A MAGNETIC SUSCEPTIBILITY STUDY OF Cu(II)–Co(II) SUPEROXIDE DISMUTASE

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1. Introduction

Cupro–zinc superoxide dismutases are dimeric enzymes containing 1 Cu(II) and 1 Zn(II) in each identical monomeric unit of mol. wt ~16 000. X-ray analysis of the bovine enzyme [1] has shown that the imidazolate group of histidine 61 is a ligand for both the copper and zinc, thus explaining the results obtained with the Co(II)–Cu(II) derivative, where 50% of the Zn had been substituted by Co(II) [2]. In fact, in this derivative, which is 100% active, the amount of EPR detectable Cu(II) is just 50% of the total copper present, suggesting a magnetic interaction between contiguous Co(II) and Cu(II) spin system. Further EPR measurements [3] indicated a lower limit for the coupling constant in the order of 100 cm^{-1}; later magnetic susceptibility work [4] on a very narrow temperature range and on a different preparation containing approx. 30% uncoupled Co(II) and about 50% of Cu(II) as Cu(II)–Cu(II) pairs, was only able to establish a lower limit in the range of 5 cm^{-1}.

The measure of the antiferromagnetic coupling constant of this system has been re-attempted here with the use of a new high resolution magnetometer [5] which permits measurements in a much wider temperature range. Moreover a cleaner preparation in terms of magnetic species was used [1,6]. No deviation from the Curie behaviour was found in the range 30–200°K. The resolution of the measurements allows us to place a new lower limit for the coupling constant $2J \gtrsim 600$ cm^{-1}.

2. Materials and methods

The cobalt enzyme was prepared as in [2]. Metal analyses were performed with a Hilger and Watts Atomspek Model H 1170 atomic absorption instrument. X-band EPR measurements were obtained with an E-9 Varian spectrometer at liquid helium temperature, using an Air Products and Chemicals CT-3-110 liquid transfer Cryo-Tip refrigerator with automatic temperature controller.

3. Results and discussion

The temperature dependence of the magnetic susceptibility of a typical sample of cobalt bovine superoxide dismutase is shown in fig.1. The measure-
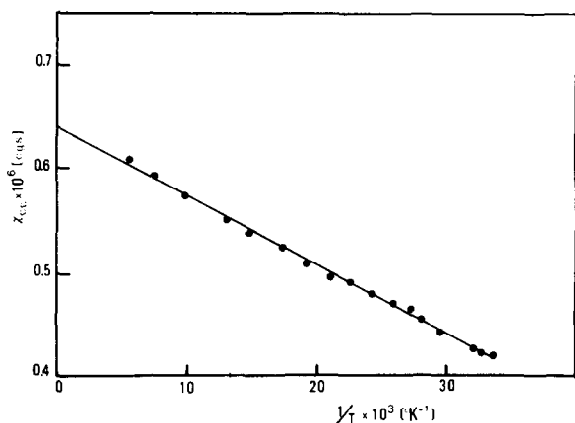
Fig.1. Temperature dependence of the volume susceptibility of Cu(II)−Co(II) bovine superoxide dismutase. See text for details on sample composition.

ment was repeated with 3 different preparations and gave identical results in all cases. In the case shown in fig.1 the sample contained 6.1×10^{-3} M copper and 3.4×10^{-3} M cobalt as evaluated by atomic absorption. Its liquid helium EPR spectrum gave a cobalt signal approx. 10% as intense as the cobalt signal observed after reduction of all the copper by ferrocyanide [3]. This means that 0.3×10^{-3} M Co(II) is not coupled to the copper for some reasons and in fact the EPR-detectable copper of the same sample was 3×10^{-3} M after double integration of the signal, recorded under non-saturating conditions, against a Cu(II)−EDTA standard.

The main features of fig.1 are:

(i) Within the limits of our resolution the behaviour is linear with respect to the reciprocal temperature.

(ii) The slope of the paramagnetic contribution as given by the best fit line, is:

$$\frac{d\chi_{cc}}{dT^{-1}} = 6.0 \times 10^{-6} \text{ cgs}^{\circ}\text{K}$$

a value much larger than in a lower temperature range [4].

The most obvious explanation for this discrepancy is the different temperature ranges explored in the two sets of measurements. The useful experimental values in [4] are in the range $1.4−5^{\circ}$K and indicate that the system is fully coupled in that temperature region.

Therefore the slope of fig.1 has to be explained by some transition occurring in the system above 5°K. Since the superoxide dismutase Co(II) appears to be high spin at all temperatures [2,3,7] it is excluded that the observed slope is due to population of the S 1 state of a Cu(II)−low spin Co(II) couple.

We can also easily exclude the possibility of observing totally uncoupled spin systems as it would give:

$$\frac{d\chi_{cc}}{dT^{-1}} = 9.3 \times 10^{-6} \text{ cgs}^{\circ}\text{K}$$

even for the minimum value (spin only value, μ_B 3.88) for the μ_{eff} of high spin Co(II). We can also exclude a situation in which the coupling constant is of the order of $2J \simeq 15$ cm^{-1} and at the same time the zero field splitting constant $\delta \simeq 15$ cm^{-1} as it would give:

$$\frac{d\chi_{cc}}{dT^{-1}} = 8.6 \times 10^{-6} \text{ cgs}^{\circ}\text{K}$$

of the same order of that calculated for the previous situation.

We are left with two alternatives (fig.2), which would in principle be indistinguishable, that is either $2J \simeq 15$ cm^{-1}, $\delta \gtrsim 600$ cm^{-1} (fig.2a) or $2J \gtrsim 600$ cm^{-1}
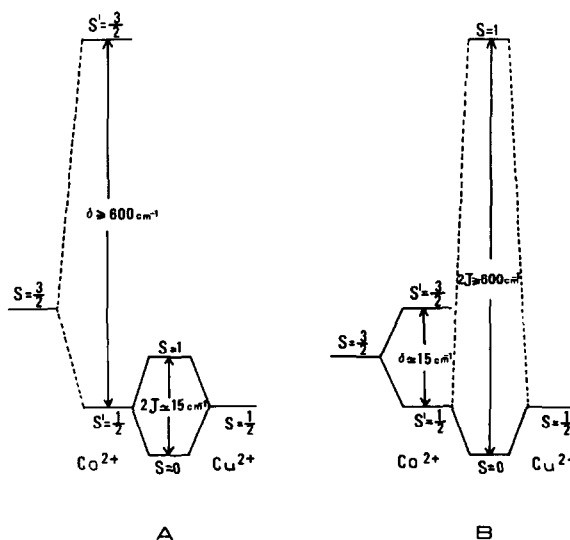

Fig.2. Two possible magnetic interactions in Cu(II)−Co(II) superoxide dismutase based on the results presented in the text.

with $\delta \simeq 15$ cm^{-1} (fig.2B). In fact in both cases we get:

$$\frac{d\chi_{cc}}{dT^{-1}} = 5.5 \times 10^{-6} \text{ cgs}^{\circ}\text{K}$$

in the temperature range above 30°K, which agrees fairly well with the experimental slope. However in the former situation such slope is due to population of the S 1 state resulting from the interaction between the Co(II) lower doublet and Cu(II), while the latter case involves the population of the higher S^1 3/2 doublet of the high spin Co(II). However, $\delta > 100$ cm^{-1} is very unlikely as values of zero field splitting constants have been reported for Co(II) in a variety of environments and range from -40 cm^{-1} to $+80$ cm^{-1} (see [4] and ref. therein). Moreover, in the case of reduced Co(II) superoxide dismutase $\delta = 23$ cm^{-1} has been estimated from EPR measurements [3]. Therefore $\delta \simeq 15$ cm^{-1}, that is very close to that observed for the Cu(I)–Co(II) protein (3), and a new lower limit for the coupling constant $2J \gtrsim 600$ cm^{-1} appears to be much more likely. It should be remarked that this conclusion is consistent with water proton relaxation measurements [8] which suggested that

the coupling between the Co(II) and Cu(II) spin system is still present at room temperature.

References

[1] Richardson, J., Thomas, K. A., Rubin, B. H. and Richardson, D. C. (1975) Proc. Natl. Acad. Sci. USA 72, 1349–1363.

[2] Calabrese, L., Rotilio, G. and Mondovì, B. (1972) Biochim. Biophys. Acta 263, 827–829.

[3] Rotilio, G., Calabrese, L., Mondovì, B. and Blumberg, W. E. (1974) J. Biol. Chem. 249, 3157–3610.

[4] Moss, T. H. and Fee, J. A. (1975) Biochem. Biophys. Res. Commun. 66, 799–808.

[5] Cerdonio, M., Mogno, F., Romani, G. L., Messana, C. and Gramaccioni, C. (1977) Rev. Sci. Instrum. 48, 300–306.

[6] Rotilio, G. and Calabrese, L. (1977) in: Superoxide and Superoxide dismutase (Michelson, A. M., McCord, J. M. and Fridovich, I. eds) pp. 193–198, Academic Press, London.

[7] Rotilio, G., Calabrese, L. and Coleman, J. E. (1973) J. Biol. Chem. 248, 3853–3859.

[8] Rigo, A., Terenzi, M., Franconi, C., Mondovì, B., Calabrese, L. and Rotilio, G. (1974) FEBS Lett. 39, 154–156.