Some Biochemical Properties of Liver Rhodanese (E. C. 2. 8. 1.1) Isolated from a Typical Marine Fish (Lutjanus goreensis)

Jack, A. S. ¹, Anosike, E. O. ², Brown, H. ³, Ben-Chioma, A. ⁴

¹, ³, ⁴Department of Medical Laboratory Science, Faculty of Science, Rivers State University of Science and Technology, Port Harcourt. ²Department of Biochemistry, College of Natural and Applied Sciences, University of Port Harcourt, Port Harcourt

Abstract: Rhodanese is a multifunctional, mitochondrial enzyme which primary function is the protection of the electron transport system from the deleterious effect of cyanide. It carries out the said primary function, by catalyzing the conversion of the cyanide to thiocyanate, a less toxic compound. The enzyme is also implicated in preventing the formation of inorganic sulfide, a highly neurotoxic substance and in the synthesis of sulfur-containing macromolecules. The activity of the ubiquitous enzyme has been reported in soil, bacteria, plants and animal tissues. The aim of this research is to establish the presence of the cyanide detoxifying enzyme, Rhodanese, in marine aquatic life, especially in the liver of a typical marine fish, Lutjanus goreensis that subsists on other fishes and shrimps which in turn, feed on planktons and some lower organisms of the aquatic food chain. The fish liver Rhodanese, purified 11.4 fold with activity recovery of 9.03%, had a specific activity of 19.7µMmin⁻¹mg⁻¹ enzyme protein. The invitro cyanide detoxifying enzyme, thiosulphate sulphur transferase (E. C. 2.8.1.1), followed a non-sequential ping-pong mechanism in the formation of thiocyanate from cyanide and thiosulphate. From the kinetic studies, the Vₘₐₓ of the enzyme catalysis was 5.0µM/minute while the Km values for thiosulfate and cyanide were 5.88mM and 11.11mM respectively. The optimum pH of the fish liver Rhodanese enzyme was 8.2, with the pH range of 8.0 to 8.4. The optimum temperature of the enzyme was 35°C. The molecular weight of the fish liver Rhodanese was 37,153 daltons, using the gel filtration technique. From the foregoing, it is evident that the Rhodanese enzyme is present in the liver of the tropical marine fish with an appreciable activity level that is possibly attributable to the feeding habit of the fish.

Keywords: Marine fish Liver, Rhodanese, cyanide and thiocyanate

1. Introduction

The metalloenzyme of Iron with the odd suffix for enzymes and named, Rhodanese (thiosulphate sulphur transferase, E. C. 2.8.1.1) was first discovered in bovine liver by Lang, (1933). From the bovine liver, the enzyme was crystalized and characterized by Sorbo, (1953). This initial isolation of the enzyme, opened the research avenue for several investigators to carry out numerous studies on the Rhodanese enzyme with the primary aim of elucidating the physiological importance of the mitochondrial enzyme. The current concept is that the iron-sulphur Centre enzyme protein is implicated in the synthesis of sulphur-containing macromolecules. The second understanding is that Rhodanese plays a vital role in the protection of cytochrome oxidase of the electron transport system from the attack of the cyanide molecule as per the accounts of the protective effect of Rhodanese on respiration by Augrica and Koj, (1975). Isom and Jaine, (1976) and Tabita et al., (1969) documented the presence of the Rhodanese enzyme in bovine liver and in bacteria, Ferrobacillus ferrooxidan, respectively. Other investigators that reported on the activity of the enzyme in various other sources include; Anosike and Jack (1982a and b) in liver and kidney of guinea pig (Lepus caniculus) and albino rat (Mus musculus), (Westley, 1959) in beef liver and kidney Rhodanese enzyme activity comparative study. Recently, Lee et al. (1995) in mouse liver, Agboola and Okonji (2004) in fruit bat liver, Ezzi et al., (2003) in Trichoderma sp, Aladesammi et al., (2009) in tortoise liver, Akinsiku et al.,(2010) in the liver of African catfish, Okonji et al.,(2011) and(2010) in mudskipper liver and in soldier termite, respectively.

In plants, the activity of the Rhodanese enzyme was reported in tapioca leaves by Chew and Boey, (1972) and by Anosike and Ugochukwu, (1981) in cassava leaf, peel and flesh where the enzyme distribution correlated with the concentration of the cyanogenic glucoside, estimated on the premise of hydrogen cyanide (HCN) released. Most recently, the activity of Rhodanese has also been reported in the leaves of bitter melon (Momordica Charantia) by Ehigie et al. (2015)

However, not much investigation has been reported in literature, concerning the presence of the cyanide detoxifying enzyme in various tropical marine aquatic lives. This study was, therefore, undertaken to demonstrate the presence of the Rhodanese enzyme in the liver of a typical marine fish, (Lutjanus goreensis) and to reveal some of the inherent biochemical properties associated with the enzyme for the advancement of knowledge on the ubiquitous Rhodanese enzyme which diverse physiological functions are increasingly being appreciated, such as preventing the formation of inorganic sulphide (a highly neurotoxic substance), the synthesis of sulfur-containing macromolecules and most importantly, the protection of the electron transport chain from the deleterious effect of cyanide.

2. Materials and Methods

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The marine fish which liver was implicated in this study was purchased from the Creek Road waterside local market in Port-Harcourt, Nigeria. All other reagents, chemicals and their sources are as listed below; Folin-Ciocalteu reagent, Bovine Serum Albumin (BSA) and Disodium hydrogen phosphate were products of E. Merch (Darstadt). Hydrochloric acid, Sodium hydroxide, potassium thiocyanate were products of East Anglia Chemicals. Sodium hydrogen phosphate, Formaldehyde, Tris (hydroxyethyl) amino methane and potassium cyanide were products of BDH.

2.1 Protein Estimation

Protein was estimated, using the method of Lowry et al., (1951) with bovine serum albumin as the standard.

2.2 Enzymes assay

For routine assay of the enzyme activity, the method of Tomati et al., (1974) was used. In which the intensity of the reddish-brown color formed in the course of enzyme catalysis, was proportional to the concentration of thiocyanate produced and was estimated spectrophotometrically at 460nm, using Bausch and Lomb spectronic 20 Spectrophotometer. The enzyme activity was expressed as µmole KCSN formed per min per mg enzyme protein.

2.3 Effect of pH on fish liver Rhodanese enzyme

The optimum pH of the marine fish liver Rhodanese was determined, using 0.01M Tris-HCl (pH 5.0-7.0) and 0.01M Sodium phosphate (pH 7.5-9.5) as buffer systems. A suitable aliquot (1.5mls) of pH buffer at a particular pH was used for assay of the enzyme activity in the manner of using 0.01M Sodium Phosphate buffer (pH 8.2) for routine enzyme assay (Tomati et al., 1974). The enzyme assay was carried out with 8.33mM of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}, 8.33mM of KCN and 2.5mM of each type of buffer.

2.4 Effect of temperature on fish liver Rhodanese

The effect of temperature on the marine fish liver Rhodanese catalyzed reaction was tested over the temperature range of 20-60°C with 5°C interval. For temperature 25°C, the reaction tubes were incubated in temperature regulated water bath by the addition of ice chips. At each temperature of the test, the tube was duplicated; the reagent blank tube with which the spectrophotometer was zeroed and the other in which the actual effect of temperature on the enzyme was measured. This nullified the formation of thiocynate by temperature rather than by enzyme catalysis.

2.5 Molecular weight of fish liver Rhodanese determination

The enzyme extract was separately applied to a column of 1cm x 34cm, packed with Sephadex G150-120 that was equilibrated for 72 hours in 10.0mM Tris-HCl buffer (pH7.6). The elution was with the same buffer and 4.0mls (42drops) was collected with the aid of a fraction collector that was the product of Rikakika company limited. Tokyo, Japan

The sephadex-packed column was calibrated with standard marker proteins of known molecular weights; Cytochrome C (13,000 Daltons), Pepsin (34,000 Daltons), Egg Albumin (44,000 Daltons) and Bovine Serum Albumin (65,000 Daltons).

3. Results

The results of kinetic study of the fish liver Rhodanese are as shown in table 1 of the double reciprocal plot. Fig 3 shows the pH profile of the fish liver Rhodanese in relation to percentage Vmax, while fig 4 shows the effect of temperature on the enzyme in relation to percentage Vmax. Fig 5 shows the plot of Log mol.wt versus elution volume with which the Mol. wt of the fish liver Rhodanese enzyme was determined by extrapolation.

<table>
<thead>
<tr>
<th>[KCN]\textsuperscript{-1}mM\textsuperscript{-1}</th>
<th>(Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3})\textsuperscript{-1}mM\textsuperscript{-1}</th>
<th>0.12</th>
<th>0.15</th>
<th>0.2</th>
<th>0.3</th>
<th>0.6</th>
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<tr>
<td>[KCN]\textsuperscript{-1}</td>
<td>V\textsuperscript{-1}</td>
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<td>1.9</td>
<td>2.4</td>
<td>3.20</td>
<td>5.9</td>
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<tr>
<td>0.12</td>
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<td>2.07</td>
<td>2.76</td>
<td>3.87</td>
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<tr>
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<td>2.52</td>
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<td>4.5</td>
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<tr>
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<td>3.0</td>
<td>3.87</td>
<td>5.2</td>
<td>9.7</td>
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Figure 1a: A plot of reciprocal initial rate versus varying concentration of [KCN]\textsuperscript{-1}mM\textsuperscript{-1}
A plot of reciprocal initial rate \((V^{-1}\mu M \cdot min^{-1})^{-1}\) of enzyme catalyzed reaction Vs reciprocal of potassium cyanide concentration \([KCN]^{-1} \text{mM}^{-1}\). At various fixed concentration of sodium thiosulphate \([\text{Na}_2\text{S}_2\text{O}_3]\) 8.33 mM; 6.67 mM; 5.0 mM; 3.33 mM.

**Figure 1b:** A plot of intercept versus varying Concentrations of \([\text{KCN}]^{-1} \text{mM}^{-1}\)

A plot of reciprocal initial rate \((V \mu M \cdot min^{-1})^{-1}\) of enzyme catalyzed reaction versus reciprocal of sodium Thiosulphate concentration \([\text{Na}_2\text{S}_2\text{O}_3]^{-1} \text{mM}^{-1}\). At various fixed concentration of Potassium Cyanide \([\text{KCN}]\) 8.33 mM, 6.67 mM, 5.0 mM, 3.33 mM, 1.66 mM.

**Figure 2a:** A plot of reciprocal initial rate versus varying concentration of \([\text{Na}_2\text{S}_2\text{O}_3]^{-1} \text{mM}^{-1}\)

**Figure 2b:** A plot of intercept versus varying Concentration of \([\text{Na}_2\text{S}_2\text{O}_3]^{-1} \text{mM}^{-1}\)
3.1 Effect of pH on fish liver Rhodanese enzyme

From the results obtained and with the plot of percentage Vmax versus pH, as shown in fig 3, it was deduced that the optimum pH for the marine fish liver Rhodanese was 8.2, with optimum pH narrow range of 8.0-8.4.

3.2 Effect of temperature on fish liver Rhodanese enzyme

From the results of the study, upon which the percentage Vmax versus temperature was plotted, as shown in fig 4, it was observed that the optimum temperature, at which point the marine fish liver Rhodanese exhibited maximum enzyme activity, was 35°C.

3.3 Molecular weight of fish liver Rhodanese

From the plot of log molecular weight against elution volume, as shown in fig 5, with the results from the study, it was observed that the molecular weight of the fish liver Rhodanese was extrapolated to be 37,153 Daltons from the elution volume of 60 mls for the enzyme.

4. Discussion

From the Kinetic study and the Lineweaver-Burk plots, as shown in figs 1a and 2a, the Vmax of the marine fish liver Rhodanese was observed to be 5.0µM/minute. This is a unique attribute of the marine fish liver Rhodanese under the condition of the study. Several investigators have reported different values of Vmax for the same enzyme from different sources. The Km values of the fish liver Rhodanese enzyme catalyzed reaction, indicated higher affinity for thiosulphate than for cyanide. This observation is consistent with the findings of Ehigie et al., (2015) for the Rhodanese enzyme from the leaves of bitter melon, Tayefi-Nasrabadi and Rahmani, (2011) for the Rhodanese from the liver of Rainbow Trout, Gafar et al., (2014) for the Rhodanese from the liver of Tilapia, Tabita et al., (1969) for the Rhodanese from Ferrobacillus ferroxidan, Chew et al., (1979) for the Rhodanese from tapioca leaf, Akinsiku et al., (2010) for the Rhodanese from the African catfish, Okonji et al., (2010) and (2011) for the Rhodanese enzymes from the soldier termites and from the mudskipper liver, respectively. However, the km values of this study, are not consistent with the reported findings of Anosike & Jack, (1982a and b) for Rhodanese enzymes from rat and guinea pig kidneys and that of Agboola and Okonji, (2004) for the Rhodanese in the cytosolic fraction of the fruit bat liver where the enzymes were found to have higher affinity for cyanide than for thiosulphate. In all cases of catalysis, the Rhodanese enzyme followed a non-sequential ping-pong mechanism as was initially reported by Sorbo, (1953) for bovine liver Rhodanese and by Saidu (2004) reviewed account on Rhodanese. The mechanism entails the binding of thiosulphate (S₂O₃⁻) to the enzyme molecule that causes an intermolecular protein conformational change which appears to break the S-S bond with the extrusion of the sulfite (SO₃⁻) radical. The sulfonite- enzyme complex (E-S), then binds with the available cyanide (CN⁻). During this process, the
sulphur group, combines with the electrophilic nitrogen centre of the cyanide (CN), forming a thiocyanate-Enzyme Complex (E-CNS) that rapidly breaks down to thiocyanate (CNS) and free Rhodanese enzyme molecule (E).

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\begin{align*}
\text{Enzyme} & \quad \text{Na}_2\text{S}_2\text{O}_3 & \quad \text{E-Na}_2\text{S}_2\text{O}_3 & \quad \text{E-S} & \quad \text{E-CNS} & \quad \text{Free enzyme (E)}
\end{align*}
\]

From the plot of the percentage maximum activity (Vmax) of the fish liver Rhodanese versus pH, as shown in fig 3, it is deduced that the marine fish liver rhodanese had an optimum pH of 8.2, with the optimum pH narrow range of 8.0-8.4. This report is in agreement with the documented finding of Okonji et al., (2010) for the soldier termite Rhodanese of optimum pH of 8.0, with a wide optimum pH range of 8-11. It is also consistent with the reports of Okonji et al.,(2011) and Yanhua et al., (2012). Both of whom reported an optimum pH value of 8.0 for Rhodanese enzyme from mudskipper liver and for the cyanide degrading enzyme from Alcaligene sp.DN25 with a narrow optimum pH range of 7.0-8.0, respectively. The optimum pH of 8.0 for the marine fish liver Rhodanese of this study, is not in agreement with the optimum pH value of 6.0, reported for giant freshwater prawn Rhodanese by Okonji et al.,(2008) and optimum pH of 6.5 reported by Lee et al., (1995) for mouse liver Rhodanese, Akinsiku et al.,(2011) for African catfish liver Rhodanese and Gafar et al., (2014) for tilapia liver Rhodanese. It is also not consistent with the reported optimum pH as high as 10.5 with the optimum pH wide range of 5-12 for the rainbow trout liver Rhodanese by Tabita et al., 2015. Both of whom documented an optimum pH range of 6.0-9.0, with an average of approximately 8.0 for the marine fish liver Rhodanese of this study, is not in agreement with the optimum pH value of 8.0 reported by Lee et al., (1995 ) and Gafar et al. (2014) respectively. While Okonji et al.,(2011) and (2010) reported 50°C and 55°C as optimum temperature for the activity of the mudskipper liver and the soldier termite Rhodanese enzymes, respectively, Chew and Boey, (1972) and Ezigie et al., (2015) documented an optimum temperature as high as 59°C and 60°C for the tapioca leaves and the bitter melon leaves Rhodanese enzymes, respectively. The observed optimum temperature of 35°C for the marine fish liver Rhodanese by this study, is within the lower optimum temperature range of 35-55°C obtained for Rhodanese enzymes in all Trichoderma strains by Ezzi et al., (2003). It is also within the upper optimum temperature range of 25-35°C reported for Bovine liver Rhodanese enzyme by Sorbo (1953). However, the value is completely not in agreement with the optimum temperature range of 50-55°C documented for cabbage Rhodanese enzyme by Tomati et al., (1974). The remarkable difference in optimum temperature of the enzymes from other sources, especially from plants, may well be as a result of the temperature difference of the environment to which the organism or the plant is adapted. This confers an appreciable higher thermal stability on the enzymes from these sources.

The molecular weight of the marine fish liver Rhodanese was found to be 37,153 daltons, using the gel filtration technique. This realization is very similar to the molecular weight of 37,154 daltons for the native soldier termite Rhodanese by Okonji et al., (2010). It is also consistent with the value of 37,000 daltons that was reported for the bovine liver Rhodanese enzyme by Lee et al., (1995), the human liver Rhodanese by Jarabak and Westley, (1974) and the guinea pig kidney Rhodanese enzyme by Anosike and Jack (1982b). It compares well with the 36000 daltons for the fruit bat liver Rhodanese reported by Agboola and Okonji, (2004) for the Rhodanese in the cytosolic fraction of the fruit bat liver that was observed to have similar optimum temperature of 35°C. However, it is in contrast with the value of 32000 daltons for rat kidney Rhodanese by Anosike and Jack (1982a), mouse liver Rhodanese by Lee et al., (1995), cabbage leaf Rhodanese by Tomati et al., (1974), the value of 33000 daltons for tapioca and cassava leaves Rhodanese.
enzymes by Chew and Boey, (1972) and Anosike and Ugochukwu, (1981), respectively, as well as with the value of 35460 daltons for the tilapia liver Rhodanese by Gafar et al., (2014). It would be inferred, therefore, that the molecular weight of the Rhodanese enzyme protein molecule, from most sources, is between 32,000 to 37,000 daltons.

5. Conclusion

This research has shown cyanide detoxifying enzyme, Rhodanese, to be present in the liver of the typical marine fish, Lutjanus goreensis (snapper) that mainly feeds on other fishes and shrimps, within the rocky and sandy estuary, which in turn subsist on proteinic remains of other aquatic lives, planktons and some lower aquatic organisms such as diatoms. So, the feeding habit of the tropical marine fish may have contributed to having a high activity of the Rhodanese enzyme protein, especially in the liver of the fish which is the detoxification organ, with the characteristic of having a higher affinity for the thiosulfate than for cyanide, the toxicant. The more hypolimion- dwelling marine fish is adapted to the prevailing warm temperature of the sandy sea bed (hypolimion) which confers a high thermal stability on the inherent Rhodanese enzyme. The alkaline optimum pH of the marine fish liver Rhodanese is an indication of the physiological optimum pH that enables the fish to thrive even at low oxygen tension where the pH could be low (hypercapnia environment).

It is not surprising, therefore, that the molecular weight of the tropical marine fish liver Rhodanese is similar to that of the human liver, soldier termites, fruit bat liver, bovine liver and guinea pig kidney Rhodanese enzymes. The two latter animals have a common feeding habit of subsisting on cyanogenic glycosides-containing elephant grass (Andropogon spp) while the termites feed on decaying wood.

References


