Intracellular Compartmentation of Metals in Aquatic Organisms: Roles in Mechanisms of Cell Injury

by Bruce A. Fowler*

The intracellular compartmentation of essential and toxic metals is of intense scientific interest because of its potential for adding to our understanding of both normal homeostatic mechanisms for metals and of the mechanisms which underlie metal-induced cell injury. High-affinity metal-binding proteins, lysosomes, and precipitates such as inclusion bodies or concretions, play major roles in the regulation of divalent-metal cation bioavailability. The contribution of a given compartment toward metal homeostasis is dependent upon the level exposure, cell type, organ, species, and life cycle of the organism. Toxic metals may move between these compartments, but the rates and determinants of such exchanges have not been characterized. Available data clearly indicate that sequestration of toxic metals in these specialized compartments can produce profound disturbances in the subcellular handling of essential metals. Further studies of the mechanisms by which metals partition and/or transfer among these compartments are essential to understand and predict toxicity of this important class of toxic agents.

Recent studies from a number of laboratories have identified several subcellular compartments as being major “sinks” for both essential and toxic metals in mammals and aquatic organisms. High-affinity metal-binding proteins (1-7), lysosomes (8-12), and precipitates such as inclusion bodies (13-19) or mineral concretions (7,20-27) all play important roles in intracellular metal homeostasis. The extent to which any one of these compartments is involved in metal binding appears to depend upon a number of factors, including dosing regimen for the metal administered, interactions with competing metals, cell type, organ, species, and life cycle. In addition, there appears to be movement of metals between these compartments, but the rates of metal exchange have not been determined. This review will focus on current knowledge of metal handling by these compartments, and the relationships that appear to exist between intracellular metal binding and toxicity in both mammals and nonmammalian aquatic species. This discussion will also attempt to suggest some needed areas for future research.

It is hoped that this examination will illustrate the scientific potential of the comparative approach to provide a better understanding of mechanisms of metal-induced cell injury. In particular, attention will be focused on the relationships that must exist between intracellular compartmentation of metals and their biological activity since this area appears to be of central importance to understanding mechanisms of injury under chronic exposure conditions.

Metal-Binding Proteins

In recent years, extensive attention has been focused on the roles of soluble metal-binding proteins in the biological activity of both essential and toxic metals in mammalian (1-6,10,11) and nonmammalian (6,28-30) organisms. While a majority of these proteins appear to share similarities with mammalian metallothionein (2,31), others do not (2). A current summary of these comparative data has been recently published (2). In addition, a nomenclature system that divides SH-mediated metal-binding proteins and other low molecular weight metal-binding molecules into three (I-III) metallothionein classes based on degree of sequence homology with equine metallothionein will be published shortly (3).

Although a number of chemical differences between metal-binding proteins from various species do exist, most of these proteins appear to function as major inducible “sinks” for metals in a manner analogous to that already well known for metallothionein (1,3,6,28). Once metals enter the cell, all of these macromolecules appear to play major regulatory roles in both essential (Zn, Cu) and toxic (Cd, Cu, Hg) metal homeostasis (Fig. 1). In this regard, both mammalian and nonmammalian proteins exhibit changes in essential metal composition following toxic metal exposure (30,32). These data suggest

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that toxic metal disruption of normal essential metal homeostasis at the molecular level may also play a role in toxicity (30) and/or repair after cell injury from these agents. In other words, sequestration of toxic metal ions by these proteins is not without consequence with respect to the normal metabolism of essential metals in either mammalian or nonmammalian cells.

In all species studied to date, the quantity of a given toxic metal not sequestered by these metal-binding proteins (free or spill-over fraction) appears to be most closely correlated with development of cell injury (10,11,28) as defined by both ultrastructural and biochemical criteria. For example, both mammalian (10,11) and nonmammalian species (28) show increased binding of cadmium to high molecular weight cytosolic proteins once the metal-binding capacity of the inducible metallothionein or metallothionein-like protein pools are exceeded. The implication of these data is that those toxic metal ions which are not under the homeostatic control of binding proteins actually produce toxicity when available as free cations which can react with other sensitive high or low molecular weight target molecules (5,11). At this point, it should be noted that nonmammalian organisms generally bind less (~30% versus ~90% for mammals) of their total cellular toxic metal burden to specialized soluble proteins than mammals (5,6). This may be a reflection of the generally lower dissociation constants ($K_d$, e.g., ~10^{-6} M for cadmium reported for nonmammalian binding proteins (6) relative to mammalian metallothionein (~10^{-10} M), a lower production rate of these proteins in nonmammals and/or greater competition between intracellular compartments in nonmammals. However, nonmammalian organisms do possess several other effective intracellular mechanisms for sequestering metals which may compensate for the lower metal-binding capacity of these soluble proteins. These will be discussed below.

**Lysosomal Binding of Metals**

Studies from a number of laboratories (9-12,27) have shown that lysosomes may play important roles in the intracellular bioavailability of metals in both mammalian and nonmammalian species (Fig. 1). Metals transported into the cell either bound to macromolecules or adsorbed to the cell membrane may be mobilized via proton displacement at the low pH of the lysosomes (10,27). Met-

*From Squibb et al. (10). Note that the primary lysosome fractions (II-IV) retain 2-3 times as much cadmium as the spiked control even 24 hr after Cd-MT injection when 70% of the injected dose was present in the cytosol. Rats were injected intravenously with 0.17 mg Cd/kg body weight as 106Cd-MT at 0.5, 3, or 24 hr prior to sacrifice. Control rats were injected with 0.9% (w/v) NaCl and 106Cd-Mt was added to the kidneys at the time of homogenization.

**SEM, n = 3.**

*Indicates the number is significantly different (p < 0.05) than the control value for the same fraction.

### Table 1. Subcellular distribution of 106Cd in rat kidney following intravenous injection of 106Cd-Mt.*

| Subcellular fraction | Centrifugation speed g × min | 0.5 hr | 3 hr | 24 hr | Control | Predominant organelles |
|---------------------|-------------------------------|--------|------|-------|---------|-------------------------|
| I                   | 1,500                         | 33.2 ± 0.6* | 30.2 ± 3.2* | 22.4 ± 4.2* | 14.2 ± 0.5 | Nuclei, large cytoplasmic bodies |
| II                  | 7,500                         | 14.3 ± 1.1* | 2.7 ± 0.3* | 2.7 ± 0.5 | 1.4 ± 0.3 | Mitochondria, lysosomes |
| III                 | 42,800                        | 18.3 ± 0.8 | 1.6 ± 0.2 | 1.7 ± 0.4 | 0.9 ± 0.1 | Mitochondria, lysosomes |
| IV                  | 292,000                       | 6.0 ± 0.6 | 1.9 ± 0.2* | 1.7 ± 0.2* | 0.6 ± 0.1 | Mitochondria, lysosomes |
| V                   | 3,600,000                     | 2.5 ± 0.1* | 1.7 ± 0.1* | 1.0 ± 0.1 | 0.4 ± 0.2 | Microsomes, small mitochondria, lysosomes |
| VI                  | 3,600,000                     | 25.6 ± 1.8* | 61.8 ± 5.4 | 70.4 ± 5.6 | 82.5 ± 1.4 | Cytoplasmic sap |

*SEM, n = 3.
Intracellular Precipitates

Intranuclear Inclusions

Morphologically distinctive intranuclear inclusion bodies have been reported in kidney proximal tubule cells following exposure to metals such as Pb (13–15), Bi (16), and Hg + Se (17). Inclusions have also been reported in hepatocytes of fish (18) following prolonged exposure to arsenic, indicating that the intranuclear inclusion phenomenon is not peculiar to mammals. Sub-
cellular distribution studies (19) and X-ray microanalytical studies have confirmed the presence of high concentrations of these toxic metals in the inclusions. The data suggest that these structures (Fig. 3) are the major "sink" for Pb in the cell (13,19). In Pb inclusion bodies, the metal appears to be precipitated upon an inducible carboxyl-rich protein (13). The exact mechanism by which the inclusions are formed is unknown, but it appears that soluble high-affinity metal-binding proteins may be initially involved in transporting Pb into the nucleus prior to induction of inclusion body protein (38). The formation of Pb inclusions is highly susceptible to the presence of other metals such as Cd \textit{in vivo} (39,40) and \textit{in vitro} (41), presumably due to competition for the initial binding proteins. These data again stress the potential importance of metal–metal interactions with regard to understanding mechanisms of intracellular bioavailability.

**Mineral Concretions**

In a number of invertebrate species (7,20–27), mineral concretions (Fig. 4) composed primarily of calcium phosphate appear to be extremely important in the intracellular handling of both essential and toxic metals. These structures, which are found in large vacuoles within parenchymal cells of the kidneys (7,20–27) of molluscs, appear to be primarily involved in the regulation of calcium. They are also capable of accumulating other essential and toxic metals into the calcium phos-

![Figure 3. Electron micrograph of a rat kidney epithelial cell showing lead-containing intranuclear inclusion body (arrow). × 14,167.](image-url)
phate matrix under both field (20,21,25) and laboratory (7,22–24,26) conditions. The exact mechanism of concretion formation is also not understood. However, it does appear that in some species (30) a membranous protein matrix (Fig. 5) is formed initially within a vacuole and is subsequently calcified. X-ray microanalytical studies (25,26) have shown that metal accumulation in these structures occurs at the periphery, suggesting metal deposition via accretion. Under conditions of high-dose metal exposure (26), cells of the scallop kidney extrude concretions into the tubule lumina, with reductions in total metal renal metal concentrations (26); this suggests a depuration mechanism. The importance of the concretions in cellular handling of metals within cells containing these structures cannot be understated, but the kinetic relationships which must exist between metal binding in concretions and other intracellular compartments such as metal-binding proteins or lysosomes.
Discussion

Some bacteria regulate the intracellular bioavailability of many essential and toxic metals via specific membrane transport systems (27,42). In contrast, most eukaryotes utilize metal-binding proteins (which organize metals into discrete multimetal clusters), organelle sequestration, and intracellular precipitation as primary mechanisms of achieving intracellular metal homeostasis (27,43–49). The possible selective advantages of this change in homeostatic control of divalent metal ion availability from the membrane to the intracellular milieu have been reviewed recently (42). As discussed elsewhere (23), each of these metal-binding mechanisms has specific chemical advantages and limitations which perhaps account for the presence of several different compartments in the same cell type. Thus, inducible metal-binding proteins may provide an initial high-affinity mechanism for control of metals within the cell. Because these proteins turn over relatively rapidly, this compartment is more responsive to changing metal levels; however, it is also more labile. On the other hand, inclusion bodies, concretions, or lysosomes are probably more stable longer term “sinks” for the storage of toxic metals.

At present, in both mammals and nonmammals, the mechanisms by which metals move between these intracellular compartments are unknown, but such data are essential to understanding relationships between total tissue burden of a given toxic metal, intracellular binding patterns, and mechanisms of cell injury. Critical to this understanding is the determination of rates at which metals move between these various compartments inside the cell (Fig. 6), and a fuller characterization of metal–metal interactions within major binding components. In addition to their relevance with regard to metal toxicity, such data are extremely important to our understanding of normal physiological interactions controlling essential metal homeostasis.

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REFERENCES

1. Kagi, J. H. R., and Nordberg, M., Eds. Metallothionein and Other Low Molecular Weight Metal-Binding Proteins. Birkhauser Verlag, Basel–Boston–Stuttgart, 1979, pp. 41–124.
2. Fowler, B. A., Ed. Proceedings of the International Conference on The Biochemistry of High-Affinity Metal-Binding Proteins in Non-Mammalian Species: Implications for Human Health. Environ. Health Perspect. 65: 3–224 (1986).
3. Kagi, J. H. R., Ed. Proceedings of the Second International Conference on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins. Birkhauser Verlag, Basel, 1986, in press.
4. Ridlington, J. W., and Fowler, B. A. Isolation and partial characterization of cadmium-binding protein from the American oyster (Crassostrea virginica). Chem.-Biol. Interact. 25: 127–138 (1979).
5. Fowler, B. A., Engel, D. W., and Brouwer, M. Purification and characterization studies of cadmium-binding proteins from the American oyster Crassostrea virginica. Environ. Health Perspect. 65: 63–70 (1986).
6. Fetering, D. H., and Fowler, B. A. Roles of metallothionein and
related proteins in metal metabolism and toxicity: problems and perspectives. Environ. Health Perspect. 65: 212–224 (1986).

7. Carmichael, N. G., Squibb, K. S., Engel, D. W., and Fowler, B. A. Metals in the molluscan kidney: uptake and subcellular distribution of 60Co, 68Zn and 65Zn by the clam Mercenaria mercenaria. Comp. Biochem. Physiol. 65A: 203–206 (1980).

8. Brun, A., and Brunk, U. Histochemical indications for lysosomal localization of heavy metals in normal rat brain and liver. J. Histochem. Cytochem. 18: 820–822 (1970).

9. Fowler, B. A., Carmichael, N. G., and Squibb, K. S. Factors affecting trace-metal uptake and toxicity to estuarine organisms. II. Cellular mechanisms. In: Biological Monitoring of Marine Pollutants (F. J. Vernberg, A. Calabrese, F. P. Thurber, and W. B. Vernberg, Eds.), Academic Press, New York, 1981, pp. 145–163.

10. Squibb, K. S., Ridlington, J. W., Carmichael, N. G., and Fowler, B. A. Early cellular effects of circulating cadmium thionine on kidney proximal tubules. Environ. Health Perspect. 28: 287–296 (1979).

11. Squibb, K. S., Pritchard, J. B., and Fowler, B. A. Cadmium metallothionein nephropathy: ultrastructural/biochemical alterations and intracellular cadmium binding. J. Pharmacol. Exp. Ther. 226: 311–321 (1984).

12. Fowler, B. A., Wolfe, D. A., and Hettler, W. F. Mercury and iron uptake by cytosomes in mantle epithelial cells of Quahog clams (Mercenaria mercenaria) exposed to mercury. J. Fish. Res. Bd. Can. 32: 1767–1775 (1975).

13. Moore, J. P., Goyer, R. A., and Wilson, M. Lead-induced inclusion bodies: solubility, amino acid content and relationship to residual acidic nuclear proteins. Lab. Invest. 29: 488–494 (1973).

14. Choie, D. D., Richter, G. W., and Young, L. B. Biogenesis of intranuclear lead-protein inclusions in mouse kidney. Brit. Pathol. 155: 197–203 (1975).

15. Fowler, B. A., Kimmel, C. A., Woods, J. S., McConnell, E. E., and Grant, L. D. Chronic low level lead toxicity in the rat. III. An integrated toxicological assessment with special reference to the kidney. Toxicol. Appl. Pharmacol. 56: 59–77 (1980).

16. Fowler, B. A., and Goyer, R. A. Bismuth localization within nuclear inclusions by X-ray microanalysis: effects of accelerating voltage. J. Histochem. Cytochem. 23: 722–726 (1975).

17. Carmichael, N. G., and Fowler, B. A. Effects of separate and combined chronic mercuric chloride and sodium selenate administration in rats: histological, ultrastructural and X-ray microanalytical studies of liver and kidney. J. Environ. Pathol. Toxicol. 3: 399–412 (1979).

18. Sorenson, E. M. B., Smith, N. K. R., and Ramirez-Mitchell, R. Electron probe X-ray microanalysis of arsenic inclusions in fish. Arch. Environ. Contam. Toxicol. 11: 469–473 (1982).

19. Oskarsson, A., and Fowler, B. A. Effects of lead inclusion bodies on subcellular distribution of lead in rat kidney: the relationship to mitochondrial function. Exptl. Mol. Pathol. 43: 409–417 (1985).

20. Doyle, L. J., Blake, N. J., Woo, C. C., and Yevich, P. Recent biogenetic phophoconite: concretions in molluscan kidneys. Science 199: 1431–1433 (1978).

21. George, S. G., Pirie, B. J. S., and Coombs, T. L. Isolation and elemental analysis of metal-rich granules from the kidney of the scallop, Pecten maximus (L.). J. Exp. Mar. Biol. Ecol. 42: 143–156 (1980).

22. George, S. G., and Pirie, B. J. S. The occurrence of cadmium in subcellular particles in the kidney of the marine mussel, Mytilus edulis, exposed to cadmium. Biochem. Biophys. Acta 580: 234–244 (1979).

23. Fowler, B. A., Abel, J., Elinder, C.-G., Hapke, H.-J., Kagi, J. H.-K., Kleiminger, J., Kojima, Y., Schoot Uterkamp, A. J. M., Silbergeid, E. K., Silver, S., Sumner, K. H., and Williams, R. J. Structure, mechanism, and toxicity. In: Changing Metal Cycles and Human Health (Dahlem Workshop Report), J. NIRAGU, Ed.), Springer-Verlag, Heidelberg, 1984, pp. 391–404.

24. Coombs, T. L., and George, S. G. Mechanisms of immobilization and detoxication of metals in marine organisms. In: Physiology and Behavior of Marine Organisms (D. L. McLoskey and A. J. Berry, Eds.), Pergamon Press, Oxford, 1978, pp. 179–187.

25. Carmichael, N. G., Squibb, K. S., and Fowler, B. A. Metals in the molluscan kidney: a comparison of two closely related bivalve species (Argopecten) using X-ray microanalysis and atomic absorption spectroscopy. J. Fish. Res. Bd. Can. 39: 1149–1155 (1982).

26. Carmichael, N. G., and Fowler, B. A. Cadmium accumulation and toxicity in the kidney of the bay scallop Argpecten irradians. Mar. Biol. 65: 35–43 (1981).

27. Wood, J. M., Chakrabarty, A. M., Craig, P. J., Forstner, U., Fowler, B. A., Heims, U., Krull, I. S., Mackay, D., Olson, G. J., Russell, D. H., Saunders, W., and Silver, S. Speciation in systems under stress. In: The Importance of Chemical Speciation in Environmental Processes (Dahlem Workshop Reports) (M. Bernhard and F. Brinkman, Eds.), Springer-Verlag, Heidelberg, 1986, pp. 425–441.

28. Engel, D. W., and Fowler, B. A. Factors influencing the accumulation and toxicity of cadmium to marine organisms. Environ. Health Perspect. 28: 81–88 (1979).

29. Fowler, B. A., and Megginson, M. M. Isolation and partial characterization of a high molecular weight Cd/Zn binding protein from the kidney of the scallop Placopecten magellanicus: preliminary studies. Environ. Health Perspect. 65: 199–204 (1988).

30. Fowler, B. A., and Gould, E. Alterations in intracellular metal distribution within kidney tubule cells of the scallop Placopecten magellanicus: I. Ultrastructural and biochemical studies following prolonged exposure to cadmium or copper. Marine Biol., in press.

31. Kagi, J. H. R., Vasak, M., Lerch, K., Gilg, D. E. O., Hunziker, P., Bernhard, W. R., and Good, M. Structure of mammalian metallothionein. Environ. Health Perspect. 54: 93–103 (1984).

32. Petering, D. H., and Fowler, B. A. Alterations of renal metallothionein metal composition by combined cadmium exposure and zinc deficiency. Environ. Health Perspect. 54: 73–81 (1984).

33. Ridlington, J. W., Winge, D. R., and Fowler, B. A. Long-term turnover and stability of cadmium-metallothionein following an initial low dose in rats. Biochem. Biophys. Acta 673: 177–183 (1981).

34. Feldman, S. L., Squibb, K. S., and Cousins, R. J. Degradation of cadmium-thionine in rat liver and kidney. J. Toxicol. Environ. Health 4: 805–813 (1978).

35. Fowler, B. A., Brown, H. W., Lucier, G. W., and Krigman, M. R. The effects of chronic oral methyl mercury exposure on the lysosome system of rat kidney. Morphometric and biochemical studies. Lab. Invest. 32: 313–322 (1975).

36. Madsen, K. M., and Christensen, E. F. Effects of mercury on lysosomal protein digestion in the kidney proximal tubule. Lab. Invest. 38: 165 (1978).

37. Fowler, B. A., Kardos, R., and Woods, J. S. Alteration of hepatic microsomal structure and function by acute indium administration. Ultrastructural, morphometric and biochemical investigations. Lab. Invest. 48: 471–478 (1983).

38. Mistry, P., Lucier, G. W., and Fowler, B. A. High-affinity lead-binding proteins from rat kidney cytosol: mediation cell-free nuclear translacation of lead. J. Pharmacol. Exp. Therap. 222: 462–469 (1985).

39. Mahaffey, K. R., and Fowler, B. A. Effects of concurrent administration of dietary lead, cadmium, and arsenic in the rat. Environ. Health Perspect. 19: 165–171 (1977).

40. Mahaffey, K. R., Capar, S. G., Gladen, B. C., and Fowler, B. A. Concurrent exposure to lead, cadmium and arsenic: effects of toxicity and tissue metal concentrations in the rat. J. Lab. Clin. Med. 98: 463–481 (1981).

41. Mistry, P., Latreri, M., and Fowler, B. A. Influence of metal ions on renal cytosolic lead-binding proteins and nuclear uptake of lead in the kidney. Biochem. Pharmacol. 35: 711–713 (1986).

42. Silver, S. Bacterial transformation of and resistance to heavy metals. In: Changing Metal Cycles and Human Health (Dahlem Workshop Report No. 29), J. O. NIRAGU, Ed.), Springer-Verlag, Berlin, 1984, pp. 199–223.

43. Oskarsson, A., Squibb, K. S., and Fowler, B. A. Intracellular binding of lead in the kidney: partial isolation and characterization of postmitochondrial supernatant lead-binding components. Biochem. Biophys. Res. Commun. 104: 290–296 (1982).
of delta-aminolevulinic dehydratase by a high-affinity renal lead-binding protein. J. Pharmacol. Exptl. Therap. 231: 66-71 (1984).

45. Goering, P. L., and Fowler, B. A. Mechanisms of renal lead-binding protein protection against lead-inhibition of delta-aminolevulinic acid dehydratase. J. Pharmacol. Exptl. Therap. 234: 365-371 (1985).

46. Goering, P. L., Mistry, P., and Fowler, B. A. A high-affinity lead-binding protein in brain attenuates lead inhibition of delta-aminolevulinic acid dehydratase: comparison with a renal lead-binding protein. J. Pharmacol. Exptl. Therap. 237: 220-225 (1986).

47. Fowler, B. A., Goering, P. L., and Squibb, K. S. Mechanism of cadmium-metallothionein-induced nephrotoxicity: relationship to altered renal calcium metabolism. In: Metallothionein (J. H. R. Kagi, Ed.), Second International Meeting on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, Birkhauser Verlag, Basel, 1986, in press.

48. Goering, P. L., and Fowler, B. A. Donation of zinc from kidney metallothionein to delta-aminolevulinic acid dehydratase. In: Metallothionein (J. H. R. Kagi, Ed.), Second International Meeting on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, Birkhauser Verlag, Basel, 1986, in press.

49. Goering, P. L., and Fowler, B. A. Mechanism of kidney metallothionein reversal of lead inhibition of delta-aminolevulinic acid dehydratase. Arch. Biochem. Biophys. 235: 48-55 (1987).