Since theory predicts[7] a substantial triplet preference for **1**—a notion qualitatively supported by our Curie plot[3]—we conclude that the spectra of Figure 1 represent absorption from (emission to) the triplet ground state of **1**. The only alternative source of these spectra would be a rapidly equilibrating singlet state of **1**. However, the data of Figure 2 were obtained at 4 K, conditions under which significant population of a thermally excited singlet is quite improbable.

Given this analysis, we can determine an oscillator strength for the transition. Thus, the biradical concentration of a typical sample was determined by a spin count, i.e., a comparison of its double-integrated ESR signal intensity with that of a free radical standard[8] at 77 K. Measurement of the absorbance of this sample provided an ϵ (506 nm) of $(8 \pm 2) \times 10^3$ M^{-1} cm^{-1}. Integration of the absorption spectrum gave an oscillator strength of $f \approx 0.025$, indicating that the transition is spin-allowed.

Our results demonstrate the remarkable changes in electronic structure obtained on going from benzene (λ_{max} = 254 nm) to its non-Kekulé isomer **1**. We have found[9] that standard PPP-CI theory predicts a transition ($^3B_{2u} \rightarrow {}^3B_{3g}$) at 501 nm with f = 0.018, in excellent agreement with experiment. Further experimental and theoretical studies of **1** and related structures are under way.

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Magnetic Susceptibility Studies of the Native Cupro-Zinc Superoxide Dismutase and Its Cobalt-Substituted Derivatives. Antiferromagnetic Coupling in the Imidazolate-Bridged Copper(II)-Cobalt(II) Pair

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Polynuclear metalloproteins are quite numerous in nature. When the metals of the active sites interact through bridging ligands, the magnetic susceptibility measurements may be one of the most powerful techniques to determine the nature and the magnitude of this interaction. So far, very few studies of this type have been carried out[1-5] and the reasons can be easily understood. Proteins are of large molecular weight and their constituents are essentially diamagnetic. The magnetic metal ions represent an almost negligible part of those molecules. The problem is to extract a very small paramagnetic effect from an overall high diamagnetic signal. Therefore the conditions required to resolve the contribution of interest are a high degree of purity and homogeneity of proteins on one hand, and owing to the important magnetic dilution, a high sensitivity and accuracy of the susceptometers on the other hand. Susceptometers utilizing quantum flux detection methods present the characteristics required for reliable magnetic susceptibility study on proteins. But in order to get absolute values of protein susceptibilities, the determination of the diamagnetic correction remains a crucial point. The only way to reach those absolute values is the direct and independent measurement of the diamagnetic susceptibility of the apoproteins when available.

These requirements have been met in the magnetic susceptibility study we have performed on the cobalt-substituted derivative, Cu_2Co_2SOD, of the native cupro-zinc superoxide dismutase, Cu_2Zn_2SOD. The latter presents the unique feature of a histidine imidazolate group bridging the copper(II) and the zinc(II) ions. A tetrahedral symmetry is achieved around the zinc(II) ion while the square-pyramidal arrangement around the copper(II) ion is completed by a water molecule in an apical position.[6,7] The cobalt(II) ion can replace the zinc(II) ion in its site and the resulting cobalt derivative Cu_2Co_2SOD contains an imidazolate bridged copper(II)-cobalt(II) pair.[8-12] The two derived apoproteins E_2E_2SOD and E_2Co_2SOD (E for empty) are also available.[11,12]

These four proteins provide a unique and perfect test in order to estimate the extent of coupling between metal ions through bridging ligands. In order to obtain a reasonable estimate of the diamagnetic contribution, we have measured the diamagnetic susceptibility of the apoprotein E_2E_2SOD, the three magnetic proteins Cu_2Zn_2SOD, Cu_2Co_2SOD, and E_2Co_2SOD differing only in their metal content. In order to have a check of the paramagnetic contribution of the two individual Cu(II) and Co(II) ions, we have measured the paramagnetic susceptibility of Cu_2Zn_2SOD and E_2Co_2SOD which contain the same but independent Cu(II) and Co(II) chromophores as in Cu_2Co_2SOD. Finally, we have measured the susceptibility of Cu_2Co_2SOD, and for the first time, we can offer a quantitative estimation of the exchange interaction in the cobalt/copper enzyme. Previous measurements of Cu_2Co_2SOD susceptibility were reported by three of us,[13] but quantitative analysis was not made possible.

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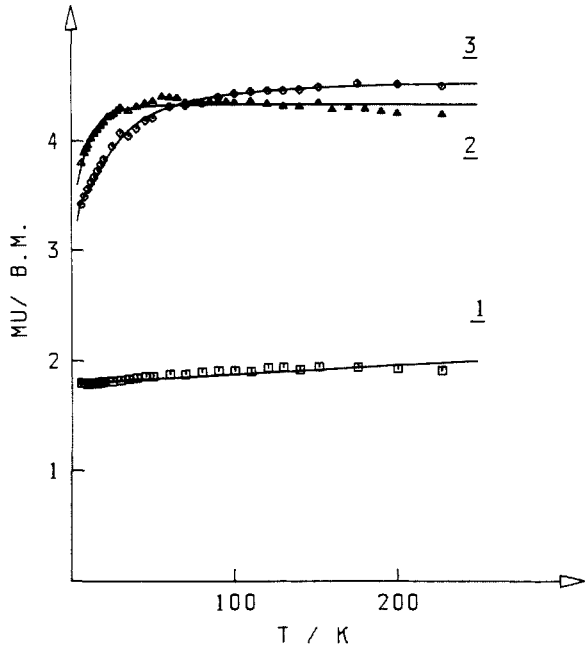


Figure 1. Magnetic moment data for **1** (Cu_2Zn_2SOD), **2** (E_2Co_2SOD), and **3** (Cu_2Co_2SOD). The solid lines represent the best fit to the data of the susceptibility equations.

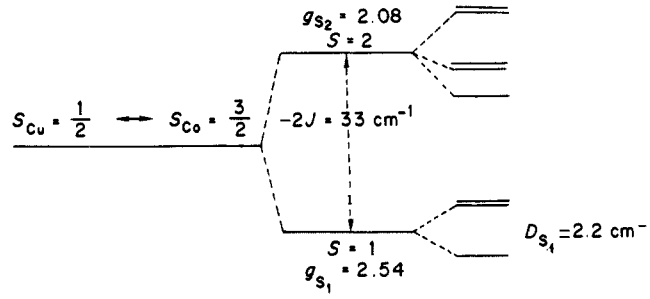
The complete approach outlined above has proved to be efficient in the field of molecular magnetism[14] and for the first time is applied to bioinorganic chemistry. For the first time also, it is applied to an imidazolate-bridged copper(II)–cobalt(II) pair since no such molecular complex exits at present. It is amazing to underline that the enzyme Cu_2Co_2SOD can be considered as a "model" for the copper(II) and tetrahedral cobalt(II) ions interaction.

The four studied proteins have been prepared from the same source, according to established procedures, and lyophilized. The measurements have been performed by using a SQUID SHE susceptometer, in the temperature range 6–300 K, on samples weighing 8 mg, with a magnetic field of 8000 G. The diamagnetic susceptibility of E_2E_2SOD has been found equal to -1.43×10^{-6} ($\pm 0.03 \times 10^{-6}$) $cm^3\ g^{-1}$.

Figure 1 shows the temperature dependence of the effective magnetic moments (in Bohr magneton units μ_B). Cu_2Zn_2SOD follows a Curie law as expected for a $S = {}^1/_2$ molecule with a moment of 1.78 ± 0.02 μ_B. E_2Co_2SOD has a magnetic moment equal to 4.27 ± 0.04 μ_B at 200K and quite constant down to 30K. Below 30 K, the moment drops to the lowest value of 3.80 ± 0.04 μ_B at 6 K, in good agreement with the depopulation of the upper Kramers doublet of the $S = {}^3/_2$ multiplet leaving the ground Kramers doublet to contribute to the magnetic moment at the lowest temperature. The comparison of the magnetic behavior of Cu_2Zn_2SOD and E_2Co_2SOD with that of Cu_2Co_2SOD rules out the possibility of magnetically independent Cu(II) and Co(II) ions in the latter enzyme. Indeed under 100 K, the observed magnetic moment is lower than the moment of E_2Co_2SOD and is indicative of an antiferromagnetic coupling. This coupling has been previously evidenced by the lack of EPR spectra[12,15] and by proton magnetic resonance spectra.[10,16]

The analysis has been made quantitative by considering the copper/cobalt antiferromagnetic coupling as an isotropic exchange interaction, approximated by the Hamiltonian $\mathcal{H}_{exch} = -J\hat{S}_{Cu}\hat{S}_{Co}$ and justified by the nonorbital degeneracy of the tetrahedral Co(II) ion. With each of the two low-lying levels $S = 1$ and $S = 2$ arising from the $S_{Cu} = {}^1/_2$ and $S_{Co} = {}^3/_2$, coupling is associated the spin Hamiltonian $\mathcal{H}_S = \beta\vec{H}(g_S)\hat{S} + \hat{S}(D_S)\hat{S}$ where the meaning of the two terms is classical and where the g_S parameters are related to the individual g_{Cu} and g_{Co} parameters according to known relations.[17] A similar spin Hamiltonian $\mathcal{H}_S$ has been associated with the $S = {}^3/_2$ level of E_2Co_2SOD. The susceptibility equations are derived by using isotropic g factors and axial zero field splitting (ZFS) parameters D. The ZFS of the excited state of Cu_2Co_2SOD has been neglected assuming that $D_{S_2} << kT$ at temperatures such that this level is populated. The best fit to the data of the susceptibility equations according to least-squares minimization procedures leads to the spin Hamiltonian parameters ($R = \sum(\chi_{obsd} - \chi_{calcd})^2/\sum(\chi_{obsd})^2$).

Owing to the low accuracy of the g factor values deduced from magnetic susceptibility measurements, the values $g_{Cu_2Zn_2SOD} = 2.06$ ($R = 9 \times 10^{-5}$) and $g_{E_2Co_2SOD} = 2.24$ ($R = 3 \times 10^{-4}$) are in good agreement with those determined from EPR spectra.[15,18] The value of 10.8 cm^{-1} for the ZFS parameter of the E_2Co_2SOD $S = {}^3/_2$ state, very close to the value of 11.5 cm^{-1} proposed for the cobalt chromophore in the reduced copper(I)/cobalt(II) derivative,[15] is in the range expected for Co(II) ions in a tetrahedral environment.[19] The spin Hamiltonian parameters describing the energy levels of Cu_2Co_2SOD are collected in Diagram I ($R = 1.7 \times 10^{-4}$).[20]



The energy gap of $-2J$ between the ground state $S = 1$ and the excited $S = 2$ level of Cu_2Co_2SOD is found equal to 33 cm^{-1}. It unambiguously indicates that the imidazolate bridge propagates the antiferromagnetic interaction between the copper(II) ion and the cobalt(II) ion in the zinc site. To corroborate this interaction, we have considered the possible presence of two magnetically independent Cu(II) and Co(II) ions. A very bad fit with a negative g_{Cu} factor has been obtained. It is interesting to compare the value of the exchange parameter $J_{CuCo} = -16.5$ cm^{-1} that we have found for Cu_2Co_2SOD with the value $J_{CuCu} = -52$ cm^{-1} proposed for the copper/copper interaction in Cu_2Cu_2SOD.[21] These experimental values follow the relation $J_{CuCo} \simeq {}^1/_3 J_{CuCu}$. Such a relation can be derived theoretically on the ground that one of the three possible exchange pathways for a copper/cobalt pair is much more efficient than the others.[22]

As a final comment, we can also suggest that the imidazolate bridge remains intact upon lyophilization, whereas it has been

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Deuterium NMR Spectra and Librational Motions of the Base Pairs in Oriented Calf Thymus DNA

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The different forms of solid DNA are usually distinguished by X-ray crystallography[1,2] and single-crystal X-ray studies have also been used to evaluate the degree of librational motion in di- and polynucleotides.[3] Similar information cannot be obtained for polynucleotides from X-ray fiber diffraction patterns, and in such cases NMR is quite useful. Phosphorus NMR studies, in particular, have linked the rate and extent of backbone motion to the degree of hydration of random[4,5] and aligned[6-8] samples of polynucleotides, but so far only limited use has been made of deuterium NMR.[4,9] Results reported here for oriented samples of Li^+ and Na^+ DNA deuterated in the adenine and guanine 8-positions demonstrate how deuterium NMR spectra of oriented samples can be used to characterize structural and dynamic properties of the bases in solid DNA.

Quadrupole echo deuteron spectra (38.4 MHz) are shown in Figure 1 for two samples (A, Na salt; B, Li salt) of oriented high molecular weight calf thymus DNA (Worthington Biochemicals). The 8-positions of adenine and guanine were deuterated at 63 °C in a D_2O buffer (pD 7.0), and oriented samples were prepared by the wet-spinning technique.[10] The samples were equilibrated with H_2O over saturated salt solutions[11] to a relative humidity of 66% for Li-DNA and 75% for Na-DNA. Spectra were recorded with the helix axes oriented parallel (top) and perpendicular (bottom) to the magnetic field, B_0. The Li-DNA spectra (Figure 1B) are characteristic of a sample with C-D bonds distributed uniformly at right angles to a common (helix) axis: at $\beta = 0°$ (top right) we observe two transitions at $\nu = \pm(69.3 \pm 0.7)$ kHz, while at $\beta = 90°$ (bottom right) we obtain a "cylindrical powder pattern" with singularities at $\pm(59.8 \pm 0.6)$ kHz and $\pm(130 \pm 2)$ kHz. The Na-DNA spectra (Figure 1A) are more complex: broad peaks at ±46 kHz as well as narrow features near ±69 kHz are predicted for a sample with base pairs tilted ~70° relative to the helix axis and a distribution of helix orientations. The relatively high intensity of the narrow doublet suggests the presence of minor amounts of B form, but except for that all four line shapes may be simulated by assuming a ±10-12° Gaussian distribution of the helix axes. X-ray diffraction patterns of these samples support our interpretation of the NMR spectra.

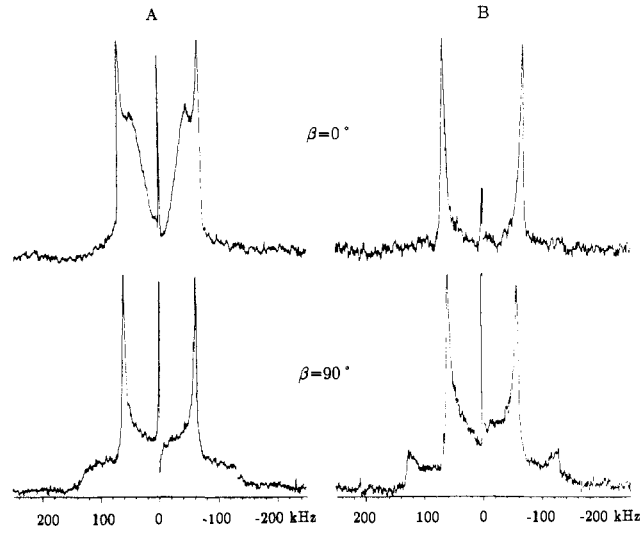

Figure 1. Deuterium NMR spectra obtained at 38.4 MHz of ca. 140-mg samples of the Na salt (A) and Li salt (B) of oriented calf thymus DNA. β is the angle between the DNA helix axis and the magnetic field. The spectra of Li-DNA at 66% relative humidity (B top and bottom) show that this sample is pure B form, while the spectra in Figure 1A of Na DNA at 75% relative humidity are characteristic of the A form, with possibly a small contribution from the B form. The sharp central peak is due to natural abundance deuterium in the water used to hydrate the samples. The spectra were obtained by using from 10 000 to 200 000 quadrupole echo pulse sequences with 2.5-μs $\pi/2$ pulses and 400-ms repetition times.

A more quantitative analysis of the B-form Li-DNA spectra in Figure 1B may be performed by considering the effect of librational motion upon the quadrupole Hamiltonian. For this purpose we used a two-step transformation[12] of the deuteron quadrupole coupling tensor (principal axis system is defined with the z axis along the C-D bond and the x axis in the purine plane), first through Euler angles (ϕ=0°,θ,χ=90°) to an intermediate frame with the z axis along the helix axis and then through angles (α,β,γ) to the laboratory frame with the z axis along B_0. We assume that the base pairs undergo librations in two planes: through $\pm\phi_0$ in the molecular plane about the base normal and through $\pm\theta_0$ in a plane perpendicular to the base pair. Assuming further that the C-D bonds are uniformly distributed within a region $-\phi_0 \leq \phi \leq \phi_0$ about $\langle\phi\rangle = 0°$ and $-\theta_0 \leq \theta \leq \theta_0$ about $\langle\theta\rangle = 90°$, we obtain the following expression for the motionally averaged deuteron transition frequencies:

$$\nu = \pm\tfrac{3}{4}(e^2qQ/h) \times [\tfrac{1}{2}(3\cos^2\beta - 1)f(\eta,\theta_0) - \tfrac{1}{2}\sin^2\beta\cos 2\gamma\, g(\eta,\theta_0,\phi_0)] \quad (1)$$

where

$$f(\eta,\theta_0) = \frac{1}{4}\left(1 - \eta - (3+\eta)\frac{\sin 2\theta_0}{2\theta_0}\right) \quad (2)$$

$$g(\eta,\theta_0,\phi_0) = \frac{1}{4}\frac{\sin 2\phi_0}{2\phi_0}\left(3(\eta-1) - (3+\eta)\frac{\sin 2\theta_0}{2\theta_0}\right) \quad (3)$$

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