THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 249, No. 11, Issue of June 10, pp. 3537-3542, 1974
Printed in U.S.A.

Equine Hepatic and Renal Metallothioneins

PURIFICATION, MOLECULAR WEIGHT, AMINO ACID COMPOSITION, AND METAL CONTENT*

(Received for publication, September 17, 1973)

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SUMMARY

An improved, simplified, and greatly shortened isolation procedure for metallothionein from tissue extracts using gel filtration (Sephadex G-50) and DEAE-cellulose chromatography is presented. When isolated by this procedure, the molecular weight of both the liver and kidney metalloproteins is found to be about 6600, as determined by equilibrium centrifugation and gel filtration. The amino acid composition of both proteins is also closely similar. Their distinctive features include the extremely high content of cysteine which comprises about 33% of all amino acid residues, and the absence of phenylalanine, tyrosine, tryptophan, and histidine. In addition, both proteins show substantial microheterogeneity even when isolated from the organ(s) of a single animal. Based on a molecular weight of 6000, the nearest integral composition for a total of 61 amino acids is Lys_6 Arg_1 Asp_3 Thr_2 Ser_7 Glu_3 Pro_3 Gly_6 Ala_6 Cys_{20} Val_2 Met_1 for both renal and hepatic proteins. All 20 cysteinyl residues are titratable with silver ions and hence the protein contains no disulfide bonds. The total metal content consisting of cadmium, zinc, and copper is close to 6 g atoms per mole for both liver and kidney metallothioneins; the liver protein contains predominantly zinc whereas that from kidney contains more cadmium. Thus, for each metal atom bound on the average 3 cysteinyl residues are available for binding.

An unusual protein, metallothionein, was first isolated from equine renal cortex by Margoshes and Vallee (3). In subsequent work it was found to contain 5.9% cadmium and 2.2% zinc and the metal-free protein, thionein, was found to contain 16.3% nitrogen and 9.3% sulfur (4). The sulfur is present as cysteine, accounting for about 30% of all amino acids present in this metalloprotein. This appears to be a fundamental feature of the molecule, since a high content of this amino acid has been found in metallothioneins isolated from a variety of species, including human kidney (5), rabbit liver (6), and rat liver (7). From all sources examined thus far, metallothionein has been reported to be a low molecular weight protein (6,000 to 10,000), and the metal composition varies with the organ from which it is isolated. The purification procedure used in the earlier studies (8) was not readily adaptable to isolation of large quantities of metallothionein, and thus it was desirable to improve and simplify the method to obtain sufficient quantities for further studies. This communication presents a new isolation procedure and some of the properties, such as molecular weight, amino acid composition, and metal content, of the metalloprotein isolated by this new method.

MATERIALS AND METHODS

Reagents and Glassware—Analytical grade chemicals and double-distilled water were used throughout. The preparation of metal-free buffers and water, and the necessary precautions to avoid metal contamination have been described (9). Solutions were stored in polyethylene containers at 4°, and all dialyses were performed in cellulose casings (Visking-Nojax, 27/32), precleaned, and treated as reported previously (8).

Preparative Gel Filtration—Filtration was carried out at 4° in a 10-liter bed of Sephadex G-50, fine beads (Pharmacia, Uppsala, Sweden). The column was constructed from a Lucite tube 120 cm in length and 10.5 cm in diameter and fitted at the bottom with a perforated Lucite plate carrying a tightly cut sintered polyethylene filter disk. The top of the column was closed by a removable O-ring sealed Lucite cover plate provided with an inlet hole in the center. Packing of the column with gel was accomplished at room temperature after replacing the cover plate by a Lucite extension tube (60 × 10 cm). In the packed column a flow rate of 250 to 350 ml per hour was maintained by a Sigma T-8 peristaltic pump. After repeated use the gel was rinsed by passing through the column under gravity-promoted flow a 2-liter volume of a solution containing 0.1 M Na_3PO_4 and 0.1% Triton X-100. After elution of the detergent from the gel the column was repacked.

Ion Exchange Chromatography—Chromatography was performed on a Whatman DE23 DEAE-cellulose, with a nominal capacity of 1.0 meq per g. The absorbant was prepared for use as indicated by the manufacturer. Packing into a Lucite column (5 × 120 cm) and equilibration with starting buffer was carried out as described by Himmelhoch and Peterson (10). Salt gradi-

* This work was supported by Grant-in-aid GM15003 from the National Institutes of Health, Department of Health, Education and Welfare. Preliminary reports of this study have been presented (1, 2).

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ents were produced with a Variograd gradient device (Buchler Company). Constant flow was maintained by Sigma T-8 peristaltic pump at about 40 ml per hour.

pH and Conductivity—pH and conductivity were measured at room temperature.

Polyacrylamide Disc Electrophoresis—Electrophoresis was performed in the standard system of Davis (11). Apparatus and material for electrophoresis were products of Canalco Corporation, Rahway, N. J. Electrophoresis was carried out (on 20-μg samples) at a constant current of 4 ma per tube and approximately 180 volts for 2 hours at 23°.

Molecular Weight Determinations—Molecular weight determinations from sedimentation equilibrium were made using the short column method of Van Holde and Baldwin (12) with 2-mm columns of protein solution in 0.1 M sodium phosphate-0.1 M potassium chloride-0.01 M 2-mercaptoethanol, pH 7.5, 20°, using FC-43 fluorocarbon to form a false bottom. All sedimentation experiments were carried out using a Spinco model E analytical ultracentrifuge equipped with Rayleigh interference optics. Results of the equilibrium runs were recorded on Kodak spectrographic plates, emulsion type IIG, nd were analyzed using a microcomparator. Initial protein concentrations were determined using a synthetic boundary-forming cell of the capillary type, and molecular weights were calculated from the slope of plots of log J, where J is the number of Rayleigh fringes, against r^2, where r is the distance to the center of rotation, using trapezoidal integration to evaluate the relevant integrals.

Solvent Densities and Partial Specific Volume—Solvent densities and partial specific volume of the protein were determined pycnometrically at 20° (13). The specific volume of the protein was determined in the buffer employed for sedimentation studies at a protein concentration of 20.7 mg per ml.

Molecular Weight Estimates—Molecular weight estimates of unfolded, oxidized polypeptide chains were made by gel filtration at 23° on a 6% agarose column (85 × 1.5 cm) (Bio-Gel A-5m, 50 to 100 mesh) equilibrated with 6 M guanidinium chloride (Schwarz-Mann Chemical Co.), pH 7.0, as described by Fish *et al.* (14). Blue dextran 2000 (Pharmacia) and dinitrophenyl-alanine (Fluka Chem. Co.) were used as reference substances. Samples containing 1 to 3 mg of protein were first oxidized with performic acid for 16 hours (15). After removal of the solvent on a rotatory evaporator the samples were taken up in 0.5 ml of 6 M guanidinium chloride containing 0.1% dinitrophenyl-alanine and 0.2% blue dextran 2000 and applied to the column. The effluent fractions were monitored by measuring absorbance at 220, 410, and 600 nm to determine the elution volumes of the polypeptide chain, V_e, of dinitrophenyl-alanine, V_t, and of blue dextran 2000, V_0. Partition coefficients, K_D, were evaluated from the relationship $K_D = (V_e - V_0)/(V_t - V_0)$. The sources and the molecular weight of proteins employed as markers are listed in Table I. The K_D value of each marker peptide chain was determined in a separate experiment. Values were reproducible within 3%.

Estimates of Stokes Radius—Molecular size estimates of native proteins were obtained by the gel filtration method of Ackers (22) at 4° on a Sephadex G-75 column (140 × 1 cm) equilibrated with 0.1 M Tris acetate, pH 7.0. Protein samples, 1 to 3 mg in 1 ml of Tris acetate buffer containing 0.2% blue dextran 2000 and 10% NaCl, were applied to the column and eluted in 2-ml fractions. Constant flow was maintained employing a Mariotte flask arrangement. The elution volumes of protein, V_e, of blue dextran 2000, V_0, and of salt, V_t, were determined by absorbance measurements at 250 nm, at 600 nm, and by conductivity measurements, respectively. Partition coefficients (K_D) (*vide supra*) of marker proteins were determined in separate experiments. K_D values were reproducible within 2%. Stokes radii of marker proteins were evaluated from known diffusion coefficients, $D_{20,w}$, (23) and are listed in Table I.

Amino Acid Analyses—Analyses were carried out in a Beckman-Spinco amino acid analyzer, model 120 B on samples oxidized with performic acid (15) and hydrolyzed in evacuated and sealed tubes in 6 N HCl for a minimum of 22 hours (24).

Protein Concentrations—Concentrations were evaluated from quantitative amino acid analysis data.

Total Reactive Mercapto Groups—Mercapto groups were titrated with silver ions (25) in a supporting electrolyte solution at pH 7.5 (26). The reagent was prepared from certified aqueous 0.1 M $AgNO_3$ (Fischer Scientific Co.).

TABLE I

Measurement of molecular weight and size by gel filtration: marker proteins

Insulin, oxidized A chain, was obtained from Mann Research Laboratories; trypsin inhibitor, type II-L and ovalbumin, grade V, were obtained from Sigma Chemical Co.; ribonuclease and chymotrypsinogen were products of Worthington Biochemical Co.

Number	Protein	Molecular weight	$D_{20,w} \times 10^7$	Stokes radius	Reference
				A	
I	Insulin, A chain	2,500	17.6	12.2	16
II	Insulin, B chain	3,500			16
III	Trypsin inhibitor (Lima bean)	8,400			17
IV	Ribonuclease	13,700	12.0	17.9	18
V	Myoglobin	17,000	11.3	19.0	19
VI	Chymotrypsinogen	25,800	9.5	22.5	20
VII	Ovalbumin	45,000	7.6	28.1	21

Metal Determinations—Cadmium, zinc, and copper were determined by atomic absorption spectrophotometry. Chromatographic fractions and aqueous solutions of metallothionein were analyzed without prior ashing since the presence of organic constituents did not interfere with the accurate determination of these metals (27). Appropriate dilutions of samples and standards were made in water.

Purification of Metallothionein—All steps were performed at 4°. Livers and kidneys were thawed for 15 to 18 hours at 4°, and cut into 250-g sections for cutting into smaller strips. With the kidney, however, the capsule is removed and only the cortex processed. The tissues divided into 80- to 90-g portions were homogenized in 2 volumes of 0.25 M sucrose-0.02 M Tris-0.005 M HCl buffer, in a Waring Blendor for 20 s. The homogenate was centrifuged at 10,000 × g for 30 min; the supernatant (approximately 500 ml) was applied to the 10-liter gel filtration column equilibrated with 0.02 M Tris-0.005 M HCl (pH 8.6, conductivity 0.45 mmho) and eluted with the same buffer at a flow rate of 250 to 300 ml per hour. The effluent was collected in 24-ml fractions and analyzed for metals and absorbance at 280 nm and 250 nm. The fractions containing the low molecular weight cadmium and zinc protein were pooled and frozen for storage. In order to accumulate sufficient material for the next step, this process, starting with 250 g of tissue, was performed 12 to 15 times. Preparations of low metal content were discarded.

For further processing all preparations of adequate metal content from the gel filtration step were thawed, pooled (10 to 15 liters), and applied to a DEAE-cellulose column (120 × 5 cm) equilibrated with 0.02 M Tris-0.005 M HCl, pH 8.6 (conductivity 0.45 mmho). After passing 1 column volume of the same buffer through the column the absorbed material was eluted using an 8-liter linear salt gradient (limit buffer: 0.2 M Tris-0.05 M HCl). Constant flow was maintained at about 300 ml per hour. Effluent fractions (24 ml) were analyzed for metals and absorbance at 280 nm and 250 nm. The fractions containing metallothionein were pooled and concentrated by pressure dialysis using a 400-ml Amicon pressure dialysis cell (UM-2 membrane). The concentrated solution (approximately 50 ml) was freed from nonvolatile salt by passing 4 liters of a solution containing 10^{-3} M $(NH_4)_2CO_3$ and 2×10^{-4} M acetic acid, pH 8.6, through the cell and then lyophilized.

If further purification is desired, 100-mg portions of the lyophilized material are redissolved in approximately 4 ml of H_2O and dialyzed 4 hours against 0.02 M Tris-0.005 M HCl, pH 8.6, and applied to a DEAE-cellulose column (21 × 2 cm) equilibrated with the same buffer. After passing 1 column volume of starting buffer through the column, elution is carried out by a 1-liter linear salt gradient (limit buffer: 0.1 M Tris-0.025 M HCl, pH 8.6, conductivity 0.45 mmho), at a flow rate of about 100 ml per hour. The fractions containing metallothionein are pooled, concentrated and desalted by ultrafiltration (*vide supra*), lyophilized, and stored at −20°.

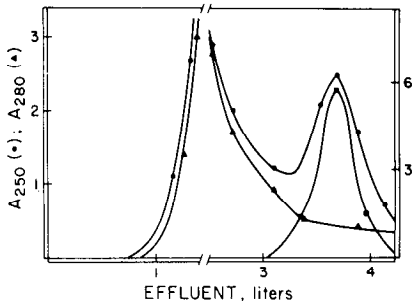

FIG. 1 (*left*). Gel filtration of the kidney homogenate supernatant on a 10-liter Sephadex G-50 column (for conditions see "Materials and Methods"). Absorbances at 250 nm (●), at 280 nm (▲), and cadmium (■) were measured. The effluent between 3.3 and 4.3 liters is combined and saved for application to the DEAE-column.

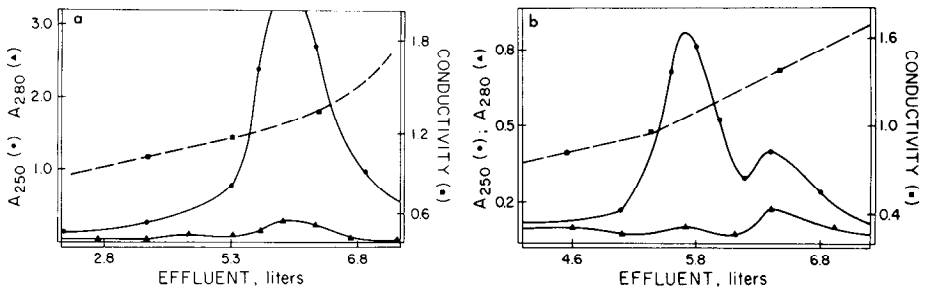

FIG. 2 (*center* and *right*). Chromatography of pooled gel filtration fractions (from 14 preparations) of kidney metallothionein (*a*) and of liver metallothionein (*b*) on a 2.3-liter DEAE-cellulose column (for conditions see "Materials and Methods"). Absorbances at 250 nm (●), at 280 nm (▲) and conductivity (■) were measured. The material between a conductivity of 1.1 and 1.4 mmho (about 1.2 liters) is collected for further processing.

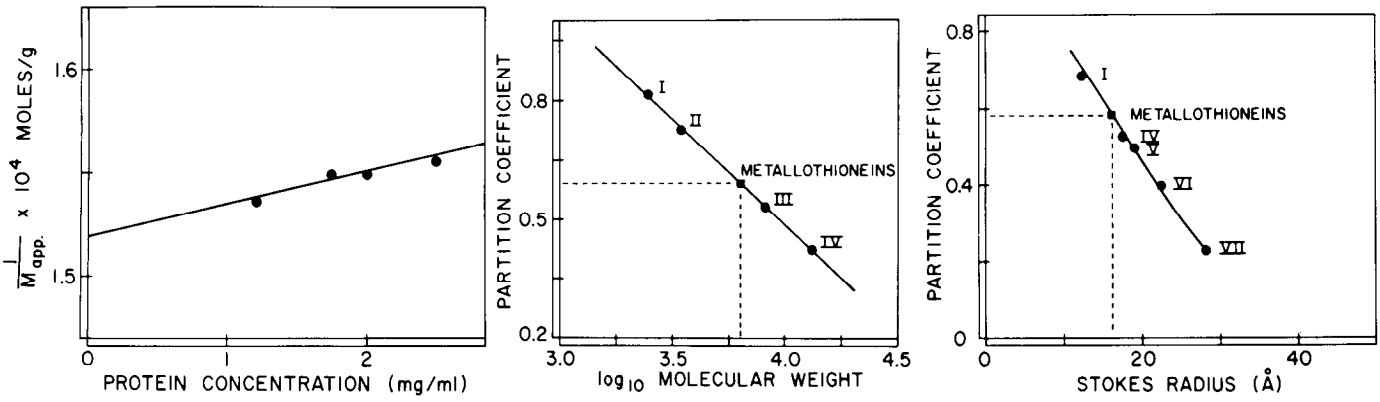

FIG. 3 (*left*). Concentration dependence of the reciprocal of the apparent molecular weight, $1/M_{app}$ of renal metallothionein in 0.1 M KCl, 0.1 M sodium phosphate, and 0.01 M 2-mercaptoethanol, pH 7.5. The molecular weight M_0 calculated from the intercept of the least square line with the ordinate is 6600.

FIG. 4 (*center*). Determination of molecular weight of performic acid-oxidized renal and hepatic metallothionein by gel filtration in 6 M guanidinium chloride on Bio-Gel A-5m. The molecular weight corresponding to the measured partition coefficient of both proteins (= 0.59) is 6500. The marker protein numbers correspond to those in Table I.

FIG. 5 (*right*). Determination of the Stokes radius of renal and hepatic metallothionein by gel filtration on Sephadex G-75. Values for the two proteins are 16.1 and 16.2 A, respectively. Stokes radii of marker proteins were calculated from diffusion constants (see "Materials and Methods"). The marker protein numbers correspond to those in Table I.

RESULTS

A representative elution pattern from the gel filtration step of the kidney homogenate supernatant is shown in Fig. 1. Either measurement of cadmium or zinc or of their mercaptide absorption at 250 nm (4) can be utilized to locate metallothionein. Similar results are obtained on gel filtration of liver extract except that zinc rather than cadmium is measured, and it should be recalled that the contribution of the former to absorption is about 4 times less than that of the latter (4).

Fig. 2 shows the elution diagram from DEAE-cellulose chromatography of the pooled main line fraction obtained by the gel filtration step of kidney and of liver metallothionein. Both proteins are eluted at the same conductivity, as indicated by the position of the main 250-nm absorption peak. As shown in Fig. 2*b*, preparations from liver occasionally contain additional small peaks, absorbing at 250 nm, emerging at higher conductivity. The fractions under the main peak are pooled, concentrated, desalted by ultrafiltration, and lyophilized. The yield of metallothionein from both kidney and liver is about 100 mg per kg wet weight. As judged by disc electrophoresis this material may still contain small amounts (<5%) of impurities. They are readily removed, however, by rechromatography on a smaller DEAE-cellulose column with a shallower salt gradient ("Materials and Methods"). The proteins isolated from kidney and liver move toward the anode and are indistinguishable in their electrophoretic mobilities from one another and from the main component of renal metallothionein obtained by the earlier procedure (4).

The partial specific volume of the renal metallothionein examined was found to be 0.635 ml per g, slightly lower than reported previously (4). Sedimentation equilibrium measurements at four concentrations yielded linear plots of log J against r^2, demonstrating weight homogeneity of the material. The apparent molecular weight, M_{app}, obtained from such data decreases slightly with increasing protein concentration. A plot of $1/M_{app}$ *versus* concentration is linear over the concentration range examined (Fig. 3), and the extrapolated molecular weight is about 6600 ± 300. This value corresponds to about 6000 for the protein moiety if a total metal content of 8.2% (*vide infra*) is taken into account.

Estimation of the molecular weight under conditions where the peptide chain exists in random coil conformation also confirms this value. Thus, gel filtration of the performic acid-oxidized polypeptide chains of renal and hepatic thionein yields a molecular weight of 6500 ± 300 (Fig. 4). Allowing for the substantial weight increase on oxidation of the cysteine side chains

the value for nonoxidized thionein may be estimated to be close to 6000.[1]

The Stokes radii of the native proteins were measured by gel filtration of Sephadex G-75 (Fig. 5). Based on comparison with marker proteins of known Stokes radius values of 16.1 ± 0.3 and 16.2 ± 0.3 are obtained for renal and hepatic metallothionein, respectively. The corresponding frictional coefficients, f, calculated from these values by Stokes' law are 3.03×10^{-8} and 3.05×10^{-8} c.g.s. u. The frictional ratio f/f_0 for native renal metallothionein is 1.39, based on a molecular weight of 6600, and $\bar{V} = 0.635$ g^{-1} ml. Assuming a degree of hydration of 0.2 the corresponding axial ratio of a prolate ellipsoid is close to 6 (29).

A very strong similarity of the amino acid composition of both renal and hepatic metallothioneins, prepared by the described procedure, is apparent (Table II). The large half-cystine content of 19 to 20 residues per molecule is the most outstanding feature, accounting for one-third of all amino acids. Along with the sole methionine residue this accounts for all of the sulfur present in the protein. This high half-cystine content is consistent with previous observations from this laboratory based on titration of mercapto groups and elementary sulfur analysis (4, 5) and with that reported for metallothioneins isolated from the livers of rabbits (6) and rats (7). All half-cystine residues occur as cysteine since there is excellent agreement between the amino acid analysis and the number of Ag^+-titratable mercapto groups (Table II). Other unusual features of this protein include the complete absence of aromatic amino acids and histidine, thus accounting for the low absorbance at 280 nm (4). The other amino acid residues occur in all preparations but most of them deviate notably from integral stoichiometry, when based on a chain molecular weight of 6000. Thus, except for methionine, aspartic acid, and glycine the number of residues per mole differs from the nearest integral value far more than would be expected from experimental variation. Two amino acids, leucine and isoleucine, are present only in fractions of mole quantities. These figures remain constant up to hydrolysis times of 134 hours.

The total metal content of metallothionein from either liver or kidney is slightly greater than 6 g atoms per mole (Table III). The metal composition of the two proteins differs, however, characteristically relative to one another. On a gram atom basis, the zinc and cadmium contents of kidney metallothionein are about equal. In contrast zinc is the predominant element in liver metallothionein, which contains only a fraction of a gram atom of cadmium. A small amount of copper is present in both proteins. Comparison of the number of cysteinyl residues present in the two proteins (Table II) with the total number of metal atoms shows that on the average both contain three mercapto ligands available for binding of each bivalent metal ion, in accord with earlier suggestions (4).

TABLE II

Amino acid composition of hepatic and renal metallothioneins

Amino acid[a]	Liver		Kidney	
	Residues %	Residues/ 6000 g	Residues %	Residues/ 6000 g
Lysine	10.41	6.31	11.06	6.66
Arginine	2.22	1.39	2.29	1.38
Aspartate	5.00	3.06	5.22	3.14
Threonine[b]	3.85	2.31	3.11	1.87
Serine[b]	11.55	6.93	11.01	6.63
Glutamate	4.47	2.68	4.27	2.57
Proline	5.10	3.06	4.29	2.58
Glycine	10.08	6.05	10.01	6.02
Alanine	9.43	5.66	9.68	5.83
Half-cystine[c]	32.62	19.60	33.58	20.22
Valine	2.57	1.54	3.11	1.87
Methionine[d]	1.50	0.90	1.68	1.01
Leucine	0.60	0.36	0.55	0.33
Isoleucine	0.63	0.44	0.17	0.10
Total[e]	100		100	
Mercapto groups	32.50	19.50	33.90	20.30

[a] The data are the average of four analyses of metallothionein oxidized with performic acid and hydrolyzed for 22 hours.

[b] Values are corrected for standard losses (24), based on hydrolysis up to 134 hours.

[c] Measured as cysteic acid, based on c value of aspartic acid.

[d] Measured as methionine sulfone, based on c value of methionine.

[e] Metallothionein contains no histidine, phenylalanine, tyrosine, and tryptophan. The absence of tyrosine and tryptophan is shown by the lack of absorbance of the metal-free protein at 280 nm.

TABLE III

Metal composition and ratio of titratable mercapto groups to metals in equine hepatic and renal metallothioneins

Metal	Liver[a]	Kidney[a]
Zinc	5.72	2.97
Cadmium	0.33	3.03
Copper	0.18	0.14
Total	6.23	6.14
Ratio of Mercapto groups[b] to total metal	3.1	3.2

[a] Gram atom metals per 6000 g of thionein.

[b] Measured by Ag^+-titration; groups per 6000 g of thionein (see Table II).

DISCUSSION

It was apparent from early investigations on metallothionein that its unusual metal content and optical properties reflect corresponding unusual characteristics of its protein moiety. Efforts to define the amino acid composition revealed an extraordinarily high cysteine content and the absence of aromatic amino acids, but difficulties in obtaining sufficient quantities of homogeneous material precluded a more decisive evaluation.

Utilizing the new method of isolation, up to 100 mg of metallothionein can now be obtained from 1 kg. of tissue. The purity of the final product is increased as demonstrated by the homogeneity of the protein when it is subjected to gel electrophoresis and gel filtration. The minor 250-nm absorption peaks observed on DEAE-chromatography of renal and hepatic metallothionein confirm the earlier observation that this protein occurs in several electrophoretic variants (4, 5).

Multiple forms of metallothionein were also observed in rat liver by Shaikh and Lucis (30) and by Nordberg *et al.* (6), who

[1] By contrast, molecular weight estimates of the metal-containing proteins by gel filtration under nondenaturing conditions on Sephadex G-50 or G-75 (28) yield values corresponding to that of a globular protein of molecular weight 10,000. Similar values were reported previously for both equine and human renal metallothionein (5).

separated two forms of this metalloprotein from rabbit liver by isoelectric focusing. Cadmium exposure has been reported to influence the proportions of cadmium-binding proteins (31), and this factor may contribute to variations of the minor peaks occasionally seen in our studies, since the tissues are selected from animals without regard to previous treatment.

The molecular weight of metallothionein is appreciably smaller than previously reported (4). The change seems to be the result of the improved isolation procedure, since gel filtration of renal metallothionein prepared by the previous procedure revealed appreciable dimer formation due to partial oxidation. The agreement shown by the present data between the molecular weight of the peptide chain measured in 6 M guanidinium chloride (Fig. 4) and that of the native protein as determined by sedimentation equilibrium analysis indicates that the protein in solution exists as a monomer of molecular weight 6600. The larger value measured by gel filtration[1] under nondenaturing conditions is readily accounted for by the marked deviation of metallothionein from globular shape, as reflected in the high value for the frictional ratio of the protein. Assuming a standard degree of hydration of 0.2 the hydrodynamic properties of metallothionein are best approximated by a prolate ellipsoid with an axial ratio of 6.

The molecular weight of 6000 as estimated from physical measurements of the protein moiety of metallothionein, thionein, is also confirmed independently by end group measurements and by sequence analysis studies currently in progress in this laboratory.[2] It is also in agreement with amino acid analysis data reported for rabbit liver metallothionein (6).

The extremely high content of cysteine, comprising about 30% of all of the amino acids, is the most striking feature of the amino acid composition of metallothionein when isolated from different sources (4–7). This is the highest cysteine content of any protein known and is approached only by certain high sulfur protein fractions of wool (32). While in the wool protein most of the half-cystine residues are in the form of cystine, in metallothionein, the close agreement between silver titratable mercapto groups and the cysteine content (Table II) demonstrates the absence of disulfide bonds.

Other unusual compositional features of metallothionein include the absence of aromatic amino acids and histidine, and the deviation of stoichiometry of most of the remaining 14 amino acids from integral values. This feature is common to the renal and hepatic proteins and is encountered to the same degree in material when isolated either from a single animal or from pooled organs from several animals. Sequence studies in progress show this to be a reflection of extensive microheterogeneity attributable to the presence of at least two different species of metallothionein in the material isolated by the present method. Efforts to resolve the mixture into its components have not been successful thus far.

The abundance of cysteine offers an obvious explanation for the metal-binding capacity of metallothionein. As shown in Table II, there is a *stoichiometric relationship* of three mercapto groups to each metal atom bound. This relationship is further supported by the complete lack of free sulfhydryl groups in the metalloprotein, as demonstrated previously (1, 4). This same ratio of mercapto groups to the sum of metal atoms present is found in preparations from all sources examined thus far,[2] and thus appears to be a fundamental feature of the metallothionein structure. The spatial arrangement of six metal binding sites, each composed of 3 cysteine residues, poses intriguing questions regarding both the linear and three-dimensional structures of metallothionein, which are presently under investigation.

A discussion of metallothionein would not be complete, of course, without attention to the decade-old question regarding its metabolic role. Its potential role in homeostatic mechanisms, catalysis, storage, immune phenomena, or detoxification has been developed (8). Its possible involvement in heavy metal detoxification has been singled out for most attention, a view which poses a number of problems, however. First, although as the result of its chemical composition, many heavy metal atoms will bind to thionein *in vitro*,[2] and cadmium exposure and the amount of metallothionein found have been correlated (7, 31, 33, 34), this protein generally does not bind heavy metals other than cadmium, zinc, and copper and perhaps mercury *in vivo* (35, 36). Second, metallothionein has been detected in significant quantities in fetal tissue (1), suggesting both a prenatal and postnatal role. These along with the realization that the metal content of the protein varies in different tissues raises questions regarding a function in metal storage or transport. Finally, since metallothionein has been found in nearly every tissue of the body,[2] a possible general role in maintaining oxidation-reduction potential and in transport, perhaps analogous to functions of glutathione, appears plausible, especially in view of the function of that peptide in amino acid transport (37, 38). In this regard, the possibility that thionein, the metal-free protein, rather than the metal-containing protein could be the metabolically active species should be considered, a proposition currently under investigation in this laboratory.

[2] J. H. R. Kägi, unpublished observation.

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J. Biol. Chem. 1974, 249:3537-3542.