results we note the similarities and differences between plant's Mn(III)- and mammalian Fe(III)-containing acid phosphatases.

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Temperature Dependence in the MCD Spectrum of Horseradish Peroxidase Compound I

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Ater considerable experimental study, the analysis of ENDOR [1, 2], and magnetic circular dichroism (MCD) spectra of horseradish peroxidase (HRP) compound I [3], as well as the MCD spectra of a series of porphyrin π-cation radical species [4] has led to the confirmation that the electronic configuration of the heme in the compound I species was that of an Fe(IV) porphyrin π-cation complex. While the MCD data of the enzyme were measured at 273 K, and these spectra can be obtained at low temperatures, the ENDOR [1] and EPR [5] signals can only be detected below 30 K. The MCD experiment is very sensitive to ground state degeneracy and in this paper spectra of HRP compound I are discussed that were obtained between liquid helium temperatures and 100 K.

Figure 1 shows how the reduction in temperature has a far more pronounced effect on the MCD spectrum of HRP compound I than on the comparable absorption spectrum. While there are only slight changes in the MCD spectrum between 273 K and

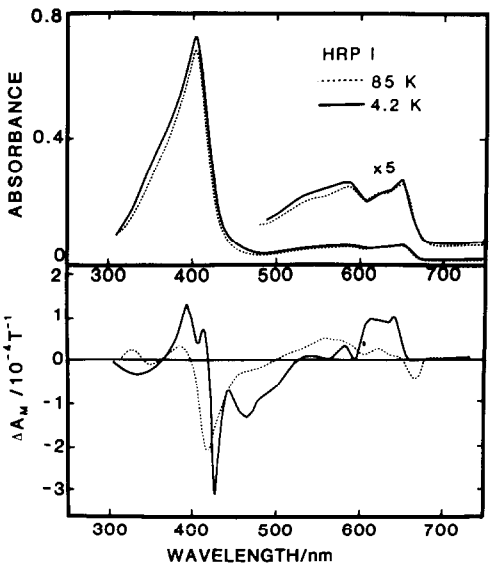

Fig. 1. The optical absorption (upper) and the MCD (lower) spectra of HRP compound I at 4.2 K and 85 K. The spectra were obtained in a 1/1 v/v glycerol/water solution. The HRP concentration was 7.3 × 10^{-5} mol l^{-1} and 4.4 mol l^{-1} in the absorption and MCD experiments, respectively. The sample path length was 0.11 cm, and the magnetic field used was 4.58 T.

30 K, there are very dramatic changes between 30 K and 4 K. Below 30 K, there is a loss in intensity at 660 nm with a corresponding increase in the 640 nm band and an increase in the dominance of the 300 nm to 500 nm region by the 420 nm and 460 nm bands. The temperature dependent band at 420 nm appears to be derived from a small impurity of the photochemical product of HRP compound I [6] which is formed during preparation of the sample and as a consequence of the visible and UV light used to measure the MCD spectra.

The most striking feature of the spectrum below 30 K is the apparent relationship between the 640 nm and 660 nm bands, and the simple intensity increase with inverse temperature that is observed in the 460 nm and 640 nm bands. The latter effect is characteristic of a C term and thus indicates the presence of an orbitally degenerate ground state. Coupling between the S = 1 iron and the S = ½ porphyrin to form the degenerate ground state as a set of three Kramers doublets has been used to explain the observations of the temperature dependence of the EPR spectrum [5]; this hypothesis can also be used to describe the appearance of the MCD C term [7]. The lack of temperature dependence above 30 K suggests that the iron and the porphyrin radical are not now strongly coupled together. These data also suggest that a structural change has occurred at the low temperatures which results in the tempera-

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ture dependence in the MCD spectrum as the result of a change in the coupling between the S = 1 iron and the S = ½ π-cation porphyrin radical, and the formation of a degenerate ground state.

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Metal Binding to Metallothioneins: a Spectroscopic Characterization

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Metallothionein proteins isolated from the livers and kidneys of animal can contain a variety of metal ions [1]. The most widely studied metallothioneins (MT) have contained cadmium, zinc and copper. Typically, Cd,Zn-MT has been isolated from livers following induction of the metallothionein by exposure of the animal to cadmium salts [2]. The UV absorption spectrum of Cd,Zn-MT exhibits a characteristic shoulder at 250 nm, which together with the absence of any significant absorbance at 280 nm, has been used as an indicator of the presence of the cadmium–thiolate group which forms the binding site in metallothionein. The UV absorption, CD and magnetic CD (MCD) spectra of Cd,Zn-MT have been shown to provide considerable information on the binding of metals to metallothioneins [3, 4]. Very recently, emission spectra have been reported for *Neurospora crassa* copper metallothionein [5] which suggests that luminescence data from metallothioneins may also yield details on the properties of the binding sites. In this paper we report absorption, CD, MCD and emission spectra for a series of metallothioneins.

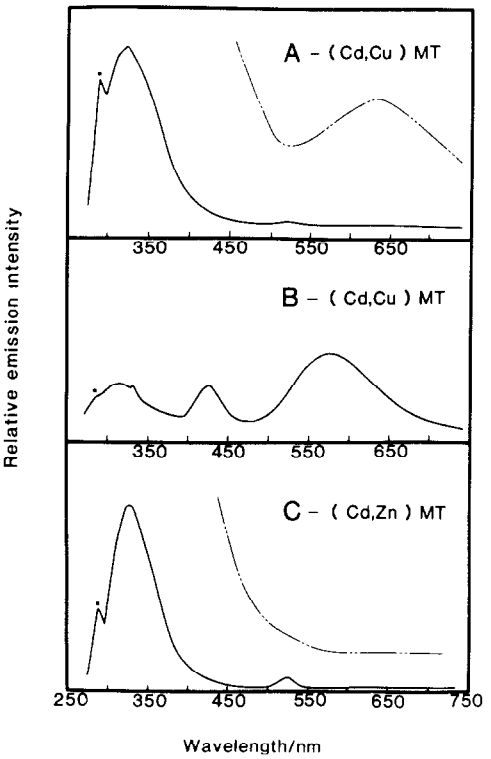

Fig. 1. Emission spectra of Cd,Cu-MT and Cd,Zn-MT. (A) Cd,Cu-MT; λ_{ex} = 260 nm for both spectra; the spectra were recorded at room temperature. (B), Cd,Cu-MT: λ_{ex} = 260 nm; the spectrum was recorded at 77 K. (C) Cd,Zn-MT; λ_{ex} = 260 nm for both spectra; the spectra were recorded at room temperature. The bands marked with a * are Raman bands from the solvent.

We discuss in this part of the paper the spectra of rat liver Cd,Zn-MT and rat kidney Cd,Cu-MT prepared as described previously [2, 6], and compare these data with Cd-MT, Cd,Cu-MT and Cu-MT formed by titration of Cd,Zn-MT with either $CdCl_2$ or $[Cu(CH_3CN)_4]^+$. Atomic absorption spectroscopy was used to determine metal loading values in the native proteins. These values are reported here as atoms of metal/molecule of protein, for Cd,Zn-MT, Cd: 2.0, Zn: 1.7 and Cu: 0.3; for Cd,Cu-MT, Cd: 1.6, Zn: 0.2 and Cu: 2.5.

Both Cd,Zn-MT and Cd,Cu-MT exhibit similar absorption and CD spectra. The 230 nm to 300 nm region is dominated by the charge transfer spectrum of the cadmium–thiolate group. The poorly resolved shoulder at 250 nm in the absorption spectrum is far clearer in the CD and MCD spectra where a derivative-shaped envelope identifies the multiple-transition nature of this band. By contrast, the absorption, CD and MCD spectra of the copper–thiolate groups within the protein are not nearly as well defined. However, the emission spectrum of copper-containing metallothioneins is distinctive