) illustrates a single very active band in the hemolymph of B. orientalis, P. americana and B. crannifer. Each band was located 5.2 mm anodically. In the heart muscle of all species, a single band was prominent but was less active than the single band found in the hemolymph. These bands appeared approximately 4.7 mm anodically (Fig. 10, A, B and C). Fig. 11 (A, B and C) demonstrates MDH banding patterns in B. orientalis, P. americana and B. crannifer, respectively in which five bands were visible in all cases. Two diffuse and overlapped bands were located 0.2 mm cathodically in a group. The other two bands, located 2.3 mm near the center of the gels
were active and sharp. The single band which formed 4.8 mm anodically was very diffuse and broad. Fig. 12 (A, B and C) illustrates multiple banding patterns of MDH in the fat body of B. orientalis, P. americana and B. crannifer respectively. Only six thin bands (two in each group of three broad bands) are demonstrable. Specific activity was absent in the control gels (D) in all figures. Figs. 11 and 12 are shown schematically below the photograph in order to illustrate the bands more clearly.

Spectrophotometrically, the rate of oxidation of NADH (decrease in optical density) per 30 sec, was used to calculate the MDH activity in the extracts. Note that in the graphs described below, (A) represents the control with no specific activity, (B) B. orientalis (C) P. americana and (D) B. crannifer. When Figs. 13 and 14 are compared (hemolymph and heart tissue respectively) results indicated that MDH activity was lower in the hemolymph than in heart tissue. MDH activity in the fat body exceeded both hemolymph and heart tissue activity (Fig. 15), however it was lower than that found in leg muscle extracts (Fig. 16).

In summary, B. crannifer tissue extracts had the highest MDH activity and B. orientalis the lowest with P. americana showing intermediate activity.

Lactic dehydrogenase activity was demonstrated spectrophotometrically in the leg muscle extracts only (Fig. 17) and relative to MDH B. crannifer (D) had the highest activity and B. orientalis (B) the lowest with P. americana (C) showing intermediate activity.
Table 1. Protein concentration in the tissue extracts of three species of cockroaches

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<th>Cockroaches</th>
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<th>Leg Muscle in mg/ml</th>
<th>Fat Body in mg/ml</th>
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<td>0.109</td>
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<td>Periplaneta americana</td>
<td>1.895</td>
<td>0.216</td>
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<tr>
<td>Blaberus crannifer</td>
<td>4.971</td>
<td>4.971</td>
<td>28.65</td>
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</tr>
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Fig. 9. Disc electrophoretic pattern of malate dehydrogenase in extracts of hemolymph from cockroaches (A) *P. orientalis*, (B) *P. americana*, (C) *B. crannifer*, and (D) control. The dark areas indicate the specific enzyme activity of the bands.
Fig. 10. Disc electrophoretic pattern of malate dehydrogenase in extracts of heart muscle from cockroaches (A) *B. orientalis*, (B) *P. americana*, (C) *B. crannifer*, and (D) control. The black disc indicates the specific enzyme activity of the bands.
Fig. 11. Original and schematic representation of the disc electrophoretic pattern of malate dehydrogenase in the extracts of cockroaches leg muscle (A Aₗ, B. orientalis; B Bₗ, P. americana; C Cₗ, B. crannifer; D Dₗ, control). The dark areas indicate the specific enzyme activity of the bands.
Fig. 12. Original and schematic representation of the disc electrophoretic pattern of malate dehydrogenase in the extracts of cockroaches fat body (A A1, B. orientalis; B B1, P. americana; C C1, B. crannifer; and D D1, control). Incubation time 60 min. The dark areas indicate the specific enzymatic activity of the bands.
Fig. 13. Malate dehydrogenase activity in hemolymph extracts. (A) *B. orientalis*, (B) *P. americana*, (C) *B. crannifer*, (D) Experimental cuvettes contained Oxalacetate (0.0076M), NADH (0.0015M) and Tris buffer (0.25M) pH 7.4. The control contained no substrate. Temperature 25 C. Total volume 3.0 ml. After adding the enzyme, changes in absorption at 340 nm were recorded at 30 sec. intervals for four min.
Fig. 14. Malate dehydrogenase activity in heart muscle extracts. (A) Control, (B) *B. orientalis*, (C) *P. americana*, and (D) *B. craniifer*. Experimental cuvettes contained Oxalacetate (0.0076M), NADH (0.0015M) and Tris buffer (0.25M, pH 7.4). The control contained no substrate. Temperature 25°C. Total volume 3.0 ml. After addition of the enzyme, changes in absorption at 340 nm were recorded at 30 sec. intervals for four min.
Fig. 15: Malate dehydrogenase activity in the leg muscle extracts. (A) Control, (B) *B. orientalis*, (C) *P. americana*, and (D) *B. crannifer*. Experimental cuvettes contained Oxalacetate (0.0076M), NADH (0.0015M) and Tris buffer (0.25M, pH 7.4). The control contained no substrate. Temperature 25 C. Total volume 3.0 ml. After adding the enzyme, changes in absorption at 340 nm were recorded at 30 sec. intervals for four min.
Fig. 16. Malate dehydrogenase activity in the fat body extracts. (A) Control, (B) B. orientalis, (C) P. americana, and (D) B. craniifer. Experimental cuvettes contained Oxalacetate (0.0076M), NADH (0.0015M) and Tris buffer (0.25M, pH 7.4). The control contained no substrate. Temperature 25 C. Total volume 3.0 ml. After adding the enzyme, changes in absorption at 340 nm were recorded at 30 sec. intervals for four min.
Fig. 17. Lactate dehydrogenase activity in the leg muscle extracts. (A) Control, (B) B. orientalis, (C) P. americana, (D) B. crannifer. Experimental cuvettes contained sodium pyruvate (0.00033M made in 10X buffer), NADH (0.000067M). The control contained no substrate. Temperature 25 C. Total volume 3.0 ml. After adding the enzyme, changes in absorption at 340 nm were recorded at one min, intervals for four min.
TIME (MIN)

$\Delta \log \frac{I_0}{I}$ (340nm)
CHAPTER V

DISCUSSION

Our results showed that MDH was present in adequate concentrations in the hemolymph and tissue extracts examined. Close similarity of isozyme patterns was demonstrated in each type of tissue removed from the different species. For example, hemolymph and heart muscle from each species had only one band whereas leg muscle and fat body had 5 and 6 bands respectively.

Relative to our work, Gilbert and Goldberg (1966) found two MDH isozymes in the mid-gut, thoracic muscle and fat body in the cockroach *L. maderae*. Our results therefore, differ with Gilbert and Goldberg when correlated with similar types of tissue (skeletal muscle and fat body). This difference could be explained on the basis of species variation, however, this explanation would conflict with our own data since the three different species studied showed similar banding patterns.

Interpretation of the data in an attempt to explain similarities and differences of multi-enzyme forms in various species is difficult. Agrell and Kjellberg (1965) suggested that a probable explanation for partial similarities in isozyme patterns of the dehydrogenases seems to be that a multiple enzyme activity in an electrophoretic band is brought about through an attachment of two or more enzyme molecules on the same carrier. Most likely, the carriers are macromolecules. They also concluded that isozyme patterns will be modified through the electrophoretic mobility of the carriers. In all probability, different organs and different animal species contain different systems of carriers.
Our spectrophotometric results demonstrated that MDH activity varied both among tissues and species. It was apparent that hemolymph showed less MDH activity than all other tissues. The leg muscle had the highest MDH activity among all of the tissues. Extracts from *B. crannifer* showed the highest MDH activity and *B. orientalis* the lowest while *P. americana* showed moderate activity.

Although no visible differences in band patterns of MDH could be observed electrophoretically among similar tissue groups, variation of activity was pronounced when analyzed spectrophotometrically. These wide variations in activity of MDH could have been due to the sensitivity of the spectrophotometric assay system. It should also be pointed out that the protein concentrations in *B. crannifer* tissue extracts were considerably higher per unit volume than *P. americana* and *B. orientalis* which would also account for the increased activity of MDH.

Results reported from the literature established the fact that LDH activity is almost absent from hemolymph and various tissues of cockroaches. According to our results, LDH was present only in leg muscle. The work of Gilbert and Goldberg (1966) reported complete absence of LDH in the fat body but its presence in midgut and ovaries. Stephen and Cheldelin (1970) reported high activity of LDH in the thoracic and appendage muscles of male and female *P. americana*, and its absence in the fat body. Chiang (1971) also reported the same results when he tested three enzymes, namely LDH, GDH and Triosephosphate isomerase (TPI), comparatively in leg, thoracic muscle and fat body of *P. americana*.

These reports correspond with our finding of moderate to high activity in leg muscle. According to our literature review, no reports
were noted concerning the LDH activity in hemolymph and heart muscle of cockroaches. The expected LDH activity may not have been observed in heart muscle and hemolymph because the concentration of this enzyme was below the resolving power of our technique.

When MDH and LDH were expressed in terms of their activity, MDH activity was very high and LDH activity was absent in aerobic tissues; the reverse was true in anaerobic tissues (Zebe and McShan, 1957; Stephen and Cheldelin, 1970; Chiang, 1971). Our results suggest that MDH is a very active enzyme system in cockroaches whereas LDH appears to be limited to the skeletal musculature. As a possible explanation, MDH activity is known to be oxygen dependent and LDH is not. Our data supports this established evidence as well as other work reported in the literature. Because of the lack of extensive work in this area of study, it would be hazardous to draw any definite conclusions from this explanation. A detailed comparative study regarding characteristics and function of MDH and LDH and their compensation with other enzymes in both aerobic and anaerobic tissues is needed.
CHAPTER VI

SUMMARY

Malic dehydrogenase

1. Hemolymph extracts of cockroaches showed a single and very active band of MDH located at 5.3 mm anodically. Spectrophotometrically, MDH appeared to be less active.

2. In the heart muscle of all cockroaches, a single band was prominent but less active and was located 4.7 mm anodically. Spectrophotometric analyses indicated more activity of MDH in heart muscles than hemolymph.

3. Leg muscles of cockroaches showed five bands of MDH. The bands were very broad and diffuse. Spectrophotometrically, MDH had the highest activity in leg muscle.

4. The tissue extracts of fat body showed six bands of MDH. The bands were found in three groups and were overlapped and diffuse. Spectrophotometrically, MDH showed less activity in fat body than in leg muscles.

Lactic dehydrogenase

5. LDH was detected spectrophotometrically only in leg muscle extracts of all species.

6. Tissue extracts of B. crannifer had the highest MDH and LDH activity of the three species examined.
LITERATURE CITED


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