This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

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ZINC:

FROM MARCO POLO TO TRANSCRIPTIONAL CONTROL

by

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For: 77: 222, Spring 2001

22. February 2001

Abbreviations:

ACTH- adrenocorticotropin, Cu- copper, GSH- glutathione (reduced), GSH-Px- glutathione peroxidase, GSSG- glutathione (oxidized), MSH- metallothionein, SOD- superoxide dismutase

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1. Abstract

Zinc –"the eighth metal"- is an element with a fascinating history encompassing many centuries and several continents. Due to a variety of ligands and complexes, zinc plays important structural and catalytic roles in biological chemistry and genetics. Zinc does not promote free radical reactions; on the contrary, it acts as a free radical scavenger and as an antioxidant in chemical systems via two possible mechanisms. Indeed, dietary Zn deficiency seemed to cause pathology that was attributable to nonspecific/peroxidative damage to tissue, apparently compromising not the primary antioxidant system, but the components of the secondary antioxidant system in various tissues.

2. Introduction

Zinc is one of the less common elements. It has been estimated to be present in the earth's crust to the extent of 0.004 % and is twenty-fifth in order of abundance of the elements [1].

The existence of zinc as an uncombined element in nature is doubtful. Zinc usually occurs in combination with sulfur or oxygen [1]. Metallic zinc was not known as early as many of the other metals, but its use as an alloy with copper to make brass antedates the earliest records of civilization. It is believed that the Romans first made brass in the time of Augustus (20 B.C. to 14 A.D.). The production of metallic zinc by indirectly heating calamine was described in the Hindu book Rasarnava, written around 1200 A.D. By 1374,the Hindus had recognized that zinc was a new metal, the eighth known at that time [2]. The account of Marco Polo's journey on the Silk Road (1272-1292) proves that the Chinese were equally advanced in the craft of making brass as well as metallic zinc. Marco Polo also described the manufacture of zinc oxide in Persia and how the Persians prepared *tutia* (a solution of zinc vitriol) for healing sore eyes [2]. Paracelsus (1493-1541) was the first European to state clearly that "zincum" was a new metal and that it had properties distinct from other known metals [2]. In 1620, the Dutch captured a Portuguese vessel with a cargo of zinc from the East Indies. The metal was sold as *spialter* from which comes the industrial name *spelter*, now applied to less pure zinc [1]. An Englishman named Isaac Lawton is said to have gone to China expressly in order to learn the method of zinc refining. Having acquired this secret, he returned to England around 1740 [2]; following this trip, a zinc smelter was erected in Bristol in 1743 [1]. In 1742, the Swedish chemist Anton von Schwab distilled zinc from calamine and, two years later, prepared it from blende. Since the vapors rose to the top of the alembic before passing into the receiver, this process was called

distillation *per ascendum*. In 1746, the German Andreas Marggraf reduced calamine from Poland, England and Hungary in closed retorts and obtained metallic zinc from all of them. He described his method in detail, therefore establishing the basic theory of zinc production. In the United States, zinc was first produced in 1838 in the Washington, D.C. arsenal [1]. The U.S. is currently the largest producer of zinc in the world. The word "zinc" may come from the Persian word "sing", meaning "stone" [2].

3. Physical and chemical properties of zinc

Zinc is a blue-white metal of moderate ductility, strength, and hardness [3]. Zinc has an atomic number of 30, an atomic weight of 65.38, a boiling point of 906 ^{o} C and a melting point of 419^{0}C. The effective radius of the bivalent ion is 0.74 A [4].

The electronic configuration of Zn^{2+} is shown in Figure 1:

Of the many chemical and biochemical properties of zinc, this paper will focus only on those that are significant in terms of free radical involvement and related issues.

4. The evolutionary and genetic aspects of zinc biochemistry

Zinc could have been incorporated to some small degree in very primitive biological systems; the living forms most closely related to early anaerobic life- prokaryotes and archaebacteria-

contain less copper and zinc than advanced eukaryotic and multicellular organisms. Subsequently, the change in the composition of the air (*i.e.*, the advent of dioxygen and the subsequent removal of H_2S some 1-2 billion years ago) dramatically increased copper and zinc availability, since evolution follows environmental change. The increase in zinc concentration allowed it to become the strongest intracellular Lewis acid. Zinc is not a redox threat to DNA; it is present in many sulfur/nitrogen centers in the cells of organisms developed after the appearance of dioxygen. Zinc is a strong Lewis acid and that makes it extremely valuable as a catalytic element, as well as in structural/regulatory functions. Its role is mainly in hydrolytic reactions of peptide and ester bonds, but it is also important in RNA synthesis and reverse transcriptase, *i.e.* in synthetic pathways, and in redox two-electron reactions of some NADH dependent enzymes. Zinc also has a role in the generation from precursor proteins of hormonal peptides (*i.e.*, ACTH and encephalins), their destruction by extracellular enzymes and in the degradation of connective tissue polymers, *e.g.* collagen. These features, all associated with signaling and growth of multicellular systems, came after the advent of dioygen. The connection with the breakdown of connective tissue in extracellular enzymes, stabilized by S-S links, led to the theory that the roles of S, Cu and Zn evolved to give a system for the mend (Cu) and cut (Zn) operations within connective tissue necessary for the controlled building of relatively rigid multicellular systems. It may well be that through metallothionein, an intracellular thiolate-rich protein, there is a joint homeostatic balance of both Zn and Cu in which lies the secret of the connective tissue and the evolution of multicellular organisms [5].

Parallel with this development in evolution, zinc became a very powerful part of regulatory genes at the transcription level, "zinc fingers" and similar proteins, whereas iron switched to translational control. Zinc also became a stabilizing cross-linker for intracellular proteins, *e.g.*

transcarbamylase. Zinc is not only associated with the synthesis and degradation of peptide hormones but also with hormone receptors that bind to DNA. The hormones concerned are the sterols, retinoic acid, thyroxines *etc.* Most of these hormones are secondary metabolites produced by iron enzymes in the cells. These systems are absent from prokaryotes. It was proposed that direct control of growth processes *via* free iron interacting with DNA in prokaryotes was replaced at a certain moment in evolution by hormonal control *via* hormones produced by iron,which interact with DNA via zinc receptors. Thus, the cell is perceived to be in a homeostatic condition which links many elements together. The connections between Zn, Cu, hormones and connective tissue are shown in Figure 2 (from [5]); the connection from calcium is *via* the vitamin D hormones produced by iron enzymes and interacting with zinc finger receptors:

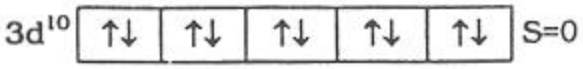

Figure 1. Electronic Configuration of Zn^{2+}. S is the net spin. From [4].

Figure 2. The connections between zinc, copper, hormones and connective tissue.

In the active sites of hydrolytic enzymes, zinc can activate water and smaller substrates such as carbonate, as in carbonic anhydrase [5]. Its main role in enzymology, however, is that of an electrophilic catalyst: that is, it stabilizes negative charges encountered during an enzyme-catalyzed reaction, as happens in the case of Cu-Zn SOD [6].

In addition to these catalytic roles, zinc may also play a structural role in some organisms. The jaws of both the locust, *Schistocerca gregaria*, and the annelid, *Nereis virens*, are loaded with high concentrations of zinc. As a percentage of total jaw dry weight, zinc content is invariant in each species; zinc may thus confer durability to invertebrate mouthparts, perhaps providing high density bridging across organic polymers of the cuticle [7]. Generally, zinc is a

constant constituent of plasma (and serum), erythrocytes and leukocytes. There is a continual tissue deposition and turnover of zinc which varies greatly with different tissues [8].

Structural or regulatory zinc is found either as a single metal ion or as part of a cluster of two or more metals. In multinuclear clusters cysteine thiolates either bridge two metal ions or serve as terminal ligands to a single metal ion [8]. The sulfur atom is a favorable zinc ligand because of its size and polarizability. The thiol side chain of cysteine (pK_a~8.5) is negatively charged as it complexes a metal ion in a protein. The hydrogen bond interactions in cysteine thiolate are important for metal-binding site organization, as well as the folding and stabilization of zinc protein structure[8].

Hydrogen bond interactions are also important for the function of histidine as a zinc ligand. The basicity of histidine towards a metal ion such as zinc is enhanced by a hydrogen bond with a negatively charged carboxylate [8].

In CuZnSOD, the histidine anion is stabilized by bridging the copper and zinc ions. Figure 3 (from [8]) shows a carboxylate-histidine-zinc triad, found in another enzyme, carboxypeptidaseA:

Figure 3. The carboxylate-histidine-zinc triad found in carboxypeptidase A.

Zinc fingers are potential metal-binding domains; they are regions of protein containing four residues of histidine and /or cysteine that are considered to bind zinc and form a loop that can take part in protein-nucleic acid interactions [6].

6. The physiological role of zinc as an antioxidant

Zinc acts as an antioxidant in systems of purified chemicals *via* two possible mechanisms. The first mechanism is the protection of sulfhydryl groups against oxidation. The mechanisms of stabilization of sulfhydryl groups in the enzyme d-aminolevulinate dehydratase- a homooctamer containing four active sulfhydral groups in each monomer- are: 1) Zn^{2+} directly binds to sulfhydryl group I and reduces its activity; 2) Zn^{2+} is chelated in close proximity to sulfhydryl group I and reduces its activity by steric hindrance; 3) Zn^{2+} binding causes a conformational change in the reactivity of sulfhydryl group I [9]. The second mechanism by which Zn^{2+} may function as an antioxidant involves the prevention of hydroxyl radical and superoxide radical production by transition metals. Since Zn^{2+} has been shown to bind ADP and NADPH as well as to inhibit NADPH oxidation, it is probable that zinc is able to inhibit oxygen-centered free radical generating systems, Fe-ADP-ascorbate and Fe-ADP-NADPH [9].

Currently, research is pursued on the effect of zinc ions and chelates on: 1) the prevention of transfer of electrons between organic molecules, b) the stabilization of organic free radicals, and c) the termination of free radical reactions.

7. Dietary zinc, zinc deficiencies and free radical metabolism

Zinc is present in a variety of alimentary sources, but it is mainly associated with protein food, such as milk, meat, fish, eggs, nuts, and oysters. Zinc is required in the diet of vertebrates in small (trace) quantities, *e.g.* 15 mg Zn/day in humans [9]. If Zn has a critical physiological role as an antioxidant, dietary Zn deficiency should cause pathology that is attributable to nonspecific/peroxidative damage to tissue. Supplementation of vitamin E to Zn deficient animals may partially alleviate the apparent deficiency lesions.

The antioxidant effect of dietary Zn may involve the fact that Zn status of the animals affects free radical metabolism. A variety of studies have suggested there is increased free radical production in tissues or isolated membranes from Zn-deficient animals. It seemed that the absence of Zn in the diet caused alterations in the membranes, which resulted in a great increase in the potential for oxidative damage. Researchers asked themselves the question whether Zn caused an inadequacy in the capacity to detoxify the free radicals generated (primary antioxidant defense system) or an inability to repair the free-radical damaged tissue components (secondary antioxidant defense system). Experimental data did not suggest that dietary Zn deficiency was involved in compromising the overall capacity of the primary antioxidant system in any tissue; however, it was stated that Zn deficiency may affect the secondary antioxidant defense system [10]. The enzymatic and non-enzymatic components of the free radical defense system are shown in Figure 4 (from [9]):

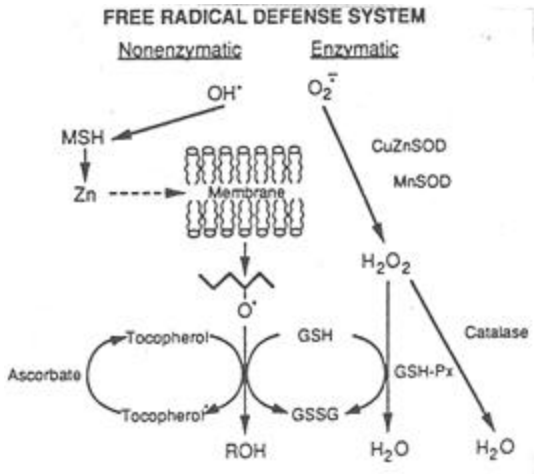

Figure 4. Enzymatic and nonenzymatic free radical defense system.

8. Summary

Zinc serves as an antioxidant in defined chemical systems and has important structural and regulatory roles that make it an element of great evolutionary and genetic significance.

9. References

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