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This is a review of hemoglobin structure and function. The role of hemoglobin as the red blood cell oxygen-transport protein is outlined. Structure is defined in terms of amino acid sequence, x-ray crystallographic structure, and subunit interaction. Function is described for reversible oxygen binding, carbon dioxide and protein mechanism.

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HEMOGLOBIN ---- For the *Macmillan Encyclopedia of Chemistry*

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Hemoglobin is a blood protein that transports oxygen in the body by binding oxygen from the air that is inhaled into the lungs, carrying it throughout the circulatory system, and releasing it to tissues where oxygen is required for respiration. In vertebrates, hemoglobin is circulated in the bloodstream inside erythrocytes (*i.e.*, red blood cells; the bright red color of hemoglobin actually gives the red blood cell its name.) Hemoglobin is the primary cellular component of mature erythrocytes. A person has approximately 25 trillion red blood cells, and each red blood cell contains approximately 280 million molecules of hemoglobin (Dickerson and Geis, 1983). This gives a total of about 70×10^{20} molecules of hemoglobin per person, thereby defining the oxygen transporting capacity needed to sustain human life.

Hemoglobin was one of the first proteins to be obtained in high enough concentration and purity to determine its amino acid composition and molecular weight (approximately 64,000 daltons). This is because of its intense, visible color and the large amount of it in the blood. The ease with which hemoglobin's color can be measured has facilitated studies of the protein's function: when oxygen is bound, *i.e.*, oxyhemoglobin, it is bright red, and when oxygen is unbound, *i.e.*,

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deoxyhemoglobin, it changes to a darker, purple color. Human hemoglobin also was one of the first three-dimensional protein structures to be solved by x-ray diffraction analysis of hemoglobin crystals, an analysis which determined that, in addition to a difference in color, there is also a difference in the molecular structure of oxy- *versus* deoxyhemoglobin (*see* Bunn and Forget, 1986, and the references cited therein).

Hemoglobin exists functionally in a well-defined, globular conformation. The unique sequence of amino acids forms helices, which fold together in a manner that stabilizes the molecule. The amino acid sequences of hemoglobins from several species have been determined. The species-to-species sequence variations create different hemoglobin structures, each of which appears to be best suited to the specific organism's function. Most information about hemoglobin structure has been obtained from human hemoglobin, and that molecule will be described in more detail here, although it should be kept in mind that there are features common to all hemoglobins.

Human hemoglobin is comprised of four separate amino acid structures, two α and two β subunits. These four protein subunits, each called a globin monomer, interact with one another to form the larger globular protein molecule known as hemoglobin (*see* **Figure 1**). One α subunit interacts strongly with one β subunit, and the $\alpha\beta$ pairs, or dimers (*i.e.*, two units), interact more weakly with a second $\alpha\beta$ dimer to form the hemoglobin tetramer (*i.e.*, four units), $\alpha_2\beta_2$. Hemoglobin requires a complete set of all four subunits to function properly. Because the

interaction between dimers is fairly weak, the hemoglobin tetramer can dissociate into $\alpha\beta$ dimers when it is outside of the red blood cell. In its concentrated environment inside the red blood cell, hemoglobin exists primarily in tetrameric form. A recent study has shown that hemoglobin's microenvironment, including the surrounding water molecules, plays an important role in the functional state of the protein (Colombo *et al.*, 1992).

Each human hemoglobin molecule contains 574 amino acids. Each α subunit is made up of 141 amino acids that fold into seven helices, the A, B, C, E, F, G, and H helices, with turns formed between the helices. Each β subunit is made up of 146 amino acids that fold into eight helices, A, B, C, D, E, F, G, and H, with turns formed between the helices. The extra D helix in the β subunit makes this subunit slightly larger.

In addition to globin, the hemoglobin molecule contains four heme prosthetic groups, one for each subunit. Heme is protoporphyrin IX with an iron atom coordinated at the center of the porphyrin. The four globin subunits plus hemes constitute the complete, functional hemoglobin molecule. The heme iron atom is bound covalently within the heme pocket in each subunit, in a cleft between the E and F helices, to a single amino acid residue, the proximal histidine of the F helix (*see* **Figure 1**). Heme is oriented within this pocket by the coordination bond to the imidazole nitrogen of the proximal histidine and by interactions with other amino acid residues lining the pocket. There are slight differences in the configuration of the heme pockets of the α and β subunits. A

schematic representation of the heme pocket of the α subunit is pictured in **Figure 2**.

Oxygen (O_2) binds to hemoglobin at each heme in the tetramer. Thus, four molecules of oxygen bind per molecule of hemoglobin. O_2 binding to the iron atom at the center of heme ring occurs reversibly, opposite to the site where the heme binds with the proximal histidine. Without O_2 bound, the iron atom is oriented toward the histidine residue on the F helix and is most stable out of the plane of the heme. When O_2 binds on the opposite side, the iron atom moves back into the plane of the porphyrin ring (*see* **Figure 2**). This movement of the iron atom during O_2 binding starts a chain of events within the protein that begins with movements in the F helix and carries on throughout the entire protein structure. As a result, at some point during the process of binding the four molecules of oxygen, with the exact point depending on the microenvironment, hemoglobin converts from one end-state structure, deoxyhemoglobin, to the other, oxyhemoglobin.

It is the structural change that brings about the change in color in hemoglobin (primarily through rearrangements in the electronic configuration of the heme). The protein dynamics also couple hemoglobin structure to function. As hemoglobin's structure changes, so does its function: that is, after O_2 is bound to a hemoglobin molecule, the probability of O_2 binding to that same molecule increases. In other words, oxyhemoglobin has a higher affinity for O_2 than does deoxyhemoglobin, a phenomenon that is called cooperativity.

Cooperativity can be illustrated as follows: when the fraction of hemoglobin with O_2 bound (*i.e.*, hemoglobin saturation) is displayed as a function of O_2 concentration or partial pressure (PO_2), one sees a sigmoidal (S-shaped) binding curve (*see* **Figure 3**). The O_2 affinity, which is less at low levels of hemoglobin saturation, increases markedly as fractional saturation increases. Thus, high affinity for O_2 at high partial pressures helps to load O_2 onto hemoglobin in the lungs, and low affinity for O_2 at low partial pressures helps to unload O_2 from hemoglobin in the tissues. This classic relationship between hemoglobin structure and function has become a model for the study of cooperative protein systems.

Molecules other than O_2 also bind to hemoglobin. Some gaseous molecules such as carbon monoxide (CO) and nitric oxide (NO) bind at the heme, at the same site as O_2. Both of these molecules bind to hemoglobin with higher affinity than O_2, and at high concentrations they can cause suffocation by displacing bound O_2 or by preventing O_2 from binding.

Molecules that bind to hemoglobin at sites other than the heme are important physiological modulators of hemoglobin function. A list of some the most important effectors includes the hydrogen ion [*i.e.*, the proton (H^+)], the chloride ion (Cl^-), 2,3-diphosphoglycerate (2,3-DPG), and carbon dioxide (CO_2). Since they are linked to O_2 binding, they are called oxygen-linked effectors.

The oxygen-linked effectors regulate O_2 binding in the following way. More hydrogen ions bind to deoxyhemoglobin than to oxyhemoglobin. Thus, as the pH of a hemoglobin solution decreases (*i.e.*, the concentration of protons increases),

such as may occur in muscles during exercise, the deoxyhemoglobin conformation is favored and the affinity of hemoglobin for O_2 decreases, and *vice versa*. This is called the Bohr effect and is an important factor for delivery of O_2 to working muscles and for picking up O_2 in the lungs. Cl^- binding works in a similar manner, but binding occurs at different sites on the hemoglobin molecule because of the negative charge of the ions. 2,3-Diphosphoglycerate is a molecule formed during the breakdown of sugar in normal human erythrocyte metabolism. It is also negatively charged and binds to hemoglobin between β subunits to lower O_2 affinity. Hemoglobin with 2,3-DPG bound releases O_2 more readily at the low O_2 tension in tissues and consequently delivers O_2 more efficiently. Carbon dioxide (CO_2) is another primary effector molecule which works in two ways: (1) CO_2 plays a role in the Bohr effect through its reversible reaction with protons in the plasma to produce bicarbonate, and (2) CO_2 binds directly to hemoglobin at the amino terminal residues of both α and β subunits. (About ten to twenty percent of the CO_2 exhaled through the lungs is transported bound directly to hemoglobin.) Overall, the strict physiological control of respiration requires a delicate balance among all of these effects. The need for O_2, the need to exhale CO_2, and the tight maintenance of blood pH are all interrelated by hemoglobin function.

Hemoglobin's fundamental role in life processes has made it one of the most studied of all proteins. Even so, the dynamics of hemoglobin's structural/functional mechanism are largely still a mystery, and this molecule continues to provide a source of fascination for scientists. More studies will be

required to define precisely the connection between hemoglobin structure and the thermodynamics of O_2 binding. Thus, understanding the intermediate binding states of hemoglobin has become a major goal in biochemical research today.

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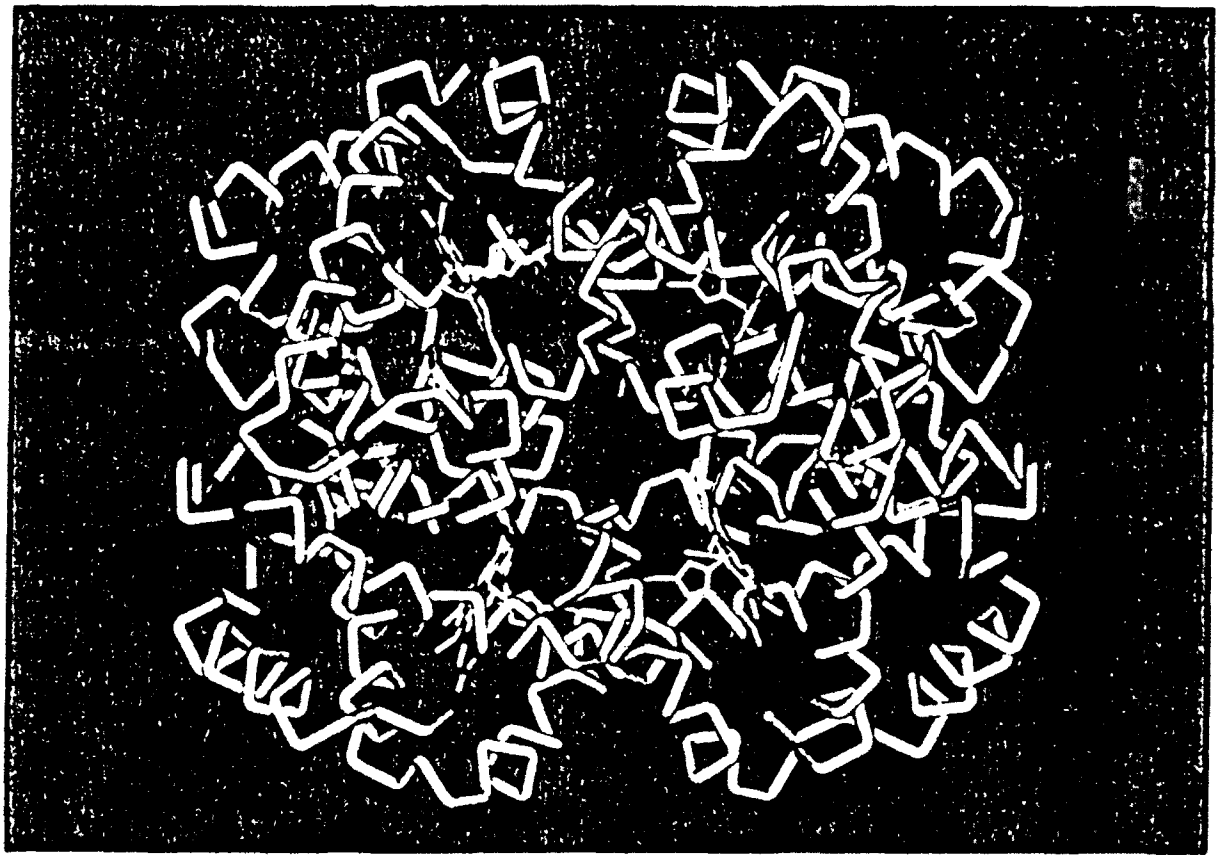
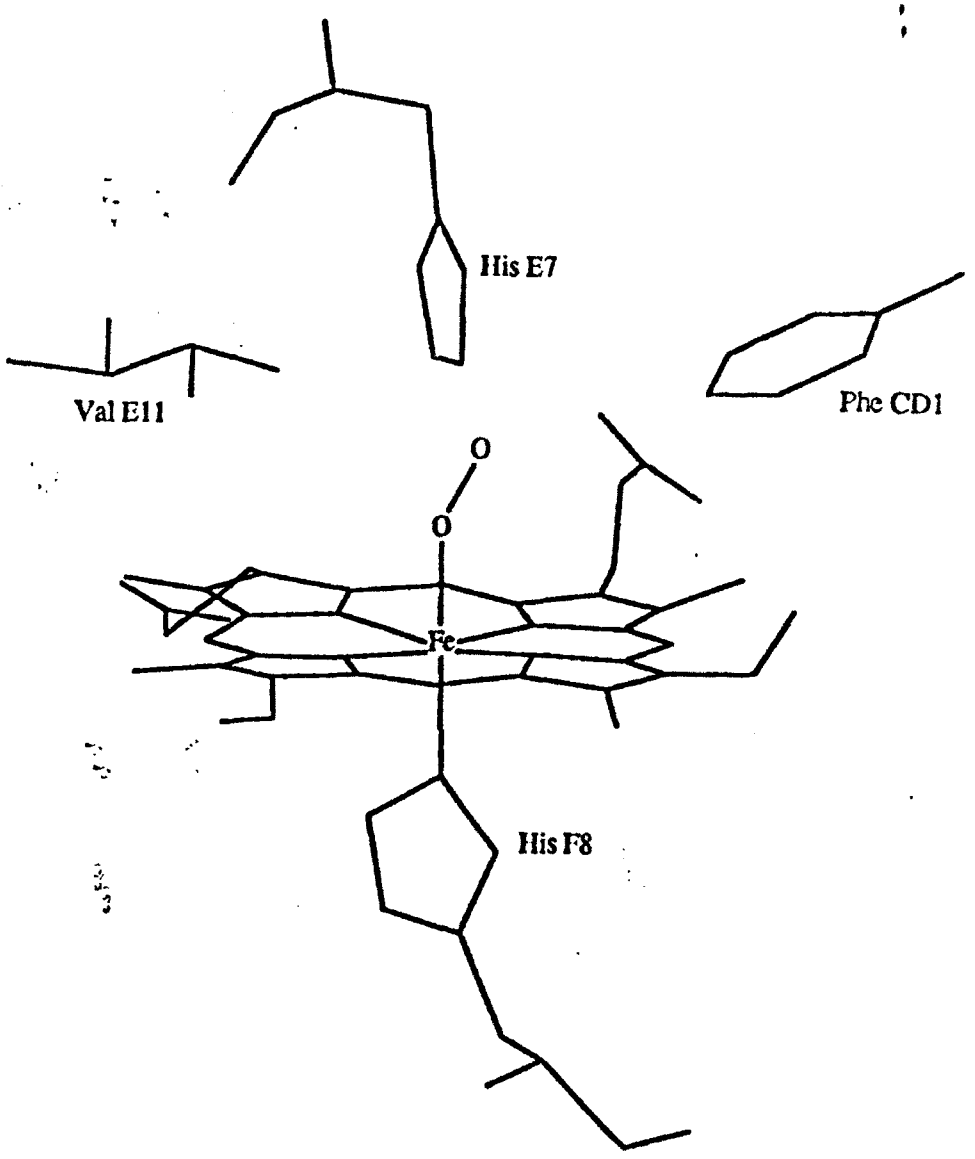
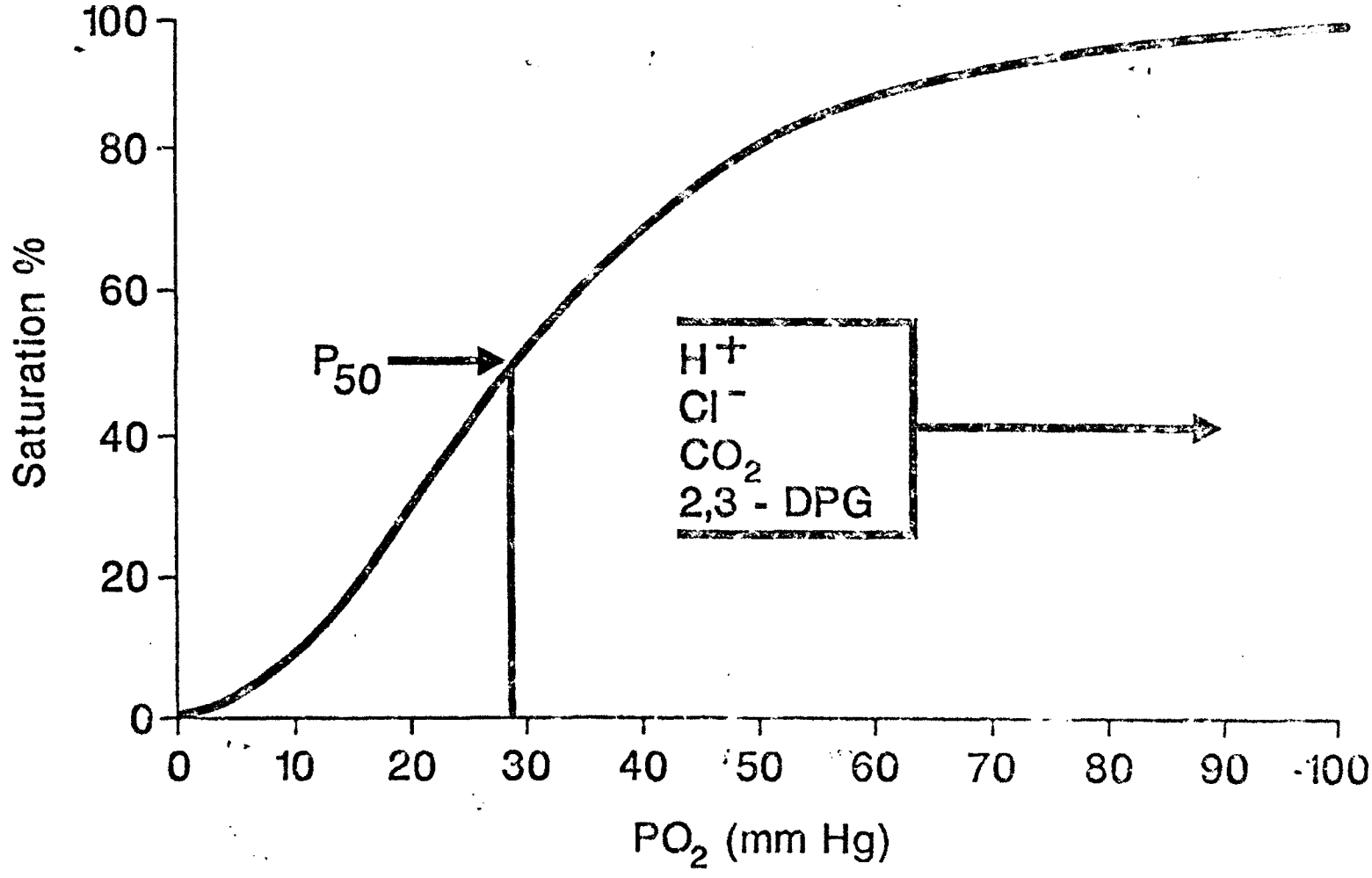


Fig. 1



α subunit

Fig. 2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80
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Saturation %
P_{50}
H^+
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CO_2
2,3 - DPG
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60
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80
90
100
PO_2 (mm Hg)

Fig. 3