Metal constitution of metallothionein influences inhibition of δ -aminolaevulinic acid dehydratase (porphobilinogen synthase) by lead

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This study was undertaken to evaluate the effect of Zn and Cd pretreatment on the inhibition of δ -aminolaevulinic acid dehydratase (ALAD; porphobilinogen synthase, EC 4.2.1.24) by Pb. Male CD rats were pretreated with 200 μ mol of Zn/kg s.c. (subcutaneously) or 18 μ mol of Cd/kg s.c., 48 and 24 h before assay of ALAD. Pretreatment with Zn resulted in activation of hepatic and renal ALAD and attenuated the inhibition of this enzyme by Pb *in vitro*. Pretreatment with Cd increased hepatic ALAD activity, and the inhibitory effect of Pb on the hepatic enzyme was attenuated in this group. In contrast with the situation in liver, pretreatment with Cd did not affect the activity of renal ALAD and did not alter the inhibitory effect of Pb on the renal enzyme. The Pb IC₅₀ (concentration causing half-maximal inhibition) values for hepatic and renal ALAD in Zn-pretreated rats and for hepatic ALAD in Cd-pretreated rats were increased above control, whereas the IC₅₀ for renal ALAD in Cd-pretreated rats was unchanged. Cytosolic binding patterns for the three metals were assessed by gel-filtration chromatography and disclosed that ²⁰³Pb was co-eluted with Zn and Cd bound to liver and kidney Zn-thioneins and liver Cd,Zn-thionein, although minimal binding of ²⁰³Pb to kidney Cd,Zn-thionein was observed. Estimation of the molar ratio of metals bound revealed Cd/Zn ratios of 2 and 5 for Cd,Zn-thioneins from liver and kidney respectively. The inhibition of purified ALAD by Pb was also attenuated by addition of purified Zn-thioneins and Cd,Zn-thioneins from liver and kidney in the following order: liver Zn-thionein = kidney Zn-thionein > liver Cd,Zn-thionein >> kidney Cd,Zn-thionein. Thus liver and kidney Zn-thioneins and liver Cd,Zn-thionein with a low Cd/Zn ratio readily decrease the free pool of Pb available to interact with ALAD. These data also demonstrate that the capacity of metallothionein to alter the intracellular distribution of Pb and mediate the inhibition of ALAD by Pb is dependent on the tissue source and relative metal constitution of the metallothionein.

INTRODUCTION

The intracellular distribution and activity of a number of essential and toxic metals are dependent, at least in part, on the binding of these cations to various high-affinity metal-binding proteins. One well-studied protein, metallothionein, has a high affinity for metals such as Cd, Zn, Cu, Hg and Ag (Nordberg & Kojima, 1979) primarily attributable to the high number of cysteine residues and the cluster arrangement of the molecule, which facilitates the association of metals with the maximal number of cysteine residues (Otvos & Armitage, 1980; Winge & Miklossy, 1982). Induction of metallothionein synthesis by pretreatment with Zn and Cd has been shown to protect against Cd-induced toxicity, including testicular damage (Parizek & Zahor, 1956), carcinogenesis (Gunn *et al.*, 1964), inhibition of microsomal mixed-function oxidases (Roberts & Schnell, 1982), mitochondrial oxidative phosphorylation (Sporn *et al.*, 1969), Cd-thionein-induced nephrotoxicity (Squibb *et al.*, 1984), hepatotoxicity (Goering & Klaassen, 1984) and lethality (Leber & Miya, 1976;

Goering & Klaassen, 1983). The mechanism of detoxication involves chelation of the toxic cation into a relatively inert intracellular metal-protein complex, effectively decreasing the pool of free metal available to interact with sensitive intracellular targets (Nordberg, 1972; Probst *et al.*, 1977; Goering & Klaassen, 1983). Metallothionein has also been suggested to play a role in the homeostasis of essential metals such as Cu and Zn. The protein has been postulated to serve as an intracellular storage pool for Zn and Cu, sequestering and donating these cations to biological processes requiring them, such as synthesis of metalloenzymes (Kojima & Kagi, 1978).

Recently, Pb inhibition of ALAD (EC 4.2.1.24, porphobilinogen synthase), a cytosolic Zn-containing enzyme involved in haem biosynthesis, has been shown to be mediated by an endogenous high-affinity Zn,Pb-binding protein in kidney (Goering & Fowler, 1984, 1985) and brain (Goering *et al.*, 1986). These proteins share several properties with metallothionein, such as high Zn content (Goering & Fowler, 1985), heat-stability and similar elution profiles when analysed by gel-

Abbreviations used: ALAD, δ -aminolaevulinic acid dehydratase (porphobilinogen synthase, EC 4.2.1.24); s.c., subcutaneous(ly); IC₅₀, concentration causing half-maximal inhibition.

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filtration, anion-exchange, and hydrophobic-interaction chromatography (DuVal *et al.*, 1986), but differ in amino acid composition and electrophoretic mobility (DuVal & Fowler, 1987). Recently it has been demonstrated that purified kidney Zn-thionein can reverse the inhibition of purified liver ALAD by Pb (Goering & Fowler, 1987a,b). The mechanism by which these metal-binding proteins mediate the effect of Pb on ALAD involves chelation of Pb away from the enzyme and donation of Zn from the protein to ALAD, resulting in enzyme activation (Goering & Fowler, 1985, 1987a,b). The present study was undertaken to examine the effects of Zn- and Cd-induced metallothionein on the inhibition of rat liver and kidney ALAD by Pb. It was also of interest to determine if a different relationship exists between metallothioneins with different metal ratios induced by an essential metal (Zn) and a toxic metal (Cd) with regard to the modulation of Pb action on ALAD and the binding of Pb to metallothionein in these tissues.

MATERIALS AND METHODS

Animals

Adult male CD rats (Charles River, Kingston, NY, U.S.A.) weighing 280–320 g were housed in plastic cages and were exposed to a 12-h light/12-h dark cycle in an environmentally controlled room. Food (NIH 31 Rat Chow) and water were provided *ad libitum*.

Metals

Solutions containing Pb were prepared by dissolving lead acetate (certified A.C.S. grade; Fisher Chemical Co., Fairlawn, NJ, U.S.A.) in 0.1 M-sodium acetate, pH 6.8. Solutions containing Cd and Zn were prepared for s.c. injection by dissolving CdCl₂ (certified A.C.S. grade; Fisher Chemical Co.) and ZnCl₂ (ultrapure; Alfa Products, Danvers, MA, U.S.A.) in 0.9% NaCl.

ALAD assay

Rats were pretreated with 0.9% NaCl (1 ml/kg, s.c.), Cd (18 μ mol/kg, s.c.) or Zn (200 μ mol/kg, s.c.) 48 and 24 h before assay of ALAD. The activity of ALAD was determined by measuring the rate of formation of product, porphobilinogen, in the 13000 g supernatant from liver and kidney homogenates by the methods of Gibson *et al.* (1955) and Baron & Tephly (1969), with modifications described by Goering & Fowler (1984, 1985). Liver and kidney postmitochondrial supernatants were incubated with Pb (0.5 and 3 μ M respectively) in capped N₂-purged tubes for 2 h at 4 °C before the 1 h ALAD assay. Solutions containing Pb were added in a 10 μ l volume to achieve the concentrations indicated in the Figures. Additional modifications were required for reaction mixtures in studies utilizing purified proteins. These reaction mixtures contained 3.3 mM-GSH in 0.1 M-sodium acetate, pH 6.8; 1 mg of purified bovine liver ALAD (Sigma, St. Louis, MO, U.S.A.) in 2.5 M-(NH₄)₂SO₄/50 mM-potassium phosphate/0.1 M-dithiothreitol, pH 6.8; and 60 μ g of purified rat kidney or liver metallothionein in 10 mM-Tris/acetate, pH 6.8. The total volume of the reaction mixture was 1.5 ml. All protein concentrations, including the metallothioneins, were estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Binding of Zn, Cd and Pb to metallothionein

In order to determine the distribution of Zn, Cd and Pb in hepatic and renal cytosol after various treatments, rats were pretreated with 0.9% NaCl (2 ml/kg, s.c.), Cd (18 μ mol/kg, s.c.) or Zn (200 μ mol/kg, s.c.) 48 and 24 h before the experiment. Livers and kidneys were removed after decapitation, and subsequently homogenized on ice with a Teflon pestle and Potter–Elvehjem glass homogenizer after addition of 1 ml of cold 10 mM-Tris/acetate, pH 7.4, per g wet weight of tissue. The homogenate was centrifuged at 105000 g for 1 h at 4 °C. Liver and kidney cytosols were incubated with 0.5 μ Ci of ²⁰³Pb/ml of cytosol (10 mCi/ml, 30 mCi/mg; New England Nuclear Corp., Boston, MA, U.S.A.) for 3 h at 4 °C. Cytosol was applied to a 60 cm $\times$ 2.2 cm Sephadex G-75 column previously equilibrated with homogenization buffer. Elution was performed with the same buffer by using gravity flow (30 ml/h), and 100 3 ml fractions were collected. The fractions were analysed for ²⁰³Pb by γ -radiation spectrometry (Packard Auto-Gamma 500 instrument) and for Cd and Zn by atomic-absorption spectrophotometry (Perkin–Elmer 305B). It is well known that endogenous rat kidney metallothionein contains Cu as well as Zn (Brady *et al.*, 1982), even after induction after Cd injection (Suzuki *et al.*, 1977). We were unable to detect Cu on kidney Zn-thionein and Cd,Zn-thionein. This observation may be due to the presence of excess Zn and Cd in kidney cytosols isolated from Zn- and Cd-pretreated rats respectively. Loss of Cu from the proteins may also have occurred during purification procedures, since the Cu–thiolate bond is susceptible to air oxidation (Nielson *et al.*, 1985).

Purification of metallothioneins

In order to examine the effect of purified Zn- and Cd-induced metallothioneins on the inhibition of purified ALAD by Pb, liver and kidney Zn-thioneins and Cd,Zn-thioneins were isolated and purified from Zn- and Cd-treated rats by using a standard protocol involving gel filtration (as described above) and anion-exchange chromatography. Eluted gel-filtration fractions which contained the thionein of interest ($V_e/V_0 = 2.2$ –2.8) were pooled and further purified on a 40 cm $\times$ 2.2 cm DEAE-Sephadex A-25 anion-exchange column equilibrated with 10 mM-Tris/acetate buffer, pH 7.4. After the pooled sample was applied and the column washed with low-salt buffer, a linear gradient of Tris/acetate (10–200 mM, pH 7.4) was initiated at a flow rate of 25 ml/h and 100 3 ml fractions collected. Fractions containing the two isometallothioneins I and II were pooled and subsequently concentrated and desalted under N₂ at 4 °C by using an Amicon cell containing a YM5 membrane (Amicon Corp., Danvers, MA, U.S.A.). Metallothioneins were relatively homogeneous, as assessed by non-denaturing polyacrylamide-gel electrophoresis (Goering & Klaassen, 1985).

Statistics

Comparisons between various groups were performed by analysis of variance, followed by a *t* test using a mean-squared-error term (Winer, 1971).

RESULTS

The effects of Zn and Cd pretreatment on the inhibition of liver and kidney ALAD by Pb are shown in

Fig. 1. Effect of Zn and Cd pretreatment on inhibition of rat liver ALAD by Pb *in vitro*

Rats were pretreated with Zn (200 μ mol/kg, s.c.) or Cd (18 μ mol/kg, s.c.) 48 and 24 h before the experiment. Liver postmitochondrial supernatants were incubated with Pb (0.5 μ M) for 2 h at 4 °C before the 1 h ALAD assay. Values represent means $\pm$ S.E.M. ($n = 5$). Values with no letters in common are significantly different ($P < 0.05$). Groups represent: (1) control; (2) Pb, Pb alone *in vitro*; (3) Zn, Zn pretreatment alone; (4) Zn + Pb, Zn pretreatment + Pb; (5) Cd, Cd pretreatment alone; (6) Cd + Pb, Cd pretreatment + Pb. Abbreviation: PBG, porphobilinogen.

Fig. 2. Effect of Zn and Cd pretreatment on inhibition of rat kidney ALAD by Pb *in vitro*

Rats were pretreated with Zn (200 μ mol/kg, s.c.) or Cd (18 μ mol/kg, s.c.) 48 and 24 h before the experiment. Kidney postmitochondrial supernatants were incubated with Pb (3 μ M) for 2 h at 4 °C before the 1 h ALAD assay. Values represent means $\pm$ S.E.M. ($n = 5$). Values with no letters in common are significantly different ($P < 0.05$). Groups are described in Fig. 1. Abbreviation: PBG, porphobilinogen.

Figs. 1 and 2 respectively. In control liver, addition of 0.5 μ M-Pb inhibited ALAD activity to 46% of control (Fig. 1). In livers from Zn-pretreated rats (no Pb), activation of ALAD was observed; activity was increased to 141% of control. In the presence of 0.5 μ M-Pb, ALAD activity in livers from Zn-pretreated rats was not different from controls, i.e. Zn pretreatment effectively attenuated the inhibition of liver ALAD by Pb. In livers from Cd-pretreated rats, ALAD was activated to 123% of control. In the presence of 0.5 μ M-Pb, ALAD activity in livers from Cd-pretreated rats was inhibited to 76% of control; however, Cd pretreatment partially prevented the inhibition of ALAD by Pb, since enzyme activity in this group was significantly higher compared with the group receiving Pb alone.

Addition of 3 μ M-Pb to control kidney reaction mixtures resulted in inhibition of ALAD to 59% of control (Fig. 2). In kidneys from Zn-pretreated rats, activation of ALAD was observed; activity was increased to 171% of control. In the presence of 3 μ M-Pb, ALAD activity in kidneys from Zn-pretreated rats was not significantly different from controls; thus Zn pretreatment attenuated the inhibition of kidney ALAD by Pb. No change in ALAD activity was observed in kidney from Cd-pretreated rats (no Pb); however, in the presence of 3 μ M-Pb, kidney ALAD activity was inhibited to 65% of control. Thus Cd pretreatment did not alter the effects of Pb on renal ALAD.

To determine if the mechanism for the observed effects of Pb on ALAD in liver and kidney after Zn and Cd pretreatment was related to differences in binding of Pb to Zn- and Cd-induced metallothionein, liver and kidney cytosols from control, Zn- and Cd-pretreated rats were

incubated with ^{203}Pb *in vitro* and fractionated by gel-filtration chromatography on a Sephadex G-75 column. A marked increase in the amount of ^{203}Pb associated with the metallothionein fraction ($V_e/V_0 = 2.2\text{--}2.8$) was observed in livers from Zn- and Cd-pretreated rats compared with controls (Fig. 3). A marked increase in the binding of Zn and Cd to metallothionein was also observed in these same groups compared with controls. The ratio of Cd and Zn bound to liver Cd,Zn-thionein was approx. 2.

In kidneys from Zn-pretreated rats (Fig. 4) the amount of ^{203}Pb associated with the metallothionein fraction was approx. 2-fold higher than the amount of ^{203}Pb bound to the endogenous Zn,Pb-binding protein from control rats previously described (Goering & Fowler, 1984, 1985). In contrast with liver from Cd-pretreated rats, binding of ^{203}Pb to metallothionein in kidney from Cd-pretreated rats was minimal. The amount of Zn and Cd bound to kidney metallothionein was increased after Zn and Cd pretreatment. The Cd/Zn ratio for kidney Cd,Zn-thionein was estimated to be 5. Binding patterns similar to those shown here have been obtained from liver and kidney of rats injected *in vivo* with ^{203}Pb after Zn and Cd pretreatment (Goering & Fowler, 1987b).

The effect of both Zn and Cd pretreatment on the IC_{50} of Pb for liver and kidney ALAD is shown in Table 1. The IC_{50} for control liver ALAD was estimated to be 0.31 μ M-Pb. After Zn pretreatment, liver ALAD was less sensitive to Pb; the IC_{50} was increased almost 3-fold to 0.82 μ M-Pb. After Cd pretreatment, the IC_{50} for liver ALAD was also higher than control and was increased almost 2-fold to 0.53 μ M-Pb. The IC_{50} for control kidney ALAD was estimated to be 3.06 μ M-Pb. After Zn pretreatment, a change in sensitivity of kidney ALAD was observed similar to that which occurred in liver; the IC_{50} was elevated almost 2-fold to 5.14 μ M-Pb. In

Fig. 3. Representative Sephadex G-75 gel-filtration elution profiles of ^{203}Pb (a), Zn (b) and Cd (c) in liver cytosol from control (—), Zn-pretreated (----) and Cd-pretreated (-.-.-) rats

Cytosols were incubated for 3 h with ^{203}Pb ($0.5 \mu\text{Ci}/\text{ml}$ of cytosol) before fractionation. Metals eluted with retention coefficients (V_e/V_0) of 2.2–2.8 were considered bound to metallothionein. The average recovery of ^{203}Pb was 93% ($n = 3$ runs). Liver Cd,Zn-thionein was estimated to have a Cd/Zn ratio of 2.

contrast with the situation in liver, the IC_{50} of Pb for kidney ALAD after Cd pretreatment was not markedly different from control; the IC_{50} was estimated to be $2.60 \mu\text{M-Pb}$.

In order to assess more rigorously the interaction of metallothionein and ALAD with respect to inhibition of the enzyme by Pb, purified Zn-thioneins and Cd,Zn-thioneins from liver and kidney were incubated with

Fig. 4. Representative Sephadex G-75 gel-filtration elution profiles of ^{203}Pb (a), Zn (b) and Cd (c) in kidney cytosol from control (—), Zn-pretreated (----) and Cd-pretreated (-.-.-) rats

Cytosols were incubated for 3 h with ^{203}Pb ($0.5 \mu\text{Ci}/\text{ml}$ of cytosol) before fractionation. Metals eluted with retention coefficients (V_e/V_0) of 2.2–2.8 were considered bound to metallothionein. The average recovery of ^{203}Pb was 95% ($n = 3$ runs). In contrast with the situation in liver cytosol, an endogenous Zn,Pb-binding protein also bound a significant amount of Pb, as shown previously (Goering & Fowler, 1984, 1985). Kidney Cd,Zn-thionein was estimated to have a Cd/Zn ratio of 5.

purified bovine liver ALAD in the presence of Pb (Table 2). Addition of $5 \mu\text{M-Pb}$ to the reaction mixture inhibited ALAD activity to 33% of control. In contrast, addition of Zn-thionein and Cd,Zn-thionein from liver and kidney affected the inhibition of ALAD by Pb to various extents. Liver and kidney Zn-thionein completely

Table 1. Estimated IC_{50} values for the inhibition of liver and kidney ALAD by Pb in 0.9% NaCl-, Zn-pretreated, and Cd-pretreated rats

Various concentrations of Pb (0.1–1.6 μM for liver, 0.625–10 μM for kidney) were added to tissue postmitochondrial supernatants from saline-, Zn- and Cd-pretreated rats, and ALAD activity was determined as described in the Materials and methods section. By using a method described by Dixon (1953), plots of inhibitor concentration versus reciprocal enzyme velocity were constructed using least-squares analysis. The point of intersection of the curve with the abscissa was defined as the IC_{50} (concentration resulting in half-maximal inhibition). The values reported represent the means $\pm$ S.E.M. of four determinations.

Organ	Pretreatment...	IC_{50} (μM -Pb)		
		0.9% NaCl	Zn	Cd
Liver		0.31 ± 0.03	0.82 ± 0.08	0.53 ± 0.06
Kidney		3.06 ± 0.42	5.14 ± 0.50	2.60 ± 0.25

Table 2. Effect of purified rat liver and kidney metallothioneins on the inhibition of purified bovine liver ALAD by Pb

Values are averages for three determinations. The amount of each thionein added to reaction mixtures was 60 μg of protein [determined by the method of Lowry *et al.* (1951)]. Abbreviation: PBG, porphobilinogen. Experimental details are given in the Materials and methods section.

	ALAD activity	
	(nmol of PBG/h per mg of protein)	(% of control)
Control	12.3	100
Pb (5 μM)	4.1	33
+ Liver Zn-thionein	12.9	105
+ Liver Cd,Zn-thionein	8.5	69
+ Kidney Zn-thionein	12.7	103
+ Kidney Cd,Zn-thionein	4.9	40

prevented inhibition of ALAD by Pb. In the presence of liver Cd,Zn-thionein, inhibition of ALAD by Pb was partially prevented; enzyme activity was 69% of control. Kidney Cd,Zn-thionein was least effective in preventing inhibition of the enzyme by Pb; activity was only 40% of control.

DISCUSSION

The present study demonstrates that the capacity of metallothionein to alter the intracellular distribution of Pb and mediate the inhibition of ALAD by Pb is dependent upon the tissue source and relative metal constitution of the protein. After pretreatment of rats with Zn, a metallothionein with Zn as the major metal constituent is formed in both liver and kidney (Nordberg

& Kojima, 1979). Results obtained in the present study demonstrated that the presence of Zn-thionein in liver and kidney prevents the inhibition of ALAD by Pb in these tissues (Figs. 1 and 2). In addition, purified liver and kidney Zn-thioneins prevented the inhibition of purified ALAD by Pb (Table 2). After pretreatment of rats with Cd, a Cd,Zn-thionein is formed in both liver and kidney, with a Cd/Zn ratio greater than unity (Nordberg & Kojima, 1979). The presence of Cd,Zn-thionein in liver attenuated the inhibition of hepatic ALAD by Pb (Fig. 1). Purified rat liver Cd,Zn-thionein also attenuated the inhibition of purified ALAD by Pb (Table 2). The mechanism responsible for these effects appears to involve chelation of Pb into metallothionein metal-binding sites (Figs. 3 and 4). This would effectively decrease the pool of free Pb ions in liver and kidney which are available to interact with ALAD. Such a mechanism is similar to the well-described role that metallothionein plays in preventing or reducing the toxicity of other metal cations, such as Cd (Nordberg, 1972; Leber & Miya, 1976; Roberts & Schnell, 1982; Goering & Klaassen, 1983, 1984; Squibb *et al.*, 1984). In contrast with the above results, the presence of Cd,Zn-thionein in kidney did not alter the inhibition of renal ALAD by Pb (Fig. 2). Similarly, purified rat kidney Cd,Zn-thionein was relatively ineffective at preventing inhibition of the purified enzyme by Pb (Table 2). This effect is most likely due to the lack of significant binding of Pb to rat kidney Cd,Zn-thionein (Fig. 4).

The high degree of Pb binding observed for liver and kidney Zn-thioneins and liver Cd,Zn-thionein and the low degree of Pb binding to kidney Cd,Zn-thionein may be dependent upon the relative amount of Zn bound to the particular metallothionein. It has been shown that Pb is capable of displacing Zn from rat liver Zn-thionein (Waalkes *et al.*, 1984; Nielson *et al.*, 1985) and displacing Zn, but not Cd, from rat liver Cd,Zn-thionein (Nielson *et al.*, 1985). The association constant of Pb for equine kidney metallothionein has been estimated to be between that of Cd and Zn (Ulmer & Vallee, 1969). Thus a higher proportion of Pb is able to bind to rat liver and kidney Zn-thioneins because it readily displaces Zn from the protein and essentially no other metal ions with higher affinities are present. In the present study, the Cd/Zn ratio for rat liver Cd,Zn-thionein was approx. 2 (Fig. 3), which corresponds to the value obtained for rabbit liver (Nettesheim *et al.*, 1985). In contrast, the Cd/Zn ratio for kidney Cd,Zn-thionein was estimated to be 5 (Fig. 4). These results suggest that a higher percentage of Pb was able to bind to liver Cd,Zn-thionein (Cd/Zn = 2), because the ratio of Cd bound compared with Zn was relatively low, and hence Pb could occupy more binding sites previously occupied by Zn. In kidney, the binding of Pb to Cd,Zn-thionein was much lower because the ratio of Cd bound to the protein compared with Zn was higher (Cd/Zn = 5), and Pb could displace proportionally less Zn.

Metallothionein has been previously postulated to function in essential-metal homeostasis, e.g. serving as a reservoir to store and release ions such as Zn and Cu to meet requirements of various metabolic processes (Kojima & Kagi, 1978). In support of this hypothesis, various enzymes which require Zn and/or Cu as cofactors, such as carbonic anhydrase, alkaline phosphatase and superoxide dismutase, have been re-activated *in vitro* after incubation with Zn- and

Cu-thioneins (Udom & Brady, 1980; Li *et al.*, 1980; Geller & Winge, 1982). Recently, it has also been shown that Zn-thionein and endogenous Zn,Pb-binding proteins in kidney and brain simultaneously regulate the interaction of Pb and Zn with ALAD by donating Zn to, and chelating Pb away from, ALAD, resulting in a reversal of Pb inhibition of the enzyme (Goering & Fowler, 1984, 1985, 1987a,b; Goering *et al.*, 1986).

The present data also support the hypothesis that metallothionein functions as a donor of Zn to metallo-enzymes in that, after Zn pretreatment alone (no Pb), a significant increase in hepatic and renal ALAD activity above control values was observed (Figs. 1 and 2). After Cd pretreatment (no Pb), a more modest increase in hepatic ALAD activity was observed. These results suggest that the Cd/Zn ratio on liver Cd,Zn-thionein was sufficiently low (Cd/Zn = 2) that an adequate amount of Zn was transferred to activate the enzyme and, in the presence of Pb, both Pb chelation and Zn donation were still operative. In contrast, no change in renal ALAD activity was observed after Cd pretreatment alone (no Pb), suggesting that an insufficient amount of Zn was available on kidney Cd,Zn-thionein (Cd/Zn = 5) to be transferred to the enzyme, and this may explain the lack of protection for the enzyme after Cd pretreatment in the presence of Pb.

It could be argued that Zn pretreatment was protecting ALAD from Pb inhibition simply by activating the enzyme or that Pb was preventing Zn activation of ALAD. This implies that the same proportion of Pb was binding to the enzyme in the Pb alone and metal pretreatment plus Pb groups. In an effort to more precisely evaluate this question, the Pb IC_{50} for hepatic and renal ALAD was estimated in control, Zn- and Cd-pretreated rats. Pretreatment with Zn effectively elevated the IC_{50} for Pb 2.6-fold and 1.7-fold higher than control (Pb alone) in both liver and kidney respectively. Pretreatment with Cd elevated the IC_{50} for Pb in liver approx. 1.7-fold, but did not alter the IC_{50} for Pb in kidney. These data would suggest that liver and kidney Zn-thioneins and liver Cd,Zn-thionein can effectively decrease the intracellular pool of free Pb. Thus it appears that both Zn donation and Pb chelation are operative in liver and kidney after Zn pretreatment. These mechanisms are less effective in liver after Cd pretreatment, owing to the presence of Cd on Cd,Zn-thionein, and are ineffective in kidney after Cd pretreatment because of the relatively high Cd/Zn ratio.

In spite of the fact that Pb readily reacts with thiol groups (Kagi & Hapke, 1984), the interaction of Pb with metallothioneins and the implications of this interaction have not been completely addressed, most likely because Pb is a poor inducer of metallothionein (Eaton *et al.*, 1980; Waalkes & Klaassen, 1985; Ikebuchi *et al.*, 1986; Maitani *et al.*, 1986). The present data demonstrate that the consequences of metallothionein interaction with a Zn-metalloenzyme, i.e. ALAD, are dependent upon the metal constitution of the metallothionein, which affects the availability of essential metal cofactors, such as Zn and/or the intracellular distribution and toxicity of metals of environmental concern, such as Pb. Liver and kidney Zn-thionein and liver Cd,Zn-thionein were shown to protect ALAD from inhibition by Pb. The mechanism for this interaction probably involves donation of Zn from metallothionein to ALAD (Goering & Fowler, 1987a,b) and chelation of Pb away

from the enzyme. Kidney Cd,Zn-thionein did not protect ALAD from inhibition by Pb, most likely because of the relatively high Cd/Zn ratio of the protein, which decreased both Zn availability and Pb-binding sites.

We acknowledge the outstanding technical support of Ms. Robin Racine.

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Received 15 September 1986/16 March 1987; accepted 25 March 1987