Domain Nature of Metallothionein*

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Metallothionein purified from the livers of rats injected with CdCl₂ was cleaved by proteolysis into a 32-residue polypeptide that contained 4 bound Cd ions. Appearance of this fragment designated α requires prior treatment of metallothionein with EDTA to remove the Zn ions and destabilize the 3-metal cysteine cluster in the other domain. The half-molecule domain was not efficiently produced by proteolysis of native metallothionein. The Cd-α fragment is asymmetric in shape, as is the parent molecule. NH₂-terminal sequence analysis revealed that the α fragment starts at Lys 30. Since the same amino acids are released from the COOH terminus of intact thionein and the α fragment by carboxypeptidase Y, the α domain generated by digestion with subtilisin therefore comprises residues 30 through 61. The amino acid composition of the α polypeptide is consistent with the structure of the 4-metal cysteine cluster proposed by Otvos and Armitage ([1980] Proc. Natl. Acad. Sci. U. S. A. 77, 7094–7098). Metallothionein appears to consist of a 3-metal cysteine domain in the NH₂-terminal half of the thionein molecule and the 4-metal cysteine domain in the COOH-terminal half.

Metallothionein is a 6800-dalton protein that contains an unusually high amount of cysteine (33 mol %) and is capable of binding a variety of metal ions including Cd, Zn, Cu, Co, Ag, and Hg (1–10). The physiological function of the protein is unknown. The number of binding sites for Zn or Cd is 7 metal ions/molecule (4–6). The protein has been isolated from various eukaryotic cells and is inducible by administration of metal ions (6–12), dexamethasone (13), food restriction (14), metallothionein (4–6). The protein has been isolated from various eukaryotic cells and is inducible by administration of metal ions (6–12), dexamethasone (13), food restriction (14), and stress (15). The induction occurs by an enhanced production of thionein mRNA (16, 17). Two polymorphic variants of M-Th’ are induced, with the isoforms differing in positions of cysteine (6), it is likely that the two-cluster arrangement of the bound metal ions is a common structural feature in all M-Th. We wanted to know whether the two clusters were formed by independent segments of the thionein polypeptide, and if so, whether the two domains could be separated. In the present study we report the resolution of the 4-metal cysteine cluster from M-Th and its characterization.

Materials and Methods

Sephadex gels and DEAE-cellulose (DE-52) were obtained from Pharmacia and Whatman, respectively. Ninhydrin and amino acid analyzer buffers were from Beckman, and methyl-Cellosolve was from Fisher. The various proteases and EDTA were purchased from Sigma. The subtilisin used was subtilisin Carlsberg from Bacillus subtilis. Fluorescamine was obtained from Pierce. Male Sprague-Dawley rats weighing between 200 to 300 g were purchased from Simmomsen and were fed commercial rat chow ad libitum.

Metallothionein was purified from the livers of rats given repeated subcutaneous injections of CdCl₂ (2.5 mg of metal/kg, body weight) as described previously (7, 24). The isoforms of CdZn-thionein separated by urea exchange chromatography were desalted, concentrated by lyophilization, and stored at –80 °C. Homogeneity was ascertained by polycrylamide gel electrophoresis, the absorption ratio of A280/A230, and amino acid analysis. These samples represented the starting material for the proteolysis studies.

Amino acid analysis performed on a Beckman 120 C analyzer was used to assess purity and in all cases to quantitate protein concentrations. Cysteine was determined as cysteic acid after performic acid oxidation (25). Cysteines in the thionein samples were also modified by carboxymethylation using iodoacetate (26). Metal analysis was carried out on a Perkin-Elmer Model 305A spectrometer. Absorption spectra were recorded on a Cary 11B spectrometer. Fluorescence measurements were made on a Perkin-Elmer 650-10S spectrophotometer of samples treated with fluorescamine (27). Samples in 1.8 ml of 0.2 M sodium borate, pH 8.8, were mixed with 0.2 ml of fluorescamine (0.5 mg/ml) and the fluorescence emission at 475 nm was measured with excitation at 390 nm. Polycrylamide gel electrophoresis was performed at pH 8.5 in 7.5% gels according to the procedure of Davis (28). Analytical gel filtration was done on a Sephacryl G-75 (superfine) column (2.5×110 cm) in 10 mM potassium phosphate, pH 7.8, at 25 °C. Protein standards and metallothionein samples were chromatographed in varying combinations. Reference proteins used to calibrate the column included albumin, CuZn-superoxide dismutase, carbonic anhydrase, myoglobin, ribonuclease, cytochrome c, and pancreatic trypsin inhibitor. Blue dextran and NaCl were added to mark

appear to be bound in two separate polynuclear metal cysteine clusters (21–23). The most convincing evidence of metal cysteine clusters in Cd-Th is from the work of Otvos and Armitage using ¹¹²Cd NMR spectroscopy (21). They observed that the ¹¹²Cd resonances are split into multiplets by ¹¹²Cd-¹³Cd spin coupling. Selective homonuclear decoupling of the multiplets permitted the identification of the ¹¹²Cd ions that were spin-coupled within the same polynuclear cluster (21). Analysis of the data revealed that the resonances arose from two distinct clusters of metal ions. One cluster contained 3 Cd ions arranged such that each ion was linked to the other two, presumably through cysteine thiolate bridges. The second cluster thus contained the remaining 4 Cd ions.

Since various M-Th sequenced to date show marked homology in the positions of cysteines (6), it is likely that the two-cluster arrangement of the bound metal ions is a common structural feature in all M-Th. We wanted to know whether the two clusters were formed by independent segments of the thionein polypeptide, and if so, whether the two domains could be separated. In the present study we report the resolution of the 4-metal cysteine cluster from M-Th and its characterization.

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The abbreviations used are: M-Th, metallothionein, implying that the metals bound are CdZn-Th; CdTh, zinc-free Cd-thionein; I and II, the two isoforms of metallothionein; α, the fragment of isoform I containing the 4-metal cysteine cluster.

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the excluded and internal volumes, respectively. The elution distribution coefficient ($K_d$) was plotted versus the logarithm of the molecular weight. Fractional ratios were calculated from the ratio of Stokes radii ($R/R_o$) where $R$ is the experimental Stokes radius from gel filtration data and $R_o$ is the calculated radius of a hydrated sphere of known molecular weight (29).

$$R_o = \left[ \frac{3M}{4\pi\eta N} (\bar{v} + \frac{\delta c}{c}) \right]^{1/3}$$

(1)

$M$ is the molecular weight, $\bar{v}$ is the partial specific volume, and $\delta$ is the degree of solvation (assumed 0.2 g of H$_2$O/g of protein). Fractional ratios were also calculated from gel filtration data using the relationship (30):

$$\frac{f/f_{\text{min}}}{f/f_{\text{min}}^{\text{ref}}} = \left( \frac{M_{\text{cal}}}{M_p} \right)^{1/3}$$

(2)

where ($f/f_{\text{min}}^{\text{ref}}$) is the fractional ratio for reference proteins and was assumed to be 1.2 $M_{\text{cal}}$ is the experimentally derived apparent molecular weight from gel filtration, and $M_p$ is the actual molecular weight of the protein from sequence data.

NH$_2$-terminal sequence analysis of the performic acid-oxidized thionein proteolytic fragment was carried out on a Beckman 890B Sequencer using a 0.1 M Quadrol program (12). Polybrene was included in each sequence run, and five pretreatment cycles were run in the presence of the dipeptide lysyl-glycine prior to loading thionein (31). Fractions recovered from each cycle were automatically converted to phenylthiodyantoin with a Sequemat P-6 converter. Phenylthiodyantoin were analyzed by high pressure liquid chromatography using a Waters SISP-790A instrument. In standard runs using sperm whale myoglobin in the absence of polybrene, fmt step yields averaged about 60% with repetitive yields around 93%.

**RESULTS**

Incubation of native rat liver M-Th (1 to 5 mg) containing 4.7 Cd ions and 2 Zn ions/molecule with 1 mM EDTA in 10 mM potassium phosphate, pH 7.8, at 25 °C for 1.5 h leads to a selective loss of thionein-bound Zn (2, 32, 33). Upon removal of the Zn-EDTA by Sephadex G-25 column chromatography, the resulting M-Th retained the 5 g atoms of Cd/mol of protein, and contained less than 0.1 g atom of Zn/mol. With higher concentrations of M-Th (10 to 15 mg), the EDTA concentration was increased up to 4 mM to remove the zinc. Incubation of the EDTA-stripped M-Th isomorph II, designated Cd-ThThII, with various proteases including elastase, subtilisin, proteinase K, and thermolysin at a protein to protease ratio of 20:1 at 37 °C in 10 mM Tris-Cl, pH 8.6, for 16 h resulted in discrete cleavage products (Fig. 1). The additional bands observed in the Cd$_2$-Th blank (lane B) appeared to represent aggregates. Trypsin, chymotrypsin, and papain did not generate fragments under similar conditions. Incubation of native Cd$_2$Zn$_2$-Th with the mentioned proteases under the same conditions for up to 48 h did not result in any apparent proteolysis. In repeated proteolysis experiments with Cd$_2$-Th, subtilisin appeared to be the most effective protease in generating a single fragment of thionein, and therefore was the enzyme used in the subsequent studies.

Chromatography of the subtilisin-digested Cd$_2$-Th on Sephadex G-75 yielded two main Cd-containing fractions designated pool 1 and pool 2 eluting with $K_d$ values of 0.54 and 0.7, respectively (Fig. 2). The elution position of pool 1 was indistinguishable from that of native Cd$_2$Zn$_2$-Th, whereas the minor Cd-containing fraction eluting ahead of pool 1 was an aggregate of M-Th. Because of the asymmetry in M-Th, the molecule elutes with an apparent $M_t$ = ~11,000 (3). Amino acid and metal analyses of pool 1 revealed that it bound 5.1 g atoms of Cd/mol of protein and had an amino acid composition identical with native M-Th. Pool 2 exhibited an apparent $M_t$ = 6,500. Amino acid analysis of pool 2 after desalting on Sephadex G-25 indicated that it was a peptide fragment of M-Th1 (Table I) and was designated fragment $\alpha$. From the minimum composition, it was apparent that $\alpha_1$ (fragment $\alpha$)

![FIG. 1](http://www.jbc.org/)

**Nondenaturing polyacrylamide gel electrophoresis of the proteolytic digestion products of Cd$_2$-Th$_i$.** Gel A is native Cd$_2$Zn$_2$-Th, whereas gels B–F from the same slab gel represent the following: B, intact Cd-Th; C, Cd-Th plus subtilisin; D, Cd-Th plus thermolysin; E, Cd-Th plus elastase; and F, Cd-Th plus proteinase K. Gels run of incubations containing only the proteases did not show any stained bands.

![FIG. 2](http://www.jbc.org/)

**Gel filtration of the subtilisin-digested Cd$_2$-Th$_i$.** Cd-Th$_i$ (15 mg) was digested with subtilisin (0.3 mg) at 4 °C for 16 h in 10 mM potassium phosphate, pH 7.8. Aliquots of the elution fractions were monitored for absorbance at 290 nm, Cd concentration, and fluorescamine reactivity.

**TABLE I**

Amino acid compositions of M-Th and a fragment from limited subtilisin digestion

| Amino acid | Rat M-Th isoforms | Rat $\alpha$ fragments | Mouse M-Th I (34) |
|------------|------------------|------------------------|------------------|
|            | Residues 1–29    | Residues 30–61         | residues/molecule|
| Aspartic acid | 4.3 4.3 3.5 3.5 3.5 | 3.5 3.5 3.5 3.5 3.5 | 3 1 |
| Threonine   | 6.6 7.2 7.2 7.2 7.2 | 7.2 7.2 7.2 7.2 7.2 | 6 3 |
| Serine      | 8.2 8.2 8.2 8.2 8.2 | 8.2 8.2 8.2 8.2 8.2 | 6 3 |
| Glutamic acid | 2.2 2.2 2.2 2.2 2.2 | 2.2 2.2 2.2 2.2 2.2 | 0 1 |
| Proline     | 2.0 2.0 2.0 2.0 2.0 | 2.0 2.0 2.0 2.0 2.0 | 1 1 |
| Glycine     | 6.1 6.1 6.1 6.1 6.1 | 6.1 6.1 6.1 6.1 6.1 | 1 1 |
| Alanine     | 3.0 3.0 3.0 3.0 3.0 | 3.0 3.0 3.0 3.0 3.0 | 1 1 |
| Cysteine    | 20.8 21.2 21.2 21.2 21.2 | 20.8 21.2 21.2 21.2 21.2 | 9 11 |
| Valine      | 2.2 2.2 2.2 2.2 2.2 | 2.2 2.2 2.2 2.2 2.2 | 0 2 |
| Methionine  | 1.0 1.0 1.0 1.0 1.0 | 1.0 1.0 1.0 1.0 1.0 | 0 0 |
| Isoleucine  | 0.2 0.2 0.2 0.2 0.2 | 0.2 0.2 0.2 0.2 0.2 | 0 0 |
| Lysine      | 7.3 7.3 7.3 7.3 7.3 | 7.3 7.3 7.3 7.3 7.3 | 2 5 |
from isoform I) was devoid of Met, contained only about half of the Cys residues, but had the same number of residues of Val and Ala as did the native molecule. Using the protein concentration calculated from amino acid analysis, the α1 fragment from the bulk preparation depicted in Fig. 2 contained 4.8 × 10^6 g atoms of Cd/mol of protein. In various preparations of α1, the Cd content varied from 3.6 to 4.1 ions/molecule. The samples were devoid of Zn.

Digestion of CdZn-Th from isoform I1 with subtilisin under the conditions mentioned gave similar results. The α1 fragment eluted from the analytical Sephadex G-75 column with a Kd of 0.7 and contained 3.5 to 4.0 Cd ions/α molecule. The minimum amino acid composition of α1 is listed in Table I. Although similar α fragments can be obtained from both isoforms of M-Th, the efficiency of a formation by subtilisin treatment is greater with isoform I compared to I1. Retreatment of Sephadex G-75 pool I with EDTA and subsequently redigestion of the desalted material with subtilisin again resulted in incomplete conversion of M-Th to α with both isoforms.

The α fragments from the two isoforms purified through Sephadex G-75 and desalted on Sephadex G-25 were electrophoresed on 7.5% polyacrylamide gels. Fig. 3 shows the Coomassie R-250-stained protein bands of the α fragments and their parent isoforms on the nonnaturing gels. The α fragments from both isoforms were applied to DEAE-cellulose columns equilibrated in 10 mM Tris-Cl, pH 8.6, and both eluted in the column wash. No further degree of purification was observed after ion exchange chromatography as evidenced by nondenaturing polyacrylamide gel electrophoresis or amino acid analysis.

After the initial characterization of the α fragment, the

![Fig. 3. Polyacrylamide gel electrophoresis of the α fragment. Gels A and B represent Coomassie-stained bands of CdZn-ThII and -I, respectively. Gels C and D are the purified α fragments from isoforms II and I, respectively.](http://www.jbc.org/content/journal/jbc/261/20/3473)

### Table II

**NH₂-terminal sequence analysis of α**

| Cycle | Residue from known sequence mouse M-Th I (34) | Residue (present data) | Yield nmol |
|-------|---------------------------------------------|------------------------|-----------|
| 1     | Lys 30                                     | Lys                    | 28        |
| 2     | Lys                                         | Lys                    | 18        |
| 3     | Ser                                         | Ser                    | 2.4       |
| 4     | Cys                                         | Cys                    | 1.0       |
| 5     | Cys                                         |                        |           |
| 6     | Ser                                         | Pro                    | 4.5       |
| 7     | Cys                                         | Val                    | 11.4      |
| 8     | Cys                                         | Gly                    | 5.3       |
| 9     | Pro                                         | Val                    |           |
| 10    | Val                                         | Gly                    |           |
| 11    | Gly                                         |                        |           |
| 12    | Cys                                         |                        |           |
| 13    | Ser                                         |                        |           |
| 14    | Lys 43                                      | Lys                    | 5.8       |

*As is well known for PTC-derivatives of cysteic acid, extraction of the Cys from the Cu is very inefficient. Therefore, yields for phenylthiohydantoin-cysteic acid was appreciable.

* Yield for phenylthiohydantooin-Ser are invariably low and difficult to analyze.

Conditions of proteolysis were investigated to determine what was required to generate α from Cd-Th. Digestion of Cd-Th with subtilisin at a 50:1 protein to protease ratio at 4 °C for 16 h yielded α fragments that exhibited properties identical with those of the α molecules generated by incubation at 37 °C and at higher concentrations of subtilisin. The proteolytic conditions may still be excessive, but the α molecule is extremely resistant to proteolysis.

The ultraviolet absorption spectra of α1 and native M-Th are featureless, but characteristic of M-Th. The A₂₅₀nm/A₃₈₀nm ratio for α was about 14, compared to over 20 for native CdZn-Th. At 250 nm the absorbance of a 1% solution of α is about 160 cm⁻¹ compared to 102 cm⁻¹ for native CdZn-Th.

The α fragment was characterized to determine the arrangement of the α polypeptide in the primary structure of M-Th. Edman degradations of the performic acid-oxidized derivative of α revealed that the NH₂ terminus was Lys 30 based on the known sequence of mouse M-Th (Table II). The first step yield as quantitated by integration of the high pressure liquid chromatography peak was only 18%, but sequence runs of CNBr-treated isoforms of thionein invariably showed first step yields of about 20% (33). The sequence of α was carried out in duplicate and the results unambiguously show α starting at residue 30.

The α fragments and intact thionein were incubated with carboxypeptidase Y to determine if subtilisin cleaved any residues from the carboxyl-terminal end. The carboxymethylated samples were incubated at 37 °C in 0.1 M pyridine acetate, pH 5.5, at a protein to carboxypeptidase Y weight ratio of 7:1. As can be seen in Fig. 4, both α1 and thionein gave a time-dependent release of Ala, carboxymethylcysteine, and Ser (i.e., SerCysAla-COOH) consistent with the known COOH-terminal sequence of the thioneins characterized to date (6). No release with carboxypeptidase Y was observed if the thionein samples were performic acid-oxidized instead of carboxymethylated. Control incubations of only carboxypeptidase Y or only thionein did not reveal any significant concentration of free amino acids.

Since the various metallothioneins sequenced to date show

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marked homology (6), alignment of the α polypeptide in the known sequence of mouse thionein (32) is justified. The α fragment generated by subtilisin cleavage represents the polypeptide portion of thionein from residues 30 through 61. In Table I, the compositions of the α samples of the two isoforms are shown, with the compositions of the comparable mouse liver thionein polypeptide calculated from the known sequence. The correlation in the amino acid composition is excellent.

Although the calculated molecular weight of Cd,α from sequence data is 3617, the apparent molecular weight from gel filtration is 6500, suggesting that the molecule exhibits asymmetry in shape. The frictional ratio (f/fmin) of Cd,α calculated according to Equation 2 under “Materials and Methods” is 1.45, whereas the ratio for native M-Th is 1.4. The frictional ratio (f/fo) calculated from Stokes radii (R/Ro) for native M-Th was 1.3 (33). It therefore appeared that the asymmetry in shape. The frictional ratio for native M-Th and the fragment. The absorbance at 250 nm was monitored as a function of pH. The absorbance at pH 8 was used as the 100% bound value. Cd,Zn-Th (30 µg) and Cd,α (18 µg) were titrated with 0.1 N HCl.

Since α represents the COOH-terminal half of the thionein molecule and binds 4 metal ions, the question arises as to the fate of the NH₂-terminal portion of the molecule. After proteolysis of Cd,α-Th with subtilisin, the elution fractions from Sephadex G-75 were monitored with the fluorescamine reaction to quantitate amino groups. As can be seen in Fig. 2, a major fluorescence peak emerged in the column internal elution volume (V0). The only source of amino groups was necessarily from thionein, since proteolysis and column elution were performed in potassium phosphate buffers. Since α was a homogeneous fragment, the NH₂-terminal polypeptide segment was presumably degraded into small peptides. Quantitation of the relative fluorescence in the α and internal volume

![Fig. 4. Release of COOH-terminal amino acids by carboxypeptidase Y. Thionein or α samples (70 µg) were incubated with 10 µg of carboxypeptidase Y at 37 °C in 0.1 M pyridine acetate, pH 5.5. At the times indicated, the samples were acidified, dried, and analyzed by amino acid analysis. No amino acids were observed if either the thionein sample or carboxypeptidase was left out of the incubations.](image)

![Fig. 5. Hydrogen ion displacement of thionein-bound Cd in native M-Th and the α fragment. The absorbance at 250 nm was monitored as a function of pH. The absorbance at pH 8 was used as the 100% bound value. Cd,Zn-Th (30 µg) and Cd,α (18 µg) were titrated with 0.1 N HCl.](image)

![Fig. 6. Reactivity of metallothionein with EDTA. Samples at a concentration equivalent to 5 µg of Cd were in 1 ml of 10 mM Tris-Cl, pH 7.5, containing 1 mM EDTA and 0.1 M KCl. The sealed cuvettes were incubated at 25 °C and absorbances at 254 nm were recorded with time.](image)
loss of absorbance, i.e., decrease in Cd content, was more pronounced in α1 compared to M-Thu. The time required for the absorbance to be reduced by 50% was twice as long for M-Thu and M-Thui compared to α1.

**DISCUSSION**

Metallothionein was cleaved by proteolysis into a 32-residue polypeptide domain that binds 4 Cd ions. The domain, designated α1, was generated with a variety of proteases, but subtilisin has been most extensively used. The α fragment exhibits properties similar to native M-Th in its ultraviolet absorption spectra, asymmetry in conformation, and pH-dependent release of Cd ions. Although the hydrogen ion displacement of Cd ions in Cd-α and Cd,Zn-Thu is similar, the reactivity of these samples toward EDTA differs. The slower reactivity of native M-Th with EDTA suggests that the juxtaposition of the two domains exerts a stabilizing effect on each other. We plan to study the actual metal-binding affinities of α and M-Th by microcalorimetry.

The α domain is efficiently produced only if native M-Th is initially treated with EDTA. Presumably the EDTA incubation removes the two Zn ions that appear to bind to the 3-metal cluster (21). Loss of the Zn ions appears to destabilize the structure of the 3-metal cluster, resulting in a random coil conformation in the N2H-terminal portion of the molecule. It is well established that removal of metal ions from M-Th results in a random coil configuration of the polypeptide (18). Proteolysis with subtilisin cleaved the NH2-terminal 29-residue polypeptide in numerous places, yielding small peptides and α that was then separated from the peptides by gel filtration. Proteolysis of native M-Th (not EDTA treated) under rigorous conditions produced only insignificant amounts of α. Therefore, under the conditions employed, isolation of the intact 3-metal cluster was not possible. Complete conversion of M-Th to α fragment was not possible with repeated digestions of either isoform, suggesting that microheterogeneity may exist in the sequence of the susceptible hinge. Proteolysis with elastase or proteinase K yielded additional charge variants of α (Fig. 1).

Otvos and Armitage (21) proposed from 111Cd NMR studies that M-Th contains two polynuclear metal cysteine clusters. They postulated structures for each cluster that would satisfy their 111Cd,115Cd spin coupling data. The 4-metal ion center contained 11 cysteines with 6 thiolate bridges connecting adjacent metal ions, and the 3-metal ion center contained 9 cysteines and 3 thiolate bridges. The 4-metal cysteine center designated cluster A by Otvos and Armitage (21) is consistent with the composition of our α fragment. Our data, therefore, supports the postulate that M-Th contains two separate metal cysteine centers. The 4-metal center (cluster A) is in the α domain and that constitutes the COOH-terminal half of the molecule. Our data on the structure of metallothionein is consistent with that depicted in Fig. 7.

Characterization of a M-Th genomic clone isolated by partial restriction nuclease digestion of mouse myeloma DNA showed that the thionein gene contained two introns (16). One intron-exon junction occurs in the coding region for Ser 32. Thus, exon 3 encodes the COOH-terminal 29 amino acids that fold into the α domain. Whereas the location of this intron supports the idea that exons represent functional units in a protein, the location of the other intron in the polypeptide segment encompassing the 3-metal center raises doubts about this hypothesis. The polypeptide segment from Thr 27 to Ser 32 may be a partially exposed hinge peptide linking the two metal-binding domains.

The isolation of the half-thionein molecule will reduce the complexities in structural studies, since the structure of a single metal cysteine cluster can now be independently investigated. Elucidation of the crystal structure of α may provide insights into the physiological role of the protein and would confirm cysteinate bridging in proteins. We are trying to successfully separate the two metal clusters from native M-Th in order to study the metal ion distribution between the two centers. In Cu-Th, 10 g atoms of Cu bind/mol of protein, unlike the 7 metal ions bound in Cd,Zn-Thu. It is conceivable that different conformations of the clusters exist to accommodate varying metal ions, and the alterations in conformation may influence function.

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**REFERENCES**

1. Kagi, J. H. R., and Vallee, B. L. (1960) J. Biol. Chem. 235, 3460-3465.
2. Kagi, J. H. R., and Vallee, B. L. (1961) J. Biol. Chem. 236, 2435-2442.
3. Kagi, J. H. R., Himmelhoch, S. R., Whanger, P. D., Bethune, J. L., and Vallee, B. L. (1974) J. Biol. Chem. 249, 3537-3542.
4. Pulido, F., Kagi, J. H. R., and Vallee, B. L. (1966) Biochemistry 5, 1768-1777.
5. Tsuono, H., Kino, K., Nakajima, H., Hata, A., Huang, I.-Y., and Yoshida, A. (1978) J. Biol. Chem. 253, 4172-4174.
6. Kagi, J. H. R., Kojima, Y., Kissing, M. M., and Leech, K. (1980) Ciba Found. Symp. 22, 223-237.
7. Winge, D. R., Premselaar, R., and Jagapalan, K. V. (1975) Arch. Biochem. Biophys. 170, 242-252.
8. Weser, U., Rupp, H., Donay, F., Linnemann, F., Voelter, W., Voetsch, W., and Jung, G. (1973) Eur. J. Biochem. 39, 127-140.
9. Zelazowski, A. J., and Piotrowski, J. K. (1980) Biochim. Biophys. Acta 625, 89-99.
10. Ohta, H., and Kaga, M. (1979) Biochem. J. 183, 683-690.
11. Prinz, R., and Weser, U. (1975) FEBS Lett. 54, 224-229.
12. Hidalgo, H. A., Koppa, V., and Bryan, S. E. (1979) Biochem. J. 170, 219-225.
13. Karin, M., and Herschman, H. R. (1980) Eur. J. Biochem. 107, 390-401.
14. Bremner, I., and Davies, N. T. (1975) Biochem. J. 149, 733-738.
15. Oh, S. H., Deagen, J. T., Whanger, P. D., and Weswig, P. H. (1978) Am. J. Physiol. 234, E282-E285.
16. Durnam, D. M., Perrin, F., and Palmer, R. D. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6511-6515.
17. Eger, M. D., Rall, L. B., and Hildebrand, C. E. (1979) Nucleic Acid Res. 7, 271-288.
18. Vasak, M., Galdes, A., Hill, H. A. O., Kagi, J. H. R., Bremner, I., and Young, B. W. (1980) Biochemistry 19, 416-425.
19. Hartmann, H. J., and Weser, U. (1977) Biochim. Biophys. Acta 491, 211-222.
20. Galdes, A., Vasak, M., Hill, H. A. O., and Kagi, J. H. R. (1978) FEBS Lett. 92, 17-21.
21. Otvos, J. D., and Armitage, I. M. (1980) Proc. Natl. Acad. Sci. U.
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22. Vasak, M. (1980) J. Amer. Chem. Soc. 102, 3953–3955
23. Sadler, P. J., Bakka, A., and Beynon, P. J. (1978) FEBS Lett. 94, 315–317
24. Winge, D. R., Geller, B. L., and Garvey, J. (1981) Arch. Biochem. Biophys. 208, 160–166
25. Hirs, C. H. W. (1967) Methods Enzymol. 11, 197–199
26. Hirs, C. H. W. (1967) Methods Enzymol. 11, 199–203
27. Bohlen, P., Stein, S., and Udenfriend, S. (1974) Arch. Biochem. Biophys. 163, 390–399
28. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
29. Tanford, C. (1961) Physical Chemistry of Macromolecules, pp. 356–360, John Wiley and Sons, New York
30. Rydén, L., and Deutsch, H. F. (1978) J. Biol. Chem. 253, 519–524
31. Hunkapiller, M. W., and Hood, L. E. (1978) Biochemistry 17, 2124–2133
32. Li, T. Y., Krakor, A. J., Shaw, C. F., and Petering, D. H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6334–6338
33. Winge, D. R., and Miklossy, K. A. (1982) Arch. Biochem. Biophys. in press
34. Huang, I.-Y., Yoshida, A., Tsunoo, H., and Nakajima, H. (1977) J. Biol. Chem. 252, 8217–8221
Domain nature of metallothionein.
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