Vol. 84, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 30, 1978 Pages 1125-1128

EVIDENCE FOR ANTIFERROMAGNETIC EXCHANGE COUPLING IN THE TETRANUCLEAR HIGH POTENTIAL IRON-SULFUR PROTEIN OF RHODOPSEUDOMONAS GELATINOSA

Haywood Blum*, J.C. Salerno*+, Michael A. Cusanovich**

*Johnson Research Foundation
Department of Biochemistry and Biophysics
School of Medicine, University of
Pennsylvania, Philadelphia, Pennsylvania 19104

and

**Department of Biochemistry
The University of Arizona
Tucson, Arizona 85721

Received September 18,1978

SUMMARY. EPR spectra of oxidized R. gelatinosa HiPIP demonstrate two kinds of temperature dependent changes which can be analyzed in terms of an excited state at 142 ± 10cm^{-1} and a second excited state at 490 ± 100cm^{-1}. These states represent further verification of antiferromagnetic exchange among the 4 irons in this tetranuclear cluster, with a value for the coupling constant of J = -44cm^{-1}. Aside from resonance Raman spectroscopic results, this is the first report of a ladder of excited states predicted for exchange coupled ions.

INTRODUCTION. Iron-sulfur proteins are vital constituents of many fundamental biochemical processes. Tetranuclear high potential iron protein (HiPIP) centers form an integral component of succinate dehydrogenase in mammalian mitochondria (1) and other organisms, and of the tricarboxylic acid cycle enzyme, aconitase (2). In Chromatium, the light induced oxidation of HiPIP appears to be linked only to the oxidation of cytochrome c_{555} in the cyclic electron flow system (3).

In this report we present the temperature dependence of the EPR spectrum of the R. gelatinosa HiPIP. The EPR linewidth at elevated temperatures is a measure of the spin-lattic lifetime, T_1 (4). T_1 in the iron-sulfur proteins appears to follow an Orbach process (5) with relaxation proceeding

+ Present address: Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina 27710

0006-291X/78/0844-1125$01.00/0

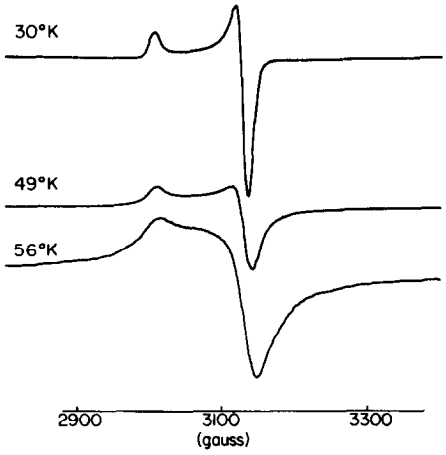


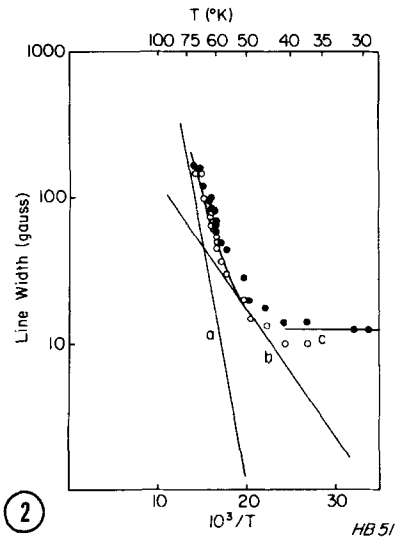
Figure 1. EPR derivative spectra of R. gelatinosa HiPIP at the temperatures indicated. Low temperature spectrum can be fit with a computer simulation with $g_{\parallel} = 2.11$ and $g_{\perp} = 2.03$. EPR conditions: microwave power, 5 mW; modulation, 10 Gauss.

through excited states (6). Thus by monitoring line widths we can deduce the energies of the excited states. Alternatively, excited levels close enough to the ground state can participate in depopulation of the ground state. Measurement of the fall-off of total intensity of the EPR spectrum with temperature thus enables us to find the positions of some excited levels by a quite different method.

MATERIALS AND METHODS. Preparation of R. gelatinosa was as described (7) using CM-cellulose chromatography. The A^{283}/A^{388} ratio of the reduced protein was 2.32 - 2.35 (8). EPR methods have been described (6).

RESULTS. Figure 1 shows the temperature dependence of the axial EPR derivative spectrum of R. gelatinosa HiPIP. The line width, taken as the magnetic field difference between the maximum of the integrated EPR spectrum and the high field half maximum value is shown in Figure 2.

In Figure 3 the integrated intensity times temperature is plotted versus reciprocal temperature. No depopulation of the ground state would give a horizontal line. From the fall-off shown we find it necessary to postulate two excited states.



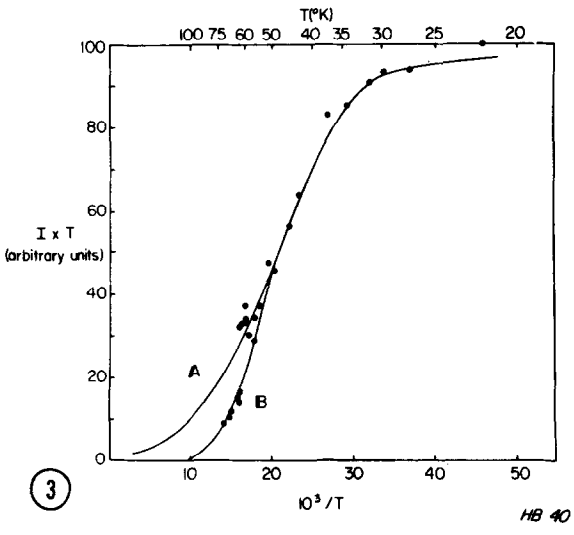


Figure 2. Line width versus reciprocal temperature of the integrated EPR spectrum, solid circles. Low temperature half-width is limited by "g strain" to 12 Gauss, line c. Lorentzian spin packet half-width necessary to broaden low temperature spectrum to fit higher temperature data, open circles. This corrected data can then be decomposed into line 'a' plus line 'b' such that $\Delta H_{LOR} = \Delta H_a + \Delta H_b$. This assumes that the lifetime, T_1, is given by $T_1^{-1} = T_{1a}^{-1} + T_{1b}^{-1}$; that is, relaxation occurs through states a and b in parallel. From the slopes of lines 'a' and 'b', $\Delta_a = 520$ cm^{-1}, $\Delta_b = 142$ cm^{-1}. The ratio of the T = ∞ intercepts is approximately 3000. The curved line is the sum of lines 'a' and 'b'.

Figure 3. Integrated intensity times absolute temperature of EPR spectrum versus reciprocal temperature, solid circles. Curve A is expected values if a single excited state exists at $\Delta_b = 142$ cm^{-1} with multiplicity relative to the ground state of 70. Curve B results if, in addition, another excited state exists at $\Delta_a = 457$ cm^{-1}, with multiplicity relative to the ground state of approximately 5×10^4. The ratio of multiplicities is 700. The multiplicities and energy exponents are straight-forwardly calculated from a semi-logarythmic plot (6) of this data.

DISCUSSION. The antiferromagnetically spin-coupled model (9) of iron-sulfur proteins has been applied to the EPR of Chromatium HiPIP (6,10). The first two excited states should be -3J and -8J above the ground state in the oxidized form of the protein if the coupling constant J is the same among all the irons. In this report we find that the ratio of energies for the first two excited states is 3.5 ± 1.0, as compared to 8/3 = 2.7, which we take as additional confirmation of this model. The relative multiplicities of the excited states is very high and does not follow from this model in any simple

way. We have noted this in other cases (6,11). The multiplicity value may represent a coupling of vibrational states, adventitiously located at the position of the excited state, to the spin states.

ACKNOWLEDGEMENTS. This study was supported by NIH grants GM-25052, GM-12202 and GM-21277 and by NSF grant PCM 75-21009. MAC is a recipient of USPHS Career Development Award EY-00013.

REFERENCES

1. Ohnishi, T., Winter, D.B., Lim, J., and King, T.E. (1976) J. Biol. Chem. 251, 2105-2109.
2. Ruzika, F.J. and Beinert, H. (1978) J. Biol. Chem., 253, 2514-2517.
3. Dutton, P.L. and Leigh, J.S. (1973) Biochem. Biophys. Acta 314, 178-190.
4. Gayda, J.P., Gibson, J.F., Cammack, R., Hall, D.O., and Mullinger, R. (1976) Biochim. Biophys. Acta 434, 458-484.
5. Orbach, R. (1961) Proc. Roy. Soc. Lond. A 264, 154-163.
6. Blum, H., Salerno, J.C., Prince, R.C., Leigh J.S., and Ohnishi, T. (1977) Biophys. J. 20, 23-31.
7. De Klerk, H. and Kamen, M.D. (1966) Biochim. Biophys. Acta 112, 175-178.
8. Tedro, S.M., Meyer, T.E., and Kamen, M.D. (1976) J. Bio. Chem. 251, 129-136.
9. Gibson, J.F., Hall, D.O., Thornley, J.H.M., and Whately, F.R. (1966) Proc. Natl. Acad. Sci. USA 56, 987-990.
10. Antanaitis, B.C., and Moss, T.H. (1975) Biochim. Biophys. Acta 405, 262-279.
11. Blum, H., Leigh, J.S., Ohnishi, T., and Salerno, J.C. (1978) in Frontiers in Bioenergetics, Vol. I, P.L. Dutton, J.S. Leigh and A. Scarpa, eds., Academic Press, New York.