Proc., Symp. on Advances and Priorities in Fisheries Technology, SOFT (I) and CIFT Cochin, 11-13 Feb:302 1998

Metallothioneins against Metal Toxicity and as Biomarker in Marine Organisms

M. GEORGE CHERIAN[1] and M.R. RAGHUNATH

Central Institute of Fisheries Technology, Cochin 682 029

Invertebrate metallothioneins (MT) provide an attractive system for studying the protective mechanisms of metal toxicity in aquatic organisms, and have use as biomarkers for metal exposure. Like mammalian MT, certain basal levels of MT are found in fish and invertebrates, depending on their developmental stage and exposure to excessive amounts of metals. They are inducible proteins and have protective effects against metal toxicity because of their high affinity for metals like zinc, copper, cadmium and mercury. Chemical properties, biological functions, protective role against metal toxicity and the potential use of MT as a biomarker of metal pollution in aquatic organisms are reviewed.

Key words: **Metallothioneins, metal toxicity, protection, biomarker, marine organisms**

Marine organisms are exposed continuously to essential and non-essential metals in the environment. Essential metals such as zinc, copper and calcium are required in certain concentrations for the optimum growth and survival of the organisms, and they also provide nutritional supplements to the consumers of marine products. However, overexposure to essential metals can cause toxic effects, similar to those caused by exposure to non-essential metals. Since marine organisms are constantly exposed to metals in water or food, they have developed various biochemical mechanisms for uptake, transport, retention and excretion of metals. On overexposure, some of these mechanisms may be affected along with other effects on general metabolism. Because of the wide diversity of species, the type of toxicity and biological response, the protective mechanisms against toxicity may also differ among marine organisms (Roesijadi, 1993; Roesijadi and Robinson, 1994). One of the common protective mechanisms in marine organisms along with the role of metallothioneins (MT) in protection against metal toxicity, and also their potential use as a biomarker of metal exposure in aquatic animals are discussed.

Protective Mechanisms

Most of the aquatic organisms have natural defence mechanisms to counteract against toxic effects of various chemicals. These include several intracellular binding ligands such as amino acids, peptides and proteins along with detoxifying enzymes such as P450 enzymes for polycyclic hydrocarbons and antioxidants. Presence of reduced glutathione in various organisms is a typical example of a naturally occurring

[1] University of Western Ontario, London, Ontario, Canada

defence mechanism. In addition, external factors such as salinity also can affect the uptake of metals in aquatic animals. High salinity and chlorides can decrease the acute toxicity of cadmium in certain fish (Lin and Dunson, 1993). On low level chronic exposures, certain inducible mechanisms can also affect the toxicity of chemicals. Thus on exposure to polycyclic hydrocarbons, mixed function oxygenase enzyme activities can be induced in mussels and fish, and they have been used as biomarkers of pollution (Garrigues *et. al.,* 1993). Similarly, on exposure to certain metals like zinc or cadmium, a metal binding protein called metallothionein (MT) can be induced in fish liver and epithelial cells of gills.

Invertebrate metallothioneins

Invertebrate MT are low molecular weight metal binding proteins with high cysteine content and less of aromatic amino acid residues, similar to their mammalian counterpart. They bind certain mono and divalent metals and are inducible on exposure to metals. These proteins have been identified in about 45 invertebrate species and they have been assigned to class I and II MT depending on their primary structure (Fowler *et. al.,* 1987). Invertebrate MT show considerable differences in their structure among various invertebrate groups and also from mammalian MT. Some properties of invertebrates MT are given below

- Low molecular weight proteins with high cysteine content and high affinity to metals.
- Aromatic amino acid residues absent.
- The primary structure varies considerably from one species to another.
- Most of the species contain more than one isoform.
- In crabmeat, they contain two 3-metal clusters where the metals are tetrahedrally co-ordinated.
- In oyster, there are two distinct forms: N-acetylated and non-acetylated methionine at the N-terminal.
- Most of these proteins are inducible upon exposure to certain metals
- Induction sites are liver and the epithelial cells of gills in fish.

The most well characterised invertebrate MT is from crab *Scylla serrata* (Otvas *et. al.,* 1982). There are two major isoforms in crab hepatopancreas with 58 amino acid residues and similar structure. The bound metals are organised into two clusters of 3 metals and they differ from mammalian MT that contains one 3-metal and one 4-metal clusters. These structural differences may be related to their different proposed cellular functions. In the crab *Cancer pagurus,* the affinity of cadmium for MT with 3 metal clusters has been shown to be lower than that for rabbit liver MT with one 3-metal and one 4-metal clusters (Highman *et. al.,* 1986). This may be due to the lower affinity of metals in the 3 metal cluster than the 4 metal cluster.

The primary structures of MT are available for 3 fishes (rainbow trout, winter flounder and plaice) and five invertebrates (sea urchin, crab, lobster, oyster and mussel). The fish MT show very close similarity at the N-terminal region while there are marked differences among invertebrates. Crab and lobster as well as oyster and mussel MT show high similarity in their primary structures.

Multiple isoforms of MT along with monomeric and dimeric forms also have been isolated from several aquatic animals. Although the significance of the various forms of MT is still unclear, their expression may depend on the binding to a specific metal such as copper or cadmium, or metal exposure levels. In oysters, both N-acetylated and non-acetylated MT have been isolated depending on their exposure to metals (Roesijadi, 1992). At high levels of cadmium exposure more non-acetylated form of MT is formed. This could be due to post-translational changes because there is only a single MT gene in oysters.

Functions of Metallothioneins

Homeostasis of essential metals like sequestering of zinc during embryogenesis and larval development and of copper and its transfer to apohemocyanin during moult cycle in crustaceans, and protection against metal/free radical toxicity are the two major functions of MT.

Homeostasis

The high expression of MT and their binding of zinc during early development suggest a homeostatic role for MT in marine organisms. The increased requirement for zinc for several enzymes and transcription factors during embryogenesis and larval development may be met by sequestration of zinc by MT which can be later transferred to several metalloproteins required for DNA, RNA and protein synthesis. Thus regulation of metal bioavailability for metal-dependant functions can be achieved by increased synthesis of MT and intracellular sequestration of metals in certain tissues during early development. The increased synthesis of MT and MT mRNA has been demonstrated in sea urchin, oyster and fish during developmental stage. These observations in aquatic animals are very similar to those reported on very high expression of both MT and MT mRNA with zinc accumulation in mammalian liver during development (Cherian, 1994).

Another function of MT has been demonstrated in crustaceans during moulting. Most of the copper from hemocyanin is transferred to MT just before the initiation of moult cycle. (Brouwer and Brouwer-Hoexum, 1992). Moulting is characterised by a cycle of degradation and resynthesis of hemocyanin that is a major copper-binding oxygen carrier molecule. During this process copper is retained as MT, and it is later transferred to the newly synthesised apohemocyanin by a glutathione mediated transfer mechanism. Thus in crustaceans, MT may have a special role to store metals during certain developmental stages including moult cycle. Ionic form of copper (Cu^{+}) can take part in Fenton reaction and release of toxic hydroxyl radical within the cell. Sequestration of copper by MT during moulting can prevent release of free copper ions from hemocyanin. In addition, it is also proposed that MT with about 30% cysteine residues can act as an antioxidant during oxidative stress (Sato and Bremner, 1993; Cherian and Chan, 1993). During oxidation the metals may be released from MT and the free sulphydryl groups can act as nucleophiles.

Protection against metal toxicity

Prevalence of toxic metals and pesticide residues in fish and invertebrates is of increasing concern to fish industry and general public. Since aquatic animals are often the first forms to come in contact with these chemicals, the cellular adaptive mechanisms, which are specific to these chemicals, are of great interest. The most toxic form of metals is the ionic form that can either inhibit certain enzymes or can inactivate certain ligands that are required for cellular function. Studies in mammalian systems have demonstrated that toxicity of certain metal ions like cadmium and mercury can be reduced following induction of MT in animals (Cherian and Chan, 1993). Resistance to metals has also been demonstrated in cultured cells with elevated levels of MT. The most convincing evidence on the protective role of MT against metal toxicity is shown in MT-null mice where MT expression is disrupted. These mice are very sensitive to cadmium toxicity (Masters *et. al.*, 1994). Studies in fish and aquatic invertebrates have demonstrated increased tolerance to metal toxicity after induction of MT by pre-exposure to copper, cadmium, mercury or zinc (Roesijadi, 1992). Induction of MT by one metal like zinc or cadmium can provide tolerance to another metal like mercury. The protective mechanism will involve immobilising the ionic form of intracellular metals by induced MT synthesis because of its high affinity and capacity to bind with metals. In invertebrates there is a lag period of days for induction of MT after exposure to metals as compared to hours only in mammals. The induction of MT and cadmium toxicity at sub-lethal concentrations of cadmium in early stages of embryonic and larval development in oysters showed that the cadmium toxicity was associated with appearance of non-acetylated form of MT (Roesijadi *et. al.*, 1996). The significance of the formation of non-acetylated form of MT in oysters is not yet understood.

Induction of MT by metals may not always result in metal tolerance. It is reported that rainbow trout held in pens in lakes with elevated zinc, copper and cadmium did not show increased metal tolerance (Roch and McCarter, 1984). This could be due to differences in the rate of MT synthesis or metal saturation of induced MT.

Biomarkers of metal exposure

Metals have long biological half-life in human and aquatic organisms after their accumulation. In low level chronic exposure, there is a long lag period between exposure and symptoms of toxicity. Therefore it is important to develop potential biomarkers of exposure using various vectors. As MT synthesis increases in cells on exposure to elevated levels of metals in the environment, MT have high potential as biomarkers of metal pollution. An added advantage is that MT in an organism can be measured at three different levels; increase in metal content of the MT pool, increase in the MT itself and increase in MT mRNA. There are a wide variety of techniques available for MT measurement ranging from biochemical binding assays to HPLC-ICP-MS techniques (Roesijadi, 1992). It has been shown that MT induction occurs when environment has elevated metal levels. Because of their sensitivity, specificity and potential for early warning before deleterious changes manifest in population, MT can serve as useful biomarkers of metal pollution. However, a detailed understanding of MT function and relationship to induction by metals is yet to emerge.

As MT synthesis in organisms can also occur for mobilisation of essential metals like zinc and copper, this needs to be differentiated from the MT induction upon exposure to toxic metals. Further, the level of MT induction in relation to pollutant concentration in environment and the ability and efficiency of MT to sequester metals, and possible competition from other metal ligands in the organism are to be known with a greater degree of accuracy before full-fledged use of MT can be made as biomarkers of metal pollution.

References

Brouwer, M. & Brouwer-Hoexum, T. (1992) *Biochemistry*, **31**, 4096

Cherian, M.G. & Chan, H.M. (1993) in *Metallothionein III* (Suzuki, K.T., Imura, I. & Kimura, K, Eds) p. 363 Birkhauser Verlag, Basel

Cherian, M.G. (1994) *Environ. Hlth. Perspect*, **102**, 131

Fowler, B.A., Hildebr. C.E., Kojima. Y. & Webb, M. (1982) *J. Biol. Chem.*, **257**, 2427

Garrigues, P., Norbonne, J.F., Lafaurie, M., Ribero, D., Lemaire, P., Raoux, C., Michel, X., Solaun, J.P., Monod, J.L., & Romeo, M. (1993) *Total. Enviorn.*, **139**, 225

Highman, D.P., Nicholson, J.K., Overnell. J. & Sadler, P.J. (1986) *Environ. Hlth. Perspect*, **65**, 157

Lin, H.C. & Dunson, W.A. (1993) *Arch. Environ. Contam. Toxicol.*, **25**, 41

Masters, B.A., Kelly, E.J., Quaife, C.J., Brinster, R.L. & Palmiter, R.D. (1994) *Proc. Natl. Acad. Sci.*, **91**, 584

Otvas, J.D., Olafson, R.W. & Armitage, I.M. (1982) *J. Biol. Chem.*, **257**, 2427

Roch, M. & McCarte, J.A. (1984) *Comp. Biochem. Physiol.*, **77**, 77

Roesijadi, G. & Robinson, W.E. (1994) in *Aquatic Toxicology* (Malins, D.C. & Ostter, G.K., Eds) p. 387, Lewis Publishers, Boca Raton, Florida, USA

Roesijadi, G. (1992) *Aquatic Toxicol.*, **22**, 81

Roesijadi, G. (1993) in *Metallothionein III* (Suzuki, K.T., Imura, I. & Kimura, K., Eds) p. 142, Birkhauser Verlag, Basel

Roesijadi, G., Hansen, K.M. & Unger, M.E. (1996) *Toxicol. Appl. Pharmacol.*, **140**, 356

Sato, M. & Bremner, I. (1993) *Free Rad. Biol. Med.*, **14**, 325