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Characterization of the cadmium(II) binding site in Cd, Zn-metallothionein by magnetic circular dichroism spectroscopy.

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SUMMARY: Absorption and magnetic circular dichroism spectra of rat liver Cd, Zn-metallothionein, and the cadmium complexes of propanethiolate and 1,2 propanedithiolate are reported. Observation of the same derivative-like MCD signal in the 250 nm region of each of these species provides experimental evidence for the assignment of the 250 nm shoulder in the Cd, Zn-metallothionein absorption spectrum as a sulfur to cadmium charge transfer band.

INTRODUCTION

A low molecular weight protein (ca. 6800) known as metallothionein (MT) containing the metal ions Cd^{2+}, Zn^{2+} and Cu^{+} can be extracted from the livers of rats following injections of aqueous solutions of $CdCl_2$ (1-6). Although a wide variety of metal ions can be incorporated into MT *in vivo* and *in vitro*, the Cd, Zn-MT has been most extensively studied spectroscopically (7-13). The slight shoulder at 250 nm in the UV absorption spectrum which is on the side of a rapidly rising background absorption is commonly used as an identifier for the presence of the Cd-metalloprotein(2,7). Owing to the lack of aromatic amino acids there is no significant absorption from metal-free MT to the long wavelength side of 240 nm(7,13). Although the absorption at 250 nm can be used to determine the concentration of solutions of Cd, Zn-MT, the resolution of the bands is so poor that a complete assignment of the transitions that give rise to the observed band envelope is very difficult (2,12). Additionally, while model compound spectra, for example, the Cd(II) mercaptoethanol complex (4), suggest that a band in this region is due to charge transfer, the 250 nm band in the protein

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is almost completely overlapped by a much more intense band to lower wavelengths (7,12,13).

Magnetic circular dichroism (MCD) spectroscopy has been shown to provide the additional assignment criteria necessary to identify specific transitions from a set of overlapping transitions as in the case of the Cd, Zn-metallothionein spectra (14). In this paper, we present absorption and MCD data that do indeed show that the MCD spectra identify the transitions that arise from sulfur to cadmium charge transfer and provide the necessary support for the assignment of the 250 nm shoulder as a transition that does involve the cadmium.

MATERIALS & METHODS

Male Sprague-Dawley rats were treated with ten daily 1 mg Cd/kg body weight injections over a two week period. The metallothionein was purified and freeze dried as previously reported (15). The MT was further purified by passage down a Sephadex DEAE column. The pH at this stage was 8.5. The pH of a solution in a cuvette was reduced by adding μL aliquots of concentrated HCl. Spectra were recorded immediately after addition of the acid. The propanethiolate and 1,2 propanedithiolate complexes of cadmium were prepared as previously described (16). Absorption spectra were recorded on a Cary 219 spectrophotometer. CD measurements were made with a JASCO CD/ORD-5 which had been modified to Sproul SS-20 specifications. Magnetic circular dichroism spectra were also recorded on the JASCO J5 spectrometer using an Oxford Instruments SM2 superconducting magnet operating at 5.5T. The spectrometer was calibrated as described previously (14). The value of $[\theta]_M$ at 510 nm for an aqueous solution of $CoSO_4$ was -61.6 deg cm^2 $dmol^{-1}T^{-1}$. The CD and MCD spectra were digitized directly from the spectrometer using a device constructed in this laboratory (14). Each MCD spectrum reported here has had the corresponding zero field CD spectrum subtracted from it.

RESULTS AND DISCUSSION

The absorption spectrum of rat liver Cd, Zn-MT recorded at pH 8.5, Fig. 1, is as previously reported (13). A distinct shoulder near 250 nm on the rising background, and very low absorption at 280 nm is characteristic of cadmium in metallothionein. Although it is difficult to determine the number of transitions buried in this shoulder, the CD spectrum of Cd, Zn-MT, Fig. 1, where the Cd/Zn mole ratio is greater than 3 (actually close to 10 in the sample used in this spectrum), does indicate the presence of two main bands. These most likely arise from the stereochemical splitting of a degenerate transition which, in a chiral

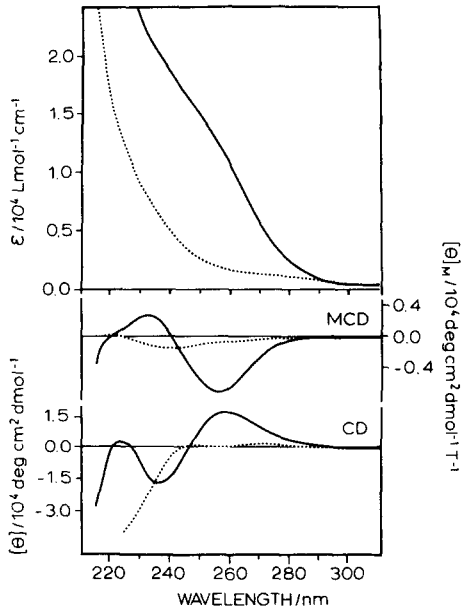

Figure 1. The absorption (top), MCD (middle) and CD (bottom) spectra of Cd, Zn-metallothionein recorded at pH 8.5 (solid line) and pH 2.3 (dotted line). The concentration of the protein was 2.79 x 10^{-5} M; a magnetic field of 5.5T was used for the MCD spectrum.

environment, give rise to a positive band at 260 nm and a negative band at 238 nm in the shoulder region; a third transition near 220 nm contributes a positive CD signal but because of the large overlap of the negative band due to the protein itself it is not possible to determine either the number of bands present or their approximate band centres.

The MCD spectrum of the Cd, Zn-MT, Fig. 1, clearly identifies two transitions under the 250 nm shoulder that result in a negative trough at 256 nm and positive peak at 232 nm. This signal could arise from either two overlapping and oppositely-signed B terms or from a positive A term (ie. positive because the positive lobe is to high energy of the cross-over point) in the event that the stereochemistry around the cadmium(II) is close to an undistorted tetrahedral symmetry (10-12,17).

Absorption and MCD spectra of two thiolatocadmate species that can be considered chromophoric models of the cadmium binding site in the metallothionein are shown in Fig. 2. These propanethiolate complexes are representitives of a large series of thiolatocadmates for which multinuclear nmr and optical data have been obtained

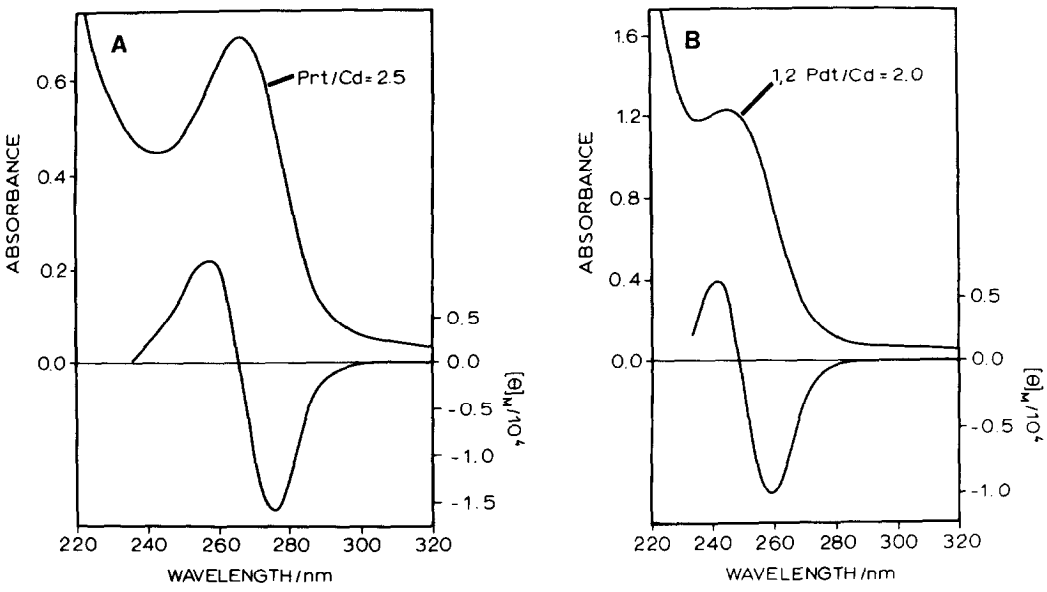

Figure 2. Absorption and MCD spectra of propanethiolate (Prt), A, and 1,2 propanedithiolate (1,2 Pdt), B, complexes with Cd^{2+}. The concentrations used were for A: [Prt] = 0.00011M and $[Cd^{2+}]$ = 0.000044M, and [1,2 Pdt] = 0.00024M and $[Cd^{2+}]$ = 0.00012M. A 1.0 cm pathlength cell was used for A and a 0.5 cm pathlength cell was used for B. The units for both MCD ordinates are deg cm^2 $dmol^{-1}$ T^{-1}.

(16). Both the monothiolate (Prt), Fig. 2A, and the dithiolate (1,2 Pdt), Fig. 2B, species exhibit new, well-resolved absorption bands near 250 nm upon complexation with cadmium(II). The MCD spectrum for both complexes approximates a positive A term which is centred on the absorption band centre of 266 nm for Prt^- and 246 nm for 1,2 Pdt^{2-}.

It is clear that the absorption spectra of the protein and the two model compounds do not match very well. This is, of course, not at all unreasonable, because the high-energy transitions (i.e. below 230 nm) arise from quite different chromophores. The MCD spectra on the other hand do match quite closely. This means that we can identify the cadmium based transitions in the protein from amongst others that do not exhibit the characteristic positive derivative signal.

Analysis of the absorption and MCD spectra of a wider range of thiolatocadmates (16) suggests that as the stereochemistry about the cadmium(II) departs from tetrahedral geometry so the MCD peak-to-trough intensity also diminishes. In

addition, the separation between the positive and negative components of the derivative signal increases as the tetrahedral geometry is lost. The MCD spectra of the thiolatocadmates closely resemble the spectra reported by Schreiner et al. (18) for a series of tetrahalomercurates, and an assignment of the transitions near 250 nm in Figs. 1 and 2 as charge transfer from 3p (S) to 5s and 5p (Cd^{2+}) seems reasonable. The CD spectrum (available only for the protein) provides further information on the number of transitions that lie in the 250 nm region. The positive and negative bands are very close in wavelength to the MCD features which suggests that the geometrical effects on the excited states may result in two bands in which the zero field splitting is such that the MCD intensity is considerably reduced from that expected for the degenerate states generated by a purely tetrahedral symmetry.

In conclusion, the MCD spectrum of Cd, Zn-MT, where the mole ratio of Cd to Zn is large, is dominated by a derivative signal that arises from sulfur to cadmium charge transfer transitions. This derivative signal is also characteristic of the spectra observed for thiolate complexes of cadmium at approximately the same wavelengths. Under the required sensitivity conditions the MCD spectrometer is not sensitive to the rising background absorption of the amino acid residues in the protein which means that the MCD spectrum provides a considerably more precise indication of the origin of the absorption intensity in the 250 nm region.

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REFERENCES

1. Margoshes, M., Vallee, B.L., (1957) J. Am. Chem. Soc., 79, 4813-4814.
2. Kagi, J.H.R., Nordberg, M., eds. (1979) in Metallothionein, Birhauser Verlag, Basel.
3. Webb, M., (1975) Biochem. Soc. Trans., 3, 632-634.
4. Kagi, J.H.R., Vallee, B.L., (1961) J. Biochem. 236, 2435-2442.
5. Cherian, M.G., (1977) J. Toxicology & Environ. Health, 2, 955-961.

6. Cherian, M.G., (1977) J. Nutr., 107, 965-971.
7. Weser, U., Rupp, H., Donay, F., Linnemann, F., Voelter, W., Voetsch, W., and Jung, G., (1973) Eur. J. Biochem., 39, 127-140.
8. Rupp, H., Voelter, W., and Weser, U. (1975) Z. Physiol. Chem., 356, 755-765.
9. Sokolowski, G., Weser, U., (1975) Z. Physiol. Chem., 356, 1715-1726.
10. Rupp, H., Weser, U. (1978) Biochim Biophys Acta, 533, 209-226.
11. Otvos, J.D., Armitage, I.M., (1979) J. Am. Chem. Soc., 101, 7734-36.
12. Vasak, M., Kagi, J.H.R., Hill, H.A.O. (1981) Biochemistry, 20, 2852-2856.
13. Law, A.Y.C., Stillman, M.J. (1980) Biochem. Biophys. Res. Commun., 94, 138-143.
14. Browett, W.R., Stillman, M.J. (1980) Biochem. Biophys. Acta, 623, 21-31.
15. Cherian M.G. (1974) Biochem. Biophys. Res. Commun., 61, 920-926.
16. Carson, G.K., Dean, P.A.W., Stillman, M.J. (1981) Inorg. Chim. Acta, in press.
17. Vasak, M., (1980) J. Am. Chem. Soc., 102, 3953-55.
18. Gunter, J.D., Schreiner, A.F. and Evans, R.S., (1975) Inorg. Chem., 14, 1589-1592