HEMOGLOBIN: A GENERAL REVIEW

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Abstract

Hemoglobin (Hb) is red blood corpuscle pigment causing red color in blood with centrally located iron atom, responsible for oxygen transport having molecular weight of a tetramer of 64,458 g/mol. The oxygen binding properties of hemoglobin are affected by pH and presence of carbon dioxide. It cause different clinical problems due to its high or low concentration in blood. Poisoning of Hb can also occur by combination of Hb with carbon monoxide resulting in formation of carboxyhemoglobin. Besides carboxyhemoglobin, sulfhemoglobin and methemoglobin are also derivatives of Hb.

Keywords: Binding, Derivative, Residue, Concentration, Ligand, Poisoning, Carboxyhemoglobin, Anemia, Bohr's effect

Introduction

Hemoglobin is red blood pigment found in erythrocytes with normal concentration of 13.5-18 g/dL in male and 11.5-16 g/dL in female and iron containing oxygen transport protein, present in the red blood cells that efficiently carries oxygen from the lungs to the tissues while it also contributes to the transport of CO_2 and hydrogen ion back to the lungs [3]. It is remarkably oxygen carrier that is able to use as much as 90% of its potential oxygen carrying capacity effectively. Hemoglobin is also found outside the red blood cells as in alveolar cells, macrophages, mesangial cells in kidney, retinal pigment epithelium, hepatocytes, endometrial cells where it acts as regulator of iron metabolism [6].

Hemoglobin has two component, the heme (non-protein part) and globin (apo-protein part). Globin has four polypeptide chains. Adult Hb (HbA1) is made up of two alpha chains and two beta chains. The red colour of Hb is due to heme. Heme consists porphyrin molecule called protoporphyrin with iron at its center.

Three dimensional structure of hemoglobin

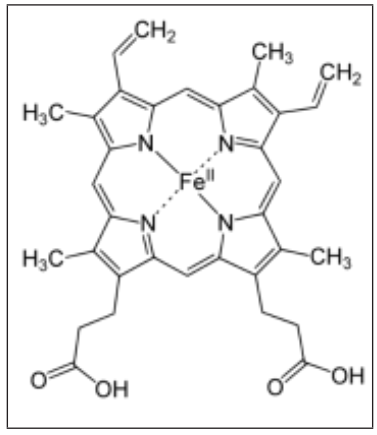

Figure1: Structure of hemoglobin

(Jeremy M. Berg, et al., Biochemistry 8th edition)

Three dimensional structure of the subunits is held together only by weak non-covalent bonds including salt bridges, H-bonds and many non-polar interactions. The polar amino acid side chains are mainly in contact with the solvent, while non polar residues generally lie in the interior of the molecules or in the regions of the contacts between subunits with some exceptions. The heme group is embedded in hydrophobic pocket which is similar for both chains and also shows few noticed differences. Most of the residue is in Vander walls contact with the heme are invariant in different mammalians Hbs. Nearly all the bonds are hydrophobic interaction which are made with the non-polar part of the protoheme. Thus confirming the role of non-polar side chains is stabilizing the Hb-complex [2]. Dissolution of Hb tetramers iinto dimers involves essentially the formation of symmetrical dimmers; this is amply substaintiated by the finding that the number of contacts between like chains is very limited and probably consists of salt bridges involving the terminal residues. On the other hand, there is large number of bonds, most of which are contributed by the non-polar residues, although some hydrogen bonds are present.

Synthesis of hemoglobin

Hemoglobin is synthesized in a complex series of reactions. Eight enzymes plays important role in heme synthesis, four enzymes work in the mitochondria whereas four enzymes work in the cytosol. The process starts in the mitochondria, where ALA (aminolevulinic acid) synthase links glycine and succinyl coenzyme A to form ALA. From second to fifth step occur in the cytosol. Then in second step, ALA dehydratase takes two molecules of ALA and produces PBG (porphobilinogen). In step third, porphobilinogen deaminase takes four molecules of PBG and produces hydroxymethylbilane [4]. Then in step four, uroporphyrinogen III co-synthase takes hydroxymethylbilane and produces uroporphyrinogen III. Next in the fifth step, uroporphyrinogen decarboxylase takes uroporphyrinogen III and produces coproporphyrinogen III. The final three steps of heme synthesis occur in the mitochondria. Then, Coproporphyrinogen III is transformed to protoporphyrinogen IX by coproporphyrinogen oxidase. The seventh step occurs when protoporphyrinogen oxidase converts protoporphyrinogen IX to protoporphyrin IX. The final step of heme synthesis is the addition of Fe to protoporphyrin IX by ferrochelatase, thus producing a heme molecule. The globin part is synthesized by libosomes in cytosol. Here, heme and globin combines together to form hemoglobin.

Each hemoglobin consists of four polypeptide chains, two identical alpha chains and two identical beta chains. The heme,that accounts for only 4% of weight of molecule is composed of a ring like organic compound known as porphrin to which an iron atom is attached[2,4].

Hemoglobin derivatives

Hemoglobin (specifically heme) has capacity to bind with different ligands and forms hemoglobin derivatives. Normally, oxyhemoglobin and deoxyhemoglobin are present. Beside these, other important derivatives are methemoglobin and carboxyhemoglobin. The Hb derivatives have characteristic colour and they can be detected by absorption spectra.

I. **Methemoglobin:** The iron in hemoglobin should remain in the ferrous (Fe^{2+}) state to carry oxygen. Normally, molecular oxygen does not oxidize Hb, it only loosely binds to form oxyhemoglobin. The oxidation of hemoglobin to methemoglobin (Fe^{3+}) occurs in living system by H_2O_2 and free radicals. The methemoglobin is unable to bind to O_2. Instead, a water molecule occupies the oxygen site in the heme of methemoglobin. Normally, occasional oxidation of hemoglobin is corrected by the enzyme, methemoglobin reductase present in erythrocytes [3].

II. **Carboxyhemoglobin:** Carbon monoxide (CO) is a toxic compound that can bind with Hb as same as O_2 binds. CO has about 200 times more affinity than O_2 for binding with Hb. The

symptoms of carboxyhemoglobin include headache, nausea, breathlessness, vomiting and irritability. Administration of O_2 through oxygen masks can help to reverse the manifestation of CO toxicity [2, 3].

III. **Sulfhemoglobin:** It is formed by the addition of a sulfur atom to the pyrrole ring of heme and has a greenish pigment. In sulfhemoglobinemia, hemoglobin tetramers usually contain only one or two sulfureted heme isomers. The affected molecules shift unaffected heme moieties towards the unliganded conformation which causes impairment of oxygen delivery to tissues.

Respiratory function of hemoglobin

Structure–Function Relations:One molecule of hemoglobin consists of four hemes. Each heme group contains a porphyrin ring and a ferrous atom capable of reversibly binding one oxygen molecule. The globin units of deoxyhemoglobin are tightly held by electrostatic bonds in a tense (T) conformation with a relatively low affinity for oxygen. The binding of oxygen imposes mechanical and chemical stresses that break these electrostatic bonds, leading to a relaxed ® conformation in which the remaining binding sites become more exposed and have an affinity for oxygen that is 500 times as high as when the molecule is in the T conformation. Cooperativity among binding sites occurs due to conformational changes, so that binding of one oxygen molecule to deoxyhemoglobin increases the oxygen affinity of the remaining binding sites on the same hemoglobin molecule. Thus, the binding curve forms a sigmoid shape, reflecting the transition from low to high affinity as more binding sites become occupied. This cooperativity during oxygen transport provided the first clear insight into how an allosteric enzyme regulates a metabolic pathway. The properties of an allosteric protein include multiple interacting binding sites, reversible noncovalent binding to a primary ligand, quaternary conformational changes induced by ligand binding (homotropic effects), and modulation of ligand binding by secondary effectors (heterotropic effects). The major heterotropic effectors of hemoglobin are hydrogen ion, carbon dioxide, and red-cell 2,3-bisphosphoglycerate [7,8].

Adaptation to High Altitude:at high altitude, oxygen transport is impaired, thus impaired loading of oxygen onto hemoglobin causes alveolar hypoxia, the desirable adjustment would be a lower P50 (p50 is the oxygen tension when hemoglobin is 50% saturated with oxygen). At moderately high altitude (3100 m), hypoxia induces an increase in the red-cell 2,3-bisphosphoglycerate concentration, which raises the P50 to approximately 29 mm Hg at rest.Heavy exercise at 3100 m induces a further increase in the P50 to approximately 38 mm Hg. This incomprehensible increase may be beneficial at rest or during submaximal exercise, as long as oxygen loading can be maintained by raising the alveolar oxygen tension through ventilator stimulation. However, under conditions of severe hypoxia or at extremely high altitude, hyperventilation cannot adequately increase the alveolar oxygen tension, but the associated respiratory alkalosis causes a large decrease in P50. The severe hypoxic ventilator stimulus leads to a profound respiratory alkalosis (arterial pH, 7.7; carbon dioxide tension, 7.5 mm Hg) and a reduction in the in vivo P50 to 20 mm Hg,32 raising the actual arterial oxygen saturation to 78 percent at the same arterial oxygen tension. Thus, in severe hypoxia, feedback control of oxyhemoglobin binding allows climbers to achieve adequate saturation for short-term survival without supplemental oxygen [7,8]. There is a strong inverse correlation between the P50 and the hemoglobin concentration. On the other hand, high oxygen affinity has a potential advantage for adaptation to high altitudes. At high altitudes in the Himalayas, native Sherpas have less hypoxia and a lower alveolar– arterial oxygen-tension gradient than acclimatized lowlanders, which may reflect higher pulmonary diffusing capacity rather than higher hemoglobin affinity.

Sickle Cell Anemia: substitution of valine for glutamic acid at position 6 of the b chain of hemoglobin leads to sickle cell anemia. When in solution, sickle cell hemoglobin has a normal

affinity for oxygen. However, whole blood from patients with sickle cell disease has a remarkably decreased affinity for oxygen as a result of intracellular polymerization of hemoglobin S and higher levels of 2,3-bisphosphoglycerate. The Bohr effect is increased in blood from patients with sickle cell disease and a given drop in tissue pH causes a greater decrease in oxygen affinity [4]. The higher P50 facilitates oxygen unloading. On the other hand, a higher P50 also favors the formation of deoxyhemoglobin, which in turn increases the polymerization of hemoglobin S and may trigger a sickling crisis.

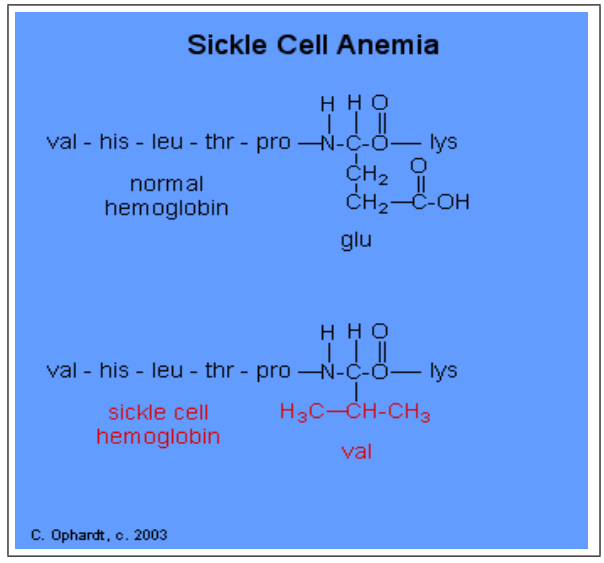

Figure 2: normal Hb vs sickle cell Hb

Effects of Carbon Monoxide: since the affinity of hemoglobin for carbon monoxide is 200 times its affinity for oxygen, hemoglobin binds alveolar carbon monoxide in preference to oxygen during pulmonary transit, whereas oxygen dissociates more readily than carbon monoxide during tissue transit, leading to an apparent blockade of oxygen diffusion in lung and muscle. Even a small concentration of carbon monoxide can be lethal if inhaled long enough. Besides reducing the oxygen-carrying capacity of blood, carbon monoxide directly increases the oxygen-binding affinity of hemoglobin and impairs oxygen extraction. Therefore, the blood carboxyhemoglobin concentration consistently underestimates the tissue oxygen deficit; any given blood carboxyhemoglobin concentration causes greater tissue hypoxia than an equivalent reduction in hemoglobin content caused by anemia. Fetal hemoglobin has a high oxygen affinity (P50, 19.4 mm Hg), which facilitates the uptake of oxygen from the hypoxic maternal uterine blood (oxygen tension, 28 mm Hg). Since the normal fetal arterial oxygen saturation is only 75 to 80% (on the steep portion of the oxyhemoglobin dissociation curve), the fetus is sensitive to small changes in oxygen tension. Even minute amounts of maternal carboxyhemoglobin can impair fetal oxygen transport [8].

Oxygen-Hemoglobin binding

The oxygen binding properties of hemoglobin are markedly affected by pH and by the presence of carbon dioxide, a phenomenon known as Bohr effect [4]. One molecule of Hb binds to four molecules of oxygen. Thus binding ability of Hb with oxygen can be studied by a graphical presentation which is called oxygen dissociation curve. The normal curve is sigmoid shape. The affinity of Hb for oxygen is very high, when Hb is exposed to increased partial pressure of oxygen or decreased

hydrogen ion concentration, the binding of oxygen to Hb increases.

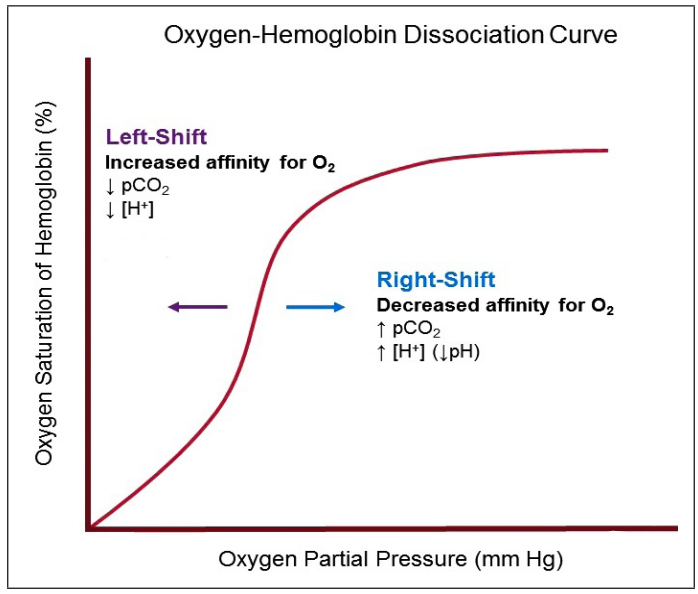

Figure 3: oxygen-hemoglobin dissociation curve

(source: U. Satyanarayan et al., Biochemistry)

If there is increased partial pressure of carbon dioxide, the affinity of oxygen to Hb is decreased. Bohr effect causes a shift in the oxygen dissociation curve to the right. This effect is responsible for release of oxygen from oxyhemoglobin to the tissue.

Hemoglobin as buffer

Hemoglobin of RBC is an important buffer (buffer is a solution that can resist pH change upon the addition of an acidic or basic components) in the respiratory regulation of pH and accounts for one-third of the mass of cell. It mainly buffers the fixed acids, besides being involved in the transport of gases (O_2 and CO_2). At tissue level, hemoglobin binds to H^+ ions and helps to transport CO_2 as HCO_3^- with a minimum change in pH (referred to as isohydric transport). In the lungs, as hemoglobin combines with O_2, H^+ ions are removed which combines with HCO_3^- to form H_2CO_3. The latter dissociates to release CO_2 to be exhaled [3,6].

Pathophysiology of carbon monoxide poisoning

The main aspects of CO poisoning pathophysiology involves interference with the transport of oxygen, from alveoli to tissues due to the binding of CO to hemoglobin [6]. CO rapidly diffuses across the alveolar-capillary membrane and binds to hemoglobin with an affinity more than 200-fold greater than that of oxygen. The degree of CO uptake depends on the duration of exposure, ventilator rate and the relative concentrations of CO. The latter effect occurs because of CO binding to hemoglobin, which changes the cooperative characteristics of oxygen binding. Approximately 10-15% of the total amount of absorbed CO is bound to extravascular proteins. Binding of CO to myoglobin causes reduced cytochromes, guanylate cyclase and nitric oxide synthase. The affinity of CO for cytochrome oxidase is relatively low. Since the rate of dissociation is slow, it is possible that the interaction may result in a relatively prolonged impairment of oxidative metabolism. This may

cause the electron transport chain to become fully reduced, and electrons may leak from mitochondrial oxidoreductase sites proximal to cytochrome oxidase. Mitochondria seems to be a source of oxygen-based free radicals within the first several hours after CO poisoning. An alternative hypothetical mechanism for mitochondrial dysfunction relates to a recent observation that CO exposure will cause a several hundred-fold increase in release of the free radical, nitric oxide, from platelets. Nitric oxide, and nitric oxide-derived oxidants such as peroxynitrite, have a high affinity for sulfide and heme-containing proteins. Peroxynitrite can inactivate mitochondrial enzymes, impair electron transport and, in some circumstances, accelerate production of reduced oxygen species. Acute mortality from CO appears to be the due to ventricular dysrhythmias caused by the hypoxic stress precipitated by COHb. The $C0_2$ produced may also increase CO toxicity. Production of hydrogen cyanide gas, which is relatively common in fires, may further complicate the hypoxic insult by direct cellular mechanisms. Neuroimaging studies suggest that the primary site of CO injury in the central nervous system is the perivascular zone and that focal pathology, which is one of the hallmarks of CO poisoning, may be due to secondary hemorrhagic necrosis.Continuous exposure to relatively high concentrations of CO will cause bradycardia and a decreased cardiac output.

Hemoglobin measurement

Hemoglobin levels are measured by blood test. The amount of hemoglobin in whole blood is expressed in grams per deciliter (g/dL). CBC includes measurement of hemoglobin level in the blood. The normal range of hemoglobin depends on age and gender of person. The normal hemoglobin level for males is 13.5-18 g/dL and that for females is 11.5-16 g/dL. The hematocrit measure the volume of red blood cells compared to total blood volume. Anemia is diagnosed if a blood test finds less than 13.5 g/dL in male or less than 11.5 g/dl in female [1,2].

Hemoglobin measurement is generally done in patients with abnormal complete blood count (CBC), signs and symptoms of hemolytic anemia (increase in unconjugated bilirubin, weakness, fatigue, decrease in hemoglobin levels, jaundice, hemoglobinuria), or a family history of a hemoglobinopathy.

Clinical problems due to hemoglobin levels

i. Low hemoglobin level

Hemoglobin deficiency can be caused by decreased amount of hemoglobin molecule or its decreased ability to bind to oxygen. The common causes of decreased hemoglobin includes active bleeding (that may be due to menstrual bleeding, gastro-intestinal ulcers, wounds, cancer of colon), bone marrow problems, nutritional deficiency, chemotherapy, sickle-cell disease, chronic diseases, etc. In general, according to size of size of red blood cells, there are three major types of anemia and they are microcytic anemia (iron deficiency anemia and thalassemia), normocytic anemia (anemia of chronic disease, kidney disease) and macrocytic anemia (pernicious anemia and anemia related to alcoholism) [3,6].

Other several forms of anemia are listed below:

Iron deficiency anemia is most common type due to inadequate dietary intake of iron. It may be caused due to chronic bleeding (colon cancer, intestinal polyps), stomach ulcers or poor gastric absorption.

Pregnancy related anemia occurs during pregnancy and childbirth because they require increased amount of iron.

Pernicious anemia occurs due to poor absorption of vitamin B_{12}.

Hemolytic anemia is inherited that occurs when red cells are broken in blood or spleen.

Sickle cell anemia is due to abnormal hemoglobin molecules that is sickle shaped and resists blood flow through small blood vessels.

Aplastic anemia is a condition where bone marrow is affected that significantly diminish production of all blood cells.

Symptoms of low hemoglobin level includes dizziness, lightheadedness, fatigue, weakness, pale skin, cold hands and feet, fast heartbeat, shortness of breath, etc.

ii. High hemoglobin level

High level of hemoglobin in blood results to a rare disease, polycythemia [5]. It can be due to increase in the number of red blood cells or decrease in volume of plasma that leads to blood clots, heart attacks and strokes. It is a serious lifelong condition that can be fatal if not treated. The emergency treatment of polycythemia is by phlebotomy.

Risk factors

Infants, young children, women of child bearing age and older population are at higher risk for iron- deficiency anemia. Vigorous exercise causes excessive breakdown of red blood cells in blood. People with chronic health conditions, liver disease, thyroid disease, inflammatory bowel disease, auto-immune disease are at high risk of developing anemia. Family history of hemolytic anemia is also a risk factor that affects people of all ages, races and sexes. Risk for aplastic anemia is higher when an individual have been exposed to toxins or under certain drugs, had chemotherapy or radiation, certain infectious disease or auto-immune disorders. Sickle cell anemia is common in people from Africa, South or Central America, Mediterranean countries, India and Saudi Arabia. Pernicious anemia can occur if an individual have type I diabetes, Grave's disease and Addison's disease.

Similarly, clinical conditions that causes high hemoglobin levels includes polycythemia vera, lung disease (COPD, emphysema), heart disease, kidney tumors, dehydration and carbon dioxide exposure [5].

Prevention of anemia

Some types of anemia are preventable. Anemia can be prevented by dietary modification. Iron rich foods such as dark green leafy vegetables, dried fruits, nuts, beefs can prevent anemia caused by iron deficiency. Other foods include meat and dairy products, citrus juices, legumes and fortified cereals [5].

Treatment

Treatment of anemia depends on cause. Dietary modification or iron supplementation in diet improves the hemoglobin level in blood. Pernicious anemia can be treated by replenishing vitamin B12 supply in the body. Treatment of aplastic anemia includes medicines to suppress the immune system, blood transfusion or bone marrow transplant. Management of sickle cell anemia is aimed at avoiding pain episodes, relieving symptoms and preventing complications. Treatment includes medications and blood transfusions. Hemolytic anemia can be treated by blood transfusions, medicines, plasmapheresis, surgery, blood and marrow stem cell transplants and life style changes. Polycythemia is a lifelong disease that can be managed with medication but has no definite cure [1].

Conclusion

Hb is main component of blood which helps for the transport of oxygen from lungs to tissues and also transport carbon dioxide and H^+ ions back to the lungs. Maintaining the level of Hb in the body prevents disease, otherwise it may increase the risk of anemia, polycythemia, etc.Hb level in blood can be balanced by the use of dietary foods (dark green leafy vegetables, dried fruits, nuts, etc.) including iron supplements.

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