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# Catalysis of Silver catfish Major Hepatic Glutathione Transferase proceeds via rapid equilibrium sequential random Mechanism

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ABSTRACT

Fish hepatic glutathione transferases are connected with the elimination of intracellular pollutants and detoxification of organic micro-pollutants in their aquatic ecosystem. The two-substrate steady state kinetic mechanism of Silver catfish (*Synodontis eupterus*) major hepatic glutathione transferases purified to apparent homogeneity was explored. The enzyme was dimeric enzyme with a monomeric size of 25.6 kDa. Initial-velocity studies and Product inhibition patterns by methyl glutathione and chloride with respect to GSH-CDNB; GSH-ρ-nitrophenylacetate; and GSH-Ethacrynic acid all conforms to a rapid equilibrium sequential random Bi Bi kinetic mechanism rather than steady state sequential random Bi Bi kinetic. α was 2.96 ± 0.35 for the model. The pH profile of $V_{max}/K_M$ (with saturating 1-chloro-2,4-dinitrobenzene and variable GSH concentrations) showed apparent pKa value of 6.88 and 9.86. Inhibition studies as a function of inhibitor concentration show that the enzyme is a homodimer and near neutral GST. The enzyme poorly conjugates 4-hydroxylnonenal and cumene hydroperoxide and may not be involved in oxidative stress protection. The *se*GST is unique and overwhelmingly shows characteristics similar to those of homodimeric class Pi GSTs, as was indicated by its kinetic mechanism, substrate specificity and inhibition studies. The rate- limiting step, probably the product release, of the reaction is viscosity-dependent and is consequential if macro-viscosogen or micro-viscosogen.

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## 1. Introduction

Glutathione transferases (GSTs; E.C.2.5.1.18), multigene family of isoenzymes, are widely distributed in animals, plants and microorganisms [39,55]. They constitute important roles in detoxification of toxic compounds [69,5] and in non-enzymatic ligand binding functions [72]. They are linked with peroxidase and isomerase activity [27] and are also involved in the inhibition of Jun N-terminal kinase [82]. These functions make glutathione transferase superfamily important target of pharmacological and toxicological studies; and are candidates for anticancer and allergy drug therapy. GSTs catalyzes the nucleophilic addition of thiol of reduced glutathione (GSH) to a wide range of electrophilic compounds [72].

GST isoenzyme families share a common fold and are obligate dimers [84]. They are either homodimers or heterodimers with subunit sizes ranging from 23 to 30 kDa and organized into two domains: GSH binding domain (at the N-terminal) and a xenobiotic substrate-binding domain at the C terminus [8,68]. GSTs are encoded by at least nine different gene families and into 15 different classes namely- alpha, beta, delta, epsilon, kappa, lambda, mu, omega, phi, pi, sigma, tau, theta, zeta, and rho in cytosol and membrane [83,57,39,42,59]. These classes are distinguished based on sequence identity, substrate specificities, antibody cross reactivity and sensitivity to inhibitors. GSTs that differ in amino acid sequences show 50% identities within a class and less than 30% between different classes [57,72]. The diversity of GST isoenzymes provides the capability to conjugate a very broad range of compounds [52].

GSTs exhibit a remarkable degree of catalytic diversity with single isoenzyme catalyzing multiple reaction type; and, are not substrate specific but show overlapping specificities among classes [16,58]. Different forms of GST differ in their catalytic properties and indicate different kinetic mechanism of detoxification [9]. Enzymological studies on GSTs have been proven pivotal to the understanding of structure-activity relationships and physiological regulations [46,55]. The kinetic mechanism of GSTs is clearly abstruse and is isoenzyme-dependent [8,44]. The Kinetic mechanism of Class Pi has been shown to be rapid equilibrium random Bi Bi kinetic mechanism [43,44] while class Mu is a steady-state random Bi Bi mechanism [45,44]. Till date, Class Alpha, historically known as ligandin, kinetic mechanism has not being established. The extents to which these different kinetic mechanisms are applicable or extended to other GST isoenzymes class still remain unclear. Despite the conjectural position, the acceptable kinetic

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mechanism, currently, is the random sequential model. Most GST kinetic mechanism models have been limited to conjugation of GSH with 1,2-dichloro-4-nitrobenzene but have not being extended to its other electrophilic substrates. Be that as it may, kinetic mechanism of GSTs may provide a veritable criterion to distinguish various GST isoenzymes classes, if properly explored exhaustively [43].

The potential physiological roles of piscine GSTs have been studied. Their economic value and relevance to aquatic toxicology as a potential pollution biomarker have been stressed [8,63]. Previous researches with GSTs in both aquatic and non-aquatic animals has indicated that the level of GST changes in response to certain environmental pollutants [9,8]. This is a step in understanding the metabolism of xenobiotic compounds that could pose serious ecologic and health problems in coastal areas exposed to industrial pollutions. Aquatic environment presents a unique situation, in terms of special types of living populations and exposure to pollution. The consequent examination of nature and function of hepatic GSTs from marine animals could provide clues to the survival of these organisms in adverse ecologic contexts. However, relatively little research has been done on GST isoenzymes from fish comparable to that of mammals [8,63]. Kinetic characterization of the cytosolic GSTs from several aquatic organisms has been described [76,2,21,9]. Fish GST kinetic study have complicated, inadvertently, by near silence and scanty details of fish GST kinetic mechanism and non-ideal kinetic mechanism for the isolated class.

Warri River is one example eutrophic ecosystem in oil rich Niger–Delta area of Nigeria that has been the focus of water pollution case study [23]. Exploitation of petroleum resources within the area has resulted into the discharge of petroleum hydrocarbon into the ecosystem [67]. The adjoining tributaries empty itself into this river with attendant deposition of tonnes of silts, finely divided agricultural matter and wastes. These accidentally endangered the aquatic and human lives in the area [1]. Silver catfish (*Synodontis eupterus*) is one of the catfish family widely distributed in Warri River with an important economic value. It is well known for its ability to withstand low dissolved oxygen and spawn readily. Catfish families play important economic roles in the lives of the community and serve as alternative and cheap source of daily protein. These economic roles have increased the activities of farming practices within and around Nigerian rivers to the extent that some farmers employ chemical poisons as a fishing method. Commercially important fish are sensitive to environmental pollutants [2]. Silver catfish (*Synodontis eupterus*) are known to be hardy and sturdy; and has high survival rate. The molecular basis of these attributes might be connected biochemical strategies adopted for its survival. This might be connected to the specialization of GSTs enzyme catalyzing detoxification reaction to cope with exogenous and endogenous chemical threats. The Silver catfish (*Synodontis eupterus*) GST is unknown. The present study was undertaken to determine the kinetic mechanism hepatic glutathione transferases of Silver catfish (*Synodontis eupterus*) employed in the detoxification of exogenous and endogenous organic micro-pollutants in its aquatic environment. From a comparative of view to other catfish family, this could add to body of models of toxicology research.

## 2. Materials and methods

### 2.1. Materials

Reduced glutathione (GSH), oxidized glutathione (GSSG), 1-chloro 2,4-dintrobenzene (CDNB), 7-chloro-4-nitrobenzene-2-oxa-1,3-diazole, ethacrynic acid, 1,2-dichloro-4-nitrobenzene (DCNB), bromosulfophthalein (BSF), ρ-nitrophenyl acetate (ρNPA), phenylmethanesulfonyl fluoride, cibracron blue, hematin are products of Sigma-Aldrich Chemical, St Louis, USA. DEAE-Trisacryl was from LKB, Villeneuve-la Garenne, France. Tributyltinacetate (TBTA) and triphenyltinchloride (TPTC) were purchased from Alfa Company (Karlerushe, Germany). Glutathione Sepahrose 4B$^{TM}$ is from Amersham Bioscience, Uppsala, Sweden. Other specialty chemicals were obtained from Sigma in the highest purity available unless noted otherwise. All other reagents were of analytical grade commercially available.

### 2.2. Methods

#### 2.2.1. Sample collection

Silver catfish (*Synodontis eupterus*) used for this study were obtained from a local "fish community" at Ikpesu market water side, Warri, Nigeria. The river has its major activities as fishing (fish farming) and sand mining/dredging by the inhabitants of the area. Silver catfish was collected from the river with the aid of fishnet. Fish were immediately transferred in a glass aquarium (120 l capacity) to the laboratory and acclimated for a week prior to the experiment. The fish were exposed to 12 h L: 12 h D and were fed with fish pellets for that period. Adult males Silver catfish (*Synodontis eupterus)* used in this project were collected in third month of the year.

#### 2.2.2. Enzyme purification

In a typical purification, the fish were killed by cervical dislocation and the livers were immediately removed and washed in ice-cold saline (9.0 g/liter sodium chloride) to remove as much blood as possible and subsequently drained of filter paper. The diced livers (38 g) homogenized with a glass homogenizer, on ice, in 5 vols of 25 mM Tris–HCl buffer, pH 8.0, containing 0.25 M sucrose, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride acid, and 0.1 mM β-mercaptoethanol. The supernatant was clarified by centrifugation at 100 000*g* for 50 min at 4 °C using Beckman-optima-LE-80k Ultra centrifuge. The supernatant filtered through a 0.45 μm Millipore filter (Millipore, Bedford. MA) was used as the crude lysate. The lysate (about ≈150 ml) was subjected to purifications.

The crude lysate was applied to a DEAE-Trisacryl column (2.0 × 30 cm) previously equilibrated with 25 mM Tris–HCl buffer, pH 8.0 and washed with the same buffer at a flow rate of 20 ml/hour and 5 ml fractions were collected and analyzed for enzyme activity and amount of protein. The bound fractions were eluted with 0–1 M salt gradient. Bound fractions containing GST activities were pooled and concentrated with 10 MWM Millipore (10 kDa cut off membrane) then filtered through a 0.45 μm Millipore filter. This was further purified using Glutathione Sepharose$^{TM}$ 4 B gel column according to the protocol supplied. The bound GST was eluted with a linear gradient of GSH (0–10 mM). The pooled fractions were immediately concentrated using 10 MWM Millipore (10 kDa cut off membrane) before kinetic assays. All purification steps were performed at 0–4 °C unless stated otherwise.

The purity of the preparation was determined by SDS/PAGE [51]. Protein concentrations were determined using BIORAD protein assay kit (Bio-Rad, Hercules, CA, USA,) based on the method of [10] with BSA as a standard. $A_{280}$ was used to monitor the protein in column effluents.

#### 2.2.3. Glutathione transferase (GST) assay

Activities with the substrates (1-chloro-2,4-dintrobenzene, 4-chloro-7-nitrobenzo-2-oxa-1,3 diazole, ρ-nitrophenylacetate, ethacrynic acid, 1,2-dichloro-4-nitrobenzene, 4-hyroxylnonenal and *p*-nitrophenyl chloride) for characterization of the GST were measured according to published procedures [37,36,61,4]. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmole of product/min/mL at 25 °C in the optimal assay condition for each substrate. Specific activity was

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