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MAGNETIZATION OF HEMOGLOBIN AND MYOGLOBIN BELOW 1K*

by

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ABSTRACT

The magnetization of methemoglobin and metmyoglobin has been measured from 2K down to 0.01 K. The data is fitted to a Curie-Weiss law with $\Delta = 2$ mK for metmyoglobin and $\Delta = 0.9$ mK for methemoglobin, in agreement with second moment calculations based mainly on interactions of the iron with protons and other iron dipoles.

The principal difference in the physical and physiological properties of the two heme proteins, hemoglobin and myoglobin, is due to the presence of four iron ions per molecule of the former and a single iron in the latter. A large variety of magnetic susceptibility studies in these proteins has been undertaken since Pauling's [1] pioneering work. They were important in elucidating details of the electronic state of iron, principally its spin state and the zero field splitting in both proteins [2-4]. None of the experiments have, to our knowledge, been extended to temperatures below 1 K where ordering phenomena could possibly be of importance.

In the present study we report on magnetization measurements in methemoglobin and metmyoglobin between 2 K and 0.01 K.

The sixth coordination position of iron in the met form of both proteins is taken up by a water molecule (Fig. 1), and the iron is in the ferric state [Fe^{+3}]. In the physiological conditions this same position is occupied by an oxygen molecule (oxyhemoglobin), or is empty (deoxyhemoglobin). In either of the latter two cases iron is in the ferrous state.

Existence of high and low spin states has been detected in the ferric hemoglobin, and thermal equilibrium distribution between these two spin states has been postulated [5]. The low spin state (S=1/2) lies lower in methemoglobin, while in metmyoglobin the high spin state (S=5/2) is the lowest one.

As a result of this difference we expect at very low temperatures to populate exclusively the $m_S = \pm 1/2$ state both in methemoglobin and metmyoglobin; in the latter case the ground state $\pm 1/2$ doublet is separated from the $\pm 3/2$ state by the crystal field splitting 2D, and by 4D from the $\pm 5/2$ state. Since 2D is of the order of 9 cm^{-1} (13K) [6], the lowest lying doublet is well isolated from the higher lying states at the temperatures of our experiment.

The g-value of the lowest doublet in the high spin state is highly anisotropic: g = 6 in the plane of the heme and g = 2 in the direction perpendicular to the heme's plane (fig. 1.) [7]. The g-values of the low spin state are: $g_x = 1.72$, $g_y = 2.22$, $g_z = 2.80$ [8].

The experimental results are shown in fig. 2. The material used was a lyophylized powder sample of sperm whale myoglobin and lyophylized rabbit hemoglobin in powder form as well, both from Sigma Chemical Company. The samples were in the shape of a cylinder 6 mm long by 2 mm diameter. The magnetization has been measured in magnetic fields of 1 Oe and 10 Oe with a SQUID magnetometer in a ^3He - ^4He dilution refrigerator [9]. The samples were cooled from room temperature to liquid nitrogen temperature over a period of approximately 12 hours.

In Figure 2, Curie's law gives an effective number of Bohr magnetons, n_e = 2.18, for hemoglobin. For low spin state, the expected value is 1.97. The small discrepancy of our experimental results from only low spin behavior can be attributed to the existence of a thermal mixture of low spin state with a small amount of high spin state. Such effects have been investigated by many groups [10, 11]. For the myoglobin, our saturation magnetization measurements at 0.01K in a field of 900 Oe (not shown here), yield $n_e = 3.6$. For a high spin state (in a powder), the expected value is $n_e = 4.36$ for the $\pm$ 1/2 doublet. Here as well, the small discrepancy could be due to a thermal mixture containing a small amount of low spin states as evidenced by our high field measurements where a two-spin Brillouin function is needed to fit the data. Similar thermal mixture effects have been reported in magnetic studies at higher temperatures [3] where $n_e = 2.57$ for hemoglobin and $n_e = 4.0$ for myoglobin.

Departures from Curie's law are observed in metmyoglobin at temperatures below .05K and in methemoglobin at temperatures below 0.025 K (Fig. 2). Brill

and Hampton [12] have calculated the dipolar contribution to the EPR line width in methemoglobim and concluded that the largest contribution is due to superhyperfine interaction and dipolar interaction between the iron 3d-electrons and the protons of the water molecule in the sixth coordination position of iron (Fig. 1). The superhyperfine interaction is comparable in magnitude to the Fe-Fe interactions, due to the small distance of about 2Å between an iron and a water molecule, as compared to a distance of about 30Å which separates the iron ions.

A simple calculation of the energy of an iron dipole in the local field of protons, nitrogen nuclear spins, and other iron dipoles (the second moment for g = 2 in hemoglobin is calculated to be 5.3 gauss [12]) yields an interaction energy of 0.9 mK for methemoglobin and of 2.0 mK for metmyoglobin (for the g = 6 case we assumed a second moment of 6.5 gauss using Ref. 12; we neglected the displacement of the iron off the heme plane in metmyoglobin). The difference in interaction energy is due to the smaller moment of the methemoglobin. Fitting our magnetization data to a Curie-Weiss type of behavior we get a Curie-Weiss Δ of 2 $\pm$ 0.2 mK for metmyoglobin and 0.9 $\pm$ 0.2 mK for methemoglobin; which is in good agreement with the above calculations. It is interesting to note that in the calculation of ref. 12, two-thirds of the calculated interaction energy comes from the 2 protons of the H_2O molecule coordinated to the iron, while iron-iron contributions are expected to be much less than a milliKelvin in both proteins for the g = 2 case.

Recent magnetization studies [13] at high fields down to 2K on high spin iron porphyrins were analyzed in terms of superexchange interactions. On the other hand, measurements of Imes et al [14] on crystals of another biologically important material, Cu (TPP), at very low temperatures showed no evidence for

such exchange interactions. Our data, even though the system is different, also show no evidence for exchange interactions.

In spite of the complexities of the molecules involved, the behavior of the iron can be explained on a simple model of dipolar and superhyperfine interactions. Our magnetization measurements show the approach towards ordering of the iron in the local field, however, lower temperatures are needed to probe the ground state behavior of these two interesting systems.

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Figure Captions

Figure 1. The structure of the heme in myoglobin and hemoglobin. The shaded circle represents the water molecule in the met form of both proteins.

Figure 2. The magnetization per unit magnetic field normalized to concentration of iron in metmyoglobin and methemoglobin, as a function of the reciprocal temperature. The dotted lines correspond to Curie behavior.

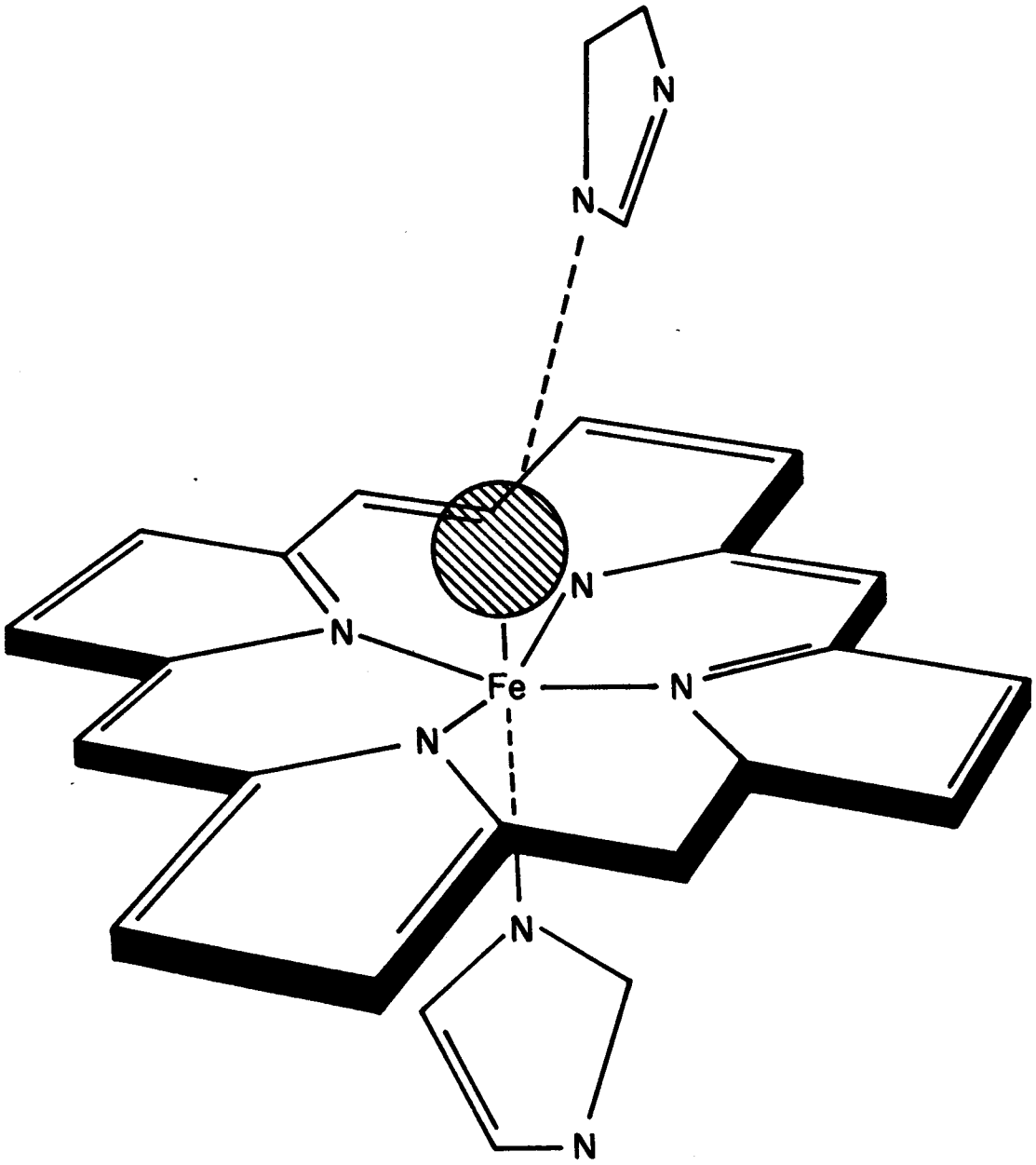

Fig. 1

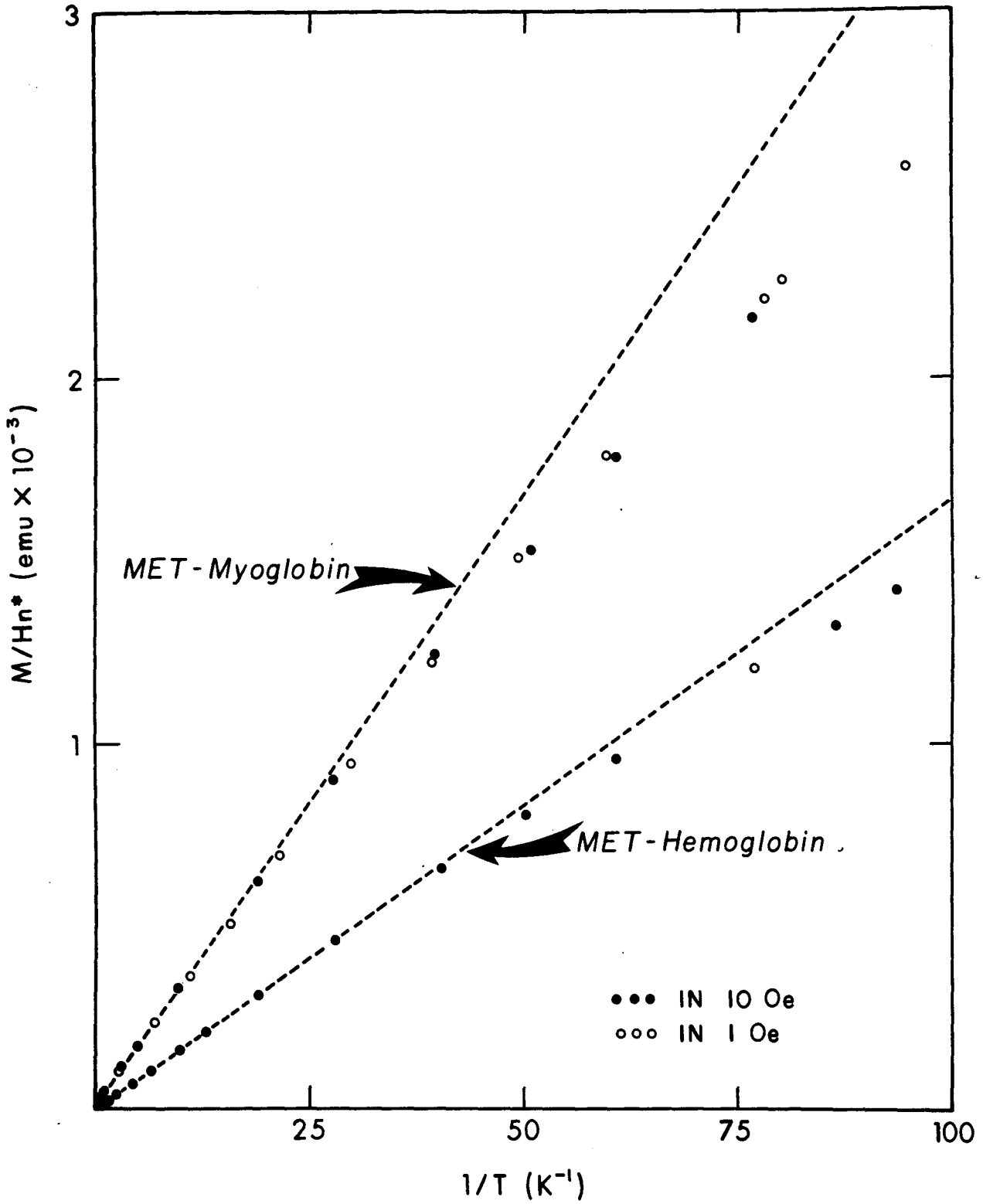
3
2
1
M/Hn* (emu X 10⁻³)
MET-Myoglobin
MET-Hemoglobin
●●● IN 10 Oe
○○○ IN 1 Oe
25
50
75
100
1/T (K⁻¹)

Fig. 2