Zinc and Health

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Zinc is a redox-inert trace element that is second only to iron in abundance in biological systems. In cells, zinc is typically buffered and bound to metalloproteins, but it may also exist in a labile or chelatable (free ion) form. Zinc plays a critical role in prokaryotes and eukaryotes, ranging from structural to catalytic to replication to demise.

metalloproteins zinc transporters metal chelators antibiotic resistance antimicrobials

1. Introduction

Zinc, an essential component of life in the three domains, follows iron as the second most abundant transition metal ion in living organisms [1][2]. About 5–6% and 9–10% of proteins from prokaryotes and eukaryotes, respectively, depend on this metal to fulfill their biological functions [1]. A bioinformatics study found that over 50% of zinc-bound proteins are enzymes, and in the vast majority of them, the metal plays a catalytic role [3]. About 20% of them use zinc as a structural component, and in a small percentage, it is a regulator or substrate of enzymatic activity [3][4][5]. The requirement of zinc in such a high number of proteins illustrates its fundamental role in numerous biological processes [6][7][8][9][10][11]. The essential nature of zinc for cellular viability, together with the toxic nature of the element in higher concentrations, led prokaryotes [12] and eukaryotes [13] to evolve export and import systems to keep ionic homeostasis.

2. Prokaryotes

2.1. Zinc as Antimicrobial

Zinc is a member of the group of metals that participate in the nonspecific mechanisms of defense against infection [14][15]. The host defenses reduce trace elements' availability to starve the infecting bacterial cells in a response known as "nutritional immunity", a term coined in the mid-70s [16]. The first hint to this defense strategy's existence occurred in the mid-40s, when the high-affinity iron-binding transferrin was discovered [17][18]. Immediately after the invasion of infecting bacteria, the body responds, reducing free iron levels in the blood and tissue (hypoferremic response) [19][20]. Posterior studies have shown that nutritional immunity is a strategy that is not limited to iron sequestration and also includes restricting the availability of other essential elements, including zinc [21][22][23][24][25]. In the face of these nutritional limitations, microorganisms evolved stratagems to scavenge sufficient quantities of trace elements necessary to support their metabolism and growth. Zinc is a component of nutritional immunity; in human serum, which contains 0.1% of the total body zinc, about 98% is bound to proteins, mainly albumin (80–85%) and alpha-2-macroglobulin (5–15%), and in marginal quantities to other proteins [26][27][28]. Additionally, zinc is

further restricted to pathogens in ongoing infections by releasing calprotectin, a protein that sequesters this metal and creates zinc-limited microenvironments [29][30][31][32]. Calprotectin, a heterodimer formed by the S100A8 and S100A9 proteins, also binds manganese and iron [31][33][34], and it has a proven effect against infection [23][33][34][35][36][37][38]. Other proteins, like the S100 family calgranulin C (S100A12) and psoriasin (S100A7), have also been shown to be able to bind zinc and could contribute to nutritional immunity response [39][40]. As zinc is a component of nutritional immunity, bacteria need to sense the intracellular concentrations and put in motion the different mechanisms involved in this element's homeostasis. Interestingly, while essential to support growth, zinc is also known to inhibit the progress of infectious processes caused by bacteria [41][42] and viruses, including SARS-CoV-2, the causative agent of COVID-19 [43][44][45][46]. Zinc also inhibits SOS-induced antibiotic resistance and horizontal transfer of antibiotic resistance genes in enteric bacteria [47][48]. Another utilization of zinc as defense by the human host is through macrophages, which use it within the phagolysosome to intoxicate invading bacterial cells [49][50][51][52][53]. A common mechanism by which zinc in excess is toxic to bacterial cells is by binding to noncognate proteins [52][54][55][56].

The effect of zinc in the progress of infection was also investigated utilizing zinc-deficient murine models [45][57][58]. Furthermore, zinc-deficient mice were found to be suitable models for more general studies of infections caused by enterotoxigenic *Escherichia coli*, *Shigella flexneri*, and *Campylobacter jejuni* and potential treatments and immunization [45][57][58].

While zinc uptake is an essential process for bacterial pathogens to cause disease, this element can also be detrimental to some infections. Since long ago, zinc has been used as treatment and prophylaxis of diarrheal diseases [59][60][61]. It was originally thought that the beneficial effect of zinc in treating enteropathogenic *E. coli* (EPEC)-produced diarrhea was entirely caused by enhancement of the immune response and inhibition of ecto-5'-nucleotidase, an enzyme that catalyzes the conversion of the 5'-AMP to the secretagogue adenosine [57][58]. However, this initial idea proved to be insufficient to explain the therapeutic effects observed [57][58][62]. Addition of zinc acetate at sublethal concentrations caused a decrease in the expression of various virulence factors [62]. The mRNA species corresponding to the *bfp* gene (bindle forming protein) and various *esp* genes (EPEC-secreted proteins) were expressed at reduced levels. Furthermore, zinc acetate lowered the bacterial cells' adherence, inhibited secretion of infection-induced fluids into ileal loops, and reduced histopathological damage in an animal model of infection [62]. Zinc acetate also had effects on the virulence of Shiga-toxigenic *E. coli* (STEC) and enteroaggregative *E. coli* (EAEC) [44][45][63]. It inhibited STEC adherence to cultured cells, expression of enterohemorrhagic *E. coli* (EHEC)-secreted protein A (EspA) and Shiga toxin. In vivo, it reduced fluid secretion and toxin levels in the loops and reduced STEC-induced histological damage [64]. In several forms like oxide, sulfate, and acetate, zinc was also used to test its effects on EAEC [42]. A decrease was observed in biofilm formation, cell adhesion, and expression of other potential or confirmed virulence factors [42]. The observation that zinc reduced the expression of *recA* suggests that inhibition of the SOS response may be one mechanism by which zinc acts on *E. coli* virulence [41]. This finding also prompted other studies to test if zinc could also reduce SOS-induced hypermutation response to antibiotics or horizontal transfer of resistance traits [48][65][66][67]. Zinc blocked the SOS-induced (hypermutation response) development of resistance in *E. coli, Klebsiella pneumoniae*, and *Enterobacter cloacae,* probably by inhibiting RecA binding to single-stranded DNA [47]. Zinc also interfered with horizontal

transfer of a β-lactamase gene from *Enterobacter* to *E. coli* strain [47]. As observed with other zinc effects [63][68], the complex zinc ionophore showed significantly higher activity than zinc salts in inhibition antibiotic-induced hypermutation [47].

Environmental enteropathy, a small intestinal disorder caused by subclinical intestinal infections, produces chronic low-grade intestinal inflammation and dysregulation of tight junctions [69][70]. A study on adults with environmental enteropathy showed disruptions that cause leakage in the patients' epithelial barrier [69]. The authors hypothesized that the sites with epithelial defects could be responsible for bacterial translocation. In vitro experiments utilizing enteropathogenic *E. coli* and *Citrobacter rodentium* showed that these bacteria induced barrier dysfunction, and treatment with numerous compounds like zinc, epidermal growth factor, colostrum, trefoil factor 3, resistin-like molecule-β, hydrocortisone, and ML7 (an inhibitor of the myosin light chain kinase) increased transepithelial resistance while reducing bacterial translocation [71]. This effect was nutrient-independent, and, of all the tested compounds, only zinc exhibited an antimicrobial activity [71].

2.2. Zinc Oxide Nanoparticles as Antibacterial

Zinc ions at concentrations higher than those needed for the cells' normal physiology are detrimental [12]. Multiple effects of excess zinc concentrations lead to bacterial cell death [12][52]. Interestingly, zinc ions at sublethal concentrations inhibit biofilm formation but do not disrupt preformed biofilms in *Actinobacillus pleuropneumoniae*, *Salmonella typhimurium*, *Haemophilus parasuis*, and at a lesser level, *E. coli*, *Staphylococcus aureus,* and *Streptococcus suis* [72]. Zinc oxide is the most common, but not the only, zinc compound used as an antibacterial [73][74], and it has attracted great interest in nanoparticle form [75][76]. Zinc oxide nanoparticles are being researched for applications not only against infections but also as drug delivery tools and as therapies for a variety of conditions [75]. The enthusiasm for nanoparticles' uses as antibacterial agents is partly fueled by their particular mechanisms of action that differ from those utilized by currently used antibiotics and have targets reducing the frequency of appearance of resistant strains [77][78]. Various research teams have tested the activity of zinc oxide nanoparticles as antibacterials against numerous species [75][76][79][80][81][82][83][84]. The mechanism by which zinc is toxic to bacterial cells is ultimately that of other forms of the ion; however, zinc oxide seems to be more effective when it is administered in nanoparticle form [80][81][85]. Zinc oxide nanoparticles release zinc in an aqueous medium, which then penetrates the cells and produces toxic effects [75]. Zinc oxide nanoparticles are robust candidates to be developed as standalone antimicrobials or as components in combination therapies against multiresistant bacterial infections.

3. Eukaryotes

3.1. Importance of Zinc in Eukaryotic Cells

The effectiveness of antibiotics against bacteria exemplifies the importance of these drugs against many human infections. Unfortunately, the same drugs that kill bacteria have similar effects on the mitochondria present within eukaryotic cells [86]. The synergistic effect of zinc with antibiotics may also be the reason why zinc itself is toxic to

eukaryotic cells at high intracellular levels because zinc enters the mitochondria and enhances production of reactive oxygen species (ROS) [87][88].

In eukaryotic cells, zinc exists in labile or free ion form (chelatable) [89], while zinc bound to proteins serve in a structural or catalytic capacity [89][90][91]. Zinc is a small ion (~0.65 angstrom) that binds nitrogen- and sulfur-containing molecules and readily exchanges ligands due to its low ligand field stabilization energy. Intracellular zinc levels range from 10^{-12} to 10^{-9} M in most cells, but zinc-enriched cells such as neurons, hepatocytes, splenocytes, and thymocytes may contain an estimated 10^{-6} to 10^{-5} M amount [92][93][94][95]. Zinc concentrations range from 10^{-9} M within the cytoplasm in most cells to 10^{-3} M in some vesicles [96]. In transgenic baby hamster kidney cells that express zinc influx transporter proteins, Palmiter and colleagues (1996) estimated that vesicular zinc concentrations reach 14 µM when these cells are exposed to high levels of exogenous zinc [97]. Cell survival in vitro is compromised when cells are exposed to extracellular zinc concentrations ($[Zn^{2+}]e$) between 225–1000 µM in neuronal cells and 7.5–200 µM in non-neuronal cells [98][99][100][101]. It is therefore essential that cells regulate their intracellular zinc concentrations through protein influxers and effluxers as well as physiological chelation by apo-thionein or other zinc-sequestering apoproteins [97][102][103][104][105].

3.2. Zinc-Rich Cells

Zinc-rich cells in mammals are found in various tissue and organs, particularly in the brain, mammary gland, intestine, pancreas, thymus, prostate gland, testes, and ovaries [89][106][107][108]. In the brain, high levels of chelatable or labile zinc is synaptically co-released with glutamate during normal neuronal communication [89], with ionic levels reaching 100–300 µM, particularly during a seizure activity [109][110]. Intracellular zinc is typically buffered but is exocytosed from neurons or secretory cells that release vesicles or granules, respectively. Zinc release in the hippocampal mossy fiber terminals is calcium-dependent whether it was evoked via potassium or kainic acid administration and spontaneous activity [109][111][112][113]. Intracellular zinc elevation may occur due to high exogenous zinc levels [114][115][116][117][118] or caused by cytoplasmic zinc release from compartments or proteins due to oxidation by ROS or nitrosylation by reactive nitrogen species [119][120][121]. Zinc overload kills neurons, and thus it is imperative that cells tightly regulate intracellular zinc concentration via zinc transporters and buffering of zinc-binding amino acids or proteins.

3.3. Zinc Transporters

High- and low-affinity uptake mechanisms for zinc have been identified with dissociation constants (Kd) of 15 and 361 µM, respectively [109]. An even higher binding affinity constant (Ka) of 0.25 µM has been reported for zinc, which is saturable, ATP-independent, and unaffected by Na^+ concentration gradient [122]. Indeed, zinc levels are strictly maintained by tissue-specific and highly conserved low molecular weight transport protein families known as the ZnTs (also known as SLC30 for solute carrier 30) and ZIPs (also known as SLC39 for solute carrier 39) [123][124][125]. Early studies in the field led to the discovery of mammalian ZnTs involved in the extrusion of zinc out of cells named ZnT1 [102] and sequestration of zinc into compartments called ZnT2 [97]. ZnT1 is mainly localized in the plasma membrane [102]. Meanwhile, ZnT2 is localized within vesicular (acidic) compartments, such as lysosomes,

but has a low affinity for zinc [97]. Another effluxer termed ZnT3 was cloned and identified to localize within synaptic vesicles of zinc-rich neurons [104]. ZnT3 is expressed in the mammalian brain, such as in the cerebral cortex and the hippocampus, and strongly detected in the dentate granule cells. Over the course of time, other members of the ZnT effluxers (ZnT4–ZnT10) and ZIP influxers (ZIP1–ZIP14) were identified through sequence similarity analyses, cloning, and functional experimentations [110][125]. More recently, transmembrane 163 protein (TMEM163; also known as SV31) [126][127] was functionally characterized as a dimeric protein that effluxes zinc [128], and one of us proposed that TMEM163 be now classified as ZnT11 as a new member of the ZnT efflux family of proteins [128]. One commonality among ZIPs and ZnTs is that histidine (H) and/or aspartic acid (D) residues, such as the HXXXD motif (where X is a nonpolar amino acid) typically located within transmembrane domain (TMD)-4 and TMD5 helices of ZIPs, as well as HXXXH motif found in TMD2 and TM5 helices of ZnTs have been shown to be responsible for tetrahedral zinc coordination [110][129]. For a relevant review on certain zinc transporters, we refer the reader to the paper by Styrpejko and Cuajungco (2021) as part of this Special Issue.

In addition to transporters, intracellular buffers offer a secondary defense mechanism to prevent intracellular zinc overload, such as the metallothioneins (MTs)—a group of low molecular weight (~6–7 kDa), single polypeptide chains with four functional mammalian isoforms (MT1−MT4) [106][130]. MTs, however, are not a long-term storage for zinc due to its short biological half-life [131]. Thus, vesicular or compartmental storage mediated by ZnTs and ZIPs provide important contributions to zinc homeostasis.

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