TEMPERATURE DEPENDENCE OF THE MAGNETIC SUSCEPTIBILITY OF COBALT(II) STELLACYANIN

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Summary: Magnetic susceptibility measurements on cobalt(II) stellacyanin (Rhus vernicifera) have been performed between 2.2 and 50 K. The effective magnetic moment of Co(II) in the protein is 3.91 ± 0.12 (μ_B). Nonlinear behavior below 3 K evidences the presence of zero-field splitting attributable to a low-symmetry component of the ligand field. The results are consistent with a structural model based on a distorted tetrahedral Co(II) site involving one or more extremely covalent metal-ligand bonds.

We have previously reported the preparation of a Co(II) derivative of the blue copper protein Rhus vernicifera stellacyanin (1). The electronic absorption spectrum of this Co(II) derivative suggests that the ground state is of the high-spin type, and further that the coordination geometry in all probability is distorted tetrahedral (1-3). Direct determination of the magnetic moment of Co(II) stellacyanin, however, is required to establish unambiguously the nature of the electronic ground state. Such an experiment should also provide important additional information concerning the geometry and metal-ligand covalency at the bonding site. For these reasons, we have measured the magnetic susceptibility of cobalt(II) stellacyanin between 2.2 and 50 K.

Methods: All magnetic susceptibility measurements were made on the Caltech superconducting quantum magnetometer (4). The superconducting magnetometer detects the sample-related flux, arising either from movement of the sample (total susceptibility measurement, χ_T) or change of sample temperature (relative susceptibility measurement,

$\chi_R(T)$). Both modes of operation were used on 17.34 mg of lyophilized cobalt(II) stellacyanin that had been prepared as described previously (1). The relative change of susceptibility as a function of temperature was measured in a magnetic field of about 160 G between 2.2 and 50 K. This $\Delta\chi_R(T)$ change is independent of the diamagnetic background from the protein host. The total suceptibility measurements made at and above 20 K were used as the reference χ_T for the relative data taken between 3 and 50 K. Similarly, the χ_T near 2.2 K was chosen to be the reference point for χ_R (T) below 3 K. Owing to experimental difficulties, relatively large uncertainties are associated with the lowest temperature data.

Results: A plot of susceptibility against inverse temperature is shown in Figure 1. The crosses represent relative $\chi_R(T)$ and the open boxes are the χ_T data. Taking the slope between 3 and 10 K, where Curie behavior clearly prevails, the effective magnetic moment is determined to be 3.91 ± 0.12 Bohr magnetons (μ_B). The rise of susceptibility just above 50 K is attributed to the presence of a small amount of antiferromagnetic impurity. The nonlinear χ vs $1/T$ curve below 3 K suggests the presence of a zero-field splitting of the ground state owing to a low symmetry distortion at the cobalt(II) site. The magnitude of this splitting, however, cannot be fixed from the present results.

Discussion: The μ_B value confirms a high-spin electronic configuration for Co(II) stellacyanin. The fact that the μ_B value is only slightly above spin-only (μ_B = 3.87) can only be reconciled with a distorted tetrahedral geometry in which the orbital contribution has been quenched (5). The magnetic moments of high-spin Co(II) complexes with coordination numbers of five and six are in general well above μ_B =4.4 (6). What is more, analysis of the absorption, CD, and MCD d-d spectra of Co(II) stellacyanin has definitely ruled out a five- or six-coordinate metal-binding site (3).

Certain distorted tetrahedral Co(II) complexes exhibit μ_B values below 4.3, particularly if the metal-ligand bonds possess appreciable

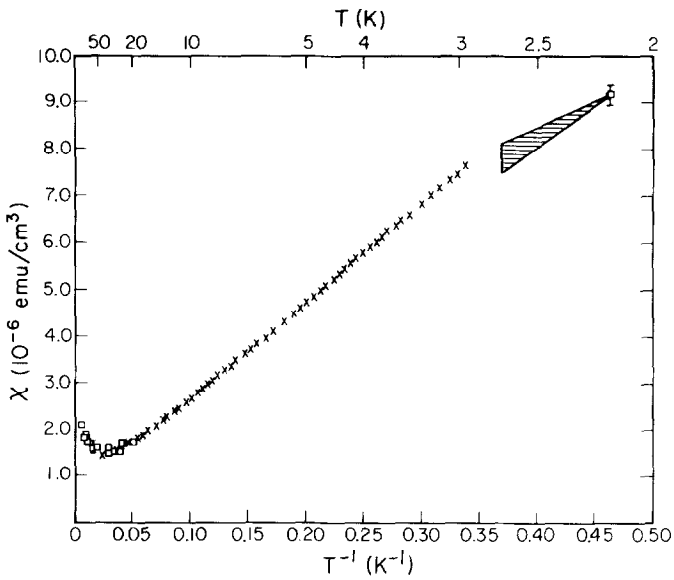

Figure 1. Temperature dependence of the magnetic susceptibility of a lyophilized sample (17.34 mg) of cobalt(II) stellacyanin: ×, $\chi_R(T)$ data; ⊡, χ_T data.

covalent character (5). From the standard expression (6) $\mu_B = 3.87\ (1-4k^2\lambda/10\ Dq)$ for a tetrahedral Co(II) center (k is the orbital reduction factor, λ is the spin-orbit coupling constant), we calculate $0 \leq k^2 \leq 0.3$ from the limits placed on μ_B for Co(II) stellacyanin. In this calculation we have taken $\lambda = -180$ (5) and $10\ Dq = 4900\ cm^{-1}$ (3).

It is apparent that an unusually high degree of metal-ligand covalency is associated with the distorted tetrahedral center in Co(II) stellacyanin. This result is not surprising, as many physical measurements on blue copper proteins and their cobalt(II) derivatives have pointed in the same direction. The very low parallel hyperfine coupling constants obtained from EPR studies of the native proteins, for example, may be cited in this connection (7). It is likely that the extremely high covalency is associated in part with Co(II) binding to the sulfur atom in the proposed N_2N^*S (N = His; N* = deprotonated peptide nitrogen; S = Cys) donor set of a type 1 site (8). Further information about the ground state of Co(II) stellacyanin may be

derived from low temperature EPR measurements, and experiments along these lines are in progress in our laboratory.

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