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Amino acid sequence and comparative antigenicity of chicken metallothionein

(vertebrate evolution/conserved cysteine residues)

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ABSTRACT **The complete amino acid sequence of metallothionein (MT) from chicken liver is reported. The primary structure was determined by automated sequence analysis of peptides produced by limited acid hydrolysis and by trypsin digestion. The comparative antigenicity of chicken MT was determined by radioimmunoassay using rabbit anti-rat MT polyclonal antibody. Chicken MT consists of 63 amino acids as compared to 61 found in MTs from mammals. One insertion (and two substitutions) occurs in the amino-terminal region, a region considered invariant among mammalian MTs. Eighteen of the 20 cysteines in chicken MT were aligned with cysteines from other mammalian sequences. Two cysteines near the carboxyl terminus are shifted by one residue due to the insertion of proline in that region. Overall, the chicken protein showed ≈68% sequence identity in a comparison with various mammalian MTs. The affinity of the polyclonal antibody for chicken MT was decreased by 2 orders of magnitude in comparison to that of a mammalian MT (rat MT isoforms). This reduced affinity is attributed to major substitutions in chicken MT in the regions of the principal determinants of mammalian MTs. Theoretical analysis of the primary structure predicted the secondary structure to consist of reverse turns and random coils with no stable β or helix conformations. There is no evidence that chicken MT differs functionally from mammalian MTs.**

Isolated and characterized some 30 years ago (1, 2), the cysteine-rich, metal-binding protein metallothionein (MT) is now recognized to exist in several tissues of virtually every vertebrate species examined (3). However, despite extensive characterization of MT, the precise biological function is not known. Similar cysteine-rich proteins have also been found in invertebrates such as sea urchin (4) and crab (5) and in microorganisms such as yeast (6) and fungi (7), suggesting that MTs are ubiquitous.

Mammalian MTs contain 30% cysteine and have no leucine, histidine, or aromatic amino acids and generally only one amino-terminal methionine. Typically, four amino acids (cysteine, serine, lysine, and alanine) can account for 70% of the residues in MT. Most mammalian MTs studied so far contain 61 amino acids, which are aligned in a highly conserved primary structure. In fact, the positions of the 20 cysteines in the sequences of all MTs from vertebrates studied so far are invariant (3), undoubtedly reflecting the importance of these residues in the coordination of metals (8, 9). Other regions of the sequence are also invariant, but the functional significance of these remains to be determined. One invariant region at the amino terminus (residues 1–5) and one homologous region (residues 20–26) have been shown to be the primary antigenic determinants for mammalian MTs (10). Presently, there are no data concerning the principal determinants for nonmammalian MTs.

MTs of many species exhibit polymorphism. Two major isoforms of MT exist in tissues from a wide variety of species ranging from humans to crabs (11–15). Interestingly, one vertebrate species, the chicken, appears to possess only one form of MT, which is similar in ionic characteristics to the major mammalian isoform I (16–18). Studies of the significance of MT polymorphism (19–22) in mammals have not established functional differences between isoforms.

In contrast to the extensive reports on the primary structure of MTs from mammalian species (23) and of organisms such as fungi (7), yeast (6, 24), crab (15), and sea urchin (4), there is little or no direct information concerning the primary structure of MTs from nonmammalian vertebrates. A partial sequence of plaice MT has been reported (25) but the complete primary structure has not been determined. As indicated previously the chicken possesses only a single form of MT. Moreover, MT from chicken liver contains histidine (16, 17, 26), an amino acid not present in mammalian MTs. Because of these unique characteristics, we initiated work to determine the primary structure of chicken MT.

EXPERIMENTAL PROCEDURES

Preparation of Metallothionein. Four-week-old male chickens were given three i.p. injections of zinc sulfate (5 mg of zinc per kg of body weight) over 3 days. Liver MT was prepared by gel filtration and anion-exchange chromatography as described (18). In brief, the purification protocol involved gel filtration of liver cytosol, with no heat treatment, on Sephadex G-75 followed directly by DEAE-Sephadex A-25 anion-exchange in columns (1.6 × 30 cm) equilibrated with filtration buffer (2 mM Tris acetate, pH 7.4). The column was washed and the sample was eluted with a 200-ml gradient of 2 mM Tris acetate, pH 7.4, to 80 mM Tris acetate, pH 8.6. We have observed that an increasing pH during gradient elution facilitates purification. The purity of the preparations was determined by nondenaturing gradient gel electrophoresis (27), reverse-phase HPLC (28), and amino acid compositional analysis (29).

Direct Sequence Analysis. Sequence analysis was conducted on S-pyridylethylated MT (PyrEt-MT). Samples of zinc MT (≈500 μg protein in 1 ml of 5 mM Tris acetate, pH 8.6) were incubated for 1 hr with 200 μl of 14 mM Tris·HCl, pH 7.4/100 mM EDTA. Following the addition of 3 μl of 2-mercaptoethanol, 9 μl of 4-vinylpyridine was added and the sample was incubated overnight. The precipitate was recovered, dissolved in 10% acetic acid, and chromatographed by HPLC as described (28). Elution was achieved with a gradient of solvent A (0.1% trifluoroacetic acid) and solvent

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Abbreviations: MT, metallothionein; PyrEt-MT, S-pyridylethylated MT; Cam-MT, S-carboxamidomethylated MT.
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B (acetonitrile containing 0.1% trifluoroacetic acid). The gradient was linear from 5% to 30% solvent B and developed over a total run time of 30 min at a constant flow rate of 2 ml/min. Amino acid compositional analysis revealed essentially complete alkylation and no free cysteine. Since all MTs studied thus far contain *N*-acetylmethionine at the amino terminus, PyrEt-MT was treated with 70% formic acid to hydrolyze the acid-sensitive bond between Asp-2 and Pro-3. The PyrEt-MT sample was dried and dissolved in 70% formic acid and incubated at 37°C for 96 hr as described (8). Following this treatment, samples were chromatographed as described above. The selective loss of methionine and aspartic acid was confirmed by amino acid analysis. Formic acid-treated PyrEt-MT (≈230 nmol) was then subjected to automated sequence analysis as described (30). Both phases (organic and aqueous) of the phenylthiohydantoin amino acid extract were analyzed by HPLC.

Limited Acid Hydrolysis. PyrEt-MT was subjected to limited acid hydrolysis as described by Schultz (31). Samples of PyrEt-MT (≈300 μg) were dried in hydrolysis ampoules and dissolved in 0.037 M HCl. The samples were then incubated *in vacuo* for 40 hr at 105°C. The resulting peptides were separated by reverse-phase HPLC as described above and were collected manually. Amino acid compositional analysis was performed on each peptide.

Tryptic Digestion. Tryptic digestion of MT was performed on S-carboxyamidomethylated metallothionein (Cam-MT). Approximately 200 μg of zinc MT was chromatographed by HPLC (as described above) to remove zinc. The resulting thionein was collected, dried under N_2, and dissolved in 500 μl of 0.012 M HCl. Five microliters of 2-mercaptoethanol was added and the sample was neutralized, under N_2, to pH 8–9 by the addition of 400 μl of 0.2 M Tris base. Sixty microliters of 1 M iodoacetamide in 0.2 M *N*-ethylmorpholine acetate buffer (pH 8.0) was then added and the sample was incubated for 20 min in the dark (at room temperature), at which time the sample pH was increased by the addition (under N_2) of 100 μl of 0.2 M Tris base. After an additional 20-min incubation, the sample was chromatographed by HPLC as described above. Amino acid compositional analysis of the resulting Cam-MT indicated essentially 100% alkylation and no losses of amino acids. One hundred micrograms of Cam-MT was then dried (under N_2) and dissolved in 200 μl of distilled/deionized water. After the addition of 200 μl of 0.2 M $(NH_4)_2CO_3$, 2 μg of trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated; Worthington) in 0.2 M *N*-ethylmorpholine acetate (pH 8.0) was added. The tube was flushed with N_2, sealed, and incubated at 37°C for 1 hr, at which time the digestion was complete. Samples were then chromatographed by HPLC using a gradient of 0% B to 20% B in 40 min at a flow rate of 2 ml/min. All peptides were collected manually and identified by amino acid composition; several were subsequently sequenced.

Sequence Analysis of Peptides. Sequence analyses of both acid and tryptic peptides were performed by the Biotechnology Institute Sequence Facility at Cornell University. A gas-phase sequenator (model 47A, Applied Biosystems) similar to that described by Hewick *et al.* (32) was employed for those analyses. Phenylthiohydantoin amino acid derivatives were determined by reverse-phase HPLC.

Radioimmunoassay. The double-antibody, competitive binding RIA was performed as described (33). The reference antigen was a 1:1 mixture of rat MT-1 and rat MT-2.

Structural Analyses. To provide information complementary to the primary sequence and results of the RIA, secondary structure and hydrophilicity analyses were performed as previously described (34), using computer programs based on the protocols of Chou and Fasman (35) to predict secondary structure and the protocols of Hopp and Woods (36) to predict hydrophilicity and regions of principal antigenicity.

RESULTS

Purification and Compositional Analysis. The isolation of chicken liver MT was accomplished by conventional gel filtration and DEAE-Sephadex A-25 ion-exchange chromatography. The purity of the isolate was determined by nondenaturing gel electrophoresis in 15% acrylamide and gradient (7.5–30% acrylamide) gels (27) and by reverse-phase HPLC. In both cases and with several amounts of sample, only a single band (peak) was observed (data not shown). Amino acid analysis (Table 1) showed a typical MT composition—i.e., 30% cysteine, no aromatic amino acids, and the presence of a single histidine. Also, throughout our purification and subsequent sequence analysis, we observed no evidence of more than one form of MT.

Sequence Analysis. Direct sequence analysis of 70% formic acid-treated PyrEt-MT unambiguously established the sequence from Pro-3 to Asn-24 (Fig. 1). Several residues beyond Asn-24 were tentatively identified (Cys-27 to Ser-29, Lys-32 to Cys-35, and Pro-39 to Gly-41) and later confirmed by sequence analysis of peptides containing these sequences. Assignment of amino-terminal residues was made as a result of changes in composition as determined by amino acid analysis subsequent to formic acid treatment of PyrEt-MT. The analysis showed the virtual absence (loss) of methionine and the loss of one aspartic acid/asparagine from the original composition.

Peptide maps from limited acid hydrolysis of PyrEt-MT and tryptic digestion of Cam-MT are shown in Fig. 2. Analysis of selected acid-hydrolyzed peptides provided unambiguous assignment of Cys-25 through Gly-41. We utilized the tentative positions of Cys-27 through Ser-29 (indicated above) to position acid peptide A_6 for these assignments. The sequence analysis of tryptic peptide T_6 provided an overlap and the extension of sequence assignments to Lys-47. Tryptic cleavage at Lys-31 was apparently not preferred, since the major peptide in T_6 (Fig. 2) contained an amino-terminal lysine. Sequence analysis of acid peptide A_5 provided an overlap of T_6 (at Cys-45 through Lys-47) and the remaining sequence. The residues in this latter segment of the protein were confirmed by compositional analysis of two additional tryptic peptides, T_4 and T_5. Although the carboxyl-terminal position was not directly determined, it was assigned from two separate sequence analyses (A_3 and A_5) and one compositional analysis (T_4) in which amino acid composition

Table 1. Amino acid composition of chicken MT

Amino acid	Mol %	Residues per mol*
Asx	9.8	6.3 (6)
Thr	1.5	1.0 (1)
Ser	13.4	8.6 (9)
Glx	3.5	2.2 (2)
Pro	5.2	3.4 (3)
Gly	6.7	4.2 (4)
Ala	9.8	6.3 (6)
Cys†	30.9	19.8 (20)
Val	1.4	0.9 (1)
Met	2.0	1.3 (1)
Ile	0.06	0.04 (0)
Leu	0.03	0.02 (0)
Phe	ND	ND (0)
Tyr	ND	ND (0)
His	1.7	1.1 (1)
Lys	9.2	5.9 (6)
Arg	4.7	3.0 (3)

ND, not detected.
*Numbers in parentheses indicate composition determined from sequence analysis.
†Determined as *S*-pyridylethylcysteine.

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1               5              10              15
Met-Asp-Pro-Gln-Asp-Cys-Thr-Cys-Ala-Ala-Gly-Asp-Ser-Cys-Ser
                20              25              30
Cys-Ala-Gly-Ser-Cys-Lys-Cys-Lys-Asn-Cys-Arg-Cys-Arg-Ser-Cys
                35              40              45
Arg-Lys-Ser-Cys-Cys-Ser-Cys-Cys-Pro-Ala-Gly-Cys-Asn-Asn-Cys
                50              55              60
Ala-Lys-Gly-Cys-Val-Cys-Lys-Glu-Pro-Ala-Ser-Ser-Lys-Cys-Ser
63
Cys-Cys-His
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FIG. 1. Amino acid sequence of chicken liver MT. Asterisks denote residues sequenced directly from 70% formic acid-treated (unblocked) PyrEt-MT. Continuous arrows indicate peptide positions established by sequence, compositional analysis, or both. Peptides were obtained either by limited acid hydrolysis (A) of PyrEt-MT or by tryptic digestion (T) of Cam-MT.

showed the presence of a single histidine residue. Also shown in Fig. 2 is a tryptic peptide map of Cam-MT (isoform 2) from rat liver, prepared under conditions identical to those described for chicken MT. It is clear from this comparison that there are distinct differences between the tryptic peptides of rat and chicken MTs.

Radioimmunoassay. The RIA of chicken MT (Fig. 3) established that the response was shifted by 2 orders of magnitude (in the direction of decreased reactivity) with respect to the response of the reference rat MTs. As previously demonstrated (10), such a shift in response is attained when either the amino-terminal segment (residues 1–5 in mammalian MTs) or the region of residues 20–26 is chemically modified. These regions have been indicated by theoretical analysis (34) to be the principal antigenic determinants

FIG. 2. HPLC peptide maps of acid- or trypsin-hydrolyzed MT. (*A*) PyrEt-MT from chicken liver, subjected to limited acid hydrolysis. Peaks representing peptides A_3, A_5, and A_6 are indicated. (*B*) Cam-MT from chicken liver, digested with trypsin. Peaks representing peptides T_1 through T_7 are indicated. (*C*) Cam-MT isoform 2 from rat liver, treated identically as chicken MT in *B*. Samples were analyzed by reverse-phase HPLC using a gradient of acetonitrile.

FIG. 3. RIA of chicken liver MT. The responses of chicken MT and of rat MT isoforms 1 and 2 in a competitive binding, double-antibody RIA (33) are shown. *Y* is the fraction of labeled antigen (^{125}I-labeled rat MT-1) bound; *Q* is the logarithm of MT mass (in pg). The 2-orders-of-magnitude displacement of the response of chicken MT to the polyclonal antibody (raised against rat MT) compared to that of the response of the reference antigen (rat MT-1 and MT-2) is an indication of a major change in the region of the two principal antigenic determinants of mammalian MTs (residues 1–5 and 20–26; ref. 10).

in mammalian MTs. Chicken MT differs significantly from other vertebrate MTs in the sequence of these analogous regions (Fig. 1) as well as the immediately adjacent regions; thus the reduced affinity for the polyclonal antibody raised against rat MT is not unexpected. The theoretical analysis of secondary structure (Fig. 4) indicates that the hydrophilicity profile is quite similar to that of other vertebrate MTs (34) and that the secondary structure is also similar, being essentially a series of reverse turns and random coils with no stable β or helix conformations.

DISCUSSION

The present study has elucidated important similarities as well as significant differences between the primary structures of chicken and mammalian MTs. The most significant difference was the presence of 63 total residues in the chicken protein. Most mammalian MTs studied so far have 61 total residues and regions of the sequence that are identical. When adjustments for the two insertions in chicken MT were performed, the positions of the cysteines were found to align with those of other species (Fig. 5). However, it is important to recognize that the insertion of Pro-54 would result in nonalignment of two cysteines in that region. This structural deviation does not appear to affect zinc binding quantitatively, since our analysis of the molar zinc/thionein ratio of zinc-induced MT showed 6.99 mol of zinc per mol of chicken thionein, a value reported previously for chicken (26) as well as for mammalian MTs (3). Therefore, the general mode of zinc coordination in chicken MT does not appear to differ from that established for mammalian MTs (9, 47, 48).

All vertebrate MTs previously analyzed by RIA have contained an invariant amino-terminal sequence (Met-Asp-Pro-Asn-Cys-Ser-Cys-). Chicken MT varies substantially from that sequence (Met-Asp-Pro-Gln-Asp-Cys-Thr-Cys-), and the inference is that the antibody, which is specific for the above-mentioned invariant sequence (a principal determinant) of mammalian MTs, has a much-reduced affinity for the sequence in chicken MT. The vertebrate MTs previously analyzed have some variation in the other principal determinant (residues 20–25). In equine MT, an arginine appears instead of lysine-25; in rat MT-1, a glycine appears instead of lysine-20; in position 23, glutamine, asparagine, or glutamic acid may appear; in calf MT, a proline appears instead of threonine-27. These substitutions have minor impact on the

Hydrophilicity Profile

Reverse Turn Profile

FIG. 4. Hydrophilicity and reverse-turn analyses of chicken liver MT. (*Upper*) Hydrophilicity indices are indicated for sequential hexapeptides according to the protocol of Hopp and Woods (36). Values are plotted in the center of the hexapeptide sequence with terminal regions completed with values for penta-, tetra-, tri-, and dipeptides. (*Lower*) The reverse-turn profile was calculated by the protocol of Chou and Fasman (35). Probabilities for sequential tetrapeptides to form a reverse turn (P_t) are plotted with values indicated in the center of the tetrapeptide. Values greater than twice the average value (0.55×10^{-4}) as cited by Chou and Fasman (35) indicate significant potential for reverse-turn conformation. Two segments that have high β-structure-forming potential (residues 5–8 and 49–51; -Asp-Cys-Thr-Cys- and -Cys-Val-Cys-, respectively) are indicated by (----) and (---). A segment that has helix-forming potential (residues 52–58; -Lys-Glu-Pro-Ala-Ser-Ser-Lys-) is denoted by (/\/\/\). The potential β and helix regions are also those of high turn potential and are predicted not to exist as stable conformations.

affinity of the antibody for the region (33, 34, 46). However, chicken MT has three residues in or immediately adjacent to the region in which arginine appears instead of lysine-25, threonine-27, and lysine-30. Since antigenic determinants are topographic (affinity is influenced by nearby residues), we infer that these substitutions in the case of chicken MT significantly reduce the affinity of the antibody for that region.

The hydrophilicity and predicted secondary structure of chicken MT (Fig. 4) are similar to those of other MTs thus far examined (34). The predicted secondary structure consists of a series of reverse turns or loops and random coils with no stable β or helix conformations. This conclusion from theory is in agreement with recent x-ray crystallographic analysis (48). The very high reverse-turn probabilities (P_t) for tetrapeptides containing residues 2–5, 22–25, and 26–29 (Fig. 4) correspond to the immunodominant segments (residues 1–5 and 20–26) of other vertebrate MTs (10). The reduced antigenicity of chicken MT is then to be associated with the numerous substitutions in these regions (see above) and not to a major change in either secondary or tertiary structure. We predict that similar (but not identical) regions (residues 1–6 and 20–28) are the principal sites of antigenic determinants in the chicken protein. The substantive difference then in the chicken protein concerns the latter region, extended in the present study to include three additional amino acids, Arg-26, Cys-27, and Arg-28.

It is not clear whether the functional characteristics of the chicken MT have been altered by the various substitutions mentioned. Metal-binding characteristics of MT in the chicken suggest that apparent function is not significantly affected. Preliminary data from our laboratory indicate, however, that copper-induced accumulation of hepatic MT in the chicken is not similar to that of rats. Parenteral copper loading of chicks results in the accumulation of two species of MT (separable by ion-exchange chromatography) that differ only in metal composition, one form containing only zinc (unpublished data). In mammals copper is preferentially bound in the β domain of MT (49, 50), a segment of MT in which there are clear differences between chicken and mammalian MTs in both structure and antigenicity.

Overall, our sequence analysis demonstrated considerable similarity between a consensus sequence of human MT-2 and that of chicken MT. When aligned to accommodate the insertions, the chicken protein differed at 17 positions (including the insertions), corresponding to 72% identity. MTs from the other species shown in Fig. 5 differ by 19.6 ± 0.6 (mean ± SEM) residues, showing an average identity of

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             1   5    10   15   20   25   30   35   40   45   50   55   60
             |   |    |    |    |    |    |    |    |    |    |    |    |
HUMAN II     MDPNCSCAAGDSCTCAGSCKCKECKCTSCKKSCCSCCPVGCAKCAQGCICKGASDKCSCCA
HUMAN IA      --------T-G----T----------N-----------MS--------------E-----
MONKEY 1     --------T-V-----D-------------------------------V-----E--N---
MONKEY 2     -------V-------------------------------------------------N---
HORSE IA     -------PT-G-------------R-------------G---R-----V------------
HORSE IB     -------V--E-----------Q-R-A---------------------V------------
SHEEP 1      -------PT-G--S-----T--A-R-P---------------------V------------
HAMSTER 1    -------ST-ST---SS--G--D------------------S------V--------T---
HAMSTER 2    --------TDG--S-------------T----------------S---V--E---------
CHICK        ---D-T-------S--------N-R-R--R--------A--NN--K--V--E--S-----H
               Q                                                P
MOUSE 1      -------ST-G----TS--A--N------------------S------V----A---T---
MOUSE 2      --------SDG--S---A----Q---------------------S------Q-----S---
RAT 1        -------ST-G----SS--G--N------------------S------V--------T---
RAT 2        --------TDG--S--------Q---------------------S------E---------
CALF 1[a]    -------PT-G-----------A-R-P---------------------V------------
CALF 2[a]    -------T--E-----------D---A---------------------V------------
CALF 2[b]    -------PT-G--S-----T--A-R-P---------------------V------------
                                                            (I)
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FIG. 5. Amino acid sequences of MTs from various mammalian species and the chicken. Standard one-letter amino acid symbols are shown. The amino acid sequence of chicken MT was aligned to accommodate the two insertions. Other sequences are human MT-II (37), human MT-I_A (38), monkey MT-1 and MT-2 (39), horse MT-IA (13), horse MT-IB (8), sheep MT-1 (40), hamster MT-1 and MT-2 (41), mouse MT-1 (42), mouse MT-2 (43), rat MT-1 and MT-2 (44), and calf MT-1 and MT-2. [[a], from Munger *et al.* (45); [b], from Winge *et al.* (46)].

67.9%. These values compare to a range of 97% to 82% for comparisons of human MT-2 to sequences of other mammalian MTs (Fig. 5) with an average value of 87.6%. The high value represents MT-2 from monkey, whereas the low value was calculated for MT-1 from hamster (and also for mouse MT-1). Interestingly, there is no greater sequence identity between isoforms of MT within a species. Sequences from monkey, horse, hamster, mouse, and rat MTs show differences ranging from 6 (monkey) to 15 (mouse and hamster) residues, with an average value for percent identity between MT isoforms of 82.3%. In a comparison of structural variation of cytochrome *c*, Margoliash and Fitch (51) reported ≈90% identity between cytochrome *c* of mammals and birds and only slightly lower values from a comparison of mammals/birds to reptiles. Also, using a value of 280 million years for the time of divergence of the mammalian and avian lines of descent, they calculated a unit evolutionary period of 26.4 million years. Kägi *et al.* (52) calculated a unit evolutionary period of 14 million years for mammalian MTs. Employing a similar time of divergence for mammals and birds as cited above, a value of 16.5 million years can be calculated for the evolution of MT from birds to humans (using 17 substitutions) as determined from the present sequence. Chicken MT, therefore, appears to show greater diversity relative to cytochrome *c* and perhaps a greater rate of evolutionary change. Again, the apparently important elements of the structure—i.e., the positions of the cysteine residues—are highly conserved.

Note. The nucleotide sequence of cloned chicken MT cDNA was completed recently by Glen K. Andrews of the Department of Biochemistry at the University of Kansas Medical Center (personal communication). The amino acid sequence of the protein deduced from this information is consistent with that given in the present report.

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1. Margoshes, M. & Vallee, B. L. (1957) *J. Am. Chem. Soc.* **79**, 4813–4814.
2. Kägi, J. H. R. & Vallee, B. L., eds. (1960) *J. Biol. Chem.* **253**, 3460–3465.
3. Kägi, J. H. R. & Nordberg, M. (1979) *Metallothionein* (Birkhauser, Basel).
4. Nemer, M., Wilkinson, D. G., Travaglini, E. C., Sternberg, E. J. & Butt, T. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4992–4994.
5. Olafson, R. W., Sim, R. G. & Kearns, A. (1979) in *Metallothionein*, eds. Kägi, J. H. R. & Nordberg, M. (Birkhauser, Basel), pp. 197–204.
6. Butt, T. R., Sternberg, E. J., Gorman, J. A., Clark, P., Hamer, D., Rosenberg, M. & Crooke, S. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3332–3336.
7. Lerch, K. (1980) *Nature (London)* **284**, 368–370.
8. Kojima, Y., Berger, C., Vallee, B. L. & Kägi, J. H. R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3413–3417.
9. Otvos, J. D. & Armitage, I. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7094–7098.
10. Winge, D. R. & Garvey, J. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2472–2476.
11. Shaikh, Z. A. & Lucis, O. J. (1971) *Experientia* **27**, 1024–1025.
12. Nordberg, G. F., Nordberg, M., Piscator, M. & Vesterberg, O. (1972) *Biochem. J.* **126**, 491–498.
13. Kojima, Y., Berger, C. & Kägi, J. H. R. (1979) in *Metallothionein*, eds. Kägi, J. H. R. & Nordberg, M. (Birkhauser, Basel), pp. 153–161.
14. Kojima, Y. & Kägi, J. H. R. (1978) *Trends Biochem. Sci.* **3**, 90–93.
15. Lerch, K., Ammer, D. & Olafson, R. W. (1982) *J. Biol. Chem.* **257**, 2420–2426.
16. Oh, S. H., Nakaue, H., Deagen, J. T., Whanger, P. D. & Arscott, G. H. (1979) *J. Nutr.* **109**, 1720–1729.
17. Klauser, S., Kägi, J. H. R. & Wilson, K. J. (1983) *Biochem. J.* **209**, 71–80.
18. McCormick, C. C. (1984) *J. Nutr.* **114**, 191–203.
19. Suzuki, K., Ebihara, Y., Akitomi, H., Nishikawa, M. & Kawamura, R. (1983) *Comp. Biochem. Physiol.* **76C**, 33–38.
20. Suzuki, K. T. & Yamamura, M. (1980) *Biochem. Pharmacol.* **29**, 2407–2412.
21. Suzuki, K. T. & Yamamura, M. (1980) *Toxicol. Lett.* **6**, 59–65.
22. Winge, D. R. & Miklossy, K.-A. (1982) *Arch. Biochem. Biophys.* **214**, 80–88.
23. Hamer, D. H. (1986) *Annu. Rev. Biochem.* **55**, 913–951.
24. Karin, M., Najarian, R., Haslinger, A., Valenzuela, P., Welch, J. & Fogel, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 337–341.
25. Overnell, J., Berger, C. & Wilson, K. J. (1981) *Biochem. Soc. Trans.* **9**, 217–219.
26. Weser, U., Rupp, H., Donay, J., Linnemann, F., Voelter, W., Voetsch, W. & Jung, G. (1973) *Eur. J. Biochem.* **39**, 127–140.
27. Lin, L.-Y. & McCormick, C. C. (1986) *Comp. Biochem. Physiol.* **85C**, 75–84.
28. Fullmer, C. S. (1984) *Anal. Biochem.* **142**, 336–339.
29. Fullmer, C. S. & Wasserman, R. H. (1979) *J. Biol. Chem.* **254**, 7208–7212.
30. Fullmer, C. S. & Wasserman, R. H. (1981) *J. Biol. Chem.* **256**, 5669–5674.
31. Schultz, J. (1967) *Methods Enzymol.* **11**, 255–263.
32. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) *J. Biol. Chem.* **256**, 7990–7997.
33. Garvey, J. S., Vander Mallie, R. J. & Chang, C. C. (1982) *Methods Enzymol.* **84**, 121–138.
34. Garvey, J. S. (1984) *Environ. Health Perspect.* **54**, 117–127.
35. Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol.* **47**, 45–148.
36. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3224–3228.
37. Kissling, M. M. & Kägi, J. H. R. (1977) *FEBS Lett.* **82**, 247–250.
38. Richards, R. I., Heguy, A. & Karin, M. (1984) *Cell* **37**, 263–266.
39. Schmidt, C. J. & Hamer, D. H. (1983) *Gene* **24**, 137–146.
40. Peterson, M. G., Lazdino, I., Danks, D. M. & Mercer, J. F. (1984) *Eur. J. Biochem.* **143**, 507–511.
41. Griffith, B. B., Walters, R. A., Enger, M. D., Hildebrand, C. E. & Griffith, J. K. (1983) *Nucleic Acids Res.* **11**, 901–910.
42. Glanville, N., Durnam, D. & Palmiter, R. (1981) *Nature (London)* **292**, 267–269.
43. Huang, I.-Y., Kimura, M., Hata, A., Tsunoo, H. & Yoshida, A. (1981) *J. Biochem.* **89**, 1839–1845.
44. Winge, D. R., Nielson, K. B., Zeikus, R. D. & Gray, W. R. (1984) *J. Biol. Chem.* **259**, 11419–11425.
45. Munger, K., Germann, U. A., Beltramini, M., Niedermann, D., Baitella-Eberle, G., Kägi, J. H. R. & Lerch, K. (1985) *J. Biol. Chem.* **260**, 10032–10038.
46. Winge, D. R., Gray, W. R., Zelazowski, A. & Garvey, J. S. (1986) *Arch. Biochem. Biophys.* **245**, 254–262.
47. Winge, D. R. & Miklossy, K. A. (1982) *J. Biol. Chem.* **257**, 3471–3476.
48. Furey, E. F., Robbin, A. H., Clancy, L. L., Winge, D. R., Wang, B. C. & Stout, C. D. (1986) *Science* **231**, 704–710.
49. Briggs, R. W. & Armitage, I. M. (1982) *J. Biol. Chem.* **257**, 1259–1262.
50. Nielson, K. B. & Winge, D. R. (1984) *J. Biol. Chem.* **259**, 4941–4946.
51. Margoliash, E. & Fitch, W. M. (1968) *Ann. N.Y. Acad. Sci.* **151**, 359–381.
52. Kägi, J. H. R., Vasak, M., Lerch, K., Gilig, D. E., Hunziker, P., Bernhard, W. R. & Good, M. (1984) *Environ. Health Perspect.* **54**, 93–103.