Araştırma Makalesi https://doi.org/10.46810/tdfd.1242764 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Purification and Charcterızatıon Glutathione S-Transferase from Chicken Liver

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(Received: 26.01.2023, Accepted: 05.03.2023, Online Publication: 27.03.2023)

Keywords
Glutathione S-transferase, Chicken liver, Purification

Abstract: In this study, the glutathione S-transferase enzyme (GST; EC 2.5.1.18) was purified with 8.35 EU/mL specific activity, 24.56 times 8% yield, from chicken liver, using ammonium sulfate precipitation and glutathione-agarose affinity chromatography. In order to control the purity of the enzyme, SDS-PAGE was performed and a single band was obtained. The molecular mass of the subunit was calculated as approximately 30.9 kDa. In addition, the optimum pH value of the enzyme (8.5 in Tris-HCl); optimum ionic strength (150 mM with Tris-HCl); optimum temperature (70 °C); stable pH value (8.5 with Tris-HCl) was determined. The K_M value for the GSH substrate of the enzyme was 0.802 mM, the Vmax value was 1.833 EU/mL; For CDNB, the K_M value was calculated as 3.6 mM and the Vmax value was calculated as 2.829 EU/mL.

Tavuk Ciğerinden Glutatyon S-Transferazın Saflaştırılması ve Karakterizasyonu

Anahtar Kelimeler
Glutatyon S-transferaz, Tavuk karaciğeri, Saflaştırma

Öz: Bu çalışmada glutatyon S-transferaz enzimi (GST; EC 2.5.1.18) tavuk karaciğerinden amonyum sülfat çöktürmesi ve glutatyon-agaroz afinite kromatografisinden yararlanılarak 8,35 EÜ/mL spesifik aktivitesine sahip olan enzim, %8 verimle 24,56 kat saflaştırıldı. Saflaştırılan enzimin saflığının kontrol edilmesi maksadıyla SDS-PAGE işlemi yapıldı ve tek bant elde edildi. Alt birimin molekül kütlesi yaklaşık olarak 30,9 kDa olarak hesaplandı. Ayrıca enzimin optimum pH değeri (Tris-HCl içinde 8,5), optimum iyonik şiddeti (Tris-HCl ile 150 mM), optimum sıcaklığı (70 °C), stabil pH değeri (Tris-HCl ile 8,5) tespit edildi. Enzimin GSH substratı için K_M değeri 0,802 mM, Vmax değeri 1,833 EÜ/mL; CDNB için de K_M değeri 3,6 mM ve Vmax değeri 2,829 EÜ/mL olarak hesaplandı.

1. INTRODUCTION

Enzymes are indispensable macroproteins for the metabolic activities of living things. Many enzymes play important roles alone or with their derivatives in animal, plant and human cells [1-3]. One of these is the glutathione S-transferase (GST) (EC.2.5.1.18) enzyme, which provides the internal balance of the body by catalyzing the first step of mercapturic acid, which is the end product of the detoxification systems developed by the body against the environmental, chemical and radioactive effects that the body is exposed to. In the detoxification system, which consists of three phases in total, the glutathione s transferase enzyme appears in the Phase II phase produced by conjugation reactions [1-6]. The first place where the glutathione S-transferase enzyme, which has a weight of about 27 kDa and consists of subunits of 229 amino acids, was purified by Boyland et al., form rat liver [7].

GST enzyme group is named as microsomal, cytosolic and mitochondrial according to their positions, mostly cytosolic in the cell [4]. The fact that this enzyme does not lose its activity for a long time under certain conditions, contains a large number of isoenzymes and has parts that bind water and substrates has made it included in many research topics. These properties allow reduced glutathione to be conjugated with many different

compounds and to catalyze substrates with different structures [4,8].

GST enzyme can only be activated by the presence of reduced glutathione. GST, which is selective about the substrate, shows partial specificity with this behavior. Apart from providing homeostasis in the body's defense, the GST enzyme plays a preventive role by preventing the microparticles in the body from combining with the toxic structures entering the body and posing a threat [7,9,10]. Enzymes are made of proteins, and their components are made up of amino acids. The type of amino acids found in enzymes is very important according to the enzyme's function. Although the GST enzyme does not contain the same amino acids in every living thing, the most similar component type is found in humans and rats. The amino acids found in common in human and rat GST enzymes are aspartic acid, glutamic acid, and leucine. Tryptophan and cysteine amino acids are only found in trace amounts in rats, unlike humans [10,11].

In this study, it was aimed to purify and characterize the GST enzyme from chicken liver tissue for the first time.

2. MATERIAL AND METHOD

2.1. Chemicals

Reduced Glutathione (GSH), 1-chloro 2,4 dinitrobenzene (CDNB), Sephadex G-150, acrylamide, coomassie brilliant blue G-250, bromine thymol blue, sodium dodecyl sulfate (SDS), ammonium persulfate, beta-mercaptoethanol used in our study, standard bovine serum albumin, N,N,N',N'-tetramethyl ethylenediamine (TEMED), glutathione agarose, glutathione acetate, glycine, glycerine, potassium bisphosphate, potassium phosphate, trihydroxymethylaminomethane (Tris), NaOH, NaCl, HCl, H_3PO_4, C_2H_5OH, CH_3OH, CH_3COOH, $NaCH_3COO$, isopropanol were obtained from Sigma Chemical Comp.

2.2. Preparation of Homogenate

Chicken livers used during the experiment were obtained fresh from Bingöl Meat and Milk Institution according to cold chain rules. Tissues brought to the laboratory were cut into small pieces and 10 grams of tissue was taken and powdered with liquid nitrogen in a mortar. The pulverized tissue was suspended in 10 mL of 50 mM Tris-HCl (pH: 7.5) buffer. The resulting suspension was then centrifuged at 13.000 rpm for one hour to precipitate tissue and cell particles. The supernatant was carefully taken with a dropper and used in the study, and these processes were carried out at +4°C [12].

2.3. Ammonium Sulphate Precipitation and Dialysis

Ammonium sulfate precipitation was made in the ranges of 0-20%, 20-40%, 40-60%, 60-80%, 80-100% in the homogenate obtained, and the interval at which the enzyme precipitated was determined. Centrifugation was carried out at 10.000 rpm for 20 minutes at each stage. The activity of the supernatant and precipitate was measured each time. Then, the thin transparent cylindrical dialysis bag was opened, knotted from the bottom, and poured into the mixture obtained as a result of precipitation, and another knot was tied in such a way that there was no air on it. Dialysis was performed against dialysis buffer (10 mM K-Phosphate, 1 mM EDTA and pH 7.5) for about 2 hours by changing the water on the magnetic stirrer every hour and the temperature was kept at +4ºC during the procedures.

2.4. Purification of Glutathione S-Transferase

Affinity chromatography technique, which is one of the most popular and efficient methods among enzyme purification techniques, was preferred for this study and enzyme purification was performed from affinity chromatography using the glutathione-agarose. First, 1 g of dry glutathione-agarose solid was weighed and washed with 200 mL of water, and the small solid particles were removed. After washing, the gel was swelled and the gel was suspended by adding buffer solution (0.05 M K-phosphate, pH: 7.4, 1 mM EDTA and 1 mM DTT), which functions as balancing, packaging and washing. The glutathione agarose affinity column was adjusted to correspond to a flow rate of 60 mL per hour by means of a peristaltic pump. Against the risk of cracking the column, the buffer was added up to 1-2 cm above the gel. First, the column was regenerated with 0.5 M NaCl and 0.1 boric acid (pH: 8.5) and 0.1 M sodium acetate and 0.5 M NaCl (pH: 4.5) buffers, and 10 mM KH_2PO_4 and 150 mM equilibrated with NaCl (pH:7.4) buffer. Then, ammonium sulfate and enzyme sample obtained after dialysis processes were applied and washing was performed with 0.05 M K-phosphate, pH: 7.4, 1 mM EDTA and 1 mM DTT buffer. After washing, the enzyme sample was eluted with 50 mM Tris-HCl buffer (pH: 9.5) containing 2.5 mM, 5 mM and 10 mM GSH solution into three mL tubes [13,14].

2.5. Measuring Enzyme Activity

Activity measurement of GST enzyme was measured spectrophotometrically according to the method of Habig et al. This method is based on the absorbance of dinitrobenzene 5-glutathione at 340 nm, which is formed in the reaction catalyzed by the GST enzyme [15].

2.6. Protein Determination

2.6.1. Qualitative Protein Determination

The qualitative determination of proteins is based on the principle that tyrosine and tryptophan amino acids, which are the amino acids present in the structure of proteins, show a maximum absorbance value at 280 nm [16].

2.6.2. Quantitative Protein Determination

The amount of protein (quantitative) at all stages throughout the study was made using the Bradford method. This method works on the basis that Coommassie Brilliant Blue G-250 reagent rapidly binds to protein and shows maximum absorbance at 595 nm. [17].

2.7. Control of GST Enzyme Purity via SDS-PAGE

The purity of the GST enzyme was checked by batch SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) with a range of 3-8% using the technique proposed by Laemmli [18].

2.8. Characterization Studies

2.8.1. Determination of Optimum pH

In order to determine the optimum pH of the glutathione S-transferase enzyme obtained from chicken liver, the pH values from potassium phosphate buffer were 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0; Tris-HCl buffer solutions were prepared at pH ranges of 7.5, 8.0, 8.5, and 9.0. The activity of the enzyme was calculated with each of these buffers separately. pH-activity graphs were drawn in order to determine the optimum pH.

2.8.2. Stable pH Determination

In order to determine the pH value at which the enzyme exhibits optimal stability; KH_2PO_4 with pH values of 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 and 7.5, 8.0, 8.5 and 9 prepared. Activity measurements were made with these prepared buffers every 24 hours for 3 days. As a result of the activity measurements, graphs were drawn to determine the stable pH of the enzyme.

2.8.3. Determination of Optimum Ionic Strength

In order to determine the optimum ionic strength of the enzyme, activity measurements were made with 50, 100, 150, 160, 170, 200 and 250 mM Tris-HCl solutions.

2.8.4. Determination of Optimum Temperature

In order to determine the optimum temperature value of the enzyme, an activity measurement was carried out at every 10ºC between 0ºC and 90ºC using optimum pH, optimum ionic strength and the mentioned buffer.

2.8.5. Kinetic Studies

First of all, 1/V-1/[S] graph was drawn by measuring the activities at the fixed concentration of GSH and 5 different suitable concentrations of CDNB, and K_M and V_{max} values for CDNB were calculated from these graphs. Then, the same procedures were performed for the fixed concentration of CDNB and the GSH substrate at 5 different appropriate concentrations of GSH [19].

3. RESULTS

A standard graph was prepared for the determination of protein amounts in homogenate, ammonium sulfate precipitate and purified enzyme solution and shown in Figure 1.

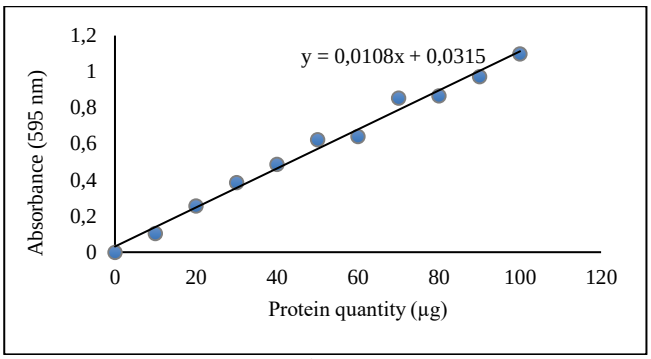

Figure 1. Standard graph obtained for quantitative protein determination

The ammonium sulfate precipitation procedure was performed as described in the Materials and Methods section, and the results are shown in Table 2.

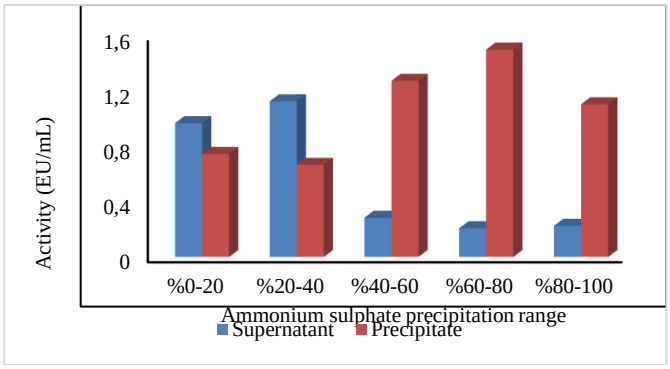

Figure 2. Ammonium sulfate precipitation interval graph

Purification of the enzyme was performed as described in the Materials and Methods section, and the specific activity, yield%, activity, total activity, protein, total protein and purification coefficient values were calculated and indicated in Table 1. As seen in the Table 1, 8.35 EU/mg GST enzyme with protein specific activity was purified with 24.56 fold and 8% yield.

Table 1. Purification results of glutathione S-transferase enzyme

Steps	Total Volume (mL)	Activity (EU/mL)	Total Activity (EU/mL)	Protein (mg/mL)	Total Protein (mg)	Specific Activity	Yield %	Purification Coficient
Homogenate	20	1,633	32,66	4,809	96,18	0,340	100	1
Ammonium Sulfate Precipitation (40-100%)	8	2,470	19,76	5,735	45,88	0,430	60	1,26
Affinity Chromatography	6	0,440	2,640	0,052	0,316	8,350	8	24,56

As mentioned in the Materials and Methods section, the activity values of the eluates after performing the gradient elution process are shown in Figure 3.

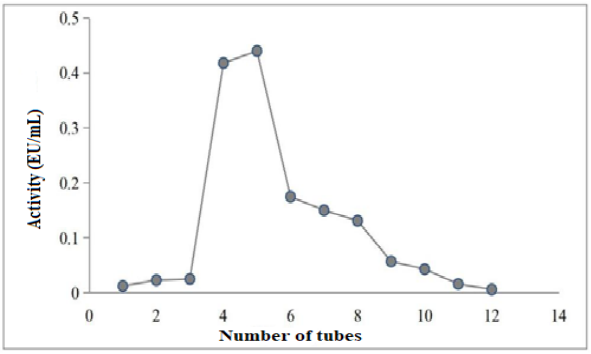

Figure 3. Elution graph obtained in the purification process

SDS-PAGE was performed as described in the Materials and Methods section, and its photograph was taken and shown in Figure 4. In addition, using the SDS-PAGE photograph in Figure 4, the R_f-Log MK graph was drawn to determine the molecular mass of the subunit of the enzyme and shown in Figure 5.

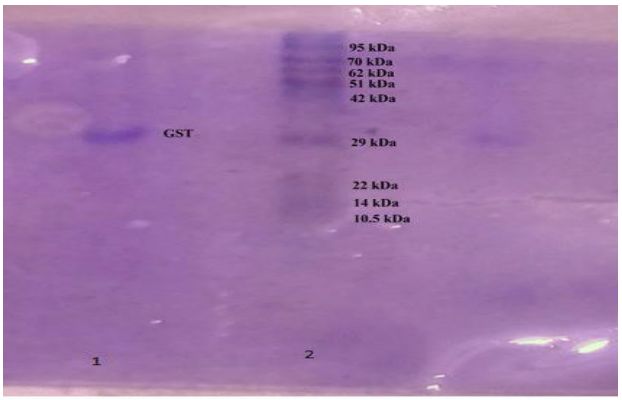

Figure 4. SDS-PAGE photograph. well 1 pure enzyme, 2 well standard proteins

As described in the materials and methods section, optimum pH determination studies were performed and is shown in Figure 5.

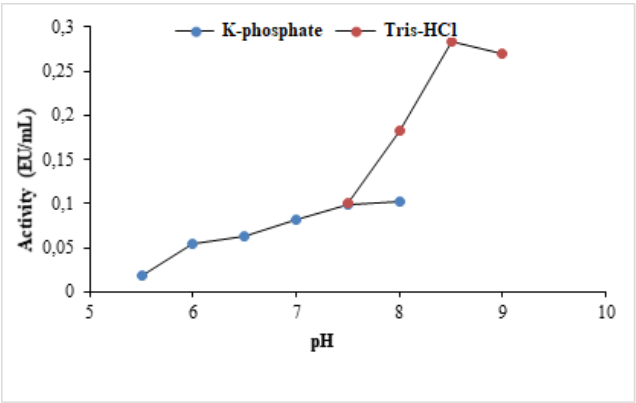

Figure 5. pH-activity plot using Tris HCl and K-Phosphate buffers

Stable pH studies with K-phosphate and Tris-HCl buffers as described in the materials and methods section are shown in Figure 6 and Figure 7.

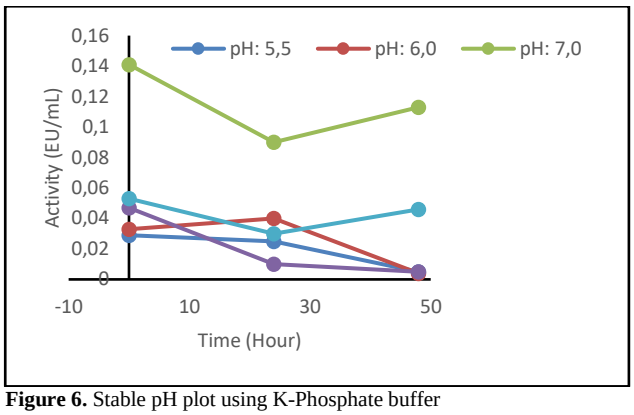

Figure 6. Stable pH plot using K-Phosphate buffer

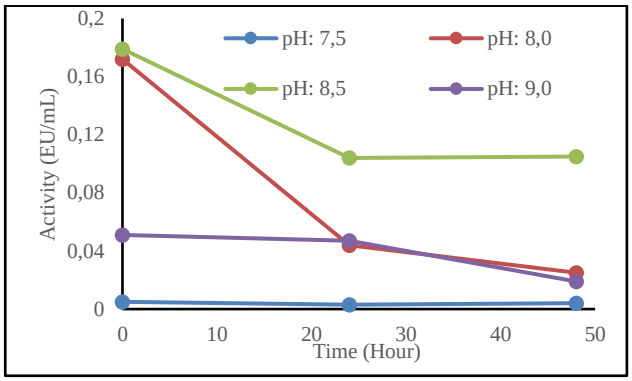

Figure 7. Stable pH plot using Tris-HCl buffer

Optimum ionic strength studies were performed as described in the Materials and Methods section, and the results were shown in Figure 8.

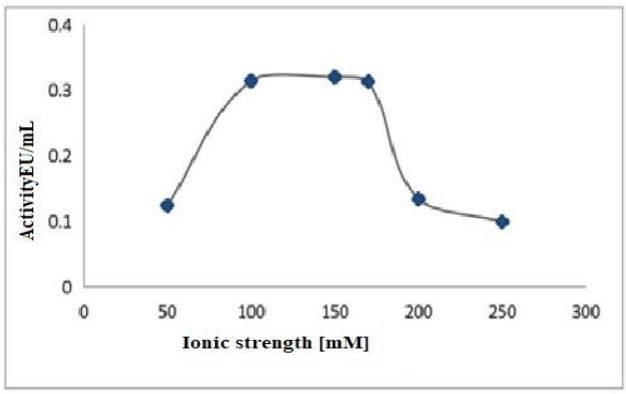

Figure 8. Optimum ionic strength graph

Optimum temperature studies are performed as described in the Materials and Methods section, and the results are shown in Figure 10.

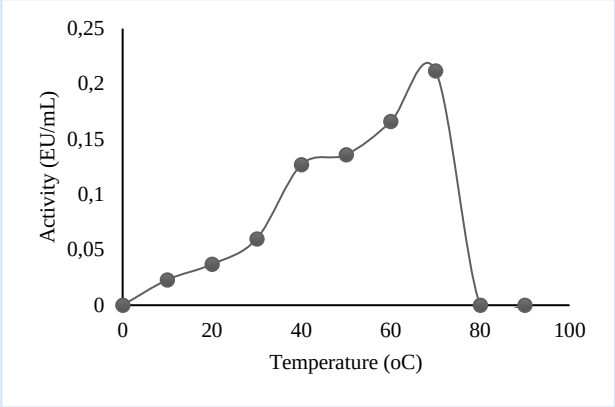

Figure 9. Optimum temperature graph

As described in the Materials and Methods section, 1/V-1/[S] graphs were drawn to calculate the K_M and Vmax values for CDNB and GSH, which are the substrates of the enzyme, and are shown in Figure 10 and Figure 11.

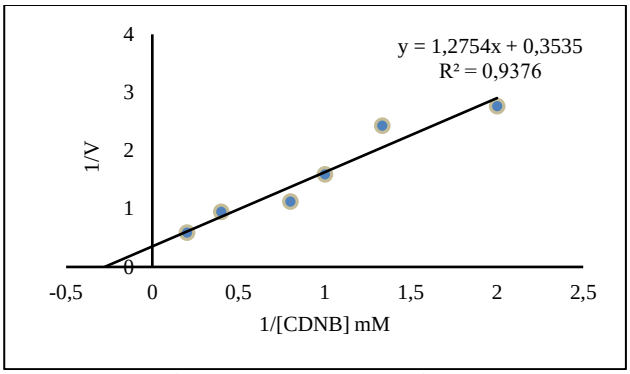

Figure 10. Graph of 1/V to 1/[CDNB] obtained for CDNB substrate

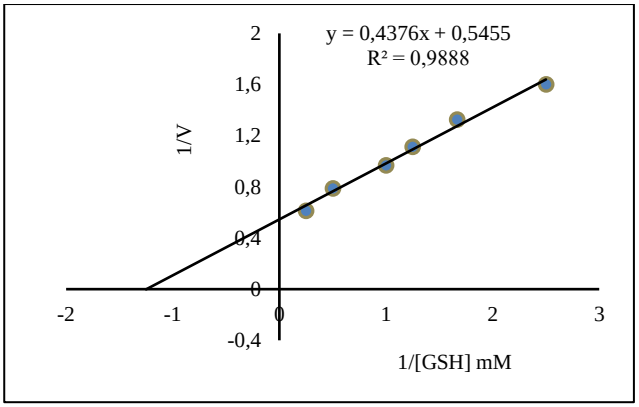

Figure 11. Graph of 1/V-1/[GSH] obtained for the GSH substrate

4. DISCUSSION AND CONCLUSION

Glutathione S-Transferase enzymes especially act as phase-II detoxification enzymes. Although they generally perform their functions in the cytosol, they also have functions such as conjugation of electrophilic substrates to glutathione. Cytosolic mammalian GST enzymes have been characterized and classified as α, μ, π, and θ. A few new classifications have also been made in non-mammalian organisms. Living organisms are exposed to many chemicals, xenobiotics, toxic and carcinogenic substances during feeding. In the last century, this threat has increased due to the chemicals thrown into the environment. Against all these threats, systems created in living things automatically activate and eliminate harmful substances. If the threats are not eliminated, serious problems arise. One of the most important substances used for the elimination of xenobiotics involved in metabolism is the GSH molecule. GSH provides conversion by making conjugate with the substances in question under the catalysis of GST enzymes. This results in a reduction of hydroperoxides. In addition to their catalytic properties, GST enzymes are enzymes with broad ligand binding properties [20-25].

Considering the above-mentioned features of the GST enzyme, it seems to be a very important metabolic enzyme. Therefore, within the scope of this study, the GST enzyme was purified from chicken liver and characterized.

Measuring the amount of protein in the samples during the purification studies is very important for the study. Protein determination was performed according to the Bradford method. The sensitivity of this method is high and it is less affected by the disturbing factors in the environment [17].

As can be seen from Figure 2, it is seen that the majority of the protein to be purified does not precipitate in the precipitation process between 0-20% and 20-40%. While the amount of enzyme precipitated increases in the range of 40-60%, it seems that the amount of precipitation reaches a maximum in the range of 60-80%. These ranges were evaluated and the precipitation range was accepted as 40-80%. This precipitation range was found to be 20-80% in quail liver and in some studies, ammonium sulfate precipitation was not performed [21,22].

In the literature, it is seen that the GST enzyme is purified from many living tissues by using some chromatographic methods. For example, the GST enzyme was purified using DEAE-cellulose anion exchange chromatography [26], CM-cellulose G-75 affinity chromatography [27], in another study using DEAE-cephagel and glutathione agarose affinity column [28]. In this study, glutathione-agarose affinity chromatography was used in terms of short purification time, high yield and low consumption of chemicals. As it can be seen from Table 1, GST enzyme with 8.35 EU/mg protein specific activity was purified from chicken liver with 8% yield as 24.56 fold in our study. With a similar method, the enzyme (with a specific activity of 11344,83 EU/mg.protein) was purified 1543.5 times from the gills of Lake Van pearl mullet with a yield of 82.25% [21]. In another study with quail liver, the GST enzyme with 15.86 EU/mg.protein specific activity was purified 46.1-fold with a yield of 12.36% [2]. While the specific activity, % yield and purification coefficient obtained in our study are lower than Zaric's study, they are similar to Taysi's study results [21,22].

Looking at Figure 3, it is seen that the enzyme is eluted from the 3rd tube. In the 3rd tube, there is no significant activity and the enzyme activity is the highest in 4.5.6.7.

and 8 tubes. Therefore, in kinetic studies 4.5.6.7. and 8th tubes were combined and used.

As seen in Figure 4, the presence of a single band in the first well of the SDS-PAGE photograph obtained after the SDS-PAGE process shows that the enzyme was obtained pure. In addition, the molecular mass of the enzyme, obtained under denaturing conditions, was found to be 30.9 kDa, with the help of the graphic obtained by using this photograph and shown in Figure 5. In the literature, this value was 25.1 kDa for quail liver [22], 22.5 kDa for *E.coli* GST enzyme [28], 26 kDa for rat liver GST enzyme [29], 23 kDa for GST enzyme for mullet liver [30], 22.3 kDa for human liver GST enzyme [31], and 27 kDa [32] for bovine brain GST enzyme, 25.2 kDa for rat erythrocyte GST enzyme [41] . It is seen that the molecular mass found in this study is close to the values found in the literature.

As seen in Figure 6, Tris-HCl with pH 7.5 8.0, 8.5 and 9.0 and pH 5.5, 6.0, 6.5, 7.0, 7.5 A graph was obtained by measuring enzyme activities in K-phosphate buffers of pH 8.0. As seen from the graph, the highest activity was obtained in Tris-HCl buffer at pH= 8.5. Therefore, the optimum pH was accepted as 8.5 in Tris-HCl buffer. Similar results were obtained in quail liver (pH= 8.5, Tris-HCl) [22], for E. coli (pH= 7.0) [28], in albus fish liver pH= 7.0-7.5 [33], in human blood serum (pH= 5.5) [34], in Lake Van fish (pH= 7.3) [21].

As can be seen from Figures 7 and 8, it is seen that the maximum activity for the enzyme in stable pH studies with K-phosphate and Tris-HCl buffers is obtained at pH= 8.5 in Tris-HCl buffer, which is the best maintained pH for 48 hours. Therefore, the pH value of the enzyme was accepted as 8.5. Similar results were found for gill GST enzyme of Lake Van fish [21], 8.5 [22] for quail liver, 8.0 [35] for rainbow trout liver.
As can be seen from Figure 9, when the activity measurements measured with various concentrations of Tris-HCl buffer are examined, it is seen that the highest activity is 150 mM. Therefore, the optimum ionic strength was accepted as 150 mM. In the literature review, this value was found to be 1400 mM [22] in quail liver and 120 mM [21] in Lake Van fish. The result we found is close to the optimum ionic strength value for Lake Van fish.

In order to find the optimum temperature, the enzyme activity was measured every 10ºC in the 0-90°C temperature range and shown in Figure 9. As seen in Figure 10, the highest activity was obtained as 70°C. Therefore, the optimum temperature was accepted as 70ºC. In the literature researches; It was found as 35 °C [21] in Lake Van fish, 35ºC [33] in albus fish, 30°C [36] in rainbow trout erythrocytes, 55 °C [22] in quail liver, 50ºC [28] for *E. coli*, 65°C [34] in human blood serum. As can be seen from these results, the temperature we found has a higher value than all those found in the literature. Since this situation is related to the amino acid sequence and the environment, it is normal for it to vary from living thing to living thing and from tissue to tissue.

It is extremely important to find the K_M and V_{max} values for an enzyme's substrates. As seen in Figures 10 and 11, Linaweaver-Burk graphs were drawn at 5 different substrate concentrations for the enzyme's substrates CDNB and GSH, and K_M and V_{max} values were found for each substrate with the help of these graphs. The K_M constants obtained for CDNB and GSH were 3.6 and 0.802 mM, respectively, and the V_{max} values were 2.829 and 1.833 EU/mL, respectively. According to these results, it can be said that the GSH substrate, which has a lower K_M constant, has a greater interest in the enzyme than the CDNB substrate. In the literature review, K_M values for quail liver in GSH and CDNB substrates were 0.114 and 0.672 mM, respectively; The V_{max} value was also 0.048 and 0.047 EU/mL [22], the K_M values for albus fish liver were 0.35 and 0.42 mM [37], respectively, the K_M values for GSH and CDNB for human blood serum were 4.11 and 2, respectively. 8 mM [34], K_M constants 0.59 and 1.057 mM [21] for Lake Van fish gill, K_M values 0.5 and 0.42 mM [37] for tilapia fish gill, 0.0395 for rainbow trout erythrocytes, respectively and 0.259 mM [36]. When our results were compared with the literature, it was seen that generally similar results were obtained.

Acknowledgement

This study was supported by Bingöl University BAP Coordination Unit. Bingöl University Projects Coordination Unit (Project number: BAP-FEF-2022.001).

REFERENCES

[1] Temel Y, Ayna A, Hamdi Shafeeq I, Ciftci M. In vitro effects of some antibiotics on glucose-6-phosphate dehydrogenase from rat (Rattus norvegicus) erythrocyte. Drug Chem. Toxicol. 2020; 43(2): 219-223.
[2] Bayindir S, Ayna A, Temel Y, Ciftci M. The synthesis of new oxindoles as analogs of natural product 3, 3′-bis (indolyl) oxindole and in vitro evaluation of the enzyme activity of G6PD and 6PGD. Turk. J. Chem. 2018;42(2): 332-345.
[3] Bayindir S, Temel Y, Ayna A, Ciftci M. The synthesis of N-benzoylindoles as inhibitors of rat erythrocyte glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. J. Biochem. Mol. Toxicol. 2018;32(9): e22193.
[4] Autrup H. Genetic polymorphism in human xenobiotica matebolizing enzymes as susceptibility factors in toxic response. Mutat. Res. 2000;464: 65-76.
[5] Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003;349:474-485.
[6] Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of nonmammalianmembers of an ancient enzyme superfamily. Biochem J. 2001;360:1-16.
[7] Özaslan MS, Demir Y, Küfrevioğlu O I, Çiftci M. Some metals inhibit the glutathione S-transferase from Va Lake fish gills. J Biochem Mol Toxicol. 2017;73:e21967.

[8] Espinoza HM, CR Williams and EP Gallagher. Effect of cadmium on glutathione S-transferase and metallothionein gene expression in Coho Salmon liver, gill and olfactory tissues. Aquat Toxicol. 2012;110: 37-44.
[9] Aksoy AS. Bazı flavonoidlerin sığır karaciğer glutatyon stransferaz enzimi üzerine etkilerinin araştırılması. (Yüksek Lisans Tezi), Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Kimya Anabilim Dalı, Balıkesir; 2018.
[10] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu. Rev. Pharmacol Toxicol. 2005;45: 51-88.
[11] Cnubben NHP, Rietjens IMCM, Wortelboer H, van Zanden J, van Bladeren J. The interplay of glutathione-related processes in antioxidant defense. Environ Toxicol Pharmacol. 2001;10:141-152.
[12] Armstrong RN. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol. 1997;10(1):2-18.
[13] Baş O. Dinitrocresol'ün (Rat rattus norvegicus) Sıçan glutatyon S-transferaz enzim aktivitesine etkisi (Yüksek Lisans Tezi) Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Bursa; 2006.
[14] Fleischner GK, Kamisaka IM, Arias IM. Immunoloanalysis of rats and human ligandins. Glutathione: Metabolism and Function, 1976; 229-235.
[15] Toribio F, Martinez-Lara E, Pascual P, Lopez-Barea J. Methods for purification of glutathione peroxidase and related enzymes. J Chromatogr B. 1996;684:77-97.
[16] Güvercin S, Erat M, Şakiroğlu H. Determination of some kinetic and characteristic properties of glutathione s-transferase from bovine erythrocytes. Protein Pept. Lett. 2008;15(1): 6-12.
[17] Laemmli DK, Lajmanovich RC, Andre´s M, Attademo AM, Peltzer PM, Junges CM. Cleavage of structural proteins during in assembly of the head of bacteriophage T4. Nature, 1970; 277, 680-685.
[18] Ahmed B. M, Temel Y, Çiftçi M. Purification and characterization glutathione S transferase enzyme from quail (Coturnix, coturnix japonica) heart and investigation the effect of some metal ions on enzyme activity. C S J. 2019;40(4):802-812.
[19] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976;72:248-251.
[20] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymmatic step in mercapturic acid formation. J Biol Chem.1974;246:7130-7139.
[21] Lineweaver H, and Burk D. The determination of enzyme dissociation constants. J Am Chem Soc. 1934;56 (3):658-666.
[22] Awasthi YC, Dao DD, Saneto RP. Interrelationship between anionic and cationic forms of glutathione S-transferases ofhuman liver. Biochem J. 1980;191:1-10.
[23] Nelson DL and Cox MM. Lehninger Principles of Biochemistry, Fourth edition. W.H. Freeman and Company, New York; 2004.
[24] Zariç Y. Glutatyon S-Transferaz Enziminin Van Gölü İnci Kefali Balığı *(chalcalburnus Tarichi)*Solungacından Saflaştırılması, Karakterizasyonu, Bazı Metal Ve Pestisitlerin Enzim Aktivitesi Üzerine Etkilerinin İncelenmesi. (Yüksek Lisans Tezi), Ağrı İbrahim Çeçen Üniversitesi Fen Bilimleri Enstitüsü, Ağrı; 2018.
[25] Taysi MŞ, Temel Y. Glutathione S-transferase: Purification and characterization from quail (Coturnix coturnix japonica) liver and the impact of some metal ions on enzyme activity. Bionanoscience. 2021;11: 91-98.
[26] Gözükara ME. Biyokimya, Nobel Matbaacılık, İstanbul; 2011.
[27] Keha E, Küfrevioglu Öİ. Biyokimya, Aktif Yayınevi, Erzurum; 2009.
[28] Dawn B, Marks Allan D, Mark, Colleen M, Smith. Basic Medical Biochemistry a clinical approach. A Wawely Company; 1996.
[29] Loscalzo J, Freedman J. Purification and characterization of human platelet glutathione-S transferase. Blood, 1986; 67, 1595-1599.
[30] Arca P, Garcida P, Hardisson C, Suarez JE. Purification and study of a bacteril glutathione S-transferase Area de Microbiologia, Fac. med.1990;1:77-79.
[31] Iizuka M, Inoue Y, Murata K, Kimura A Purification and some properties of glutathione S-transferase from escherichia coli B. J Bacteriol Res. 1989;6039-6042.
[32] Hiratsuka A, Sebata N, Kawashima K, Okuda H, Ogura K, Watabe T, Satoh K, Hatayama I, Tsuchida S, Ann Ishikawa TA. New class of rat glutathione Stransferase Yrs-Yrs inactivating reactive sulfate esters as metabolites of carcinogenic arylmethanols. J B C. 1990;265(20):11973-11981.
[33] Barcena JAE, Martinez-Lara SG, George J, Lopez-Barea. Purification and characterization of multiple glutathione s transferase isoenzymes from grey mullet liver. CMLS. 1997;759-768.
[34] Novoa-Valinas MC, Perez-Lopez M, Melgar MJ. Compparative study of the prufication and characterization of the cytosolic glutathione S-transferase from two salmonid species: Atlantic salmon (Salmo solar) and Brown trout (Salmo Trutta). C B P. part C, 2002;131:207-213.
[35] Young PR, and Briedis AV. Purification and kinetic mechanism of the major glutathione S-transferase from bovine brain. Biochem J. 1989;257:541-548.
[36] Huang Q, Liang L, Wei T, Zhang D, Zeng QY. Purification and partial characterization of glutathione transferase from the teleost monopterus albus. C B P Part C. 2008;147:96-100.
[37] Türkanoğlu A. Human serum arylesterase and glutathione S-transferase activities in patients with ischemic stroke compared to healthy controls. Approval of the Thesis Master of Science in Biochemistry Department, Middle East Technical University, Ankara; 2007.

[38] Tekman B, Ozdemir H, Senturk M, Ciftci M. Purification and characterization of glutathione reductase from rainbow trout (Oncorhynchus mykiss) liver and inhibition effects of metal ions on enzyme activity. C B P Part C. 2008;148:117-121.
[39] Çomaklı V, Çiftçi M, Küfrevioğlu Öİ. Gökkuşağı alabalık eritrositlerinden glutatyon S-transferas enziminin saflaştırılması ve bazı antibiyotiklerin enzim aktivitesi üzerine etkilerinin incelenmesi. Hacettepe J Biol Chem. 2011; 39 (4): 413-419.
[40] Hamed RR, Maharem TM and Guinidi RAM. Glutathione and its related enzymes in the nile fish. Fish Physiol Biochem. 2004;30 (3-4): 189-199.
[41] Ayna A, Khosnaw L, Temel Y, Ciftci M. Antibiotics as inhibitor of glutathione S-transferase: biological evaluation and molecular structure studies. Curr Drug Metab. 2021; 22(4):308-314.