

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 $\sim 16\,000$. X-ray analysis of the bovine enzyme [1] has shown that the imidazolite 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 $2J \gtrsim 600\text{ cm}^{-1}$.

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-

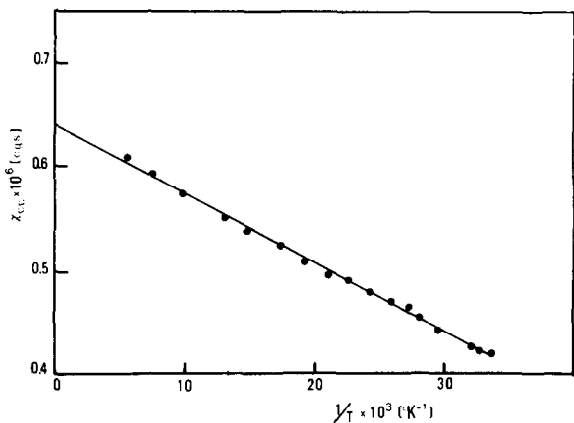


Fig.1. Temperature dependence of the volume susceptibility of Cu(II)–Co(II) bovine superoxide dismutase. See text for details on sample composition.

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°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 $2J \approx 15 \text{ cm}^{-1}$ and at the same time the zero field splitting constant $\delta \approx 15 \text{ cm}^{-1}$ 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 $2J \approx 15 \text{ cm}^{-1}$, $\delta \approx 600 \text{ cm}^{-1}$ (fig.2a) or $2J \approx 600 \text{ cm}^{-1}$

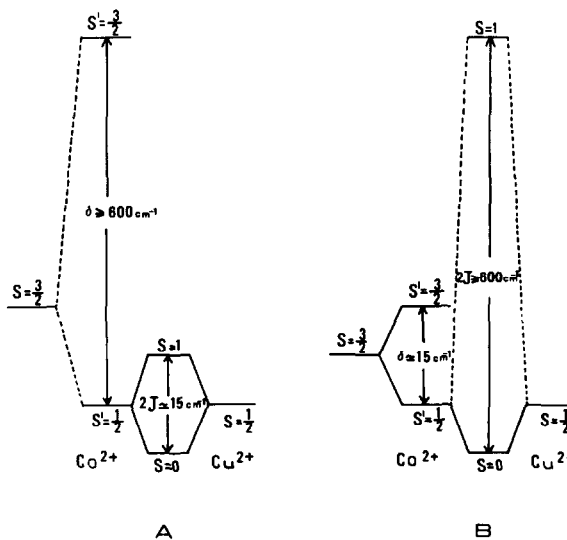


Fig.2. Two possible magnetic interactions in Cu(II)–Co(II) superoxide dismutase based on the results presented in the text.

with $\delta \approx 15 \text{ cm}^{-1}$ (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 $S=3/2$ doublet of the high spin Co(II). However, $\delta > 100 \text{ cm}^{-1}$ 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 $+80 \text{ cm}^{-1}$ (see [4] and ref. therein). Moreover, in the case of reduced Co(II) superoxide dismutase $\delta = 23 \text{ cm}^{-1}$ has been estimated from EPR measurements [3]. Therefore $\delta \approx 15 \text{ cm}^{-1}$, that is very close to that observed for the Cu(I)-Co(II) protein (3), and a new lower limit for the coupling constant $2J \gtrsim 600 \text{ cm}^{-1}$ 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.

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