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

WILLIAM R. BROWETT, ZBIGNIEW GASYNA[+] and MARTIN J. STILLMAN*

Department of Chemistry, University of Western Ontario, London, Ont., N6A 5B7, Canada

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 π-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.

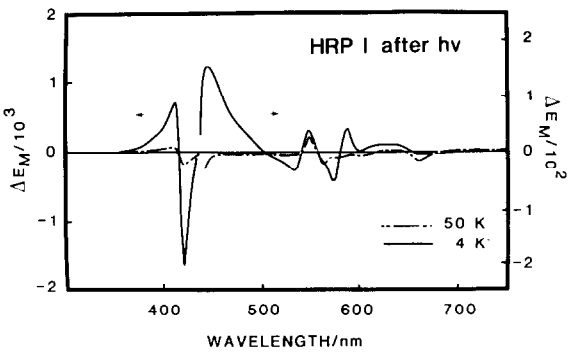

Fig. 1. MCD spectra of the product of HRP compound I photolysis in 1:1 v/v glycerol:water solution at 80 K. The HRP concentration was 4.4×10^{-5} mol·l^{-1}, the sample path length was 0.11 cm, and the magnetic field used was 4.58 T. The signal intensities are expressed in units of L·mol^{-1}·cm^{-1}·T^{-1}.

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 α band regions of the MCD spectrum of the heme in the photochemical product. The almost linear

[+]Permanent address: Institute of Radiation Chemistry, Technical University, Lodz, Poland.

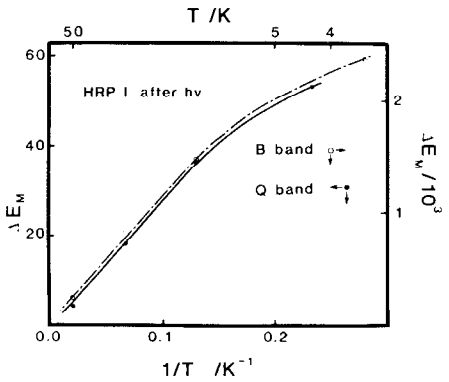

Fig. 2. The temperature dependence of the MCD peak-to-trough intensity for the B or Soret band at 416 nm (right hand ordinate scale), and the Q or α band at 580 nm (left hand ordinate scale) of the photolysed HRP compound I. The signal intensities are expressed in units of $L \cdot mol^{-1} \cdot cm^{-1} \cdot T^{-1}$. The experimental conditions were the same as in Fig. 1.

relationship between the intensity and the inverse of the absolute temperature suggests that the ground state in the iron porphyrin species which is formed photochemically is orbitally degenerate. The non-linearity of this relationship at very low temperatures and the high magnetic field (4.58 T) used in this study most likely arises from simple Boltzmann saturation effects.

Various structures have been suggested for the product of the photolysis of HRP compound I [2, 4, 6, 7]. The close similarity between the optical absorption and MCD spectra of the photolysis product and HRP compound II suggests that the heme retains the S = 1 Fe(IV) porphyrin electronic structure following photolysis and that the porphyrin is reduced from the 17 π-electron cation radical of compound I to the stable 18 π electron configuration as in compound II [8, 9]. In addition, the strong temperature dependence of the MCD intensity observed in the spectra of the photochemical product indicates that significant coupling takes place between the paramagnetic iron and the diamagnetic porphyrin π system.

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O5

The Copper of Dopamine β-Monooxygenase: High Accessibility and Rapid Exchange

TORE SKOTLAND, TORGEIR FLATMARK

Department of Biochemistry, University of Bergen, Årstadveien 19, N-5000 Bergen, Norway

and TORBJØRN LJONES

Department of Chemistry, College of Arts and Sciences, University of Trondheim, N-7055 Dragvoll, Norway

Dopamine β-monooxygenase (dopamine β-hydroxylase; EC 1.14.17.1) catalyzes the reaction:

$$\text{Dopamine} + \text{ascorbate} + O_2 \rightarrow$$

$$\text{noradrenaline} + \text{dehydroascorbate} + H_2O$$

$$(RH + 2e^- + 2H^+ + O_2 \rightarrow ROH + H_2O)$$

The purified water-soluble enzyme from bovine adrenal medulla contains 4 copper atoms per enzyme tetramer of 290,000 daltons. These copper atoms are essential for enzymic activity, and they most probably participate in both electron transfer and binding of O_2 [1].

The copper in dopamine β-monooxygenase can be classified as type 2 copper according to its EPR spectrum, with a large hyperfine splitting and the low absorption in the visible spectrum (ϵ = 40 $M_{Cu}^{-1} \cdot cm^{-1}$ at 680 nm, the maximum of the Cu(II)-band) [1]. This enzyme-bound copper is, however, different from type 2 copper in the blue oxidases by other criteria, especially by showing a high accessibility of the copper sites. Thus, we have shown that the copper atoms of dopamine β-monooxygenase can be rapidly removed by chelators at non-denaturing conditions both in the reduced and oxidized states, and the inactive apoenzyme is reactivated in less than 2s by addition of $CuSO_4$ [2, 3].