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Kinetic characteristics of *Synodontis membranaceus* gills and liver rhodanese

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Abstract

The biochemical reaction catalyzed by cyanide:thiosulphate sulfurtransferase commonly referred to as rhodanese is the most significant mechanism for cyanide detoxification. This investigation's aim is to establish the presence of the enzyme rhodanese in *synodontis membranaceus* and to elucidate its kinetic parameters in the liver and gills of the *synodontis* specie. Rhodanese activity was assayed by measuring the amount of thiocyante formed in micromoles at 37°C and pH 8.2. The results revealed that the km and Vmax values recorded for *Synodontis membranaceus* liver rhodanese were 11.12+2.58 and 0.15umol/min+0.01 for cyanide and 27.78mM+5.15 and 0.22umil/min+0.00 for thiosulphate. Also the, km and Vmax values for gills rhodanese were 15.38mM+1.38 and 0.13umol/min+0.00 for cyanide and 25mM+3.65 and 0.19umol/mind were for thiosulphate. Substrate specificity studies revealed thiosulphate as the best sulphur donor. Both liver and gills rhodanese showed maximum activity at pH 8.0. The liver enzyme showed maximum activity at 30°C, while the gills enzyme showed maximum activity through 30-35°C. Rhodanese activity is present in the *synodontis* specie investigated with properties comparable to rhodanese present in other aquatic organisms.

Keywords: Rhodanese; Cynide; *Synodontis membranaceus*; Liver; Gills

1. Introduction

Cyanogenic glycosides are produced by cyanogenic plants with potential defensive properties against herbivores and pathogens (Boter & Diaz, 2023). When broken down by the enzymes β-glucosidases and α-hydroxynitrile lyase, cyanogenic glycosides release the toxic compound hydrogen cyanide (Arnaiz et al., 2022). Cyanide is a potent inhibitor of the cytochrome c oxidase or complex IV of the mitochondrial electron transport chain. Thus, cyanide being readily absorbed in the cell, prevents the use of oxygen in tissues, which can eventually cause death (Broderick *et al*., 2006).

Eukaryotic cells have a well established mechanism for cyanide detoxification. Cyanide:thiosulphate sulfurtransferase (rhodanese) catalyzed reaction is the most significant mechanism for cyanide detoxification in most organisms. The enzyme is a ubiquitous enzyme distributed widely living things. Rhodanese catalyses the biotransformation of toxic cyanide to thiocyanate, a less toxic product, using thiosulphate as sulphur donor (westly 1981) as shown in the reaction below. To detoxify cyanide, sulphur atom is transferred to rhodanese from a donor substrate, and then the sulfur-substituted enzyme further transfers the sulphur atom to cyanide (Aminlari & Vaseghi, 2006).

$$S^2O_3^{2-} + CN \longrightarrow SO_3^{2-} + SCN^-$$

Not many investigations have been done and reported on the characteristics of rhodanese in aquatic organisms. However, investigations on the characteristics of rhodanese have been reported in *Oreochromis niloticus* by Okonji et

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al. (2010), African catfish by Akinsiku et al. (2010), mudskipper liver by Okonji et al. (2011), *Tilapia Zillii, Sarotherodon Galilaeus* and *Hepsetus Odoe* by Falode et al. (2014) *Lutjanus goreensis* liver by Jack et al. (2015), rainbow trout liver by Tayefi-Nasrabadi and Rahmani (2012) and in *Synodontis schall* liver, by Wodu et al. (2022). To this end therefore, this investigation was done to establish the presence of the ubiquitous enzyme rhodanese in *synodontis membranaceus* and to elucidate the physicochemical properties of the enzyme so as to add to the existing body of knowledge.

2. Materials and Methods

2.1. Preparation of tissue extract

Five grams (5g) each of liver and gills of *Synodontis membranaceus* were homogenized in 25mls of phosphate buffer (pH 8.2). The suspension was centrifuged for 15 min at 4,000 rpm and 4°C to obtain the clear supernatant, which was used as the source of crude enzyme

2.2. Protein and enzyme assay

The method by Bradford (1976) was used to estimate the concentration of protein. *Synodontis membranaceus* liver and gills rhodanese assayed was done by the method of Agboola and Okonji (2004) with some modifications. The reaction mixture of 1ml contains 10mM $Na_2S_2O_3$, 10mM KCN, 0.25mM borate buffer (pH 8.2) and enzyme solution (10µl). Reaction time was 1min at 37ºC. The reaction was stopped by the introduction of 0.5ml of formaldehyde (15%). The colour was developed by the addition of Sorbo reagent (Sorbo, 1953) and the absorbance taken at 460nm. The enzyme activity was defined as the concentration of thiocyanate (µmoles) formed per minute under specified conditions.

2.3. Determination of kinetic constant

Kinetic parameters (Km and Vmax) estimation were done with varied concentrations of KCN at fixed concentration (10mM) of $Na_2S_2SO_3$ and vice versa. Double reciprocal plots were used to obtain the kinetic parameter (Lineweaver and Burk, 1934).

2.4. Substrate specificity of Sulfur Donor substrates

Sulphur compounds used in this work were ammonium sulphate, 2-mercaptoethanol, and sodium metabissulphite. They were used in place of sodium thiosulphate in rhodanese assay. Km and Vmax of the enzyme for the sulphur donor compound were estimated using Linewaever-Burk plots.

2.5. Temperature and pH effects

Temperature range of 10-60ºC was used to study how temperature modulates rhodanese activity. The assay mixture was incubated at each test temperature for 10mins. The enzyme was equilibrated at each test temperature before adding it to the reaction mixture to initiate the reaction.

The effect of pH on *Synodontis membranaceus* liver and gills rhodanese was done using 50mM concentration each of citrate buffer (pH 4-6.5), potassium phosphate buffer (pH 7.0-8.5) and borate buffer (pH 9-10). Rhodanese assay was done as described above using the different buffer solutions to replace the assay buffers.

2.6. Statistical Analysis

Statistical analysis was done using SPSS software.

3. Results

The cyanide detoxifying property of *Synodontis membranaceus* liver and gills rhodanese was investigated and their kinetic characteristics elucidated. Lineweaver-Burk plots (figures 1-4) were used to estimate the kinetic constants summarised in table 1. The results established the presence of rhodanese in *Synodontis membranaceus*.

Mean±sd of km and Vmax for *Synodontis membranaceus* liver and gills rhodanese are presented in table 1 as estimated from Lineweaver-Burk plots shown in figures 1-4. The km and Vmax values recorded for *Synodontis membranaceus* liver rhodanese were 11.12+2.58 and 0.15umol/min+0.01 for cyanide and 27.78mM+5.15 and 0.22umil/min+0.00 for thiosulphate. On the other hand, km and Vmax values for gills rhodanese were 15.38mM+1.38 and 0.13umol/min+0.00 for cyanide, while 25mM+3.65 and 0.19umol/mind were recorded for thiosulphate.

While evaluating the substrate specificity with regards to sulphur donor substrate, thiosulphate appeared to be the most suitable donor substrate. The km values for thiosulphate was smallest for liver (27.78mM+5.15) and gills (25.51mM+3.65) rhodanese compared to ammonium sulphate (liver: 31.42mM±6.92; gills: 29.52mM±7.24), sodium metabossulphate (liver: 37.04mM±5.24; gills: 41.63mM±9.52), and 2-mercarptoethanol (liver: 52.83mM±15.17; gills: 48.50mM±10.72). Also, the catalytic efficiency (Vmax/km) for the liver (0.0079) and gills (0.0074) rhodanese was highest with thiosulphate compared to the other possible sulphur donor substrates as can be seen in table 3. Rhodanese activity in liver and gills were highest when thiosulphate was used as sulphur donor compared to the other substrates as can be seen in table 2.

Optimum pH for both liver and gills rhodanese was 8.0. The liver enzyme showed maximum activity at 30°C, while the gills enzyme showed maximum activity through 30-35°C.

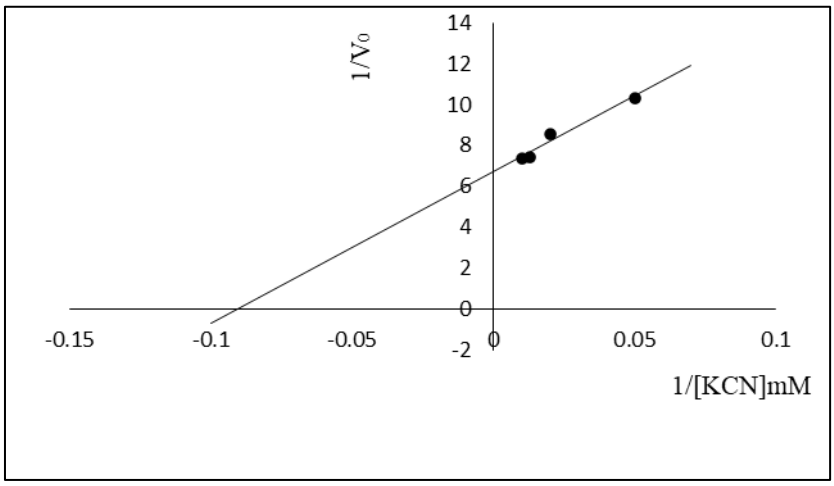

Figure 1 Lineweaver-Burk plot at a fixed concentration of $Na_2S_2O_3$ (10mM) for *S. membareacues* liver rhodanese

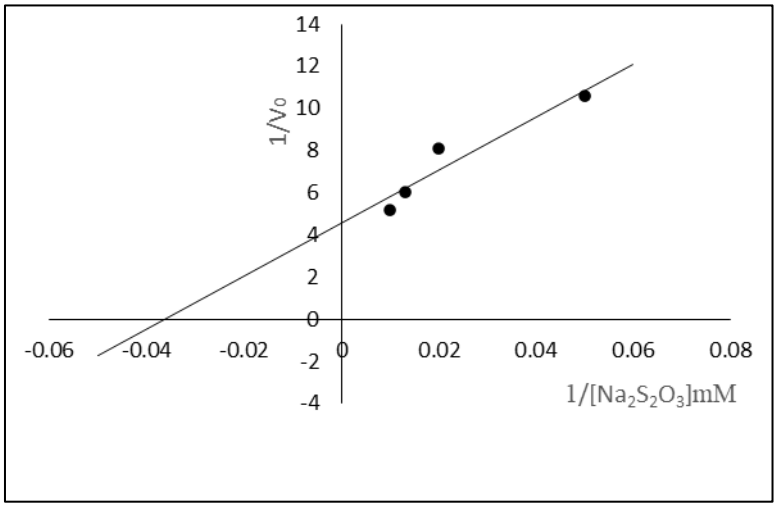

Figure 2 Lineweaver-Burk plot at a fixed concentration of KCN (10mM) for *S. membareacues* liver rhodanese

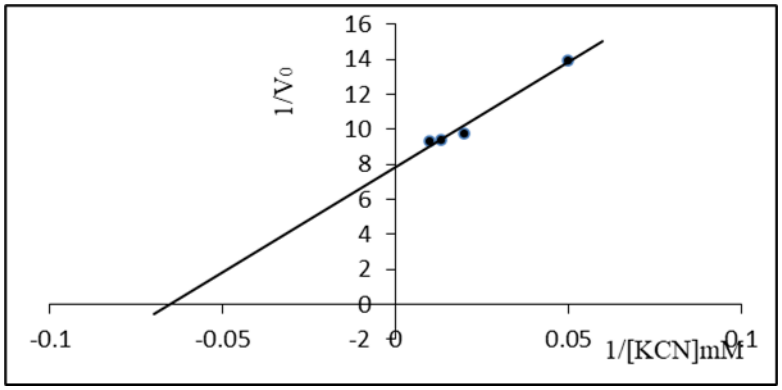

Figure 3 Lineweaver-Burk plot at a fixed concentration of $Na_2S_2O_3$ (10mM) for *S. mem bareacues.* gills rhodanese**.**

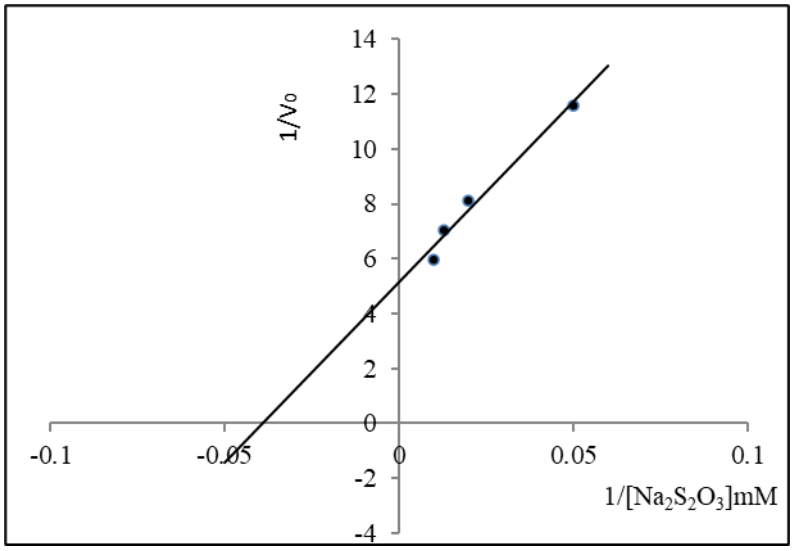

Figure 4 Lineweaver-Burk plot at a fixed concentration of KCN (10mM) for *S. membareacues* gillsrhodanese

Table 1 Kinetic Parameters of liver and gills rhodanase

	Substrate	**Km**	**Vmax**	**Temp. Optimum(^{0}C)**	**pH Optimum**
Liver	$Na_2S_2O_3$	27.78±5.15	0.22±0.00	30	8.0±2.91
	KCN	11.11±2.58	0.15±0.01		
Gills	$Na_2S_2O_3$	25.51±3.65	0.19±0.00	30-35	8.0±1.37
	KCN	15.38±1.36	0.13±0.00		

Table 2 Percentage activity of sulfur donor substrate

Substrate	**Liver**	**Gills**
Sodium thiolsulphate ($Na_2S_2O_3$)	100.0	100.0
Ammonium sulphate ($(NH_4)_2SO_4$)	65.31	59.73
2-mercaptoethanol ($CH_2(SH)CH_2(OH)$)	36.42	41.85
Sodium metabissulphate ($Na_2S_2O_5$)	48.36	52.58

Table 3 Substrate specificity of sulphur donor substrate of liver and gills rhodanese

Substrate	Liver			Gills		
	Km(mM)	Vmax	Vmax/ km	Km(mM)	Vmax	Vmax/ km
Sodium thiolsulphate ($Na_2S_2O_3$)	27.78±5.18	0.22±0.00	0.0079	25.51±3.65	0.19±0.00	0.0074
Ammonium sulphate ($(NH_4)_2SO_4$)	31.42±6.92	0.20±0.01	0.0064	29.52±7.24	1.08±0.53	0.0061
2-mercaptoethanol ($CH_2(SH)CH_2(OH)$)	52.83±15.17	0.18±0.01	0.0034	48.50±10.72	0.22±0.03	0.0045
Sodium metabissulphate ($Na_2S_2O_5$)	37.04±5.24	0.17±0.00	0.004	41.63±9.52	0.21±0.02	0.0050

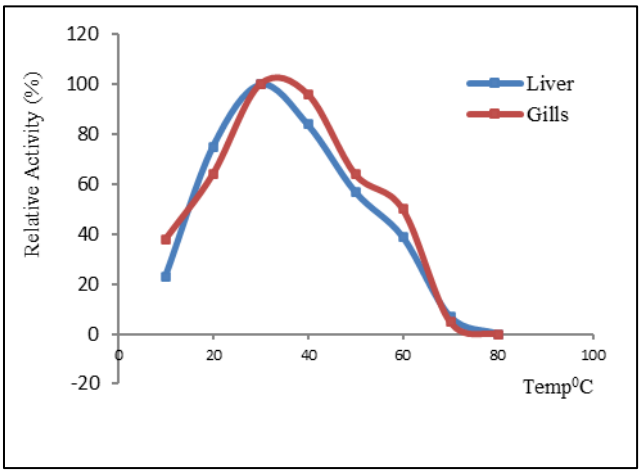

Figure 5 A plot of relative activity versus temperature^{0}C

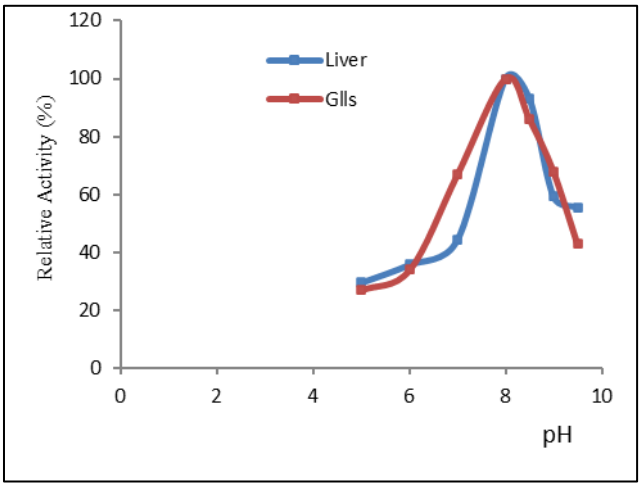

Figure 6 A plot of relative activity versus pH

4. Discussion

The presence of rhodanese in gills and liver of *Synodontis membranaceus* have been established in the present study. The enzymes kinetic constants and characteristics were similar to rhodanese isolated from other aquatic organisms. In the present study, rhodanese extracted from liver and gills of *Synodontis membranaceus* exhibited higher affinity for cyanide than thiosulphate. In contract however, the studies conducted by Jack et al. (2015), Wodu and Frank-Oputu

(2022) and Agboola et al. (2025) reported higher affinity for thiosulphate than cyanide. Thiosulphate in the present study still remains the best sulphur donor substrate as a result of its low km values and high Vmax/km, indicating that both liver and gills rhodanese have highest catalytic efficiency with thiosulphate as sulphur donor substrate in cyanide detoxification. This observation is similar to the report by Wodu et al. (2022) and Wodu and Frank-Oputu (2022) for *Synodontis schall* gills and liver rhodanese respetively. Rhodanese have been reported to have the ability to utilise different sulphur containing compounds as donors (Okonji et al., 2008) for cyanide detoxification. To an appreciable extent, ammonium sulphate may be used as donor substrate in rhodanese catalysed reactions in vitro.

As deduced from the plot of relative activity versus pH (figure 6), optimum pH of 8.0 reported in the present study, was also reported by Agboola et al. (2025) and Wodu and Frank-Oputu (2022) for *Coptodon zillii* rhodanese and *Synodontis schall* gills rhodanese respectively. Jack et al. (2015) reported a range of 8.0-8.4 for *Lutjanus goreemsis* liver rhodanese.

Temperatures optima reported in the present study for *Synodontis membranaceus* liver and gills were also reported for other aquatic organisms (Jack et al., 2015; Wodu and Frank-Oputu, 2022). Observations from the relative activity versus temperature plot in figure 5 gills rhodanese had maximum activity at 30-35°C before and after which temperatures are followed by rapid decline in activity. The liver enzyme on the other hand, had optimum temperature of 30°C.

5. Conclusion

This study has shown the presence of rhodanese in the gills and liver of *Synodontis membranaceus*. The *Synodontis* specie has an established cyanide detoxifying mechanism, thus it can survive in its natural aquatic habitat. Rhodanese extracted from liver and gills of the *Synodonti spp* had higher affinity for cyanide than thiosulphate. Also, thiosulphate is the best sulphur donor for both gills and liver rhodanese compared to the other donor substrates investigated. *Synodontis membranaceus* has kinetic properties similar to those of other aquatic organism. Although this work attempts to compare the extracted enzyme with those other aquatic species, the kinetic characteristics of rhodanese from *Synodontis membranaceus* have kinetic properties that compare with those of other organisms beyond aquatic habitat.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Approval was obtained from the Research and Ethic Committee of the Faculty of Basic Medical Sciences Niger Delta University, Bayelsa state. Nigeria.

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