Characterization of Rhodanese Extracted from Synodontis schall Liver

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Abstract: Rhodanese, a cyanide detoxifying enzyme was extracted from the liver of Synodontis schall and its characteristics investigated. Crude enzyme preparation was prepared and the enzyme was assayed by measuring the activity of rhodanese in RU min⁻¹ mg⁻¹. The results revealed that rhodanese extracted from Synodontis schall liver had km values for Na₂S₂O₃ and KCN were 12.23mM±1.36 and 8.45mM±1.05 respectively. The enzyme had higher affinity for KCN than Na₂S₂O₃. Dithio oxiamide, 2-mercaptoethanol and sodium metabisulfite were not capable of replacing Na₂S₂O₃ as sulfur donors. Na₂S₂O₃ had the highest relative activity followed by ammonium sulphate. Synodontis schall liver rhodanese had optimum activity at pH 8.0 and 45°C. Relative activities of cations tested showed that none had any significant effect on Synodontis schall liver rhodanese. Rhodanese present in the liver of Synodontis schall had properties similar to those from other sources.

Keywords: Rhodanese, cyanide, cations, Synodontis schall, liver, detoxification.

INTRODUCTION

Cyanide is a potent poison. It is readily absorbed and causes death by preventing tissue utilization of oxygen (Okalie and Osagie, 2000; Okafor et al., 2002; Sousa et al., 2002). It exists as hydrogen cyanide gas, water soluble sodium and potassium cyanide salts and poorly water-soluble mercury, gold, silver and copper cyanide salts (Patel et al., 2014). Cyanide is contained in a lot of naturally occurring substances and in industrial products (Li et al., 2000). Animals are more frequently exposed to cyanide through ingestion of plants containing cyanogenic glycosides (Nobrega et al., 2006). Upon hydrolysis of these cyanogenic glycosides toxic cyanide is released, thus the need for its detoxification.

Rhodanese (EC.2.8.1.1) is an enzyme involved in biotransformation of cyanide. The enzyme converts toxic cyanide to less toxic thiocyanate by transferring sulfur atom from a sulfur donor to cyanide. Rhodanese catalysed cyanide detoxification is such that the enzyme accepts sulfur atom from a sulfur containing anion; the sulfur-substituted enzyme then transfers the sulfur atom to strongly thiolphile cyanide (Aminlari and Vaseghi, 2006). During the catalytic cycle of rhodanese, the enzyme exists in two forms, the free enzyme and the persulfate-containing form (Domenica et al., 2000). Rhodanese is ubiquitous in nature and its activity is found in all living organisms. The biological functions of rhodanese include cyanide detoxification (Koj and Frenod, 1962), formation of iron-sulfur centers (Cerletti, 1986) and participation in energy metabolism (Ogata and Volini, 1990).

Rhodanese activity has been studied and reported in many organisms by several researchers. Its activity has been reported in bacteria (Itakorode et al., 2019), plants (Anosike and Ugochukwu, 1981; Okonji et al., 2017; Ehigie et al., 2019), animals (Anosike and Jack, 1982; Eskandarzade et al., 2012; Wodu, 2015; Okonji et al., 2015; Ehigie et al., 2019; Wodu et al., 2021). However, rhodanese in Synodontis schall is yet to be elucidated. Thus, the aim of this research work is to characterize crude extract of rhodanese from Synodontis schall.

MATERIALS AND METHODS

All reagents used were of analytical grade and did not need any further purification.

Sample collection
The liver of the Synodontis schall used in the investigations was excised from the fish and stored in a refrigerator at -4°C until required.

Preparation of tissue extract
Tissue extract was prepared by homogenizing 10g (w/v) of the liver in 3 volumes of homogenization...
buffer (phosphate buffer, pH 8.2). The suspension was centrifuged for 20 min at 4,000 rpm in a refrigerated centrifuge (Model universal 320R). The supernatant was used as crude enzyme source.

**Protein and enzyme assay**

Protein concentration was estimated using the method of Bradford (1976). Bovine Serum Albumin was used as standard. Rhodanese was assayed by the method of Agboola and Okonji (2004) with slight modifications. The reaction mixture contained 10mM Na₂S₂O₃, 10mM KCN, 0.25mM borate buffer, pH 8.2 and 10μl of enzyme solution in a final volume of 1.0ml. The reaction was carried out for 1min at 37°C and stopped by adding 0.5ml 15% formaldehyde. Exactly 1.5ml of Sorbo reagent (which is made up of ferric nitrate solution containing 0.025g Fe(NO₃)₃,9H₂O in 0.74ml water and 0.26ml concentrated nitric acid) to develop the colour. Absorbance was measured at 460nm. The unit of enzyme activity was defined as the amount of thiocyanate formed in micromoles per minute at 37°C and pH 8.2.

**Determination of kinetic constant**

The kinetic constants (Km and Vmax) were determined by varying the concentrations of KCN between 2mM and 10mM at fixed concentration of 10mM Na₂S₂O₃. Also, the concentration of Na₂S₂O₃ was varied between 2mM and 10mM at fixed concentration of 10mM KCN.

Kinetic parameters were estimated from the double reciprocal plots (Lineweaver and Burk, 1934). Primary and secondary plots were made for kinetic constant estimation.

**Substrate Specificity**

The substrate specificity of the enzyme was determined using different sulphur compounds namely dithio oxamide, 2-mercaptoethanol, sodium metabisulphite and ammonium sulphate in a typical rhodanese assay. The kinetic parameters of the different compounds were estimated using the double reciprocal plot (Lineweaver and Burk, 1934), by varying the concentrations of the individual substrates between 2mM and 10mM at a fixed concentration of 10mM KCN.

The activity was also expressed as a percentage activity of the enzyme using sodium thiosulphate as the control.

**Effect of pH on Rhodanese Activity.**

The effect of pH on rhodanese extracted from the liver of *Synodontis schall* was determined using 50mM citrate buffer (pH 4-6.5), 50mM potassium phosphate buffer (pH 7.0-8.5) and 50mM borate buffer (pH 9-11). Rhodanese activity was assayed as described in the assay section with the assay buffer being replaced by these buffers.

**Effect of temperature on Rhodanese Activity**

Rhodanese was assayed at temperatures between 20°C to 60°C to elucidate the effect of temperature on the enzyme activity. The assay mixture was first incubated at the test temperature for ten (10) minutes before initiating reaction by the addition of the enzyme which had been equilibrated at the same temperature. Rhodanese activity was assayed as in the standard procedure described in the assay section at the different temperatures.

**Effect of Salts on the Enzyme Activity**

The influence of various cations on *Synodontis schall* was investigated using the following salts: BaCl₂, SnCl₂, CoCl₂, HgCl₂, MgCl₂, MnCl₂, and CuCl₂ at 0.05mM, 0.1mM and 0.2mM in a typical rhodanese assay mixture. The reaction mixture without the salts was taken as control with 100% activity.

**Statistical Analysis**

The SPSS statistical analysis system was used for analysis of the data. All the assays of were in triplicate determinations. The data collected were presented as means ± standard deviations and also relative activity in percentage (%). The statistical significance was assessed by one-way analysis of variance. Significant differences (P ≤ 0.05) among means were detected using Duncan’s multiple range tests.

**RESULTS AND DISCUSSION**

Crude extract of rhodanese from *Synodontis schall* was investigated to elucidate its characteristics. Double reciprocal plots for kinetic constants estimation and bisubstrate mechanism deduction for *Synodontis schall* liver rhodanese are presented in Figures 1-4 and Table 1. The km of 8.45mM and 12.23mM for Na₂S₂O₃ and KCN respectively for rhodanese investigated compare well with what had been reported for rhodanese from other sources (Table 2). Although very low km values of 0.408mM and 0.316mM had been reported by Ehigie et al., (2019) for Na₂S₂O₃ and KCN respectively for rhodanese extracted from cane rat kidney. Also, Wodu et al., (2021) reported very high km value of 50mM for KCN for rhodanese from ram liver.

From the observation in the present investigation, *Synodontis schall* liver rhodanese had higher affinity for KCN than Na₂S₂O₃ as indicated in the lower km value of 8.45mM for KCN as against 12.23mM for Na₂S₂O₃ (table 1). Similar results for rhodanese from other sources have been recorded by some researchers. Higher affinity for KCN was reported by Agboola and Okonji, (2004) for fruit bat rhodanase, Okonji et al., (2015) for garden snail hepatopancreas, Wodu et al., (2021) for ram kidney rhodanese and Ehigie et al., (2019) for cane rat kidney. Higher affinity for Na₂S₂O₃ substrate by rhodanase has been reported by Sorbo (1953) for bovine liver rhodanase.

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In a bid to investigate the sulfur donating property of some possible sulfur donors in rhodanese catalyzed cyanide detoxification, it was observed that *Synodontis schall* liver rhodanese had preference for Na$_2$S$_2$O$_3$ than for other sulfur donors (Tables 3 and 4). Of all the sulfur donors investigated, Na$_2$S$_2$O$_3$ had the lowest km of 12.2mM which indicates that *Synodotis schall* rhodanese had higher affinity for it. Dithiooxiamide, 2-mercaptoethanol and sodium metabisulfite were not capable of replacing Na$_2$S$_2$O$_3$ as sulfur donors.

*Bacillus cereus* rhodanese activity had been reported to be inhibited by 2-mercaptoethanol (Chew and Boey, 1972; Itakorode *et al.*, 2019) and sodium metabisulfite (Itakorode *et al.*, 2019). Rhodanese has the ability to use different sulfur containing compounds as sulfur donors (Okonji *et al.*, 2008). The result in the present investigation indicates that ammonium sulphate may be used as sulfur donor in the cyanide detoxifying mechanism of rhodanese. The percentage residual activity shown in table 3 revealed that Na$_2$S$_2$O$_3$ had highest activity followed by ammonium sulphate.

*Synodintis schall* catalyzed cyanide detoxification followed a double displacement (ping pong) bisubstrate mechanism (Figures 1 and 3). *Synodintis schall* liver rhodanese followed the same bisubstrate mechanism used by bacterial rhodanese reported by Atkinson (1975). Sorbo (1953) however, reported that bovine liver rhodanese catalyzed cyanide detoxification using ordered sequential mechanism.
Fig 3: Primary Plot of $1/V_0$ versus $1/[\text{KCN}]$ at fixed concentration of $\text{Na}_2\text{S}_2\text{O}_3$

Fig 4: A Secondary Plot of Intercept versus $1/[\text{Na}_2\text{S}_2\text{O}_3]$

Table 1: Kinetic Parameters of *Synodontis schall* Rhodanese

|       | $K_m$ (mM) | $V_{max}$ (RU ml$^{-1}$ min$^{-1}$) | pH Optimum | Temp. Optimum ($^\circ$C) |
|-------|------------|-------------------------------------|------------|-------------------------|
| KCN   | 8.44±1.05  | 0.083±0.00                          | 45.0±2.0   | 8.0±1.0                 |
| $\text{Na}_2\text{S}_2\text{O}_3$ | 12.23±1.36 | 0.089±0.001                          |            |                         |

Values are recorded as mean±SE of the mean.
Optimum rhodanese activity in Synodinitis schall was observed at pH 8.0 and 45°C (table 1 and figures 5 and 6). Both pH and temperature optima of Synodinitis schall liver rhodanese conforms to findings reported by several researchers. Optimum temperature of 40ºC had been reported for rhodanese extracted from African cat fish (Akinsiku et al., 2010) and Bacillus previs (Oyedeji et al., 2013). Temperature optimum range of 35ºC - 55ºC was reported for rhodanese from different strains of Trichoderma by Ezzi et al., (2003).

Optimum pH of 8.0 estimated for Synodinitis schall liver rhodanese had been reported by several researchers for rhodanese from different sources (Okonji et al., 2010; Okonji et al., 2015; Ehigie et al., 2019). Most of the reported findings have shown that optimum pH of rhodanese falls within the range of 6.0 - 11.0 (Chew and Boey, 1972; Ferni and Raphael, 2003; Agboola and Okonji (2004); Akinsiku et al., (2009); Hossein and Reza, 2011).

Table 2: Kinetic parameters of rhodanese from different species

| Source                        | Km (mM) | KCN | Na$_2$S$_2$O$_3$ |
|-------------------------------|---------|-----|-----------------|
| Synodontis schall$^a$         | 8.44    | 12.23 |                |
| Bovine liver$^b$              | 19.0    | 6.5  |                |
| Mouse Liver$^c$               | 12.5    | 8.3  |                |
| Bat liver$^d$                 | 13.36   | 19.15 |                |
| Garden snail hepatopancreas$^e$ | 9.1    | 12.3 |                |
| Cat liver$^f$                 | 25.4    | 18.6 |                |
| Ram liver$^g$                 | 50      | 12.5 |                |

$^a$ =present work; $^b$= Sorbo (1953); $^c$ = Lee et al., (1995); $^d$=Agboola and Okonji (2004); $^e$ = Okonji et al., (2015); $^f$= Akinsiku et al., (2009); $^g$ = Wodu et al., (2021).

Table 3: Kinetic Parameters of Possible Sulphur Donor Substrates in Rhodanese Catalysed Reaction

| Substrate                   | Km (mM)   | Vmax (RU min$^{-1}$ mg$^{-1}$) |
|-----------------------------|-----------|--------------------------------|
| Sodium thiosulphate         | 12.23±1.36| 0.089±.001                     |
| Dithio oxamide              | 67.62±4.93| 0.092±0.022                    |
| 2-mercaptoethanol           | 63.33±15.14| 0.163±0.101                   |
| sodiummetabisulphite        | 75.65±14.90| 0.120±0.036                   |
| Ammonium sulphate           | 25.38±8.73| 0.065±0.012                    |

Values are recorded as mean±SE of the mean

Table 4: Relative enzyme activity for different sulphur donor substrates

| Substrate                   | Residual Activity (%) |
|-----------------------------|-----------------------|
| Sodium thiosulphate         | 100±7.18              |
| Dithio oxamide              | 32.62±4.93            |
| 2-mercaptoethanol           | 23.76±5.17            |
| Sodium metabisulphite       | 31.44±14.90           |
| Ammonium sulphate           | 67.43±8.25            |

Values are recorded as mean±SE of the mean

Results for the modulation of Synodinitis schall liver rhodanese activity by some cations presented in figure 7 showed that all the cations investigated did not have any significant effect on the enzyme activity at the concentrations studied. This observation is similar to the findings reported by Fagbohunka et al., (2004), Okonji et al., (2011), and Okonji et al., (2015) for rhodanese from giant snail hepatopancreas, mudskipper liver, garden snail hepatopancreases respectively. In the present investigation, Hg$^{2+}$ did not show any inhibitory effect on Synodinitis schall liver rhodanese; Agboola and Okonji, (2004), Okonji et al., (2010), Ehigie et al., (2019) and Wodu et al., (2021) however, reported inhibition of rhodanese extracted from fruit bat liver, soldier termites, cane rat liver and ram (liver and kidney) respectively by Hg$^{2+}$.

The fact that the metal ions investigated in the present research work had no significant effect on Synodinitis schall liver rhodanese activity may be because of the concentrations used and/or the fact that Synodinitis schall may have been exposed to the salt of these metals over a long time.
CONCLUSION

In summary, this investigation showed the presence of rhodanese activity in the liver of Synodontis schall, whose characteristics are similar to rhodanese extracted from other sources. The results showed that Synodontis schall liver rhodanese had higher affinity for cyanide. Also, other sulfur donors may be exploited in the rhodanese assay. Cyanide detoxification is key to
the survival of Synodontis schall in its natural habitat, hence that the fish is able to survive even after consuming food containing cyanogenic glycosides means that it has a functional cyanide detoxification mechanism implicated by the presence of rhodanese. Finally, there is every likelihood that the organism under investigation had been exposed to several metal salts over time that made these metal salts not to affect certain physiological functions such as cyanide detoxification by rhodanese.

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