Astyanax scabripinnis (PISCES: CHARACIDAE) HEMOGLOBINS: STRUCTURE AND FUNCTION

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ABSTRACT

Electrophoretic patterns of hemoglobins, Root effect, Bohr effect in blood and stripped hemoglobin, Hb-O_2 affinity GTP modulation of *Astyanax scabripinnis* (lambari), caught at three different altitudes in Ribeirão Grande, near Campos do Jordão (São Paulo), are described. All populations showed the same electrophoretic patterns: two cathodal components in starch gel. Normal Bohr effect values were found in these three populations both in blood (ϕ = –0,11) and stripped hemoglobin (ϕ = –0,12). Different blood O_2 affinities collected in fish of these 3 populations were detected. GTP has a large influence on Hb-O_2 binding properties in *A. scabripinnis*. Stripped hemoglobin shows small Root effect. The addition of triphosphated nucleotides increases this effect. GTP is more effective than ATP on enhancing Root effect. Oxygen availability in water can be the factor responsible for differences found in blood O_2 affinity.

Key words: lambari, oxygen availability, organic phosphates, Bohr effect, altitude.

RESUMO

Hemoglobinas de *Astyanax sacabripinis* (Pisces: Characidae): estrutura e função

Padrão eletroforético de hemoglobina, efeito Root, efeito Bohr em sangue e em Hb *stripped* e efeito do GTP na afinidade Hb-O_2 de *Astyanax scabripinnis* (lambari) coletados em três altitudes diferentes no Ribeirão Grande, na região de Campos do Jordão (São Paulo), são descritos. Todas as populações mostraram os mesmos padrões eletroforéticos: dois componentes catódicos em gel de amido. Efeito Bohr normal foi encontrado para as três populações, tanto em sangue (ϕ = –0,11) quanto em Hb *stripped* (ϕ = –0,12). Detectamos afinidades diferentes da hemoglobina ao O_2 entre as três populações estudadas. GTP possui forte influência nas propriedades de ligação da hemoglobina ao oxigênio em *Astyanax scabripinnis.* Em hemoglobina *stripped*, o efeito Root é baixo. A adição de nucleotídeos trifosfatados aumenta esse efeito. GTP é mais efetivo que ATP no aumento desse efeito. A disponibilidade de O_2 na água pode ser o fator responsável pelas diferenças encontradas nas afinidades da hemoglobina pelo oxigênio.

Palavras-chave: lambari, disponibilidade de oxigênio, fosfato orgânico, efeito Bohr, altitude.

INTRODUCTION

Hemoglobins are particularly important in studies of fish adaptations because the hemoglobin molecule is poised between the metabolism of the organism and the environment and so it has to cope with both metabolic requirements and environmental constraints (Riggs, 1979).

The *Astyanax scabripinnis* individuals studied here, live in river headwater, and in mountains, forming separated populations that are able to migrate down, but very seldom to it.

Krogh & Leitch (1911) showed that blood respiratory properties could be related to availability of environmental oxygen. After this report, many authors showed that differences exist in functional properties of hemoglobin in fishes from different environments.

The present paper compares functional properties of unfractionated blood, hemoglobin electrophoretic pattern, functional properties of stripped hemoglobin and the influence of organic phosphates in O_2 affinities, from three populations living at three different altitudes of *Astyanax scabripinnis* in Campos do Jordão, SP, Brazil.

MATERIAL AND METHODS

Seventy two *Astyanax scabripinnis* individuals caught at ponds located at three altitudes of Ribeirão Grande were analyzed: 29 specimens from 1,920 m, 26 from 1,800 m and 17 from 700 m.

Blood was obtained by caudal puncture with heparinized syringes.

While still in the field just after bleeding a suspension of unfractionated blood and yeast in a buffered solution (bis-tris and tris 0.1 M) was placed in a YSI Oxygen Monitor (model 53) and the O_2 consumption was plotted by a recorder. Oxygen dissociation was determined at 20ºC as described by Johansen *et al.* (1978a). The Bohr factor (ΔlogP50/ΔpH) was calculated from oxygen dissociation curves obtained at different pH values.

The red blood cells were washed 3 times with 0.9% NaCl, and then tris-EDTA 0.015 M, pH 8.0 was added to the packed erythrocytes in a 2:1 ratio. Hemolysis was achieved by freezing and thawing three times. To free the hemolysate of cellular debris, the sample was centrifuged at 12,000 g for 15 min at 4°C.

The hemoglobin solution was frozen at –20°C for further analysis in the laboratory.

In order to check if some variation was present in the samples, electrophoresis of individual hemolysates was carried out on starch gel as described by Smithies (1955, 1959) and modified by Val *et al*. (1981).

For spectrophotometric oxygen equilibria and Root effect experiments, the supernatant was then stripped of salt and organic phosphates by passage through a 2.5 cm x 30 cm column of Sephadex G-25 resin equilibrated in 0.1 mM Tris-HCl, pH 8.0. Methemoglobin, when present, was reduced with dithionite in a Sephadex G-25 column according to Schwantes *et al*. (1976), modified in the following way: the sample was applied only after no dithionite solution remained at the top of the resin; due to change in the sample colors, reduction and reoxygenation could be accompanied visually as the sample passes through the column.

Oxygen equilibria of stripped hemoglobin were carried out at 20°C by the spectrophotometric method described by Riggs & Wolbach (1956). The effect of GTP was measured by addition of appropriate volumes of solutions of ATP dissodium salt to stripped hemoglobin solutions. GTP was tested in a molar ratio of 1:8 (Hb: NTP). The P50, Bohr effect and *n* values (Hill plot) were interpolated from linearized logarithmic plots of the O_2 equilibrium data.

The Root effect was performed as described by Farmer *et al*. (1979) and the data were divided into four arbitrary categories based on approximate percentages: 0, less than 10% deoxygenation; +, 10%-20% deoxygenation; ++, 20%-40% deoxygenation; +++, more than 40% deoxygenation. The amount of GTP and ATP used in these experiments was calculated as described above for GTP.

RESULTS

The hemolysates from several adult individuals were characterized eletrophoretically. The analysis of 20 specimens (not all samples could be submitted to electrophoresis due to the very low amount of blood obtained) showed two hemoglobins components, one major and another minor band. This pattern was constant from one individual to another (Fig. 1).

Oxygen equilibria determined from unfractionated blood samples of *Astyanax scabripinnis* are shown in Fig. 2. The results obtained from blood for *A. scabrippinis* at the three studied altitudes showed normal Bohr effect, with increasing O_2 affinity in more alkaline pHs. Little difference in oxygen affinities from blood samples from fish caught in any of these three altitudes were found. However the higher the altitude, the lower the affinity (Table 1).

The oxygen equilibria data on stripped hemoglobin, Bohr effect value was $\phi = -0.10$ and GTP presence increased slightly this effect, resulting in $\phi = -0.14$ (Fig. 3). Cooperativity values (*n*) for the *Astyanax scabripinnis* were approximately 1.0, showing low or no cooperativity, with or without GTP.

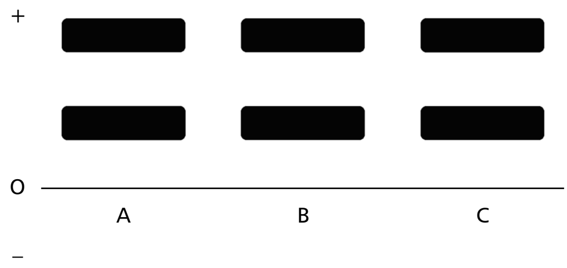

Fig. 1 — Diagrammatic representation of the electrophoretic patterns of unfractionated hemolysate of *Astyanax scabripinnis* on starch gel electrophoresis. A. Fish caught at 1,920 m; B. 1,800 m; C. 700 m.

TABLE 1

Unfractioned blood, Hb functional properties, temperature and O_2 available in water at three altitudes.

	700 m	1,800 m	1,920 m
Unfractioned blood Bohr effect	–0.11	–0.10	–0.11
Log P50 (pH 7.0)	1.12	1.2	1.31
Water temperature (°C)	21	18	16
Oxygen available (mg/L)	9.5	10.2	10.4

The Root effect analysis shows that ATP also modulates negatively Hb-O_2 affinity, but at a lower intensity than GTP (Table 2).

DISCUSSION

Electrophoresis is usually used in structural pattern characterizations of fish hemoglobins, i.e. number of components and relative concentrations. The support and system of buffers used varies and the choice of electrophoretic migration media depends on resolution power, costs, and speed, among other factors.

In this work, we used starch gel as a migration media for determining species electrophoretic patterns due to excellent resolution of this support for fish hemoglobin. Beside the samples obtained at the sites mentioned here, *A. scabripinnis* hemoglobin from other sites in the Campos do Jordão area were submitted to electrophoresis and showed an identical pattern. So this species is probably monomorphic for hemoglobin.

For the analyzed species, we observed that GTP decreases Hb-O_2 affinity showing that this phosphate acts negatively on Hb-O_2 affinity, and confirming the fact that GTP is the main modulator in fresh water fishes. These phosphates exert the greatest effect on P50 at 10 mM concentration and GTP seems to depress oxygen affinities more strongly than ATP at the same concentration (seen in Root effect experiments). The Root effect analysis shows that ATP also modulates negatively Hb-O_2 affinity, but less intensely than GTP does.

According to Weber *et al.* (1979), the concentration of GTP decreases more than ATP during hypoxia. Probably this happens with *Astyanax scabripinnis* too, since GTP is more effective than ATP as a negative modulator.

Studies of blood functional properties can be of the great value in understanding fish habitat and habits (Wood & Johansen, 1972; Powers, 1974; Greaney & Powers, 1978; Val *et al.,* 1986). Many researches have demonstrated that fish species living in low oxygen concentration sites show greater blood affinity to this gas than those living in well oxygenated sites (Johansen *et al.*, 1978a,b; Powers *et al.*, 1979a,b; Powers, 1985; Val *et al.*, 1986, 1990).

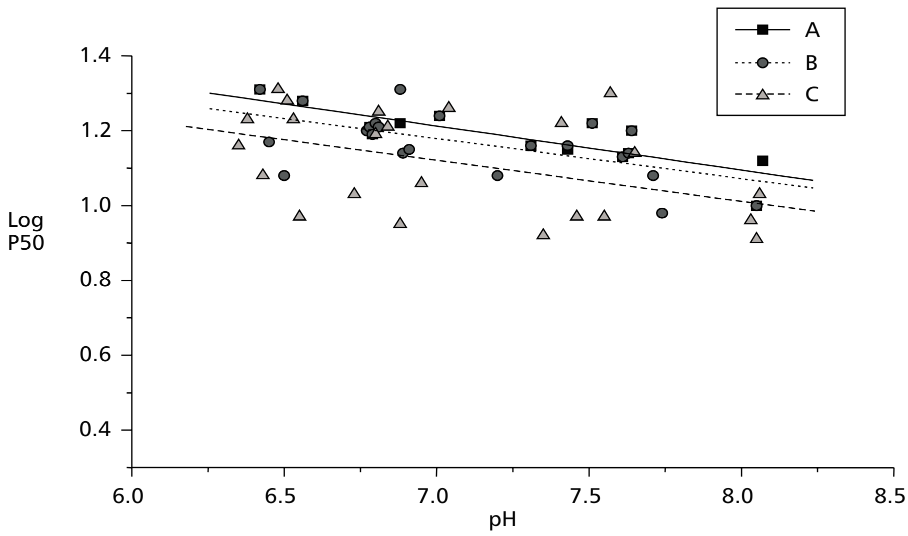

Fig. 2 — Hemoglobin oxygen equilibria in blood: P50 X pH from *Astyanax scabripinnis* at 20ºC. A. 1,920 m, B. 1,800 m, and C. 700 m.

TABLE 2

The Root effect in the presence and absence of phosphates (ATP and GTP).

Hb	% deoxygenation	Farmer
Stripped	12	+
Stripped + ATP	22	++
Stripped + GTP	34	++

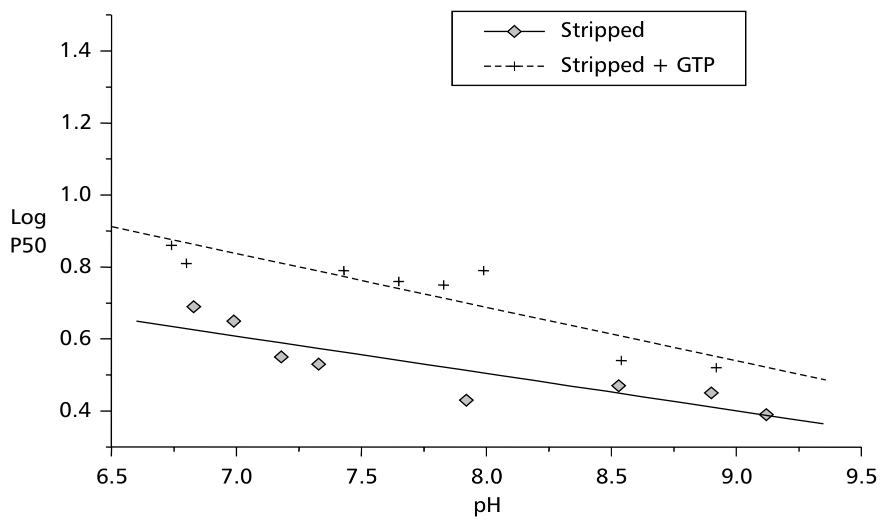

Fig. 3 — The pH effect on oxygen affinity of stripped hemoglobin from *A. scabripinnis* in the presence and absence of GTP at 20ºC.

The data obtained on oxygen affinities and their pH dependence from unfractionated blood of *Astyanax scabripinnis* in three studied altitudes showed normal Bohr effect. This is positive for these fishes, since it facilitates O_2 release at the tissular level and uptake by the gills. However the data obtained on blood-O_2 affinity shows slight differences depending on the altitude at which the sample was obtained (Table 1).

The different O_2 affinities in individuals caught at the three altitudes, can be due to oxygen availability and/or water temperature. At low altitudes (700 m) the water is warmer and oxygen concentration lower than at the others (1,800 and 1,920 m) (Table 1).

The hemoglobin Hb-O_2 affinity of vertebrates is regulated by allosteric effectors, such as chlorides (Cl^-), protons (H^+), carbon dioxide (CO_2), and several types of organic phosphates, besides temperature and salinity (reviewed by Weber & Jansen, 1988). In general, the increase of any of these factors or physical parameters within erythrocytes causes a drop in Hb-O_2 affinity.

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