ELECTROPHORETIC VARIATION IN HAEMOGLOBINS OF SOME FRESHWATER AND ESTUARINE FISHES OF GHANA

S. AMISAH, BSc, Dip Ed, M.Phil, MSc, PhD
Institute of Renewable Natural Resources, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana.

ABSTRACT

Electrophoresis has been performed on the haemolysates of some freshwater and estuarine fishes in and around Cape Coast in the Central Region of Ghana. The electrophoresis was performed on the haemolysates of Sarotherodon melanotheron (Ruppel) Tilapia zilli (Gervais), Heterobranchus isopterus (Burchell)., Clarias gariepinus (Valenciennes), Chrysichthys nigrodigitatus (Valenciennes), Channa obscura (Walbaum), and Carassius auratus L.

All the fishes studied had multiple haemoglobins. Nine haemoglobin bands were found in T. zilli and s. melanotheron. Four bands each were observed in C. gariepinus and H. isopterus. Three bands were found in Carassius auratus whilst Channa obscura and Chrysichthys nigrodigitatus were characterised by four and three bands respectively. The multiple haemoglobins show striking similarities among related species but also display considerable differences among the different taxonomic families.

Keywords: *electrophoresis; fishes; haemoglobins, haemolysates.*

INTRODUCTION

Haemogobin contained in erythrocytes is an iron-containing conjugate protein, which conveys oxygen from the lungs or gills to the tissues and partly facilitates the return of carbon dioxide from the tissues back to the lungs or gills (1) Most studies of haemoglobins have been confined to the blood and muscle of mammals (2) As a result, the knowledge of the functional and structural properties of haemoglobins of lower vertebrates such as fish has been fragmentary. The respiratory requirements of lower vertebrates vary over an enormous range (2) giving rise to many interesting adaptations.

The blood volume of fish is relatively small, and counts of blood cells seem to show that larger fish require an increased oxygen carrying capacity though this is yet to be established (3). In fishes, the effects of carbon dioxide can be so pronounced that the haemoglobin does not get saturated at partial pressures of oxygen levels in the body. This extreme effect of carbon dioxide is called Root effect (4, 5). Fish are the most numerous vertebrates and they constitute about 42.6% of the entire vertebrate population and with about 20000-23000 species (6). They live in varied environments and it would be interesting studying their haemoglobins. Generally, fishes have multiple haemoglobins and it is very rare to find a fish with only one type of haemoglobin It may well be that the possession of multiple haemoglobins enables the fish to adapt to a varied and dynamically unstable environment (7).

Ontogenetic changes in fish haemoglobins are widespread and have been observed in four species of salmon and in the herring and sprat (4). Changes in haemoglobins in a number of fish occur during their life cycle. This has been found in the herring, *Clupea harengus* L. (2), where in the juveniles two types of haemoglobins are initially present. One of the haemoglobins is gradually replaced over a period of four years, whilst a third type is added just prior to full maturity of the fish (2, 8). A pre-requisite for haemoglobin to have adaptive value is the difference in the functional properties of the various haemoglobin types (9).

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Fish haemogobin electrophoresis

When fish haemoglobin is subjected to electrophoresis, it often separates into bands moving at different velocities, the proportion of which have been used to identify different races or stocks within a single species (10). Electrophoresis therefore provides an important method for measuring the genetic discreteness of fish stocks and for the study of genetic relationships among stocks.

This work is a preliminary study of the electrophoretic variation in the haemoglobins of some local fishes in Ghana. Haemolysates from freshwater and estuarine fishes in and around Cape Coast, Ghana, have been surveyed by cellulose acetate paper electrophoresis to provide their haemoglobin variation, and particularly to describe the haemoglobin patterns of the fishes in the locality with a view to measuring the genetic discreteness of the fish stocks in the area.

MATERIALS AND METHODS

Collection of Fish Samples

Fish samples belonging to several genera were caught from local freshwaters and lagoons in and around Cape Coast in the Central region of Ghana using cast and gill nets. Some fish were also caught from some estuaries along the main trunk road linking Cape Coast and Takoradi in the Western region. The fishes in all cases were transported live in portable aquaria to the laboratory and bled within one to two hours of collection.

Bleeding of Fishes

Bleeding of the fishes was done according to the method described by Sharp (11). A 2.0ml aliquot of anti-coagulant consisting of 0.5 M Dibasic sodium phosphate (Na_2HPO_4) and 0.5 M Sodium citrate ($Na_2C_6H_5O_7$) was drawn into a 10 ml syringe equipped with a hypodermic needle. The fish was placed on its side and held firmly while blood was drawn from the caudal vein into the anti-coagulant-filled syringe. Alternatively, blood samples were obtained by severing the peduncle or tails of the fish using a sharp knife and allowing the blood to collect in anti-coagulant in labelled centrifuge tubes.

Blood samples were centrifuged for 5 minutes at speed 2 using the MSE Minor Centrifuge. The supernatant plasma was decanted leaving the precipitated erythrocytes at the bottom of the tubes. The erythrocytes were washed thrice with a 2% (w/v) saline. The red blood cells were then haemolysed by adding one part distilled water and one part carbon tetrachloride. The haemolysate was centrifuged for another 5 minutes and with the aid of a pipette dropper, the supernatant was carefully collected for electrophoresis. The process was repeated for all the fishes collected.

Electrophoretic Techniques

Electrophoresis was performed on the fish haemolysates according to the method described by Sharp (11). A 0.5M buffer solution of pH 8.4 was prepared from Supre Heme Tris EDTA-borate (Helena Laboratories, Texas, USA) by dissolving it in a litre of distilled water. A Titan III 5.70 x 7.62 cm cellulose acetate strip was floated on some of the buffer solution and allowed to impregnate for 15 minutes. A Shandon electrophoretic bath was also filled with some of the buffer solution of Supre-Heme Tris-EDTA Borate at both anode and cathode compartments. Both compartments were filled to the same level to avoid a siphoning effect on the separation. Contact was then established between the anode and cathode by means of wicks prepared from filter papers.

The impregnated cellulose acetate strip was dried by pressing gently between two filter papers. The blood samples were then applied on the strip using a sample applicator. The strip was carefully placed upside down and horizontally on the bridge with the plastic surface up. The wicks were used to establish contact between the buffer solution in each compartment and the cellulose acetate strip. The Shandon electrophoretic bath was connected to a Vokam power pack, which had been connected to the mains. The electrophoresis was run for 15 minutes.

Staining

The cellulose acetate strip was removed and transferred into a protein fixative, 5% (v/v) trichloroacetic acid for 5 minutes. Afterwards, the strip was transferred into a staining solution of 0.2% Ponceau S in 5% trichloroacetic acid for 15 minutes. The excess stain was washed off in three changes of 5% glacial acetic acid and finally washed with distilled water. the strip was then observed and the number of haemoglobin bands present in each case was recorded. The strips were dried and stored or kept in 10% methyl alcohol. The experiment was repeated for all the samples.

RESULTS AND DISCUSSION

Electrophoresis was performed on the haemolysates of fishes from four taxonomic families represented by seven genera. The number of haemoglobin bands (Hb) in each fish and their direction of migration were recorded (Table 1). A schematic representation of the direction of migration of haemoglobin bands of the fishes during the electrophoresis is illustrated in Fig. 1. It was further observed that within a particular species of fish there were no differences in the number and direction of migration of the haemoglobin variants. The results of this study have shown that all the fishes examined had multiple haemoglobins. There was no fish with just one haemoglobin type. For the fishes examined the number of haemoglobin bands ranged between 3 and 9 with a mean of 5. *S. melanotheron*, obtained from a lagoon and also from some estuaries (brackish waters), gave 9 bands of haemoglobin just as *T. zilli* obtained from the freshwaters of river Kakum. These two fishes are phenotypically alike (6) *C. auratus*, obtained from the freshwater ponds of Cape Coast University however had 3 bands all of which were anodal. It therefore does not appear that the environment determines the number of haemoglobins the fish can have.

Heterobranchus and Clarias species, which are both morphologically similar (6) and belong to the family Clariidae, were found to have 4 bands each. They were obtained from the freshwaters of the River Kakum. *Chrysichthys, nigrodigitatus* (Bagridae), also obtained from River Kakum had 3 bands of haemoglobin, two of which were anodal and one cathodal.

Table 1: Haemoglobin variation in some freshwater and estuarine fishes of Ghana

Family	Fish species	No. Of fish	No. of Hb per fish	*Anodal Hb*	Cathodal *Hb*
Cichlidae	**Sarotherodon melanotheron**	40	9	9	0
	Tilapia zilli	40	9	9	0
	Carassius auratus	40	3	0	3
Clariidae	**Heterobranchus isopterus**	40	4	4	0
	Clarias gariepinus	40	4	4	0
Bagridae	Chrysichthys nigrodigitatus	40	3	2	1
Channidae	Channa obscura	40	4	2	2

Mean Hb count for fishes examined = 5

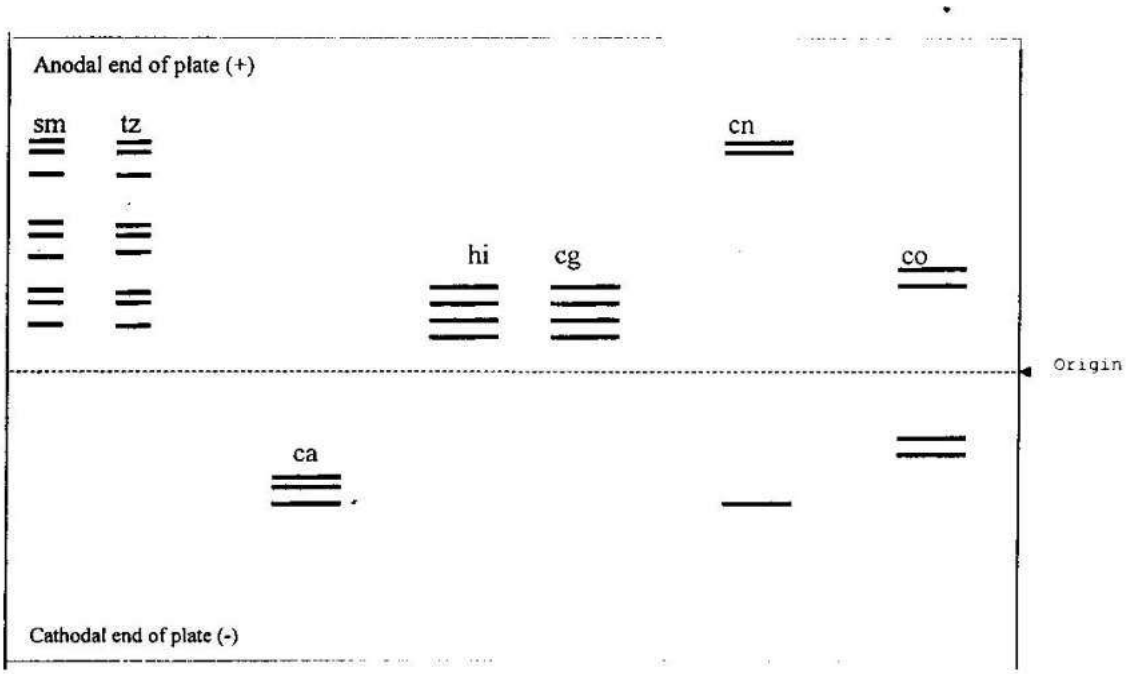

Fig. 1.: ***Schematic representation of the electrophoretic migration of haemoglobin bands of some freshwater and estuarine fishes of Ghana. (Notation: sm – Sarotherodon melanotheron; tz – T. zilli; ca – Curasius auratus; hi – Heterrobranchus isopterus; cg – Clarias gariepinus; cn – Chrysichthys nigrodigitatus; co – Channa obscura.***

The family Channidae, represented by *Channa obscura* (snakehead) was found to have four haemogobins: 2 anodal and 2 cathodal. In the Clariidae there were no observable differences in the velocity of protein migration, distribution, orientation and number of haemoglobin bands amongst the individuals, suggesting the possibility of some biochemical and/or genetic similarities. A similar observation was made for *T. zilli* and *S. melanotheron*. Protein migration was also found to be slower for the Clariidae than for the Cichlidae, suggesting the possibility of a higher molecular weight for the Clariidae

The present results are in consonance with those of Riggs (9) and Bonaventura *et al.* (12), who in their studies of haemoglobin heterogeneity in tropical Amazonian fishes and in temperate freshwater fishes found a mean of 4.0 and 5.0 haemoglobin bands respectively for the fishes

Different types of haemoglobin in a species might endow the species with some biological advantages. Hochachka and Somero (13), have suggested two evolutionary strategies for catalytic proteins adapting to variable conditions. one has selection favouring an enzyme capable of functioning under more adverse conditions; the second has selection favouring the acquisition of additional enzyme variants, each with slightly different sensitivities to the working conditions. The occurrence of multi-haemoglobins in the haemolysates examined in this study may well be a type case of such dualism in adaptive strategy.

Riggs (9), in his review of mainly temperate fish species, stated that "haemolysates with only one single component are quite exceptional" The current findings are consistent with this statement. Fishes live in freshwater, brackish and marine environments. Others travel between freshwater and marine environments. It may well be that the possession of multiple haemoglobins is an adaptation to cope with a variable environment. The almost universal oc-

currence of multiple haemoglobins in fish suggests that some important physiological factors may favour multiplicity and that the possession of multiple haemoglobins confers some advantage.

The biological significance of this multiplicity is not certain but the hypothesis has been advanced that it represents an adaptation to cope with a varied and dynamically unstable environment (4). A basic requirement for haemoglobin variation to have adaptive value is differences in the functional properties of the various haemoglobins. This aspect was not investigated in this work. This work needs to be followed with studies on oxygen binding characteristics of isolated haemoglobins from these fishes from the various environments to look for functional differences.

CONCLUSION

The electrophoresis of the fish haemolysates has revealed that all the fishes have more than one type of haemoglobin. There is considerable electrophoretic variation in the haemoglobins of the fishes examined. Some have a net positive charge whilst others have a net negative charge. There were no observable differences in the haemoglobin patterns of *S. melanotheron* and *T. zilli*. It may well be that the two species are evolutionarily related though the former lives in both brackish and fresh waters whilst the latter is found in freshwaters only. The same observation was made for *Heterobranchus* and *Clarius* and these may also share some evolutionary relationship. Both inhabit the freshwaters.

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REFERENCES

1. Schmidt-Nielsen, K. Animal Physiology: Adaptation and Environment (3rd edn). pp. 70-94. Cambridge Univ. Press, Cambridge. 1983
2. Riggs, A. Properties of fish haemogobins. In Fish Physiology (W. S. Hoar and D. J. Randall eds) Vol. IV pp. 209-252. Academic Press, New York. 1970.
3. Gordon, M. S. Animal Physiology: Principles and Adaptations. 2nd ed. 592 pp. McMillan, New York, 1972.
4. Brett, J. R. The metabolic demand for oxygen in fish, particularly the salmonids and a comparison with other vertebrates. Respiratory Physiology 14:151-170. 1972.
5. Tetens, V. And Lykkeboe, G. Blood respiratory properties of rainbow trout, *Salmo gairdneri:* Responses to hypoxia acclimation and anoxic incubation of blood in vitro. J. Comp. Physiol. 145:117-125. 1981.
6. Jordan, E. L. Chordate Zoology (2nd ed). pp 469-600. Chand and Co. New Delhi, 1968.
7. Hill, R. W. Comparative Physiology of Animals: An Environmental Approach, pp. 374-377, Harper & Row Publ. New York, 1976.
8. Randall, D. J. Gas exchange in fish in Fish Physiology (W. S. Hoar and D. J. Randall eds). pp. 253-292. Academic Press, New York. 1970.
9. Riggs, A. Functional properties of haemoglobins. Physiol Rev. 45:619-673. 1965.
10. Riggs, A. and Gillen, R. G. Structure and function of isolated haemoglobins of American eel, *Anguilla rostrata*. J. Biol. Chem. 248L:1961-1969.
11. Sharp, G. D. Electrophoretic study of Haemoglobins of Scombrid fishes and related forms. 135pp. McGraw Hill, New York. 1965.
12. Bonaventura, J.; Bonaventura, C., and Sullivan, B. Non-heme oxygen transport proteins. In Oxygen and Physiological functions (Jobsis, F. F. ed), pp. 177-220. Dallas: Professional Information Library, 1977.
13. Hochachka, P. W. and Somero, G N Strategies of Biochemical Adaptation, 534pp. Saunders, Philadelphia. 1973.