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Investigation of the Effects of Some Metals on Glutathione Reductase Activity Purified of from Rainbow Trout (Oncorhynchus Mykiss) Erythrocytes

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Abstract

The enzyme glutathione reductase (GR; *E.C.1.6.4.2) was isolated from the erythrocytes of rainbow trout. GR enzyme was produced with a yield of 13.36 percent and a purification efficiency of 7135.2 EU/mg protein specific activity. SDS-PAGE was used to purity-check the enzyme after it had been purified. At a wavelength of 340 nm, enzyme activity was assessed spectrophotometrically using Carlberg and Mannervik technique. All kinetic investigations and purification processes followed this technique. On the activity of the isolated enzyme, it was examined how certain metal ions (Cu^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}, Co^{2+}, Mg^{2+}, Cr^{2+}, and Al^{3+}) reacted *in vitro*. Metal ions that had an inhibitory impact have their IC_{50} values determined. To identify the kind of inhibition, Lineweaver-Burk plots were created, and the plot's K_i constants were extracted from it. The GR enzyme activity isolated from rainbow trout erythrocytes was found to be unaffected by Al^{3+} ion, whereas Cr^{2+} ion activates the enzyme.

Keywords: *GR, Metal Ion, Inhibition, Erythrocyte*

1. Introduction

Rainbow trout (Oncorhynchus mykiss), which belongs to the Salmonidae family, is a fish with high commercial value and consumption potential in the world and in our country, thanks to its taste and satiety among freshwater fish [1]. When these fish, which are consumed a lot due to their nutritive properties, are exposed to toxicity by xenobiotics, they engage their detoxification metabolism to tolerate it like all living organisms. However, sometimes the deficiency or inhibition of enzymes involved in detoxification metabolism disrupts the functioning of this system. Glutathione Reductase enzyme is required for the continuity of glutathione, which is the substrate of Glutathione S transferase, which is one of the phase II enzymes of detoxification metabolism [2]. In light of all these factors, we sought to investigate metals that are toxic to organzymes and induce bioaccumulation as well as to examine the harm these metals do to detoxification and metabolism from a new angle.

The glutathione reductase (GR) enzyme (EC 1.8.1.7) is a member of the disulfide oxidoreductase family that converts oxidized glutathione to its reduced form [3,4]. The mechanism by which the enzyme is catalyzed is schematic below (Figure 1).

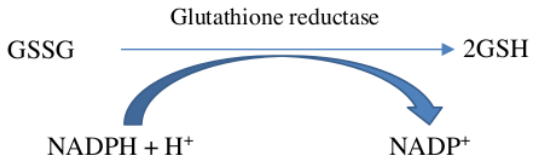

Figure 1. Catalysis mechanism of glutathione reductase enzyme [2]

While GR converts oxidized glutathione to a reduced state, electrons from NADPH are not directly transferred to the disulfide bond of oxidized glutathione. They are first transferred to the tightly bound FAD, then transferred

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to oxidized glutathione by transfer to the disulfide bridge located between the two cysteines in the subunit [5-7]. This enzyme has been purified from numerous sources (fungi, spinach, wheat, corn, peas cyano bacteria, many mammalian tissues, characterized and amino acid sequence determined [3,6,8-13]. GR is a cytoplasmic and mitochondrial enzyme.

Because of the deleterious impacts of metals, water, an essential component of life, has an adverse impact on the universe. Since humans are at the top of the food chain, many metals over time accumulate in the environment, plants, and animals before reaching us.Depending on each metal's characteristics, different metals have different harmful effects [14-15]. In general, when heavy metals interact with organic substances, harmful consequences result. In order for biological molecules to carry out their duties, part of their qualities are lost, which leads to the death of the afflicted cells [14-15].

2. Materials and Methods

2.1. Chemicals

The following items were acquired from Sigma: $CuCl_2$, $Zn(NO_3)_2$, $Al(NO_3)_2$, $Pb(NO_3)_2$, $Co(NO_3)_2$, $Cr(NO_3)_2$, $MgSO_4$, $Fe(NO_3)_2$, NADPH, GSSG, GSH, protein assay reagent, and chemicals for electrophoresis. Pharmacia was used to acquire 2′,5′-ADP Sepharose 4B. All further compounds were analytical-grade and were bought from Sigma or Merck.

2.2. Preparation of hemolysate

Fish samples were taken from the Department of Aquaculture, Faculty of Agriculture, Atatürk University's rainbow trout farm. The caudal vein of the fish was taken for blood using a 10 mL plastic heparinized syringe (5 IU/mL). It was then put into tubes and centrifuged for 15 minutes at 3000 x g using a Hettich Micro 22 R refrigerator centrifuge. A drip was used to extract the plasma. The samples were centrifuged three times at a rate of 3000 x g for 15 minutes to separate the supernatants after the package of red blood cells had been rinsed three times with KCl solution (0.16 M). The erythrocytes were centrifuged (+4°C, 13,000xg) for 30 minutes after being hemolyzed with 5 liters of ice-cold water. The precipitate was then thrown away. The supernatant was taken away [14].

2.3. Purification of glutathione reductase enzyme

At 30-70 percent precipitation of ammonium sulfate, the enzyme was shown to precipitate [15]. The purification procedure was changed to fit the needs of our laboratory. The DEAE-Sephadex A50 anion exchange column (3 cm^2x30 cm), was loaded with the enzyme solution. After the column had been cleaned with a buffer solution, elution was carried out using a linear gradient of 0-1.5 M KCl. At 340 nm, the eluted fractions were collected, and the enzyme activity was assessed. Enzyme-active tubes were joined. At 4°C, all purification processes were carried out. The affinity column was loaded with the previously obtained dialyzed enzyme solution, and the flow rate was set to 20 mL/h. A series of equilibrate buffer washes were performed on the column until the ultimate absorbance difference was 0.05. Elution solution was used to elute the enzyme at the end [15]. Final fractions were used to test the enzyme activity, and the tubes that had that activity were gathered. The temperature was maintained at +4°C during all processes [14, 15]. In the resulting solution, the protein was found with Bradford's technique [16]. To control enzyme purity was used by Laemmli's technique [17].

2.4. In vitro effects of metal ions

The modified Carlberg and Mannervik technique was used to assess the glutathione reductase activity spectrophotometrically at 25°C [18,19]. Cu^{2+} (0.01-0.2 mM), Zn^{2+} (0.03-0.2 mM), Pb^{2+} (0.1-0.55 mM), Fe^{2+} (0.01-0.2 mM), Co^{2+} (0.005-0.4 mM), and Mg^{2+} (1.42-78.1 mM) were added to the reaction medium in order to study the effects of metal ions on trout erythrosite GR. To get IC_{50} values, plots of activity (percent) and metal

ion concentration were employed. Lineweaver and Burk diagrams (1934) were produced using 1/V vs. 1/[S] observations, and the K_i constant was calculated from these graphs [20].

3. Results and Discussion

Purification of glutathione reductase enzyme from rainbow trout erythrocytes was performed in four steps. By using two chromatographic techniques one after the other, GR enzyme was obtained with 7135.2 fold and 13.36% yield (Table 1). Ulusu and Tandoğan, using a method similar to our study, stated that they purified GR enzyme from bovine liver [21]. When the results were compared, rainbow trout erythrocyte GR was obtained with a higher purification coefficient. However, the yield is less. Tekman et al. purified GR enzyme from rainbow trout liver [15]. According to our study, it was purified with a lower purification coefficient.

Table 1. Purification steps of glutathione reductase enzyme

Purification stages	Total Protein (mg)	Total activity (EU)	Specific activity (EU/mg)	%Yield	Purification Coefficient
Hemolysate	1290.3	2.208	0.0017	100	1
Ammonium Sulfate Precipitation	299.28	0.384	0.0013	21.7	0.76
DEAE-Anion Exchange	13.248	1.62	0.122	73.36	71.76
2'5' ADP-Sepharose 4B	0.0243	0.295	12.13	13.36	7135.2

Inhibition type and K_i constants was determined as Cu^{2+} 0.008533±0.0031 mM, Fe^{2+} 0.12800±0.03900 mM, Co^{2+} 0.140333±0.0040 mM, Pb^{2+} 0.154333±0.055 mM, Zn^{2+} 0.049667±0.0055 mM and Mg^{2+} 0.4993±0.071 mM from the obtained graph (Table 2). It was determined that Al^{3}+ ion did not show an inhibitory effect on the GR enzyme activity purified from rainbow trout erythrocytes, while Cr^{2+} ion activates the enzyme.

Table 2. Inhibitory effect of metal ions showing on glutathione reductase enzyme activity

Inhibitor	K_i (mM)	Total activity (EU/mL)
Cu^{2+}	0.008533±0.0031	Uncompetitive
Fe^{2+}	0.128±0.039	Competitive
Co^{2+}	0.140333±0.004	Uncompetitive
Pb^{2+}	0.15433±0.055	Uncompetitive
Zn^{2+}	0.049667±0.0055	Competitive
Mg^{2+}	0.498333±0.071	Competitive

It has been reported that Cd^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Fe^{3+} and Al^{3+} ions have an inhibitory effect on glutathione reductase enzyme activity purified from rainbow trout liver in vitro conditions [15]. Inhibitory effects of Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+} and Al^{3+} metal ions on glutathione reductase enzyme activity purified from human erythrocytes have been reported [14]. Although it was stated that it inhibited Al^{3+} in both studies, it did not show an inhibitory effect in our study. This is proof that the enzyme structure and its response to inhibitors differ from living thing to living thing and even from tissue to tissue. This is thought to be due to post-translational modifications.

The information gathered for this study was crucial in shedding light on the detrimental impact of metal ions in water and soil due to growing industry on the detoxification processes in living things. It is also a strong indication that an enzyme's sensitivity to an inhibitor might differ depending on the organism or tissue.

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