# The Role of Water in Deoxygenated Hemoglobin Solutions

THOMAS L. FABRY

AND

HASKELL A. REICH

IBM Watson Laboratory, Columbia University, New York, New York

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Although the structure of hemoglobin is well known there is still no conclusive evidence about the identity of the ligand in the sixth coordination position in deoxyhemoglobin. In the hemoglobin molecule the iron is located at the middle of the heme part, four of the coordination positions being taken up by the nitrogens of the porphyrin ring. The globin residue is attached to the fifth coordination position and the sixth coordination position is usually taken up by a ligand. Haurowitz[1] presented circumstantial evidence, that in the dry, deoxygenated form of hemoglobin there is a water molecule in the sixth coordination position. George and Lyster[2] pointed out that there may be other plausible explanation for these results. Presently, no X-ray data are available to show whether there is a water molecule in this position. A literature survey indicates divided opinions on this question.

We decide to investigate the matter using a nuclear magnetic resonance technique. In an aqueous solution the spin-lattice relaxation rate of the solvent protons is the sum of the relaxation rates due to the different mechanisms of relaxation. Since the relaxation due to the magnetic fields of unpaired electrons is very efficient, in the case of an aqueous solution of paramagnetic ions a principal contribution to the relaxation of the water protons comes from collisions with the paramagnetic ions. This effect has been used previously to determine the position of paramagnetic ions in macromolecules.[3] The solvent protons

are also relaxed by water-water and water-protein collisions.

According to the studies of Pauling and Coryell[4] deoxyhemoglobin and methemoglobin are paramagnetic, oxyhemoglobin and carboxyhemoglobin are diamagnetic. They concluded that in hemoglobin the iron is present as ferrous ion with four unpaired electrons. One would expect, therefore, greatly decreased proton relaxation times in solutions of deoxyhemoglobin and methemoglobin compared with the relaxation times in the solutions of the diamagnetic forms provided that the paramagnetic ions are in contact with the water molecules. Further methemoglobin should relax the water protons about sixteen times faster than deoxyhemoglobin[5] since $\mu_{eff}(Fe^{+++})/\mu_{eff}(Fe^{++}) = 4$. The water proton relaxation time in solution of methemoglobin has been previously measured.[3] From the measurements for methemoglobin we expect a measurable effect in deoxyhemoglobin solution provided the paramagnetic ion in the heme and the water molecules could interact.

Hemoglobin was prepared from the freshly drawn blood of an adult male by the Drabkin technique. Relaxation times were measured on the apparatus of Garwin and Reich at 2.5 Mc/sec.[6] The measurements were carried out at room temperature (21-22°C). The hemoglobin sample (1.5 ml) was placed in a small test tube fitted with a stopcock. At first, the relaxation rate of oxyhemoglobin solution was determined. It was then deoxygenated by bubbling oxygen free nitrogen, saturated with water to avoid changes in the concentration, through the sample. After measuring the relaxation rate in deoxyhemoglobin solution we converted the hemoglobin into carboxyhemoglobin by bubbling deoxygenated wet carbonmonoxide through the solution. Since oxygen gas is paramagnetic, it also contributes to the relaxation rate of the solution. It is possible to correct for this effect by deducting the relaxation rate due to the dissolved oxygen in the hemoglobin solution. As a final check we also measured the relaxation rate in a methemoglobin solution by oxidizing the hemoglobin with potassium ferricyanide.

We have done three sets of measurements on three preparations of hemoglobin. A typical set of measurements is shown on Fig. 1. These measurements were carried out at four different concentrations. Since the relaxation rate increases as a linear function of concentration,

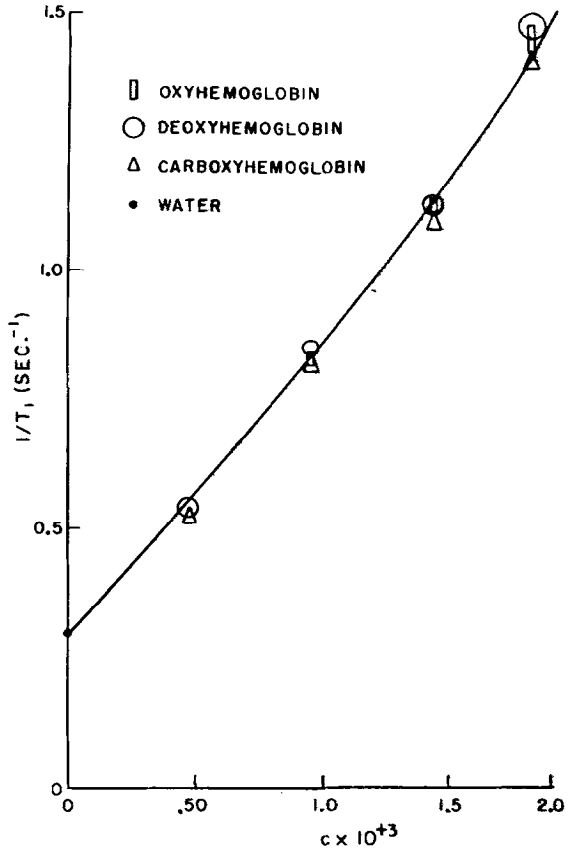


Fig. 1. Relaxation rate as a function of hemoglobin concentration.

the dissolved protein itself enhances the relaxation rate. But no differences within experimental error are observable between the relaxation rate of oxyhemoglobin, carboxyhemoglobin and deoxyhemoglobin. The relaxation rate of methemoglobin is so much larger than the other relaxation rates that it is not indicated on the graph. For the most concentrated methemoglobin solution we observed $1/T_1 = 3.42/\text{sec}$, compared with 1.45/sec for hemoglobin. Since the water molecules in the hydration sphere of the protein undergo rapid exchange with those of the solvent water, one expects the observed relaxation rate to depend

on a correlation time, $\tau_c$, characteristic of the protein molecule, including the globin and the heme part. This assumption can be verified by studying either the frequency or the temperature dependence of the relaxation rate. We intend to carry out further work along these lines. Since the relaxation rate is observed to be independent of the magnetic state of the iron, $\tau_c$ can be identified with the rotational Brownian motion time, and not the electron spin relaxation time. This also implied that the protons in the hydration sphere of the protein do not exchange readily with whatever ligand occupies the sixth coordination position of the iron. The large increase in relaxation time observed upon oxidation of hemoglobin suggests that there is an accompanying change in configuration which allows water molecules to enter the sixth co-ordination position.

Our results suggest that in deoxyhemoglobin solution the sixth coordination position is either not occupied by water, or that the water is so firmly bound that no exchange is possible with the bulk of the water molecules.

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