A COMPARISON OF ELECTROPHORETIC BANDING PATTERNS OBSERVED FOR PROTEINS FROM TWO SPECIES OF THE GENUS DOROSOMA

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ABSTRACT

Polyacrylamide gel electrophoresis was used for the taxonomic comparison of lactate dehydrogenase, esterase, and general proteins of Dorosoma petenense Gunther and Dorosoma cepedianum LeSueur in Center Hill Lake. The fish were separated by sex and grouped by age into one, two, and three year old classes. The zymograms for the two species were exactly the same for the general muscle protein and similar for esterase and lactate dehydrogenase. No sex differences were noted in the banding patterns. The banding patterns for esterase and lactate dehydrogenase were found to vary with age. Some electrophoretic differences that may be of taxonomic value in distinguishing the two species were noted for esterase and lactate dehydrogenase.

INTRODUCTION

Specimens of gizzard shad, Dorosoma cepedianum LeSueur, and threadfin shad, Dorosoma petenense Gunther, are sometimes difficult to distinguish from each other by conventional taxonomic methods, particularly when fry are used. This difficulty is compounded by the fact that there is evidence for natural hybridization between the two species (Minkley and Krumholz, 1960; Shelton and Grinstead, 1972). The position of the mouth is very helpful in identifying the species when the anal fin rays are not fully developed. The mouth of threadfin shad is at the anterior end of the head. This position is considered to be terminal. In gizzard shad, a portion of the snout extends beyond the mouth, which is in a subterminal position. Upon maturation, gizzard shad has 30-33 anal fin rays while threadfin shad has 20-25 anal fin rays.

In recent years, analysis of the electrophoretic banding patterns of isozymes has been shown to be quite useful as an aid in making taxonomic distinctions between various groups of fish (Avise, 1974; Smith et al., 1976; Utter et al., 1974). The purpose of this study was to assess the utility of this parameter for distinguishing between the two species and to compare electrophoretic banding patterns observed for proteins from gizzard shad with those of threadfin shad in order to obtain additional information concerning the taxonomic relationship between them. Esterase, lactate dehydrogenase, and general muscle proteins were examined.

Esterase

The esterases are a very complex family of enzymes with overlapping specificities, multiple tissue sources, and genetic variations in some species. Various classes of esterases may be distinguished on the basis of differential substrate and inhibitor specificities. Four main types of soluble esterases have been observed in vertebrate tissue extracts. They are carboxylesterase (E.C. 3.1.1.1), arylesterase (E.C. 3.1.1.2), acetylesterase (E.C. 3.1.1.6), and cholinesterase (E.C. 3.1.1.8) (Aldridge, 1954; Bergmann et al., 1957; Augustinsson, 1960; Holmes et al., 1968).

Lactate dehydrogenase

The enzyme lactate dehydrogenase (E.C. 1.1.1.27) exists in many organisms in isozymic form (Markert and Moller, 1959). It is a tetramer with a molecular weight of 140,000 (Markert, 1963). Two different subunits, A and B, join to form the tetramer. The subunits are specified by two different genes (the A locus and the B locus), and have five arrangements designated LDH-1 (A₁B₁), LDH-2 (A₁B₂), LDH-3 (A₂B₁), LDH-4 (A₁B₄) and LDH-5 (A₅). The most anodal band is LDH-1, while LDH-5 is the most cathodal band. The LDH-1 and LDH-5 isozymes are often termed homotetramers, and the heterogeneous combinations of subunits (LDH-2, LDH-3 and LDH-4) are referred to as allotetramers. The LDH subunits are not found in equal quantities in all tissues. The A subunit is produced in large amounts in white skeletal muscle, while the B subunit predominates in heart and brain tissue (Wilson et al., 1964).

A third LDH gene (the E locus) has also been found to be expressed in higher teleosts (Markert and Faulhaber, 1965). Originally postulated to be active only in retinal tissue and the eye lens, it has subsequently been observed in liver and other tissues (Shaklee et al., 1973).

General muscle protein

Muscle tissue contains a heterogeneous mixture of proteins. The basic structural proteins of muscle are actin and myosin. Also present are smaller and more soluble proteins such as parvalbumins (Pechere and Pantel, 1974). Thick filaments of muscle are composed almost exclusively of myosin, and this protein constitutes about 50 percent of myofibrillar protein. Myosin is a long slender molecule which consists of two helically twisted polypeptide chains with a molecular weight of 225,000 each. At regular intervals along the filament, globular head proteins are present (Lehninger, 1973). Red and white skeletal muscle proteins have been found to show tissue variations (Sarkar et al., 1971). On the basis of amino acid differences, it has
been concluded that the myosin of red muscle and that of white muscle are under separate genetic control (Hazar and Elzingier, 1972).

**Materials and Methods**

Collection of samples

All fish were collected by Tennessee Cooperative Fishery Research Unit personnel from the area between Toch Aukas Bia- logical Station and the Prospect Fork on the Clinch River. Most of the fish were collected by electroshocking, but nets were used on several occasions to capture the desired species. Collected fish were immediately packed in ice for transport to the laboratory where they were scored in a freezer.

Preparation for electrophoresis

Several scales were removed from each fish and impressions were obtained in plastic, using a modification of the method described by Greenbank and O'Donnell (1950). Scale impressions were ob- served on an Eberbach projector, using well's (1955) method for determining the age of shad. In addition to age and total length, the sex of each fish was recorded whenever an accurate determination could be made by examination of gonads.

Skeletal muscle from each fish was used for extraction of protein. Heart and optic nerve tissues were also used in some cases. With very small specimens, the digestive tract of the fish was removed to avoid interference by the liver and other digestive food particles. A 1:15 mixture (W/V) of 0.1 M tris-EDTA-borax buffer at pH 8.0, plus 1 mg/ml of homogenized iminogen, 10 mg/ml of menadione, 0.1 mg/ml of sodium azide, and 0.02 mg/ml of sulfur was adjusted to a pH of 8.0. The homogenate was centrifuged at 20,000 x g for 10 minutes. The resulting supernatants were adjusted to 0.3 M with respect to NaCl and used directly for electrophoresis.

**Electrophoresis**

Electrophoresis was accomplished utilizing a vertical slab polyacrylamide gel apparatus, manufactured by the E. A. Apparatus Company. All proteins were separated on 7.5% acrylamide gels, utilizing 0.1X Tris, 0.1X Borate, and 0.1X EDTA as the running buffer. Each gel was composed of 200 ml of 0.1 M tris-EDTA-borax acid buffer, pH 8.4, 2 ml of glycerol, 1 mg/ml of menadione, and 0.2 g of am- inoacid added to the running buffer. A continuous buffer utilizing 0.1 M tris-EDTA-borax acid buffer, pH 8.4, was used for all separations. Following electrophoresis, each gel was stained for the appropriate enzyme. Only those enzymes were used to the anode of the electrophoretic gels were examined.

**Staining procedures**

The staining solution was composed of 138 ml of distilled water, 1 g of 0.2 M phosphate buffer, pH 7.4, 1 ml of 1 percent Coomassie blue in 50 percent acetic acid (W/V), and approximately 150 mg of amido black. The solution was filtered through glass wool onto the gels, which were immersed in this solution for five hours. The gels were then placed in distilled water and scored the next day.

**Results**

All fish examined in this study were obtained from the same area of Clinch River. For both sexes, from males in each age class (one, two, and three year old) were sampled. Except for the males, the isozyme banding patterns differed very little from one individual to another within the same species. Therefore, it was just simple to classify the isozymes by taking the banding patterns and the age of the organism. In the general muscle protein banding patterns observed for the gizzard and the threadfin shad were the same. The patern did not vary with the sex or age of the organism and the bands was uniform throughout the sample.

Examination of the lactate dehydrogenase isozyme banding patterns observed for gizzard and threadfin shad muscle tissue (Figure 1) reveals several cases where the bands overlapped. A comparison of isozyme banding patterns for one year old fish demonstrates that band 1 overlapped for gizzard shad dehydrogenase band 3 and the threadfin shad band 1. A comparison of banding patterns for two year old fish shows that band 2 of the gizzard shad dehydrogenase overlaps the threadfin shad band 2. The three bands overlaps the dehydrogenase band 3. The only LDH band for the three year old shad is the gizzard shad band 3 and the threadfin shad band 3. The patterns did not vary with the sex of the organism, but the migration distance did vary with the age of the organism. It is of interest to note that all of the fish examined had three lactate dehydrogenase bands. The differences in migration distances of the bands.

**Discussion**

The most striking difference in banding patterns ob- served for the two species is in the heart muscle lactate dehydrogenase banding patterns. The pattern for giz- zard shad heart muscle tissue (Figure 3) has a fast migrating anodal band which is not present in thread- fin shad heart muscle tissue. This difference may be useful in differentiating these two species, and should be studied further.

Although the esterase banding patterns were the most irregular of the three proteins examined, some con- sistent differences between the two species were ob- served. The pattern of the lactate dehydrogenase banding pattern has a band which migrates to the anode faster than any other esterase band for the one year old threadfin shad. The two year old threadfin shad esterase banding pattern has four bands, none of which migrates to the anode faster than any of the threadfin shad esterase banding patterns observed for individuals of the same age. This shows promise as a means for differentiating these species; however, it should be noted that the aging differences observed absolutely require that individuals of comparable age be used. For example, bands 1 and 2 observed for the three year old threadfin shad band 1 and 2 for one year old threadfin shad.

When comparing the banding patterns, a number of similarities may be observed. General muscle protein rates of migration for gizzard shad and threadfin shad were identical in every respect. Lactate dehydrogenase skeletal muscle banding patterns did not vary greatly when compared with each other. All of the fish examined had three lactate dehydrogenase bands, with the differences involving the comparative migration distances.

Even for the esterase skeletal muscle banding
patterns, several similarities were noted. For example, in comparing three year old individuals of the two species, two bands were observed to overlap. As noted above, when banding patterns for different age groups are compared, a number of similarities between the bands for the two species are observed. In comparing the three year old gizzard shad esterase banding pattern with the two year old threadfin shad esterase banding pattern, one notes that the gizzard shad bands 1 and 2 overlap the threadfin shad bands 1 and 2. Also, when one compares the two year old gizzard shad esterase banding pattern with the three year old threadfin shad esterase banding pattern, one notes that the gizzard shad bands 3 and 4 overlap the threadfin shad bands 1 and 2. From examination of the electrophoretic banding patterns in this study, the similarities suggest that the two species are closely related on a taxonomic basis.

Additional studies are needed in order to clearly establish the utility of electrophoresis to taxonomically distinguish between these two species. The studies could include the following areas of research: use of different proteins or enzymes, use of cathodal banding patterns (in this study only anodal patterns were examined), use of different buffer systems, and use of two dimensional electrophoresis.

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LITERATURE CITED


