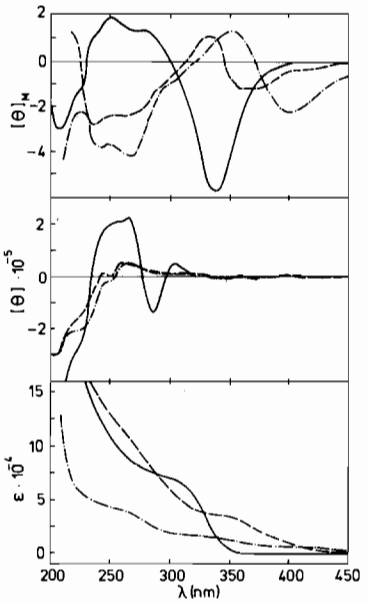

Fig. 1. MCD (top), CD (middle) and absorption (bottom) spectra of Hg(II)$_7$-metallothionein (———), Pb(II)$_7$-metallothionein (- - - - -) and Bi(III)$_7$-metallothionein (– · –). Units employed: ϵ (M^{-1} cm), θ (deg cm^2 dmol^{-1}), θ_M (deg cm^2 dmol^{-1} gauss^{-1}).

bolism, storage and detoxification of essential and nonessential trace metals. The vertebrate forms characterized to date contain a single polypeptide chain of 61 amino acid residues, among them 20 cysteines providing the ligands for 7 metal-binding sites. Native metallothioneins are usually heterogeneous in metal composition with Zn, Cd, Cu and occasionally other metals occurring in varying proportions [1]. However, forms containing only a single metal species, *i.e.*, Zn, Cd, Ni, and Co, have been prepared by reconstitution from the metal-free apoprotein [2, 3]. By spectroscopic analysis of such derivatives it was established unambiguously that all cysteine residues provide their thiolate ligands for metal binding, that each metal ion is bound to 4 thiolate ligands, that the symmetry of each complex is tetrahedral, and that in order to satisfy the requirements of the overall Me_7-$(Cys^-)_{20}$ stoichiometry, these ceomplexes are combined to form metal–thiolate clusters [4].

Mercury and bismuth are also occasional constituents of native metallothionein [5]. To explore the mode of binding of these and related ions, we have now prepared derivatives containing Hg(II), Pb(II) or Bi(III). With Hg(II) and Pb(II) different types of complexes are formed depending on pH and metal-to-protein ratio. At a stoichiometry of 7 metal ions per molecule with each of the three metals unique complexes are obtained in which all cysteine residues are likely to participate in metal binding. The absorption, circular dichroism (CD) and magnetic circular dichroism (MCD) spectra of these forms are highly characteristic (Fig. 1) and can be interpreted in terms of Jørgensen's optical electronegativity theory of electron transfer (CT) transitions of metal ligand complexes [6]. From the coincidence of the location of the first CT-transition of Hg_7- and Pb_7-metallothionein with those predicted theoretically for T_d-thiolate coordination of these metals, it appears very likely that in these derivatives of metallothionein, too, these metals are bound in T_d microsymmetry. The close similarity of the spectra of Bi_7- and Pb_7-metallothionein allows, furthermore, the inference that Bi(III) which is isoelectronic with Pb(II) is also bound in this fashion. Since T_d coordination is highly unusual for these metals, it appears that as in the earlier case of Ni_7-metallothionein [3], this geometry is imposed by the rigidly maintained thiolate-binding sites of the protein. That all three derivatives have the same overall structure is suggested independently by the observation that their Stokes' radii are identical.

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Metal–Protein Interaction in *Carcinus Maenas* Hemocyanin

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Hemocyanin (Hc) is the respiratory pigment next in importance to hemoglobin for its distribution in two large phyla of Invertebrates: Arthropoda and Mollusca. The characteristic of Hc as oxygen carrier has overshadowed interest in other properties of possible physiological meaning such as the pseudo-

TABLE I.

	Shell	Gill	Vas Def.	Heart	Epider.
Co	15.12	1.19	5.16	3.12	2.97
Mn	54.87	12.23	5.11	2.10	4.07
Zn	55.82	21.89	47.38	36.94	83.21

	Hepatop.	Muscle	Hc[a]	Serum[a]
Co	1.48	2.60	1.27	0.38
Mn	1.88	8.52	0.37	
Zn	40.51	115.48	22.08	0.88

[a] μg/ml; for all other values μg/g w.w.

TABLE II.

	n	k_A (M^{-1})
Co^{2+}	3	2.7×10^3
Mn^{2+}	8	7.8×10^3
Zn^{2+}	4	1.6×10^4

TABLE III.

Metal concentration	10^{-5} *M* F/F°	10^{-4} *M* F/F°
Co^{2+}	0.68	0.51
Mn^{2+}	1.18	1.16
Zn^{2+}	0.65	0.41

catalasic, the pseudoperoxidasic and pseudophenolasic activities, the possible contribution to the osmotic equilibrium *etc.*

Recently we have obtained indications that Hc could function in Crustacea in transporting metal ions from the external environment to the tissues [1]. In this communication we present preliminary data on the interaction between Hc and some metal ions. In Table I the quantitative distribution of Zn^{2+}, Co^{2+} and Mn^{2+} in the blood, the shell and tissues of *Carcinus maenas* is reported.

Equilibrium dialysis was carried out and the experimental data were used for calculating, according to the Scatchard procedure [2], the number of sites and the binding constants of Hc with the metal ions.

At metal ion concentration higher than 10^{-4} *M* the shape of the Scatchard plot indicates the presence of binding sites with lower affinity.

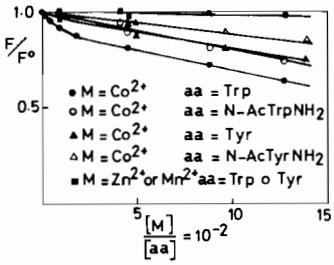

Fig. 1.

The binding of Co^{2+} and Zn^{2+} to Hc leads to a quenching of protein intrinsic fluorescence ($\lambda_{exc.}$ = 280 nm, emission λ_{max} = 330 nm).

The results are reported in Table III where F° is the value of the fluorescence intensity in the absence of metal.

In contrast, the fluorescence increases by about 10% when the protein binds Mn^{2+}.

To assess the nature of these changes, the effect of Co^{2+}, Zn^{2+} and Mn^{2+} (chloride salts were used) on the fluorescence properties of tyrosine and tryptophan have been studied in unbuffered water solutions. Zn^{2+} and Mn^{2+} do not exert any influence on the fluorescence emission of these aromatic aminoacids (Fig. 1).

As the binding of Zn^{2+} and Co^{2+} with aminoacids have similar values [3], the significant fluorescence quenching observed upon binding of Co^{2+} to Tyr and Trp can be attributed only to a paramagnetism of the cobalt ion. The absence of a 'paramagnetic ion' effect in the case of Mn^{2+} is probably due to very low values of the binding constant of Mn^{2+} with aminoacids as compared with those of the other ions [3].

The almost equal quenching values for Co^{2+} and Zn^{2+} (Table III) leads to the conclusion that, in our experimental conditions, Hc fluorescence quenching by both ions is due to a conformational modification of the protein, as a consequence of the metal binding. The opposite effect observed in the case of Mn^{2+} can be attributed to binding in different sites of the protein which causes a different conformational modification.

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