Approaches to Issues and Concepts of Heavy Metals in Biochemical and Biosynthetic Pathways

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Abstract

The molecular mechanisms in which small molecules contaminate the environment and are etiological agents of perturbative physiological impacts are unknown regarding the targets and mechanisms involved. Potentially hazardous heavy metal levels have permeated and disseminated into subsurface sediment and groundwater in several metal-contaminated sites representing a challenge for environmental restoration. The effective bioremediation of these sites necessitates full knowledge and information of genetic pathways of resistance and biotransformation due to component organisms with a microbial community. Derangement of metal ion homeostasis may result in oxidative stress state whereby elevated production of ROS degrades body-antioxidant shielding and, consequently induces inter alia DNA disruption, lipid peroxidation, protein denaturation or alterations which are pathognomic for several disorders. Although, several metals are essential for microbial function, the heavy metals present toxic effects on cellular metabolism, biochemical and biosynthetic pathways.
Keywords: heavy metals, biosynthetic pathways, toxicity, remediation, microorganisms

Introduction

Heavy metal environmental pollution is currently very expansive from industry, old paints, chemicals, pipes and emanating from erstwhile contaminants accumulating in the food chain (Schauder et al 2010). The molecular mechanisms in which small molecules contaminate the environment and are aetiological agents of perturbative physiological impacts are unknown regarding the targets and mechanisms involved (Chauhan et al 1997).

The production, emission and dissemination of metals have increased exponentially since the industrial revolution culminating in superimposed natural cycles of metals in numerous ecosystems. Metals display an expansive array of physico-chemical properties such as, essential versus non-essential and redox-active versus non-redox-active. Generally, all metals can accumulate in toxicity and oxidative stress when imbibed in excessive quantities, thus constituting adverse threat to the environment and human health (Jozefczak et al 2012, Chukwuma 2014). In this regard, trace elements, i.e. heavy metals and metalloids are of concern as environmental pollutants, and several of them are toxic at low concentrations. Primary sources of pollution of the biosphere include fossil fuel burning, mining and smelting of metalliferous ores, organochemicals, sewage and municipal wastes as well as natural mineral deposits containing large reserves or deposits of heavy metals in mineral-enriched regions of the world (Memon, Schröder 2009). Mixed pollution containing trace elements and organic industrial components characterizes numerous spill areas and dumping sites (Schröder et al 2009).

Therefore, potentially hazardous heavy metal levels have permeated and disseminated into subsurface sediment and groundwater in several metal-contaminated sites representing a challenge for environmental restoration. The effective bioremediation of these sites necessitates full knowledge and information of genetic pathways of resistance and biotransformation due to component organisms with a microbial community. Although, several metals are essential for microbial function, the heavy metals present toxic effects on cellular metabolism (Nies 1999), biochemical and biosynthetic pathways.

Heme

Iron, Fe is a heavy metal on which an appreciable iron demand of the body is necessary for heme synthesis and assembly, but iron is also needed for Fe-S cluster proteins and several redox enzymes. Heme is an essential, iron-binding molecule that acts as a prosthetic group of hemoproteins or as a regulator in biosynthetic pathways. Pb, Ga, Cu, Kd, Hg and Al compete with Fe on transporters, decrease the cellular Fe pool, bind to proteins and are etiological agents in physical and mental aberrations as well as their exposure on heme synthesis as the major iron-sequestering process of the human body. Heavy metals cause impairment of diverse modalities of the heme biosynthesis pathway such as, enzyme activity, gene expression and iron integration into protoporphyrin IX. It is relevant to elucidate associated risk factors and impacts on iron-dependent processes as to enhance public awareness in the enhancement of public awareness in heavy metal dissemination in the environment as well as the debilitative exposure impacts, albeit in low doses (Schauder et al 2010).

The essential human enzyme, porphobilinogen synthase (HsPBGS, 5-aminolevulinate dehydratase, ALAD) is used in heme biosynthesis. The catalytic activity of HsPBGS is allosterically regulated through an equilibrium of inactive hexamers and active octamers. It has been shown that specific drugs and drug-like small molecules are able to inhibit HsPGBS in vitro by the stabilization of the hexamer (Chauhan et al 1997). The hexamer-stabilizing inhibitors are suggested to potentiate lead poisoning and PGBS dysfunction associated with

an inborn error of metabolism named ALAD porphyria. Allosteric regulation activity through n equilibrium of alternate oligomers are proposed for several proteins. Derangement of associated oligomeric equilibria by small molecules such as environmental contaminants and culminating pollutants are termed mechanisms of toxicity.

Depending on the organism, the heme biosynthesis enzyme, delta-aminolevulinic acid dehydratase (ALAD(needs zinc or magnesium for activity and the heme moiety contains Fe. In this regard, metals are crucial for heme production in at least two disparate manners. Bradyrhizobium japonicum, ALAD* is a formulated derivative of wild-type ALAD that needs Zn2+ for activity instead of Mg2+ (Chauhan & O'Brian. 1995). The pH optimum for ALAD* activity was more than 3.5 units less than that of the wild-type enzyme, and ALAD* activity was inhibited by Pb and Cd, this has also been reported for animal Zn-containing dehydratases. Moreover, ALAD* was markedly more thermostable than ALAD, with temperature optima being 50 and 37 degrees centigrade, respectively (Chauhan et al 1997).

It is suggested that the metal influences both catalysis and structure in ALADs generally. Inasmuch as Fe did not impact on the activity of the preformed protein, it is clear that enzyme assays and immunoblot analysis depicted that the Fe levels in which the cells were grown had a significant positive correlation on ALAD activity and the protein concentration. RNase shielding analysis demonstrated that the transcript quantity of hemB, the gene encoding for ALAD was Fe-dependent, with Fe regulating hemB at the level of mRNA. hemB mRNA induction elicited by Fe was observed to be fast, thus suggesting that the factor (s) required for the mediation of iron control was available in Fe-limited cells, and was not required to be synthesized de novo. ALAD protein concentrations and enzyme reactions were identical in cells of the wild type and a heme-defective strain that is indicative of Fe control and not an indirect effect of the status of the cellular heme. Thus, the heme biosynthetic pathway is coordinated with cellular Fe concentrations and that this control may obviate toxic porphyrin intermediates aggregation (Chauhan et al 1997).

Oxidative Stress
Plants respond to heavy metal toxicity through diverse trajectories. These responses are immobilization, exclusion, chelation and compartmentalization of the metal ions, and the presentation of expansive general stress response mechanisms as depicted in ethylene and stress proteins (Cobbett 2000). The main response to these metals are shielding from oxidative stress such as up-regulation of manganese-dependent superoxide dismutase soda. Glutathione s-transferase, thioredoxin, glutaredoxins, and DNA repair enzymes were significantly influenced by cadmium and chromate. The cadmium and chromium stress response was determined on the production of the intracellular metal levels with multiple efflux pumps used to eliminate cadmium, whereas a sulphate transfer was down-regulated to decrease nonspecific chromium uptake. In addition, membrane proteins were up-regulated as elicited by most of the tested metals. A dual component signal transduction system associated with the elicitation of the uranium was determined. Multiple disparately regulated transcripts from previously non-established regions for the encoding of proteins were identified, thus depicting the benefits for the evaluation of transcripts involving whole genome microarrays (Hu et al 2005).

Detailed studies have demonstrated that redox active metals such as, Fe, Cu, Cr and other metals redox cycling reactions with the capability to generate reactive radicals like superoxide anion radical and nitric oxide in biological systems. Derangement of metal ion homeostasis may result in oxidative stress state whereby elevated production of ROS degrades body-antioxidant shielding and, consequently induces inter alia DNA disruption, lipid peroxidation, protein denaturation or alterations which are pathognomic for several disorders (Sharma et al (2014) such as, cancer, cardiovascular disorders, diabetes, neurological disorders and chronic inflammation. The undergirding mechanisms of action of these metals are associated with the generation of superoxide radical via Fenton reaction and other ROS culminating in mutagenic and carcinogenic malondialdehyde (MDA), 4-hydroxynonenal (HNE) and other exocylic adducts of DNA. The redox inactive metals such as, Cd, As and Pb exhibit their toxicity by binding to sulphydryl groups of proteins and glutathione depletion (Jomova, Valko 2011).

The redox inert metal Zn is an essential component of several proteins associated with defense against antioxidant stress as well as impact on the immune system and contains neuroprotective attributes. DNA disruption by impairments of DNA repair mechanisms (Jomova, Valko 2011; Valko et al 2005). The mechanism of metal-induced production of free radicals is inextricably influenced by cellular antioxidant actions. Numerous low molecular weight antioxidants such as, ascorbic acid (vitamin C), alpha tocopherol (vitamin E), glutathione (GSH), flavonoids and carotenoids can chelate metal ions resulting in the decrease of their catalytic action to produce ROS. A newfangled therapeutic strategy to extinguish or suppress oxidative stress is premised on the production of dual function antioxidants comprising both chelating and scavenging properties. Ironically, two main antioxidant enzymes, catalase and superoxide dismutase possess as an integral portion of their active sites certain metal ions to shield against toxic effects of metal-induced free radicals (Jomova, Valko 2011; Valko et al 2005).

The metallothioneins

The metallothioneins (MT) are small, cysteine-rich heavy metal-binding proteins associated in an expansive array of protection stress response (Andrews 2000). Thus, these metallothioneins are a family of multifunctional protein which participate inter alia in stress response (Egg et al 2009). Even though, one single essential function of MT has not been established, MT of higher eukaryotes evolved as a mechanism for the regulation of zinc concentrations and dissemination within cells and organisms. These MT proteins are also protective against certain toxic metals and oxidative stress-inducing agents. In mice, the MT-I and MT-II genes are most largely expressed among the four determined MT genes (Andrews 2000). The gene transcription is accelerated and markedly up-regulated when elicited by zinc and cadmium including response to aetiological agents of oxidative stress and/or inflammation. The metalloregulatory functions of metal-response element-binding transcription factor-1 (MTF-1) have been partly mapped to its six significantly conserved zinc fingers (Jiang et al 2003). The six zinc-finger metal responsive transcription factor MTF-1 is associated in a pivotal role in transcriptional activation of the MT-I gene as a result of elicitation by metals and oxidative stress. MTF-1 gene mutation extinguishes these responses, and MTF-1 is elicited to bind to the metal response elements proximal to MT promoter in zinc-treated cells or as oxidative stress presents. It is suggested that the DNA-binding activity of MTF-1 both in vivo and in vitro is invariably reversibly activated by zinc interactions with the domain of the zinc finger (Andrews 2000). This depicts heterogeneity in the structure and function of the six zinc fingers.

It has been demonstrated that the metal-elicited augmentation of the MTF-1 to the MT-I promoter is a rate-limiting step in its metalloregulatory fnction and, that an integrated zinc finger domain is necessary for this recruitment or augmentation in the ability of zinc finger deletion mutants of mouse MTF-1 to regulate the endogenous metallothionein-1 (MT-1) gene in cells devoid of endogenous MTF-1. It is indicated that accelerated derangement of nucleosome structure at the MT-I promoter involves zinc-responsive recruitment of an active MTF-1-coactivator complex (Okumura et al 2011). It is suggested that mouse MTF-1 exhibits polymorphisms (Jiang et al 2003); thus mouse MT-I gene regulation by metals and oxidative stress has participatory multiple signaling pathways depending on the metal ion species and the magnitude of the oxidative stress (Andrews 2000). It pertains that these would be essential tools to predict the potential for plasticity and niche-specific adaptation of stress-responsive genes in populations of increasing alterations of environmental conditions (Egg et al 2009) and heavy metal challenge (Otsuka et al 2007) as well as action in zinc homeostasis, cadmium detoxification and protein and protection against reactive oxygen species, ROS (Okumura et al 2011).

Also, the impact of growth hormone (GH) and Cd on metallothionein (MT) expression in fish hepatoma cells of two isoforms MT-A and MT-B was that cystolic $Ca2+$ is essentially associated with MT-A regulation; as well as that $Ca2+$ and tyrosine phosphorylation are required for Cd induction and GH/Cd synergy on MTs. It is suggested that the synergy is due to interactions in disparate signaling pathways culminating in a differential treatment of MTF-1 and AP-1 transcription factors (Vergani et al 2007). The interference between heavy metals and GH on cell signaling on the isoforms depict that all heavy metals induce MT-A more expressively than MT-B, but disparities existed as metals were coupled with GH (Vergani et al 2009). Disparate signaling pathways may be mechanisms depicting the differential regulation of metallothioneins with either $Ca2+$, $Cu2+$, $Hg2+$, $Zn2+$ or $Cd2+$ and GH, via a differential recruitment of transcription factor (Vergani et al 2009).

Gastropods can tolerate fluctuating availabilities of nonessential trace elements such as, cadmium via induction of Cd-specific metallothionein isoform (Cd-MT) expression. The induction mechanism is by the binding of metalo-regulatory transcription factors (MTF-1 or MTF-2) to metal response elements (MREs) in the MT promoter regions. It is suggested that transient Cd-MT transcription superimposed on $Cd2+$ exposure in Helix pomatia results from inhibitory action of the distal MRE cluster (Höckner et al 2009

Remediation and Restoration of Contaminated Sites

Phytoextraction is an environmentally friendly clean-up procedure for toxic metal contaminated sites. GSH plays a central role in this process due to the high affinity of metals to thiols and as precursor for phytochelatins (PCs) and that GSH is an essential metal chelator (Seth et al 2012). Also, as redox couple, oxidized and reduced GSH can transmit specific information, tuning cellular signaling pathways during environmental stress conditions. Phytoextraction can be enhanced by employing transgenic plants in plant-associated microorganisms (Seth et al 2012).

Caulobacter crescentus and related.stalk bacterial species have distinctive ability to occupy low-nutrient ambients, thus developing a feature of a vast array of heavy metal-contaminated sites. Caulobacter cresecentus constitutes a model organism for cell cycle regulation presenting well-developed genetics. Identification of the pathways responding to heavy metal toxicity in C. crescentus provides insights for application of Caulobacter to environmental restoration. Exposure of C. crescentus to the heavy metals, Cr, Cd, Se and Ur followed by analysis of genome-wide transcriptional activities postexposure by employing an Affymetrix Genechip microarray, it was observed that C. crescentus displayed marked tolerance to uranium; a

possible mechanism for the formation of extracellular calcium-uranium-phosphate precipitates (Hu et al 2005).

These areas usually support certain plant species thriving in highly mineralized areas. Although, a large variety of plant species obviate metal uptake from these soils; other species accumulate significantly elevated levels of toxic metals even exceeding the soil contents. The natural phenomenon of heavy metal tolerance in certain of these plants has been of interest and concern to environmentalists, ecologists, botanists and physiologists to investigate inter alia the physiology and genetics of metal tolerance in hyperaccumulators (Chukwuma, Memon, Schröder 2009) with particular emphasis on transcriptomics of heavy metal accumulators and to identify functional genes attributed in tolerance and detoxification (Memon, Schröder 2009).

Discussion and Conclusion

Depending on their chemical and physical attributes, three disparate molecular mechanisms of heavy metal toxicity can be established, viz: (i) formation of reactive oxygen species by antioxidation and Fenton reaction, that is commonplace for transition metals such as, and Fe; (ii) the blockage of essential functional groups in biomolecules observed for non-redox-reactive heavy metals like Cd and Hg; (iii) dislodgment of essential metal ions from biomolecules as depicted in a variety of heavy metals. There are review papers assessing the mode of action and role of antioxidants as shields from heavy metal stress in roots, mycorrhizal fungi and mycorrhizae (Schützendübel &, Polle 2002). It is suggested that mycorrhizal fungi protect via GSH because elevated levels of this thiol were detected in pure cultures of the fungi than in unprotected roots. The production of coupled stress tolerant plant-myccorrhizae may assist as a new strategy for phytoremediation and site remediation (Schützendübel &, Polle 2002). Thus, GSH has been implicated in metal homeostasis, antioxidant defense, and signal transduction during metal stress (Jozefczak et al 2012). The varied functions of GSH emanate from the sulphydryl groups as cysteine, thus creating the latitude for GSH to chelate metals and be involved in redox cycling. Stress reactions, reactive oxygen species formation and antioxidant depletion cause changes in xenobiotic detoxification with the necessity of the ability of plants to detoxify chlorophenols via glutathione conjugation in a mixed pollution environment (Schröder et al 2009).

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