THE EFFECT OF AL^{3+} AND HG^{2+} ON GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM *CAPOETA UMBLA* KIDNEY

KIRICI, M.[1,*] - KIRICI, M.[2] – DEMIR, Y.[3] – BEYDEMIR, S.[3] – ATAMANALP, M.[4]

[1]*Department of Fisheries, Faculty of Agriculture, Bingöl University, 12000-Bingöl, Turkey.*

[2]*Department of Chemistry, Faculty of Arts and Science, Bingöl University, 12000-Bingöl, Turkey.*

[3]*Department of Chemistry, Faculty of Science, Atatürk University, 25240-Erzurum, Turkey.*

[4]*Faculty of Fisheries, Atatürk University, 25240-Erzurum, Turkey.*

**Corresponding Author:*
e-mail: muammerkirici@hotmail.com
(phone: +90 536 891 7150; fax: +90 426 216 0029)

(Received 23rd Jul 2015; accepted 16th Feb 2016)

Abstract. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49; G6PD) is an important enzyme found in all mammal tissues, and produces NADPH in the metabolism. NADPH provides a reductive potential to maintain a balanced redox state within the cell. The aim of this study was to purify G6PD from *Capoeta umbla* kidney and determination of inhibition or activation effects of aluminium and mercury on enzyme activity. In this purpose, glucose 6-phosphate dehydrogenase was purified from *Capoeta umbla* kidney by using preparation of homogenate, ammonium sulphate precipitation and 2',5'-ADP Sepharose 4B affinity chromatography. Molecular weight of the enzyme was determined on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and the purified enzyme showed a single band on the gel with a molecular weight of 75 kDa. Moreover, K_i constants of Al^{3+} and Hg^{2+} were found as 0.98 ± 0.084 and 0.57 ± 0.019 mM, respectively. In conclusion, affinity of the Hg^{2+} to the enzyme was higher than Al^{3+}

Keywords: *Capoeta umbla, fish, glucose 6-phosphate dehydrogenase (G6PD), purification, metals, inhibition.*

Introduction

Heavy metals are readily released to agricultural ecosystem causing an opposite effect due to widespread human activities. Agricultural ecosystem has a close relation with human health; hence, heavy metal pollution of agricultural ecosystem has been of attention throughout the world (Pandey and Pandey, 2008; Bermudez et al., 2012). Heavy metals are extremely toxic, non-degradable and bio-accumulative. Although some heavy metals such as zinc (Zn) and copper (Cu) are necessary elements for plants and humans as catalytic components of proteins and enzymes, a great majority of them do not have any useful physiological function, and their extreme accumulation in the human body can cause many diseases (Godt et al., 2006). For instance, accumulation of cadmium (Cd) in the human body can give rise to kidney, bone and pulmonary damage (Godt et al., 2006); lead (Pb) can harm the central nervous system, kidneys and blood system (Tong et al., 2000), etc.

Heavy metal pollution is constantly caused by waste water irrigation, solid waste disposal, vehicular exhaust, fertilisation, industrial activities, etc. (Khan et al., 2008; Liu et al., 2012). Among these pollution sources, industrial activities are the dominant

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

sources of heavy metals near factories. Kabala and Singh (2001) reported that, in the vicinity of a Cu smelter in Poland, the concentrations of Cu, Pb and Zn in the surface soils were significantly higher than their concentrations in the subsurface soils. It has reported that industrial waste can give rise to heavy metal pollution of the surrounding soils and water.

Mercury (Hg) is found in comestible seafood, such as fish. Hg is a neurotoxin which can give rise to numerous effects in humans such as memory loss, disrupted coordination, sight disturbances, cardiovascular problems, etc. It also influences the thyroid gland, digestive system, liver and skin (Nigam et al., 2009). The toxicity of Hg exposure is partly a function of enhanced oxidative stress (OS). Enhancement of OS probably arises from the inhibition of antioxidant enzymes and the consumption of thiol compounds (especially GSH) (Franco et al., 2007) giving rise to cell injury, damage to biomolecules, and lipid peroxidation (Leonard et al., 2004).

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49; G6PD) is the first enzyme of the pentose phosphate metabolic pathway (Beydemir et al., 2003). It catalyses the conversion of glucose 6-phosphate into 6-phosphogluconate (Ciftci et al., 2007). The most important function of this enzyme is the production of ribose 5-phosphate and NADPH which are necessary for membrane lipids synthesis, reductive and nucleic acid synthesis (Kuo et al., 2000; Ceyhun et al., 2010). In addition, NADPH is a coenzyme which is involved in the synthesis of some amino acids, fatty acids and steroids, protecting the cells against the oxidant and detoxification of xenobiotics through the glutathione reductase peroxidase system (Ceyhun et al., 2010; Guler et al., 2013).

The genus *Capoeta* of Cyprinids is distributed in southern China, northern India, Turkmenistan, Lake Aral, the Middle East and Anatolia (Turkmen et al., 2002). The species diversity of *Capoeta* was last revised by Karaman (1969). While textbooks such as Geldiay and Balık (2007) recorded 7 species in *Capoeta* (plus five subspecies) from Turkey, Ozulug and Freyhof (2008) recorded 17 species from this area. In the last years, five new *Capoeta* species have been described from Turkey (Turan et al., 2006; Ozulug and Freyhof, 2008; Turan et al., 2008). Turkey is clearly the centre of diversity of this genus which comprises about 23 species (Kucuk et al., 2009). One of the well known species is *Capoeta umbla* (*C. umbla*). *C. umbla*, Transcaucasian barb, inhabits Euphrates-Tigris River Systems. It is also known as "lake fish or stream fish" locally and it is the most commercially valued fish for the local people (Coban et al., 2013) around Murat River. Murat River is one of the most important large and long (722 km) tributary of the Euphrates River in South East Anatolia of Turkey. The distribution area of Murat River is upper basins systems of the Euphrates and Tigris River (Koyun, 2011).

In the present study we have purified G6PD from *C. umbla* kidney and determination of inhibition or activation effects of aluminium (Al^{3+}) and mercury (Hg^{2+}) ions on enzyme activities.

Materials and Methods

Chemicals

2',5'-ADP Sepharose 4B was purchased from Pharmacia. $NADP^+$, glucose-6-phosphate, protein assay reagent, and chemicals for electrophoresis were purchased from Sigma. All other chemicals used were of analytical grade and were purchased from Merck.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

Animal and experimental procedure

Ten *C. umbla* (healty, adult fish-weighing 150-250 g) were caught from Murat River (Turkey, Bingöl). All procedures were conducted in strict compliance with the guidelines established by the Animal Care and Use Committee. The fish were decapitated and their kidneys were extracted and stored at -80°C.

Preparation of the homogenate

For analyses, the frozen kidney was thawed and cut into small pieces by using a scalpel. Kidney simples (10 g) were washed three times with 0.9% sodium chloride solution. These samples were homogenized gently for about 45 sec. and suspended in standard homogenizator buffer, containing 50 mM KH_2PO_4, 1 mM PMSF, 1 mM EDTA and 1 mM DTT. The homogenates was centrifuged for 2 h at 13.500 rpm. The supernatant was collected and kept for analysis.

Activity determination

In accordance with the Beutler (1971) method, enzyme activity was spectrophotometrically measured at 37^{o}C. This method is based on the fact that NADPH, which is formed as a result of reducing $NADP^+$, yields absorbance at 340 nm. One enzyme unit was described as the enzyme amount reducing 1μmol $NADP^+$ per minute.

Ammonium sulphate fractionation and dialysis

G6PD enzyme homogenate was exposed to ammonium sulphate precipitation at 0–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80% ranges; and the precipitation range of the enzyme was determined. During each precipitation process, centrifugation was carried out at 13.500 rpm for 15 min. After ammonium sulphate, the precipitate was obtained and dissolved in 50 mM KH_2PO_4 (pH 7.2) buffer. Enzyme activity was measured in the precipitate and supernatant for each time. Then, the enzyme solution was dialysed at 4^{o}C in 10 mM KH_2PO_4 including 1 mM EDTA (pH 7.2) for 2h with two changes of buffer (Ninfali et al., 1990).

2',5'-ADP Sepharose 4B affinity chromatography

For 10 ml of bed volume, 2 g of dry 2',5'-ADP Sepharose 4B was washed several times in 400 ml of distilled water. During several washings, the impurities were removed and the gel was conditioned. After the removal of the air in the gel, it was resuspended in the buffer (0.1 M K-acetate + 0.1 M K-phosphate, pH 6.0) at a ratio of 25% buffer to 75% gel and was packed in a column (1 x 10 cm). Precipitation of the gel, it was equilibrated with the same buffer by means of a peristaltic pump (flow rate: 50 ml/h). After the dialyzed enzyme solution was loaded on the column which was equilibrated with buffer (0.1 M K-acetate + 0.1 M K-phosphate, pH 6.0) and the flow rate was regulated to 20 ml/h. The column was respectively washed with 25 ml of 0.1 M K-acetate + 0.1 M K-phosphate (pH 6.0) and 25 ml of 0.1 M K-acetate + 0.1 M K-phosphate (pH 7.85). Eventually, the enzyme was eluted with a solution of 80 mM K-phosphate + 80 mM KC1 + 0.5 mM $NADP^+$ + 10 mM EDTA (pH 7.8). The enzyme activity was measured, and the activity-containing tubes were collected together (Ninfali et al., 1990).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

Protein determination

Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method, with bovine serum albumin used as a standard (Bradford, 1976).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

The control of enzyme purity was carried out using Laemmli's procedure (Laemmli, 1970) in 3% and 8% acrylamide concentrations for running and stacking gel, respectively. Gel was stained with Coomassie Brilliant Blue R-250.

In vitro effects of metal ions

Al^{3+} (5, 8, 10, 12.5 and 20 mM) and Hg^{2+} (0.5, 1, 2, 5 and 8 mM) were used as inhibitors. Assays were carried out under standard conditions with varying concentrations of Al^{3+} and Hg^{2+} metal ions. The inhibition of enzyme by Al^{3+} and Hg^{2+} was further examined by varying G6-P concentrations at a fixed NADP concentration and at six different constant concentrations of each metal ion. The activity of control cuvette in the absence of an inhibitor was taken as 100%. All compounds were tested in triplicates for each concentration. For each inhibitor, an activity %-[Inhibitor] graph was drawn. Metal ions concentrations that produced 50% inhibition (IC_{50}) were calculated from the regression graphs.

To determine the K_i values, three different inhibitor concentrations (Al: 7, 10 and 12.5 mM; Hg: 0.5, 2 and 5 mM) were tested for each metal ion. In these experiments, G6-P was used as substrate at five different concentrations (0.03, 0.06, 0.09, 0.15 and 0.27 mM, respectively). Inhibitor (metal ions) solutions were added to the reaction medium, resulting in three different fixed concentrations of inhibitors in 1 ml of total reaction volume. All assays were repeated three times. Lineweaver–Burk graphs (1934) were drawn by using 1/V vs. 1/[S] values. K_i constant and the inhibitor type were calculated from these graphs.

Results and Discussion

In this study, G6PD was purified from *C. umbla* kidney tissues by using ammonium sulphate precipitation and 2',5'-ADP Sepharose 4B affinity gel chromatography. As a result of the two consecutive steps, the enzyme was purified 402.14-fold in a yield of 22.7% with a specific activity of 11.26 U/mg (*Table 1*). Purity of the enzyme was determined by SDS-PAGE and showed single band on the gel (20 µl of the sample was loaded onto SDS-PAGE gel) (*Fig. 1*). Rf values were calculated for standard proteins and G6PD according to Laemmli's procedure from Rf–Log MW graph and molecular weight of protein was 73.8 kDa. For each metal, Lineweaver-Burk graphs were drawn and are shown in *Figs. 4 and 5*. K_i constants were determined as 0.98 ± 0.084 and 0.57 ± 0.019 mM from the graphs for Al^{3+} and Hg^{2+}, respectively (*Table 2*).

In addition, [Metal] vs. activity % graphs were drawn for the metals and are shown in *Figs. 2 and 3*. IC_{50} values were calculated as 7.22 and 3.12 mM from the graphs for Al^{3+} and Hg^{2+}, respectively (*Table 2*). Both Al^{3+} and Hg^{2+} inhibited the G6PD activity *in vitro* and showed competitive inhibition (*Figs. 2-5*). Hg^{2+} was a stronger inhibitor than Al^{3+}. Furthermore, Hg^{2+} had higher affinity for G6PD than that of Al^{3+}.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

Table 1. *Purification scheme of G6PD from C. umbla kidney*

Purification Step	Activity (U/ml)	Protein (mg/ml)	Total Volume (ml)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg protein)	Purification Factor	Yield (%)
Hemolysate	0.274	9.78	31	8.494	303.18	0.028	1	100
Ammonium Sulphate Precipitation and Dialysis	0.462	14.2	6	2.772	85.2	0.033	1.18	32.6
2',5'- ADP Sepharose 4B Affinity Chromatography	0.642	0.057	3	1.926	0.171	11.26	402.14	22.7

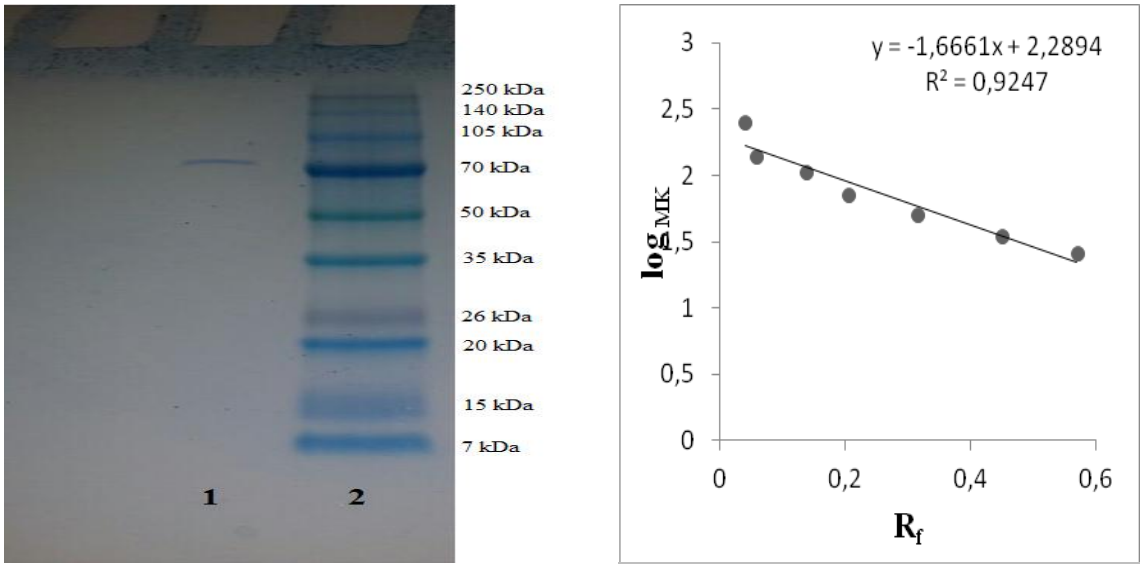

Figure 1. *SDS–polyacrylamide gel electrophoresis of purified G6PD. Lane 1: standard proteins and Lane 2: C. umbla kidney G6PD.*

G6PD has been purified previously with chromatographic methods from many different sources, such as, humans (Yoshida, 1966; Cho and Joshi, 1990; Ozmen et al., 2005), animals (Beydemir et al., 2003; Erat, 2005), plants (Coban et al., 2002; Esposito et al., 2005; Wei-Fu et al., 2007) and microorganisms (Heise and Opperdoes, 1999; Ibraheem et al., 2005). Inhibitory effects of many metal ions on G6PD enzyme activities in different animal species have been reported in many investigations (Velasco et al., 1994; Comaklı et al., 2013; Hu et al., 2013).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

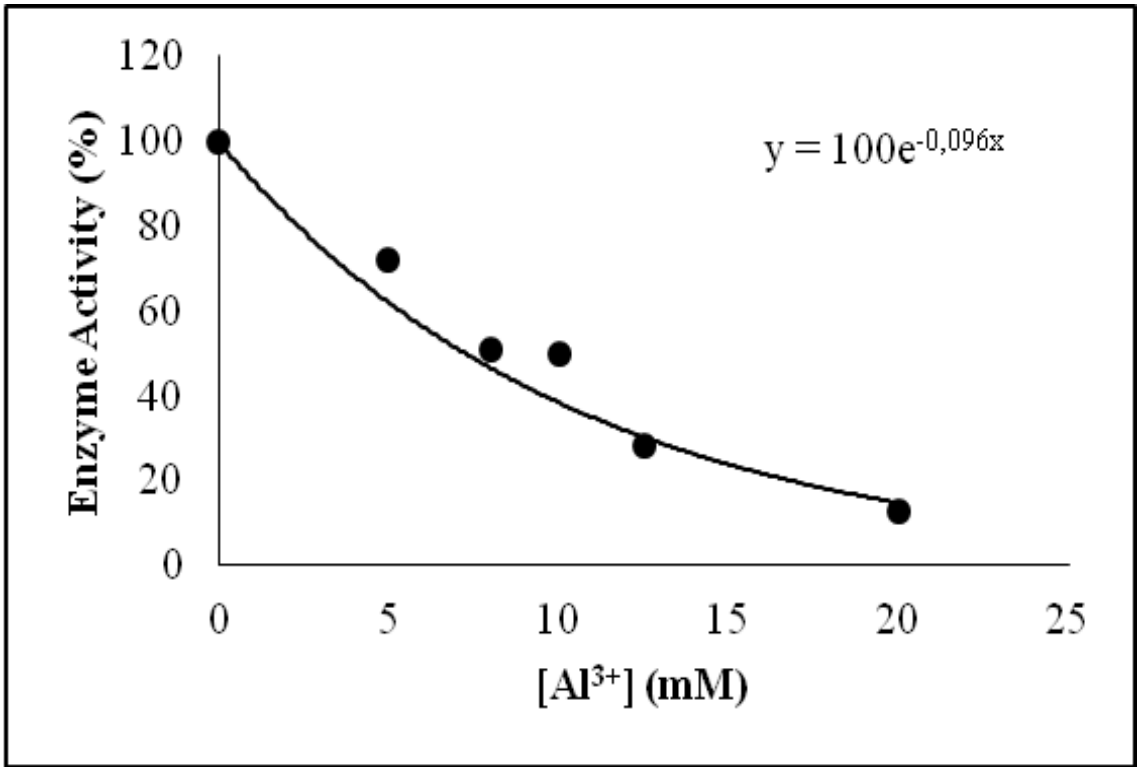

Figure 2. *Effect of Al^{3+} at five different concentrations on C. umbla G6PD activity*

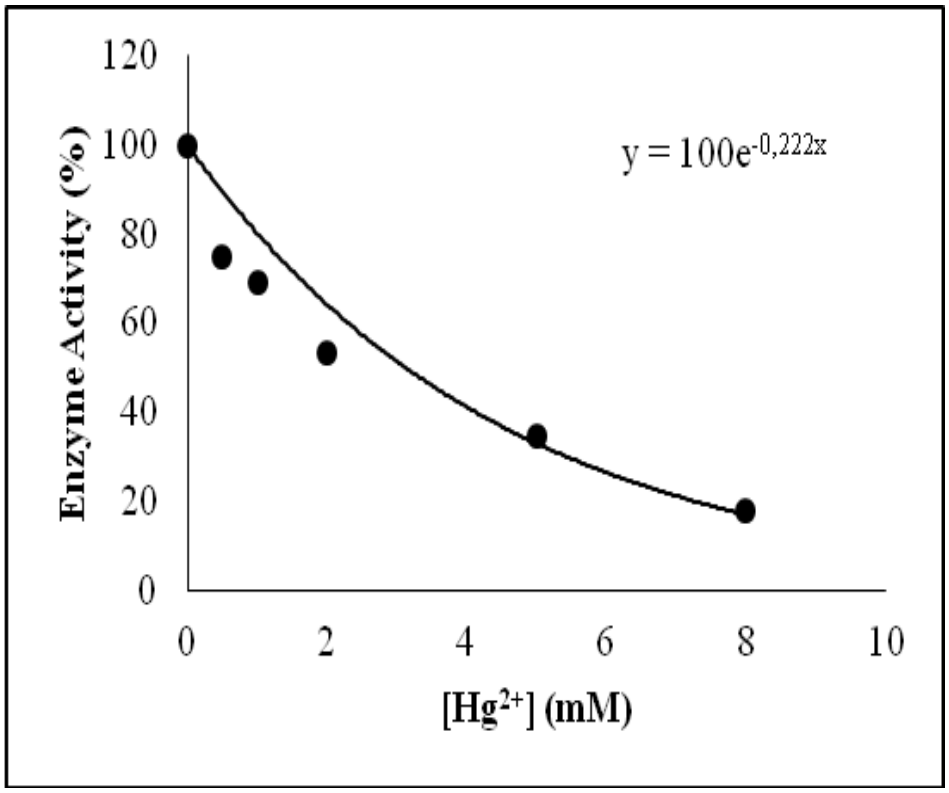

Figure 3. *Effect of Hg^{2+} at five different concentrations on C. umbla G6PD activity*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

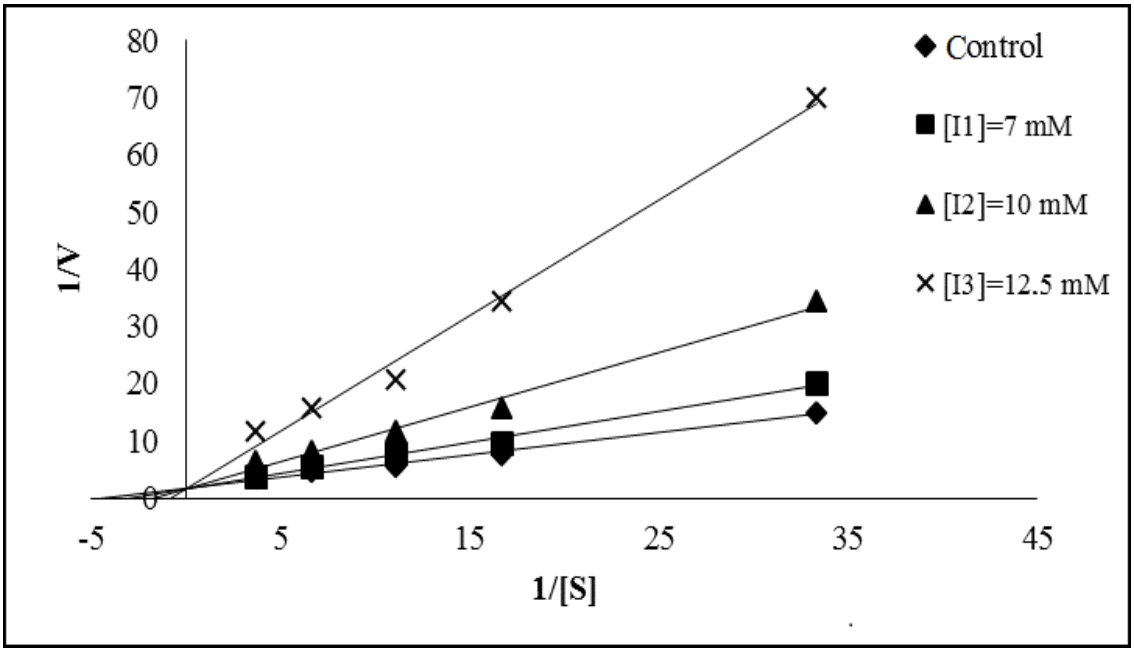

Figure 4. *Lineweaver–Burk graph in five different substrate concentrations and in three different Al^{3+} concentrations for the determination of K_i.*

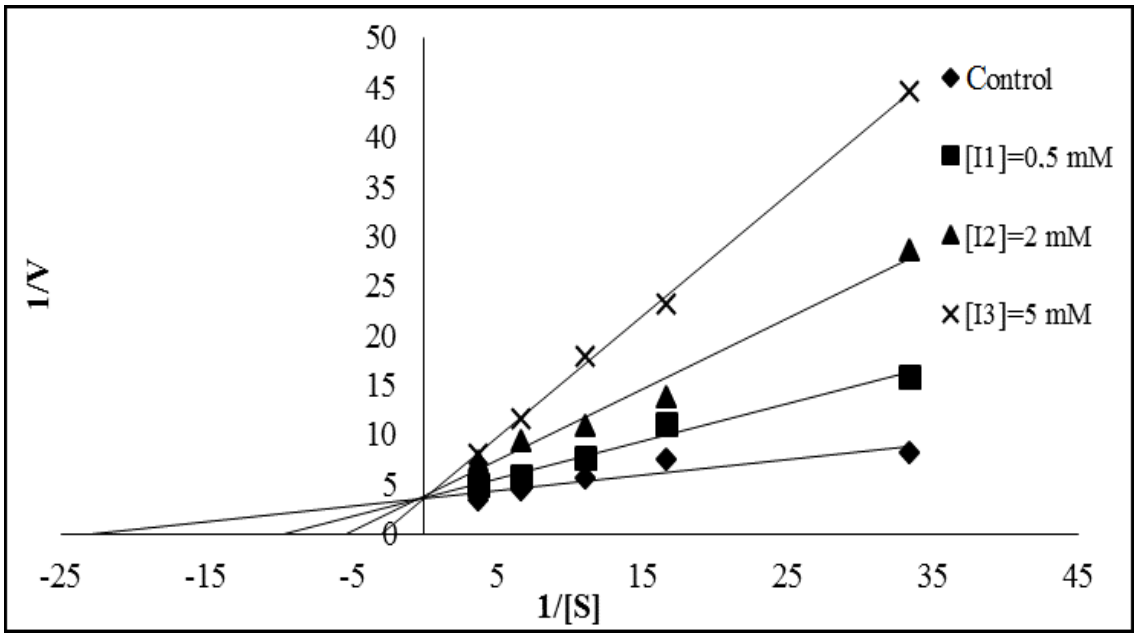

Figure 5. *Lineweaver–Burk graph in five different substrate concentrations and in three different Hg^{2+} concentrations for the determination of K_i.*

In the present study, specific activity of the enzyme was determined as 11.26 U/mg protein, which was lower than those in chicken erythrocytes (20.86 U/mg protein, Yilmaz et al., 2002), rainbow trout liver (36.25 U/mg protein, Cankaya et al., 2011), rat kidney (32 U/mg protein, Adem and Ciftci, 2012), grass carp (18 U/mg protein, Hu et al, 2013), but higher than that in sheep lens (0.15 U/mg, Charlton and Heyningen, 1971)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

and bovine lens (2.64 U/mg, Ulusu et al., 1999). The observation of different specific activities for G6PD from different sources was not uncommon, depending on several factors such as NADP, salt, etc.

Table 2. *The results of the activity of G6PD; K_i, I_{C50} values and inhibition types*

Metals	IC_{50} (mM)	K_i (mM)	Inhibition Type
Al^{3+}	7.22	0.98 ± 0.084	Competitive
Hg^{2+}	3.12	0.57 ± 0.019	Competitive

A molecular weight of G6PD was 75 kDa with SDS-PAGE in this study, which was similar to that reported in chicken erythrocytes (73.2 kDa, Yilmaz et al., 2002), but higher than bovine lens (69.2 kDa, Ulusu et al., 1999), human placenta (54 kDa, Ozer et al., 2001), dog liver (52.5 kDa, Ozer et al., 2002), buffalo erythrocyte (67.6 kDa, Ciftci et al., 2003), rainbow trout (60 kDa, Erdogan et al., 2005), rainbow trout liver (48.5 kDa; Cankaya et al., 2011), rat kidney (68 kDa, Adem and Ciftci, 2012) and grass carp (71.85 kDa, Hu et al., 2013).

Inhibition of some significant enzymes, which play a key role in a metabolic pathway, may give rise to pathologic conditions or disorders. In the literature, effects of various drugs and chemical substances on the catalytic activity of the G6PD enzyme were investigated. K_i values of these substances are higher than the values calculated for the coumarin derivatives. Ki values of isepamicin sulphate, omeprazole, morphine sulphate, vancomycin, magnesium sulphate, metamizol and granisetron hydrochloride were reported as 1.7 mM, 8.2 mM, 25.9 mM, 2.71 mM, 13.2 mM, 6.3 mM, 4.5 mM, respectively (Ozmen and Kufrevioglu, 2004; Ozmen et al., 2005).

Hopa et al. (2014) investigated the inhibition effects of IC_{50} and K_i parameters of coumarin derivatives for G6PD. IC_{50} values of OPC (6,7-Dihydroxy-3-(2-methylphenyl)-2H-chromen-2-one), MPC (6,7-dihydroxy-3-(3-methylphenyl)-2H-chromen-2-one) and PPC (6,7-dihydroxy-3-(4-methylphenyl)-2H-chromen-2-one) were 0.305, 0.769 and 0.790 mM and the K_i constants were 1.37 mM, 0.734 mM 0.269 mM, 0.835 mM, respectively.

Hu et al. (2013) purified G6PD from grass carp (*Ctenopharyngodon idella*) and determined the inhibition effects of Zn, Mn, Al, Cu and Cd on G6PD activity *in vitro*. They found IC_{50} values as 0.42, 0.54, 0.94, 1.20, and 4.17 mM, respectively. K_i constants were determined as 0.52, 1.12, 0.26, and 4.8 mM, respectively. Cankaya et al. (2011) reported that the IC_{50} values of Fe, Pb, Hg, Cu, Zn, and Cd on the purified G6PD activity of trout liver was 0.39, 0.78, 0.87, 1.19, 1.97, 2.16 mM and the K_i constants were 0.197,0.213, 0.542, 1.721, 2.034, 2.770 mM, respectively.

Fish meat is a precious food of animal source for human depletion. Accumulation of metals in fish may be considered as an important warning signal for fish health and human consumption. Metal ions accumulated in fish as a toxic concentration will be hazardous for human health. For this reason, great efforts and cooperation between different authorities are need to protect the aquatic resources from metal pollution. To avoid the aquatic life loss there is need to use the advanced technologies generating less metal pollution to environment. Briefly, concentration of metal ion in contaminated lakes and rivers must be decreased.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

REFERENCES

[1] Adem, S., Ciftci, M. (2012): Purification of rat kidney glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glutathione reductase enzymes using 2', 5'-ADP Sepharose 4B affinity in a single chromatography step. - Protein Expression and Purification 81: 1–4.

[2] Bermudez, G. M. A., Jasan, R., Plá, R., Pignata, M. L. (2012): Heavy metals and trace elements in atmospheric fall-out: their relationship with topsoil and wheat element composition. - Journal of Hazardous Materials 213-214: 447-456.

[3] Beutler, E. (1971): Red Cell Metabolism Manuel of Biochemical Methods. - Academic Press, London.

[4] Beydemir, S., Gulcin, I., Kufrevioglu, O. I., Ciftci, M. (2003): Glucose 6-phosphate dehydrogenase: *in vitro* and *in vivo* effects of dantrolene sodium. - Polish Journal of Pharmacology 55: 787-792.

[5] Beydemir, S., Yilmaz, H., Ciftci, M., Kufrevioglu, O. I. (2003): Purification of glucose 6-phosphate dehydrogenase from goose erythrocytes and kinetic properties. - Turkish Journal of Veterinary and Animal Sciences 27: 1179-1185.

[6] Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Analytical Biochemistry 72: 248-254.

[7] Cankaya. M, Sisecioglu, M, Ciftci, M., Ozdemir, H. (2011): Effect of some metals on metal ions on trout liver G6PD. - Reserch Journal of Environmental Toxicology 5(6): 385–391.

[8] Ceyhun, S. B., Sentürk, M., Ekinci, D., Erdogan, O., Ciltas, A., Kocaman, E. M. (2010): Deltamethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. - Comparative Biochemistry and Physiology C-Toxicology and Pharmacology 152(2): 215-223.

[9] Charlton, J. M., Heyningen, R. (1971): Glucose-6-phosphate dehydrogenase in the mammalian lens. - Experimental Eye Research 11: 147–160.

[10] Cho, S. W., Joshi, J. G. (1990): Characterization of glucose-6-phosphate dehydrogenase isozymes from human and pig brain. - Neuroscience 38(3): 819-828.

[11] Ciftci, M., Beydemir, S., Yilmaz, H., Altikat, S. (2003): Purification of glucose 6-phosphate dehydrogenase from Buffalo (*Bubalus bubalis*) erythrocytes and investigation of some kinetic properties. - Protein Expression and Purification 29: 304–310.

[12] Ciftci, M., Turkoglu, V., Coban, T. A. (2007): Effects of some drugs on hepatic glucose 6-phosphate dehydrogenase activity in Lake Van fish (*Chalcalburnus tarischii* Pallas, 1811). - Journal of Hazardous Materials 143(1-2): 415-418.

[13] Coban, M. Z., Gunduz, F., Demirol, F., Ornekci, G. N., Karakaya, G., Turkgulu, I., Alp, A. (2013): Population dynamics and stock assessment of Capoeta umbla (Heckel, 1843) in Lake Hazar, Elazığ, Turkey. - Turkish Journal of Fisheries and Aquatic Sciences 13(2): 221-231.

[14] Coban, T. A., Ciftci, M., Kufrevioglu, O. I. (2002): Purification and investigation of some kinetic properties of glucose-6-phosphate dehydrogenase from parsley (*Petroselinum hortense*) leaves. - Preparative Biochemistry and Biotechnology 32(2): 173-187.

[15] Comaklı, V., Akkemik, E., Ciftci, M., Kufrevioglu, O. I. (2013): Purification and characterization of glucose 6-phosphate dehydrogenase enzyme from rainbow trout (*Oncorhynchus mykiss*) liver and investigation of the effects of some metal ions on enzyme activity. - Toxicology and Industrial Health 1-9.

[16] Erat, M. (2005): Purification of 6-phosphogluconate dehydrogenase from chicken liver and investigation of some kinetic properties. - Preparative Biochemistry and Biotechnology 35: 53-69.

[17] Erdogan, O., Hisar, O., Köroglu, G., Ciltas, A. (2005): Sublethal ammonia and urea concentrations inhibit rainbow trout (*Oncorhynchus mykiss*) erythrocyte glucose-6-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

phosphate dehydrogenase. - Comparative Biochemistry and Physiology C-Toxicology and Pharmacology 141(2): 145–150.

[18] Esposito, S., Guarriero, G., Vona, V., Di Martino R. V., Carfagna, S., Rigano, C. (2005): Glutamate synthase activities and protein changes in relation to nitrogen nutrition in barley: The dependence on different plastidic glucose-6P dehydrogenase isoforms. - Journal of Experimental Botany 409: 55-64.

[19] Franco, J. L., Braga, H. C., Nunes, A. K. C., Ribas, C. M., Stringari, J., Silva, A. P., Pomblum, S. G., Mora, A. M., Bohrer, D., Santos, A. R. S., Dafre, A. L., Farina, M. (2007): Lactational exposure to inorganic mercury: Evidence of neurotoxic effects. - Neurotoxicology and Teratology 29: 360–367.

[20] Geldiay, R., Balik, S. (2007): Türkiye Tatlısu Balıkları [Freshwater Fish of Turkey]. - Ege Üniversitesi Su Ürünleri Fakültesi Yayınları, Izmir.

[21] Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., Groneberg, D. A. (2006): The toxicity of cadmium and resulting hazards for human health. - Journal of Occupational Medicine and Toxicology 1: 1–6.

[22] Guler, M., Kivanc, M. R., Turkoglu, V., Basi, Z., Kivrak, H. (2013): *In vitro* determination of 6PGD enzyme activity purified from Lake Van fish (*Chalcalburnus tarichii* Pallas, 1811) liver exposed to pesticides. - The Bulletin of Environmental Contamination and Toxicology 91(5): 560-564.

[23] Heise, N., Opperdoes, F. R. (1999): Purification, localisation and characterisation of glucose 6-phosphate dehydrogenase of *Trypanoma brucei*. - Molecular and Biochemical Parasitology 99: 21-32.

[24] Hopa, E., Basaran, I., Sinan, S., Turan, Y., Cakir, U. (2014): *In vitro* inhibition effects of some coumarin derivatives on human erythrocytes glucose 6-phosphate dehydrogenase activities. - Journal of Enzyme Inhibition and Medicinal Chemistry 29(5): 728-732.

[25] Hu, W., Zhi, L., Zhuo, M., Zhu, Q., Zheng, J., Chen, Q., Gong, Y., Liu, C. (2013): Purification and characterization of glucose 6-phosphate dehydrogenase from grass carp (*Ctenopharyngodon idella*) and inhibition effects of several metal ions on G6PD activity *in vitro*. - Fish Physiology and Biochemistry 39: 637–647.

[26] Ibraheem, O., Adewale, I. O., Afolayan, A. (2005): Purification and properties of glucose 6-phosphate dehydrogenase from *Aspergillus aculetaus*. - Journal of Biochemistry and Molecular Biology 38(5): 584-590.

[27] Kabala, C., Singh, B. R. (2001): Fractionation and mobility of copper, lead, and zinc in soil profiles in the vicinity of a copper smelter. - Journal of Environmental Quality 30: 485-492.

[28] Karaman, M. S. (1969): Süßwasserfische der Türkei. 7. teil. revision der klein- und vorderasiatischen arten des Genus Capoeta (Varicorhinus, partim). - Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 66(1): 17-54..

[29] Khan, S., Cao, Q., Zheng, Y., Huang, Y., Zhu, Y. (2008): Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. - Environmental Pollution 152: 686–692.

[30] Koyun, M. (2011): First record of Dogielius forceps (Monogenea) on Capoeta umbla (Pisces, Cyprinidae) to Turkey, from Murat River. - Aquaculture, Aquarium, Conservation, Legislation - International Journal of the Bioflux Society 4(4): 469-473.

[31] Kucuk, F., Turan, D., Sahin, C., Gulle, I. (2009): Capoeta mauricii n. sp., a new species of cyprinid fish from Lake Beyşehir, Turkey. - Zoology in the Middle East 47(1): 71-82.

[32] Kuo, W., Lin, J., Tang, T. K. (2000): Human glucose 6-phosphate dehydrogenase (G6PD) gene transforms NIH 3T3 cells and induces tumors in nude mice. - International Journal of Cancer 85: 857-864.

[33] Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature 227: 680-685.

[34] Leonard, S. S., Harris, G. K., Shi, X. (2004): Metal-induced oxidative stress and signal transduction. - Free Radical Biology and Medicine 37: 1921-1942.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

[35] Lineweaver, H., Burk, D. (1934): The determination of enzyme dissociation constants. - Journal of The American Chemical Society 56: 658-666.
[36] Liu, J., Wang, J., Qi, J., Li, X., Chen, Y., Wang, C., Wu, Y. (2012): Heavy metal contamination in arable soils and vegetables around a sulfuric acid factory, China. - Clean – Soil Air Water 40: 766–772.
[37] Nigam, R., Linshy, V. N., Kurtarkar, S. R., Saraswat, R. (2009): Effects of sudden stress due to heavy metal mercury on benthic foraminifer *Rosalina leei*: laboratory culture experiment. - Marine Pollution Bulletin 59: 362-368.
[38] Ninfali, P., Orsenigo, T., Barociani, L., Rapa, S. (1990): Rapid purification of glucose 6-phosphate dehydrogenase from mammal's erythrocyte. - Preparative Biochemistry and Biotechnology 20: 297-309.
[39] Ozer, N., Aksoy, Y., Ogus, I. H. (2001): Kinetic properties of human placental glucose 6-phosphate dehydrogenase. - International Journal of Biochemistry and Cell Biology 33: 221-226.
[40] Ozer, N., Bilgi, C., Ogus, I. H. (2002): Dog liver glucose 6-phosphate dehydrogenase: purification and kinetic properties. - International Journal of Biochemistry and Cell Biology 34: 253-262.
[41] Ozmen, I., Kufrevioglu, O. I. (2004): Effect of antiemetic drugs on glucose 6-phosphate dehydrogenase and some antioxidant enzymes. - Pharmacological Research 50: 499–504.
[42] Ozmen, I., Kufrevioglu, O.I., Gul, M. (2005): Effects of some antibiotics on activity of glucose 6-phosphate dehydrogenase from human erythrocytes *in vitro* and effect of isepamicin sulfate on activities of antioxidant enzymes in rat erythrocytes. - Drug and Chemical Toxicology 28: 443-445.
[43] Ozulug, M., Freyhof, J. (2008): Capoeta turani, a new species of barbel from River Seyhan, Turkey (Teleostei: Cyprinidae). - Ichthyological Exploration of Freshwaters 19(4): 289-296.
[44] Pandey, J., Pandey, U. (2008): Accumulation of heavy metals in dietary vegetables and cultivated soil horizon in organic farming system in relation to atmospheric deposition in a seasonally dry tropical region of India. - Environmental Monitoring and Assessment 148: 61-74.
[45] Tong, S., Von Schirnding, Y. E., Prapamontol, T. (2000): Environmental lead exposure: a public health problem of global dimensions. - Bulletin of The World Health Organization 78: 1068-1077.
[46] Turan, D., Kottelat, M., Ekmekci, G. (2008): Capoeta erhani, a new species of cyprinid fish from Ceyhan River, Turkey (Teleostei: Cyprinidae). - Ichthyological Exploration of Freshwaters 19(3): 263-270.
[47] Turan, D., Kottelat, M., Kirankaya, S. G., Engin, S. (2006): Capoeta ekmekciae, a new species of cyprinid fish from northeastern Anatolia, (Teleostei: Cyprinidae). - Ichthyological Exploration of Freshwaters 17(2): 147-156.
[48] Turkmen, M., Erdogan, O., Yildirim, A., Akyurt, I. (2002): Reproduction tactics age and growth of C. c. umbla Heckel, 1843 from the Aşkale Region of the Karasu River, Turkey. - Fisheries Research 54(3): 317-328.
[49] Ulusu, N. N., Kus, M. S., Acan, N. L., Tezcan, E. F. (1999): A rapid method for the purification of glucose 6-phosphate dehydrogenase from bovine lens. - International Journal of Biochemistry and Cell Biology 31: 787-796.
[50] Velasco, P., Barcia, R., Ibarguren, I., Sieiro, A. M., Ramos-Martinez, J. I. (1994): Purification, characterization and kinetic mechanism of glucose 6-phosphate dehydrogenase from mouse liver. - International Journal of Biochemistry 26(2): 195-200.
[51] Wei-Fu, K., Jian-Ye, C., Zhi-Xia, H., Peng-Fei, W., Ji-Cheng, Z., Quihong, P., Wei-Dong, H. (2007): Activity and subcellular localization of glucose 6-phosphate dehydrogenase in peach fruits. - Journal of Plant Physiology 164: 934-944.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264

[52] Yilmaz, H., Ciftci, M., Beydemir, S., Bakan, E. (2002): Purification of glucose 6-phosphate dehydrogenase from chicken erythrocytes and investigation of some kinetic properties. - Preparative Biochemistry and Biotechnology 32: 287-301.
[53] Yoshida, A. (1966): Glucose 6-phosphate dehydrogenase of human erythrocytes. I. Purification and characterization of normal (B+) enzyme. - The Journal of Biological Chemistry 241: 4966-4976.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1402_253264