Isolation and partial characterization of the low-molecular-mass zinc/cadmium-binding protein from the testes of the patas monkey (*Erythrocebus patas*)

Distinction from metallothionein

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The mammalian testes are generally quite susceptible to cadmium. A deficiency of metallothionein (MT), a metal-binding protein linked to Cd tolerance, has been observed in rat testes and may explain the sensitivity in rats. Little is known about the metal-binding proteins in primate testes. Thus this study examined the nature of these proteins in a non-human primate species, the patas monkey (*Erythrocebus patas*). In all cases proteins isolated from testes were compared with authentic MT isolated from the liver of a zinc-treated monkey. A low-molecular-mass Zn/Cd-binding protein was seen in testicular and hepatic cytosol after gel filtration. Neither protein had substantial amounts of associated copper. These proteins could be partially purified from both sources by heat treatment and acetone precipitation. When such extracts were further separated by reverse-phase h.p.l.c., four hepatic forms were isolated, all of which proved to be authentic MT by amino acid analysis. However, only two testicular forms were separated by h.p.l.c., both of which had amino acid compositions quite unlike that of MT, having a much lower cysteine content and amino acids which are absent from MT (leucine and phenylalanine). The testicular protein appeared to be uninducible by Zn treatment. These results suggest that the low-molecular-mass Cd/Zn-binding proteins in the patas testes are not MTs and further support the hypothesis that a MT deficiency may be an important determinate of the marked testicular sensitivity to Cd toxicity.

INTRODUCTION

The mammalian testes are highly susceptible to the toxic effects of Cd, exposure to which rapidly results in severe haemorrhagic necrosis (Girod, 1964; Gunn *et al.*, 1965; Gunn & Gould, 1970; Lohiya *et al.*, 1976). A loss of tubular elements occurs, along with a functional loss, as evidenced by sterility and sex-accessory-tissue regression (Gunn & Gould, 1970). Interstitial tissue then regenerates, which can result in a high incidence of testicular interstitial-cell tumours (Gunn *et al.*, 1963; Gunn & Gould, 1970). Although the effects of Cd are most extensively documented in rodents, several studies have indicated that similar effects can occur in primates (Kar, 1961; Girod & Chauvineau, 1964; Girod, 1964; Lohiya *et al.*, 1976).

Metallothionein (MT), a low-molecular-mass metal-binding protein, is thought to play a major role in the biological detoxification system for Cd (Probst *et al.*, 1977; Goering & Klaassen, 1984). MTs are distinguished by a high cysteine content (approx. 30%; Sobocinski *et al.*, 1978; Webb, 1979; Wong & Klaassen, 1979). MTs are also devoid of the aromatic amino acids, display a remarkable heat-stability and have an extremely high affinity for Cd (Kagi & Vallee, 1961; Sobocinski *et al.*, 1978; Webb, 1979). Synthesis of MT is markedly increased by Cd or Zn (Onosaka & Cherian, 1981, 1982),

and a correlation between MT concentration and decreased Cd toxicity has been well documented (Probst *et al.*, 1977; Goering & Klaassen, 1984). MT appears to decrease 'free' Cd by high-affinity sequestration and thereby decrease its toxic potential (Webb, 1975). It is suspected that MT decreases Cd carcinogenicity in a similar manner (Sunderman, 1979).

Several reports, based on physical characterizations (i.e. molecular mass, charge properties etc.), indicate the presence of high basal amounts of MT in rodent testes (Nordberg, 1971; Chen *et al.*, 1974; Chen & Ganther, 1975; Webb, 1975; Brady & Webb, 1981; Chellman *et al.*, 1984, 1985). A testicular MT-like protein has also been described in primates (Wisniewska-Knypl *et al.*, 1971; Nomiyama & Nomiyama, 1982). Studies using the Cd-haemoglobin assay for MT quantification indicate high constitutive contents of MT-like proteins in rat testes (Onosaka *et al.*, 1978; Onosaka & Cherian, 1981, 1982; Eaton & Toal, 1982). However, the specificity of this technique has only been shown for liver (Eaton & Toal, 1982). High basal contents of MT mRNA have also been detected in mouse testes, although, unlike all other tissues tested, these amounts are not increased by Cd treatment (Durnam & Palmiter, 1980).

Evidence has been presented that rat testes are actually deficient in MT, as attempts to isolate MT results in isolation of a protein which is physically similar, but

Abbreviations used: MT, metallothionein.

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chemically quite distinct (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986). Although a protein of similar molecular mass and charge to MT is found in rat testicular cytosol, it is very different in amino acid composition (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986), with much lower cysteine content, and, therefore, probably a lower affinity for Cd (Waalkes & Perantoni, 1986). As MT is thought to detoxify Cd by high-affinity sequestration, the MT deficiency in the rat testes might explain the remarkable target-site specificity of Cd for this tissue.

Very little is known about the nature of the Cd-binding proteins in the testes of primates. Therefore this study was undertaken to characterize the low-molecular-mass metal-binding proteins in testes of a representative species of Old World monkey, *Erythrocebus patas*, to determine if testicular deficiency of MT occurs in species more closely related to humans.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless otherwise noted. Radioactive cadmium (^{109}Cd) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Trifluoroacetic acid and acetonitrile for h.p.l.c. were obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.) and J. T. Baker Chemical Co. (Phillipsburg, NJ, U.S.A.) respectively.

Animals and sample preparation

The monkeys used were from a colony held under contract by Sema Inc., Rockville, MD, U.S.A. Two feral-origin males, acquired as adults from Primate Imports (New York, NY, U.S.A.), which had been imported from Nigeria 92–126 months previously, and one colony-born male, 123 months old, were used. All animals were ex-breeders, estimated at between 123 and 198 months old, and were housed individually in cages providing space equal to or in excess of recommendations. The environment was maintained at $22 \pm 2^\circ\text{C}$ ($72 \pm 4^\circ\text{F}$), with a relative humidity of $50 \pm 10\%$ and with a minimum of 12 complete room air changes without recirculation per hour and a 12 h photoperiod. Monkeys were allowed free access to potable water and were fed on commercially available dry primate diet (Ralston Purina Co., St. Louis, MO, U.S.A.) twice daily, supplemented with fresh fruit.

Preliminary studies were performed on a testis removed aseptically by scrotal excision under sodium thiamylal anaesthesia. The excised testis was decapsulated and then rapidly processed at 4°C as a source of proteins from fresh tissue. Further studies were performed with tissues obtained at necropsy, which was immediately placed in screw cap vials, frozen in liquid N_2 and stored at -20°C or colder until processing within 1 month. Such storage did not affect the results of gel filtration, as elution profiles were similar with fresh or frozen tissues. Animals were killed by sodium pentobarbital overdose (25 mg/kg, intravenously) after ketamine hydrochloride (12.5 mg/kg, intramuscularly)-induced anaesthesia.

Hepatic MT was induced in one animal by subcutaneous injection of 50 mg of zinc acetate/kg in

saline (5 ml) in the lower posterior thoracic region, opposite sides being used on alternate days. Injections were made 72, 48 and 24 h before death.

For sample preparation, tissue was homogenized (1:4, w/v) with a Potter–Elvehjem glass homogenizer and a motor-driven Teflon pestle in 20 mM-Tris/HCl (pH 8.6 at 4°C) containing 5 mM-2-mercaptoethanol and 250 mM-sucrose. Cytosol was derived by centrifugation at 10000 g for 10 min, followed by 100000 g for 45 min.

A method for the partial purification of MT from hepatic cytosol (Sobocinski *et al.*, 1978) was applied to testicular and hepatic cytosols. Cytosol was heated to 85°C for 10 min and centrifuged (15 min at 27000 g). The heat-stable protein was then subjected to selective acetone precipitation (0–40%, 40–60%, 60–80%) with centrifugation (27000 g for 15 min) between each step. The 80%-acetone precipitate was stored under argon until reconstitution in: (a) 20 mM-Tris/HCl, pH 8.6, containing 5 mM-2-mercaptoethanol and 250 mM-sucrose for gel-filtration chromatography; (b) 0.1% trifluoroacetic acid containing 3 M-guanidine hydrochloride for reverse-phase h.p.l.c.; or (c) 5 mM-Tris/HCl, pH 7.4, for anion-exchange h.p.l.c.

Gel-filtration chromatography

Testicular cytosol was either used as prepared or saturated with Cd by addition of $1.25\text{ }\mu\text{mol}$ of Cd/g wet wt. of tissue equivalent. Hepatic cytosol was saturated with $5.0\text{ }\mu\text{mol}$ of Cd/g wet wt. equivalent. Samples of 80%-acetone precipitate from heat-treated cytosol were reconstituted in 2.0 ml of buffer/g wet wt. equivalent and then saturated with Cd. Samples (no greater than 1.0 g wet wt. equivalent) were applied to a column ($1.5\text{ cm} \times 40\text{ cm}$) of Sephacryl S-200 previously equilibrated with 20 mM-Tris/HCl, pH 8.6, containing 50 mM-NaCl, 5 mM-2-mercaptoethanol and 0.02% NaN_3 , and were eluted with a flow rate of 36 ml/h. Fractions ($40 \times 5\text{ min}$) were collected and assayed for Cd, Zn, Cu, Cr, Co and Fe contents by atomic-absorption spectrophotometry.

Reverse-phase h.p.l.c.

The method of Klauser *et al.*, (1983), with slight modification (Waalkes & Perantoni, 1986), which previously has been employed for isolation of hepatic MT isoforms, was used. Proteins were separated by using an Aquapore RP-300 column (Brownlee Laboratories, Santa Clara, CA, U.S.A.) with a 65 min linear gradient of 25–40% acetonitrile in 0.1% trifluoroacetic acid (pH 2.0). Flow rate was 1.0 ml/min, and 60 1 min fractions were collected. Elution as described was accompanied by a loss of metal-binding capacity in both testicular and hepatic preparations.

Anion-exchange h.p.l.c.

Separations were performed by the method of Suzuki (1980), by using a Waters Protein Pak DEAE 5 PW anion-exchange column, on samples of hepatic or testicular low-molecular-mass metal-binding proteins partially purified by either acetone extraction and solubilization in 5 mM-Tris/HCl (pH 7.4) or gel filtration followed by dialysis against 5 mM-Tris/HCl (pH 7.4). Approx. $3\text{ }\mu\text{Ci}$ of $^{109}\text{CdCl}_2$ was added to the samples (approx. 1.0 g wet wt. equivalent). Samples were loaded on to the column, which was then washed for 10 min with 5 mM-Tris/HCl, pH 7.4, at a flow rate of

1.0 ml/min. Proteins were then eluted with a linear gradient of 5–200 mM-Tris/HCl, pH 7.4.

Amino acid analysis

Fractions containing each separate hepatic and testicular metal-binding protein peak after elution by reverse-phase h.p.l.c. were combined, divided in two and freeze-dried. One of each pair was hydrolysed for 20 h at 110 °C in 6 M-HCl containing 0.1% phenol. The other sample was first oxidized in performic acid for 4 h at 4 °C, then diluted with water, frozen, freeze-dried and hydrolysed in 6 M-HCl containing 0.1% phenol for 20 h at 110 °C. Hydrolysates were analysed for amino acid content with a Dionex D-500 amino acid analyser and ninhydrin detection.

Heat-stable-protein Cd-binding capacity

An assay originally designed to measure tissue concentration of MT (Onosaka *et al.*, 1978), which uses the heat-stability of MT for separation of bound and free Cd, was applied to cytosol derived from control testes and the testes and liver of the Zn-treated monkey. Cytosol was prepared by homogenization in 10 mM-Tris/HCl, pH 7.4, and centrifugation. Cytosol (100 μ l) was incubated for 10 min with radioactive Cd (100 μ l; 2 μ g of Cd/ml, 3 μ Ci/ml in Tris buffer) before addition of 50 μ l of 2% (w/v) haemoglobin (bovine; Sigma type I) to absorb free Cd. The samples were then heated for 1 min at 100 °C, cooled on ice, and centrifuged at 10000 g for 1 min to eliminate Cd bound to non-heat-stable proteins. The haemoglobin addition and all subsequent steps were repeated once. A sample of the final supernatant was removed and ^{109}Cd radioactivity was determined.

Estimation of metal-binding-site number

The number of metal-binding sites was estimated by determining the ratio of Zn to protein contents of hepatic MT and of the testicular metal-binding protein. Hepatic and testicular cytosol was subjected to gel-filtration chromatography as described above, and the fractions containing the low-molecular-mass Zn-binding protein peak (V_e/V_0 approx. 1.8–2.3) were pooled and protein content was measured by the method of Bradford (1976). The pooled fractions were then extensively dialysed against distilled water in Spectrapor membrane tubing (cut-off M_r 3500; American Scientific Products, McGraw Park, IL, U.S.A.). Zn was determined in the resulting material after digestion (1:1, v/v) with ultrapure HNO_3 (Ultrex; J. T. Baker Chemical Co.), by using a Perkin-Elmer model 500 atomic-absorption spectrophotometer by direct aspiration in the flame mode. Molecular masses of the proteins concerned were estimated by using the average of the values for the several forms as determined by amino acid analysis. Results reported are averages of two separate samples for the testes and of the single hepatic sample.

RESULTS

A representative gel-filtration elution profile of endogenous cytosolic metals from testes of untreated patas monkey is shown in Fig. 1. Results indicated that, of the metals associated with the low-molecular-mass protein in testicular cytosol, the major endogenously bound cation was Zn. Approx. 44% of the total cytosolic

Fig. 1. Representative gel-filtration elution profile of endogenous Zn, Cu, and Fe in testicular cytosol

Cytosol was derived from an untreated patas monkey. The Sephacryl S-200 column was eluted with 20 mM-Tris/HCl, pH 8.6, containing 50 mM-NaCl, 5 mM-2-mercaptoethanol and 0.02% NaN_3 , at a flow rate 48 ml/h. Fractions (5 min) were collected and assayed for Zn (—), Cu (---), Fe (●—●), Co and Cr. Co and Cr were not detectable in any fraction.

Table 1. Amounts of heat-stable Cd-binding protein in patas-monkey liver and testes

Zinc treatment consisted of three separate injections (subcutaneous) each of 50 mg of zinc acetate/kg 72, 48 and 24 h before the animal was killed.

Tissue	Treatment	Heat-stable Cd-binding capacity (nmol of Cd bound/g wet wt.)
Testes	Control	34.9, 45.4
Testes	Zinc	35.2
Liver	Zinc	2770

Zn was contained within this peak. A modest amount of Cu was also detected in association with this protein, but never exceeded 20% of the total metal bound. Fe, Cr and Co were not detected in association with this protein. This testicular protein had a relative elution volume ($V_e/V_0 = 2.1$) very similar to that of patas-monkey hepatic MT ($V_e/V_0 = 2.0$).

The low-molecular-mass protein in testicular cytosol bound Cd after saturation *in vitro* and subsequent gel filtration. Hepatic MT likewise bound Cd that was added to cytosol before gel filtration. These Cd-binding proteins from testicular or hepatic cytosol could be partially purified by a technique utilizing heat treatment and sequential acetone precipitation. After use of this procedure, the prominent low-molecular-mass Cd-binding protein was detected by gel filtration but a loss

Fig. 2. Further purification of partially purified low-molecular-mass testicular Cd/Zn-binding protein by reverse-phase h.p.l.c.

Samples partially purified by heat treatment and acetone precipitation were reconstituted in 1.0 ml of 0.1% trifluoroacetic acid containing 3 M-guanidine hydrochloride/g wet wt. equivalent, and 250 μ l of this solution was injected on the column. Chromatography was performed at 1 ml/min with a linear gradient of 25–40% acetonitrile (linear plot) in 0.1% trifluoroacetic acid. Two major peaks (T_1 and T_2) were detected. Peaks eluted before 20 min were due to traces of constituents of the isolation buffer (Tris, 2-mercaptoethanol).

Fig. 3. Further purification of partially purified low-molecular-mass hepatic Cd/Zn-binding protein by reverse-phase h.p.l.c.

Chromatography was performed as described in Fig. 2. Four major peaks (L_1 , L_2 , L_3 and L_4) were detected. Peaks before 20 min were due to traces of constituents of the isolation buffer (Tris, 2-mercaptoethanol).

Fig. 4. Anion-exchange-h.p.l.c. elution profile of the hepatic Cd/Zn-binding protein

Hepatic preparations of low-molecular-mass Cd/Zn-binding protein were isolated by initial gel filtration. After application, protein samples were eluted with a linear gradient (linear plot) of Tris/HCl (pH 7.4; 5–200 mM) at a flow rate of 1.0 ml/min. The upper panel represents A_{220} , and the bottom panel shows Cd binding as determined by γ -radiation spectrometry. In both cases four major peaks, eluted between 23 and 40 min, were detected.

of other, higher-molecular-mass, Cd-binding proteins from cytosol of either hepatic or testicular origin occurred.

An assay of cytosolic heat-stable-protein Cd-binding capacity, typically used to determine MT concentrations, was applied to the testes of the untreated animals and the Zn-treated animal (Table 1). The results show that amounts of heat-stable Cd-binding protein in the testes were comparable regardless of treatment, indicating a lack of inducibility. Very high contents were, however, detected in the liver from the Zn-treated animal.

Preparations of the low-molecular-mass metal-binding proteins partially purified from patas testes cytosol by heat treatment and sequential acetone precipitation were further purified with reverse-phase h.p.l.c. (Fig 2). The testicular protein was separated into two major isoforms

Fig. 5. Anion-exchange-h.p.l.c. elution profile of the testicular Cd/Zn-binding protein

Testicular preparations of low-molecular-mass Cd/Zn-binding proteins were isolated by initial gel filtration. Chromatography was performed as described in Fig. 4. The upper panel represents A_{220} , and the lower panel shows Cd binding as determined by γ -radiation spectrometry. Results indicate a major peak at 70 min of A_{220} and Cd binding occurring after application of limit buffer and a single peak of Cd binding at 50 min.

eluted at 47 and 56 min. In contrast, when preparations from patas liver were separated by reverse-phase h.p.l.c. (Fig. 3), the low-molecular-mass metal-binding protein was partitioned into four distinct forms, eluted between 55 and 71 min. Testicular and hepatic preparations were also admixed and applied to a reverse-phase h.p.l.c. column (results not shown). Separation of the first testicular form, but not the second, from the several liver forms was readily accomplished.

Marked tissue-dependent differences became evident when these proteins were subjected to anion-exchange h.p.l.c. Four major forms of Cd-binding protein were eluted by the Tris gradient from preparation of hepatic low-molecular-mass metal-binding protein initially partially purified by gel-filtration chromatography (Fig. 4). All four hepatic forms were eluted between 23 and 39 min. Similar results (not shown) were seen with

Table 2. Amino acid composition of patas-monkey hepatic metallothionein

Expressed as residues per molecule with values in parentheses as the nearest whole number. Designations of L_1 , L_2 etc. are given by order of elution from reverse-phase h.p.l.c. as shown in Fig. 3. Compositions are given relative to methionine content.

Amino acid	L_1	L_2	L_3	L_4
Asp	4.2 (4)	4.7 (5)	4.3 (4)	4.3 (4)
Thr	2.3 (2)	2.4 (2)	2.3 (2)	2.2 (2)
Ser	7.8 (8)	6.9 (7)	5.9 (6)	5.3 (5)
Glu	4.0 (4)	4.3 (4)	4.3 (4)	3.8 (4)
Pro	3.2 (3)	2.8 (3)	5.3 (5)	4.5 (5)
Gly	3.5 (4)	4.7 (5)	4.9 (5)	4.8 (5)
Ala	4.9 (5)	7.0 (7)	6.9 (7)	6.7 (7)
Val	1.6 (2)	2.5 (3)	2.4 (2)	2.5 (3)
Half-Cys*	17.7 (18)	19.2 (19)	18.1 (18)	16.8 (17)
Met	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)
Ile	—	—	—	—
Leu	—	—	—	—
Tyr	—	—	—	—
Phe	—	—	—	—
His	—	—	—	—
Lys	9.4 (9)	8.9 (9)	8.6 (9)	8.0 (8)
Arg	—	—	—	—
Total residues	61	61	63	61
Minimum molecular mass (Da)	6020	6040	6410	6040

*Determined as cysteic acid after performic acid oxidation.

hepatic preparations partially purified by heat treatment and acetone extraction. In sharp contrast, testicular preparations partially purified by gel filtration contained only one broad peak of protein-bound Cd much later in the Tris gradient (50 min), with no corresponding absorbance peak (Fig. 5). In fact most of the Cd was eluted after the limiting buffer was applied in full strength (200 mM). Essentially identical results (not shown) were obtained with testicular protein extracted by heat treatment and selective acetone precipitation.

Amino acid compositions of the proteins isolated by reverse-phase h.p.l.c. confirmed the presence of MT in the liver (Table 2). All four liver isoforms had high cysteine contents and significant amounts of serine and lysine. However, the hepatic proteins did not contain leucine, tyrosine, isoleucine, phenylalanine, histidine or arginine. The amino acid contents of the testicular metal-binding proteins purified by reverse-phase h.p.l.c. (Table 3) were distinctly different from that of MT. The most notable difference was that the two testicular proteins contained very little cysteine (1 residue/molecule). Other differences included the detection in the testicular proteins of amino acids which were not present in liver protein (phenylalanine, isoleucine, leucine and arginine).

Estimation of the number of metal-binding sites (see the Materials and methods section) indicated that in hepatic metallothionein 6.09 Zn atoms were associated with each molecule of protein. Similar ratios have been seen with rat hepatic Zn metallothionein (6.44; Waalkes

Table 3. Amino acid composition of patas-monkey testicular metal-binding protein

Expressed as residues per molecule with values in parentheses as the nearest whole numbers. Forms (T₁, T₂) are defined by order of elution after reverse-phase h.p.l.c. (see Fig. 2). Compositions are given relative to methionine content.

Amino acid	T ₁	T ₂
Asp	5.2 (5)	4.9 (5)
Thr	3.2 (3)	5.1 (5)
Ser	4.8 (5)	4.1 (4)
Glu	12.4 (12)	10.9 (11)
Pro	4.5 (5)	3.9 (4)
Gly	3.3 (3)	3.0 (3)
Ala	3.3 (3)	4.0 (4)
Val	1.1 (1)	0.7 (1)
Half-Cys*	1.2 (1)	0.9 (1)
Met	1.0 (1)	1.0 (1)
Ile	2.0 (2)	2.9 (3)
Leu	2.5 (3)	2.6 (3)
Tyr	—	—
Phe	1.2 (1)	1.2 (1)
His	—	—
Lys	8.1 (8)	8.4 (8)
Arg	1.1 (1)	1.7 (2)
Total residues	54	56
Minimum molecular mass	5960	6180

*Determined as cysteic acid after performic acid oxidation.

et al., 1984c). In contrast, the ratio of Zn to metal-binding protein in the patas testes indicated that only approx. 0.842 atom of Zn was associated with each protein molecule.

DISCUSSION

The results of the present study indicates that the low-molecular-mass Cd/Zn-binding protein in the patas-monkey testes is not a MT. Although this protein has some characteristics in common with MT, a comparison of amino acid contents reveals several major differences, of which differences in cysteine content are the most prominent. Regardless of source, approx. 30% of the amino acids in MT are cysteine (Sobocinski *et al.*, 1978; Webb, 1979; Wong & Klaassen, 1979; Waalkes *et al.*, 1984a; Waalkes & Perantoni, 1986), an observation confirmed in the present study with patas liver MT. In contrast, the protein isolated from the patas testes contained only one cysteine residue per molecule. Furthermore, the monkey testicular protein contained isoleucine, leucine, phenylalanine and arginine, amino acids not present in patas liver MT. The absence of leucine and aromatic amino acid are typical of MTs (Webb, 1979; Nordberg & Kojima, 1979). The patas testicular protein also contains much more glutamate than does MT. Similar differences in amino acid contents have been detected between MT and the testicular Cd-binding proteins in several strains of rats (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986). In primates, two studies have suggested the presence of MT in the testes (Wisniewska-Knypl

et al., 1971; Nomiya & Nomiya, 1982), based on molecular-sieve chromatography and not amino acid analysis. In all cases in which the testicular 'MT-like' protein has been subjected to amino acid analysis, major distinctions from MT have been seen (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986). Marked differences also occurred between the patas testicular protein and MT when number of Zn-binding sites were compared. Therefore it appears that the low-molecular-mass testicular Cd-binding proteins of these two distantly related species are not MT and that a deficiency of MT may be a consistent finding in mammalian testes.

MT is thought to constitute a high-affinity storage protein in Zn homeostasis (Bell, 1979; Wong & Klaassen, 1979; Brady, 1982) or in Cd detoxification (Probst *et al.*, 1977; Goering & Klaassen, 1984). The high affinity of MT for these metals is due to the preponderance of cysteine residues in MT, a preferred bioligand for Zn and Cd (Jacobson & Turner, 1980). However, low amounts of cysteine are present in these testicular proteins (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986; the present study), and alternative binding moieties must therefore be considered. In this regard, glutamate is the most prominent amino acid in the monkey testicular protein, and in the rat testicular proteins it is also one of the most common amino acids (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986). Carboxy groups, as in glutamate, are thought to provide secondary low-affinity binding sites for Cd (Jacobson & Turner, 1980). Association of Cd with free amino acids is generally greater than with amino acids in polypeptide structures, owing to the free carboxy groups of amino acid monomers (Jacobson & Turner, 1980). Zn also interacts with carboxy groups, but with less avidity than with cysteine (Jacobson & Turner, 1980). Hence carboxy-group binding appears to be a possible mechanism for the binding of metal cations to these testicular proteins.

Regardless of the exact binding moiety, low cysteine contents in these testicular proteins indicate they probably have a lower binding affinity for Cd and Zn than does MT. Thus they may normally provide a low-affinity storage depot for Zn. Such a low-affinity pool might be more rapidly mobilized and more responsive to changes in Zn demand. Demand for Zn in the testes is quite high (Halsted *et al.*, 1974; Prasad, 1979), in part owing to the high rates of testicular DNA, RNA and protein synthesis, processes which all require Zn (Halsted *et al.*, 1974; Brady, 1982). Indirect evidence also indicates that the testicular pool of Zn is very labile relative to that of other tissues. Dietary Zn deficiency in rodents rapidly affects testicular Zn concentrations, decreasing both testicular size and function but having much less effect on other tissues (Halsted *et al.*, 1974; Prasad, 1979). Examples of Zn deficiency decreasing testicular function in primates are also well documented (Prasad, 1979). The possibility that the testes of rat and monkey are deficient in MT, a high-affinity binder of Zn, and in its place appear to have a much lower-affinity binding protein, is consistent with this observed lability of testicular Zn relative to other tissues (Halsted *et al.*, 1974; Prasad, 1979).

The apparent deficiency of MT in the testes observed in the present and previous studies (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes &

Perantoni, 1986) is also consistent with the remarkable sensitivity of this organ to Cd. In both rodents (Gunn *et al.*, 1965; Gunn & Gould, 1970) and primates (Girod, 1964; Girod & Chauvineau, 1964; Lohiya *et al.*, 1976), Cd causes pronounced acute toxic effects in testicular tissue, culminating in a marked haemorrhagic necrosis, and in those species observed for prolonged periods, a high incidence of testicular tumours (Gunn *et al.*, 1963). The apparent testicular deficiency of MT, a protein widely believed to confer tolerance to Cd, is consistent with observed testicular sensitivity to Cd. Toxicokinetic evidence also indicates the defence mechanisms of other tissues may not be completely functional in testes, as Cd-induced lesions occur despite the very small fraction (< 0.2%) of a Cd dose that actually localizes in the testes (Gunn & Gould, 1970). MTs appear to detoxify Cd by sequestration through high-affinity binding (Kagi & Vallee, 1961; Probst *et al.*, 1977; Goering & Klaassen, 1984), thus making less metal available for interactions with more sensitive bioligands (Webb, 1975; Goering & Klaassen, 1984). The inducibility of MT synthesis is also thought to be a key factor in biological adaptation to the presence of Cd through creation of a greater capacity for sequestration of the toxic metal (Probst *et al.*, 1977). However, the testicular protein isolated in the present and previous studies (Waalkes *et al.*, 1984a,b; Deagen & Whanger, 1985; Waalkes & Perantoni, 1986), with its probable lower affinity for Cd, may be unable to provide the stable binding seen with MT, and, in combination with an apparent lack of inducibility, may allow Cd greater access to its molecular targets. Hence the apparent deficiency of MT in the testes may be an important determinant of the high sensitivity of this tissue to Cd-induced lesions.

In conclusion, the results of the present study indicate that the testicular low-molecular-mass Cd/Zn-binding protein of the patas monkey, *Erythrocebus patas*, is not MT. A deficiency of MT in mammalian testes has been a consistent finding, and may be an important factor in testicular Zn metabolism as well as a key determinant of the susceptibility of this tissue to Cd poisoning.

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