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AN ELECTROPHORETIC TAXONOMIC STUDY ON THE HAEMOGLOBINS OF CYPRINID FISH

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Few studies have been done concerning the haemoglobin of Cyprinid fish (Ohkuba *et al.*, 1993). Moreover, on the haemoglobins of *Capoeta trutta, Capoeta capoeta umbla, Leuciscus cephalus orientalis,* and *Acanthobrama marmid* have not been electrophoretically studied. In the present investigation, haemoglobin of these fishes has been analyzed by native - PAGE technique and thus resemblances and differences between these species have been established.

Materials and Methods

The haemoglobins of *Capoeta trutta, Capoeta capoeta umbla, Leuciscus cephalus orientalis* and *Acanthobrama marmid* belonging to Cyprinidae family were examined by native-PAGE and gel filtration techniques. The fish species were collected in Karakaya Dam Lake (Malatya, Turkey). The fish were alive when they were transported to the laboratory. 2-3 ml blood was taken from the dorsal aorta of fishes into tubes, which contain EDTA. The blood samples were centrifuged at 1500xg for 10 min at +4°C. Then the erythrocytes were lysed with equal volumes of cold distilled water. Cell debris were removed by centrifugation at 20.000 g for 20 min and the clear haemoglobin solution was used for the PAGE. The haemoglobin concentrations were determined spectrophotometrically by Drabkin (1946) method at 540 nm.

Native-PAGE was performed according to the Laemmli (1970) and O'Farrell (1975) methods. Native haemoglobins were separated on 16 x 10 cm dimension and 1 mm thick slab gel. Proteins are stocked in stacking gel and running gel separates them. Running and stacking gel contains 10% and 4% acrylamide, respectively. Running gel was polymerized for 12 hours before stacking gel was poured on it. For native-PAGE, 40µl of sample was loaded on the stacking gel and 200 V voltage are given to system until bromo phenol blue come to lowest side of running gel. Following electrophoresis, the haemoglobin bands were stained with solution which contains 0.125% commassie brilliant blue R-250 in 40% ethanol and 7% acetic acid. After that, by using acetic acid, the running gel was cleared off excess stains.

Get filtration chromatography was carried out using a sephadex G-100 column (2 x 100 cm) eluted with 0.2 M potassium phosphate, pH 7.6 Column was calibrated for molecular weight studies with private kinase (237 kD). 10 mg per 250 µl of haemoglobin in the hemolysate was applied in sephadex G-100 column.

Results and Discussion

Total number of the haemoglobin bands of *Capoeta trutta* and *Acanthobrama marmid* was only one, the total number of the haemoglobin bands of *Capoeta capoeta umbla* and *Acanthobrama marmid* were four and two respectively. In the present study, each fish have a single haemoglobin peak in gel filtrations. Molecular weights of haemoglobin bands of *Capoeta trutta, Capoeta capoeta umbla, Leuciscus cephalus orientalis,* and *Acanthobrama marmid* obtained from gel filtration were calculated to be 85, 54, 91 and 85 kD, respectively.

Electrophoretic patterns of haemoglobin of freshwater breams *Abramis brama, A. ballerus* and *A. sapa* belonging to Cyprinidae family were investigated by Arefyev and Karnauchov (1989) and reported that each species showed two types of haemoglobin (Hbl and HbII), which differ from each other on relative protein content and electrophoretic mobility of components; greatest differences between Hbl and HbII were found in *A. Brama*. In this study haemoglobin band patterns of three species have shown a similarity to haemoglobin band patterns of *Leuciscus cephalus orientalis.* However, haemoglobin band patterns of the other fish studied were found to be different. The molecular weight and the number of the haemoglobin bands among four fish species were different, they were distinguished from each other taxonomically. It is concluded that comparison of haemoglobin might have practical importance into toxonomic classification of species belonging to the same family.

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