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The Significance of Zinc in Antioxidant Processes

by

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Abbreviations Used

EPR	Electron paramagnetic resonance
LH	Lipid
$L^{\cdot}$	Lipid radical
$LO^{\cdot}$	Lipid alkoxyl radical
$LOO^{\cdot}$	Lipid peroxyl radical
LOOH	Lipid hydroperoxide
MT	Metallothionein
NADP	Nicotinamide adenine dinucleotide phosphate
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances

Outline

Abstract

Various trace elements have very important roles in living organisms. Zinc is one such element. It is a non-redox active element with remarkable chemical properties that allow it to coordinate with proteins and other biological molecules. It is essential for the function of many enzymes and research has shown that it contributes to antioxidant processes. While it has been demonstrated that excessive amounts of zinc have destructive effects on tissues, zinc deficiency has been connected with oxidative damage in a number of disease states. This paper will examine the chemistry and biochemistry of zinc; especially its antioxidant properties.

Introduction

Zinc is a metal well known for its importance in anti-rust applications and as a nutrient for the human body. It has been used for over two thousand years in brass production; however, zinc was not recognized as an element until the early 19th century[1]. Biologically, zinc is very important in antioxidant processes, working as a binding metal in some antioxidant enzymes and blocking the binding of potentially harmful metals to other proteins and lipids. This work will discuss the chemistry of zinc, (especially its significance as an antioxidant), some of the biological effects of zinc deficiency, and several forms of detecting zinc.

Chemistry

Zinc is an element with atomic number 30 and a molecular weight of 65.37. Its natural state is a positive ion with an oxidation state of 2+. Zinc is found in the earth's crust and natural waters as zinc oxide (ZnO) and zinc sulfide (ZnS). Other common forms of zinc include zinc carbonate ($ZnCO_3$), zinc sulfate ($ZnSO_4$), and zinc chloride ($ZnCl_2$). Zinc is often used for its anticorrosive properties. Nails and other iron products are often coated with a thin film of zinc in a process called galvanization to prevent rust[1].

Zinc is a Group IIb element and has two electrons in its outer shell. Although zinc is located in the d-block of the period table amongst the transition metals, it has peculiar behavior. It does not participate in redox reactions like its neighbor copper or the very redox active iron. Zinc is positioned on a boundary of the periodic table, on the cusp of the transition metals and covalent-bonding elements. It usually forms covalent bonds with other molecules and has the ability to react as both an acid and a base [1,2]. All transition metals in their ground states have partially filled d-orbitals. Zinc has an electronic configuration of [Ar]$4s^2 3d^{10}$. The two valence electrons of the outer *4s* orbital are often lost to leave zinc with a 2+ charge and a full d-orbital which will not

[1] Web Elements Periodic Table. "Zinc." http://www.webelements.com/webelements/elements/text/Zn/key.html. Accessed 11.Feb.2005.

react with other molecules. The Zn^{2+} ion is very stable, not wanting to release or gain any electrons[2]. Zinc's relative inactivity, as compared with other transition metals, is one reason why it is so important biologically and can function as an antioxidant.

Biological considerations

Zinc is an essential nutrient for plants, animals and microorganisms [11]. It is necessary for the correct function of nearly 300 enzymes [3]. The average human being has about three grams of zinc in their body nearly 3% of the proteins encoded by the genomes of living things contain zinc fingers [4]. Free zinc ions are not found at large concentrations in human cells; rather, zinc is kept bound by a several proteins including α_2-macroglobulin, albumin and metallothionein [4,5].

The average human being consumes 5-16 milligrams of zinc every day in the form of vegetables and lean meats [2,5]. Normal zinc levels in humans range from 10-20 μmol L^{-1}. Zinc compounds can also be poisonous to humans in high concentrations [6]. Acute exposure to 77 mg/m^3 of zinc oxide for up to thirty minutes can result in reduced pulmonary function while prolonged exposure to 3.9 mg/m^3 for two hours a day can cause sore throats [2]. Zinc deficiency occurs when serum zinc concentrations fall below 8 μmol L^{-1} and the resulting syndrome is marked by diarrhea, hair loss, dermatitis, and mental changes [5].

Biochemistry

The antioxidant activity of zinc can differ depending upon the amount of time that zinc is administered in an organism. Acute exposure (an increase in zinc concentration over a short period of time) or through chronic exposure (continual elevation of zinc concentration over an extended period of time) has different effects on antioxidant activity [10,11].

[2] "Transition Metal". Wikipedia Online Encyclopedia. http://en.wikipedia.org/wiki/Transition_metal Accessed 23.2.2005

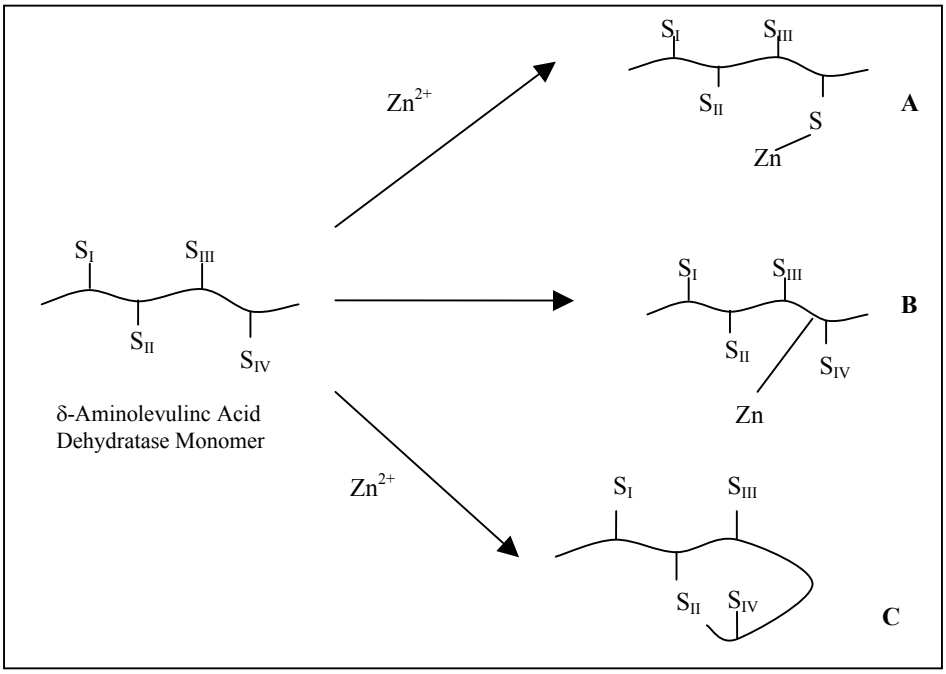

Figure 1. Zinc protects an enzyme from oxidation in three ways: A) by binding directly to Thiol IV, **B)** by sterically hindering Thiol IV, **C)** or by changing the protein conformation. Adapted from [7].

The acute antioxidant effects of zinc can be seen in two ways: sulfhydryl stabilization and blocking redox-active transition metals. Sulfhydryls (also known as thiol groups or -SH) are present on many enzymes and proteins as they are the functional groups on the amino acid cysteine. According to Gibbs *et al.* [7], zinc prevents the oxidation of these proteins in three ways: 1) directly binding to the sulfhydryl group, 2) creating a steric hindrance by binding to a neighboring protein site and 3) changing the conformation of a given protein by binding to another site on the protein. For example, the enzyme δ-aminolevulinate dehydratase (EC 4.2.1.24) catalyzes the dimerization of δ-aminolevulinic acid and this activity is strongly dependent on the sulfhydryl groups. The three methods of sulfhydryl stabilization of this enzyme are shown in **Figure 1**. There are many other enzymes that are dependent on sulfhydryl groups for their activity including DNA zinc fingers[8] and tubulin[9].

Another acute antioxidant effect of zinc is the reduction in the amount of reactive oxygen species (ROS) produced by redox-active transition metal catalysts. Copper and iron are two

transition metals that have been found to aid oxidative damage in cellular lipids and proteins [10]. Both of these metals help to catalyze the Fenton and Haber-Weiss reactions by donating electrons that can react with lipids to create ROS (**Figure 3**). Zinc could play an important role as an antioxidant, by replacing those redox metals and preventing the following reactions in **Figure 3** from occurring [10, 11].

$$LOOH + Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$$

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+}$$

$$^1O_2 + LH \rightarrow LOOH \xrightarrow{Fe^{2+}} LO^{\bullet}$$

Figure 2. Several reactions involving transition metals result in the production of reactive oxygen species. Copper (Cu^{2+}) can also donate electrons in this same manner to catalyze similar reactions. Zinc has the potential to replace these redox active metal ions to give a benign result [10].

As shown in **Figure 3**, zinc could stop the cycle of site-specific protein oxidation. Copper and iron are abundant in cells, however, they are usually only found bound to proteins, lipids and DNA at biological conditions. Since these ions are bound to larger biological molecules, they are not free to roam and catalyze reactions. Instead, the metals participate in what is known as site-specific radical formation [11] where proteins are damaged. Zinc demonstrates the ability to stop these site-specific reactions in copper and iron catalyzed DNA damage [12] and ROS production by xanthine oxidase and NADPH oxidase [13]. A mechanism has been proposed to explain zinc's inhibitory effects on the site-specific reactions (**Figure 3)**. This mechanism is known as the "push vs. pull" which in effect says that zinc is able to displace the redox-active metals that are bound to proteins, allowing them to precipitate out of solution and get pumped outside of the cell [10,11,14].

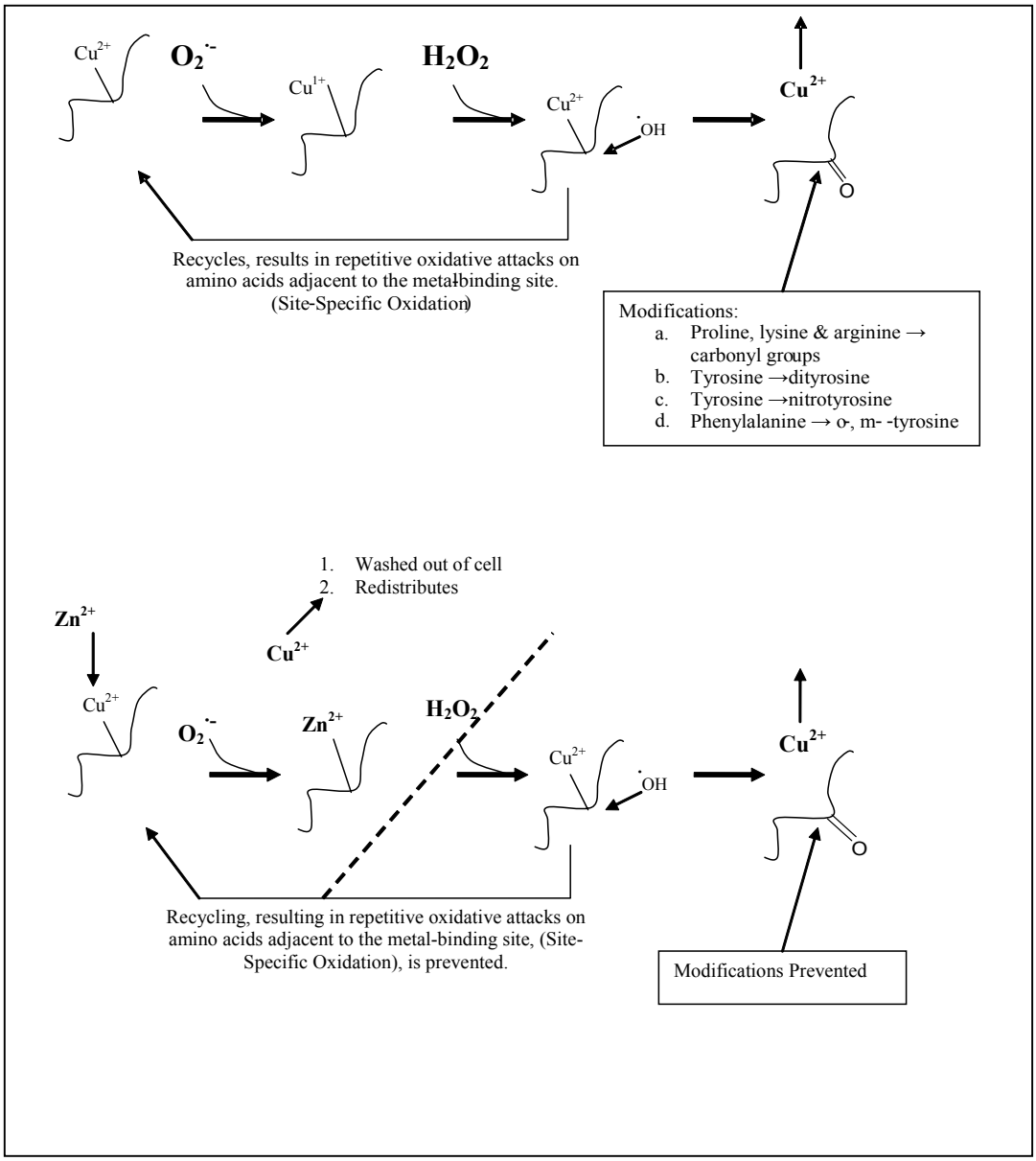

Figure 3. Site -specific protein oxidation is catalyzed by copper (A) and can be inhibited by zinc (B) [10].

Chronic antioxidant effects of zinc exposure are seen through enzymatic and other protein function. Many proteins and enzymes require zinc for proper folding and function such as copper- and zinc- containing superoxide dismutase (CuZnSOD) which is also known as SOD1. Zinc and copper are required at the catalytic site of CuZnSOD (EC 1.15.1.1) [15]. Superoxide dismutases are antioxidant enzymes that catalyze the reaction that removes superoxides as seen in **Reaction 1** below. The CuZnSOD molecule is a dimer with two active sites. Each active site contains a copper

and a zinc cation and they are bridged by an imidazolate. The coordination environment of zinc in this enzyme is an irregular tetrahedron. The ligands surrounding Zn(II) are two histidine imidazoles, one aspartate carboxyl group and the imidazolate [5].

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2 \qquad \textbf{(1)}$$

It has been established that most of the zinc present in cells is found in metalloproteins and metalloenzymes like metallothionein (MT). There are at least 17 human MT proteins. They are stable (with a rate constant, k=10^{-3} mol L^{-1}), have low molecular weights, and they bind seven zinc atoms [4]. Many scientists believe that MT's main purpose is to store and regulate zinc. **Figure 4** shows how MT helps the body maintain constant levels of zinc by releasing zinc in areas of low zinc concentration or when MT is oxidized, becoming Thionein. Thionein can bind zinc again when concentrations increase [3]. The MT proteins have 60-68 amino acid residues and twenty of these residues are cysteine. Zinc's ability to coordinate with the sulfhydryl groups of δ-aminolevulinate dehydratase was discussed previously. In the same manner, zinc helps to protect the twenty cysteine residues of MT from strong oxidants. Once again, zinc indirectly protects biological molecules from oxidative damage.

Figure 4. The binding and release of zinc ions by metallothionein provides regulation of available zinc [3].

Detection

Zinc levels in human plasma can be detected using inductively-coupled plasma absorption emission spectroscopy [16]. TBARS analysis can detect relative amounts of lipid hydroperoxidation products in order to quantify the influence zinc ions have on oxidative stress in

cells. CuZnSOD levels can be measured using a nitrobluetetrazolium chloride (NBT) assay [17, 18]. dimethylsulfoxide (DMSO) a source of superoxide radical and (NBT) as superoxide scavenger. Electron paramagnetic resonance (EPR) has been used to detect synthetic Cu(II)Zn(II) metal complexes which are being studied as a model for CuZnSOD activity [18].

Recently, fluorescent methods have been refined in such a way that free zinc(II) can be detected on the picomolar scale. Thompson *et al.* developed a method to exploit the highly selective human enzyme apocarbonic anhydrase II (CA). Zinc is the metal at the active site of CA and the fluorescent intensity is measured at 365 nm by fluorescent microscopy [19].

Conclusion

Zinc carries out a number of tasks in antioxidant processes. It does not exhibit redox activity, so when it competes with transition metals for binding sites on proteins, it is preventing oxidative damage. Zinc is also responsible for protecting sulfhydryl groups on cysteine residues of proteins by steric hindrance, and coordinating with the sulfhydryl group. Some antioxidants rely on zinc for deficiency can exacerbate existing diseases. Finally, metalloproteins complexed with zinc are sources of electrons for oxidizing molecules. Zinc's chemistry, or lack of reduction-oxidation chemistry, is what allows it to be so versatile in its antioxidant processes.

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