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Original Research Article

Characterization of Rhodanese Extracted from *Synodontis schall* Liver

Ebizimor Wodu[1*], Ayibaene Frank-Oputu[1], Oghenerume F. Asheshemi[1], Chinwe I. Ozomah[1]

[1]Department of Biochemistry, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

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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 $Na_2S_2O_3$ and KCN were 12.23mM±1.36 and 8.45mM±1.05 respectively. The enzyme had higher affinity for KCN than $Na_2S_2O_3$. Dithio oxiamide, 2-mercaptoethanol and sodium metabisulfite were not capable of replacing $Na_2S_2O_3$ as sulfur donors. $Na_2S_2O_3$ had the highest relative activity followed by ammonium sulphate. *Synodontis schall* liver rhodanese had optimum activity at pH 8.0 and 45ºC. 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.

Keyword: 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 thiophilic 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 Frendo, 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 *Synodintis 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^{0}C until required.

Preparation of tissue extract

Tissue extract was prepared by homogenizing 10g (w/v) of the liver in 3 volumes of homogenization

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

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 $Na_2S_2O_3$, 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 37ºC 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 $Fe(NO_3)_3$,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 37ºC 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 $Na_2S_2O_3$. Also, the concentration of $Na_2S_2O_3$ 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 20ºC to 60ºC 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: $BaCl_2$, $SnCl_2$, $CoCl_2$, $HgCl_2$, $MgCl_2$, $MnCl_2$, and $CuCl_2$ 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 ($P \leq 0.05$) among means were detected using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Crude extract of rhodanese from *Synodintis schall* was investigated to elucidate its characteristics. Double reciprocal plots for kinetic constants estimation and bisubstrate mechanism deduction for *Synodintis schall* liver rhodanese are presented in Figures 1-4 and Table 1. The km of 8.45mM and 12.23mM for $Na_2S_2O_3$ 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 $Na_2S_2O_3$ 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, *Synodntis scall* liver rhodanese had higher affinity for KCN than $Na_2S_2O_3$ as indicated in the lower km value of 8.45mM for KCN as against 12.23mM for $Na_2S_2O_3$ (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 $Na_2S_2O_3$ substrate by rhodanese has been reported by Sorbo (1953) for bovine liver rhodanese, Lee *et al*.,

(1995) for mouse liver rhodanese, Akinsiku *et al*., (2009) for cat liver rhodanese and Wodu *et al*., (2021) for ram liver rhodanese.

In a bid to investigate the sulfur donating property of some possible sulfur donors in rhodanese catalyzed cyanide detoxification, it was observed that *Synodntis schall* liver rhodanese had preference for $Na_2S_2O_3$ than for other sulfur donors (Tables 3 and 4). Of all the sulfur donors investigated, $Na_2S_2O_3$ had the lowest km of 12.2mM which indicates that *Synodotis schall* rhodanese had higher affinity for it. Dithio oxiamide, 2-mercaptoethanol and sodium metabisulfite were not capable of replacing $Na_2S_2O_3$ 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 ppercentage residual activity shown in table 3 revealed that $Na_2S_2O_3$ 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.

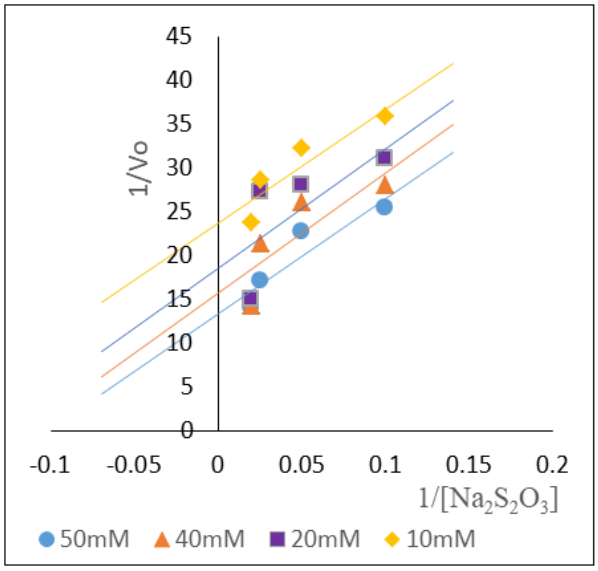

Fig 1: Primary Plot of 1/Vo versus I/[$Na_2S_2O_3$] at fixed concentration of KCN

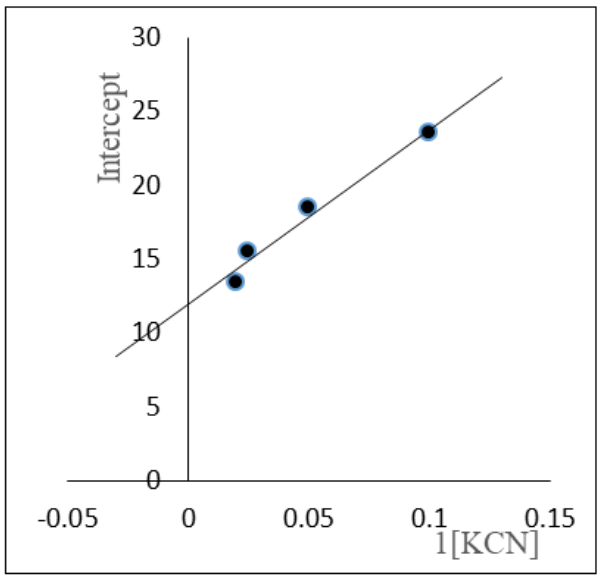

Fig 2: A Secondary Plot of Intercept versus 1/[$Na_2S_2O_3$]

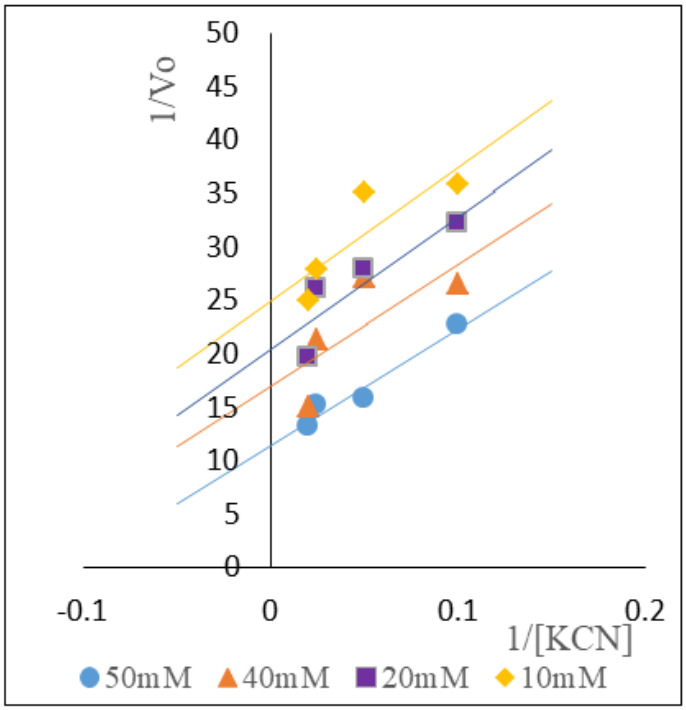

Fig 3: Primary Plot of 1/Vo versus 1/[KCN] at fixed concentration of $Na_2S_2O_3$

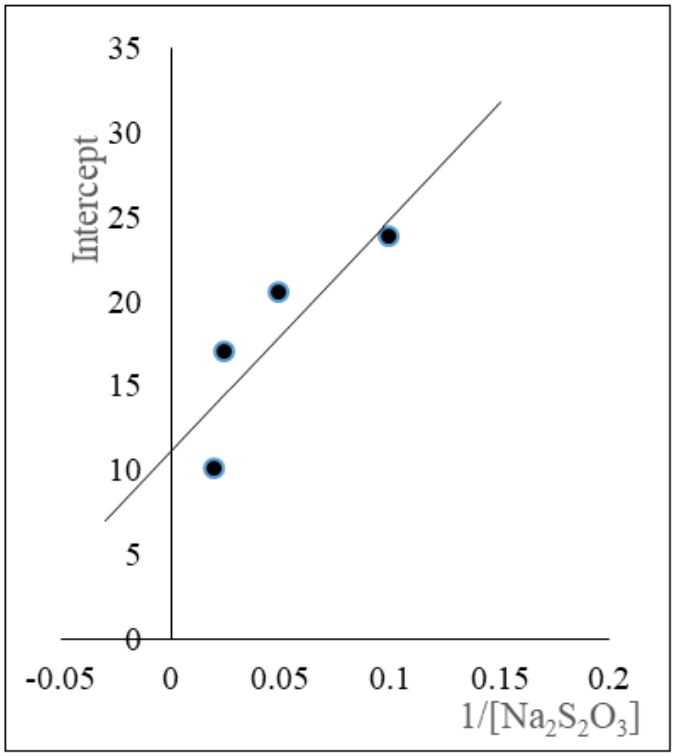

Fig 4: A Secondary Plot of Intercept versus 1/[$Na_2S_2O_3$]

Table 1: Kinetic Parameters of *Synodontis schall* Rhodanese

	Km (mM)	Vmax (RU ml^{-1} min^{-1})	pH Optimum	Temp. Optimum (°C)
KCN	8.44±1.05	0.083±0.00	45.0±2.0	8.0±1.0
$Na_2S_2O_2$	12.23±1.36	0.089±.001		

Values are recorded as mean±SE of the mean

Optimum rhodanese activity in *Synodintis schall* was observed at pH 8.0 and 45ºC (table 1 and figures 5 and 6). Both pH and temperature optima of *Synodintis 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 *Synodintis schall* liver rhodanese had been reported by several researchers for rhodanese from different sources (Okonji *et al*., 2010; Okonji *er 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; Femi 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	$Na_2S_2O_2$
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
sodiummetabisulfite	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 metabisulfite	31.44±14.90
Ammonium sulphate	67.43.38±9.25

Values are recorded as mean±SE of the mean

Results for the modulation of *Synodintis 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 hepatopancreas respectively. In the present investigation, Hg^{2+} did not show any inhibitory effect on *Synodintis 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 *Synodontis schall* liver rhodanese activity may be because of the concentrations used and/or the fact that *Synodontis schall* may have been exposed to the salt of these metals over a long time.

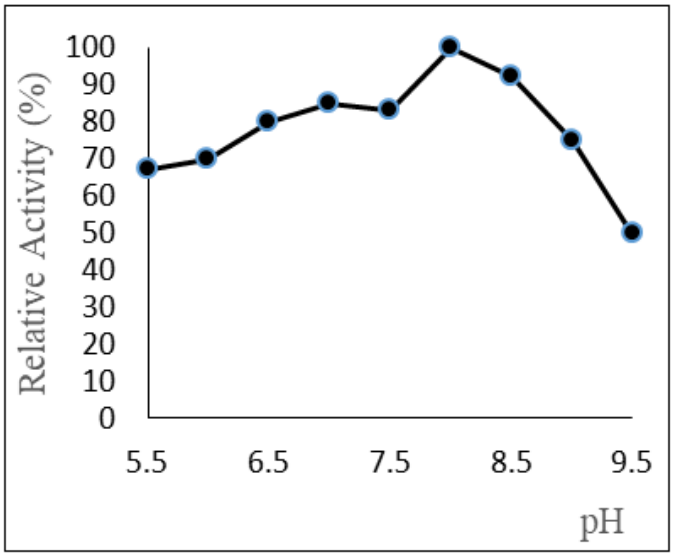

Fig 5: Effects of pH on Rhodanese Activity

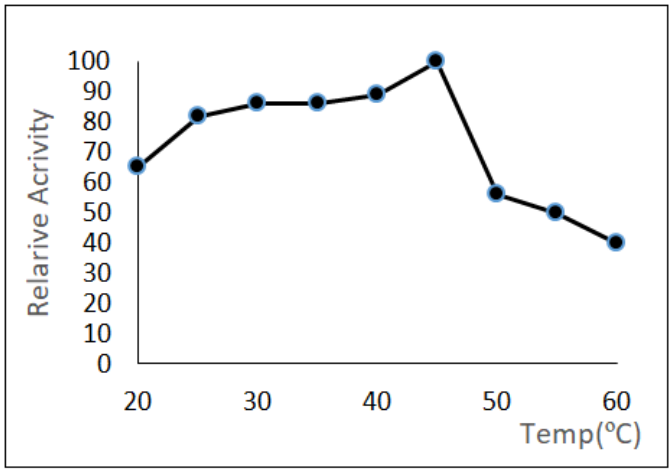

Fig 6: Effects of Temperature on Rhodanese Activity

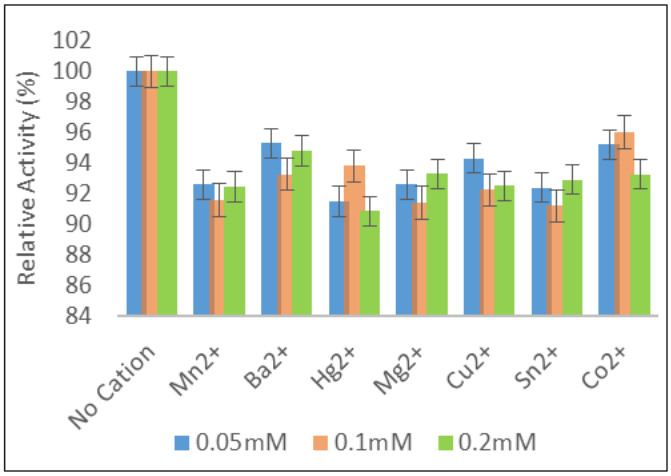

Fig 7: Effects of Cations on Rhodanese Activity

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 explioted 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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