# Biosynthesis of metallothioneins under cadmium stress, zinc and iron supplementation in liver and kidney of male albino rats

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**Abstract**— Cells react to stressful environments with a broad range of diverse homeostatic responses. Among an array of responses stress response proteins, including metallothionein (MT) play an important role against metal induced stress. The present study is carried out to know the role of MT in detoxification of cadmium (Cd) toxicity in rats before and supplementation with trace elements such as zinc (Zn) and iron (Fe). Male albino rats were treated orally with Cd at a dose of 1/10th of LD50 / 48h (i.e. 22.5 mg/kg) for 7, 15 and 30 days (d) long sojourn. 15d Cd treated rats were then subjected to trace element supplementations of Zn (12mg/kg) and Fe (40mg/kg) individually and in combination for another 7, 15 and 30d time intervals. After specific time intervals, rats were decapitated and tissues like liver and kidney were isolated and used for metallothionein studies. Purification and quantification of MT protein was carried out by the standard methods. Purified MT protein containing samples were subjected to SDS-PAGE. Clear visible bands were observed in the test tissues approximately at 6.5 KDa against standard low range molecular weight protein marker (Cat. No. M 3546). Further, MT protein levels were significantly elevated in the test tissues during Cd treatment and also after supplementation with Zn and / or Fe. Maximum MT protein synthesis was observed in 30d rat kidney under combined supplementation of both Zn and Fe. Thus, tissues that contain an excess amount of MT are resistant to Cd toxicity.

**Index Terms**— Cadmium, zinc and iron supplementation, metallothionein, Purification and quantification, liver, kidney, rat.

## 1 INTRODUCTION

MT is a molecular weight (6-7 kDa), Cysteine (Cys) - rich and stress response protein with a high affinity for divalent heavy metals such as Cd[1,2]. MT protein that is induced by other divalent metal cations (e.g. mercury, cadmium,) protects essential cellular functions[3] and enhances the survival of both cells and whole organisms that are exposed to toxic heavy metals. These MTs are ubiquitously present in a large variety of prokaryotic and eukaryotic species as well as in all mammalian organs and tissues examined so far both in animal and plant kingdom and are increasingly being demonstrated to play a vital role in metal homeostasis[4].

In mammals, four isoforms of MT have been reported (MT-I, II, III and IV). MT-I and II are found in the liver and kidney tissues. MT – III has been detected in mouse and human brain, and MT – IV has been found in certain stratified epithelia[5,6,7,8]. The biological function of MT is likely related to the physiologically relevant metals (Zn, Cu and Fe) that this protein binds. In mammals, among MT isoforms, MT-I is found to bind Zn and Fe under normal physiological conditions. Both Zn and Fe are essential trace elements in biology as components of wide variety of metalloproteins and wealth of enzyme families[9,10]. Zn is known to be the most effective MT inducer and Fe is categorized as indirect MT inducer[11]. Fe binds significantly less tightly to MT than Zn[12]. The relative affinities of these metal ions to sulfur ligands are well established from inorganic thiolate complexes[13] MT is primarily synthesized on free polysomes[14]. Based on their synthesis, MT protein has been often considered to function exclusively as an intracellular protein. While MT lacks the signal peptide sequences or other protein trafficking signals that would result in proteins entering the traditional secretory pathway, it is nevertheless detected in serum, urine, pancreatic secretions and other biological fluids as well as in branchoalveolar spaces, liver sinusoids and other extra cellular spaces. This pool of extracellular MT may originate from necrotic cell death that may accompany some forms of stress but it is also possible that MT may be selectively released from stressed cells by non-traditional secretory pathway[15]. Regardless of its origin, there is compelling evidence that the pool of extracellular MT protein has interesting roles of its own to play. One possible role of extracellular MT protein is as a distribution mechanism for both essential and toxic heavy metals. Another aspect of MTs extracellular influences is its influence on immune capacity. Recent studies have produced strong evidence to support the idea that the MT functions as a metal chaperone for the regulation of gene expression and for synthesis and functional activity of proteins such as metalloproteins and metal-dependent transcription factors[16,17]. MT could thus serve as a reservoir of essential metals such as Zn, Se, Fe and Cu. This might dictate dual functions of MTs:

1. Preventing metal toxicity under over load conditions and
2. Donating metals to apo-metlloproteins under physiological conditions.

A large amount of subsequent work has been shown that MT to serve in many cell types in the management of essential divalent metal cations, to interfere with the toxic effects of xenobiotics, heavy metals and free radicals. It also serves as a regulator of specific transcription factors14. As a consequence of these various activities, MT synthesis has an impact on developmental processes and on the cellular responses to stressful conditions under heavy metal burden[18]. In vitro studies indicate that the transfer of metals from MT to an acceptor is possible and GSH has been known to facilitate such a transfer. Most of these metal transfer studies have focused on MT regu-