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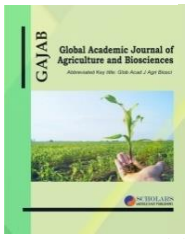

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Original Research Article

Physicochemical Characterization of *Synodontis schall* Gills Rhodanese

Ebizimor Wodu[1*] and Ayibaene Frank-Oputu[1]
[1]Department of Biochemistry, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

***Corresponding Author**
Ebizimor Wodu
Department of Biochemistry, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

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Abstract: The cyanide detoxifying enzyme, rhodanese was extracted from *Synodontis schall* gills and some physicochemical properties investigated. Activity of the enzyme preparation was assayed by measuring the activity of rhodanese in RU min^{-1} mg^{-1}. The results revealed that *Synodontis schall* gills rhodanese had km values for KCN and $Na_2S_2O_3$ as 22.73±4.12 and 16.67±5.31 respectively. The enzyme had higher affinity for $Na_2S_2O_3$. Only ammonium sulphate displayed possible sulfur donating property but was less effective than thiosulphate. *Synodontis schall* gills rhodanese displayed maximum activity at pH 8.0 and 35°C. *Synodontis schall* gills rhodanese was significantly ($p<0.05$) inhibited by $PbCl_2$, $BaCl_2$ and $HgCl_2$ in a concentration dependent manner. Gills rhodanese of *Synodontis schall* was similar in properties to rhodanese extracted from other sources.

Keywords: *Synodontis schall*, Gills, Rhodanese, Cyanide, Km and Vmax.

INTRODUCTION

Rhodanese (EC.2.8.1.1) a ubiquitous enzyme in cyanide detoxification is very crucial to the survival of aquatic organisms since they are exposed to cyanogenic glycosides released from plants and industrial waste. Some plants contain cyanogenic glycosides that release hydrogen cyanide gas upon ingestion (Conn, 2008). Frequently, animals are exposed to this toxic substance through consumption of cyanogenic glycosides (Nobrega *et al*., 2006). Toxic cyanide released from cyanogenic glycosides, must be detoxified for the continuous existence of the organism. Rhodanese detoxifies cyanide to form thiocyanate by transferring sulfur from a donor, usually thiosulphate to cyanide. In course of its catalytic cycle, rhodanese exists in the free form of the enzyme and the form that contains persulfate (Domenica *et al*., 2000).

Although significant concentration of rhodanese can be found in the kidney and other tissues, mammalian liver has been reported to have the highest concentration of rhodanese, (Cipollone and Visca, 2007). It has been reported that rhodanese plays a role in detoxifying cyanide (Koj and Frendo, 1962) as well as formation of iron-sulfur centers (Cerletti, 1986) and energy metabolism (Ogata and Volini, 1990). Although rhodanese activity has been reported in a number of living things, this work attempts to report on the physicochemical characteristics of crude enzyme extract of rhodanese from *Synodontis schall* gills as it compares with rhodanese from other sources.

MATERIALS AND METHODS

Sample collection

The gills of the *Synodontis schall* implicated in this study were removed from the fish and stored at -4°C until required.

Preparation of tissue extract

Tissue (5g) was homogenized in 30mls of phosphate buffer of pH 8.2. The homogenate was centrifuged for 20 min at 4,000 rpm and 4°C. The supernatant served as the crude enzyme.

Protein and enzyme assay

Concentration of protein was estimated by Bradford (1976) method. Rhodanese was assayed by the method of Agboola and Okonji (2004) with some modifications. The 1ml reaction mixture contains 10mM $Na_2S_2O_3$, 10mM KCN, 0.25mM borate buffer of pH 8.2 and 10µl of enzyme solution. Reaction time was 1min at 37ºC. Exactly 0.5ml of formaldehyde (15%) was used to stop the reaction. Exactly 1.5ml of Sorbo reagent (Sorbo, 1953)) was added and optical density was read at 460nm. The unit of enzyme activity was defined as the amount of thiocyanate formed in micromoles per minute at 37ºC and pH 8.2.

Determination of kinetic constant

Km and Vmax were elucidated by varying the concentrations (2mM and 10mM) of one at fixed concentration (10mM) of the other.

Km and Vmax were obtained from the Linewaever-Burk plots (Lineweaver and Burk, 1934).

Effects of Possible Sulfur Donor

Different sulphur compounds namely ammonium sulphate, 2-mercaptoethanol, dithio oxamide and sodium metabisulphite were used to replace sodium thiosulphate in rhodanese assay. Linewaever-Burk plots were used to evaluate km and Vmax for the different compounds.

Effect of pH.

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

Effect of temperature

Rhodanese was assayed at different temperatures (20 - 60ºC) to investigate the effects of temperature on its activity. The assay mixture was incubated at each test temperature for 10mins. The reaction was then initiated by adding the enzyme which had been equilibrated at that same temperature.

Effect of Salts

Effects of various metallic salts on *Synodontis schall* gills rhodanese was done with the following salts: $PbCl_2$, $BaCl_2$, $SnCl_2$, $CoCl_2$, $HgCl_2$, $MgCl_2$ and $CuCl_2$ at 0.05, 0.1 and 0.2mM in the rhodanese assay mixture. The reaction in the absence of salts represented 100% activity.

STATISTICAL ANALYSIS

The SPSS software was used to analyse all data. All assays of were done in triplicate.

RESULTS

Kinetic parameters (km and Vmax) reported in this investigation for *Synodontis schall* gills rhodanese as estimated from Lineweaver-Burk plots (figures 1 and 2) are presented in table 1. Km for KCN and $Na_2S_2O_3$ were estimated to be 22.73±4.12 and 16.67±5.31 respectively, while Vmax for KCN and $Na_2S_2O_3$ were 0.182±0.013 and 0.106±0.011 respectively. *Synodontis schall* gills rhodanese appeared to have higher affinity for $Na_2S_2O_3$ than KCN.

Rhodanese extracted from *Synodontis schall* gills exhibited maximum activity at 35^0C and pH 8.0. Of the possible sulphur donor substrate tested, only ammonium sulphate showed the capacity to act as a substrate in rhodanese assay. Dithio oxamide, 2-mercaptoethanol and sodium metabisulphite however, did not show any capacity to replace $Na_2S_2O_3$ in *in vitro* rhodanese assay.

The results from the effects of metal ions showed that the activity of *Synodontis schall* gills rhodanese was significantly ($p<0.05$) inhibited by $PbCl_2$, $BaCl_2$ and $HgCl_2$. The inhibition was concentration dependent. $SnCl_2$, $CoCl_2$, $MgCl_2$, and $CuCl_2$ on the other hand showed no significant ($p\geq0.05$) inhibition on the enzyme activity.

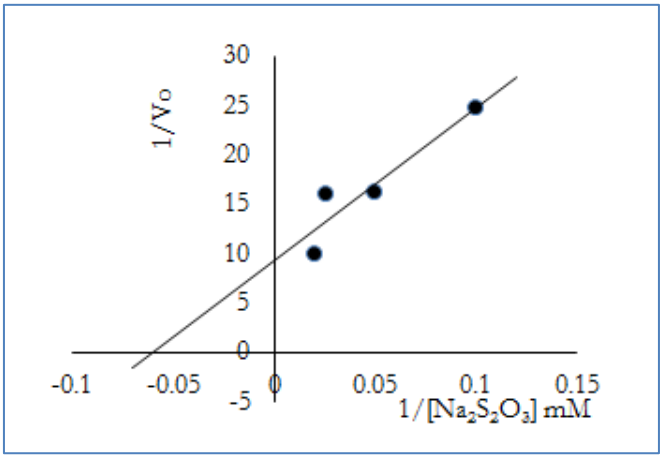

Fig-1: Lineweaver-Burk plot at fixed concentration of $Na_2S_2O_3$

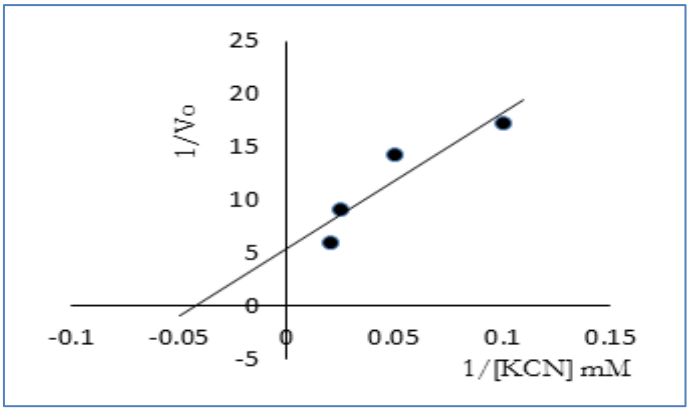

Fig-2: Lineweaver-Burk plot at fixed concentration of KCN

Table-1: Some characteristics of *Synodontis schall* Gills Rhodanese

	Km (mM)	Vmax (RU ml^{-1} min^{-1})	pH Optimum	Temp. Optimum (^{0}C)
KCN	22.73±4.12	0.182±0.013	35.0±5.2	8.0±2.1
$Na_2S_2O_3$	16.67±5.31	0.106±0.011		

Values are mean±SE of the mean

Table-2: Km and Vmax of Sulphur Donors

Substrate	Km (mM)	Vmax (RU min^{-1}.mg^{-1})
$Na_2S_2O_3$	16.67±5.31	0.106±0.011
Ammonium sulphate	42.65±6.08	0.014±0.000

Values are mean±SE of the mean

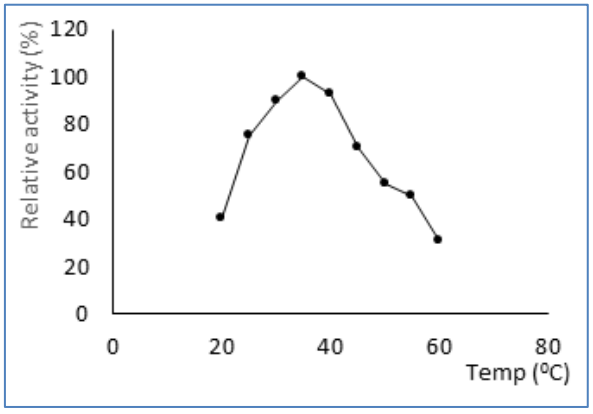

Fig-3: Plot of relative activity versus temperature (^{0}C)

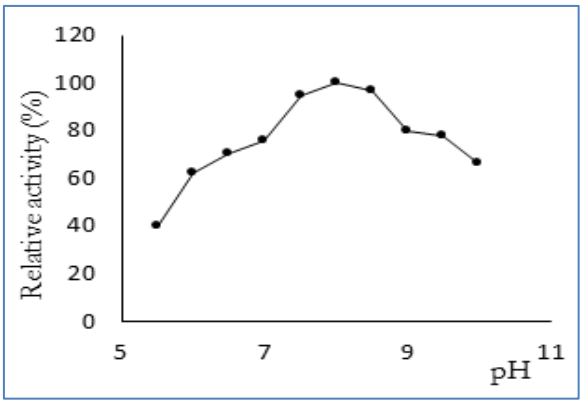

Fig-4: Plot of relative activity versus pH

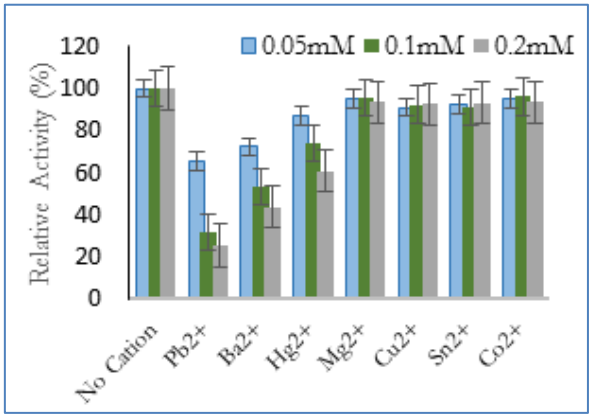

Fig-5: Effects of cations on gills rhodanese

DISCUSSION

The *Synodontis schall* gills rhodanese showed properties similar to those reported by researcher for rhodanese from other sources.

Km values for KCN and $Na_2S_2O_3$ in the present study were higher than 8.45mM and 12.23mM for $Na_2S_2O_3$ and KCN reported by Wodu *et al*., (2022) for rhodanese in the liver of *Synodontis schall*. This indicates that the liver isoenzyme had more affinity for both substrates and that cyanide detoxification is a major biological function of the enzyme in *Synodontis schall* in addition to other reported functions. Km values estimated in the present investigation compare well with 25.4mM and 18.6mM for KCN and $Na_2S_2O_3$

respectively reported by Akinsiku *et al*., (2009) for catfish liver rhodanese. *Synodontis schall* gills rhodanese exhibited higher affinity for $Na_2S_2O_3$ than KCN. The liver enzyme however, had higher affinity for KCN (Wodu *et al*., 2022).

Higher affinity of rhodanese for the substrate $Na_2S_2O_3$ was reported by Sorbo (1953), Lee *et al*., (1995), Akinsiku *et al*., (2009) and Wodu *et al*., (2021) for bovine liver, mouse liver, catfish liver and ram liver rhodanese respectively.

Optimum temperature and pH in the present study were in line with other reported findings. The optimum temperature of 35^0C for *Synodontis schall* gills rhodanese was also reported by Agboola and Okonji (2004) for liver rhodanese of fruit bat. Also, this value falls within the range of 35-55^0C reports by Ezzi *et al.*, (2003) for rhodanese in all strains of *Trichoderma*. Optimum pH of 8.0 estimated in the present work have been reported for rhodanese extracted from various sources. Some works that reported optimum pH of 8.0 for rhodanese are Okonji *et al*., (2010) for Soldier Termite, Okonji *et al*., (2015) for hepatopancreas of garden snail, Ehigie *et al*., (2019), for tomato and Wodu *et al*., (2022) for *Synodontis schall* liver.

Studies on the effects of metallic chlorides on *Synodintis schall* gills rhodanese activity showed that $PbCl_2$, $BaCl_2$ and $HgCl_2$ caused a significant ($p<0.05$) decrease in the enzyme activity. Similar inhibition by mercury was reported by Okonji *et al*., (2010) for soldier termites rhodanese. Agboola and Okonji (2004) and Ehigie *et al*., (2019) reported the inhibition of bat liver and tomato rhodanese respectively by $BaCl_2$ and $HgCl_2$. Also, Wodu *et al*., (2021) reported inhibition of ram (liver and kidney) by $HgCl_2$, and $PbCl_2$. $SnCl_2$, $CoCl_2$, $MgCl_2$ and $CuCl_2$ did not influence the activity of *Synodintis schall* gills rhodanese to any appreciable extent, which may be due to prolong exposure of the fish in its natural environment.

CONCLUSION

The presence of the activity of rhodanese is established in *Synodontis schall* gills and the characteristics measured well with rhodanese reported by other researchers. *Synodontis schall* gills rhodanese had higher affinity for $Na_2S_2O_3$. The presence of the activity of rhodanese in the gills of *Synodontis schall* indicate that apart from functional cyanide detoxification mechanism, rhodanese is also involved in other physiological functions.

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