Near-Infrared Magnetic Circular Dichroism of Catalase and Heme Octapeptide: Comparison with other Hemoproteins

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Near-infrared magnetic circular dichroism studies of bovine liver catalase and heme octapeptide from equine cytochrome c indicate high-spin character for the former and mixed-spin character for the latter. It is suggested that the ligand-field strength of phenolate is roughly comparable with that of water and hydroxyl anion.

Keywords magnetic circular dichroism; near-infrared; catalase; heme octapeptide; high-spin; low-spin; ligand

Spectroscopic data on magnetic circular dichroism (MCD) of hemoproteins and iron porphyrins have been accumulated over the past 15 years. As a result, it is recognized that MCD is more sensitive than normal absorption spectra to the spin and oxidation states of central metals and to the kind of apical ligand.[1] However, mainly because of instrumental limitations, few reports have appeared on the near-infrared (NIR) MCD of these compounds. In the case of ferric porphyrins, the bands in the NIR are generally assigned to charge-transfer (CT) from porphyrin a_{1u} and a_{2u} (π) to iron e_g ($d\pi$) orbitals ($E_u \leftarrow A_{1g}$) under the D_{4h} approximation.[2] Indeed, we and others have shown that the NIR MCD of ferric hemoproteins[3] and synthetic iron porphyrins[4] support this assignment. In contrast to the bands in the UV-visible region, the CT transition in the NIR mixes much less with π–π^* transitions. Accordingly, more direct information can be obtained from the data in this region. The aim of the present study was to examine the electronic structure of a catalase and heme octapeptide by NIR MCD and to compare the results with those of other hemoproteins.

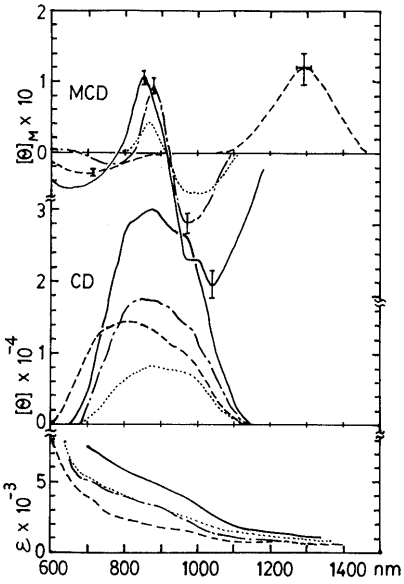

Fig. 1. NIR MCD, CD, and Absorption Spectra for Native (Fe(III)) Bovine Liver Catalase (——) and Its Azide (·—·), Cyanide (------), and Imidazole (· · · ·) Derivatives

Concentrations; 4.77×10^{-4}, 2.53×10^{-4}, 2.33×10^{-4}, and 2.34×10^{-4} M, respectively. Concentrations of NaN_3, KCN, and Im were 0.4, 0.2, and 1.2 M, respectively. Magnetic field, path length, and temperature were 1.47 Tesla, 10 mm, and 0 °C, respectively. All samples were centrifuged at 3000 rpm for about 15 min just prior to use. ——, catalase-D_2O; ·—·, catalase-N_3^-; ------, catalase-CN^-; · · · ·, catalase-Im.

Experimental

Materials Bovine liver catalase (type II) and heme octapeptide (H8PT) from equine heart cytochrome c were obtained from Sigma Chemical Co. and used without further purification. Complete oxidation of H8PT was attained by adding a slight excess of potassium hexacyanoferrate (III). Excess hexacyanoferrate was removed by passage through a column of Sephadex-G25 (medium). Measurements at room temperature were carried out in 1/15 mol dm^{-3} ^2H_2O potassium phosphate buffer (p^2H 6.96). For temperature variation experiments, the buffer solution was diluted with ethylene glycol(UV-visible region) or hexadeuterated ethylene glycol (NIR region) at 60 (v/v) percent. Catalase derivatives were prepared by adding solid NaN_3, KCN, or imidazole (Im) to appropriate concentration.

Measurements Absorption spectra were measured with a Hitachi EPS-3T spectrophotometer, MCD being recorded on JASCO J-200 and JASCO J-500 spectropolarimeters equipped with electromagnets which afford up to 1.47 and 1.14 T magnetic field, respectively. MCD magnitude is expressed by the molar ellipticity per Tesla ($[\theta]_M/10^4$ °$mol^{-1}dm^3m^{-1}T^{-1}$ or 10 °$m^2mol^{-1}T^{-1}$). The path lengths of cells were 10 mm for the measurement at ambient temperature and 3 and 1 mm for that at cryogenic temperature. Temperature was monitored with an alumel–chromel thermocouple inserted in the cell placed in a quartz Dewar vessel, and controlled by a stream of cold gaseous nitrogen evaporated from liquid nitrogen. Each scan was started 30 min after the temperature had reached a constant value.

For further details, see the caption to each figure.

Results and Discussion

Figure 1 shows absorption, natural circular dichroism (CD), and MCD spectra of catalase and its derivatives in the NIR region. The intensity of absorption decreases towards longer wavelength, although curvature is seen at around 700—1100 nm. Natural CD spectra exhibit positive bands in the same region for all derivatives. Native catalase shows a CD peak at 880 nm with $[\theta] = 30000$ and a shoulder at 970 nm. At room temperature the magnitude of the CD bands decreases in the order of native > azide > cyanide. The relatively small anisotropy factors (*ca.* 0.002 at 880 nm), defined as four times the rotation strength divided by the dipole strength,[5] imply that the bands correspond to magnetic-dipole forbidden transitions. Since CT transitions are magnetic-dipole forbidden, the above result is consistent with the assignment that NIR bands are associated with CT transitions. On the other hand, the catalase derivatives display, except for the cyanide, S-shaped MCD (Faraday *A* terms) with crossover points at around 920 nm. Such an MCD pattern and crossover wavelength are characteristic of ferric high-spin species.[3,4] Since the sinusoidal curves of azide and imidazole derivatives are more symmetrical on the $[\theta] = 0$ axis than the curve of native catalase, it can be concluded that the effective symmetry of iron in the former two derivatives is higher than that in the latter. The non-symmetricity of the MCD curve of native catalase

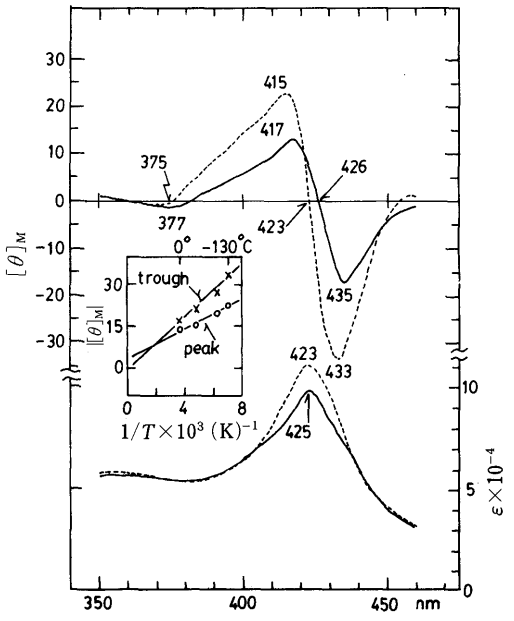

Fig. 2. Temperature Dependence of the MCD and Absorption Spectra of the Cyanide Derivative of Bovine Liver Catalase in the Soret Region

Protein concentration = 1.28×10^{-4} M. Magnetic field = 1.14 T and path length = 1 mm. The inset shows the dependence of the Soret MCD peak and trough on the reciprocal of the absolute temperature. Catalase (III)-CN^-: ——, 273 K; ------, 143 K.

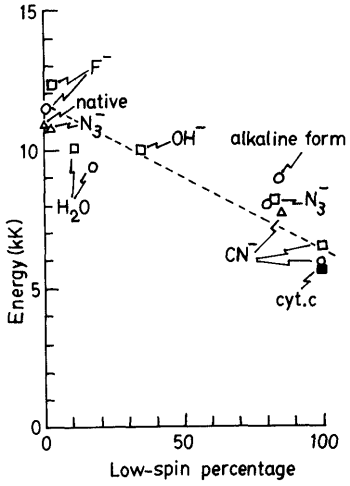

Fig. 3. Plots of Position of Maximum Intensity (Peak) of NIR MCD Bands of HRP, Myoglobin, and Catalase and Their Derivatives

Data on myoglobin derivatives were taken from ref. 3*b*). ○, HRP; □, myoglobin; △, catalase.

is probably due to a larger contribution of Faraday *C* term[3e] at around 1000 nm. The cyanide alone showed a positive peak at around 1290 nm, indicative[2,3] of a ferric low-spin component. However, this peak lies to the blue compared with that of sperm whale metmyoglobin cyanide (1530 nm)[3b] or horseradish peroxidase (HRP) cyanide (1680 nm).[3c] In order to confirm that the cyanide derivative of catalase is a low-spin complex, temperature variation experiments were carried out in the Soret region (Fig. 2). As shown in the inset, the MCD intensity at both the peak and trough of the Soret MCD shows clear linear dependence on the reciprocal of the absolute temperature, indicating that this compound is indeed in the Fe^{III} low-spin state.[6]

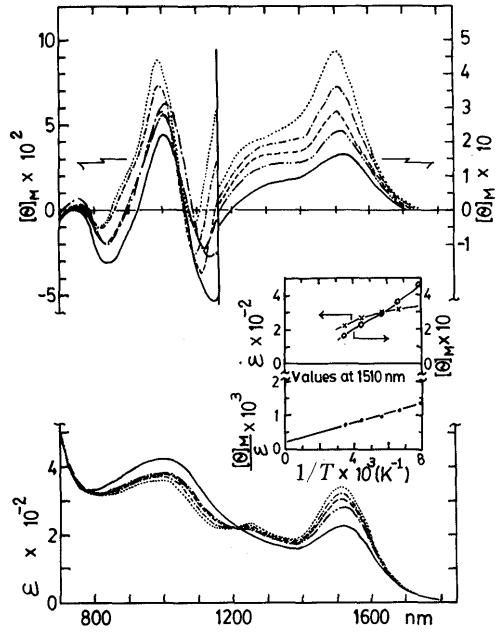

Fig. 4. Temperature Dependence of the MCD and Absorption Spectra of H8PT in the NIR Region

H8PT concentration = 3.80×10^{-3} M, cell length = 3 min, and magnetic field = 1.47 T. The inset shows the dependence of ε, $[\theta]_M$, and $[\theta]_M/\varepsilon$ at 1510 nm on the inverse of absolute temperature. ····, −138 °C; ·—·—, −123 °C; ------, −97 °C; —··, −50 °C; ·—·, 0 °C; ——, 20 °C.

Figure 3 displays the relationship between the position of the NIR band and the low-spin percentage of various hemoproteins and their derivatives. The effective Bohr magneton numbers for bovine liver catalase and its azide and cyanide are 5.96, 5.86, and 2.63, respectively.[7] Accordingly, the low-spin components in these derivatives are estimated[8] as 0, 2.2, and 93.6%, respectively. These data are also included in this figure. There is an approximately linear relationship between low-spin percentage and the position of the CT band: the higher the content of a low-spin component, the lower the energy of the CT band (since the NIR CT bands correspond to transitions from porphyrin a_{1u}, a_{2u} (π) to iron e_g (dπ) orbitals, their positions depend mostly on the energy levels of e_g (dπ) orbitals which vary more sensitively with the axial ligation mode than those of the porphyrin π orbitals).[2] Obviously, high-spin contents in catalase derivatives are higher than those in the corresponding derivatives of other hemoproteins. Since the low-spin content in hemoprotein is affected by the sum of the ligand-field strength (LFS) of the two axial ligands,[2] this implies that one of the axial ligands of catalase is different from that in other hemoproteins. According to the X-ray crystallographic data,[9] the fifth ligand of catalase is phenolate of tyrosine, while the hemoproteins in Fig. 2 are known to contain imidazole as the 5th ligand. When ligands with relatively strong LFS such as cyanide, azide, imidazole, and thiolate coordinate at the 6th position of hemoproteins whose 5th position is occupied by imidazole, the low-spin state becomes predominant (see also Fig. 3). Accordingly, the fact that the imidazole and azide derivatives (6th ligands) of catalase are in a practically pure high-spin state indicates that phenolate is a ligand with relatively weak LFS. Comparing the NIR band position of the imidazole derivative of catalase with that of the aqua or

hydroxy forms of myoglobin and HRP (*ca.* 1050 and 1000,[3b] and 1040 and 1050 nm,[3c] respectively), it can be concluded that the LFS of phenolate is approximately comparable with that of water and hydroxyl anion.

Figure 4 shows the NIR absorption and MCD spectra of H8PT. Two absorption peaks appeared at 1510 and 1000 nm at 20 °C, while another peak was discerned at *ca.* 1250 nm at cryogenic temperature. On lowering the temperature, the intensity at 1000 nm decreased while that at 1510 nm increased. In the MCD spectrum, a set of dispersion-type curves was observed corresponding to the absorption peak at around 1000 nm, while the spectroscopic pattern between *ca.* 1100 and 1800 nm is similar to that of the absorption spectrum. With decreasing temperature, the MCD magnitude between *ca.* 1100 and 1800 nm increased linearly with the reciprocal of absolute temperature (the inset), and the extrapolation of the temperature dependence at 1510 nm approached zero at infinitely high temperature, indicating that this band is composed mainly of Faraday *C* terms.[3d,e] From these observations, the absorption at 1510 and 1250 nm can be assigned as CT bands by low-spin components, and the absorption peak at 1000 nm to a CT band by high-spin components. The aqua and hydroxy forms of H8PT are known to be in thermal equilibrium between high- and low-spin states.[10] The above dependence of absorption intensity on temperature indicates that H8PT is in thermal equilibrium between high- and low-spin states even in the cryogenic solvent. Judging from the energy difference (1.38 K cm^{-1}), the peak at 1250 nm appears to be a vibrational component of the band at 1510 nm. Thus the NIR MCD of H8PT supports the result obtained in the UV-visible region. In addition, the position of the NIR band of the low-spin component (1510 nm) suggests that the sum of LFS of the 5th and 6th ligands is smaller than that in typical low-spin complexes such as cytochrome c (1720 nm),[3d] and the cyanide form of myoglobin (1530 nm)[3b,e] or HRP (1680 nm).[3c]

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