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Review Article

Zinc ions as effectors of environmental oxidative lung injury

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ABSTRACT

The redox-inert transition metal Zn is a micronutrient that plays essential roles in protein structure, catalysis, and regulation of function. Inhalational exposure to ZnO or to soluble Zn salts in occupational and environmental settings leads to adverse health effects, the severity of which appears dependent on the flux of Zn^{2+} presented to the airway and alveolar cells. The cellular toxicity of exogenous Zn^{2+} exposure is chazracterized by cellular responses that include mitochondrial dysfunction, elevated production of reactive oxygen species, and loss of signaling quiescence leading to cell death and increased expression of adaptive and inflammatory genes. Central to the molecular effects of Zn^{2+} are its interactions with cysteinyl thiols, which alters their functionality by modulating their reactivity and participation in redox reactions. Ongoing studies aimed at elucidating the molecular toxicology of Zn^{2+} in the lung are contributing valuable information about its role in redox biology and cellular homeostasis in normal and pathophysiology.

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Contents

Abbreviations: AP-1, adaptor protein 1; ASL, airway surface liquid; CCO, cytochrome *c* oxidase; CF, cystic fibrosis; COX-2, cyclooxygenase 2; Csk, c-src kinase; DMEM, Dulbeccos' minimal essential medium; DMT1, divalent metal ion transporter 1; DUOX, dual oxidase; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinases; GDP, guanosine diphosphate; GSH, glutathione; GSK-3β, glycogen synthase kinase 3 beta; GSSG, oxidized glutathione; GTP, guanosine triphosphate; HO-1, heme oxygenase 1; IkB, inhibitor of kappa B; IKK, IkB kinase; IL-1, interleukin 1 (6, 8, etc.); JNK, c-jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen activated protein kinase; MAPKK, mitogen activated protein kinase kinase; MAPKKK, mitogen activated protein kinase kinase kinase; mPTP, mitochondrial permeability transition pore; MT, metallothionein; MTF-1, metal-responsive transcription factor 1; Myc, myelocytomatosis gene; NADH, nicotinamide dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NFkB, nuclear factor kappa B; NOX, NADPH oxidoreductase; NRAMP, natural resistance-associated macrophage protein; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PDK1, pleckstrin homology domain kinase; PI3K, phosphatidylinositol-3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKB, protein kinase B; PKC, protein kinase C; PM, particulate matter; PP, protein phosphatase; PTEN, phosphatase and tensin homolog; PTP, protein tyrosine phosphatase; RNS, reactive nitrogen species; roGFP, redox sensing green fluorescent protein; ROS, reactive oxygen species; Ser, Serine; SHP-1, src-homology region 2 domain-containing phosphatase 1; SLC, solute-linked carrier; TNF, tumor necrosis factor; TPEN, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridinylmethyl)-1-2-ethanediamine; TRPA1, transient receptor potential with ankyrin repeats; Zip, Zinc transporter (SLC 39); ZnT, Zinc transporter (SLC 30).

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Introduction

Following the seminal discoveries of the late Professor Bert Vallee and his colleagues and students [1], zinc (like iron) has been recognized to be an abundant and essential transition metal in biological systems. The average total cellular Zn content in mammalian cells is estimated to be ~250 μM (10^8 atoms per cell). However, like ionized Fe, free Zn^{2+} ions are potentially toxic and therefore require buffering by Zn-binding proteins such as metallothioneins (MT), and/or sequestration in microvesicles within the cytosol ("zincosomes") or within organelles. Zn^{2+} binding to anionic histidyl, aspartyl, glutamyl, and thiolate groups give it an important role in protein structure and activity, in enzymatic function, in secretory granule formation, and in synaptic transmission. Although (unlike Fe) Zn is redox inert, its interaction with cysteine thiolates confers an important role in redox signaling by ROS and RNS, and in kinase/phosphatase-based signal transduction as well [2–7]. Indeed, as proposed by Wolfgang Maret, Zn^{2+} is increasingly accepted as having a "second messenger" role in cell metabolism [8].

Costello et al. have summarized evidence that Zn^{2+} is normally transferred directly from "donor" to "acceptor" ligand molecules without necessarily involving release of Zn^{2+} ions [9]. The normal 10–18 μM plasma total zinc concentration is 98% bound to albumin and other proteins while 2% is bound to ultrafilterable small organic molecules such as citrate and amino acids. The lower protein concentration in extracellular fluid decreases total Zn to ~5 μM. Costello et al. point out that this heterogeneous pool of bound Zn involves compounds with formation constants $< 10^{10}$, albumin having a formation constant of only $\sim 10^{6.5}$ while the small organic compounds have higher formation constants. A 5 μM concentration of "loosely" bound Zn is consistent with K_m values for Zn uptake transporters in the plasma membrane (Zip1 and Zip2) of ~5 μM [9]. Although normal concentrations of free Zn^{2+} in cytoplasm as well as extracellular fluid are thought to be subnanomolar [3,10] the application of low micromolar concentrations of Zn^{2+} to cultured cells is functionally similar to normal total extracellular fluid Zn concentrations and is generally well tolerated by the cells. Higher Zn^{2+} concentrations are often encountered in toxicologic studies and enhancement of cellular penetration by addition of Zn ionophores such as pyrithione causes Zn toxicity at relatively low extracellular Zn^{2+} levels. It seems clear that Zn^{2+} toxicity generally requires entry of Zn ions into the cell at a rate that precludes effective buffering of Zn ions by intracellular proteins such as metallothionein and/or sequestration of Zn^{2+} into intracellular vesicles and organelles. This view of Zn^{2+} transfers does not however preclude the physiologically localized release of intracellular Zn ions from Zn-binding proteins (e.g., following oxidation or nitrosation of cysteinyl residues) which can inactivate critical enzymes with high affinity for Zn^{2+} such as receptor protein tyrosine phosphatases [11].

Zinc in physiology

Proteins involved in Zn^{2+} transport and buffering are well represented in biological systems and in the genome. In terms of transport two well-conserved families of transmembrane Zn channel proteins are present in metazoa, solute-linked carrier (SLC) 39A (Zip family) and SLC30A (ZnT family). There are 14 Zip and 10 ZnT proteins in the human genome. Zip proteins facilitate Zn^{2+} entry into the cytosol, either from extracellular fluid or from intracellular organelles. ZnT transporters on the other hand remove Zn^{2+} from the cytosol, either by extrusion across the plasma membrane (ZnT1) or by transfer into intracellular organelles. ZnT proteins, usually as homodimers, function as specific Zn^{2+}/H^+ exchangers [12–15]. Electroneutral symport (cotransport) of Zn^{2+} with two HCO_3^- ions has been established for Zip8 and Zip14 [16–18]. Zip8, which is prominent in lung, also transports nonphysiologic toxic divalent metals such as Cd^{2+} [19]. Zip8 and Zip14 are also transporters of Fe^{2+} and other divalent metals of physiologic significance [20–22]. The constitutive expression of specific members of the Zip and ZnT families in different cell types varies widely. Furthermore, their expression may be modified by depletion/abundance of Zn and also by effects of mediators (e.g., cytokines) acting on their membrane receptors.

The metallothioneins (MT) are a family of proteins of small cysteine-rich proteins capable of binding up to 7 Zn atoms per MT molecule. MT1 and MT2 expression is stimulated by increased abundance of Zn via Zn^{2+} activation of the MTF1 (metal-responsive transcription factor 1) transcriptional regulator. MT-3 is limited to the central nervous system (CNS) and is expressed constitutively [6,23–25]. Zn^{2+} plays an important and specialized role in CNS function and pathology [26–28].

Certain Ca^{2+} channels may allow Zn^{2+} transport in some circumstances. Intracellular Zn^{2+} activates the transient receptor potential (TRP) with ankyrin repeats (TRPA1) [29,30] that also allows uptake of extracellular Zn^{2+} as well as Ca^{2+} [30,31]. The proton-coupled divalent metal ion transporter (DMT1, also known as NRAMP2 and SLC11A2) would be a possible candidate for Zn^{2+} transport out of lysosomes (where it is an important Fe^{2+} transporter [32]), but this has not been supported experimentally [33]. Nonphysiologic Zn^{2+} ionophores such as pyrithione and clioquinol are important and useful chemicals. They greatly enhance Zn^{2+} entry from extracellular fluid and therefore enhance Zn^{2+} toxicity [34–36].

The physiologic role of Zn in airways epithelia has been of particular interest to several investigators. Daren Knoell's group has demonstrated the importance of Zip8 transport of Zn^{2+} in epithelial cell resistance to TNFalpha-induced apoptosis [37–39], and also the role of Zip8 in Cd^{2+} toxicity [19]. A possible mechanism for resistance to TNFalpha-induced apoptosis may involve Zn-induced inhibition of the PTEN lipid phosphatase resulting in enhancement of the PI3kinase-Akt pathway [40,41], as well as by activation of NFkB. Peter Zalewski's group showed that apical cytoplasm of ciliated cells stains intensely with the fluorescent "labile Zn" indicator, Zinquin, in a vesicular pattern along with ZnT4 protein, suggesting that ZnT4 plays a role in sequestering excess cytosolic Zn^{2+} in zincosomes. Labile Zn and ZnT4 protein in the basolateral aspect of the bronchial epithelium were decreased in a mouse model of allergic inflammation [42–44]. Zn levels in sputum were found to be decreased in asthma [45] but increased in patients with cystic fibrosis (CF) and non-CF bronchiectasis [46].