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Is Zinc an Antioxidant ?

by

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Abstract

Zinc is critical to the protein synthesis and nucleic acid metabolism. It serves as a catalytic component of over 200 enzymes. Also, it may function as a structural component of antioxidants. Past studies indicate that dietary zinc deficiency seems to exert a negative effect on oxidative stress. The purpose of this report is to assess the relationship between zinc status and oxidative stress. Most experiments described in this report support Zn^{2+} has an antioxidant role in biochemical systems. These data may further encourage extended studies with Zn^{2+} and suggest that more antioxidants and prooxidants should be included.

Introduction

Zinc is found in every human tissue and tissue fluid with different concentrations. It is present in nuclear, mitochondrial, and supernatants of all cells. Table 1 shows approximately 90 % of zinc in the body resides in muscle and bone [1].

Table 1. Comparison of estimated zinc concentrations in some human tissues [1].

This table compares the estimated zinc concentrations of several tissues in a hypothetical 70-kg man [1].

Tissue	Zinc (μg/g wet wt)[a]	Zinc (mg/organ)	Zinc (% of total body, 70-kg man)
Adrenal	6	0.9	—
Aorta	26	2.6	0.1
Bladder	22	4.4	0.2
Blood	1	6.0	0.3
Bone	66	660.0	28.5
Brain	13	18.0	0.8
Gastrointestinal tract	21	25.2	1.1
Heart	27	8.7	0.4
Kidney	48	19.8	0.9
Liver	27	40.5	1.8
Lung	14	16.6	0.7
Muscle	48	1,420.0	62.2
Ovary	12	0.3	—
Prostate	87	1.7	0.7
Skin	6	30.0	1.0
Spleen	19	3.8	0.2
Testes	13	0.8	—
Thyroid	25	0.4	—
Whole body	33	—	—
TOTAL	—	2,259.7	98.9

Zinc is also an important constituent of red blood cells, representing approximately 10 times the amount of zinc founding serum. Leukocytes contain more zinc than erythrocytes and it contain the zinc-dependent enzymes alkaline phosphatase and peptidase. Zinc in serum is always bound to some ligands, as illustrated in Figure 1. Approximately 43 % of the zinc circulating in blood serum is bound to albumin [1].

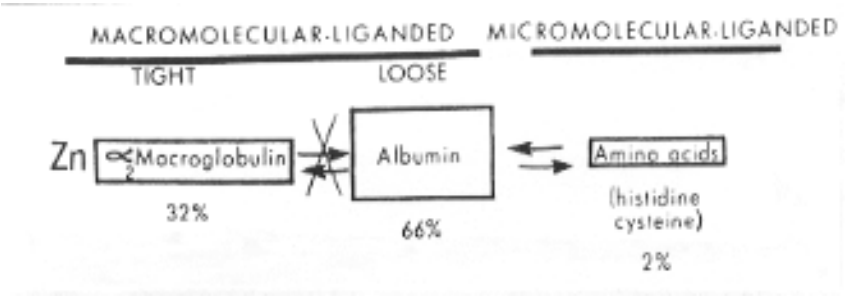

Figure 1. Binding of serum zinc [1].

The zinc-albumin complex is called the major macromolecular zinc ligand that comprises about 98 % of the circulating zinc. The albumin-bound zinc is called micromolecular zinc ligand that comprises about 2 % of the circulating zinc. This latter group of ligand is almost some form of the amino acids histidine and cysteine [1].

The purpose of this report is to describe major aspects of the biochemistry and possible antioxidant function role of zinc in humans.

Biochemical properties

Zinc participates in enzymatic pathways of metabolism through its association with enzymes. The zinc atoms in metalloenzymes are essential for catalytic activity, and in conjunction with other reactive residues of the protein, thus determine enzymatic specificity. The stability constants of a number of metallocarboxypeptidases, measured by equilibrium dialysis, follow the order $Hg^{2+} >> Cd^{2+} > Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$ and reflects the highly specific nature of the zinc binding site of the protein. The zinc atom is bound to the SH function of the sole cysteine residue and apparently to the α-amino group of the N-terminal asparagines residue of γ- or δ-carboxypeptidase [2].

A catalytic role of bound zinc in DNA polymerase is shown in Fig 2. In this mechanism, zinc coordinates with the 3'-OH primer terminus, thereby facilitating its deprotonation and preparing it for a nucleophilic attack on the α-phosphorus atom of the incoming nucleotide. The deprotonation of the 3'-OH group could be assisted by a nearby general base [3].

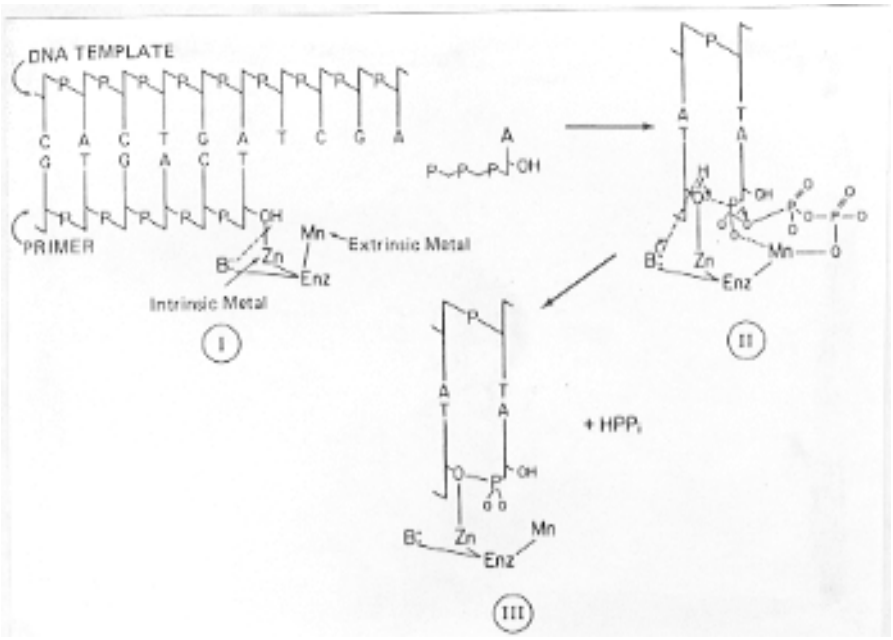

Fig 2. Proposed catalytic role of intrinsic zinc ion of DNA polymerase I from *E.coli* [3].

In RNA polymerase, the zinc content of the renatured subunits is given in Table 2. Zinc contents of subunits separated by phosphocellulose chromatography. In the native RNA polymerase, one zinc ion is located in the β' subunit while the other may be located in either β or β' subunit, or at the contact domain between these two subunits [3].

Preparations	Zinc content[a] (g-atoms/mol protein)
Holoenzyme	2.1 ± 0.2
Core enzyme	2.0 ± 0.1
σ	<0.1
α	<0.1
β	0.6 ± 0.3
β'	1.4 ± 0.5 (1.5 ± 0.3)
$\alpha_2\beta$	0.7 ± 0.3 (0.6 ± 0.2)

Table 2. Tightly bound zinc ions in RNA polymerase and its subunits [3].

Possible antioxidant functions

An antioxidant can be defined as any substance which prevents the transfer of electrons to and from molecular oxygen and organic molecules, stabilizes organic free radicals, or terminates organic free radical reactions. Zn may have an antioxidant role and two mechanisms have been proposed [4]. The first mechanism is about the protection of sulfhydryl groups against oxidation, as shown in Figure 3 and Fig 4.

The human enzyme δ-aminolevulinate dehydratase is an octamer of eight identical subunits of MW 31.000-35,000. Each monomer contains 4 reactive sulfhydryl groups. When either apoenzyme or holoenzyme was mixed with a 50-fold molar excess of 5,5'-dithiobis-(2-nitrobenzoic acid (Nbs_2) with or without 100 μM-Zn^{2+} respectively in a stopped flow spectrophotometer, reaction progress curves were obtained in Fig 3. In the presence of Zn^{2+} (holoenzyme), the reactivity decreased to about 12 ~16 % of that of the apoenzyme. This result postulat for the Zn stabilization of sulfhydryl groups in the enzyme δ-aminolevulinate dehydratase [4].

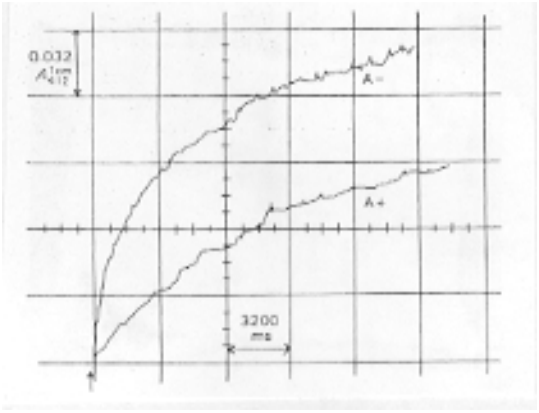

Figure 3. Stopped-flow spectrophotometric observation of the reaction between Nbs_2 and 5-aminolaevulinate dehydratase [4].

The enzyme (10 μM initial concentration) was mixed 1:1 with Nbs_2 at 25˚C in 0.1 M-Tris/HCL buffer, pH7.1. The curves of apoenzyme alone or holoenzyme in the presence of 100 μM-Zn^{2+}. The time scale was 3200ms/division. The arrow indicates the point of mixing. The vertical scales represent 0.5 molar equivalent of Nbs^- released [4].

Dihydroorotase is an enzyme in the biosynthetic pathway of the pyrimidines, and catalyzes the following cyclization: N-carbamyl-L-aspattate^{-2} + H^+ ↔ dihydro-L-orotate^{-1} + H_2O. The isolated enzyme contains 1 tightly bound essential zinc atom (subunit), and loosely binds 2 additional Zn^{2+} or Co^{2+} ions (subunit) which modulate catalytic activity. Figure 4 shows 3 Zn and 1 Zn, $2Co^{2+}$ subunit dihydrootase are stabilized relative to 1 Zn subunit dihydroorotase against activity loss by air oxidation. Thus, Zn may protect the sulfhydryl group of the enzyme dihydroorotase [5].

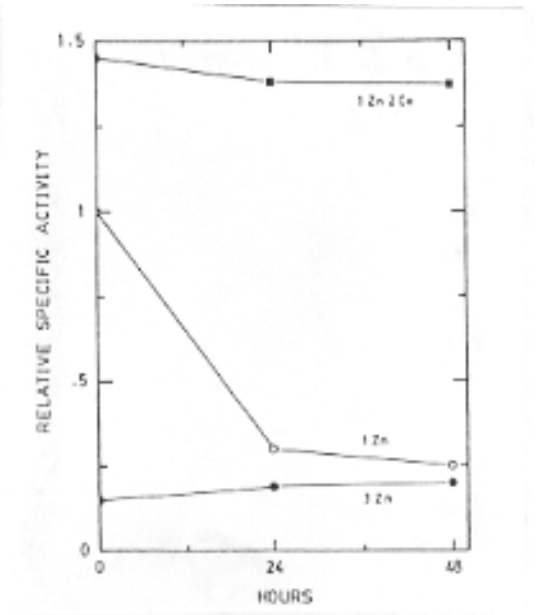

Figure 4. Loosely bound metals stabilize *E.coli* dihydroorotase against air oxidation [5].
Dihydroorotase containing one tightly bound Zn subunit (○) was supplemented with zinc (●) or cobalt (■), both of which can occupy two additional sulfhydryl-associated metal sites. Metal content was determined by atomic absorption spectroscopy before air oxidation, and dihydroorotase activity during the dialysis. Specific activities are relative to the initial value for 1 Zn subunit dihydroorotase [5].

The second mechanism by which Zn may function as an antioxidant involves the prevention of $HO^{\bullet}$ and $O_2^{\bullet -}$ production by transition metals. This mechanism involves the competition of Zn with Fe for chelation by the organic ligand cysteine. Because the cysteine-bound Fe can transfer electrons to O_2 and the produce $HO^{\bullet}$, Zn inhibits $HO^{\bullet}$ dependent processes such as lipid peroxidation [6].

Zn has also been shown to inhibit NADPH by binding to NADPH. An elution pattern of NADPH from the Sephadex G-10 column equilibrated with $ZnCl_2$ is shown in Figure 5. This elution pattern indicates that zinc inhibits NADPH oxidation by binding to NADP, because a dip occurs in the Zn^{2+} concentration which corresponds to the amount of Zn^{2+} bound to NADPH [7].

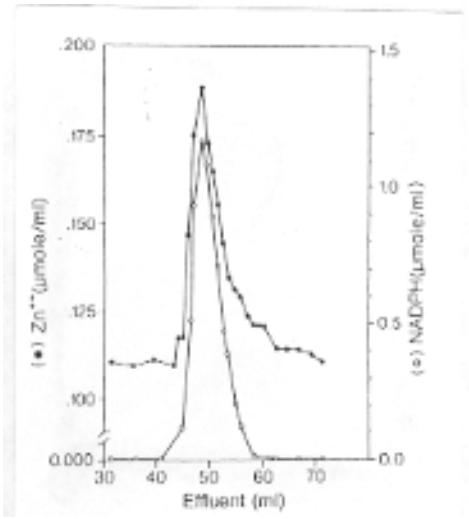

Figure 5. Elution pattern of Zn and NADPH [7].

The Sephadex G-10 column was equilibrated with 111μM ZnCl (●—●) in HEPES buffer and 13.3 μ moles of NADPH (○—○) was added and eluted with the zinc solution [7].

Zinc deficiency and oxidative stress

The effect of Zn deficiency on the primary antioxidant system can be divided into enzymatic and nonenzymatic components, as shown in Figure 6. Metallothionein (MSH) levels are depressed in liver but not lungs of Zn-deficient rats. Glutathione (GSH) levels appear to be variable in the liver of Zn-deficient rats. CuZnSOD increased in the lungs of Zn-deficient rats and in the plasma of Zn-deficient chicks. However, CuZnSOD activity unchanged in the liver, heart, and in erythrocytes of Zn-deficient rats and the erythrocytes of Zn-deficient chicks. MnSOD, catalase, and glutathione peroxidase (GSH-Px) activities are unchanged in the liver, lung, and heart of Zn-deficient rats. Although the overall changes in the free radical defense system were small, the antioxidant defense system may be important to the overall survival of the Zn-deficient animal [6].

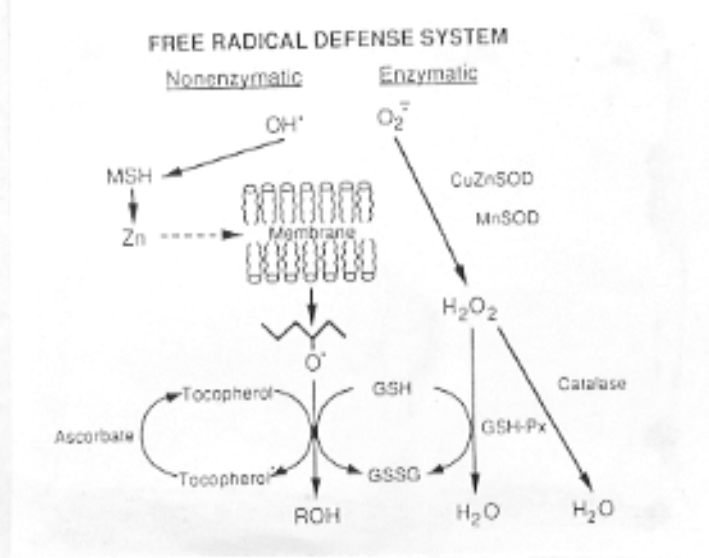

Figure 6. Enzymatic and nonenzymatic free radical defense system [6].

MSH-metallothionein; GSH-glutathione (reduced); GSSG-gluthathione (oxidized); GSH-Px-glutathione peroxidase; SOD-superoxide dismutase [6].

Conclusions

Above experiments and mechanisms by which Zn can function as an antioxidant should be pursued in other areas. First is the effect of Zn ions on the prevention of transfer of electrons to or from oxygen and organic molecules, and on the stabilization of organic free radicals as well as on the termination of free radical reactions. Second is the effect of Zn status on the metabolism, function and interaction of antioxidants such as vitamin E, glutathione, metallothionein, polyamines, taurine, and plasmalogenic phospholipids. Some Zn related compounds that can function as prooxidants such as Fe, Cu, Pb, Hg and some xenobiotics also need to be examined. Third is the effect of Zn deficiency on the gene expression and enzymatic activity such as extracellular SOD (EC-SOD) and DNAases. Fourth is the effect of Zn on inflammation and immune function. Thus, the antioxidant ability of Zn to alter free radical generation, defense and repair in different tissues can be elucidated [6].

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