

## CAPOETA CAPOETA UMBLA VE ACANTHOBRAMA MARMID'İN HEMOGLOBİN ALTÜNİTELERİ ÜZERİNE YAŞA BAĞLI ELEKTROFORETİK DEĞİŞİKLİKLER

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### ÖZET

Bu çalışmada, *Capoeta capoeta umbla* ve *Acanthobrama marmid*'in kanından hemoglobinler saflaştırılarak sodyum dodesil sülfat-poliakrilamid jel elektroforezi (SDS-PAGE) yöntemi ile yaşa bağlı olarak subüniteleri izole edilmiştir. 2 ve 3 yaş grubundaki *Capoeta capoeta umbla*'da 2 globin bandı ve 4 yaş grubundakilerde 1 globin bandı, *Acanthobrama marmid*'de ise 2 yaş grubundakilerde 4 globin bandı ve 4 yaş grubundakilerde 3 globin bandı elde edilmiştir. Aynı zaman da, bu iki balığın globin zincirlerinin molekül ağırlıklarında da farklılıklar gözlenmiştir.

Sonuç olarak, bu balıklarda yaşa bağlı olarak hemoglobinin moleküllerinin yapısının, subünite sayısı ve gerekse de subünitelerinin molekül ağırlıkları bakımından değiştiğini göstermektedir. Yaşa bağlı olarak gerçekleşen bu değişiklikler, muhtemelen bu balıkların bulunduğu ortama adaptasyon sağlaması ve/veya vücudun oksijen ihtiyacını dengeleme amacıyla kaynaklandığı kanısına varıldı.

**Anahtar Kelimeler:** *Cyprinidae*, *Capoeta capoeta umbla*, *Acanthobrama marmid*, Polimorfizm, Hemoglobin, SDS-PAGE

### ELECTROPHORETIC CHANGES OF HEMOGLOBIN SUBUNITES OF *CAPOETA CAPOETA UMBLA* AND *ACANTHOBRAMA MARMID* IN RELATION AGE

#### Abstract

In this study, hemoglobins from erythrocytes of II-IV age groups *Capoeta capoeta umbla* and *Acanthobrama marmid* (II,IV age groups) were purified and subunits of hemoglobins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Subunits of hemoglobins in SDS-PAGE, 2 globin bands from II and III age groups and 1 globin band of IV age groups *Capoeta capoeta umbla* were separated. 4 globin bands from II age groups and 3 globin bands from IV age groups *Acanthobrama marmid* were distinguished. At the same time, molecular weights of globin chains of these two fishes were found to be different.

As a result, hemoglobin structures related to age in these fishes show differences both in subunit numbers and in molecular weights. It is concluded that these varieties related to age were probably resulted from environment adaptation and/or from the balance of oxygen requirement of fish body.

**Key words:** *Cyprinidae*, *Capoeta capoeta umbla*, *Acanthobrama marmid*, polymorphism, hemoglobin, SDS-PAGE.

## INTRODUCTION

In contrast to mammals, fish show a large polymorphism in the number and molecular weight of hemoglobin (Hb) components, which relates to their ability to adapt to different environmental conditions [1].

Many researchers reported that globin chains of various fish species change from one species to another, electrophoretically [2,3,4,5]. In a research, two hemoglobins (HbI and HbII) in Antarctic notothenioid fish *Gobionotothen gibberifrons* were found and, hemoglobins of these fish consist of four globin chain (two  $\alpha$  and two  $\beta$  -chains) [6]. Among cartilaginous fish, *Bathyraja eatonii* and *Raja hyperborea* have single major hemoglobins [7]. In an investigation by Pellegrini et al. [8], it was found that the conger eel *Conger conger* hemoglobin comprises of three hemoglobins components. Electrophoretic patterns of *Asyanax scabripinnis* belonging to Characidae family have showed two cathodic hemoglobins [9]. Unfortunately, only few studies have been done, concerning the hemoglobins of Cyprinid fish [1,10,11]. Moreover, no study has been done on hemoglobins of *Capoeta capoeta umbla* and *Acanthobrama marmid*, electrophoretically.

In the present study, globins of *Capoeta capoeta umbla* and *Acanthobrama marmid* have been analyzed by SDS-PAGE technique and thus resemblance and differences between these two species has been tried to be established.

## MATERIALS AND METHODS

In the study, *Capoeta capoeta umbla* and *Acanthobrama marmid* belonging to Cyprinidae family were used. The fish species were collected from Karakaya Dam Lake (Malatya, TURKEY). The fish were alive when they were transported to the laboratory. 2-3 ml blood has been taken from the dorsal aorta of fishes injected into tubes, which contain EDTA. The blood samples were centrifuged at 1500g for 10 min at +4 0C. Then the erythrocytes were collected and washed three times with three volumes of 0.9 % NaCl. After that, the erythrocytes were lysed with equal volumes of cold distilled water. Cell debris was removed by centrifugation at 20.000 g for 20 min and the

clear hemoglobin solution was used for SDS-PAGE. The hemoglobin concentrations were determined spectrophotometrically by Drabkin [12] method at 540 nm.

SDS-PAGE was performed according to the Laemmli [13] and O'Farrell [14] methods. Denaturated hemoglobins were separated on 16x10 cm dimension and 1 mm thick slab gel. Proteins were stocked in slab gel consisting stacking gel and in running gel part on which proteins separate. Running gel (contains 12 % acrylamide) was polymerized 12 hr before the electrophoresis and stacking gel (contains 4 % acrylamide) was poured and polymerized 2 hr before the sample application. Following electrophoresis, the globin bands were stained with 0.125 % commassie brilliant blue R-250 in 40 % ethanol and 7 % acetic acid, and then destained in acetic acid. Pepsin (34.7 kD) and  $\beta$ -lactoglobulin (18.4 kD) were used as protein standard in SDS-PAGE. MW of globins in SDS-PAGE were calculated according to Weber et al. method [15].

## FINDINGS AND DISCUSSION

The hemoglobins of *Capoeta capoeta umbla* and *Acanthobrama marmid* are analysed by SDS-PAGE. As seen in figure 1, In the PAGE, total number of the globin bands of *Capoeta capoeta umbla* in II-III age groups were 2 and globin chains of IV age groups were 1. Molecular weights (MW's) of globin bands of *Capoeta capoeta umbla* in II-III age groups were found dimeric structure as 26.1 and 24.8 kD ( $1\alpha 1, 1\beta 1$ ). MW of globin band in IV age group was seen to be 26.1 kD ( $2\beta 1$ ). As seen in figure 2, total number of the globin bands of *Acanthobrama marmid* in II age groups were 4 and, globin bands of IV age groups were 3. MW's of the globin bands in II age groups were obtained to be a tetrameric structure as 25.4, 24.4, 18.7 and 18.0 kD ( $1\alpha 1 \alpha 2, 1\beta 1 1\beta 2$ ). MW's of the globin bands in IV age groups were found as 25.4, 24.4 and 18.0 kD ( $2\alpha 1, 1\beta 1 1\beta 2$ ). 18.7 kD globin band in IV age groups wasn't observed.

There has been considerable increasing interest in fish hemoglobins in recent years. They exhibit the essential features of mammalian hemoglobins, cooperative ligand binding and heterotropic responses to a variety of ionic species, but they display an astounding variety of functional behaviors. These different

properties are of interest as examples of evolutionary adaptation to differing physiological and environmental needs [16].

Many researchers have been studied on fish hemoglobins, electrophoretically. The hemoglobins of the black-chinned tilapia in different population have been analyzed by acidic urea polyacrylamide gel electrophoresis and detected five different  $\alpha$ -chains and four different  $\beta$ -chains. These variations were based on genetic variations at the globin chain coding loci [5]. In a research carried out by Arefyev and Karnachov [1], electrophoretic patterns of hemoglobin of freshwater breams *Abramis brama*, *A. ballerus* and *A. sapa* were investigated and they reported that each species showed two types of hemoglobin (HbI and HbII), which differ from each other on relative protein content and electrophoretic mobility of components; greatest differences between HbI and HbII are found in *A. brama*. Researchers have noted these findings as important, taxonomically. Similarly, the hemoglobins and globins of *Synbranchus marmoratus* have been studied by agar gel-starch electrophoresis and three distinct hemoglobin bands were found, with HbI being of the cathodic type. In cellulose acetate electrophoresis, the presence of four globin chains denoted  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$  were indicated. The probable tetrameric constitution of the hemoglobin of *Synbranchus marmoratus* was suggested [17]. Three hemoglobin components in carp (*Cyprinus carpio*) designated CI, CII, and CIII were obtained by DEAE-Toyo-pearl ion-exchange chromatography. Then, tetrameric globin chains of these hemoglobins were analyzed [11]. In other study, an ontogenic variation with specific embryonic, juvenil and adult electrophoretic patterns of chinook salmon, *Oncorhynchus tshawytscha* were investigated. The embryonic and juvenil patterns were monomorphic whereas the adult pattern occurred in three phenotypes [18]. In a research by Southard et al. [19], the nine hemoglobin from blood *Salmo clarki* by ion-exchange chromatography and preparative isoelectric focusing were purified. Then, subunit structures of eight of the purified hemoglobins were applied to electrophoresis and, six of these hemoglobins have  $\alpha_2\beta_2$  tetramers while two appear to be heterotetramers of the type  $\alpha\alpha_1\beta_2$  and  $\alpha\alpha_1\beta_1$ .

## RESULTS

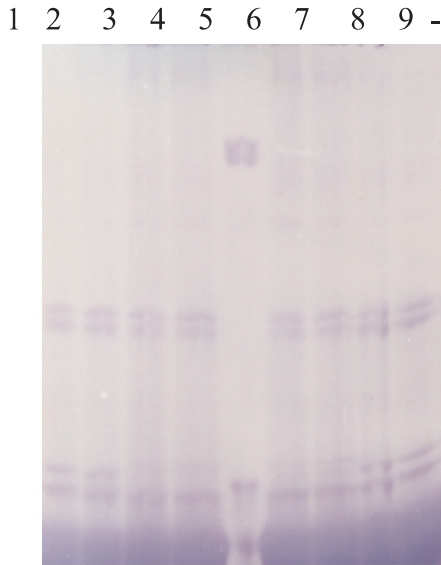
As a result, globin chains of the present study have found to be different from other fish globins previously investigated. Nevertheless, The hemoglobin structures related to age in these fishes show differences both in subunit numbers and molecular weights. These differences can be attributed to differences of these fishes in their gene level. Thus, this study may be important for the partly clarification of genetic background of these fishes. Moreover, it is concluded that these varieties related to age probably resulted from environmental adaptation and/or from the balance of oxygen requirement of fish body.

### Figure legends

1 2 3 4 5 6 7 8 9 -



**Figure 1.** Electrophoregram of the globin chains of *Capoeta capoeta umbla* hemoglobins in SDS-PAGE. The globin chains of *Capoeta capoeta umbla* in II and III age groups (1-2nd and 8-9th). 5th lane standard proteins. The globin chains of *Capoeta capoeta umbla* in IV age groups (3-4th and 6-7th).



**Figure 2.** Electrophoregram of the globin chains of *Acanthobrama marmid* hemoglobins in SDS-PAGE. The globin chains of *Acanthobrama marmid* in II age groups (1-2nd and 8-9th). 5th lane standard proteins. The globin chains of *Acanthobrama marmid* in IV age groups (3-4th and 6-7th).

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