Hemoglobin: Model Systems Shed Light on Oxygen Binding

Hemoglobin, the oxygen-carrying molecule of red blood cells, has probably been studied more than any other protein. Most biochemists might agree that more is known about the chemical structure and function of hemoglobin than about any other protein. Nevertheless, there are still large gaps in our knowledge about this compound. Most important, little has been known about the kinetics and mechanism of the binding of oxygen to protoheme, the iron-containing active site constituent of hemoglobin.

Within the last year and a half several groups of investigators have developed simplified model systems that mimic the reversible binding of oxygen characteristic of hemoglobin. One of these groups, moreover, has now for the first time measured the rates of oxygen binding and dissociation for isolated protoheme in water at room temperature. Work with these systems has confirmed some aspects of current theories about the mechanism of hemoglobin action, but it has also produced rather sharp revisions in other aspects. Many unanswered questions about hemoglobin still remain, but a fuller understanding of its action now seems much closer than before.

Hemoglobin constitutes about 95 percent of the solid components of the red blood cell; an average sized human might contain about 1.5 kilograms of hemoglobin. Its principal function is the transport of oxygen from the lungs to other tissues. Hemoglobin also participates in the transfer of carbon dioxide from tissues to the lungs, but this role is less important.

Hemoglobin is a tetramer with a mass of about 64,500 daltons. Each monomer is composed of a protein (globin) and a heme, which is a complex of divalent iron [Fe(II)] and protoporphyrin; protoporphyrin is a large aromatic ring formed from four substituted pyrrole rings linked by methene groups (Fig. 1). In the most common form of hemoglobin, two of the monomers contain 141 amino acids (alpha chains) and two contain 146 (beta chains).

Much of the present knowledge about hemoglobin is derived from the x-ray crystallographic studies of Max F. Perutz, John C. Kendrew, and their associates at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. On the basis of these studies, Perutz has presented* a convincing mechanism for the cooperative interaction of the hemoglobin monomers, the phenomenon in which binding of one oxygen molecule to the tetramer lowers the activation energy for binding of the second, third, and fourth molecules. But this mechanism says little about the binding process itself.

The hemoglobin structure of Perutz and Kendrew shows that the protoporphyrin-iron complex fits into a hydrophobic cleft in each monomer. Although some 60 amino acids of the globin contact the protoporphyrin ring, the ring is covalently bound only to one histidine residue (known as the proximal histidine) that is linked through its imidazole moiety to the heme iron. A second histidine residue (known as the distal histidine) is located near the iron atom on the opposite side of the ring. Both histidine residues are believed to participate in the binding of oxygen.

The chief impediment to the study of oxygen binding in the isolated heme is the rapid oxidation of Fe(II) to trivalent iron [Fe(III)]. Fe(III) binds so tightly to hydroxyl ion and certain other ligands that it cannot bind oxygen. The various investigators have thus attempted to simulate the local geometry and the electronic and solvent environment of the hemoglobin active site in simple heme compounds in an effort to impede such oxidation. Since these compounds are all monomers, though, they might more correctly be considered to be models of myoglobin, the oxygen-binding constituent of muscle tissues. Myoglobin contains an iron-protoporphyrin complex and a chain of 153 amino acids; it is very similar to hemoglobin, but there is no cooperativity of oxygen binding.

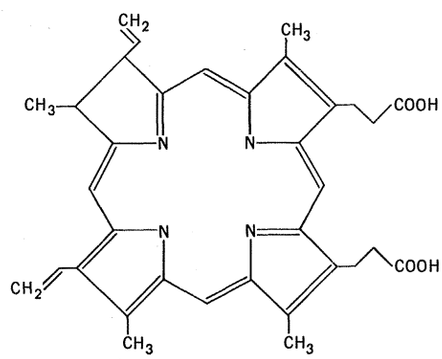


Fig. 1. The protoporphyrin ring of hemoglobin.

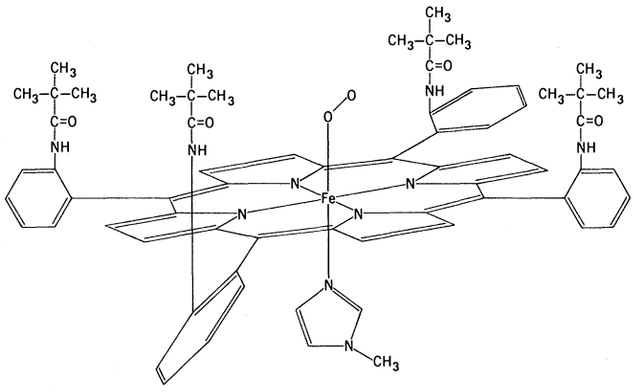
The oxidation of hemes to Fe(III) is believed to proceed through an intermediate in which two iron-porphyrin complexes are covalently linked in a face-to-face configuration by an oxygen molecule. James P. Collman and his associates at Stanford University, Stanford, California, thus reasoned that attachment of bulky substituents to one side of the porphyrin ring might prevent the intimate contact necessary for oxidation. (In all of the model systems, oxygen binding can be accomplished only when a base—generally a nitrogen base such as imidazole—is covalently bound to the heme iron. This base blocks the other side of the ring.)

Collman's group thus synthesized a derivative of tetraphenylporphyrin in which four bulky tertiary-butyl groups are substituted on one side of the ring (Fig. 2). Contrary to his expectations, the *t*-butyl groups did not prevent even bulky nitrogen bases from forming complexes with the iron, and Collman thus obtained hexacoordinate Fe(II) compounds in which two bases are bound to the iron atom. Nonetheless, the compounds proved resistant to oxidation in dilute solution and were shown to bind oxygen reversibly.

One bulky derivative could also be isolated in crystalline form, and in this form it was shown to bind oxygen reversibly in gas-solid reactions. This oxygen complex, like oxyhemoglobin and oxymyoglobin, is diamagnetic, and its Mössbauer spectra suggests that its electronic configuration is very like those of the natural complexes.

X-ray crystallography of the crystalline complex by Ward Robinson and Gordon Rodley of the University of Canterbury in New Zealand provided the first evidence for the conformation of bound oxygen in hemoglobin or myoglobin. Although there were difficult crystallographic problems, the results confirm Linus Pauling's prediction made in 1936 that oxygen is coordinated to iron in an angular fash-

* M. F. Perutz, *Nature (Lond.)* **228**, 726 (1970).



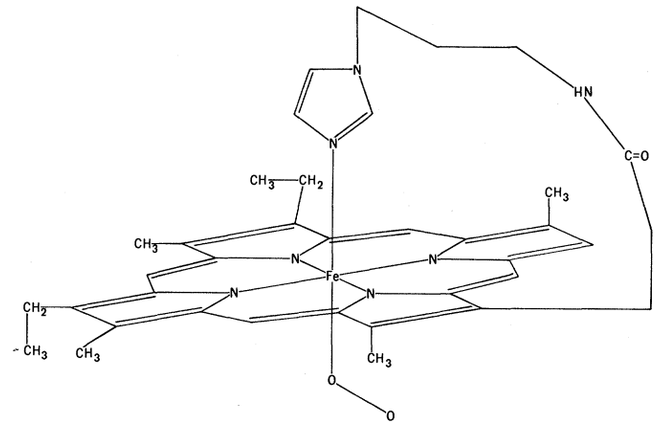


Fig. 2 (left). The hindered tetraphenylporphyrin synthesized by J. P. Collman and his associates. Fig. 3 (right). The myoglobin model synthesized by T. G. Traylor and C. K. Chang.

ion. That is, the iron-oxygen bond is perpendicular to the plane of the porphyrin ring and the Fe–O–O angle is about 136°. Robinson and Rodley also found that the Fe–O distance is slightly smaller than was expected, suggesting that there is some π-bonding between these atoms.

Collman and Harry Gray of the California Institute of Technology, Pasadena, measured the infrared stretching frequency of oxygen in the complex at −175°C, the temperature at which it is most prominent, and found it to be 1385 cm^{-1}. This value is about 250 cm^{-1} above that expected for complexes of superoxide, the form of oxygen that has been thought to exist in hemoglobin. Collman and Gray suggest that the observed value is more characteristic of a coordinated singlet oxygen. But Winslow Caughey of Colorado State University, Fort Collins, has reported that the oxygen stretching frequency of oxyhemoglobin is 1107 cm^{-1}, a value more characteristic of superoxide. The source of this discrepancy is unknown, and the electronic configuration of bound oxygen must remain a mystery for now.

Jack E. Baldwin and his colleagues at the Massachusetts Institute of Technology, Cambridge, have synthesized a hindered myoglobin model similar to Collman's and have shown that it will bind oxygen reversibly in solution. They have not yet been able to isolate it in crystalline form, however. Neither Baldwin nor Collman has examined the kinetics of oxygen binding in the model systems, partly because of the problem of separating the rates of oxygen binding and dissociation from the binding and dissociation rates of the nitrogen base. Teddy G. Traylor and C. K. Chang of the University of California at San Diego have solved this problem by synthesizing a model compound (Fig. 3) in which imidazole is covalently bound to a porphyrin ring in a geometry identical to that of the proximal histidine in hemoglobin.

This model compound is somewhat less resistant to oxidation than is Collman's, but it does bind oxygen reversibly and the kinetics can be studied in aqueous solution. The compound, Traylor says, has oxygenation kinetics and equilibria similar to those of the alpha chains of hemoglobin and the same binding constant as myoglobin. This observation seems to refute previous suggestions that the hydrophobic environment provided by the globin moiety increases the binding constant. It now seems, rather, that the major contribution of the globin is to prevent oxidation of the heme iron.

By varying solvent composition and the identity of the proximal base, Traylor and Chang have been able to vary the oxygenation and deoxygenation rates for the model compound independently to duplicate the kinetics of all steps in hemoglobin functioning. Their results suggest that, contrary to prior theories, increases in polarity of the environment around the heme stabilize the oxygen complex.

Their results also show that increasing the basicity of the proximal base increases the rate of oxygenation and thus improves binding. It therefore seems possible that changes in the environment on the distal (oxygen) side of the porphyrin ring may have as much influence on oxygen binding as the movement of the proximal imidazole group postulated by Perutz. The results also suggest that protonation of the proximal imidazole will influence oxygen binding. Many of Traylor and Chang's observations have been confirmed in the same system by Baldwin.

Traylor and Chang have also confirmed some of their results by studying the kinetics of reversible oxygen binding with isolated protoheme under physiological conditions. Such studies have never been performed before because of the rapid oxidation of protoheme, but Traylor and Chang succeeded by adapting flash photolysis techniques developed by Q. H. Gibson of Cornell University, Ithaca, New York. In essence, they found that coordination of a protoheme-imidazole complex with carbon monoxide, which binds to hemoglobin more than 100 times as tightly as oxygen, protects the complex from oxidation. Microsecond pulses of light dissociate the carbon monoxide and, under some conditions, the rates of oxygenation and deoxygenation can be measured before the iron oxidizes.

In the limited kinetic studies they have performed so far, Traylor and Chang's most important observation is that the isolated protoheme in water binds oxygen faster and more firmly than does hemoglobin itself, again suggesting that the globin contributes little to oxygen binding. But the most significant aspect of this research, Traylor contends, is that perfection of the techniques involved makes it possible to study the oxygenation kinetics of any heme in any solvent.

Some scientists argue that the lifetime of the isolated protoheme is so short that it is not necessarily a good model, and that other model systems may be more useful. William S. Brinigar of Temple University, Philadelphia, and Chang have synthesized a disubstituted porphyrin in which the imidazole chain shown in Fig. 3 is substituted on both sides of the ring. They have shown that this system will complex with oxygen reversibly at −45°C in dimethylformamide and dimethyl sulfoxide. Independently, they and Baldwin have also shown that a complex

of porphyrin and *N*-alkylimidazoles will bind oxygen reversibly under the same conditions.

Fred Basolo of Northwestern University, Chicago, has demonstrated that complexes of tetraphenylporphyrin and imidazole, piperidine, or pyridine will bind oxygen reversibly at −78°C in dichloromethane and other solvents. And Richard J. Kassner of the University of Illinois at Chicago Circle has shown that a complex of protoheme and *t*-butylamine will bind oxygen reversibly in 1-butanol at −70°C. The observations with *t*-butylamine and piperidine suggest that π-bonding from imidazole or aromatic bases is not necessary for oxygen binding, but that it increases stability of the oxygen complex.

Kinetic studies of each of these systems have been limited because of the difficulties of separating oxygen binding from binding of the nitrogen base. But the data that are available corroborate the observation that increasing polarity of the solvent increases the rate of binding of oxygen and the stability of the oxygen complex. The studies also point out the importance of low temperatures and the exclusion of water in preventing oxidation of the heme iron in the absence of globin. It thus seems apparent that the hydrophobic pocket of the globin moiety of hemoglobin or myoglobin may actually interfere somewhat with the binding of oxygen, but that this interference is counterbalanced by the protection from oxidation.

Because of the importance of excluding water from the model systems and in order to mimic more closely the hydrophobic environment produced by the protein, many of the investigators are studying their systems in the gas phase. Many of Collman's results, for example, are obtained from gas-solid reactions with the crystalline, hindered porphyrins. Traylor and Chang have incorporated their model compound into a polystyrene film to study gas-solid reactions. And Basolo and Robert L. Burwell, Jr., of Northwestern University have developed a new system in which an iron-tetraphenylporphyrin complex coordinates to 3-imidazolylpropyl groups covalently bound to silica gel. Preliminary results in the gas phase show that this material is stable in oxygen at room temperature and that it binds oxygen weakly at 0°C, more strongly at −78°C, and irreversibly at −127°C. Further studies with all of the systems are continuing.

While the mechanisms of hemoglobin and myoglobin are still not yet fully understood, knowledge about them is increasing rapidly, and the day when they will be understood seems in sight. This understanding should be of great value in the study and treatment of the abnormalities of hemoglobins and the blood diseases in which they are involved. Perhaps even more important, the duplication of the function of myoglobin with such simple model compounds suggests that other types of protein catalysis can be duplicated with protein-free small molecules.

—THOMAS H. MAUGH II

Sociobiology (II): The Evolution of Social Systems

Sociobiologists, who emphasize the genetic basis of social behavior, are taking a view of social systems that is becoming increasingly popular among ethologists who do field studies. These investigators believe that social systems evolve to increase the genetic fitness of individuals in specific environments. By combining studies of animal behavior with those of ecology, sociobiologists are beginning to understand why different social systems may have evolved.

The reconstruction of the evolution of social systems of vertebrates gained impetus soon after John Crook (University of Bristol, Bristol, England) and others described the social life of birds. These investigators observed that the social systems of different bird species in the same environment are often similar and that the social systems of closely related species in unlike environments are often different. These observations stimulated students of animal behavior to search for general rules governing the ways that environments affect social systems. Investigators are now trying to ascertain what kind of behavior would allow an animal or its close relatives to rear the largest number of offspring in a given environment and then to see how such fitness strategies change in response to changes in the environment and changes in the animal's interactions with members of its social group.

Spacing patterns among sunbirds in East Africa have been analyzed by Larry Wolf (Syracuse University, Syracuse, New York) and Frank Gill (Academy of Natural Sciences, Philadelphia). They report that male sunbirds, and sometimes females and immature individuals, often subdivide a field of flowers among themselves, each individual occupying its territory and defending it during the nonbreeding season so that only he can feed there.

Although such behavior is commonplace among birds and other animals, Wolf and Gill found two conditions in which male sunbirds will not set up territories: Territories will not be formed when nectar is so sparsely distributed that it would require an inordinate amount of energy for a male to defend a territory with enough food for himself. Nor will territories be formed when nectar is so densely distributed that it is a waste of energy to defend a territory. Wolf and Gill were able to predict whether territories would be formed and how large the territories would be by plotting the distribution of nectar in a field and knowing the metabolic energy required by a sunbird. If territories were formed, each contained slightly more nectar than was necessary to support one bird for 24 hours. Wolf and F. G. Stiles (University of Costa Rica) found, moreover, that their analysis of sunbirds could be applied to hummingbirds of the Americas—a group of species that also eat nectar.

The prediction that territories will not be formed in very sparse or very rich habitats has been tested by investigators who compared the social systems developed by related species living in different environments. For example, David Barash (University of Washington, Seattle) finds that marmots, which are large rodents, set up territories when they live in a moderate environment where the growing season is long but live in colonies in very harsh environments with short growing seasons. When marmots live in environments that are intermediate between moderate and very harsh, they are intermediate in territoriality (animals live in colonies but have ranges for foraging).

When food is randomly distributed in patches, each of which contains enough food for many members of a species, animals often form colonies instead of setting up territories or living independently of each other. The potential advantage of this form of social organization was mathematically modeled by Henry Horn (Princeton