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


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## O3

## Metal Binding to Metallothioneins: a Spectroscopic Characterization


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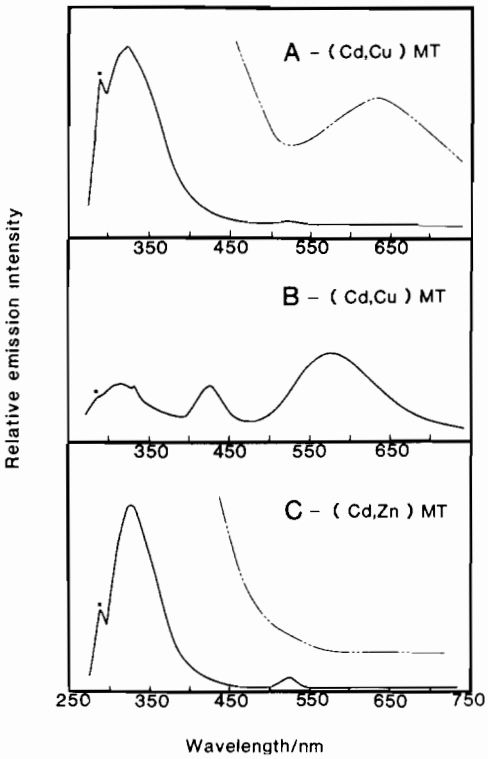


Fig. 1. Emission spectra of Cd,Cu-MT and Cd,Zn-MT. (A) Cd,Cu-MT; $\lambda_{ex}$ = 260 nm for both spectra; the spectra were recorded at room temperature. (B), Cd,Cu-MT: $\lambda_{ex}$ = 260 nm; the spectrum was recorded at 77 K. (C) Cd,Zn-MT; $\lambda_{ex}$ = 260 nm for both spectra; the spectra were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

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.

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

and can serve as an identifier for the copper. Figure 1 shows the emission spectra of Cd,Zn-MT and Cd, Cu-MT. The room temperature spectrum of Cd, Cu-MT, Fig. 1A, is in two parts: excitation at 260 nm results in a strong emission at 320 nm, whereas using a sensitivity 200 times greater and exciting at either 260 nm or 305 nm we observe a broad band near 600 nm. Figure 1B shows the spectrum of Cd, Cu-MT in an aqueous glycerol glass at 77 K. The intensity of the 580 nm band has increased dramatically and the band centre has blue-shifted by about 30 nm. Figure 1C shows the spectrum of Cd,Zn-MT recorded at room temperature under the same conditions as Fig. 1A. Although the 320 nm band is the same as that found for Cd,Cu-MT, there is no intensity in the 600 nm region. When emission spectra are recorded during the titration of this native rat liver Cd,Zn-MT with aliquots of copper(I) we observe the gradual appearance of a new band at 600 nm. At the same time, the absorption and CD spectra show the loss of first the zinc, and then the cadmium from the metallothionein binding sites.

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## O4

## Low Temperature MCD Study of the Species Formed by Photolysis of Horseradish Peroxidase Compound I

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During the reaction of the enzyme horseradish peroxidase (HRP) with hydrogen peroxide, a highly oxidized species is formed which is known as compound I. The electronic structure of HRP compound I is considered to involve an Fe(IV) porphyrin $\pi$-cation radical [1]. Previous studies of horseradish peroxidase compound I have shown that light accelerates the spontaneous conversion of compound I to compound II at room temperature, yielding, finally, the native enzyme [2]. However, photolysis at low temperatures (*i.e.* those less than the glassing temperature of the solvent) produces a photochemical product with optical properties which are close to, but not identical to, those of compound II [2–4]. Although, magnetic circular dichroism (MCD) spectroscopy is closely related to optical absorption spectroscopy, the data obtained provide considerably more information about the ground and excited state electronic configurations than is obtained from the absorption spectrum alone. In this paper we describe MCD data recorded between 4 K and 50 K following the photolysis of HRP compound I at 80 K.

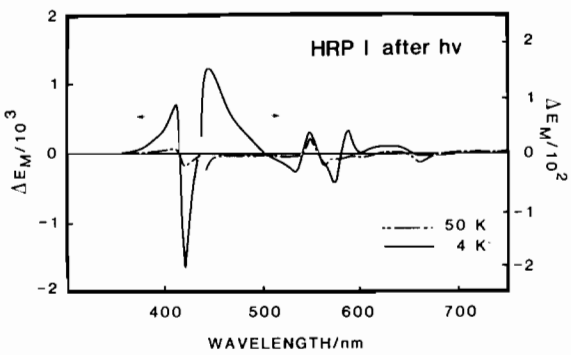


Fig. 1. MCD spectra of the product of HRP compound I photolysis in 1:1 v/v glycerol:water solution at 80 K. The HRP concentration was $4.4 \times 10^{-5}$ mol·l$^{-1}$, the sample path length was 0.11 cm, and the magnetic field used was 4.58 T. The signal intensities are expressed in units of L·mol$^{-1}$·cm$^{-1}$·T$^{-1}$.

Figure 1 shows the 4 K and 50 K MCD spectra of the HRP compound I photochemical product which was prepared by an exhaustive photolysis of compound I at 80 K. The 50 K spectrum is very similar to the MCD spectra of HRP compound II which have been previously reported for 127 K and 208 K [5]. Both sets of spectra in the visible region contain features which are quite unlike those of a ferric heme with either a S = 5/2 or S = ½ spin state. However, like the MCD spectra of ferric hemes, the spectrum of the photochemical species is very temperature dependent and is dominated by Faraday C terms. Figure 2 shows the temperature dependence of the peak-to-trough intensity for the major features in the B or Soret regions and the Q or $\alpha$ band regions of the MCD spectrum of the heme in the photochemical product. The almost linear

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