ISSN 0029-3865

CBPF-NF-003/88

EFFECT OF CONFORMATION ON SPIN LATTICE RELAXATION IN AZIDE METHEMOGLOBIN

by

Eliane Wajnberg and George Bemski

Centro Brasileiro de Pesquisas Físicas - CNPq/CBPF
Rua Dr. Xavier Sigaud, 150
22290 - Rio de Janeiro, RJ - Brasil

ABSTRACT

Spin lattice relaxation of Fe^{3+} has been measured as a function of temperature in azide methemoglobin in natural and heat denaturated condition. Of the two spin states present (S=1/2, and S=5/2) the high spin state always follows an Orbach mechanism of relaxation with a characteristic energy dependent on the protein conformation. In the absence of thermal equilibrium of the two spin states, the S=1/2 state follows a Raman relaxation process whose exponent, n, is related to the conformation of the protein.

Key-words: Spin lattice relaxation hemoglobin.

INTRODUCTION

The first measurements of spin-lattice relaxation times in biological systems attempted to identify different radical or ion species in the samples. They looked at the interaction among these components through their effects on the half saturation power (1-3). The recent interest centers on the determination and understanding of the dominant spin lattice relaxation mechanism in proteins (4,5). Deviations from the expected T^9 dependence of the Raman process have been widely observed in iron containing proteins. This was the case in the low spin state (LS), S=1/2, Fe^{3+} in myoglobin and cytochrome complexes, etc. (6-8). A few theoretical models were proposed (9-13) to explain these unexpected results based on anomalous vibrational density state. Experimental and theoretical arguments support the relation between the anomalous Raman exponent and the protein fractal dimension and conformation (9-12), although a more conventional theoretical approach that relates the anomaly to the existence of non central molecular forces, cannot be discarded (13).

We have previously suggested (14), that the Raman mechanism with T^n, where $n \sim 6.3$ is predominant only in the pure LS heme proteins. An Orbach-like relaxation mechanism was observed for the LS Fe^{3+} ion in ferric methemoglobin and metmyoglobin powdered samples where the high spin state (HS), S=5/2, coexists with the LS in thermal equilibrium. The HS obeys always the Orbach mechanism (14,15).

In the present work azide methemoglobin (methbN_3^-) was used in the native condition and in temperature denatured samples, in order to further clarify the effect of mixed spin states and conformational changes on the relaxation processes.

EXPERIMENTAL DETAILS

Hemoglobin was obtained by hemolysis of human blood. The solution was reduced with a 20-fold excess of ferricyanide and reacted with 40-fold excess of sodium azide, followed by dialysis. Samples were mixed with glycerol in 1:1 (v:v) ratio.

Electron Paramagnetic Resonance (EPR) experiments were performed with a X band spectrometer (Varian E-9) between 6K and 26K using a helium flux cryostat (Helitran LTD-3-110). Temperatures were measured with a Au-Fe vs Chromel thermocouple placed just above the sample.

The progressive saturation method was employed and the half saturation powers, $P_{1/2}$ obtained as previously described (14).

The fraction of HS ions, N_{HS}, relative to the low spin ones, N_{LS}, K^{-1} was calculated using the following expression:

$$K^{-1} = N_{HS}/N_{LS} = I_{HS}/I_{LS} \times (\Delta H_{HS}/\Delta H_{LS})^2 \times G \qquad (1)$$

where ΔH is the linewidth and G a gain factor. For the HS form only the low field line (g=6) and for the LS form the two low field lines (fig.1) were considered. When necessary, the intensity I was corrected due to saturation effects.

RESULTS AND DISCUSSION

MethbN$_3^-$ is known as a pure low-spin state ferric hemoglobin. This can be seen in the typical LS spectra (g around 2) observed at liquid nitrogen temperature. Nevertheless, at lower temperatures

we have observed the two spin states, as shown in the spectrum of fig. 1a. The denaturation process leads to an irreversible change in the conformational state in which the modified environment of iron gives rise to an altered EPR spectrum which contains broadened lines with different g values and line shapes from those of the native samples (fig. 1b and 1c). The population distribution between the two spin states has also a different temperature dependence in the denatured samples as compared to the unheated ones. Although the HS population of the native protein increases as the temperature decreases, the LS form is predominant even at very low temperatures, in the temperature range observed.

The fraction of HS in the sample varies between 4% at 77K and as much as 50% at 9K. The values of K^{-1} depend on the freezing rate of the samples but the temperature behavior does not. In contrast, the fraction of HS in a denatured sample does not vary as a function of temperature.

Frauenfelder et al.(16) postulated the existence of a large number of conformational substates in thermal equilibrium that regulate ligand binding to hemeproteins. After the denaturation the protein acquires a physiologically inactive conformation. The absence of thermal equilibrium between the two spin states can possibly be related to the loss of function of the protein.

Fig. 1b and 1c show the difference in the spectra which result from 5min. and 5 hour exposure to 80°C. The spectra reflect the differences in the Fe environment in hemoglobin. The HS component disappears after the 5 min. treatment, but reappears in the 5 hr heated sample.

An Orbach process predominates in either case, but the value of Δ, the low lying energy level characteristic of the process, de-

creases from $\Delta=32 \pm 0.5 cm^{-1}$ in the unheadted to $\Delta=16.4 \pm 0.3 cm^{-1}$ in the denaturated sample (fig.2). The energy Δ is the zero field splitting energy (ZFS) determined by the symmetry and intensity of the crystal field. The change in the value of Δ is thus expected in view of the conformational change due to denaturation.

The dominant relaxation mechanism of the LS form depends on the state of denaturation of the sample. Since we are only interested in the dependence of T_1 on temperature, absolute values are not relevant and the data of the two LS lines measured in each samples are superimposed (fig.3 and 4).

The natural sample shows an exponential dependence (fig.3). The characteristic energy in this case is $33 \pm 0.7 cm^{-1}$, very close to that measured for the HS state of this sample. This can be interpreted by a relaxation mechanism via the HS state induced by the high-low spin interconversion rate as already observed in methemoglobin (14). This interconversion is also reflected in the temperature dependence of the relative populations of spin states, K^{-1}.

The temperature dependence of relaxation of the LS lines in both denatured samples (fig.4) shows that above about 13K it has the form: $T_1 \sim T^{-n}$, with $n = 5.3 \pm 0.3$. In the theory of fractals this value of n can be related to the fracton dimensionality (9,17), d_{fr} of 1.15 ± 0.2 in contrast to 1.55 ± 0.5 observed for azide metmyoglobin (9,11).

Helman et al. (12) consider connections between different portions of the chain by hydrogen bonds, and their effect on the fracton dimension. They propose that if the number of bridges is large enough, the fractal dimension, d, equals d_{fr} and only then it is related to the Raman exponent n by $n=3+2d$. As there is no experimental value for methbN_3^- we use the calculated d of 1.50 (11) as this protein's d_{fr} to be compared with 1.15 obtained for the dena-

turated samples. On the other hand, if there are no bridges, that is, for a linear chain, d_{fr} =1 independently of d. In this case n=5 is predicted (12). This n value is in good agreement with our experimental value, considering that heating drastically reduces the number of bridges.

Elber and Karplus (10) consider that the relevant interactions in the evaluation of d_{fr} are those between amino acids along the chain. The absence of α helices and connectivity, in general, between amino acids lower d_{fr}, so that loosening of the secondary structure on heating lowers d_{fr}, in qualitative agreement with our results.

Although, detailed calculations and further studies are called for to determine the exact mechanism responsible for the observations, our results support the models which proposes that the Raman relaxation mechanism in low spin state can be tied to d_{fr} and related to the protein conformation, in absence of thermal equilibrium between the LS and HS states.

FIGURE CAPTIONS

Fig. 1 - E.P.R. Spectra of methbN_3^-.
(a) unheated protein solution, T=6.2 ± 0.3K; (b) denatured protein at 80°C for 5 min, T=6.2 ± 0.3K and (c) denatured protein at 80°C for 5 hours, T=6.0 ± 0.2K.

Fig. 2 - Temperature dependence of the spin lattice relaxation time T_1, of the high-spin state of Fe^{3+} (line g=6.0). Unheated protein (x), denatured protein 5 hrs at 80°C (o).

Fig. 3 - Temperature dependence of the spin lattice relaxation time T_1, of the low spin state of Fe^{3+} in unheated protein. (lines g=2.24 and 2.84).

Fig. 4 - Temperature dependence of the spin lattice relaxation time, T_1, of the low spin state of Fe^{3+} in denatured methbN_3^- (lines g=2.20 and 2.78) 5 min at 80°C (x) and (lines g=2.2 and 2.92) 5 hrs at 80°C (o).

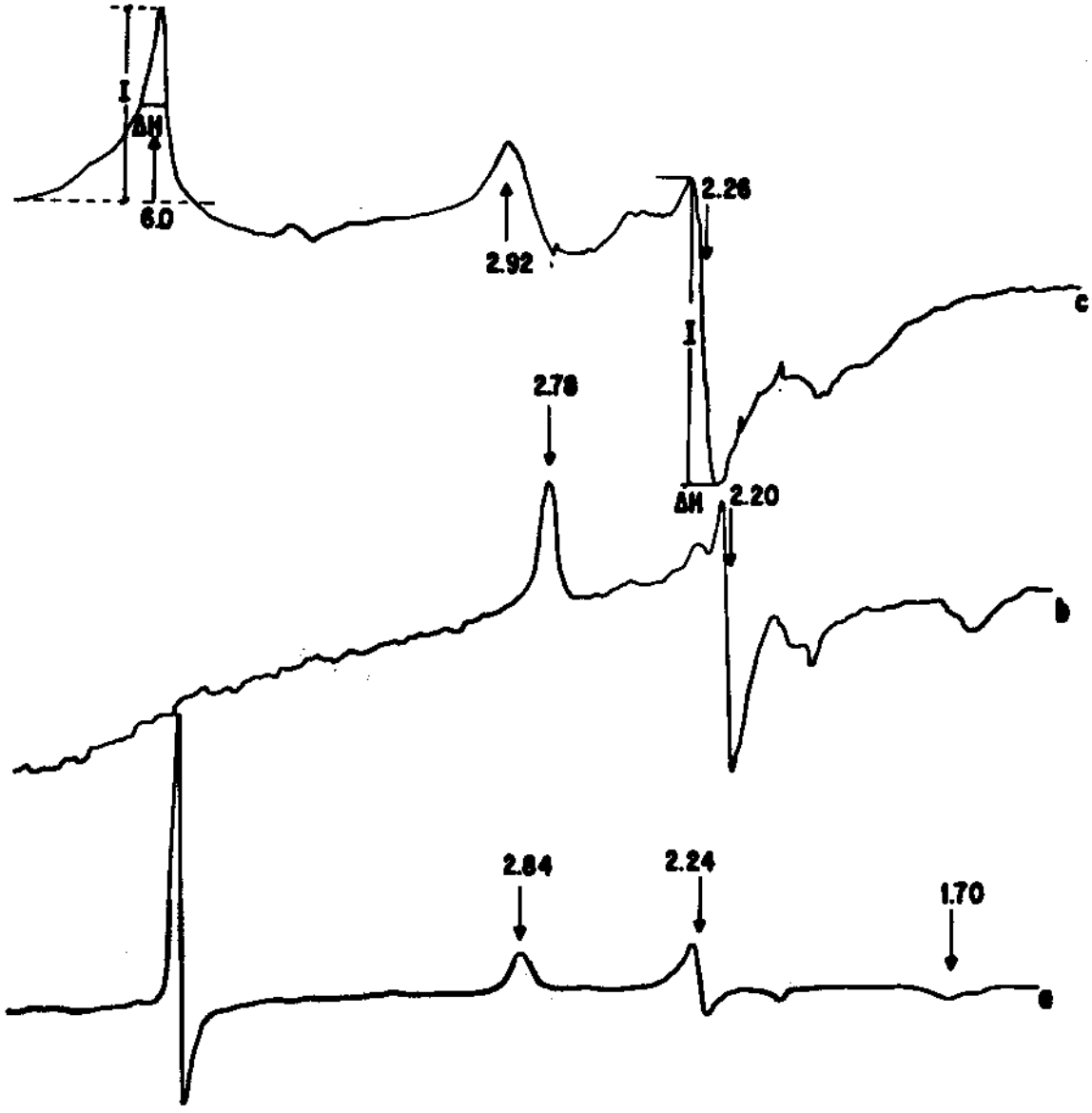

Fig. 1

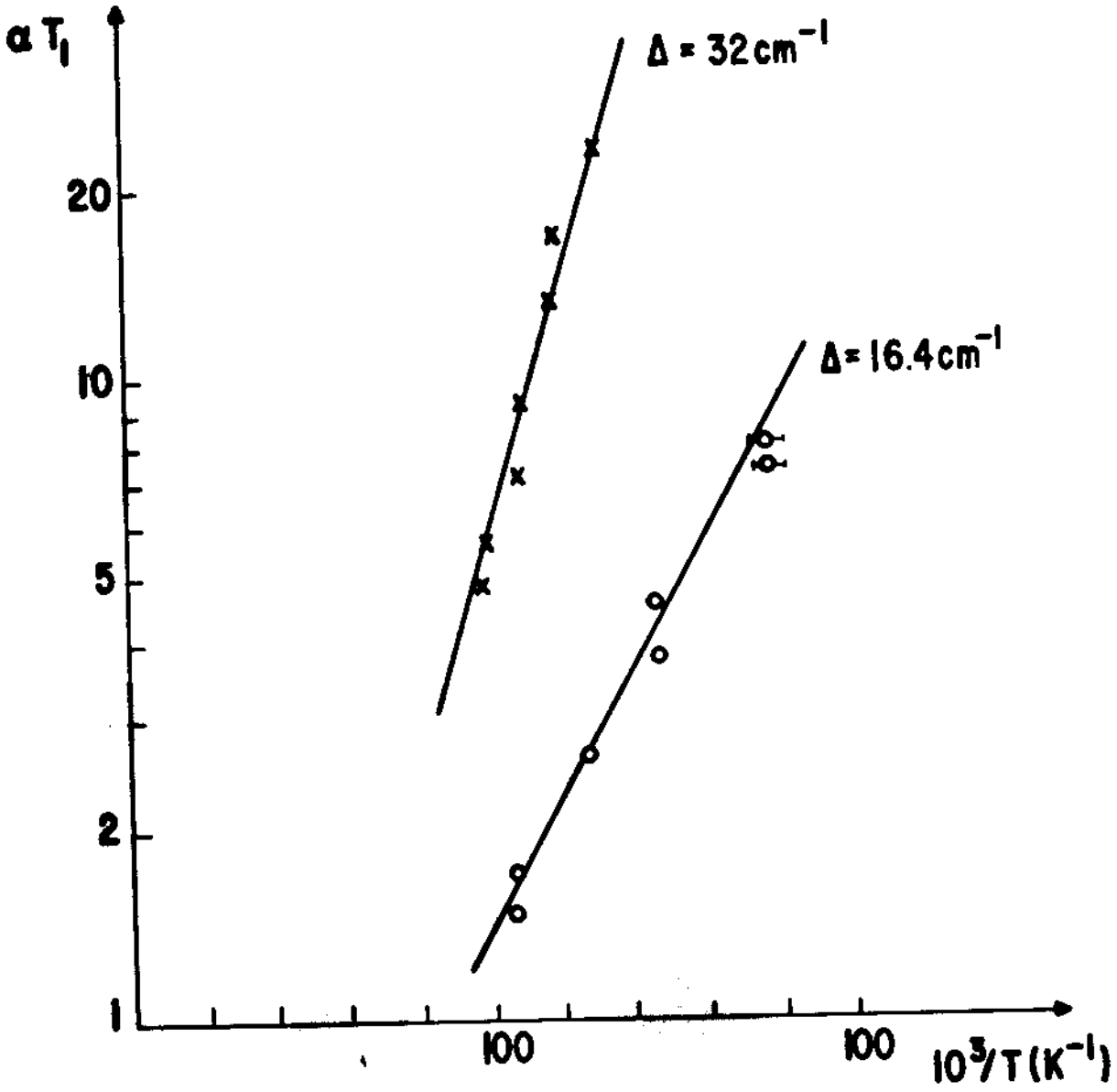

Fig. 2

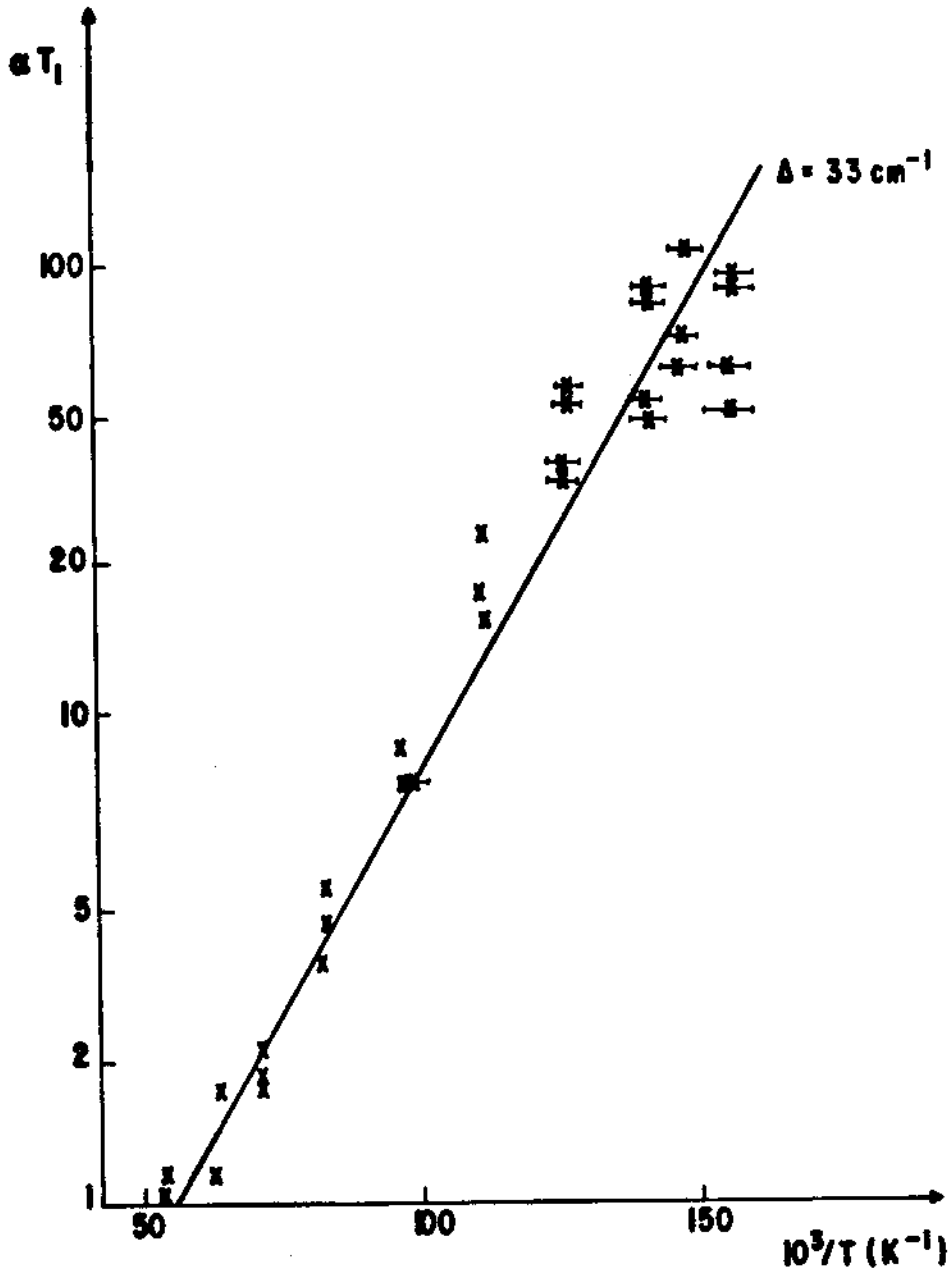

Fig. 3

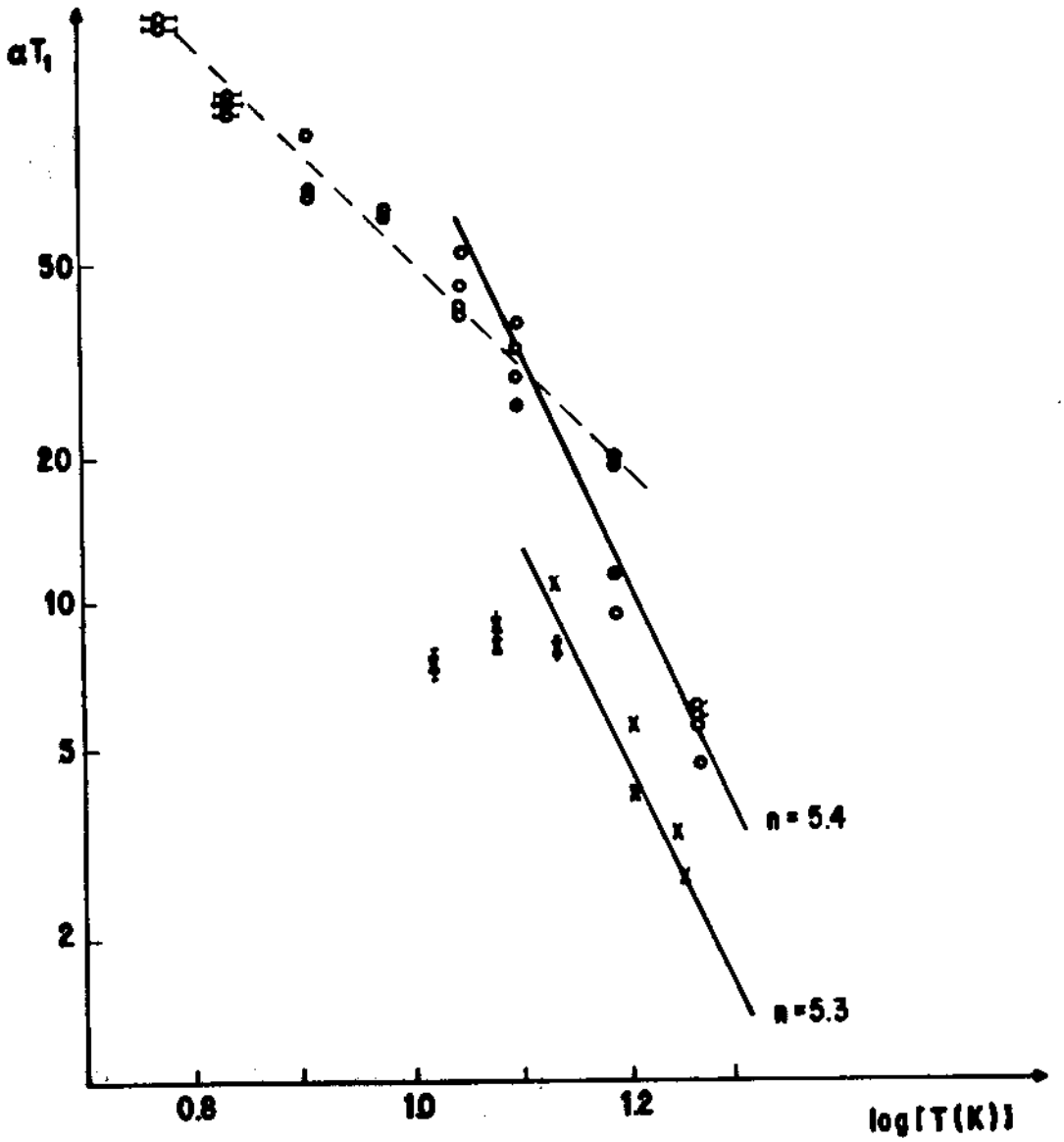

Fig. 4

REFERENCES

1- H. Beinert and B. Kok. Biochim. Biophys. Acta 88, 278-288 (1964). An attempt at quantitation of sharp light-induced electron paramagnetic resonance signal in photosynthetic materials.

2- K.V. Rajagopalan, P. Handler, G. Palmer and H. Beinert. J. Biol. Chem. 243(14), 3784-96 (1986). Studies of Aldehyde Oxidase by Electron Paramagnetic Resonance Spectroscopy.

3- G.D. Case, T. Ohnishi and T.S. Leigh, Jr. Biochem. J. 160, 785-95 (1976). Intramitochondrial Positions of Ubiquinone and Iron-Sulphur Centres Determined by Dipolar Interactions with Paramagnetic Ions.

4- J.P. Gayda, J.F. Gibson, R. Cammack, D.O. Hall and R. Mullinger. Biochim. Biophys. Acta 434, 154-63 (1976). Spin Lattice Relaxation and Exchange Interaction in a 2-Iron, 2-Sulphur protein.

5- P. Bertrand, J.P. Gayda and K. Krishna Rao. J. Chem. Phys. 76 (10), 4715-19 (1982). Electron Spin Lattice Relaxation of the (4Fe-4S) ferredoxin from B. Stearothermorphilus. Comparison with other iron protein.

6- R.C. Herrick and H.J. Stapleton. J. Chem. Phys. 65(11) 4778-86 (1976). Anomalous T^7 Raman spin-lattice relaxation rate of low spin cytochrome P-450 from pseudomonas putida.

7- C.P. Scholes, R. Janakunamah, H. Taylor and T.E. King. Biophys. J. 45, 102-30 (1984). Temperature dependence of the electron spin-lattice relaxation rate from pulsed EPR of Cu_A and heme a in cytochrome c oxidase.

8- J.P. Allen, J.T. Colvin, D.G. Stinson, C.P. Flynn and H.J. Stapleton. Biophys. J. 38, 299-310 (1982). Protein Conformation from electron spin relaxation data.

9- H.J. Stapleton. Comments Mol. Cell. Biophys. 3(4), 321-46 (1986). A Fractal Model of Electron Spin Relaxation in Proteins.

10- R. Elber and M. Karplus. Phys. Rev. Lett. 56(4) 394-7 (1986). Low-frequency modes in proteins: use of effective-medium approximation to interpret the fractal dimension observed in electron spin relaxation.

11- J.T. Colvin and H.J. Stapleton.J. Chem. Phys. 82(10), 4699-706 (1985). Fractal and spectral dimensions of biopolimer chains: solvent studies of electron spin relaxation rates in myoglobin azide.

12- J.S. Helman, A. Coniglio. Phys. Rev. Lett. 53, 1195 (1984). Fractons and the fractal structure of proteins.

13- J.A. Krumhansl. Phys. Rev. Lett. 56(25), 2696-8 (1986). Vibrational anomalies are not generally due to fractal geometry: Comments on proteins.

14- E. Wajnberg, H.J. Kalinowski, G. Bemski and J.S. Helman. Biophys. J. 49, 1195-8 (1986). Spin lattice relaxation of iron in mixed state hemoproteins.

15- C.P. Sholes, R.A. Isaacson and G. Feher. Biochim. Biophys. Acta 244, 206-10 (1971). Determination of zero-field splitting of Fe^{3+} in heme proteins from the temperature dependence of the spin-lattice relaxation rate.

16- Frauenfelder, G.A. Petsko and D. Tsernoglou. Nature 280 (5723), 558 (1979). Temperature-dependent X-ray diffraction as a probe of protein structural dynamics.

17- S. Alexander and R. Orbach. J. Physique Lett. 43, L625 (1982). Density of states on fractals: "fractons".