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## Zinc(II), Cadmium(II), and Mercury(II) Thiolate Transitions in Metallothionein<sup>†</sup>

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**ABSTRACT:** The metal-specific absorption envelopes of zinc-, cadmium-, and mercury-metallothioneins and of complexes of these metal ions with 2-mercaptoethanol have been analyzed in terms of Jørgensen's electronegativity theory for charge-transfer excitations by using the spectra of zinc(II), cadmium(II), and mercury(II) tetrahalides as references. By Gaussian analysis the difference absorption spectra of the various forms of metallothionein vs. thionein and of the corresponding 2-mercaptoethanol complexes vs. 2-mercaptoethanol were resolved into three components. For each metal derivative the location of the lowest energy band is in good agreement with the position of the first ligand-metal charge-transfer (LMCT) transition (type  $t_2 \rightarrow a_1$ ) predicted from the optical electronegativity difference of the thiolate ligands and of the central metal ion by assuming tetrahedral coordination. There is also a correspondence between the

effects of the metal ion on the position of the first LMCT band and the binding energy of the 2p electrons of the sulfur ligands as found by X-ray photoelectron spectroscopic measurements [Sokolowski, G., Pilz, W., & Weser, U. (1974) *FEBS Lett.* 48, 222]. Due to the lack of exact structural information, the assignment of the two other resolved metal-dependent bands remains conjectural, but it is likely that they include a second LMCT transition (type  $t_2 \rightarrow a_1$ ) analogous to that occurring in tetrahalide complexes of group-2B metal ions. The simplicity of the resolved thiolate spectra and their correspondence to those of tetrahedral models support the view that the various metal-binding sites of metallothionein are chemically similar and that the coordination environment of the metal ion has a symmetry related to that of a tetrahedron [Vašák, M. (1980) *J. Am. Chem. Soc.* 102, 3953].

**M**etallothionein is a cysteine- and metal-rich protein which was first isolated from the equine renal cortex by Margoshes & Vallee (1957). The same protein has since been found in the liver, kidney, and intestine of numerous other vertebrate

species and recently also in a eukaryotic microorganism (Lerch, 1979). All mammalian forms characterized to date contain a single polypeptide chain with 20 cysteinyl residues in a total of 61 amino acid residues and have a chain weight of 6100. The best studied mammalian forms contain a total of 6-7 g-atoms of zinc or of a mixture of zinc and cadmium per mole (Kojima & Kägi, 1978). All metallothioneins are devoid of aromatic amino acids and histidine. The biosynthesis of the protein occurs in the liver, kidney, and intestinal wall and is

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specifically induced by the administration of zinc and cadmium salts, implicating a regulatory function of these proteins in metal metabolism, homeostasis, or detoxification (Nordberg & Kojima, 1979).

Spectroscopic measurements (Kägi & Vallee, 1961) and studies by X-ray photoelectron spectroscopy (Weser et al., 1973) have shown that, on addition of cadmium or zinc ions to the metal-free protein, all cysteinyl side chains are deprotonated, suggesting that the metal ions are bound by thiolate coordination. The amino acid sequence shows appreciable regularity in the occurrence of the cysteinyl residues. Thus, within the polypeptide chain, 14 of the 20 cysteines (Cys) occur 7 times in Cys-X-Cys sequences, where X stands for an amino acid residue other than Cys. These structures have been suggested to serve as primary binding sites for the metals. Once formed, these primary complexes are thought to be completed by additional thiolate ligands that are brought into position by appropriate chain folding (Kojima et al., 1976). Recent  $^{113}\text{Cd}$  NMR measurements suggest, moreover, that these complexes are joined to clusters by bridging cysteinyl sulfur ligands affording tetrathiolate coordination for each metal ion (Otvoš & Armitage, 1979). This is also confirmed by optical and ESR<sup>1</sup> studies of cobalt(II)-metallothionein (Vašák, 1980). Judged from the spectral data of the cobalt complexes, all metal sites exhibit similar pseudotetrahedral symmetry.  $^1\text{H}$  NMR measurements in the region of the amide proton resonances also indicate that native metallothioneins possess a well-defined spatial structure (Vašák et al., 1980).

Direct evidence for the existence of cadmium(II)- and/or zinc(II)-thiolate complexes in metallothionein comes from the ultraviolet absorption spectra which show as their most conspicuous features characteristic shoulders at 4.00 and 4.55  $\mu\text{m}^{-1}$  and which are typical of cadmium and zinc thiolate ligation, respectively (Kägi & Vallee, 1961). The cadmium-specific shoulder at 4.00  $\mu\text{m}^{-1}$  has also been observed in the spectra of horse liver alcohol dehydrogenase (Drum & Vallee, 1970) and aspartate transcarbamoylase (Rosenbusch & Weber, 1971) after the replacement of zinc(II) by cadmium(II). Based on their spectral position and their intensity, the long-wavelength shoulders were attributed tentatively to charge-transfer transitions of the metal-thiolate complexes (Kägi & Vallee, 1961). However, no detailed analysis of these far-ultraviolet absorption bands has been made thus far. The broadness of the metal-induced absorption profiles and the complexity of the circular dichroism spectra associated with them (Rupp & Weser, 1978; Bühler & Kägi, 1979) suggest the existence of several close-lying transitions.

In the present study, an analysis of the absorption envelope of various forms of metallothionein and of model metal thiolates is undertaken, and the data are interpreted in a manner analogous to that used for the interpretation of the spectra of zinc(II), cadmium(II), and mercury(II) tetrahalides (Day & Seal, 1972) incorporating Jørgensen's concept of optical electronegativity (Jørgensen, 1970).

## Materials and Methods

Equine metallothioneins were isolated from liver or kidney by established methods (Kägi et al., 1974; Kojima et al., 1976). The purity of each preparation was checked by determining the amino acid composition and by metal analysis using atomic absorption spectroscopy (Instrumentation Laboratory Model IL 157). The cadmium-reconstituted form of metallothionein

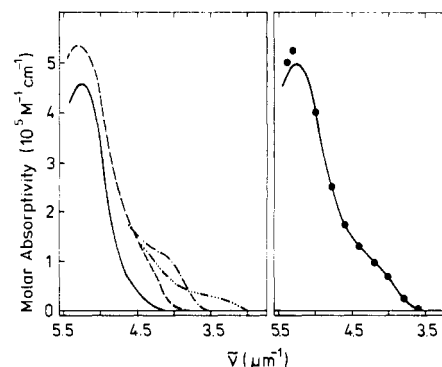


FIGURE 1: UV-absorption spectra of metallothionein. (Left) Thionein (—) in 0.1 M HCl; zinc-metallothionein (---), cadmium-metallothionein (-.-), mercury-metallothionein (....), in 0.001 M sodium phosphate buffer, pH 7.5. (Right) (Cadmium, zinc)-metallothionein (65 mol % Cd, 35 mol % Zn) in 0.001 M sodium phosphate buffer (pH 7.5) (solid line). The dots reflect the sum of the molar absorptivities of solely zinc- or cadmium-containing forms of metallothionein when taken in proportion to the relative abundance of the two metals in the preparation.

was obtained by dialysis of equine kidney (cadmium, zinc)-metallothionein against excess of cadmium ions, at pH 5.4, as described elsewhere (Kägi & Vallee, 1961). The mercury-reconstituted form of metallothionein was prepared by addition of the stoichiometric amounts of mercury(II) sulfate to zinc-metallothionein at pH 7.5. It resulted in mercury-metallothionein possessing 7 g-atoms of mercury(II)/mol (R. H. O. Bühler and J. H. R. Kägi, unpublished results). Zinc-metallothionein was obtained from equine liver (Kägi et al., 1974). The absorption spectra in the region above 200 nm were recorded by means of a Perkin-Elmer Model 340 spectrophotometer using 0.2 cm pathlength quartz cells. The region below 200 nm was measured in a Cary 15 spectrophotometer purged with nitrogen using 0.02 cm pathlength quartz cells.

Thionein (apoprotein) was prepared by dialyzing metallothionein against three changes of 0.1 M HCl. The dialysis tubing employed had a molecular weight cutoff of 3500 (Spectrapor Co.). Spectrophotometric quantitation of thionein concentration was carried out in 0.1 M HCl at 220 nm by using a molar absorptivity of 47 300 (Bühler & Kägi, 1979). Absorption spectra of metallothionein were recorded on solutions containing 0.001 M sodium phosphate buffer, pH 7.5. Difference absorption spectra of cadmium- and zinc-metallothionein vs. thionein were obtained with solutions containing the same concentration of metallothionein in 0.1 M NaCl and 0.01 M sodium phosphate buffer, pH 7.5 (sample cell), and in 0.1 M HCl (reference cell). Since at pH 7.5 thionein displays some thiolate absorption, the fully protonated form (in 0.1 M HCl) was chosen as a reference in the difference absorption spectra of metallothionein. The zinc(II) and cadmium(II) complexes with 2-mercaptoethanol were prepared by adding 0.12  $\mu\text{mol}$  of  $\text{CdCl}_2$  or  $\text{ZnSO}_4$  and 20  $\mu\text{mol}$  of 2-mercaptoethanol to 3 mL of 0.03 M sodium phosphate buffer, pH 7.5. Difference absorption spectra were recorded vs. a reference solution containing the same concentration of 2-mercaptoethanol but no metal. Measurement of stoichiometry by the method of molar ratios resulted in 2.5–3 mercapto ligands/metal ion (J. H. R. Kägi, unpublished results). For the line shape analysis, a Du Pont 310 curve resolver was employed.

## Results and Discussion

**Absorption Spectra.** Figure 1 (left) reveals the absorption spectra of fully protonated thionein (apometallothionein) and

<sup>1</sup> Abbreviations used: ESR, electron spin resonance; LMCT, ligand-metal charge transfer.

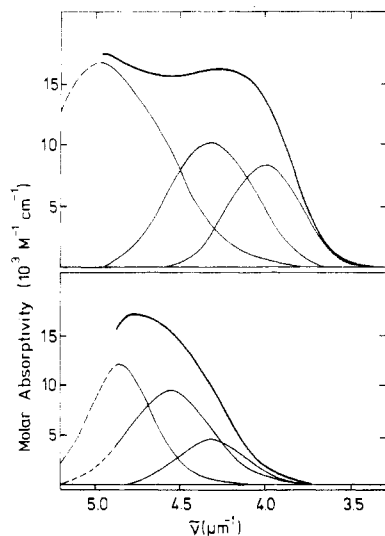


FIGURE 2: UV-difference absorption spectra of cadmium-metallothionein (top) and zinc-metallothionein (bottom) vs. thionein with Gaussian analysis. The molar absorptivity refers to the metal. For conditions, see Material and Methods.

those of metallothioneins containing the group-2B metals zinc, cadmium, and mercury. The distinguishing features of thionein are its sole absorption band at  $5.25 \mu\text{m}^{-1}$  and the total absence of absorbance below  $4.16 \mu\text{m}^{-1}$  ( $>240 \text{ nm}$ ) which reflects the lack of aromatic amino acid residues in the protein. The  $5.25\text{-}\mu\text{m}^{-1}$  band is thought to include in addition to the strong  $\pi\text{-}\pi^*$  amide transition near  $5.40 \mu\text{m}^{-1}$  a weak  $n\text{-}\pi^*$  amide transition near  $4.55 \mu\text{m}^{-1}$  and the lowest thiol transition of the cysteinyl side chains near  $5.13 \mu\text{m}^{-1}$  (Gratzner, 1967).

The binding of group-2B metal ions to the apoprotein intensifies the far-UV absorption and introduces at the red edge characteristic absorption shoulders whose positions differ as a function of the metal. The zinc- and cadmium-induced shoulders at  $4.55$  and  $4.08 \mu\text{m}^{-1}$ , respectively, are also observed in thiolate model complexes of the same metal ions and have on this evidence been postulated to arise from complexes of cadmium(II) and zinc(II) with polydentate thiolate binding sites of thionein (Kägi & Vallee, 1961). By analogy, the mercury(II)-induced shoulder at  $3.30 \mu\text{m}^{-1}$  can also be attributed to the binding of mercury(II) to the same polydentate thiolate binding sites.

Many naturally occurring metallothioneins contain both zinc and cadmium (Kojima & Kägi, 1978). This heterogeneous and varied metal composition is also reflected in their absorption spectra. Figure 1 (right) shows the spectrum of a preparation of equine renal metallothionein with a relative metal composition of 65% cadmium and 35% zinc. Also included in the figure are values for the theoretical molar absorptivities of this material calculated from the spectra of solely zinc- or cadmium-containing metallothionein and from the relative abundance of the two metals in the preparation.

**Difference Absorption Spectra.** Since at energies below  $5.00 \mu\text{m}^{-1}$  ( $>200 \text{ nm}$ ) the absorption of a polypeptide chain of the composition of thionein will not be changed appreciably by conformational changes which may accompany metal binding, it is feasible to assess the absorptive features of the metal-thiolate complexes from the difference absorption spectra of metallothionein vs. thionein. Such difference spectra obtained with cadmium- and zinc-metallothionein are shown in Figure 2. Both spectra reveal composite absorption profiles typical for each metal and similar to difference absorption spectra of the corresponding complexes of the two metal ions formed in the presence of excess 2-mercaptoethanol (Figure 3).

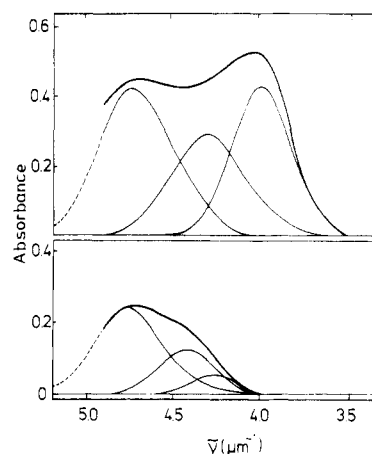


FIGURE 3: UV-difference absorption spectra of complexes of cadmium(II) (top) and zinc(II) (bottom) formed in the presence of a large excess of 2-mercaptoethanol vs. 2-mercaptoethanol with Gaussian analysis. For conditions, see Material and Methods.

To resolve some of the contributory transitions, we submitted the difference absorption spectra to an empirical line shape analysis assuming Gaussian shape for the component bands. As shown in Figures 2 and 3, each absorption profile can be fitted to a minimum of three Gaussian bands. Thus, the difference spectrum of cadmium-metallothionein can be resolved into bands with maxima at  $4.00$ ,  $4.33$ , and  $4.97 \mu\text{m}^{-1}$  (Figure 2, top). Similarly, for the complex of cadmium(II) with 2-mercaptoethanol, the maxima are at  $3.98$ ,  $4.30$ , and  $4.74 \mu\text{m}^{-1}$  (Figure 3, top). The existence of at least three cadmium-induced bands in this spectral region is also supported by circular dichroism (Rupp & Weser, 1978) and magnetic circular dichroism (MCD) data. Cadmium-metallothionein exhibits a negative MCD band at  $3.92 \mu\text{m}^{-1}$  and two positive MCD bands at  $4.30$  and  $4.60 \mu\text{m}^{-1}$ , respectively (M. Vašák, unpublished experiments).

In the zinc-thiolate difference absorption spectra, the resolved three bands are spaced with maxima at  $4.33$ ,  $4.57$ , and  $4.87 \mu\text{m}^{-1}$  for zinc-metallothionein (Figure 2, bottom) and at  $4.25$ ,  $4.45$ , and  $4.80 \mu\text{m}^{-1}$  (Figure 3, bottom) for the complex with 2-mercaptoethanol. In both zinc complexes the band of lowest energy is hidden in the descending portion of the difference absorption envelope and has, for this reason, escaped notice until now. It is noteworthy, though, that the occurrence of a zinc-induced absorption band in this region has been postulated in order to account for the positive ellipticity band centered near  $4.20 \mu\text{m}^{-1}$  (Bühler & Kägi, 1979).

Preliminary measurements (not shown) indicate that the Gaussian analysis of the metal-induced absorption shoulder of mercury-metallothionein (Figure 1, left) requires the use of more than three components. The low-energy region alone ( $<4.00 \mu\text{m}^{-1}$ ) can be fit to three Gaussian bands with maxima near  $3.3$ ,  $3.4$ , and  $3.6 \mu\text{m}^{-1}$ .

**Assignment of Metal-Dependent Transitions.** The similarity of the metal-induced absorption shoulders and of their resolved Gaussian components in complexes of group-2B metal ions with thionein and 2-mercaptoethanol (Table I) documents their common origin in metal-thiolate coordination (Kägi & Vallee, 1961). An analogous strong metal ion dependency of the position of the lowest energy absorption bands is seen in complexes of group-2B metal ions with many other ligands and is thought to be diagnostic of a ligand-metal charge-transfer (LMCT)<sup>1</sup> origin of these bands. The extent of the shift has been connected by Jørgensen with the concept of the electronegativity of the atoms linked in the complex (Jørgensen, 1970). According to this theory, the predicted

Table I: Resolved Bands in Charge-Transfer Region of Different Metal Complexes of Equine Liver Metallothionein and of 2-Mercaptoethanol and Their Comparison with Calculated Values Based on Optical Electronegativities for  $T_d$  Symmetry Complexes<sup>a</sup>

metal	spectral position of metal-induced shoulder in metallothionein [ $\mu\text{m}^{-1}$ (nm)]	contributing bands as suggested by Gaussian analysis		calcd position of LMCT bands <sup>e,f</sup> [ $\mu\text{m}^{-1}$ (nm)]	individual transitions
		metallothionein [ $\mu\text{m}^{-1}$ (nm)]	2-mercaptoethanol [ $\mu\text{m}^{-1}$ (nm)]		
zinc <sup>b</sup>	4.55 (220)	4.33 (231)	4.25 (235)	4.31 (232)	first second
		4.57 (219)	4.45 (225)	4.68 (214) (A)	
		4.87 (205)	4.80 (208)	or 4.97 (201) (B)	
cadmium <sup>c</sup>	4.08 (245)	4.00 (250)	3.98 (251)	4.01 (249)	first second
		4.33 (231)	4.30 (232)	4.38 (228) (A)	
		4.97 (201)	4.74 (211)	or 4.67 (215) (B)	
mercury <sup>d</sup>	3.30 (304)	~3.3 (303)	no data	3.21 (312)	first second
		~3.4 (294)	available	3.58 (280) (A)	
		~3.6 (278)		or 3.87 (258) (B)	

<sup>a</sup> For details, see Materials and Methods. <sup>b</sup> This form of metallothionein contained >96% zinc (Kägi et al., 1974). <sup>c</sup> Reconstituted sample contained 100% cadmium. <sup>d</sup> R. H. O. Bühler and J. H. R. Kägi, unpublished results. <sup>e</sup> Both transitions are of the type  $t_2 \rightarrow a_1$ . <sup>f</sup> The calculated position of the second LMCT band in thiolate complexes was obtained by adding to the position of the first LMCT band the average energy separation of the first two LMCT transitions of the tetrahedral bromide complexes (A) or of the tetrahedral iodide complexes (B) with the group-2B metals. The average energy difference for (A) is  $0.37 \mu\text{m}^{-1}$  and for (B)  $0.66 \mu\text{m}^{-1}$  (Day & Seal, 1972).

wavenumber  $\sigma$ , expressed in  $\mu\text{m}^{-1}$ , of the first allowed charge-transfer band of posttransition group complexes is given by the empirical expression

$$\sigma = (3.0 \mu\text{m}^{-1})[\chi_{\text{opt}}(\text{X}) - \chi_{\text{opt}}(\text{M})]$$

where  $\chi_{\text{opt}}(\text{X})$  and  $\chi_{\text{opt}}(\text{M})$  are the optical electronegativities of ligand (X) and metal (M), respectively. It follows from this relationship that for a set of homologous complexes of a given metal ion the relative position of the lowest transition is determined solely by the optical electronegativity of the ligand. Thus, from a plot of the transition energies of the first LMCT band of well-characterized reference complexes of a given posttransition metal vs. the optical electronegativities of their ligands, the positions of the first LMCT band of any homologous complex can be deduced provided the optical electronegativity of the ligand is known. For thiolate ligands the value  $\chi_{\text{opt}}(\text{X})$  reported is close to 2.6 (McMillin, 1978), yielding for complexes with group-2B metal ions LMCT band energies intermediate between those of the corresponding iodide and bromide complexes (Figure 4).

As shown in Table I, the positions predicted for the first LMCT band of thiolate complexes of zinc(II), cadmium(II) and mercury(II) (Figure 4) coincide closely with the spectral location of the first resolved Gaussian bands of zinc-, cadmium-, and mercury-metallothionein and of the corresponding complexes of these metal ions with 2-mercaptoethanol. The results identify these bands as the first Laporte-allowed electron-transfer transition. They are also supporting our view that in metallothionein the metal ions are bound in a tetrahedral type of coordination (Vašák, 1980).

From the measured energies of the first LMCT band of the metallothioneins containing zinc(II), cadmium(II), or mercury(II) and from  $\chi_{\text{opt}}(\text{RS}^-) = 2.6$ , one obtains by the above equation for the optical electronegativities of the metal  $\chi_{\text{opt}}(\text{M})$  the values 1.15 for zinc(II), 1.27 for cadmium(II), and 1.50 for mercury(II). Following the order of recalculated Pauling electronegativities (Allred, 1961), they are a measure of the relative magnitude of the electron-withdrawing effect of these metal ions on the ligand (Livingstone, 1965). Thus, the decrease in the energy of the first LMCT band on going from zinc- to cadmium- and mercury-metallothionein is a corollary of the increase in covalency of the ligand-metal bonds. It is also in agreement with X-ray photoelectron spectroscopic studies of metallothionein which showed metal-specific chemical shifts of the unresolved sulfur 2p levels. Following the

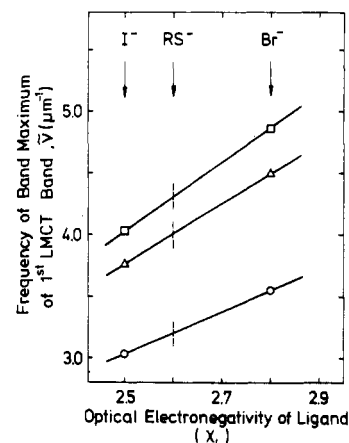


FIGURE 4: Graphical determination of first LMCT maximum of tetrahedral thiolate ( $\text{RS}^-$ ) complexes with zinc, cadmium, and mercury ions using the first LMCT bands of tetrahedral halide complexes of zinc ( $\square$ ), cadmium ( $\Delta$ ) and mercury ( $\circ$ ) ions as reference (Day & Seal, 1972). The optical electronegativity values ( $\chi_L$ ) used were 2.5 and 2.8 for iodide and bromide, respectively (Jørgensen, 1970), and 2.6 for thiolate (McMillin, 1978).

order of increasing optical electronegativity of the group-2B metal ions, the binding energies of the 2p electrons increase from 161.3 eV in zinc-metallothionein to 161.7 eV in cadmium-metallothionein and 162.7 eV in mercury-metallothionein (Sokolowski et al., 1974). Interestingly, the metal-induced increments in binding energy of the core electrons match closely the differences in energy between the first LMCT bands of these metallothioneins (Figure 5).

The unambiguous identification of the first resolved absorption band of the various metallothioneins as an LMCT transition suggests that some of the other bands may have a similar origin. The studies of Day & Seal (1972) have indicated that the lowest energy region of the absorption spectra of tetrahedral halide complexes of group-2B metal ions contains at least two LMCT transitions of the type  $t_2 \rightarrow a_1$ . According to the molecular orbital (MO) scheme employed by these authors, the first one corresponds to the electron transfer from the  $\pi$  MO of mainly ligand character to the lowest empty  $3a_1$  MO primarily located on the metal ( $3t_2 \rightarrow 3a_1$ ). The second allowed LMCT transition is thought to originate from a primarily ligand-located  $\sigma$  MO to the same metal-centered acceptor MO ( $2t_2 \rightarrow 3a_1$ ). Comparison of the positions of the two bands in tetrahalides of zinc(II), cad-

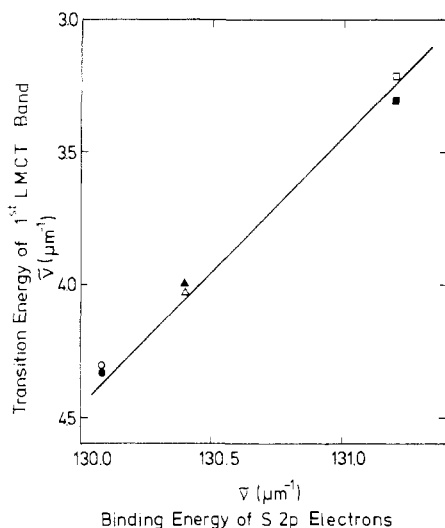


FIGURE 5: Correlation of electron binding energy of the 2p electrons of sulfur (unresolved  $2p_{1/2,3/2}$  doublet) in different metallothioneins with transition energy of the first LMCT excitation. Zinc-metallothionein (○), cadmium-metallothionein (Δ), mercury-metallothionein (□). Filled symbols refer to the observed first LMCT maximum; empty symbols refer to the position of the first LMCT maximum derived from Figure 4. The electron binding energies were taken from X-ray photoelectron spectra reported by Sokolowski et al. (1974) and were converted to wave number units.

mium(II), and mercury(II) (Day & Seal, 1972) shows that both are equally affected by the electronegativity of the central ion and that the average separation of the bands is typical of the ligand.

Based on the analogies between closed-shell thiolate anions and halide ligands, it is reasonable to predict that the spectra of the tetrahedral thiolate complexes likewise exhibit a second  $t_2 \rightarrow a_1$  LMCT band. When the assumption that the energy separation of the two thiolate LMCT bands also lies between that reported for tetrahedral bromide complexes ( $0.37 \mu\text{m}^{-1}$ ) and tetrahedral iodide complexes ( $0.66 \mu\text{m}^{-1}$ ) of group-2B metal ions (Day & Seal, 1972) is made, it is readily seen that either one of the two other metal-dependent Gaussian bands resolved in the difference absorption envelope of cadmium- or zinc-metallothionein (Figure 2) and of the complexes of zinc(II) and cadmium(II) with 2-mercaptoethanol (Figure 3) are possible candidates for the second  $t_2 \rightarrow a_1$  LMCT transition (Table I).

However, it is clear that for a more definite assignment of the empirically resolved high-energy bands additional spectroscopic and structural information is required. In the light of the recent finding that in metallothionein the metal complexes are organized as oligonuclear clusters with some of the cysteinyl side chains serving as bridging ligands, it remains to be determined how such thiolate bridges feature in the far-ultraviolet absorption profile (Dance, 1979; Otvos & Armitage, 1979). It is also an open question as to what degree ligand-internal transitions (Mastropaolo et al., 1977) and band-splitting caused by distortion of the tetrathiolate complexes affect the spectrum. The latter possibility seems especially likely, in view of the recent observation that cobalt(II)-substituted metallothionein exhibits a  $d \rightarrow d$  absorption profile that is typical of pseudotetrahedral coordination (Vašák, 1980).

In conclusion, we have obtained a satisfactory interpretation of the lowest energy band of the metal-thiolate absorption spectra of metallothionein in terms of Jørgensen's semi-empirical theory for charge-transfer excitations. The depen-

dency of the energy of the first LMCT transition ( $t_2 \rightarrow a_1$ ) on the central metal ions shows good correlation to the effect of the metals on the binding energy of the sulfur 2p core electrons. Due to the lack of exact structural information, the assignment of the remaining metal-dependent bands remains conjectural, but there is little doubt that they include a second LMCT transition, also  $t_2 \rightarrow a_1$  in  $T_d$  symmetry. The simplicity of the spectra and the similarity to those of model complexes reaffirm the view that the various metal-binding sites of metallothionein are geometrically and chemically similar (Kägi & Vallee, 1961). The reasonable correspondence of the resolved spectra with those of tetrahedral halide complexes is in line with recent suggestions that in metallothionein each metal ion is bound to sulfur ligands to yield a coordination environment with a site symmetry related to that of a tetrahedron (Otvos & Armitage, 1979; Vašák, 1980).

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