New insights on old players: specialisations and plasticity of fish hemoglobins

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Introduction

Hemoglobin (Hb) is the oxygen transporting metalloprotein present in the erythrocytes of all the vertebrates. Hb is one of the most studied proteins and it also has a role in maintaining the shape of RBC. Although, it is a carrier protein, the functional properties of Hb such as allosteric regulation are similar to that of enzymes. They exist in different forms in the same organism itself. Still, all the hemoglobin types in the whole spectra of vertebrates remain mostly unknown. Hemoglobin is an important target in adaptive research and fish Hbs can evoke interest in every biologist as fishes exhibit a high level of species and habitat diversity. When fishes are exposed to spatio-temporal variations in dissolved oxygen, temperature and pH, their hemoglobins respond by expressing polymorphic Hb molecules.

An Article titled "New insights on old players: specialisations and plasticity of fish hemoglobins" by Tincy Varghese and Mishal, P. discussed about: Hemoglobins are oxygen transporting metalloproteins present in vertebrate RBC, with allosteric regulation similar to that of enzymes. Adaptive diversity and plasticity of Hbs enabled the fishes to adapt to extreme habitats. Fishes exhibit root effect in addition to bohr effect unlike other vertebrates and it helps in swim bladder inflation Evolutionary adaptation in Antarctic ice fish (Notothenid) led to the loss of globin gene and resulted in colourless circulation. The multitude of Hb isoforms would pave way for future research on developing methods based on Hbs to identify evolutionary origin and environment of fish.

Structure of fish hemoglobins

Teleost hemoglobins are tetrameric with quaternary structure- $\alpha_2\beta_2$, having each peptide linked with a heme group. Globins are the protein part of hemoglobins, comprising alpha and beta globins and heme is the non-protein part. Other oxygen carrying globins present in vertebrate tissues other than RBC are myoglobins, neuroglobins and cytoglobins. Organization of globin gene in vertebrates also vary, as in mammals, they are present in different chromosomes, with *alpha* globin on 16^{th} chromosome and *beta* globin on 11^{th} chromosome. In amphibians, they are present in same chromosome while fishes (carp, salmon, zebrafish) have them on same chromosomes and adjacent in position. Symmetric arrangement of globin chains comprises 2 pairs of identical globin genes while asymmetric arrangement includes 3 different classes of globin genes.

Chemical and physical properties

Oxygen binding and transport are the most important function and property of all types of hemoglobin molecules. The binding is reversible which facilitate the release of oxygen into the tissues. Each heme-peptide will bind to one oxygen molecule and thus a tetrameric hemoglobin can bind to 4 oxygen molecules, as binding is attributed to the presence of Fe^{2+} in the heme. The process is called oxygenation which is different from the normal chemical oxidation reaction. Hemoglobin shows allosteric interactions, which include homotropic and heterotropic interactions. Homotropic interactions are their co-operativity in binding, i.e., when one atom of oxygen binds to one of hemoglobin's four binding sites, the oxygen affinity of the remaining three binding sites increases. Heterotrophic allosteric interactions are with the molecules such as hydrogen ions, organic phosphates- BPG, ATP, GTP, IPP, which reduces oxygen affinity.

Bohr effect and root effect

When hemoglobin gets oxygenated, protons are released from the molecule, which indicates that a basic or physiological higher pH facilitates the oxygenation process. This is called alkaline Bohr effect where the affinity of hemoglobin for O_2 decreases at low pH or increasing pCO_2. However, in the case of acidic Bohr effect, protons are taken up during oxygenation at low pH. This elucidates that the pH modulates oxygen affinity. This is due to the conformational changes in those part of hemoglobin molecule which are distant from the heme group. Root effect is the decrease in capacity of hemoglobin for O_2 due to decreasing pH or increasing pCO_2 (extreme Bohr effect). Root effect signifies an extreme alkaline Bohr effect which occurs in the absence of a normal acid Bohr effect. Root effect results in the loss of oxygen affinity and oxygen binding capacity at low pH which is irreversible. However, bohr effect is completely reversible where only the oxygen affinity is being lowered. All the root effect hemoglobin is characterised by the presence of a Ser residue at position F9 (94) β and this has got a primary importance. Many other types of hemoglobin also possess Ser residue and at the same time they do not exhibit root effect. This critical Ser residue was found to stabilise the deoxygenated Hb at low pH. There is also a loss of co-operativity in the root effect hemoglobins. The physiological role of root effect hemoglobins is said to be linked with swim bladder oxygenation and the necessity to oxygenate the

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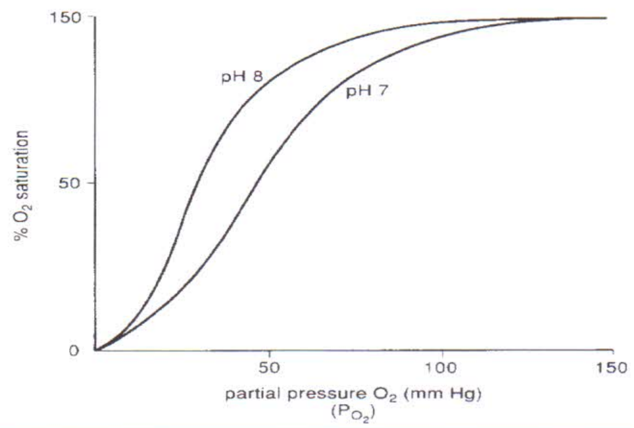

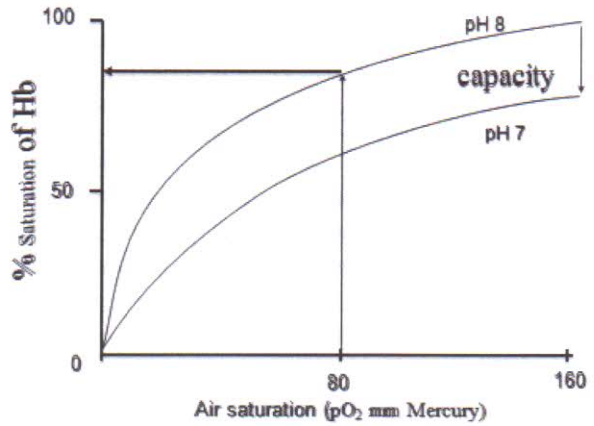

Fig. 1. Oxygen dissociation curve showing Bohr effect. At a selected partial pressure, the oxygen saturation is much lower at pH 7 when compared to pH 8. However, a high partial pressure reverses the effect. (Adapted from Moyle and Chech, 1988)

Fig. 2. Oxygen dissociation curve showing root effect. At a selected partial pressure, the oxygen saturation is much lower at pH 7 when compared to pH 8. The capacity of Hb to hold oxygen is lost, as its air saturation is not increasing even at high partial pressure (Redrawn from Moyle and Chech, 1988)

ill-vascularised retina of many fishes.

Plasticity of fish hemoglobins

Many of the environmental parameters have negative or positive effect on Hb oxygen affinity. Hypoxia reduces organic phosphate concentration in blood, i.e. low ATP/GTP production in absence of oxygen which increases the oxygen affinity of hemoglobin. Decreasing pH decreases hemoglobin affinity for O_2 which is often associated with high carbon dioxide level. The increase in CO_2 drives off O_2 (Bohr effect) which causes a decrease in blood pH, which ultimately magnifies Bohr effect. Also an increase in temperature decreases oxygen affinity and capacity, which results in fish having narrow temperature tolerances. Organic phosphates such as ATP reduce O_2 affinity and urea increases O_2 affinity of Hb.

Hemoglobin isoforms

Hemoglobin types/Iso-hemoglobins can be broadly classified into two, cathodic and anodic hemoglobins. Anodic hemoglobins are normal Hbs with marked Bohr and root effect while cathodic hemoglobins are without Bohr effect. The isoforms are formed when there is a change in the habitat parameters. For instance, Atlantic cod shows different polymorphic hemoglobins when there is a change in the latitude. Root effect hemoglobins have positive a role in osmoregulation of marine teleosts. Gene duplication is the process which is mainly responsible for these polymorphic hemoglobins. The hemoglobin polymorphism can be visualised by electrophoretic methods, where it is separated either by charge or molecular mass. There are different analytical methods for identifying and separating hemoglobin isoforms, those include Gel electrophoresis in starch/cellulose acetate gel –pH- 8.2, Citrate agar- pH -6.2, IEF and FPLC (Fast protein liquid chromatography). For instance, an analysis of common carp hemoglobins has shown 3 bands in starch gel, 4 bands in IEF and 9 peaks with FPLC.

Specific roles of hemoglobin in fish

The cathodic hemoglobins have a role in maintaining RBC consistency. As the cathodic hemoglobins will not deoxygenate properly, they will remain in the dissolved form inside the RBC. This will avoid the precipitation of deoxy-hemoglobin and thus maintain normal viscosity and consistency of hemoglobin. They also have role in the osmoregulation of marine teleosts. Marine teleost fish secrete bicarbonate (HCO_3^-) into the intestine to aid osmoregulation. Intestinal HCO_3^- secretion is associated with an equimolar transport of protons (H^+) into the blood. Highly sensitive root effect hemoglobin release oxygen to intestine which helps to carry out aerobic cell respiration. This help to meet osmoregulatory energy demand.

Hemoglobinless fishes

Polar regions, namely Arctic and Antarctic regions have cold, oxygen rich waters. Fishes living in this thermally stable environment has lost the oxygen carrier protein, hemoglobin. *Chaenocephalus aceratus* (family: Channichthyidae) is an ice fish which has no hemoglobin in the blood which makes their blood appear white. The high oxygen solubility and availability renders them without the need of an oxygen carrying molecule. The oxygen is physically dissolved in the plasma of ice fish blood. The reason is the loss of globin gene during the evolution in the ice cold environment. Oxygen carrying capacity of blood without hemoglobin is only about 10% of the normal blood. However, these fishes have specific cardiovascular adaptations which help them to maintain with normal supply of blood to the tissues. These adaptations include large heart, high cardiac output and exceptionally large blood vessels.

Conclusive remarks

Among vertebrates, fish exhibit highest number of multiple hemoglobins and there are fishes without any hemoglobins in the blood. Adaptive diversity and plasticity of fish hemoglobins and their allosteric regulations resembling true enzymes is worth to carry out detailed studies in this area. Developing marker for genetic selection based on hemoglobin heterogeneity and prediction of habitat of fish are the potential applications of hemoglobin multiplicity. One of the most interesting facts about fish hemoglobins are they exhibit root effect in addition to Bohr Effect. This may also pave way for future research on developing methods based on hemoglobins to identify evolutionary origin and habitat of fish.

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