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ZINC-mediated gene expression offers protection against H_2O_2-induced cytotoxicity

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Abstract

The ability of zinc to mobilize defense against reactive oxygen species (ROS) and H_2O_2-induced apoptosis was studied using a primary culture of rainbow trout gill cells. Gill cells were pretreated for 24 h with 100 μM $ZnSO_4$ followed by 24-h exposure to 100 or 200 μM H_2O_2, or were subjected to 100 μM $ZnSO_4$ together with 100 or 200 μM H_2O_2. Metallothionein-A (MTA) and metallothionein-B (MTB) mRNA levels were increased after treatment with zinc or H_2O_2, separately or in combination. Similarly, mRNA for glutathione S-transferase (GST) and glucose 6-phosphate dehydrogenase (G6PD) were increased in response to either zinc or H_2O_2, or after sequential treatments with zinc followed by H_2O_2. The stimulatory effects of zinc or H_2O_2 on MTA, MTB, GST, and G6PD mRNA levels could be blocked by addition of the membrane permeable zinc chelator, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), suggesting that H_2O_2-induced upregulation of these genes is zinc-dependent. Pretreatment with zinc protected the cells from subsequent cell damage and apoptosis, as assessed by lactate dehydrogenase leakage, mitochondrial dehydrogenase activity (MTT assay), caspase-3 activity, and DNA fragmentation. In contrast, when gill cells were coincubated with zinc and H_2O_2 at the same time, H_2O_2 toxicity was higher than after treatment with H_2O_2 alone. It is concluded that zinc had a direct pro-oxidant effect when administered together with H_2O_2, but that pretreatment of zinc inhibited cytotoxicity and apoptosis through an indirect antioxidant action. We propose that the antioxidant action is manifested through zinc-dependent expression of several genes encoding antioxidant proteins (e.g., MTA, MTB, G6PD, and GST). Furthermore, the apparent zinc-dependency of H_2O_2-induced expression of antioxidant genes suggests that zinc might act as a physiological signal to mediate the response to oxidative stress.

Keywords: Metallothionein; Zinc; TPEN; Hydrogen peroxide; Oxidative stress

Introduction

Zinc stimulates transcription of specific genes by binding to Metal regulatory Transcription Factor-1 (MTF-1), which upon activation binds to metal-responsive elements (MRE) located in the 5′ flanking region of the target genes (Andrews, 2001). It was previously thought that gene regulation by zinc was more or less restricted to genes that are involved in zinc homeostasis. However, more recent research has provided evidence for a much wider involvement of zinc-regulated gene activation (Cousins et al., 2003; Egli et al., 2003; Lichtlen et al., 2001). Through global mapping of MRE distribution in the human and pufferfish (*Fugu rubripes*) genomes, it was found that many genes with antioxidant ontology annotations possess multiple MRE copies (N. Hill, P. Cunningham, C. Hogstrand and P. Kille, unpublished). In Fugu, 28 antioxidant genes have two or more MRE sequences within 2 kb upstream of the open reading frame and these included genes with key roles in antioxidant defense, such as glucose-6-phosphate dehydrogenase (G6PD), glutathione *S*-transferase (GST), glutathione peroxidase-1 (GPX1), Cu/Zn-superoxide dismutase (SOD1), Mn-superoxide dismutase (SOD2), catalase (CATA), glutamate cysteine ligase catalytic subunit (GCLC), and metallothionein (MT). This led us to speculate that the well-known protective effect of zinc against reactive oxygen species (ROS; Truong-Tran et al., 2001) might

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involve zinc-induced expression of some of these genes. In support of this idea, expression of not only MT, but also those of GST and GCLC have been reported to be zinc-dependent (Günes et al., 1998; Hogstrand et al., 2002; Lichtlen et al., 2001).

ROS, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$), are generated in vivo from the incomplete reaction of oxygen during aerobic metabolism, stimulated host phagocytes, or from exposure to environmental agents (Ames, 1983; Babior, 1992; Cerutti, 1985). ROS may damage critical processes in the cell and lead to a plethora of toxic consequences. However, cells have an extensive endogenous defense system against this hazard. It consists of anti-oxidant enzymes, such as SOD, CAT, and GPX and of small anti-oxidants, such as glutathione (GSH), MT, vitamins C and E (Fridovich, 1999; Kling and Olsson, 2000; Mates et al., 1999). GST and G6PD enzymes help in the detoxification of reactive oxygen species by decreasing peroxide levels (GST), or by maintaining a supply of reducing power in form of nicotinamide adenine dinucleotide phosphate (NADPH) (G6PD) (Sun, 1990). Moreover, there are indications that exogenous antioxidants like ascorbate and α-tocopherol offer some degree of protection against this type of DNA damage (Anderson et al., 1994; Panagiotidis and Collins, 1997). H_2O_2 is a common intermediate in oxidative processes and it has been shown to be able to induce DNA-damage and to decrease cell viability in living cells (Hartley et al., 1993; Imlay and Lin, 1988; Jagtap et al., 2003; Roig et al., 2002).

Caspases are a family of proteases that cleave a variety of critical cellular proteins containing a caspase recognition site; Asp-Glu-Val-Asp (DEVD) in the case of caspase-3 and -7 (Nicholson and Thornberry, 1997). Caspase-3 activity is an important mammalian cell death gene and a hallmark of apoptosis (Nicholson et al., 1995; Tewari et al., 1995). The inhibition of caspase-3 in vitro by physiologically reasonable concentration of zinc has been suggested to be an important mechanism behind the ability of zinc to suppress apoptosis (Perry et al., 1997; Stennicke and Salvesen, 1997). The concentration of exchangeable zinc in a cell is regulated by MT, which is a family of sulfhydryl-rich, low-molecular weight proteins found in most organisms and tissues (Mckenna et al., 1998; Moffatt and Denizeau, 1997). In addition to regulating zinc, MT itself is involved in protecting against oxidative stress by virtue of its remarkably high content of cysteinyl residues (Andrews, 2000; Moffatt and Denizeau, 1997; Vallee, 1995). Accordingly, the synthesis of MT is induced by transition metal ions and by oxidative stress (Cavaletto et al., 2002; Kling and Olsson, 2000; Mckenna et al., 1998; Moffatt and Denizeau, 1997). The antioxidant properties of MT have been demonstrated in fish cells. Specifically, expression of MT in salmonid cells, either through zinc treatment or ectopic overexpression under a viral promoter, results in an increased resistance to H_2O_2 (Kling and Olsson, 2000).

Experimental evidence exists to suggest that zinc can have either positive or negative effects on physiology of cells depending on the local concentration, localization, and/or state (Chen and Liao, 2003). Excess free zinc can be detrimental and exacerbate neuronal damage, in vivo and in vitro (Choi et al., 1988; Lees et al., 1990; Yokoyama et al., 1986). In contrast, zinc appears to protect against DNA damage and apoptosis caused by oxidative stress (Leccia et al., 1999). Zinc is tightly regulated as it serves many essential roles in cells (Berg and Shi, 1996; Vallee and Auld, 1990). Several hundred enzymes are known to require zinc for their proper functions. Also, well over a thousand transcription factors contain zinc-binding sequences (Heximer and Forsdyke, 1993; Klug, 1999; Prasad, 1991; Sukegawa and Blobel, 1993). Cellular zinc is described as an inhibitor of apoptosis, and its depletion induces death in many cell lines. The decrease in intracellular zinc concentration induces characteristic apoptosis, with apoptotic body's formation and nuclear DNA condensation and fragmentation (Adler et al., 1997; Hyun et al., 2000).

Given the many antioxidant genes with multiple MRE and the conflicting information in the literature regarding the influence of zinc on oxidative stress, we speculated that zinc might protect cells indirectly against reactive oxygen species through activation of key genes involved in oxidant defense. This idea was tested in the present study through a series of experiments involving treatments of isolated rainbow trout gill cells with zinc and H_2O_2 individually or in different combinations. We further examined the role of intracellular zinc in mediating expression of MT, GST, and G6PD in response to H_2O_2.

Materials and methods

Reagents

Aqueous solution of H_2O_2 (30%), LDH kit (Sigma DG1340-UV), fetal bovine serum (FBS), NaCl, KCl, Na_2HPO_4, KH_2PO_4, $ZnSO_4 \cdot 7H_2O$, TPEN and MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] were purchased from Sigma. Liebovitz (L-15) medium with L-glutamine and phenol red, penicillin and streptomycin (PEST), fungizone reagent, gentamicin reagent solution liquid and trypsin-ethylenediaminetetraacetic acid (T-EDTA) were obtained from Gibco BRL. Cell strainers 100 μm (Falcon), cell inserts 0.4 μm (12 well; Falcon), tissue culture insert companions (12 well; Falcon), and tissue culture plate (6 well) were purchased from Marathon. Powerscript Reverse Transcriptase was obtained from Clonetech. Oligo$(dT)_{15}$ primer and random Hexamers were sourced from Promega.