

Hemoglobin and the Heme Group: Metal Complexes in the Blood for Oxygen Transport

Inorganic Synthesis Experiment



Authors: Rachel Casiday and Regina Frey
Revised by: C. Markham, A. Manglik, K. Castillo,
K. Mao, and R. Frey
Department of Chemistry, Washington University
St. Louis, MO 63130



For information or comments on this tutorial, please contact Kit Mao at mao@wustl.edu.

Key Concepts:

- Oxygen Transport in the Blood
- Metal Complexes
 - Ligands
 - Coordination numbers
- Hemoglobin Protein
 - Protein Subunits
 - Alpha-helix Structural Motif
 - Heme Group
 - Conformational Changes
- Spectroscopy and the Color of Blood
- Effect of CO₂ and H⁺ on O₂ Binding
 - Integration of Physiological and Chemical Views

Related Tutorials:

- [*Blood, Sweat, and Buffers: pH Regulation During Exercise*](#)
 - [*Energy for the Body: Oxidative Phosphorylation*](#)
 - [*Iron Use and Storage in the Body: Ferritin*](#)
 - [*Maintaining the Body's Chemistry: Dialysis in the Kidneys*](#)
-

Introduction to the Chemistry and Physiology of Blood

Our bodies consist of cells that are organized into many specialized organs and tissues that perform a variety of functions. For instance, our stomachs digest food so that the nutrients contained in the food can be distributed to the rest of the body; our lungs take in the oxygen needed by the body from the air and release carbon dioxide as a waste product; our brains coordinate all of these (and many other) activities of the body. These processes are based upon numerous chemical reactions. The sum total of the chemical reactions in the body is known as

the body's **metabolism**. Human metabolism includes the reactions needed for normal activities such as eating, sleeping, and studying.

All of our specialized organs are united by their fundamental need for a particular chemical environment that will enable the body's metabolic reactions. This environment must supply oxygen and nutrients to furnish the building blocks for cells, enable biochemical reactions, and provide energy for the body. Additionally, it must be able to eliminate the waste products of the body's metabolic activities.

Blood distributes oxygen and nutrients to the many different cells in the body, carries CO₂ generated by the cells to the lungs for exhalation, and carries other waste products to the kidneys and liver for processing and elimination. Many finely tuned chemical processes occur in the blood to allow it to perform all of these functions and provide for the needs of the body. The tutorials in Chem 151 and 152 will describe several of these vital chemical processes, such as the release of iron in controlled amounts to the blood, the removal of waste products from the blood, and the regulation of the levels of CO₂ and H⁺ to control the pH of the blood. In this tutorial, we will focus primarily on one of the most important functions of blood: the transport of oxygen from the lungs to the other cells of the body (e.g., muscle cells) that perform metabolic functions.

Oxygen Transport via Metal Complexes

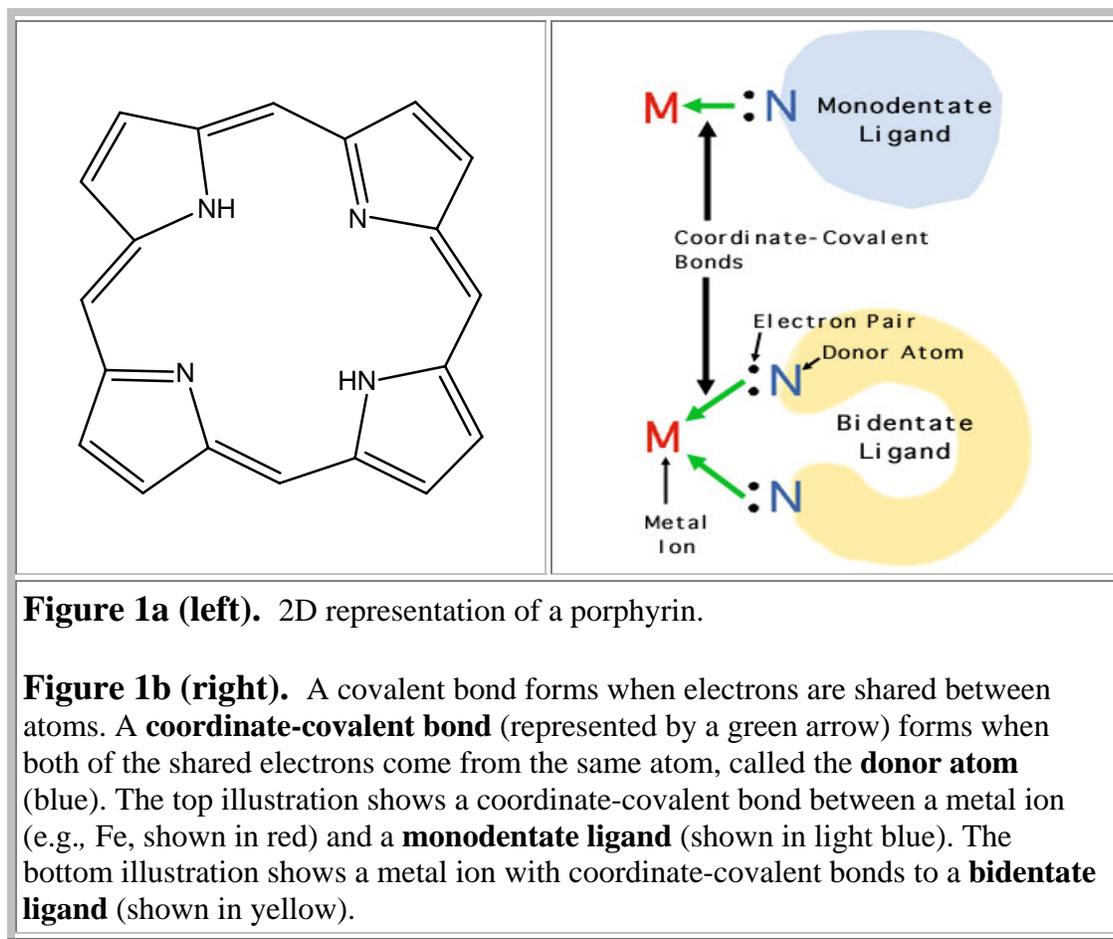
An adult at rest consumes the equivalent of 250 mL of pure oxygen per minute. This oxygen is used to provide energy for all the tissues and organs of the body, even when the body is at rest. The body's oxygen needs increase dramatically during exercise or other strenuous activities. The oxygen is carried in the blood from the lungs to the tissues where it is consumed. However, only about 1.5% of the oxygen transported by the blood is dissolved directly in the blood plasma. In fact, most of the oxygen is carried via a more sophisticated mechanism that utilizes the metal complex **heme**. This mechanism is capable of transporting the large amount of oxygen required by the body and allowing it to leave the blood when it reaches the tissues that demand the most oxygen.

Metal Complexes in the Body

Metal ions are ideal for use in biological systems due to their ability to coordinate with (bind) and then release ligands. The most common metal used in the body is iron, which plays a central role in almost all living cells. For example, iron complexes are used in the transport of oxygen in the blood and tissues.

Metal-ion complexes consist of a metal ion that is bonded via *coordinate-covalent bonds* (Figure 1) to a small number of anions or neutral molecules called *ligands*. For example, the ammonia (NH₃) ligand used in this experiment is a *monodentate ligand*; i.e., each monodentate ligand in a metal-ion complex occupies only one site in the coordination sphere of a metal ion. Some ligands have two or more electron-pair-donor atoms that can simultaneously coordinate to a metal ion and occupy two or more coordination sites; these ligands are called *polydentate ligands*. They are also known as chelating agents (from the Greek word meaning "claw") because they appear to grasp the metal ion between two or more electron-pair-donor atoms. One of the most important classes of chelating agents in nature is the **porphyrins** (Figure 1a). A porphyrin molecule can coordinate to a metal using its four nitrogen atoms as electron-pair donors, thus

prophyrins are tetradentate ligands. The **coordination number** for a metal refers to the total number of occupied coordination sites around the central metal ion (i.e., the total number of metal-ligand bonds in the complex).



Oxygen-Carrying Protein in the Blood: Hemoglobin

Hemoglobin is the protein that transports oxygen (O_2) in human blood from the lungs to the tissues of the body. Proteins are formed by the linking of amino acids into polypeptide chains. An individual amino acid in a protein is known as a **residue**. The arrangement and interactions of the amino-acid residues within the protein determine the protein's shape and contribute substantially to its function. Hemoglobin is a **globular protein** (i.e., folded into a compact, nearly spherical shape) and consists of four subunits, as shown in Figure 2. Each protein subunit is an individual molecule that joins to its neighboring subunits through intermolecular interactions.

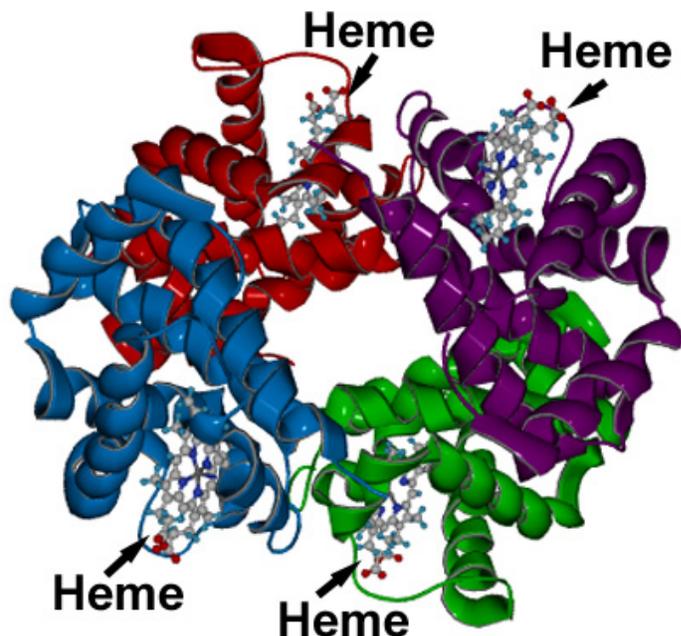


Figure 2¹

This is a molecular model of hemoglobin with the subunits displayed in the ribbon representation which traces the backbone atoms of a protein. The four heme groups are displayed in the ball-and-stick representation and is used to represent the 3-D structure of the protein.

View this Molecule
Interactively

Note: To view the molecule interactively,
please use [Chime](#).

To understand the oxygen-binding properties of hemoglobin, we will focus briefly on the structure of the protein and the metal complexes embedded in it.

The Protein Subunit

Each subunit in Figure 2 contains regions with a coiled shape. Many of the amino acids that make up the polypeptide chain interact to form this particular structure, called an **alpha helix**. In an alpha helix (Figure 3), each amino acid is *hydrogen-bonded* to the amino acid that is four residues ahead of it in the chain. In hemoglobin, the hydrogen-bonding interaction occurs between the H of an -NH group and the O of a -CO group of the polypeptide backbone chain; the amino-acid side chains extend outward from the backbone of the helix. Approximately 75% of the amino-acid composition of hemoglobin adopts an alpha-helical structure. Another common structural motif is the beta-pleated sheet, in which amino acids line up in parallel rows.

¹ The coordinates for the hemoglobin protein were determined using x-ray crystallography, and the image was rendered using SwissPDB Viewer and POV-Ray.

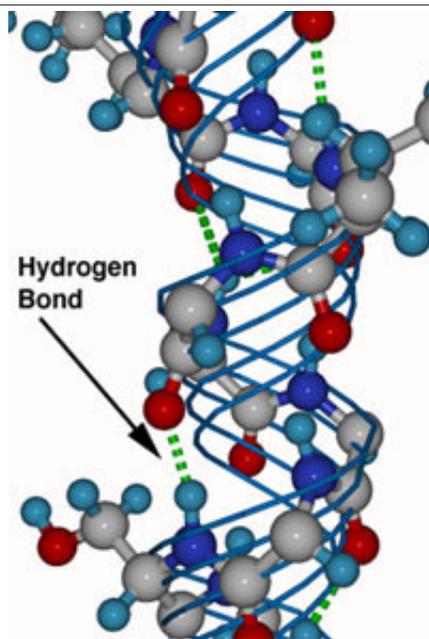


Figure 3

This is a molecular model of the alpha-helix structure in a subunit of hemoglobin. The blue strands are shown in the ribbon representation to emphasize the helical structure. The green dotted lines show the hydrogen bonding between the -NH and -CO functional groups. **Note:** To view the molecule interactively, please use [Chime](#).

View this Molecule
Interactively

View the
Movie

Download
QuickTime

The Heme Group

In hemoglobin, each subunit contains a heme group, which is displayed using the ball-and-stick representation in Figure 2. Each heme group contains an iron atom that is able to bind to one oxygen (O_2) molecule. Because hemoglobin contains four heme groups, each hemoglobin protein can bind four oxygen molecules.

In the body, the iron in the heme is coordinated to the four nitrogen atoms of a porphyrin and also to a nitrogen atom of a histidine amino-acid residue in the hemoglobin protein (Figure 4). The sixth position (coordination site) around the iron of the heme is occupied by O_2 when the hemoglobin protein is oxygenated.

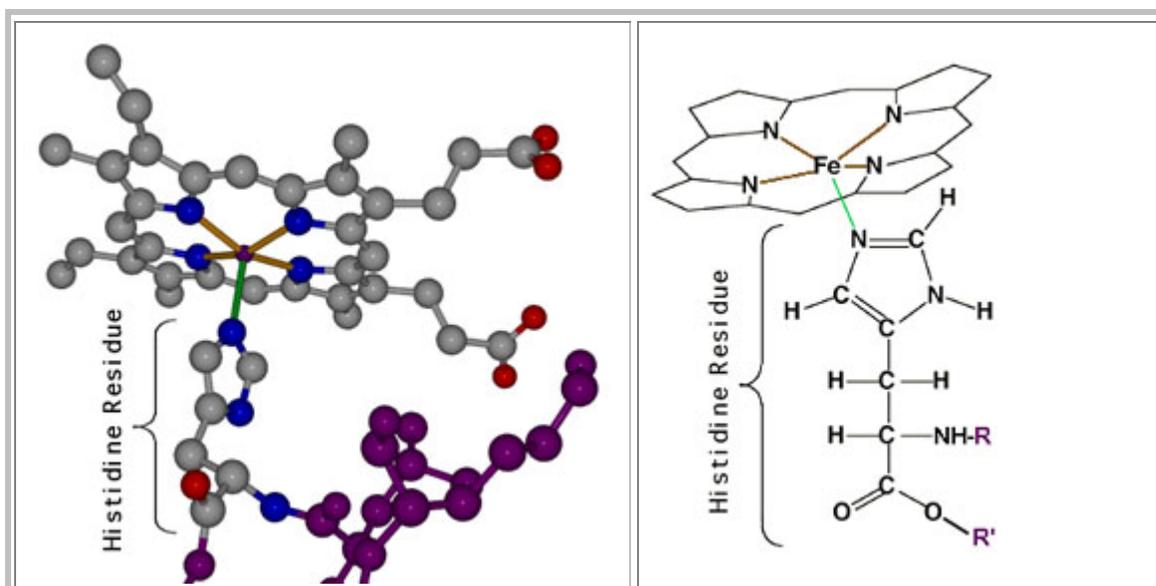


Figure 4

On the left is a three-dimensional molecular model of heme coordinated to the histidine residue (a monodentate ligand) of the hemoglobin protein. On the right is a two-dimensional drawing of heme coordinated to the histidine residue, which is part of the hemoglobin protein. In this figure, the protein is deoxygenated; i.e., there is no oxygen molecule bound to the heme group.

Note: The coordinate-covalent bonds between the central iron atom and the nitrogens from the porphyrin are shown in gold; the coordinate-covalent bond between the central iron atom and the histidine residue is shown in green. In the three-dimensional model, the carbon atoms are gray, the iron atom is dark red, the nitrogen atoms are dark blue, and the oxygen atoms are light red. The rest of the hemoglobin protein is purple.

View this Molecule Interactively

Note: To view the molecule interactively, please use [Chime](#).

Conformational Changes Upon Binding of Oxygen

Careful examination of Figure 4 demonstrates that the heme group is *nonplanar* when in its *deoxygenated* state; the iron atom is pulled out of the plane of the porphyrin toward the histidine residue to which it is attached. This nonplanar configuration is characteristic of the deoxygenated heme group and is commonly referred to as a “domed” shape. The valence electrons in the atoms surrounding the iron in the heme group and the valence electrons in the histidine residue form “clouds” of electron density. (Electron density refers to the probability of finding an electron in a region of space.) Because electrons repel one another, the regions occupied by the valence electrons in the heme group and in the histidine residue are pushed apart. Hence, the porphyrin adopts the domed (nonplanar) configuration in which the Fe is out of the plane of the porphyrin ring (Figure 5, left). However, when the heme group is in its *oxygenated* state, the porphyrin ring

adopts a *planar* configuration in which the Fe lies in the plane of the porphyrin ring (Figure 5, right).

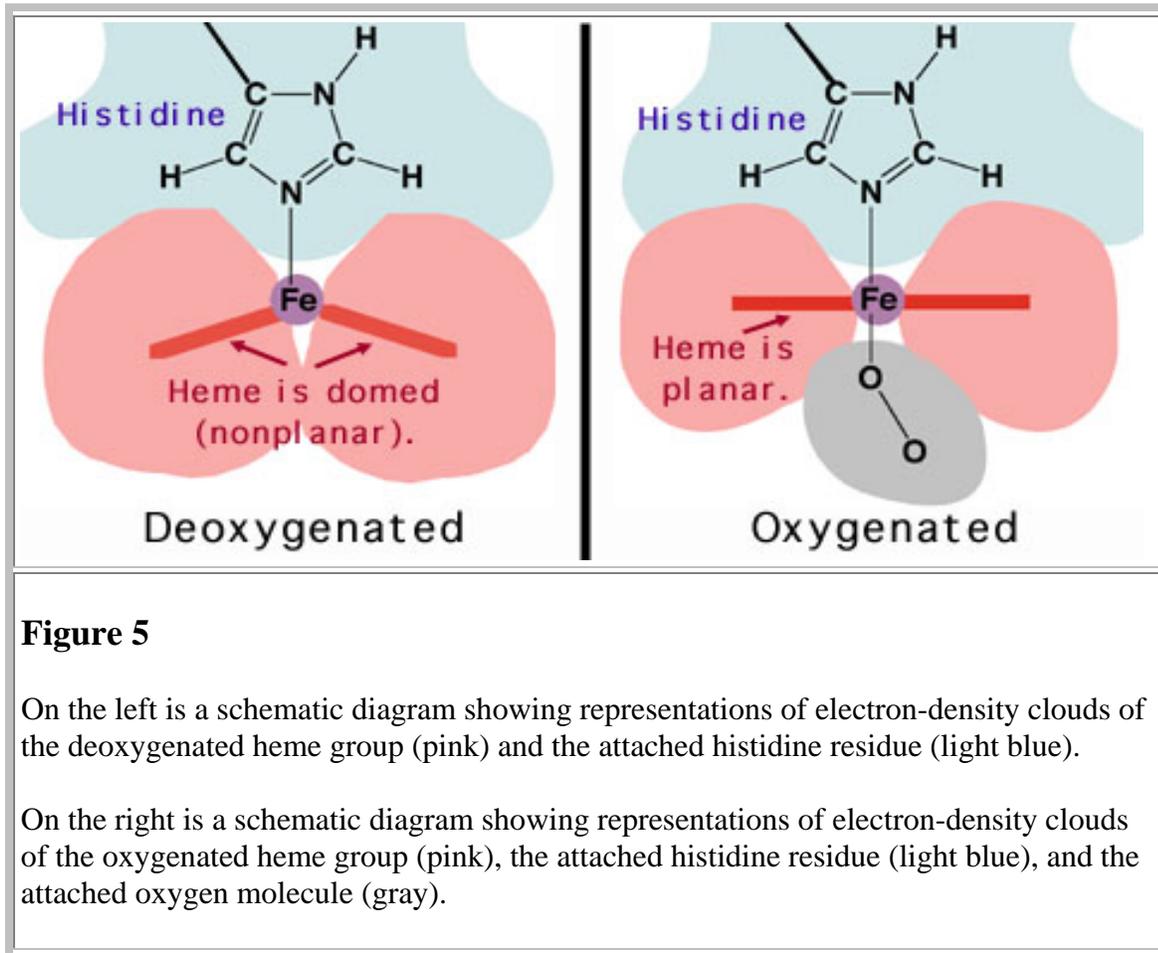


Figure 5

On the left is a schematic diagram showing representations of electron-density clouds of the deoxygenated heme group (pink) and the attached histidine residue (light blue).

On the right is a schematic diagram showing representations of electron-density clouds of the oxygenated heme group (pink), the attached histidine residue (light blue), and the attached oxygen molecule (gray).

The planar and nonplanar configurations of the heme group have important implications for the rest of the hemoglobin protein. When the iron atom moves into the porphyrin plane upon oxygenation, the histidine residue to which the iron atom is attached is drawn closer to the heme group. This movement of the histidine residue shifts the position of other amino acids that are near the histidine (Figure 6). When the amino acids in the protein are shifted by the oxygenation of one of the heme groups, the structure of the interfaces between the four subunits is altered. This causes the whole protein to change its shape. In the new shape, it is easier for the other three heme groups to become oxygenated. Thus, the binding of one molecule of O₂ to hemoglobin enhances the ability of hemoglobin to bind more O₂ molecules. This property of hemoglobin is known as **cooperative binding**.

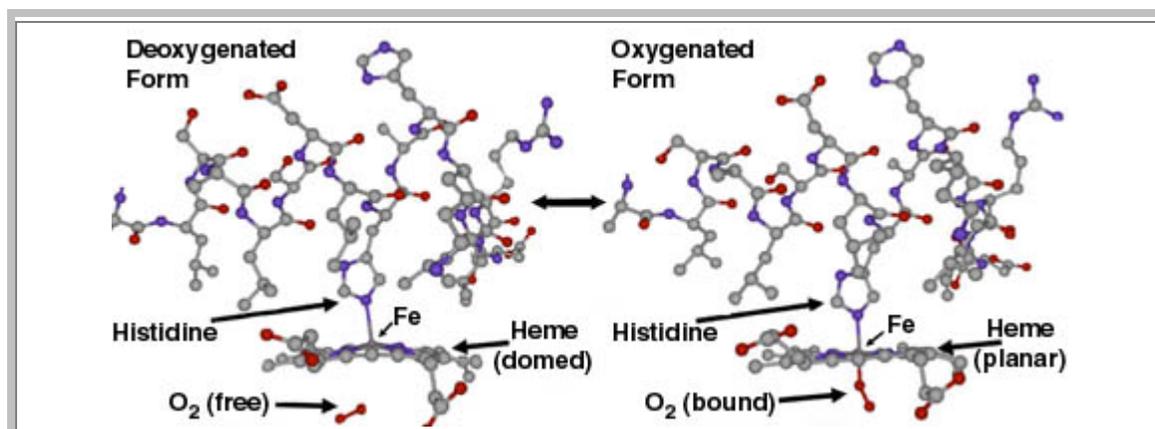


Figure 6

This figure shows the heme group and the portion of the hemoglobin protein that is directly attached to the heme. As shown in the figure, the conformational change in the heme group upon oxygenation causes the entire hemoglobin protein to change its conformation as well. Please click on the pink button below to view a QuickTime movie showing how the amino acid residues near the heme group in hemoglobin shift as the heme group converts between the nonplanar (domed) and the planar conformation by binding and releasing a molecule of O_2 .



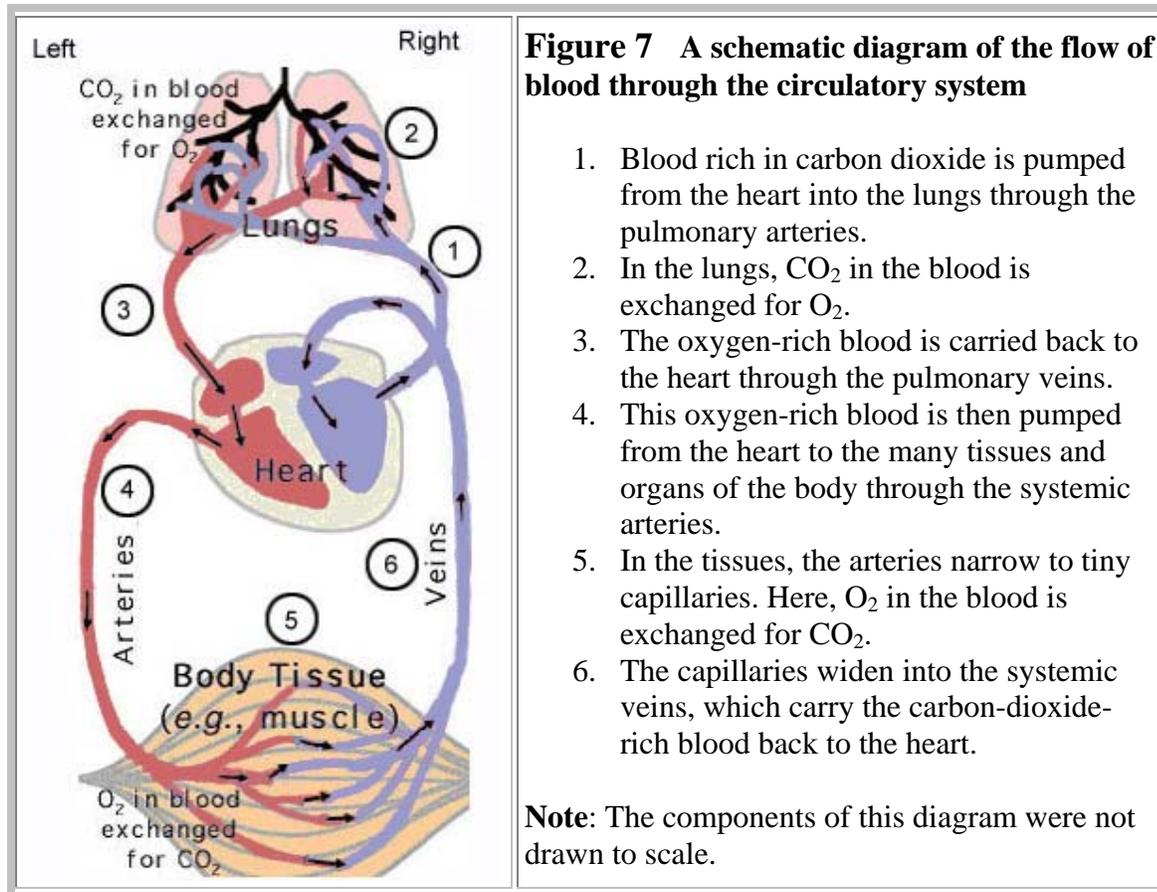
Spectroscopy and the Color of Blood

The changes that occur in blood upon oxygenation and deoxygenation are visible not only at the microscopic level, as detailed above, but also at the macroscopic level. Clinicians have long noted that blood in the systemic arteries (traveling from the heart to the oxygen-using cells of the body) is red-colored, while blood in the systemic veins (traveling from the oxygen-using cells back to the heart) is blue-colored (Figure 7). The blood in the systemic arteries is oxygen-rich; this blood has just traveled from the lungs (where it picked up oxygen inhaled from the air) to the heart. The heart then pumps the blood throughout the body so that it can deliver its oxygen to the body's cells. The blood in the systemic veins, on the other hand, is oxygen-poor; it has unloaded its oxygen to the body's cells (exchanging the O_2 for CO_2 , as described below), and must return to the lungs to replenish its supply of oxygen.

What causes this color change in the blood? We know that the shape of the heme group and the hemoglobin protein changes, depending on whether hemoglobin is oxygenated or deoxygenated. Therefore, these two conformations must have different light-absorbing properties. Recall that the color of a substance is the complementary color of what it absorbs. The oxygenated conformation of hemoglobin absorbs light in the blue-green range, and it reflects red light. This property accounts for the red appearance of oxygenated blood. On the other hand, the deoxygenated conformation of hemoglobin absorbs light in the orange range, and it reflects blue light. This property accounts for the bluish appearance of deoxygenated blood. We could use a spectrophotometer to examine a dilute solution of blood and determine the wavelength of light absorbed by each conformation.

The Effect of CO₂ and H⁺ on O₂ Binding

In 1904, Christian Bohr discovered that increased concentrations of CO₂ and H⁺ promote the release of O₂ from hemoglobin in the blood. This phenomenon, known as the Bohr effect, is a highly adaptive feature of the body's blood-gas exchange mechanism. The blood that is pumped from the heart to the body tissues and organs (other than the lungs) is rich in oxygen (Figure 7) because these tissues require oxygen for their metabolic activities (e.g., muscle contraction). It is necessary for oxygen to remain bound to hemoglobin as the blood travels through the arteries so that it can be carried to the tissues. At the same time, the oxygen must also be easily removable when the blood passes through the capillaries that feed the body tissues. CO₂ and H⁺ are produced from metabolic activities of the body, and so the concentration of these species is high in the metabolically active tissues of the body. Thus, the tissues that perform the most metabolic activity (and therefore require the largest amount of O₂) produce large quantities of CO₂ and H⁺, facilitating the release of O₂ from the blood where the O₂ is most needed. In the lungs, the reverse effect occurs: high concentrations of O₂ cause the release of CO₂ from hemoglobin.



How do CO₂ and H⁺ promote the release of O₂ from hemoglobin? These species help form interactions between amino-acid residues at the interfaces of the four subunits in hemoglobin. These interactions are called **salt bridges** because they are formed between positively-charged and negatively-charged amino-acid residues on different subunits of the same protein (Figure 8). When salt bridges form, the subunits of hemoglobin are held in a position that "tugs on" the histidine that is attached to the heme iron. (Figure 5.) This favors the domed configuration, the deoxygenated form of hemoglobin.

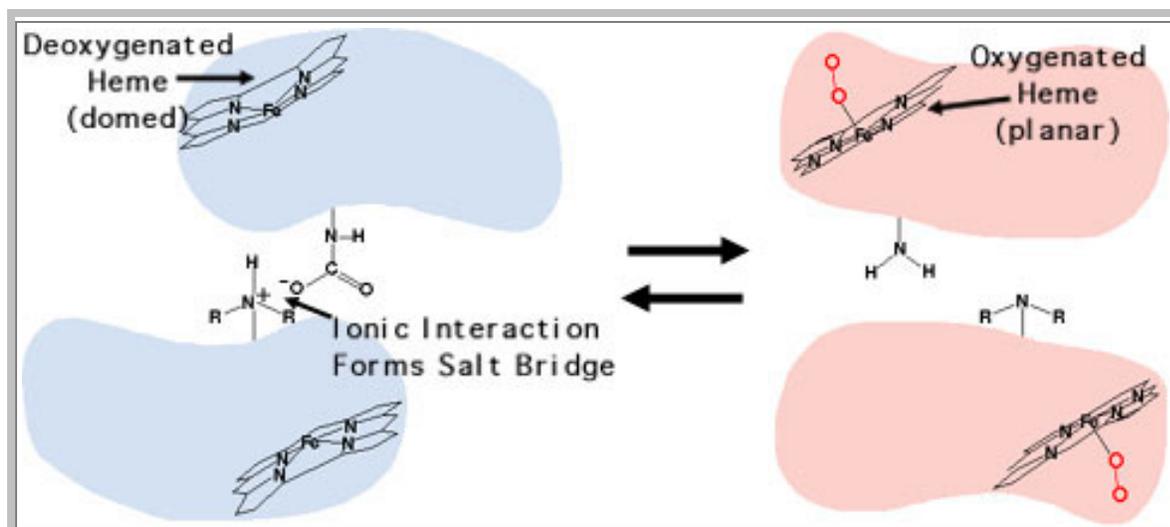
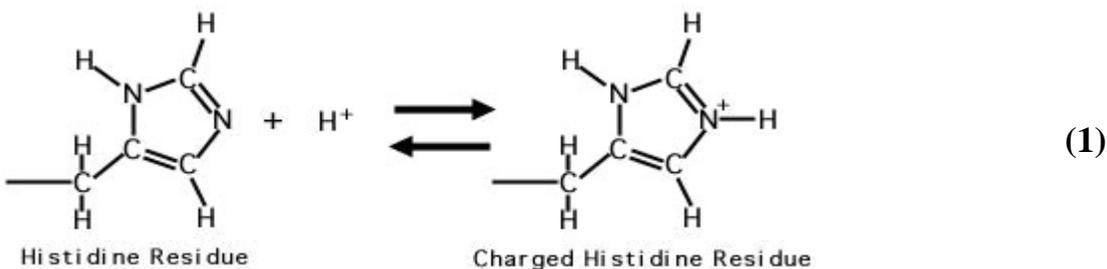


Figure 8

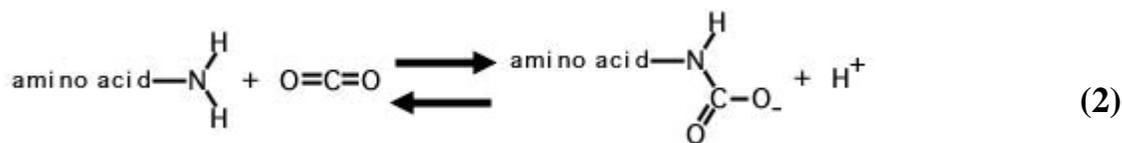
On the left is a schematic diagram of the interface of two subunits of the deoxygenated hemoglobin protein. In the presence of CO_2 and H^+ (e.g., in the muscles), charged groups are formed on the amino-acid residues lining the subunit interface. These charged groups are held together by ionic interactions, forming salt bridges between the two subunits and stabilizing the deoxygenated form of hemoglobin. When blood passes through the alveolar capillaries of the lungs, CO_2 and H^+ are removed from the hemoglobin, and the oxygenated configuration is favored (right).

Note: The components of this diagram are not drawn to scale.

When the concentration of protons (H^+) is low (pH 9), positive charges do not form on the residues at the subunit interfaces, so the salt bridges cannot form (right image in Figure 8). However, at pH 7, histidine residues at the subunit interfaces (not the histidine residues that bind the heme groups) can accept an additional proton (H^+) and hence become positively charged (Equation 1). When salt bridges form by the interaction of these interfacial histidine residues and nearby negatively charged amino-acid residues, the deoxygenated hemoglobin structure is favored, and oxygen is released (left image in Figure 8).



The number of negatively charged residues in the salt bridges is increased in the presence of carbon dioxide. CO_2 binds to the amino ($-\text{NH}_2$) group of certain amino acid residues at the subunit interfaces to produce a negatively charged group ($-\text{NHCOO}^-$) on the residue (Equation 2). This negatively charged group can form salt bridges with the positive charges on the protonated histidines described above. The H^+ produced by Equation 2 can also be accepted by histidine residues at the subunit interfaces (via Equation 1).



Thus, the changing of the overall protein structure regulates hemoglobin's biological function. This structure is altered by the binding or releasing of CO₂ and H⁺ to the interfaces of the subunits in hemoglobin (Figure 8).

Summary

Blood is an amazing and vitally important part of the body because it contains many finely tuned chemical systems that allow it to maintain the chemical environment needed for the body's metabolism. One of the most important functions of blood is delivering O₂ to all parts of the body by the hemoglobin protein. The heme group of the hemoglobin protein carries the O₂ molecule. The heme group (a component of the hemoglobin protein) is a metal complex, with iron as the central metal atom that can bind or release molecular oxygen. Both the hemoglobin protein and the heme group undergo conformational changes upon oxygenation and deoxygenation. When one heme group becomes oxygenated, the shape of hemoglobin changes in such a way as to make it easier for the other three heme groups in the protein to become oxygenated as well. This feature helps the protein to pick up oxygen more efficiently as the blood travels through the lungs. Hemoglobin also enables the body to eliminate CO₂, which is generated as a waste product, via gas exchange in the blood (CO₂ is exchanged for O₂ in the lungs, and O₂ is exchanged for CO₂ in the muscles). The species generated as waste by the oxygen-consuming cells actually help to promote the release of O₂ from hemoglobin when O₂ is most needed by the cells. Hence, hemoglobin is an example of a sophisticated chemical system of the body that enables the blood to deliver necessary molecules to cells and remove waste products from those cells.

CHIME Files

To view the molecules interactively, please use [Chime](#). To download the PDB files for viewing and rotating the molecules shown above, please click on the appropriate name below or on the "interactive" button below each molecular-model figure in the text.

- [Alpha-helix structure \(alphahelix.pdb\)](#)
- [One subunit from Hemoglobin \(subunit.pdb\)](#)
- [Hemoglobin \(hemoglobin.pdb\)](#)
- [Heme group bound to histidine residue \(heme.pdb\)](#)

Additional Links:

- [Naming Coordination Compounds](#) summarizes the steps and provides examples of naming compounds containing coordination complexes.
- For additional information on hemoglobin, see the [hemoglobin tutorial](#) by Eric Martz of the University of Massachusetts.

Note: You need CHIME to view this tutorial. You can download [Chemscape Chime](#) here.

- To learn about porphyrin and other [chelating agents](#), see this "Chemical of the Week" site from the University of Wisconsin.
 - Another porphyrin molecule, similar to heme but containing Mg rather than Fe as the central atom, is [chlorophyll](#). You can learn about the structure, function, and spectroscopy of [chlorophyll](#) from this site. (**Note:** You will be studying chlorophyll in lab at the beginning of Chemistry 152.)
-

References:

Guex, N. and Peitsch, M.C. *Electrophoresis*, **1997**, *18*, 2714-2723. (SwissPDB Viewer) URL: <http://www.expasy.ch/spdbv/mainpage.htm>.

Ji, X. *et al.* "Positive and negative cooperativities at subsequent steps of oxygenation regulate the allosteric behavior of multistate sebacylhemoglobin," (1996) *Biochemistry*, **35**, 3418. Hemoglobin PDB coordinates, *Brookhaven Protein Data Bank*.

Kavanaugh, J.S. *et al.* "High-resolution x-ray study of deoxyhemoglobin Rothschild 37beta trp->arg: a mutation that creates an intersubunit chloride-binding site," (1992) *Biochemistry*, **31**, 4111. Deoxyhemoglobin PDB coordinates, *Brookhaven Protein Data Bank*.

Kilmartin, J.V. "Interaction of haemoglobin with protons, CO₂, and 2,3-diphosphoglycerate," (1976) *Br. Med. Bull.*, **32**, 209.

Persistence of Vision Ray Tracer (POV-Ray). URL: <http://www.povray.org>.

Royer Jr., W.E. "High-resolution crystallographic analysis of co-operative dimeric hemoglobin," *J. Mol. Biol.*, **235**, 657. Oxyhemoglobin PDB coordinates, *Brookhaven Protein Data Bank*.

Stryer, L. *Biochemistry*, 4th ed. W.H. Freeman and Co., New York, 1995, p. 154-168.

Acknowledgements:

The authors thank Greg Noelken for creating the chime script files. They also wish to thank Dewey Holten, Michelle Gilbertson, Jody Proctor and Carolyn Herman for many helpful suggestions in the writing of this tutorial.

The development of this tutorial was supported by a grant from the Howard Hughes Medical Institute, through the Undergraduate Biological Sciences Education program, Grant HHMI# 71199-502008 to Washington University.

Copyright 1998, Washington University, All Rights Reserved.

Revised August 2007.

**RETURN TO
COURSES PAGE**

**RETURN TO LAB
TUTORIALS PAGE**