Roles of Lead-Binding Proteins in Mediating Lead Bioavailability

Bruce A. Fowler

Program in Toxicology, University of Maryland, Baltimore, Maryland

The intracellular bioavailability of lead (Pb) at low dosage levels in major target organs such as the kidney and brain appears to be largely determined by complexation with a group of low molecular weight proteins. These proteins are rich in aspartic and glutamic dicarboxyl amino acids. The proteins are chemically similar but not identical across all species examined to date and the brain protein appears to be different from that found in the kidney. These proteins possess dissociation constant values for Pb on the order of 10^{-8} M and appear to normally bind zinc. In rats, these proteins attenuate the Pb inhibition of the heme pathway enzyme δ-aminolevulinic acid dehydratase by a mechanism involving both Pb chelation and zinc donation to this highly Pb-sensitive zinc-dependent enzyme. Other studies in rats have shown that the kidney protein facilitates the intranuclear movement of Pb *in vitro* followed by chromatin binding, suggesting that this protein may be involved in alterations of the pathognomonic Pb intranuclear inclusion bodies in renal gene expression associated with the mitogenic effects of Pb in the kidney. — *Environ Health Perspect* 106(Suppl 6):1585–1587 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1585-1587fowler/abstract.html*

Key words: lead, lead-binding proteins, lead bioavailability, neurotoxicity, renal toxicity, low dose risk assessments, heme biosynthetic pathway, alterations in gene expression

All models for predicting blood lead values from soil or dust exposures require the movement of lead (Pb) in a soluble form between the gastrointestinal tract, lungs, blood, major organs, and urinary or fecal routes of excretion. The models also assume that blood Pb values are useful predictors of Pb toxicity for all segments of the population. The movement of Pb in blood and its transport into hard and soft tissues implies binding to soluble carriers such as metal-binding proteins. It has been known for some time that other metals such as iron are transported in blood via proteins such as transferrin and that cadmium and zinc are transported bound to metallothionein. Soluble Pb-binding proteins (PbBPs) in blood (*1,2*) and target tissues (*3–8*) appear to be involved in mediating both the bioavailability and the toxic potential of Pb. Protein-mediated mechanisms of toxicity for metals such as calmodulin and calcium have been studied extensively (*9,10*), and research from this laboratory has shown that PbBPs from the rat are capable of both attenuating the effects of Pb on δ-aminolevulinic acid dehydratase (δ-ALAD) (*11–13*) and mediating the intranuclear movement and chromatin binding (*14,15*) of Pb in target cell nuclei from the kidney. These latter effects appear to be quite important in regulating alterations in renal gene expression patterns (*16,17*) and possibly Pb-induced renal cancer (*18*). PbBPs may also be involved in the formation of Pb-containing intranuclear inclusion bodies in target tissues (*19–23*), as they appear to undergo aggregation after addition of Pb *in vitro* (*24*).

The following discussion reviews the literature on these molecules and examines how they influence individual susceptibility to Pb toxicity in humans and other species.

This paper is based on a presentation at the Workshop on Model Validation Concepts and Their Application to Lead Models held 21–23 October 1996 in Chapel Hill, North Carolina. Manuscript received at EHP 16 January 1998; accepted 17 August 1998.

Address correspondence to B.A. Fowler, Program in Toxicology, University of Maryland School of Medicine,1450 South Rolling Rd., Baltimore, MD 21227. Telephone: (410) 455-6311. Fax: (410) 455-6314. E-mail: bfowler@umbc.edu

Abbreviations used: δ-ALAD, δ-aminolevulinic acid dehydratase; K_d, dissociation constant; Pb, lead; ^{203}Pb, lead-203 isotope; PbBP, lead-binding protein; SDS, sodium dodecyl sulfate.

Lead-Binding Proteins

Rats

Kidney. Studies by Oskarsson et al. (*4*) demonstrated that the rat kidney possessed several low molecular weight protein peaks from rat kidney cytosol. One of these proteins had an estimated molecular mass of 63,000 daltons, which may be a tetramer of the lower molecular weight peak, and showed stable binding of ^{203}Pb on sodium dodecyl sulfate (SDS) gels, indicating a high degree of stability. Subsequent studies (*11–13*) demonstrated that both molecular forms were capable of attenuating the inhibitory effects of Pb^{2+} on δ-ALAD activity by a mechanism involving both Pb chelation and donation of zinc to this zinc-activated enzyme. Pb- binding studies demonstrated that the low molecular weight protein had an apparent dissociation constant (K_d) for Pb of 10^{-8} M, with extremely stable binding. Cell-free nuclear translocation studies (*14,15*) demonstrated time- and temperature-dependent nuclear uptake of ^{203}Pb-labeled PbBP with subsequent chromatin binding. *In vitro* metal competition studies (*15*) demonstrated that cadmium and zinc were the most effective competitors for inhibiting Pb binding to the PbBP. These data are of interest, as they are consistent with the results of an *in vivo* lead × cadmium × arsenic feeding study (*25*) that demonstrated that concomitant exposure to cadmium abolished formation of Pb intranuclear inclusion bodies. Biochemical characterization studies (*5*) identified the rat renal PbBP as $\alpha_{2\mu}$-globulin, which is a member of the retinol-binding protein supergene family. These studies also confirmed the apparent K_d value of 10^{-8} M Pb for binding to the purified protein. Other studies (*24*) also showed that addition of Pb to the purified protein *in vitro* also resulted in an apparent aggregation phenomenon, indicated by the appearance of higher molecular weight bands on SDS gels as a function of metal and incubation time. These findings suggest the soluble PbBP in rat kidney cytosol may be involved in the formation of pathognomonic Pb intranuclear inclusion bodies (*18–23*). These inclusions (*20,22–23*) contain acidic proteins with estimated molecular masses of 30,000 daltons.

Brain. The other major target organ in the rat also showed two protein peaks with higher molecular masses than those found in the kidney (*3*); Western blot analysis studies using polyclonal antibodies to the rat renal PbBP did not cross-react with the brain PbBP. Preliminary characterization studies (*3*) showed that, like the kidney, brain PbBP was an acidic protein with a high content of glutamic and aspartic amino acids and ion exchange chromatographic

characteristics similar to those of renal PbBP. The exact identity of this protein is currently unknown.

Humans

Blood. Ragavan and Gonick (*2*) reported earlier the increased presence of low molecular weight PbBPs in blood of PbBP-exposed workers. Subsequent studies by Lolin and O'Gorman (*1*) also showed the presence of soluble proteins in workers with moderate Pb exposure. The identity of these proteins is currently unknown.

Kidney. Recent studies by Smith and co-workers (*7,8*) have shown that there are two PbBPs in human kidney and that these are identified as diazepam-binding inhibitor and thymosin β_4. These proteins were found to exhibit K_d values of approximately 10^{-8} M, which is similar to that reported for the rat (*15*).

Brain. Studies by Quintanilla-Vega et al. (*6*) showed that human brain also contained Pb endogenously bound to thymosin β_4. 203Pb-binding studies also showed an apparent K_d value of 10^{-8} M.

Other Species

Preliminary studies in monkeys (*26*) showed that this species has kidney and brain PbBPs proteins with chromatographic characteristics similar to those of humans. The major bands on the gels were enriched in glutamic and aspartic amino acids, but the exact identity of these proteins is currently unknown. Studies on the PbBP from catfish liver (*27*) also showed similar highly anionic characteristics and the capacity to attenuate the direct inhibitory effects of Pb on δ-ALAD (*28*). The chemical and functional similarities between the PbBPs in different species appear to be highly conserved, although the proteins seem to be of different molecular masses. This could suggest that there are conserved binding regions of these various proteins across species that act in a similar manner.

Relevance of Molecular Handling of Lead to Kinetic Modeling of Lead *in Vivo*

Clearly, intracellular partitioning of Pb in target organs may be involved in mediating the low dose effects of Pb. These proteins are chemically similar but vary between species and target organs, with K_d values for Pb on the order of 10^{-8} M. *In vitro* studies suggest that the binding of Pb to these proteins is highly stable and that they represent an important Pb pool in the cytosolic compartment. Thus, they appear to be important molecular factors for regulating the bioavailability of Pb to sensitive molecular processes such as δ-ALAD activity in kidney and brain, and gene regulation in the kidney. From the perspective of kinetic modeling, they probably represent a highly stable intracellular compartment that mediates the toxic potential of Pb to the important processes noted above. It is hypothesized that differences in tissue levels of these proteins play a major role in determining individual variation in intracellular Pb bioavailabilty and hence susceptibility to toxicity from this metal. A diagram of this concept is presented in Figure 1, which shows the hypothesized regulation of Pb movement between the target tissue-specific Pb-binding proteins, sensitive effector molecules such as calmodulin, δ-ALAD, or chromatin-binding sites, and (at higher dose levels) the Pb intranuclear inclusion bodies. This last point is central to improving the predictive value of kinetic modeling for Pb toxicity. As noted above, accurate prediction of blood values is only a surrogate approach for estimating toxic potential of Pb; individuals vary greatly in their susceptibilty to Pb toxicity for equal blood Pb values. It is our hypothesis that the soluble Pb-binding proteins in major target tissues such as the brain and kidney are involved in mediating these individual differences in susceptibility, particularly at low-dose exposures. Further research is needed to test this hypothesis and to delineate how differences in tissue levels of these molecules influence the kinetics of Pb retention and bioavailability to other sensitive molecular processes.

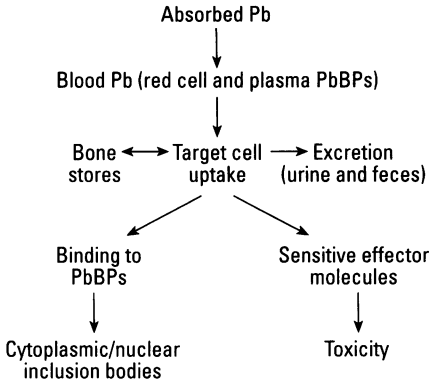

Figure 1. Diagram of hypothesized roles of target tissue-specific PbBPs in modulating the kinetics of absorbed Pb between the blood compartments, bone stores, and molecular target tissue effector molecules.

REFERENCES AND NOTES

1. LolinY, O'Gorman P. An intra-erythrocyte low molecular weight lead-binding protein in acute and chronic lead exposure and its possible protective role in lead toxicity. Ann Clin Biochem 25:688–697 (1988).
2. Ragavan SRV, Gonick HC. Isolation of low-molecular weight lead-binding protein from human erythrocytes Proc Soc Exp Biol Med 155:164–167 (1977).
3. DuVal GE, Fowler BA. Preliminary purification and characterization studies of a low molecular weight, high affinity cytosolic lead-binding protein in rat brain. Biochem Biophys Res Commun 159:177–184 (1989).
4. Oskarsson A, Squibb KS, Fowler BA. Intracellular binding of lead in the kidney: partial isolation and characterization of post mitochondrial supernatant lead-binding components. Biochem Biophys Res Commun 104:290–298 (1982).
5. Fowler BA, DuVal GE. Effects of lead on the kidney: roles of high affinity lead-binding proteins. Environ Health Perspect 91:77–80 (1991).
6. Quintanilla-Vega B, Smith DR, Kahng MW, Hernanadez JM, Albores A, Fowler BA. Lead-binding proteins in brain tissue of environmentally lead-exposed persons. Chem-Biol Interact 98: 193–209 (1995).
7. Smith DR, Kahng MW, Quintanilla-Vega B, Fowler BA. Lead-binding polypeptides in human kidney cytosol: diazepam binding inhibitor and thymosin β_4. Toxicologist 14:84 (1994).
8. Smith DR, Khang MW, Quintanilla-Vega B, Fowler BA. High affinity renal lead-binding proteins in environmentally exposed humans. Chem-Biol Interact 115:39–52 (1998).
9. Mills JS, Johnson JD. Metal ions as allosteric regulators of calmodulin. J Biol Chem 260: 15100–15105 (1985).
10. Suzuki Y,Charo S-H, Zysk JR, Cheung WY. Stimulation of calmodulin by calcium ion. Arch Toxicol 57:205–211 (1985).
11. Goering PL, Fowler BA. Regulation of lead inhibition of delta aminolevulinic acid dehydratase by a high affinity renal lead-binding protein. J Pharmacol Exp Ther 231:66–71 (1984).

12. Goering PL, Fowler BA. Mechanisms of renal lead binding protein protection against lead inhibition of δ-aminolevulinic acid dehydratase. J Pharmacol Exp Ther 234:365–371 (1985).
13. Goering PL, Mistry P, Fowler BA. A high affinity lead-binding protein in brain attenuates lead inhibition of δ-aminolevulinic acid dehydratase: comparison with a renal lead-binding protein. J Pharmacol Exp Ther 237:220–225 (1986).
14. Mistry P, Lucier GW, Fowler BA. High affinity lead-binding proteins in rat kidney cytosol: mediate cell-free nuclear translocation of lead. J Pharmacol Exp Ther 232:462–469 (1985).
15. Mistry P, Mastri C, Fowler BA. Influence of metal-ions on renal cytosolic lead-binding proteins and nuclear uptake of lead in the kidney. Biochem Pharmacol 35:711–713 (1986).
16. Hitzfield B, Planas-Bohne F, Taylor D. The effect of lead on protein and DNA metabolism of normal and lead-adapted rat kidney cells in culture. Biol Trace Element Res 21:87–95 (1989).
17. Fowler BA, Mistry P, Victery WW. Ultrastructural morphometric studies of lead inclusion body fromation in kidney proximal tubule cells: relationship to altered renal protein synthetic patterns [Abstract]. Toxicologist 5:53 (1985).
18. Fowler BA, Kahng MW, Smith DR. Role of lead binding proteins in renal cancer. Environ Health Perspect 102:115–116 (1994).
19. Goyer RA, Rhyne BC. Pathological effects of lead. In: International Review of Experimental Pathology, Vol 12 (Richter GW, Epstein MA, eds). New York:Academic Press 1973.
20. Moore JF, Goyer RA, Wilson MW. Lead-induced inclusion bodies: solubility, amino acid content, and relationship to residual acidic nuclear proteins. Lab Invest 29:488–494 (1973).
21. Oskarsson A, Fowler BA. Effects of lead inclusions on subcellular distribution of lead in rat kidney: the relationship to mitochondrial function. Exp Mol Pathol 43:409–417 (1985).
22. Shelton KR, Egle PM, The proteins of lead-induced inclusion bodies. J Biol Chem 257:11802–11807 (1982).
23. Shelton KR, Todd JM, Egle PM. The induction of stress proteins by lead. J Biol Chem 261:1935–1940 (1986).
24. DuVal GE, Jett DA, Fowler BA. Lead-induced aggregation of alpha U globulin *in vitro* [Abstract]. Toxicologist 9:98 (1989).
25. Mahaffey KR, Capar SG, Gladen BC, Fowler BA. Concurrent exposure to lead, cadmium and arsenic: effects on toxicity and tissue metal concentrations in the rat. J Lab Clin Med 98: 463–481 (1981).
26. Fowler BA, Kahng MW,Smith DR, Conner EA, Laughlin NK. Implications of lead-binding proteins for risk assessment of lead exposure. J Exp Anal Environ Epidemiol 3:441–448 (1993).
27. Conner EA, Fowler BA. Preliminary purfication and partial characterization studies of a low-molecular weight cytosolic lead-binding protein in liver of the channel catfish (*Ictalurus punctatus*). Aquat Toxicol 28:29–36 (1994).
28. Conner EA, Fowler BA. Biological and immunological properties of fish hepatic-aminolevulinic acid dehydratase (*Porphobilinogen synthetase*). Aquat Toxicol 28:37–52 (1994).