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SPIN RELAXATION OF IRON IN MIXED STATE HEMOPROTEINS

by

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Abstract

In pure states hemoproteins the relaxation of iron depends on its spin state. We found that in both mixed state met-hemoglobin and met-myoglobin, the low and high spin states relax through an Orbach-like process. Also, very short (~ 1 ns) and temperature independent transverse relaxation times T_2 were estimated. This peculiar behavior of the relaxation may result from the unusual electronic structure of mixed state hemoproteins that allows thermal equilibrium and interconversion of the spin states.

Key-words: Hemoproteins; Relaxation times; Mixed state hemoprotein.

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The low temperature dependence of the spin lattice relaxation time T_1 of iron in ferric hemoproteins has been extensively studied¹⁻⁴ and it is now well established that the relaxation mechanism depends on Fe^{3+} spin state. In the high spin form the relaxation is dominated by an Orbach process¹ whose characteristic energy Δ is identified with the zero field splitting $2D$ of the 6A_1 state. In the low spin form the dominant relaxation mechanism involves two phonons (Raman process). The bizarre temperature dependence $1/T_1 \propto T^{6.3}$ observed in this case has been explained in terms of the fractal form of the protein.²⁻⁴

In this letter we report on a new temperature dependence of the spin-lattice relaxation time T_1 of the low spin Fe^{3+} ion in ferric methemoglobin (hb) and metmyoglobin (mb) observed in powdered samples in which both the low and high spin states co-exist.⁵ Also an estimate of the transverse relaxation time T_2 of the two spin states in hb and mb is given.

The samples were prepared from sperm whale mb (Sigma Chemical Co.) and from freshly prepared horse hb. The electron spin resonance (ESR) measurements were performed using the continuous saturation technique with an X-band standard equipment, in the temperature range from 4.2K to 25K. The $g=6$ and $g=2$ ESR lines of the high spin state and the $g=2.25$ line of the low spin state were monitored. The half saturation power, $P_{1/2}$, and the inhomogeneity parameter, b , were obtained by fitting the experimental data with the expression⁶

$$I = \frac{I_{\max} \sqrt{P}}{(1+P/P_{1/2})^{b/2}} \quad (1)$$

where P is the incident microwave power, I is the intensity of the derivative of the ESR absorption line and I_{\max} is the limiting value of I at very low power. T_2 was estimated from the value of b and the linewidth measurements.

Typical results for met hb are shown in Fig. 1. Similar results are obtained for met mb. They reveal a temperature independent T_2 , and a T_1 which depends exponentially on temperature ($T_1 \sim \exp(\Delta/kT)$) for both low and high spin states, pointing to an Orbach process as the common dominant relaxation mechanism. The corresponding values of Δ are given in Table I. Our results for the high spin state are in good agreement with previous work.¹⁻⁷ However, as mentioned above, for the low spin state the Raman processes are expected to dominate since the closest excited state, necessary for the operation of Orbach processes, is too far above, at about 1000 cm^{-1} . In this sense it is noteworthy that the Orbach like behavior of the low spin state is only observed in samples where the high spin state is also present.

The similarity of the results obtained in mixed state mb and hb for the spin relaxation of Fe in the low spin state suggests that the relaxation mechanism is associated with single heme complexes. This is expected because the magnetic interaction between Fe ions of different heme groups, which are about 30 \AA apart,⁸ is very small; in fact, spin susceptibility measurements show that the Curie law remains valid down to temperatures of the order of 20 mK.⁹ Thus, cross-relaxation between different paramagnetic centers, of the kind found in other biological systems,¹⁰⁻¹¹ is very improbable in this case. The magnetic interaction between an Fe ion and its neighboring nuclear

spins is also too small to account for the observed relaxation rates.¹² We speculate that in mixed state hemoproteins the dominant relaxation mechanism of low spin Fe is induced by a local, dynamic low spin-high spin interconversion rate.¹³

In mixed state Fe compounds the crystal field is close to a critical value such that the low and high spin states have comparable free energies and can coexist.¹⁴ It is a situation of dynamical equilibrium where small variations of the crystal field (produced by vibrational modes) together with the spin-orbit interaction, induce the interconversion of low and high spin states of Fe. Dynamic spin interconversion has been studied in ferric myoglobin hydroxide¹³ and in other six-coordinated complexes of Fe,¹⁵⁻¹⁶ where interconversion rates of the order of $10^7-10^8 \text{ sec}^{-1}$ were measured at about room temperature. Since the g-factors of the low and high spin states are quite different, the dynamic interconversion would produce a phase smearing of the precessing spins leading to a relaxation time T_2 of the order of the reciprocal interconversion rate. Unfortunately, there seem not to be independent measurements of interconversion rates at low temperatures to allow a direct comparison with our surprisingly short values. However, the fact that a mixed state is present in the whole temperature range considered in this work, indicates that the activation energy for interconversion is small and therefore an almost temperature independent T_2 of the order of those measured at room temperature may also be expected in this case.

Although a low spin-high spin transition may always relax the transverse magnetization through the difference in g-fac-

tors, it will not in general relax the longitudinal magnetization. An interconversion transition with conservation of the longitudinal magnetization is already spin forbidden ($S=1/2 \rightarrow S=5/2$) and it must be assisted by the spin-orbit interaction. Thus, interconversion with longitudinal relaxation would involve terms of higher order in the spin-orbit interaction. More probably, the low spin state relaxes to the lattice by interconversion to a high spin state which subsequently suffers an Orbach relaxation before interconverting back to the low spin state; this is suggested by the observation of very similar energies Δ for the two spin states. However, since the magnitude of T_1 for low spin states is 100 times larger than T_1 for high spin, the efficiency of this process must be substantially smaller than one. As the interconversion transitions involve small displacements of the iron in and out the plane of the heme,⁸ it could happen that the interconversion transition leaves the high spin Fe in an excited vibrational state from which Orbach relaxation is less probable than from the ground vibrational state. In that case the longitudinal relaxation rate would not simply depend on the time the Fe spends in the high spin state.

Although at present these considerations are purely speculative, they are susceptible of being tested experimentally and they contemplate aspects of the problem which necessarily will have to be taken into account in an elaborate model.

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Figure Caption

Fig. 1 - Temperature dependence of the spin-lattice relaxation time T_1 and the transverse relaxation time T_2 in met-hb. T_1 : \square high spin with $g=2$, \blacksquare high spin with $g=6$, \times low spin with $g=2.25$. T_2 : \circ high spin with $g=2$, \bullet high spin with $g=6$, $+$ low spin with $g=2.25$.

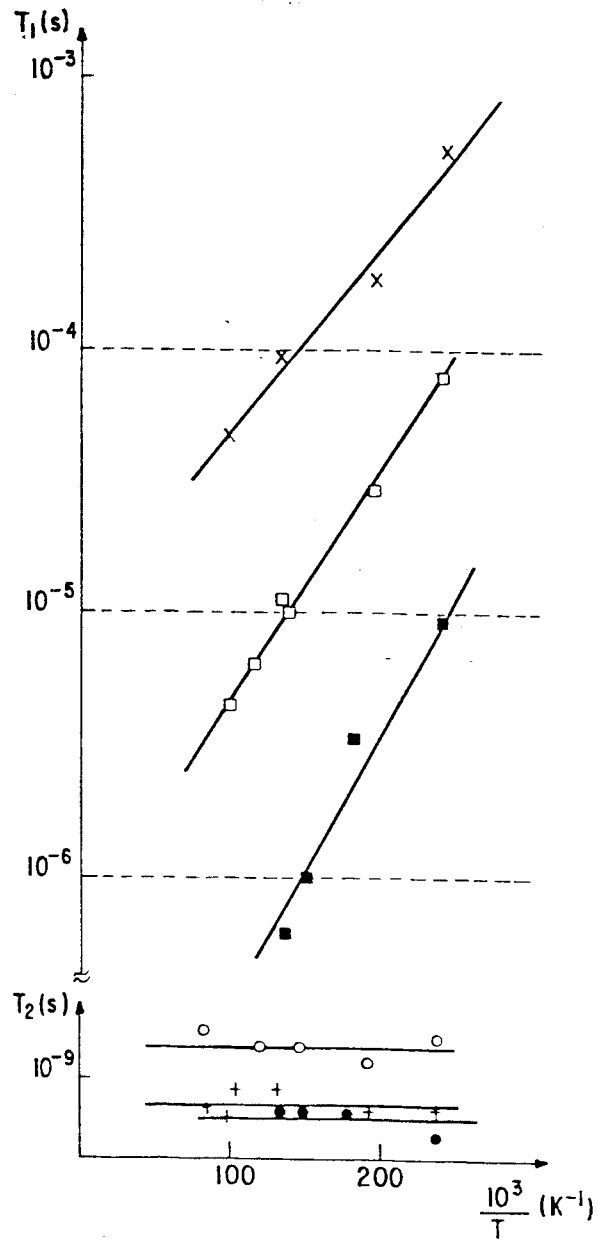


Fig. 1

Table I- Characteristic energy Δ of the Orbach relaxation process

| spin state | Protein g-value | mb (cm^{-1}) | hb (cm^{-1}) |
|------------|--------------------|----------------------------|----------------------------|
| high | 2 | 17.1 ± 1.5 | 14.2 ± 1.5 |
| high | 6 | 14.6 ± 1.5 | 16.7 ± 1.5 |
| low | 2.25 | 10.7 ± 1.5 | 13.4 ± 1.5 |

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