SCIENTIFIC NOTE

AN ELECTROPHORETIC VARIATION OF THE MYOGLOBIN IN LOACHES, *MISGURNUS ANGUILLICAUDATUS*

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Received May 26, 1976

Although a number of genetic variations of hemoglobin have been found in various species of vertebrates, only few informations on such variation of myoglobin are obtainable. In fishes, myoglobin polymorphism is known to occur in two species, *Clupea harengus* (de Ligny 1972) and *Coregonus pollan* (Ferguson 1975). In the present paper an electrophoretic variation of myoglobin in the loach will be presented.

The loaches *Misgurnus anguillicaudatus* were used in this experiment. Their hemoglobin electrophoretic patterns were identical and were classified as Hb B (Kimura, 1976). The fishes were killed by exsanguination and were stored at -20°C for three months before they were examined. Lateral line muscle was homogenized in an equal volume of distilled water. Method for preparing homogenate and starch gel electrophoretic procedure were same as those previously used for the chicken cardiac muscle phosphoglucomutase isozyme (Kimura 1974). The tris-EDTA-borate buffer system as described by Cooper *et al.* (1969) was used. The gel was placed horizontally at 5°C and a stabilized voltage of 25 V/cm was established across the gel for three hours. After electrophoresis, the gel was stained for myoglobin by a benzidine solution which was composed of 0.2 g benzidine acetate, 0.5 ml acetic acid and 0.5 ml of 30% hydrogen peroxide in 100 ml of distilled water.

Three types of myoglobin pattern were observed (Fig. 1). Two of them were designated Mb A and Mb B in order of decreasing mobility. Each of these two types consisted of two bands, a major band in the cathodic region and a minor band in the anodic region. The third type designated Mb AB corresponded to a mixture of Mb A and Mb B.

The observed number of Mb A, Mb B and Mb AB in the loach group used in this experiment were 3, 17 and 6, respectively. Judging from the shape of the pectoral fins, four out of six Mb AB fishes were male and the other two were female.

In the preceeding paper the author reported that the loach hemoglobin was electrophoretically separated into two regions, one migrated from the origin toward the cathode and the other toward the anode (Kimura, 1976). The mobility of the major band differed from those of the two hemoglobins or their derivatives (met form and cyanomet form), but the minor band occurred on the same region as that of the anodally migrating

Fig. 1. Myoglobin patterns of loach lateral line muscle extracts.

Fig. 2. Myoglobin patterns of the loach.
met Mb: met form Mb was prepared by adding NaNO_2 to the muscle extract.

hemoglobin.

Conversion of the major band to met form or cyanomet form had no effect on its mobility. Similar observation was obtained in the pollan myoglobin by Ferguson (1975). He reported that the treatment with carbon monoxide gas had no effect on the number or mobility of the myoglobin bands. On the other hand treatment of the loach minor band with sodium nitrate altered its mobility. The met form minor band migrated more rapidly toward the cathodic end than the major band (Fig. 2). It seems improbable that the minor band is derived from the blood remained in the muscle, as the loach hemoglobin is very unstable under storage and as the hemoglobin of the loach group used in the present study was monomorphic. The major band may be myoglobin, but the minor band is remained unidentified.

Ferguson (1975) reported in the pollan that the same myoglobin phenotype was found in both the heart and the lateral line muscle of an individual. Similar result on tissue specificity of myoglobin was obtained by Ann Baker (1966). She examined electrophoretically five species of bird and found that myoglobin was common to more than one organ or tissue and was qualitatively identical in all the muscles studied within one species although marked quantitative tissue-specific differences occurred. de Ligny (1972) found in the herring that five electrophoretic myoglobin variants could be distinguished in heart extracts from adult as well as young herring and that the analysis of a group of 1-year old herring did not reveal ontogenetic changes of the myoglobin patterns. Boyer *et al.* (1963) observed in man that storage of samples for several months produced no obvious alteration in the electrophoretic properties of skeletal muscle myoglobin. Ann Baker (1966) also showed that the samples which were frozen over a period of 18 months and used immediately after thawing gave invariably uniform electrophoretic resolution of proteins, including myoglobin. These facts support a possibility that myoglobin polymorphism may provide an useful and convenient marker for genetical investigation.

LITERATURE CITED

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