Development of a radioimmunoassay for rat liver metallothionein-I and its application to the analysis of rat plasma and kidneys

Rajesh K. MEHRA and Ian BREMNER*

Trace Element Biochemistry Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

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A sensitive radioimmunoassay for rat liver metallothionein-I has been developed using avid and high-titre antibodies obtained from sheep that were immunized with a conjugate of metallothionein and rabbit immunoglobulin G. The assay was specific for metallothionein-I, and did not depend on the particular metal bound to the protein. There was no significant cross-reaction with rat liver metallothionein-II. The use of the assay to measure metallothionein concentrations in rat plasma and kidneys is described.

Metallothionein is an ubiquitous protein of low molecular weight (6100) that is characterized principally by its high cysteine and metal content (see Kagi & Nordberg, 1979). It is believed that the protein plays important roles in the metabolism of Cu, Zn, Cd and other metals, but its precise physiological functions are not fully understood. Elucidation of these roles has been hindered by the lack of sensitive and rapid methods for assay of the protein. In most investigations, protein concentrations have been estimated from the metal content of the protein, after its chromatographic separation, by polarography (Olafson & Sim, 1979), or by the use of ^{203}Hg or ^{109}Cd -binding assays, which are of low sensitivity and of uncertain specificity (Zelazowski & Piotrowski, 1977; Chen & Ganther, 1975). Although r.i.a. procedures for the protein have recently been described (Vander Mallie & Garvey, 1979; Tohyama & Shaikh, 1981; Garvey *et al.*, 1982), these have differed in sensitivity and, more important, have failed to distinguish between the various isoproteins of metallothionein.

The present paper describes procedures for the generation of high-titre antibodies that are specific for rat liver metallothionein-I and can be used in a sensitive r.i.a.

Experimental

Materials

NSHPP was obtained from Sigma (London) Chemical Co. (Poole, Dorset, U.K.), Freund's

Abbreviations used: r.i.a., radioimmunoassay; NSHPP, *N*-succinimidyl 3-(4-hydroxyphenyl)propionate; IgG, immunoglobulin G.

* To whom requests for reprints should be addressed.

complete adjuvant from Gibco Bio-cult Ltd. (Paisley, Renfrewshire, Scotland, U.K.), and Sephadex G-25 and G-75 and DEAE-Sephadex A-25 from Pharmacia (G.B.) Ltd. (Hounslow, Middx., U.K.). Carrier-free Na^{125}I (100 mCi/ml) was obtained from Amersham International Ltd. Donkey anti-(sheep IgG) antiserum was kindly supplied by the Scottish Antibody Production Unit, Carlisle, Lanarkshire, Scotland, U.K.

Rabbit IgG was purified from normal rabbit serum by a combination of $(\text{NH}_4)_2\text{SO}_4$ precipitation and ion-exchange chromatography on DEAE-Sephadex A-25 or A-50 (Harboe & Ingild, 1973).

Hooded Lister rats (Rowett Institute strain) were used in all experiments. Cd^{2+} , Cu^{2+} and Zn^{2+} were always injected in the form of the sulphates in 0.9% NaCl solution.

Isolation of metallothionein

The two main isoproteins of Zn-metallothionein (I and II) were isolated from the livers of rats that had been injected intraperitoneally (i.p.) for 3 days with Zn^{2+} (10 mg of Zn^{2+} /day per kg body wt.). The isoproteins were purified by the standard procedure described by Bremner & Davies (1975). Purity of the proteins was established by horizontal slab-gel electrophoresis in 7.5% (w/v) polyacrylamide using the LKB 2117 Multiphor apparatus with Tris/glycine buffer, pH 8.9, as described by Fehrstrom & Moberg (1977).

Gels were stained with Coomassie Blue. Purified Cd-metallothioneins I and II were obtained in the same way from the livers of rats that were injected i.p. with 0.5 mg of Cd^{2+} /kg body wt. on day 1, followed by five injections of 2 mg of Cd^{2+} /kg body wt. on alternate days. The rats were killed 24 h

after the last injection. The Cd/Zn molar ratios in the purified Cd-metallothioneins I and II were 1.7:1 and 1.4:1 respectively. Purified Cu-metallothioneins I and II were obtained as described by Bremner & Young (1976) from rats that were injected i.p. with 2mg of Cu^{2+} /body wt. and killed 18h thereafter. The amino acid composition of all samples of Zn-metallothioneins I and II were similar to those described by Bremner & Davies (1975). The concentrations of solutions of the isoproteins were estimated from their metal contents, assuming that seven atoms of metal were present/molecule of Cd- or Zn-metallothioneins and ten atoms of metal/molecule of Cu-metallothionein (Kagi & Nordberg, 1979).

Immunization procedure

Cd-metallothionein-I (6mg) and rabbit IgG (12mg) were dissolved in 50mM-sodium phosphate, pH 7.5 (1.0ml), and polymerization was initiated by the addition of 3.8 μ l of 25% (w/v) glutaraldehyde. After reaction for 2h at room temperature, the conjugation mixture was diluted with 1.4ml of phosphate buffer, to give a final concentration of 7.5mg of protein/ml. The extent of conjugation of metallothionein-I, as determined by gel filtration of the product on Sephadex G-75 and measurement of Cd distribution, was about 35%.

The conjugation mixture of Cd metallothionein-I and IgG was emulsified with an equal volume of Freund's complete adjuvant and the emulsion was injected into two sheep (4ml/sheep), four intradermal and four intramuscular sites being used. Booster injections were given after 6, 16 and 24 weeks, using emulsions prepared with a 1:1 mixture of Freund's complete and incomplete adjuvant. The sheep were bled from the jugular vein 8–20 days after each booster injection and the antisera heated at 56°C for 30min. Sera were stored at +1°C after the addition of NaN_3 (0.1%) as preservative.

Preparation of ^{125}I -labelled metallothionein-I

The absence of tyrosine or histidine residues in metallothionein precluded its radioiodination by the conventional chloramine-T reaction (Greenwood *et al.*, 1963). Tracers of Zn-metallothionein-I of high immunoreactivity were therefore prepared by using the conjugation technique of Bolton & Hunter (1973) as described by Bolton (1977). Iodinated NSHPP was prepared by allowing NSHPP (0.2 μ g) to react with 1.0mCi of carrier-free ^{125}I using the chloramine-T reaction. ^{125}I -NSHPP was extracted into benzene and the solvent removed with a stream of dry N_2 . The product was mixed with Zn-metallothionein-I (5 μ g) in 10 μ l of 0.1M-borate buffer, pH 8.5, at 0°C. After 25min, 0.5ml 0.2M-glycine was added, followed after a further 5min by 0.5ml of 50mM-Tris/HCl buffer, pH 8.0, containing

0.1% gelatin and 0.1% NaN_3 (gelatin buffer). The ^{125}I -Zn-metallothionein-I was separated by gel filtration on Sephadex G-25 with gelatin buffer as eluant. The specific radioactivity of the ^{125}I -labelled protein was generally 25–50 $\mu\text{Ci}/\mu\text{g}$. The product was stored in portions at –20°C, at concentrations of 40–80ng/ml.

Detection of antibody and r.i.a. procedure

It was not possible to detect the presence of antibody to metallothionein in serum samples by double-diffusion techniques. Antibodies were therefore detected by their specific binding to ^{125}I -Zn-metallothionein-I. Titration curves were prepared as follows. Doubling serial dilutions (e.g. 1:500–1:128000; 400 μ l) of test sera were prepared in gelatin buffer containing 0.2% heated normal sheep serum. These were then incubated with 100 μ l of ^{125}I -labelled protein (10000 c.p.m.) in gelatin buffer containing 50mM-2-mercaptoethanol. After 18h at 4°C, antigen-antibody complexes were precipitated by the addition of 60 μ l of an appropriate dilution of donkey anti-(sheep IgG) antiserum. (The amount of serum required to precipitate all the bound tracer was determined in preliminary experiments.) Incubation at 4°C was continued for a further 18h, whereupon 1.0ml of cold Tris/HCl buffer (50mM, pH 8.0) was added. The mixtures were centrifuged at 1500g at 1°C for 1h, the supernatants were decanted and the precipitates counted for radioactivity in a gamma counter with NaI crystal. Corrections for non-specific binding of ^{125}I -Zn-metallothionein-I were calculated from tubes containing normal sheep serum in place of antiserum. Percentage binding was calculated as described by Vander Mallie & Garvey (1979).

In the r.i.a., all dilutions of antisera, standards and unknowns were made in gelatin buffer, but dilutions of ^{125}I -labelled metallothionein-I were made in gelatin buffer containing 50mM-2-mercaptoethanol. Sufficient carrier serum from an untreated sheep was added along with the antiserum to give a final concentration of 0.1% serum. The unknown samples or solutions containing standard amounts of Zn-metallothionein-I (100–24000pg) were preincubated at 4°C for 24–30h with diluted antiserum (usually about 1:35000 in the assay mixture) in a final volume of 400 μ l. After the addition of 100 μ l of a solution of ^{125}I -labelled metallothionein-I containing 10000 c.p.m. and further incubation at 4°C for 16–24h, bound antigen was separated by the second antibody procedure described above.

Data were expressed as $[(b/b_0) \times 100]$ versus log concentration, or as logity versus log concentration, where b_0 = radioactivity (c.p.m.) precipitated in the absence of unlabelled antigen, b = radioactivity (c.p.m.) precipitated in the presence of standard or

unknown, and $\log y = \log[(100 \times b)/(b_0 - b)]$. Both b and b_0 were corrected for non-specific binding.

Concentrations of metallothionein-I in rat plasma

The effects of Cu^{2+} , Zn^{2+} and Cd^{2+} administration on the levels of metallothionein-I in plasma were studied in several groups, each of five female rats (about 200g) which were injected i.p. with Cu^{2+} or Zn^{2+} (3.0mg/kg body wt. on two consecutive days) or with Cd^{2+} (0.5 mg/kg body wt. on day 1 and 2.0mg/kg body wt. on day 2). Blood was collected by cardiac puncture from 1 to 7 days after the second injection, whereupon the rats were killed. Up to 100 μl of heparinized plasma was used for the metallothionein-I assay, using the procedure described above. Control experiments showed that this amount of plasma did not interfere with the assay.

Concentrations of metallothionein-I in rat kidneys

The effects of Fe-induced Cu deficiency on kidney metallothionein-I concentrations were studied in eight groups, each of six male rats (60–95g), which were maintained for 2–4 weeks on a diet (Williams & Mills, 1970) containing 3 mg of Cu^{2+} and 50, 250 or 500 mg of Fe^{2+} /kg. Samples of tissue were homogenized with 10 vol. (w/v) of 10mM-Tris/acetate, pH 7.4, and centrifuged at 1500g for 15 min. The supernatant fractions were diluted with gelatin buffer and metallothionein-I concentrations were measured by using the r.i.a. procedure described above. The amount of gelatin buffer added as diluent was sufficient to bring the metallothionein-I concentration into the linear region of the standard curve for the r.i.a.

Results

Both of the sheep immunized with the Cd-metallothionein-I-IgG conjugate produced antibodies of high titre after the third booster dose (Fig. 1). With the 'better' antiserum, 35% of the tracer was bound to the antibody at a final dilution of serum of 1:64 000. However a final dilution of 1:35 000 was used in the r.i.a., since this resulted in 50% binding of tracer, which is generally regarded as optimal for both precision and sensitivity in r.i.a. procedures.

Routine assays of unknown samples could easily be made by using a slight modification of the logit-log transformations described by Rodbard & Lewald (1970), since linear plots were consistently obtained over the range 100–11 000 pg of protein (Fig. 2). Intra- and inter-assay coefficients of variation were 2–4% and 15% respectively. A slight decrease in sensitivity occurred with aging of the tracer, because of a decrease in binding in zero-dose tubes. Typically a decrease of 30–40% in sensitivity

Fig. 1. Titration curve for sheep anti-(Cd-metallothionein-I) serum

Serial doubling dilutions (1:500–1:128 000) of sheep anti-(Cd-metallothionein-I) serum, which was collected 10 days after the third booster dose of immunogen, were allowed to react with approx. 150 pg of ^{125}I -Zn-metallothionein-I (10 000 c.p.m.). The percentage binding of ^{125}I to the antibody was calculated as described in the text.

Fig. 2. Standard curve for rat liver Zn-metallothionein-I

Known amounts of pure rat liver Zn-metallothionein-I (92–12 000 pg) were incubated with serum containing anti-(metallothionein-I) for 30 h at 4°C. Approx. 150 pg of ^{125}I -Zn-metallothionein-I (10 000 c.p.m.) were then added and the incubation was continued for a further 16 h at 4°C. The procedures used for separation of the bound fraction and for determination of the extent of binding, expressed as logit y, are described in the text.

occurred 5 weeks after iodination, although this varied with the particular batch of tracer. In most cases, tracers could be used up to 4 weeks after their preparation.

The specificity of the antiserum was established by using the different isoforms of Zn-, Cd- and Cu-metallothioneins in competitive binding assays with ^{125}I -Zn-metallothionein-I as tracer. There was no difference between Zn-metallothionein-I, Cd-metallothionein-I and Cu-metallothionein-I in their reaction with the antiserum, and the inhibition curves for the three proteins overlapped completely (Figs. 3 and 4). However, neither Zn-metallothionein-II, Cd-metallothionein-II (Fig. 3) nor Cu-metallothionein-II (not shown) cross-reacted to a significant extent with Zn-metallothionein-I. For example, about 200 ng of Cd-metallothionein-II was required to produce the same inhibition of ^{125}I -Zn-metallothionein-I binding (50%) as 1.2 ng of Cd- or Zn-metallothionein-I.

Concentrations of metallothionein-I in the plasma of control rats were extremely low (1–2 ng/ml), but increased dramatically after injection of either Cd^{2+} or Cu^{2+} (Table 1). Concentrations were greater after Cd^{2+} injection and declined relatively slowly after maximum values were attained at 3 days post-injection. The decline in protein concentrations after Cu^{2+} injection was much more rapid, and values of only 2–3 times control values occurred at 5 and 7 days post-injection. Injection of Zn^{2+} caused only a minor and transient increase in metallothionein-I levels, and values were not significantly different from control values from 3 days post-injection onwards.

Marked variations occurred in kidney metallothionein-I concentrations in the rats given the Fe^{2+} -supplemented diets. Concentrations were highest in the control animals given the diet with only

50 mg of Fe^{2+} /kg and increased with age (Table 2). Concentrations decreased by 50–75% in rats given the diet with 250 mg of Fe^{2+} /kg and by up to 80% in those given 500 mg of Fe^{2+} /kg diet. Kidney metallothionein-I concentrations were directly related to kidney copper concentrations (Fig. 5), and could be expressed by the equation:

$$\text{MT-I} = 2.49 \text{Cu} - 6.93$$

where MT-I and Cu are the concentrations, in $\mu\text{g/g}$

Fig. 3. Cross-reactivity of Cd- and Zn-containing forms of rat liver metallothioneins I and II

Various amounts (100–25 000 pg) of rat liver Zn-metallothionein-I ($\square$), Cd-metallothionein-I ($\triangle$), Zn-metallothionein-II ($\blacksquare$) and Cd-metallothionein-II ($\blacktriangle$) were incorporated in the inhibition assays, with ^{125}I -Zn-metallothionein-I as tracer and anti-(Cd-metallothionein-I) antibody. See the text for details.

Fig. 4. Cross-reactivity of Cu- and Zn-containing forms of rat liver metallothionein-I

Various amounts (100–25 000 pg) of rat liver Zn-metallothionein-I ($\square$) and Cu-metallothionein-I ($\triangle$) were incorporated in the inhibition assays, with ^{125}I -Zn-metallothionein-I as tracer and anti-(Cd-metallothionein-I) antibody. See the text for details.

Table 1. Effects of injection of Cd^{2+} , Cu^{2+} and Zn^{2+} on plasma concentrations of metallothionein-I in rats

For experimental details, see the text. Rats were injected with 3.0 mg of Cu^{2+} or Zn^{2+} /kg body weight on consecutive days, or with 0.5 mg of Cd^{2+} on day 1 and 2.0 mg of Cd^{2+} /kg on day 2. Blood samples were collected 1–7 days after the second injection. The average recovery of metallothionein-I added to plasma was $109 \pm 9\%$.

Time (days)	Metal ion injected	[Metallothionein-I] (ng/ml of plasma)		
		Cd ²⁺	Cu ²⁺	Zn ²⁺
1		32.4 ± 5.9	19.5 ± 6.8	5.0 ± 1.1
2		34.2 ± 4.0	17.8 ± 4.0	2.5 ± 0.9
3		38.9 ± 11.8	8.1 ± 1.1	1.6 ± 0.2
5		21.9 ± 2.6	4.2 ± 0.3	1.5 ± 0.2
7		18.9 ± 2.5	4.1 ± 1.0	1.6 ± 0.4

Table 2. Effect of dietary Fe^{2+} supplementation on kidney Cu^{2+} and metallothionein-I concentrations in rats
For experimental details, see the text.

Time (weeks)	Dietary [Fe^{2+}] ($\mu g/g$)	Kidney [Cu^{2+}] ($\mu g/g$ wet wt.)	Kidney [metallothionein-I] ($\mu g/g$ wet wt.)
2	50	6.89 ± 0.36	11.3 ± 1.7
	250	4.65 ± 0.19	4.26 ± 0.35
	500	4.15 ± 0.26	2.42 ± 0.17
4	50	9.03 ± 0.49	15.2 ± 1.6
	250	4.46 ± 0.14	3.88 ± 0.40
	500	4.32 ± 0.15	4.53 ± 0.28

Fig. 5. Relationship between the concentrations of Cu^{2+} and of metallothionein-I in the kidneys of rats given diets with various amounts of Fe^{2+}

Concentrations of metallothionein-I in the kidney homogenates were measured by using the r.i.a. procedure described in the text.

wet weight, of the metallothionein and metal respectively.

Discussion

The r.i.a. procedure described here offers considerable advantages over those described previously (Vander Mallie & Garvey, 1979; Tohyama & Shaikh, 1981; Garvey *et al.*, 1982). The antisera were raised in sheep rather than in rabbits, and consequently supply of the antiserum is almost unlimited. More important, the production of anti-metallothionein was more consistent in sheep than in rabbits, as all the sheep that we have immunized

with metallothionein-IgG conjugates (including those of metallothionein-II) have produced high-titre antibodies. This contrasts with the report of Garvey *et al.* (1982) that several rabbits may have to be immunized to obtain one animal with suitable antibody response. The titre of the antiserum obtained from sheep, used at a final dilution of 1:35 000 for 50% binding of antigen, was appreciably better than those obtained in rabbits. For example, Vander Mallie & Garvey (1978) typically used dilutions of 1:4000 for 31% binding of antigen, Garvey *et al.* (1982) used dilutions of 1:20 000 for 30% binding of antigen, and Tohyama & Shaikh (1981) used dilutions of only 1:2500 for 25% binding.

However, the principal advantage of the current procedure is that it permits, for the first time, specific and sensitive determination of one of the major isoproteins of metallothionein, the amount of cross-reaction with Cd-, Zn- or Cu-metallothionein-II being extremely low. This contrasts with the findings of both Garvey *et al.* (1982) and Tohyama & Shaikh (1981), who found complete cross-reaction between all isoforms and metalloforms of metallothionein. Furthermore, Vander Mallie & Garvey (1979) reported virtually complete cross-reaction between apo-thionein and the holo protein, whereas the addition of EDTA to our Zn-metallothionein-I assay mixtures resulted in 70% loss of reaction with the antibody.

This suggests that the conformational changes which occur on the binding of metal to thionein (Vasak *et al.*, 1980) are crucial to the recognition and binding of the antigen by our antibody. There is, however, no distinction between the forms of metallothionein-I containing Cd, Zn or Cu, which means that concentrations of the isoprotein can be determined in the absence of information on metal binding. The cross-reactivity between rat liver metallothionein-I and the equivalent isoprotein from other species has yet to be established.

There are no obvious reasons why the antibodies raised against the conjugates of IgG and rat liver metallothionein-I in sheep should exhibit such specificity compared with the antibodies described by other authors. It may be significant that the Cd-metallothionein-I that was used as antigen was purified solely by chromatographic procedures at low temperature (Bremner & Davies, 1975), whereas others have included a heat-coagulation step to remove other hepatic proteins (Garvey *et al.*, 1982). This may have had some effect on the conformation of the protein. Production of antibodies to metallothionein has proved in the past to be difficult, perhaps because of the high degree of structural homology between the proteins from various sources and their low molecular weight. It has generally been necessary to increase the immunogenicity of metal-

lothionein by forming a polymeric derivative with glutaraldehyde. Thus soluble polymers of metallothionein and rabbit serum albumin and both soluble and insoluble self-polymers of metallothionein have previously been used as immunogens (Tohyama & Shaikh, 1981; Garvey *et al.*, 1982). In our experience, conjugation of the protein to rabbit IgG was far superior to either of these methods for raising high-titre antibodies in sheep, at least when the pH of the reaction and the concentration of glutaraldehyde were adjusted so as to produce soluble polymers. Since no rabbit serum was used in the r.i.a., the presence of rabbit IgG in the immunogen did not interfere with metallothionein estimations.

The sensitivity and linear working range of the current assay, 100pg–10ng of protein/assay tube, is similar to that described by Garvey *et al.* (1982) (200pg–10ng) and superior to that of Tohyama & Shaikh (1981). This reflects both the high titre of the antisera and the quality of the tracer that was prepared. Thus more than 80% of the ^{125}I -labelled tracer was bound by excess antibody in the current procedure (Fig. 1), compared with only 60–65% (Chang *et al.*, 1980) and 40–45% (Tohyama & Shaikh, 1981) in other r.i.a. procedures. The use of a double-antibody technique to separate free and bound antigen also contributed to the quality of the r.i.a., since non-specific binding of ^{125}I never exceeded 4% of total radioactivity counts. This compares with equivalent non-specific binding of 11% that was obtained when $(\text{NH}_4)_2\text{SO}_4$ was used as precipitant (Tohyama & Shaikh, 1981). Such high blank values limit the reliability of the assay if zero-dose tubes bind only 25% of tracer (Chard, 1980).

It has been suggested that the development of Cd-toxicosis in man can be monitored by the measurement of metallothionein in plasma or urine (Shaikh & Hirayama, 1979; Chang *et al.*, 1980), and it is clearly important that a sensitive assay should be available for this purpose. The detection of as little as 1–2ng of metallothionein-I/ml of plasma from control rats illustrates the extreme sensitivity of our r.i.a. and confirms the observations of Garvey & Chang (1981) that small amounts of the protein normally circulate in rat plasma. These concentrations are considerably less than those of more than 60ng/ml reported by Tohyama & Shaikh (1981). Injection of comparable amounts of Cd^{2+} , Cu^{2+} and Zn^{2+} , all of which induce synthesis of metallothionein in liver and other tissues, increased the concentrations of metallothionein-I in the plasma, although the response depended on the inducing metal. The lowest and most short-lived increase was obtained with Zn^{2+} , and the greatest and most prolonged increase with Cd^{2+} , concentrations after 7 days being more than 10 times those

in control plasma. The changes after Cu^{2+} injection were intermediate between those caused by Cd^{2+} and Zn^{2+} . These results are similar to those reported by Garvey & Chang (1981) for total plasma metallothionein in rats given several injections of Cd^{2+} or Zn^{2+} . The differences in the levels of circulating metallothionein after injection of different metals probably reflect: (i) the variations in the capacity of these metals to induce metallothionein synthesis in the liver and other tissues (Durnam & Palmiter, 1981), (ii) the influence of the metals on its degradation (Bremner & Mehra, 1983), (iii) the influence of the metals on the metabolism of the protein subsequent to its secretion into plasma (Bremner *et al.*, 1978), and (iv) the different degrees of tissue damage induced by the metals.

Further validation of the r.i.a. was demonstrated in the study of the effect of Fe^{2+} on kidney concentrations of metallothionein-I. This treatment can induce Cu deficiency in rats and cause large decreases in kidney, liver and plasma Cu concentrations (Bremner & Young, 1981; I. Bremner & B. W. Young, unpublished work). The decreases in kidney Cu were accompanied by parallel decreases in kidney metallothionein-I concentrations and there was a linear correlation between the concentrations of the metal and of the protein. This is consistent with previous studies on the occurrence of metallothionein in rat kidneys, where the protein was separated by chromatographic techniques (Bremner *et al.*, 1981). These indicated that about 40% of the renal Cu in rats usually occurred as metallothionein. If it is assumed that isoprotein-I constitutes 40% of the total kidney metallothionein and that there are 6g-atoms of Cu and 4g-atoms of Zn/mol of protein (Bremner *et al.*, 1981), then the slope of the regression curve would imply that 39% of the total Cu was present as metallothionein

$$\left(2.49 \times \frac{1}{0.4} \times \frac{6 \times 63.5}{6100} \times 100 \right).$$

This is extremely close to the value predicted from the chromatographic studies and confirms that the r.i.a. procedure is capable of measuring metallothionein-I concentrations in tissues and fluids with sensitivity, selectivity and accuracy.

Antibodies to rat liver metallothionein-II have also been raised in sheep by using the same immunization procedures, and preliminary results indicate that there is again little cross-reactivity between the two main isoproteins (R. K. Mehra & I. Bremner, unpublished work). The availability of specific antibodies to metallothionein should now permit detailed comparison of the biological properties of the isoproteins and further our understanding of the functions of this important class of metal-binding proteins.

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