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Comparative Studies on Glutathione S-Transferases from Chicken Liver and Heart

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Abstract

Glutathione S-transferases from chicken liver and heart have been purified (22 and 34-fold) at 44% and 56% yield from chicken liver and heart, respectively, and the specific activity for glutathione S-transferase was 85 mole/min/mg protein for chicken liver, while 80% for chicken heart GST enzyme when 1-chloro-2,4-dinitrobenzene was used as the second substrate. The affinity purified enzyme from chicken liver and heart displayed a native molecular weight of about 55,000 and 54,000, respectively, as determined by gel filtration using Sephadex G-100 column.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate analysis of the purified enzyme showed two different-size subunits. The subunit molecular weights of enzymes purified from chicken liver and heart were 28,000 and 27,000. Native polyacrylamide gel electrophoresis analysis of the enzyme from the examined tissue demonstrated a single protein band, and all the glutathione S-transferase activity was associated with this protein band. The chicken liver and heart enzyme showed a pH optimum of about 6.8 and low sensitivity to heat and lost about 50% of its activity after 7.0 days of storage at room temperature.

The purified chicken liver and heart glutathione S-transferase preferred 1-chloro-2,4-dinitrobrnzene as a second substrate but also used 1,2 epoxy-3-(nitrophenoxy) propane and ethacrynic acid at different rates.

The chromatographic technique resolves the glutathione S-transferase of chicken liver into eight distinct isoenzymes with apparent pI of 9.6, 9.0, 8.6, 8.4, 8.2, 8.0, 7.96, and 7.93, while the chicken heart glutathione s- transferase isoenzymes have four apparent pI of 6.8, 6.2, 5.4, and 4.5, respectively. The GST is composed of two semi-identical subunits of molecular weight 28,000 and 27,000, and they are immunologically similar.

The effect of various inhibitors and the substrate activity of the chicken liver and heart were tested.

Keywords: *Glutathione S-Transferase (GST); 1-Chloro -2,4-Dinitrobenzene (CDNB); Affinity chromatography; Chromatofocusing; Inhibitors; Isoenzymes; Xenobiotics; Biomarkers; Cancer resistance.*

1. Introduction

Glutathione-S-transferase (GST, EC 2.5.1.18) is a multifunctional protein that plays a pivotal role in cellular detoxification processes, protecting organisms from the harmful effects of xenobiotics and oxidative stress. GSTs catalyze the conjugation of glutathione (GSH) to electrophilic substrates, facilitating their excretion and reducing cellular damage [7,12]. Beyond their catalytic functions, GSTs are involved in non-catalytic activities, such as ligand binding, modulation of signaling pathways, and regulation of cellular redox homeostasis [17,18]. These enzymes are ubiquitously expressed across a wide range of organisms, from bacteria to mammals, underscoring their evolutionary conservation and biological importance [12,17].

The role of GSTs in mitigating oxidative stress and damage is particularly critical in tissues exposed to high levels of reactive oxygen species (ROS) and environmental toxins. In mammals, GSTs are highly expressed in the liver, where they contribute to the detoxification of drugs, carcinogens, and endogenous metabolic byproducts such as lipid peroxides [7,12]. Habig and Jakoby were among the first to systematically characterize GST isozymes, identifying a diverse array of forms in the liver tissues of rats, mice, and humans [7,12]. Recent studies have expanded our understanding of GST diversity, revealing species-specific and tissue-specific variations in isozyme expression and function [10,15]. These findings highlight the adaptability of GSTs in responding to different metabolic and environmental challenges [17,18].

Research on GSTs in rat liver cytosol has demonstrated the presence of at least 12 distinct electrophoretic forms, each with unique substrate specificities and kinetic properties [19]. Among their many functions, GSTs are implicated in the metabolism of bilirubin, a byproduct of heme catabolism [14]. The high affinity of GSTs for bilirubin and their liver-specific expression suggest a role in bilirubin detoxification and transport [14]. Recent structural and biochemical studies have provided insights into the binding of bilirubin to GSTs at sites distinct from the catalytic active site, further supporting their involvement in bilirubin metabolism [14,15].

In addition to their role in detoxification, GSTs have been implicated in the regulation of cell proliferation, apoptosis, and inflammation, making them potential targets for therapeutic intervention in diseases such as cancer, neurodegenerative disorders, and metabolic syndromes [21]. For example, overexpression of GSTs in cancer cells has been linked to drug resistance, highlighting the need for GST inhibitors as adjuvants in chemotherapy [21]. Awasthi et al. [1] and Norberg & Ericson [20] have demonstrated the inhibitory effects of bromophenol blue on GST activity, providing a foundation for the development of GST-targeted therapeutics.

2. Materials and Methods

2.1 Materials

2.1.1 Animals and tissue

Ten one-month-old female white chickens (Gallus domesticus) were obtained from the Yarmouk University Animal House. Immediately after euthanasia, fresh liver and heart tissues were removed, packed in ice, and transported to the laboratory. The tissues were either processed directly or stored at -20 °C until further use. For antibody production, four-month-old white rabbits raised and bred at the Yarmouk University Animal House were used.

This study was carried out with approval from Irbid National University Research Ethics Committee (INUREC); Serieal number: IRB0005(17-2-2025)).

The euthanasia was performed in full accordance with the established guidelines and protocols outlined above.

2.1.2 Chemicals

Reduced glutathione (GSH), reduced glutathione-linked agarose, gel filtration protein standards, SDS-electrophoresis protein standards, sodium dodecyl sulfate (SDS), Coomassie Brilliant Blue R-250, Tris-base, bovine serum albumin (BSA), complete and incomplete Freund's adjuvants, sodium azide, β-mercaptoethanol, acrylamide, N,N'-ethylene bisacrylamide, and Sephadex G-100 were purchased from Sigma Chemical Company (MO, USA). 1-Chloro-2,4-dinitrobenzene (CDNB) and bromophenol blue were obtained from BDH Laboratory Reagents (England).

Polybuffer Exchanger 94 and Polybuffer 74 were ordered from Pharmacia Fine Chemicals (Uppsala, Sweden). Tetramethylethylenediamine (TEMED) was purchased from Bethesda Research Laboratories (USA). Barbital buffer was obtained from Gelman (USA). The herbicide (oxadiazolone) was acquired from Supelco Inc. (Switzerland), and the insecticide (diazinon) was obtained from Société des Usines Chimiques Rhône-Poulenc (France). All other chemicals were obtained from commercial sources and of high purity.

2.2 Methodology

2..2.1 Preparation of Tissue Extract

A 20 g portion of chicken liver and heart was finely chopped into small pieces and separately homogenized with 0.1 M potassium phosphate buffer (pH 6.5) in a 1:2 (w/v) ratio. Homogenization was performed for one minute at maximum speed using a Waring blender. All subsequent steps were carried out at 0–4 °C unless otherwise stated. The homogenates were centrifuged at 37,000 ×g for 30 minutes using an MSE Europa 24 M centrifuge. The pellet was discarded, and the supernatants were filtered through glass wool to remove residual lipids.

2.2.2 Enzyme Assay

Glutathione S-transferase (GST) activity was determined spectrophotometrically using a PYE Unicam SP 8-400 spectrophotometer. The enzyme's activity toward substrates including 2,4-dichloronitrobenzene, 1,2-epoxy-3-(p-nitrophenoxy) propane, p-nitrobenzyl chloride, 1,2-chloro-2,4-dinitrobenzene, p-nitrobenzoyl azide, ethacrynic acid, and 5-androstan-3,17-dione was measured as described by Habig et al. (1974). Enzyme activity was expressed as µmol/min, and specific activity was calculated as µmol/min/mg of protein. Protein concentration was determined using the method of Lowry et al. (1951), with bovine serum albumin as the standard.

2.2.3 Purification of Glutathione S-Transferase from Chicken Liver and Heart

Reduced glutathione agarose beads were swollen in 0.1 M potassium phosphate buffer (pH 6.5) and packed into a 1.5 cm column as a slurry. The column was equilibrated with the same buffer at a flow rate of 30 mL/h. A 20 mL aliquot of the 37,000 ×g supernatant (containing approximately 400 mg of protein) was loaded onto the column at a flow rate of 20 mL/h. The column was washed extensively with the equilibration buffer until the absorbance at 280 nm returned to baseline. Non-specifically bound proteins were removed by washing the column with 100 mL of the same buffer containing 0.2 M NaCl. The bound GST enzymes were eluted using 50 mM Tris-HCl buffer (pH 9.6) containing 10 mM reduced glutathione. Fractions (2 mL each) exhibiting the highest enzyme activity were pooled and dialyzed for 14 hours against 40 volumes of 0.1 M potassium phosphate buffer (pH 6.5). The dialyzed sample was concentrated using an Amicon PM 30 membrane ultrafiltration system.

2.2.4 Chromatofocusing of Chicken Liver and Heart Isoenzymes

Chromatofocusing was performed according to the manufacturer's instructions (Pharmacia). The GST enzymes purified from chicken liver and heart by affinity chromatography were concentrated to approximately 2 mL using an Amicon PM 30 membrane and applied separately to a chromatofocusing column (0.9 × 30 cm) packed with Polybuffer Exchanger-94 gel. The column was pre-equilibrated with 0.025 M Tris-HCl buffer (pH 9.4). The enzymes were eluted with 270 mL of Polybuffer 94 diluted in water (pH 4.0). Fractions (2 mL each) were collected, and enzyme activity and pH were measured immediately. Fractions with the highest enzyme activity were pooled, and the Polybuffer was removed by affinity chromatography.

2.2.5 Molecular Weight Determination

The subunit molecular weights of the purified glutathione S-transferases from chicken liver and heart were estimated using 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). The following protein standards were used for calibration: phosphorylase B (94,000 Da), bovine serum albumin (67,000 Da), egg albumin (43,000 Da), carbonic anhydrase (30,000 Da), soybean trypsin inhibitor (20,100 Da), and lactalbumin (14,400 Da).

2.2.6 Antibody Production

Antibodies against purified glutathione S-transferases (GSTs) from chicken liver and heart were produced in rabbits, following the method described by Hunaiti and Kolattukudy (1984). Approximately 350 µg of the isolated enzymes were emulsified with an equal volume (1:1 v/v) of Freund's complete adjuvant. The resulting emulsion (0.9 mL) was injected into the rabbits at a dose of 125 µg per injection. Booster injections, prepared with Freund's incomplete adjuvant (1:1 v/v), were administered at two-week intervals. Two weeks after the second injection, blood was collected from the rabbits, and the antiserum was separated by centrifugation at 4,000 ×g for 10 minutes.

The effect of the rabbit anti-enzyme serum on GST activity was determined by immunotitration. Aliquots of the enzyme (20 µg) were incubated with varying concentrations of antiserum, adjusted to a final volume of 15 µL using control rabbit serum. After incubation for 5 hours at 4 °C, the mixtures were centrifuged, and the enzymatic activity in the supernatants was

measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, as described in the enzyme assay procedure above.

2.2.7 Ouchterlony Double Immunodiffusion

Ouchterlony double immunodiffusion was performed on microscope slides using 1% agarose prepared in 75 mM barbital buffer (pH 8.8) containing 0.02% sodium azide. The agarose solution was boiled, poured onto the slides, and allowed to solidify. Wells with diameters of 2 mm or 4 mm were punched into the gel. Crude extracts of chicken liver and heart (10 µL) were added to the outer wells, while 15 µL of antisera was added to the central well. The slides were incubated in a humid chamber for 24–48 hours to allow for immunoprecipitation. After incubation, the slides were washed with 0.9% NaCl in distilled water and stained with 0.1% Coomassie Brilliant Blue R-250 in 5% acetic acid.

2.2.8 Inhibition Study

The inhibitory effects of sodium azide, bromophenol blue, and bisacrylamide on GST activity were investigated. Stock solutions of each inhibitor were prepared in ethanol and tested at final concentrations of 1 mM and 2 mM. A reaction mixture containing 20 µg of enzyme, 0.1 mM reduced glutathione, and the inhibitor in 0.1 M potassium phosphate buffer (pH 6.5) was incubated for 10 minutes at room temperature. The reaction was initiated by adding 1 mM CDNB, and the enzyme activity was measured as described previously. Control experiments were performed in the absence of inhibitors to account for baseline activity.

3. Results

3.1 Glutathione S-Transferase Activity

Glutathione S-transferase (GST) activity was detected in crude extracts of chicken liver and heart. The specific activity of the enzymes was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. The specific activities of GST in chicken liver and heart were found to be 3.80 µmol/min/mg protein and 2.37 µmol/min/mg protein, respectively.

3.2 Purification of Glutathione S-Transferase

To further characterize the enzyme, GST was purified from chicken liver and heart tissues using a rapid affinity chromatography method with glutathione-linked agarose. When the 37,000 ×g supernatants from chicken liver and heart were applied directly to the affinity column, all GST activity was retained, while the majority of non-specific proteins were not bound (Fig. 1).

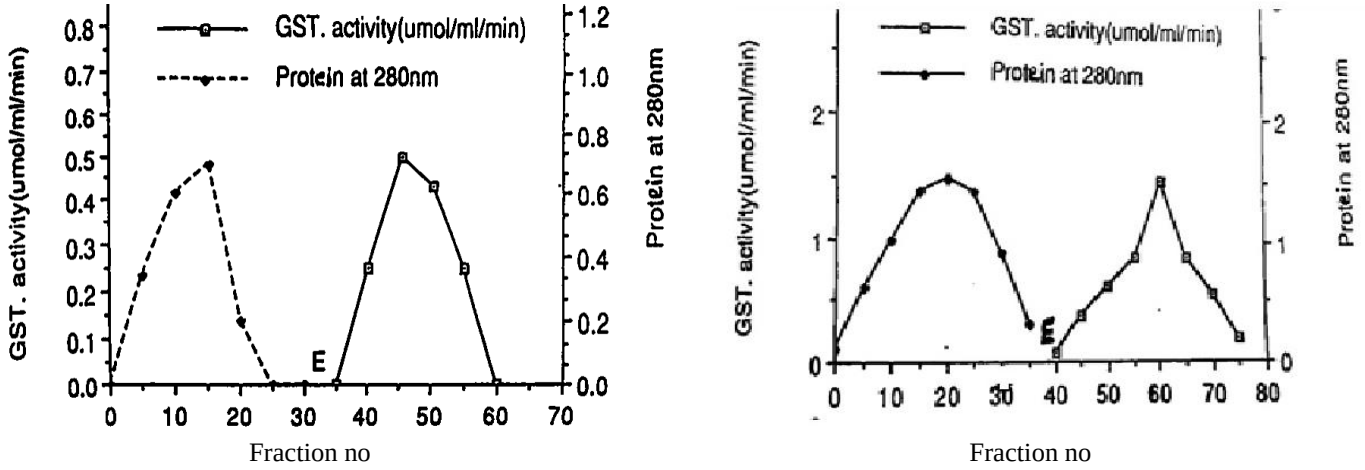

Fig 1.A and B: GST of Chicken liver and heart were purified by glutathione- linked agarose affinity chromatography. Protein was measured at 280nm after 1:40 dilution with 0.1M potassium phosphate buffer pH 6.5 E: elution buffer - Other experimental details are given in the text.

The enzymes were successfully eluted in a single sharp peak using 10 mM reduced glutathione in 50 mM Tris-HCl buffer (pH 9.6), as shown in Figures 1 and 2. This purification procedure yielded a recovery of over 44% and 56% of the applied glutathione S-transferase (GST) activity from chicken liver and heart, respectively. Additionally, the purification fold was approximately 22 for liver and 34 for heart, as summarized in Table 1.

Table 1: Purification of glutathione S-transferase from Chicken liver and heart. Experimental details are given in the text.

30 µg of enzyme in 3 ml of 0.1 M potassium phosphate buffer pH 6.5 was preincubated for 2 minutes at room temperature with inhibitors and I mM GSH. I mM l-chloro-2,4-dinitrobenzene was then added to start the reactions.

Purified glutathione S-transferase (GST) from chicken liver and heart exhibited high specific activity. The results indicated that the GST enzyme constituted approximately 4.6% of the total soluble protein in these tissues. The elution profile of glutathione S-transferase from liver tissue, as obtained through affinity chromatography, is presented below. (Fig.2).

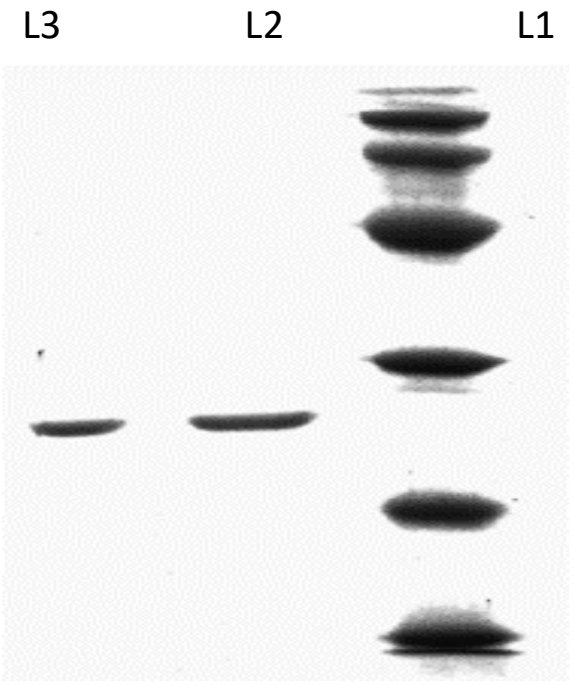

Figure 2: 12% SDS- PAGE-gel electrophoresis of purified Chicken liver and heart glutathione S-transferase. Lane1is standard protein markers, Lane2 and Lane3 are purified Chicken liver and heart enzymes. Experimental details are given in the text.

3.3 Effect of pH on Glutathione S-transferase activity.

The effect of pH on the rate of glutathione conjugation with CDNB was studied for purified glutathione S-transferase (GST) from chicken liver and heart. In all cases, GST activity was low at pH levels below 5. As the pH increased, the reaction rate increased, reaching an optimal pH of approximately 6.5 for the enzyme from both chicken liver and heart tissues. (Fig3).

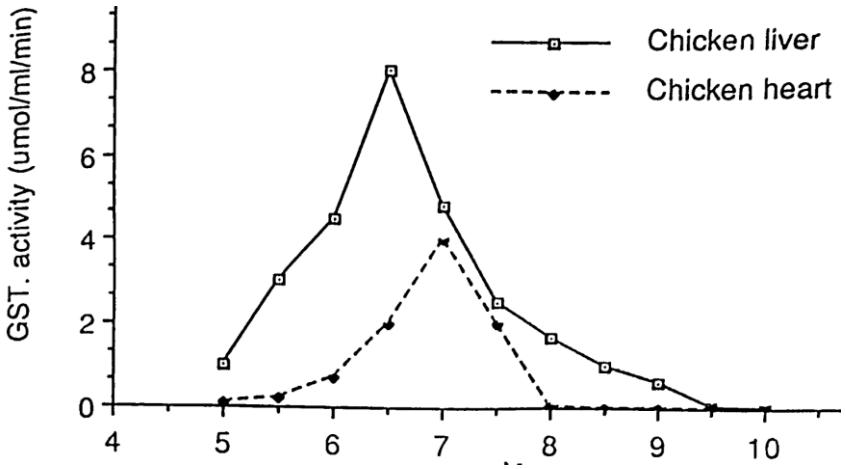

Fig.3: The effect of pH on the Chicken liver and heart GST activity.

3.4. Subunit Composition of Glutathione S-Transferase

The purity of glutathione S-transferase (GST) obtained from the affinity column was evaluated using 12% SDS-polyacrylamide gel electrophoresis. As shown in Fig. 3, the GST enzyme from chicken liver and heart displayed two distinct protein bands on the 12% SDS-polyacrylamide gel. The molecular weights of these bands, as determined from a linear plot of log molecular weight versus the mobilities of standard proteins, were approximately 28,000 and 27,000 (Fig. 4).

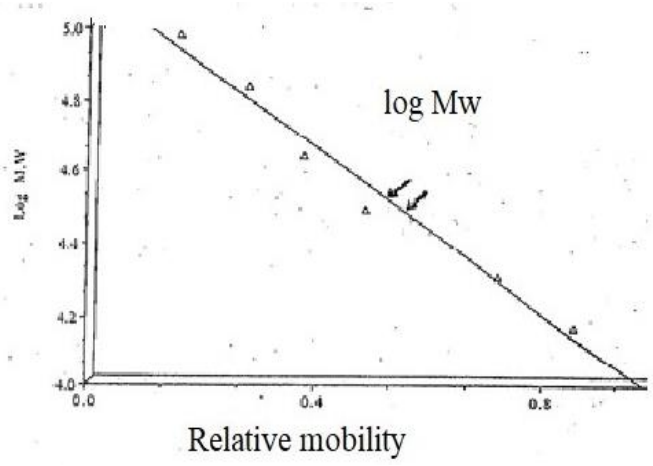

Fig.4: Determination of GST subunits molecular weight.

3.5 Isoenzymes resolution

The transferase of chicken liver was divided into eight and four peaks of activity by an isoelectric fractionation using Sephadex polybuffer exchanger 94 (Fig. 5.a&b). At a pH of roughly 8.7, the majority of the GST isozymes found in chicken liver and heart showed significant activity.

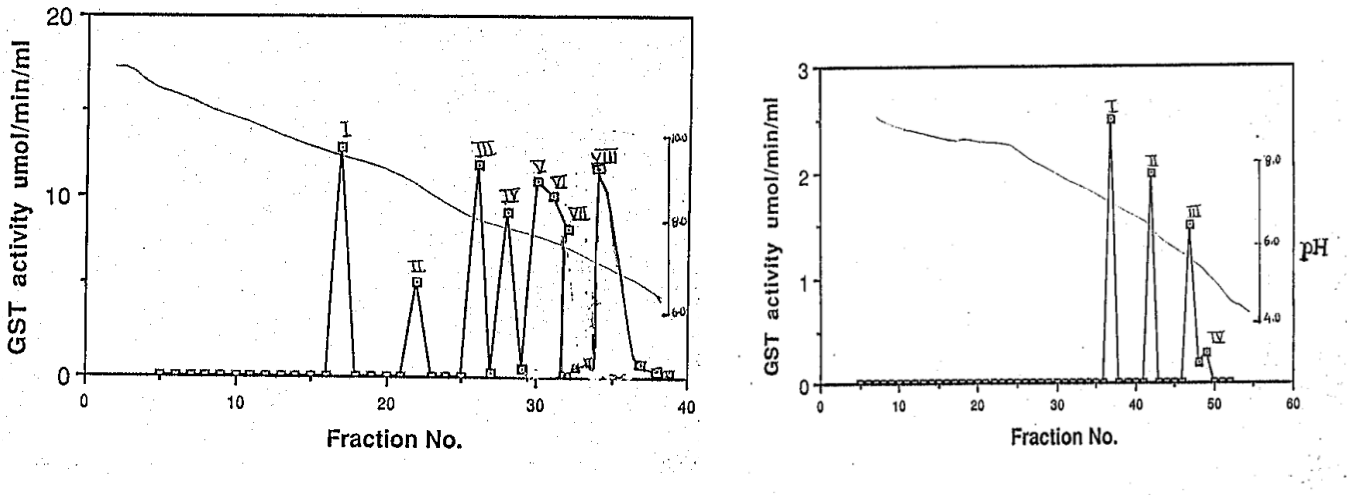

Fig5a: *Purification of various isoenzymes of Chicken liver GST by chromatofocusing Peak1 pI 9.6), Peak II (pI 9.2) , Peak III (pI 8.6) , Peak IV (pI 8.3), (Peak V (pI 8.1), Peak VI (pI 8.05), Peak VII , peakVIII(pI 8.5).*
Fig.5b*: Purification of various isoenzymes of Chicken liver GST- by chromatofocusing Peak1 (pI 6.8), Peak II(pI 6.2) , Peak III (pI 5.4) , Peak IV (pI).*

3.6 Substrate specificity

As shown in Table 2, the primary isoenzyme of chicken liver and heart (pI 8.4) utilized various substrates at lesser rates but demonstrated maximum rates of conjugation with GSH when 1–chloro-2,4-dinitrobenzene was utilized as a substrate. For 1,2-epoxy-3-(p-nitrophenoxy) propane and ethacrynic, no discernible rate was found. The other isoenzymes' restricted availability prevented their substrate specificity from being discovered.

Table 2 shows the substrate specificity of glutathione S-transferase, the main isoenzyme of chicken liver and heart.

Substrate	Concentration (mM)	Chicken liver and heart	% relative activity
1-chloro-2,4-dinitrobenzene	1.0	100	100
1,2-epoxy-3-nitrophenoxy) propane	5.0	6.8	18
Ethacrynic	0.2	0.95	17.5

Enzyme (30µg) was preincubated for 2 minutes at room temperature with inhibitors and 1 mM GSH in 3 ml of 0.l M potassium phosphate buffer pH 6.5. The reactions were then started by adding 1 mM 1-chloro-2,4-dinit robenzene.

3.7 Catalytic properties:
For a minimum of 7 minutes and 10 µg of protein per mL, the quantified activity of the refined enzyme was linear. As a result, only the starting velocities were employed in all following tests, which were all conducted within the linear range of time and protein.

3.8Ouchterlony:
The primary peak obtained from the chromatofocusing column (pI 8.7) was detected by the rabbit antibody developed against it, according to the Oucherlony double-diffusion study. either crude or pure enzyme was utilized as the antigen, As shown in Figure 6, it generated a solitary precipitin line. The antibody was also producing a single precipitin line for each of the remaining six enzyme peaks that were divided by chromatofocusing. Based on the completely merged immunoprecipitin lines that were obtained and the absence of spurs, the seven electrophoretic forms obtained by chromatofocusing seem to be immunologically related.

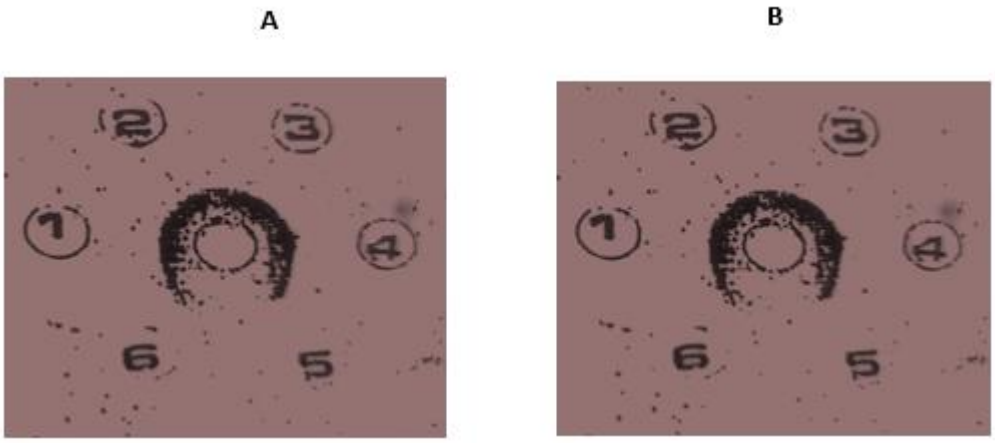

Fig. 6 A&B: Ouchterlony double-diffusion analysis. A: The centre well contained 20µ1 of rabbit antiserum raised against the major isoenzyme of chicken liver (pI 8.7), and outer wells 1, 2 and 3 ,4, 5, 6 contained increasing amount of purified isoenzyme While B figure:The centre well contained 20µ1 of rabbit antiserum raised against the major isoenzyme of chicken heart (pI 5.5), and outer wells 1, 2 and 3 ,4, 5, 6 contained increasing amount of purified isoenzyme.

Inhibitors	(%) of chicken liver GST inhibition	% of chicken heart GST inhibition
Sodium azide 1 mM	40.8	40
Sodium azide 2 mM	63.6	55
Bromophenol blue 1 mM	79.5	65
Bromophenol blue 2 mM	84.3	75
Bisacrylamide 1mM	33.4	25
Bisacrylamide 2mM	53.2	42

Table 3: Effect of inhibitors on purified Chicken liver and heart glutathione S-transferase.

3.9 Inhibitors
The rabbit enzyme was used to investigate inhibitors known to affect GSH-S-transferases from various sources (Table 3). While the other reagents shown inhibition at relatively high concentrations, the thiol-directed reagent iodoacetamide and the putative substrate N-(4-bromobutyl) phthalimide considerably hindered the enzyme activity (Table 3)

4. Discussion

The purification and characterization of glutathione S-transferase (GST) from chicken liver and heart offer a unique opportunity to explore the structural, functional, and evolutionary aspects of this enzyme family across species. GSTs are critical components of cellular detoxification systems, catalyzing the conjugation of glutathione (GSH) to electrophilic substrates, including xenobiotics and endogenous metabolites, thereby facilitating their excretion and reducing cellular damage [7,12]. This study provides a comparative perspective on GSTs from chicken tissues relative to those from mammalian sources, shedding light on both conserved and divergent features [2,10].

The GSTs purified from chicken liver and heart are heterodimeric proteins with molecular weights of approximately 54,000 and 53,000, respectively. This contrasts with the heterotrimeric structure of GSTs isolated from rat liver, which consist of subunits with molecular weights of 22,000, 23,500, and 25,000 [5,19]. The heterodimeric nature of chicken GSTs aligns more closely with GSTs from sheep and human livers, which also exhibit heterodimeric forms [2,15]. This structural similarity suggests a conserved functional role across species, particularly in detoxification and oxidative stress management [17,18].

The divergence in subunit composition between chicken and rat GSTs may reflect evolutionary adaptations to different metabolic demands or environmental pressures [10,13]. For instance, the heterotrimeric structure of rat liver GSTs could provide additional functional versatility, enabling the enzyme to interact with a broader range of substrates or regulatory molecules [5,19]. Further structural studies, including X-ray crystallography or cryo-electron microscopy, could elucidate the molecular basis for these differences and their functional implications [15,16].

The identification of at least eight distinct electrophoretic variants of GST in chicken liver and four forms in the heart underscores the tissue-specific expression and functional specialization of these enzymes [2,22]. The higher diversity of GST isoforms in the liver likely reflects its central role in detoxification and metabolism, necessitating a broader substrate range and regulatory capacity [10,15]. In contrast, the fewer isoforms in the heart may indicate a more specialized role, potentially related to the management of oxidative stress in a highly aerobic tissue [16,22].

The electrophoretic profiles of chicken GSTs share immunological similarities with human liver isoenzymes, as demonstrated by the cross-reactivity of antibodies raised against major chicken liver GST forms [23]. This suggests that certain epitopes and functional domains are conserved across species, despite differences in subunit composition and tissue distribution [15,23]. However, the evolutionary relationships among GST isoforms in chicken tissues remain incompletely understood. Observations of GST variations in chicken tissues resemble the isoenzyme C-4 in sheep liver, hinting at potential evolutionary connections [2,22].

The purification process enabled the characterization of GST isoforms through isoelectric focusing, revealing activity peaks at pI values of 8.7 for liver enzymes and 5.5 for heart enzymes [2,22]. These differences in isoelectric points likely reflect variations in amino acid composition and post-translational modifications, which may influence substrate specificity, enzyme stability, and interactions with other cellular components [16,22].

Kinetic studies of chicken GSTs have demonstrated high catalytic efficiency for the conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (CDNB), a model substrate commonly used to assay GST activity [2,22]. The kinetic parameters (Km and Vmax) of chicken GSTs are comparable to those of mammalian GSTs, further supporting their conserved functional role [2,15]. However, subtle differences in substrate affinity and catalytic efficiency may reflect adaptations to species-specific metabolic pathways or environmental challenges [10,13].

Bromophenol blue has been identified as a potent inhibitor of GST, with potential therapeutic applications in GST inhibition studies [1,20]. Inhibition of GST activity could enhance the efficacy of chemotherapeutic agents by reducing drug detoxification in cancer cells, where GST overexpression is often associated with drug resistance [21]. Further studies are needed to explore the structural basis of bromophenol blue binding to GST and its potential as a lead compound for drug development [1,20].

The conservation of heterodimeric GST structures across species, including chickens, sheep, and humans, suggests that these enzymes play a fundamental role in cellular physiology [2,15,22]. The similarities between chicken GSTs and the isoenzyme C-4 in sheep liver further highlight potential evolutionary connections and functional parallels [2,22]. Comparative genomic and proteomic analyses could provide deeper insights into the evolutionary history of GSTs and their adaptation to different physiological and environmental contexts [10,13].

5. Conclusion

The isolation and purification of glutathione S-transferase (GST) from chicken liver and heart have enabled a comparative analysis of this enzyme with GSTs from other mammalian sources. In rat liver, GSTs are heterotrimeric proteins composed of subunits with molecular weights of 22,000, 23,500, and 25,000. In contrast, GSTs from chicken liver and heart exhibit a heterodimeric structure, with subunits weighing approximately 28,000 and 27,000. This subunit composition is somewhat similar to that of GSTs from rabbit, human, and sheep livers, suggesting a degree of structural and functional conservation across species.

Using chromatofocusing, at least eight distinct electrophoretic forms of GST were identified in chicken liver, compared to four forms in the heart. Cross-reactivity studies using antibodies raised against the major electrophoretic forms revealed a functional and immunological link between these isoforms. This suggests that the multiple forms of GST in chicken tissues may share common epitopes or structural motifs, despite differences in their electrophoretic mobility.

Furthermore, the primary form of isoenzyme C-4 in sheep liver shows notable similarities to GSTs from chicken liver and heart, indicating potential evolutionary and functional parallels. These findings highlight the importance of comparative studies in understanding the structural diversity, functional specialization, and evolutionary relationships of GSTs across species

CRediT authorship contribution statement

Ahmad Al Hadidi: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. Abdelraheem Hunaiti: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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