ERYTHROCYTIC ORGANIC PHOSPHATES

IN THE RED BLOOD CELLS

OF FISH OF THE AMAZON

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Abstract

Fish of the Amazon are exposed to continuous changes in dissolved oxygen. They respond to these changes by a wide spectrum of compensatory and respiratory mechanisms directed towards facilitating both oxygen uptake and oxygen unloading to tissues. Adjustment of erythrocytic levels of organic phosphates is one of these mechanisms and is improved as soon as the animal detects any change in dissolved oxygen. These phosphates are negative modulators of haemoglobin oxygen affinity and changes in their concentration result in changes in oxygen transfer to tissues. The present paper examines the diversity of organic phosphates in the erythrocytes of fish of the Amazon, their effects on haemoglobin oxygen affinities and the time course of their adjustments when the animals are exposed to deep hypoxia, estimating the effects of these changes on P_{50} over time of exposure.

Introduction

According to Randall *et al.* (1981) hypoxia and anoxia have been common constraints during the evolutionary history of tropical fresh waters. In parallel to seasonal changes in dissolved oxygen, the fish of the Amazon are regularly exposed to variations in the concentration of this gas that tend to occur in a very short period of time. In many lakes of the Amazon, oxygen levels may drop to zero at night and reach over-saturated levels at noon the very next day. Short-term changes in dissolved oxygen force, indeed, rapid respiratory adjustments

directed towards enhancing oxygen transfer to tissues. These adjustments include behavioural, morphological, physiological and biochemical characteristics. Part of these adjustments may require also secondary adjustments, as is the case of oxygen unloading to tissues when the animals migrate out of unfavourable environments (Val and Almeida-Val, 1995).

The physiological importance of red cell phosphates for oxygen transfer from the environment to tissues has been recognized since the classical papers by Benesch and Benesch (1967) and Chanutin and Curnish (1967). Fish haemoglobins, with few exceptions, are sensitive to organic phosphates. They bind to haemoglobin decreasing oxygen affinity, i.e., the higher the proportion of haemoglobin to organic phosphate the higher the P_{50} (the lower the affinity to O_2). Because fish experience both changes in environmental oxygen availability and in their oxygen needs, adjustment of the proportion of Hb:organic phosphates is of significant adaptive advantage.

The availability of organic phosphates for reacting with haemoglobin can be altered by complex formation with other components. In the red blood cells they can readily complex with magnesium, for example, what greatly reduces their effect on Hb-O_2 affinity. Compared to marine fish, the red blood cells of fish of the Amazon have low levels of divalent cations, in particular magnesium (Mangum *et al*., 1978), and this, although related to the Amazon ion-poor waters, may be important for oxygen transfer.

The present paper reviews the occurrence of the major organic phosphates in the erythrocytes of fish from a phylogenetic perspective and analyses the effect of the adjustment of their erythrocytic concentration on P_{50} over time in fish of the Amazon exposed to deep hypoxia.

Diversity and phylogenetic occurrence

ATP and GTP are the most common organic phosphate found in fish erythrocytes. In addition to these compounds, several other organic phosphates have been described, such as 2,3DPG in *Hoplosternum littorale*, IPP in *Arapaima gigas*, IP_2 and UTP in *Lepidosiren paradoxa* (see Val, 2000 for references). The effect of organic phosphate on haemoglobin oxygen affinity of fish decreases in the following order: IHP(IPP)>GTP>ATP>2,3DPG.

The most primitive fish of the Amazon are the freshwater stingrays (Potamotrigonidae). The red blood cells of these animals have both ATP and GTP, both of them modulators of Hb-O_2 affinity. The following group, the Osteoglossomorpha, includes the air-breather *Arapaima gigas* and two species of *Osteoglossum*, *O. bicirrhosum* and *O. ferreirai*. All these three species have ATP and GTP in their erythrocytes. In addition to these compounds, the red blood cells of adult *Arapaima* present high levels of inositol pentaphosphate (IPP), a compound also described in the erythrocytes of two marine elasmobranches (Borgese and Nagel, 1978). The presence of IP_2 and UTP in the red blood cells of Dipnoi (Bartlett, 1978) possibly represents a novelty; no other fish species so far analysed have these compounds in their red blood cells. Among the Osteichthyes, the appearance of 2,DPG in the red blood cells of *Hoplosternum* and *Cychlasoma* is of evolutionary significance. 2,3DPG was first described in fish erythrocytes by Rapoport and Guest (1941) in *Amiurus* and *Micropterus.*

These data show that ATP, GTP and 2,3DPG, the three main modulators of Hb-O_2 affinity, appeared among fish and have been conserved during the evolution of vertebrates and are all found in the red blood cells of Tetrapoda. Inositol pentaphosphate, also appeared among fish, and is currently present in red cells of birds and turtle.

Erythrocytic organic phosphates in fish exposed to deep hypoxia

A decrease in the levels of ATP and GTP in the red blood cells of fish experiencing hypoxia has been reported for all Amazonian species so far analysed. Because these animals face rapid changes in dissolved oxygen in nature, the immediate question is how fast they can adjust the levels of these modulators, and so Hb-O_2 affinity, improving oxygen transfer to tissues. Previous analysis of representatives of the species *Osteoglossum bicirrhosum*, *Liposarcus pardalis*, *Mylossoma duriventris* and *Pygocentrus nattereri* indicate that they are able to reduce the levels of GTP down to 50% of the initial values within the first 15 minutes under hypoxia and to reach a new steady state (*ca* 75% reduction in GTP levels) after 30 minutes at acute hypoxia. Similar results have been observed for juveniles of *Colossoma macropomum*. Reduction of ATP levels, however, was a much slower process. Reduced levels of ATP are observed only after 60 minutes at deep hypoxia. Recovery of NTP levels is also a fast process.

Anaemia caused either by reduction in circulating red blood cells or by conversion of haemoglobin to methaemoglobin also results in internal hypoxia. In contrast to environmental hypoxia, anaemia elicits an increase in ATP and GTP in the red blood cells (Val *et al*., 1994). The time course for the adjustments of ATP and GTP in anaemic animals is also a rapid process.

These two situations suggest that fish can actively adjust the levels of ATP and GTP in the red blood cells to increase either oxygen uptake at the gills or oxygen unloading to tissues. In both cases, the mechanisms controlling the levels of ATP and GTP in the red blood cells are unknown.

Erythrocytic phosphates in fish exposed to hyperoxia

Fish of the Amazon are regularly exposed to high levels of dissolved oxygen. Surprisingly, the effect of hyperoxia on erythrocytic levels of organic phosphates in fish is known for a reduced number of species and the data is conflicting. Long-term exposure of *Colossoma macropomum* resulted in a decrease in NTP levels, similar to the result reported by Wood *et al*. (1975) for *Pleuronectes platessa*. These results contrast with that observed for *Astronotus ocellatus* that exhibited no changes in NTP levels after 15 days of exposure to Po_2=300 mmHg (Marcon and Val, 1996). Short-term exposure to hyperoxia (6 hours), however, elicited an inverse response. Specimens of *Serrasalmus rhombeus* and *Oxydoras niger* presented a significant increase in GTP levels and no changes in ATP. In general, fish exposed to hyperoxia exhibit a decrease in ventilation volume as the Po_2 increases. This response would help to keep tissue oxygen supply under control; thought high levels of oxygen at the tissue level would result in severe disturbances. More data on the control of erythrocytic levels of GTP and ATP in animals exposed to hyperoxia are needed before a clear picture can be presented. It would be reasonable, however, to postulate that an increase in the levels of Hb-O2 modulators would help to reduce oxygen unloading in the tissue in animals exposed to short-term hyperoxia.

Effect of ATP and GTP on Hb-O_2 affinity

Changes in erythrocytic cofactor levels in tambaqui affect Hb-O_2 affinities what safeguards oxygen loading at the gills. GTP, as in several other fish species of the Amazon, is stronger than ATP in decreasing the Hb-O_2 affinity in tambaqui. At pH 7.0, P_{50} values change from 0.71 for stripped haemolysate to 6.17 mm Hg when 1mM GTP is added to the sample (1:1 Hb:GTP). An intermediary P_{50}

figure was observed in the presence of 1mM ATP. Interestingly, this effect of GTP on Hb-O_2 affinity (compared to ATP) is also associated with faster changes of its intraerythrocytic level when the animal is exposed to deep hypoxia, which results in an almost immediate adjustment of oxygen transfer to tissues according to environmental oxygen availability. This rapid adjustment of ATP and GTP levels is important when the animal is skimming the water surface and is exposed to higher temperatures.

Concluding Remarks

The analysis of the erythrocytic phosphates in fish of the Amazon clearly indicates that all main modulators of Hb-O_2 affinity known to occur in the erythrocytes of advanced vertebrates have appeared early in the evolution, among fishes. All Amazonian fish species so far analysed exhibit a significant decrease in the levels of GTP and ATP within 30 minutes of exposure to hypoxia. Short-term exposure to hyperoxia, however, elicits an increase in ATP and GTP. This adjustment helps to reduce oxygen unloading to the tissues in animals transiently exposed to hyperoxia.

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