Near-infrared Magnetic Circular Dichroism Studies on Iron(III) Horse Heart Cytochrome c

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(Received June 30, 1980)

Synopsis. Magnetic circular dichroism (MCD) spectra in the near-infrared region reported first for oxidized cytochrome c in its neutral (pH 5.58), alkaline (pH 11.62), and acidic (pH 1.88) forms exhibit temperature dependence, indicating that they are composed of Faraday C terms, except for the acidic form. The relation between the near-infrared MCD and the heme vicinity is discussed in terms of the structures for the heme chromophore of cytochrome c.

Near-infrared MCD spectra for heme chromophores are very sensitive to the oxidation, spin and ligand states of hemoproteins.[1,2] This is due to the fact that the bands ascribable to the charge-transfer transitions among iron, porphyrin and axial ligand, and d-d transitions of the iron ion can be easily resolved because of the absence of strong π-π^* transitions.[3,4] However, only a few studies[1,2,5-7] have been reported on the observation of near-infrared MCD, especially its temperature dependence, because of technical difficulties. The difficulties were overcome by use of a near-infrared MCD spectropolarimeter and deuterated solvents. We wish to report the accurate temperature dependence of near-infrared MCD for several forms of iron(III) cytochrome c.

Experimental

Materials. Cytochrome c from horse heart (Sigma type VI) was dissolved in deuterium oxide without further purification. Complete oxidation was attained by adding slight excess of freshly prepared deuterium oxide solution of potassium hexacyanoferrate(III). For the temperature variation experiments, an appropriate solution was diluted with hexadeutrated ethylene glycol at 60 (v/v) percent. The pH was adjusted by adding the least amount of concentrated sodium hydroxide or hydrochloric acid solution with stirring. The concentrations were determined on the basis of the heme molar concentrations from the visible absorption spectra.

Measurements. Absorption spectra were measured with a Hitachi EPS-3T spectrophotometer, MCD being recorded on a JASCO J-200 spectropolarimeter equipped with an electromagnet which affords up to 1.47 T magnetic field. MCD magnitude is expressed by the 10^{-4} molar ellipticity per Tesla ($[\theta]_M/10^{-4}$ °mol^{-1} dm^3 m^{-1} T^{-1} or 10^{-7} °m^2 mol^{-1} T^{-1}). Temperature was controlled by means of a stream of cold nitrogen gas. The path lengths of cells were 10 mm for the measurement at ambient temperature and 3 mm for that at cryogenic temperature.

Results and Discussion

MCD and absorption spectra are shown for iron (III) cytochrome c at neutral and at alkaline pH solutions in Figs. 1 and 2, respectively. The general spectral patterns of MCD are similar to those of absorption spectra, being characteristic for iron(III) low-spin heme complexes such as metmyoglobin cyanide.[1,6] The MCD spectra show the largest absorption peak. The positions of the strongest MCD peaks for neutral cytochrome c and alkaline cytochrome c are at 1720 and 1460 nm, respectively. The cyanide derivatives of metmyoglobin[1] and peroxidase[2] reveal MCD peaks at 1530 and 1680 nm, respectively. Since the iron (III) heme bands in the near-infrared region have been assigned as charge-transfer transitions from porphyrin a_{1u}, $a_{2u}(\pi)$ to $e_g(d\pi)$ orbitals ($E_u \leftarrow A_{1g}$) in D_{4h} symmetry, the energy differences in the peak positions for these low-spin species depend mostly on the energy levels of $e_g(d\pi)$ orbitals which vary more sensitively with the axial ligation mode than those of the porphyrin π orbitals. Theoretical consideration

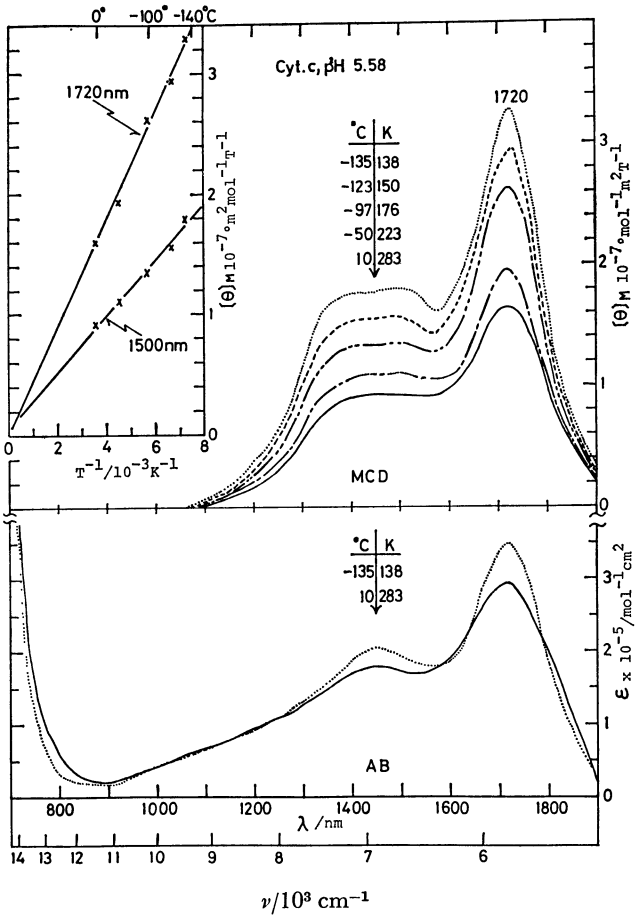

Fig. 1. MCD and absorption spectra of iron(III) cytochrome c at p²H 5.58. Cell length; 0.3 cm, concentration; 4.5×10^{-3} mol dm^{-3}, field; 1.47 T. The inset shows plots of the MCD magnitudes at 1720 and 1500 nm *vs.* the reciprocal of the absolute temperature.

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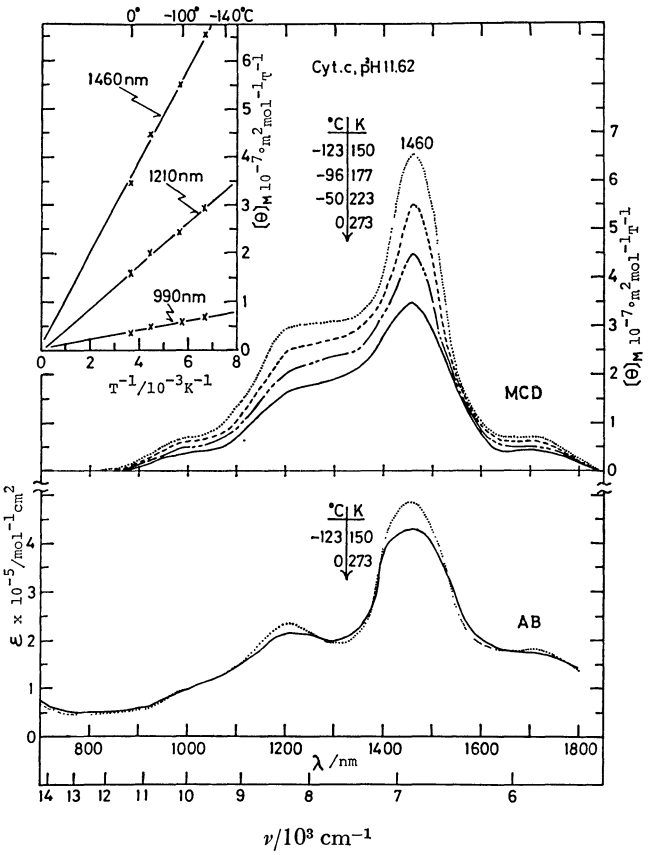

Fig. 2. MCD and absorption spectra of iron(III) cytochrome c at p²H 11.62. Other conditions are the same as for Fig. 1. The inset shows the MCD magnitudes of the peaks at 1460, 1210, and 990 nm *vs.* the reciprocal of the absolute temperature.

on the spectra of iron(III) low-spin heme derivatives[8] indicates that these peaks are composed mainly of the vibrational overtones superposed on the two electronic (charge-transfer) transitions.

The effect of wider temperature range on the near-infrared MCD of iron(III) cytochrome c is shown in Figs. 1 and 2. For metmyoglobin cyanide,[1] the shape of MCD did not change with lowering in temperature as in the Soret bands, while the MCD magnitude increased linearly with the reciprocal of absolute temperature. The extrapolation of temperature dependence at the peaks around 1720, 1500, 1460, 1210, and 990 nm approaches zero at infinite temperature, indicating that these bands are composed of pure Faraday C terms.

Figure 3 shows the MCD and absorption spectra of iron(III) cytochrome c in an acidic solution. A dispersion type MCD, characteristic of iron(III) high-spin heme, was obtained corresponding to the absorption peak at 1000 nm. For native metmyoglobin the

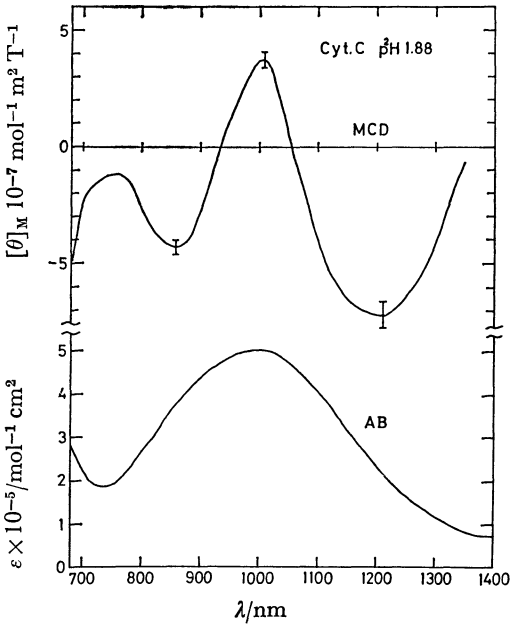

Fig. 3. MCD and absorption spectra of iron(III) cytochrome c at p²H 1.88. Cell length 1.0 cm. Concentration; 0.6×10^{-3} mol dm^{-3}. Temperature; 20 °C.

curve is non-symmetrical with respect to the line of $[\theta]_M=0$, differing from metmyoglobin fluoride which displays a clear S-shaped dispersion.[1] The MCD spectrum showed a slight change in magnitude with lowering in temperature. The band was confirmed to consist predominantly of Faraday A terms plus B terms. A similar spin state is suggested between this species and metmyoglobin from the near coincidence of the crossover points and relative intensities of the MCD peak (1010 nm) and trough (1210 nm).

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