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Properties of rhodanese from the liver of tilapia, *Oreochromis niloticus,* in Asejire Lake, Nigeria

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The study investigates the purification and characterisation of rhodanese from the liver of the tilapia fish (*Oreochromis niloticus*) collected from Asejire Lake in Nigeria. This was with a view to understanding the biochemical basis of the survival of the fish in cyanide polluted water. Rhodanese was isolated and purified from liver tissue homogenate of tilapia using CM-Sephadex ion exchange chromatography and Sephadex G-75 gel filtration. The specific activity of the enzyme was 56.86 U/mg. The K_m values for KCN and $Na_2S_2O_3$ as substrates were 0.1240 ± 0.0021 mM and 0.0516 ± 0.0097 mM, respectively. The apparent molecular weight was estimated by gel filtration on a Sephacyl S-400 column to be 35,460 Da. The optimal activity was found at pH 6.5 and the temperature optimum was 40°C. The rhodanese enzyme showed that the activity of the enzyme was not affected by $MgCl_2$, KCl, NH_4Cl, $MnCl_2$ and $CaCl_2$ while $AlCl_3$, inhibited the enzyme.

Key words: Cyanide, detoxification, tilapia, liver, rhodanese.

INTRODUCTION

Cyanide is a potent health hazard for human and ecosystem (Gupta et al., 2010). The toxic effect of cyanide (CN) is predominantly attributed to the inhibition of cytochrome c oxidase, a terminal oxidase of the mitochondrial respiratory pathway resulting in a condition of histotoxic anoxia.

Tolerance of organisms to cyanide contaminated environment and/or feed are due to a number of cyanide detoxification pathways, among the enzymatic pathways are 3-mercaptopyruvate sulphurtransferase (EC 2.8.1.2) and rhodanese (thiosulphate: cyanide sulphurtransferase, EC 2.8.1.1) which are widely distributed in living organisms. A major mechanism which removes CN^- from the body by enzymatic conversion to the less toxic thiocyanate (SCN) in the presence of a sulfur donor is by rhodanese (Rh) (Way, 1983; Petrikovics et al., 2010).

Rhodaneses are highly conserved and widespread enzymes, currently regarded as one of the most effective mechanism evolved for cyanide detoxification. *In vitro*, rhodanese catalyzes the irreversible transfer of a sulphur atom from a suitable donor (i.e. thiosulphate) to cyanide, leading to formation of less toxic sulphite and thiocyanate (Gupta et al., 2010). The enzyme has been purified and characterised from a number of animal tissues (Lee et al., 1995; Agboola and Okonji, 2004; Akinsiku et al., 2010) some of which are actively exposed to cyanide contaminated environments or food. The specific activity of rhodanese in many animal tissues is present in Table 1

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Table 1. Specific activity of rhodanese (U/mg protein) in selected tissues of human and some domestic animals.

Tissue	Specie								
	Sheep[a]	Cattle[a]	Goat[b]	Camel[a]	Horse[a]	Pigc	Dog[a]	Chicken[d]	Human[e]
Liver	5.21	4.95	2.93	1.7	1.7	0.56	0.18	0.31	0.15
Rumen				0.28					
Epithelial Layer	16.3	10.3	3.31						
Muscular Layer	0.09	0.05	0.01						
Kidney				0.08	1.4		0.06	0.08	0.96
Cortex	1.72	0.82	0.94			0.4			
Medullar	0.24	0.05	0.25			0.1			
Brain	0.48	0.36	0.47	0.13	0.15	ND	0.32	ND	0.03
Lung	0.24	0.13	0.36	0.24	0.03	0.04	0.02	0.01	0.02
Stomach					0.1		0.07		0.01
Abomasal Fundus	0.09	0.04	0.35	0.07		0.13			
Abomassal Pylorus	0.1	0.04	0.31	0.02		0.006			
Muscular layer of proventriculus								0.09	
Epithelial layer of proventriculus								0.59	
Muscle	0.04	0.02	0.08	0.05	0.15	0.07	0.04	ND	0.01

*ND = Not determined; [a]Aminlari et al., 1989; [b]Nazifi, 2003; [c]Aminlari et al., 2002; [d]Aminlari et al., 1997; [e]Aminlari et al., 2007.

Asejire Lake (07º 21'N 04º 05'E), the source of the tilapia used in this study, is a manmade lake on River Osun, has an impounded area of 2342 ha and located about 30 km east of Ibadan, Southwest Nigeria. River Osun is one of the series of West African rivers which do not drain into Niger system but discharge into coastal lagoons and creeks bordering the Atlantic Ocean (Ayoade et al., 2006).

River Osun constantly received cyanide contaminants through agricultural and industrial activities (Olajire and Imeokparia, 2001) yet the river and lake support aquatic life (Ayoade et al., 2006; Adeyemo and Akomolafe, 2011). Tilapia *Oreochromis niloticus* is the most widely harvest freshwater fish in the Lake.

The survival nature of the fish is suspected to be due to the biotransformation of cyanide to thiocyanate by rhodanese. In early report from our laboratory we described the physicochemical and kinetic characterization of rhodanese from the liver of *Clarias gariepinus* Burchell in Asejire lake (Akinsiku et al., 2010), this paper describes the isolation and characterization of rhodanese from the cytosolic fraction of tilapia liver from the lake.

MATERIALS AND METHODS

CM-Sephadex C-50, Sephadex G-75 and Sephacyl S-400 were obtained from Pharmacia Fine Chemical, Uppsala, Sweden. All other reagents were of analytical grade and were obtained from either Sigma or BDH. The tilapia fishes, *Oreochromis niloticus*, were obtained from Asejire Lake located in the outskirt of Ibadan, Oyo State, Nigeria.

Enzyme and protein assays

The activity was expressed in rhodanese unit (RU). One rhodanese unit was taken as the amount of enzyme, which under the given condition produced an optical density reading of 1.08 at 460 nm per min (Sorbo, 1953). Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Enzyme isolation and homogenisation

Scalpels were used to open up the abdomen. The liver was quickly removed and stored in the freezer until required. The frozen liver was thawed at room temperature and rinsed with cold saline (0.9% NaCl, pH 7.4) to remove blood and other impurities. Fifty grams (50 g) of the liver (obtained from 15 fishes) was minced with a pair of scissors into smaller pieces and homogenized in two volumes of 0.1 M acetate glycine buffer, pH 7.8 containing 1 mM ε-amino-n-caprioc acid, and 10 mM sodium thiosulphate for 5 min with a Warring Blender. The homogenate was centrifuged for 60 min at 20,000 rpm. The crude enzyme was assayed for protein and enzyme.

C+M-Sephadex ion exchange chromatography

CM-Sephadex C-50 was pretreated according to Agboola and Okonji (2004). The resin was packed into a 2.5 × 40 column and equilibrated with 50 mM citrate buffer, pH 5.0 containing 10 mM $Na_2S_2O_3$. The protein from the preceding step was layered on the column. The column was first washed with 200 ml of the equilibration buffer to wash the unbound protein, followed by elution with a 250 ml linear gradient of 0-0.5 M NaCl in same buffer. Fractions of 4 ml were collected at a rate of 24 ml/h. The protein profile was monitored at 280 nm. The fractions were also assayed for enzyme. The active fractions were pooled and dialysed against 50% glycerol.

Gel filtration on sephadex G-75

Sephadex G-75 gel filtration resin was prepared by swelling 40 g in boiling distilled water for 3 h. The resin was then washed with several changes of 0.1 M phosphate buffer containing 10 mM

Table 2. Purification processes.

Fraction	Total activity (mg)	Total protein (mg)	Specific activity (U/mg protein)	Percentage Yield	Purification fold
Crude extract	3113.43	2700.00	1.15	100	1.00
CM Sephadex ion exchange	2087.60	123.16	16.95	67.00	14.74
Sephadex-G75 Gel filtration	457.01	8.02	56.98	14.68	49.55

Each purification procedure is as described in the materials and methods section. Protein concentration was determined using Bradford and activity was determined by the rate of formation of thiocyanate. 1U of activity is defined as the amount of enzyme, which under the given conditions, produced an absorbance reading of 1.08 at 460 nm per min (Sorbo, 1951).

sodium thiosulphate, pH 7.2 and then packed into 2.5 × 70 cm glass column and equilibrated with the same buffer.

Forty millilitres (40 ml) of the dialyzed protein from the ion-exchange step was layered on the column. Fractions (4 ml) were collected from the column at a rate of 15 ml per hour. Protein profile was monitored at 280 nm and assayed for rhodanese activity. The active fractions were pooled and dialysed against several changes of 50% glycerol for storage.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis and subunit molecular weight determination in the absence of sodium dodecyl sulphate (SDS) was carried out according to the procedure of Weber and Osborn (1975) on 7.5% acrylamide gel. Destaining was carried out with a solution containing 25 ml of methanol, 37.5 ml of glacial acetic acid made up to 500 ml with distilled water.

SDS-polyacrylamide gel electrophoresis in 10% acrylamide gel was used for the determination of the subunit molecular weight. Standard proteins that were used for the calibration of the gel were bovine serum albumin (66,000 Da), ovalbumin (45,000 Da), pepsin (34,700 Da), trypsinogen (24,000 Da) and β-lactoglobulin (20,100 Da). The gels were run at 8 mA per gel at room temperature and stopped after 5 h. The staining and destaining were performed as described earlier. R_f values of the standards calculated were then plotted against the logarithms of their molecular weight. The molecular weight of the enzyme preparation was then intrapolated from the curve.

Determination of apparent molecular weight

The native molecular weight was determined on a Sephacyl S-400 column (1.5 × 100 cm) using standard proteins (BSA (67,000; 3 mg/ml), α-chymotrypsinogen (25,000; 3 mg/ml) and ovalbumin (45,000; 3 mg/ml)). 3 ml of each standard protein was layered on the column. The column was eluted with 10 mM phosphate buffer, pH 7.0 at a flow rate of 17 ml/h. The void volume (V_o) of the column was determined by the elution volume of Blue Dextran (2 mg/ml). 4 ml of the pure enzyme preparation was then passed through the same column and the elution volume (V_e) estimated as describe earlier. Partition coefficient (K_{av}) was calculated from V_o and (V_e). A plot of logarithm of the molecular weight of the standard proteins against K_{av} was made, the molecular weights of the enzyme preparation was then intrapolated from the curve.

Determination of kinetic parameters

The kinetic parameters (K_m and V_{max}) of the enzyme were determined by varying concentrations of KCN between 12.5 and 50 mM at fixed concentration of 50 mM $Na_2S_2O_3$. Also, the concentration of $Na_2S_2O_3$ was varied between 12.5 and 50 mM at fixed concentration of 50 mM KCN. The kinetic parameters were estimated from the double reciprocal plots (Lineweaver and Burk, 1934).

Effect of pH on the enzyme activity

The enzyme was assayed using the buffers of different pH values: 50 mM citrate buffer (pH 4.0-6.5), 10 mM phosphate buffer (pH 7.0-8.5), and 50 mM borate buffer (pH 9.0-11). A reaction mixture of 1 ml contained 0.5 ml of the respective buffer, 0.2 ml of 0.25 mM KCN, 0.2 ml of 0.25 mM of $Na_2S_2O_3$ and 0.1 ml of enzyme solution.

Effect of temperature on the enzyme activity

The enzyme was assayed at temperatures between 0 and 70°C. The assay mixture was first incubated at the indicated temperature for 10 min before initiating reaction by the addition of an aliquot of the enzyme which had been equilibrated at the same temperature. The residual enzyme was then assayed.

Determination of heat stability

The heat stability of the enzyme was also determined by incubating 0.6 ml of the enzyme for 1 h at 30, 40, 50, 60 and 70°C, respectively. 0.1 ml was withdrawn at 10 min interval and assayed for residual activity. The activity at 30, 40, 50, 60 and 70°C was expressed as a percentage of activity of the enzyme incubated at 30°C which was the control.

Effect of chloride salts on the enzyme activity

The salts include $MnCl_2$, NH_4Cl, $CaCl_2$, $AlCl_3$ and $MgCl_2$ at the final concentrations of 0.5 mM and 1.0 mM in a typical enzyme assay reaction mixture.

RESULTS

The summary of the result of the purification of rhodanese from the liver of tilapia is summarized in Table 2. The specific activity of the pure enzyme was 56.86 U/mg protein. The elution profile of the enzyme in ion-exchange chromatography on CM-Sephadex C-50 is shown in Figure 1. The peak was obtained in the breakthrough volume without the salt gradient elution. The elution profile on Sephadex G-75 is shown in Figure 2. Only one protein band was observed after gel electrophoresis of the purified rhodanese either in the presence or absence of sodium dodecyl suphate (figures not shown).

The calibration curve on Sephacyl S-400 for the determination of the native molecular weight is shown in Figure 3. The molecular weight of rhodanese from the liver of tilapia after the gel filtration was estimated to be

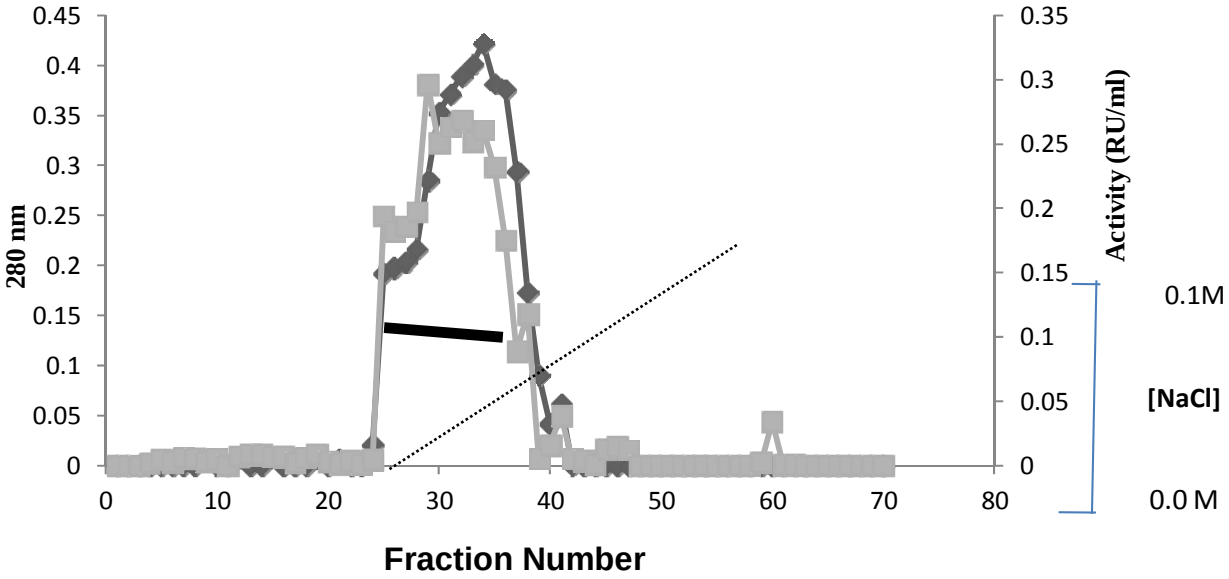

Figure 1. CM-Sephadex C-50 ion exchange chromatography elution profile of tilapia rhodanese. The column was equilibrated with 50 mM citrate buffer, pH 5.0 containing 10 mM Na_2SO_3. The dialysed protein was then layered on the column. The column was washed firstly with 200 ml buffer followed by elution with a 250 ml linear gradient of 0-0.5 mM NaCl in elution buffer. 4 ml fractions were collected at a rate of 24 ml/h. Protein profile was monitored using Bradford at 595 nm. ▬ Pooled Fraction; ····· Linear NaCl Gradient; Enzyme activity; ▯ OD◆$_{280}$

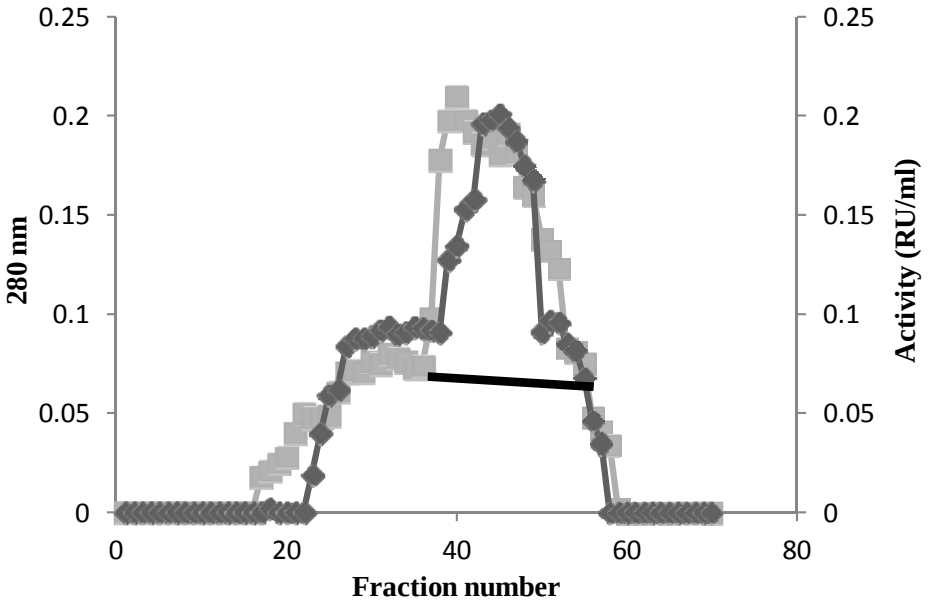

Figure 2. Sephadex G-75 Gel chromatography elution profile of Tilapia rhodanese. 40 ml of the dialysed protein from the ion exchange step was layered on a 1.5 × 40 cm column. 4 ml fractions were collected at a flow rate of 15 ml/h. Protein concentration was determined using Bradford at 595 nm. ▬, Pooled fraction; , Enzyme activity; ◆, OD_{280}

35,460 ± 450 daltons. The calibration curve obtained for polyacrylamide gel electrophoresis on 10% acrylamide gel electrophoresis is shown in Figure 4. The molecular weight was estimated to be 33,700 ± 714 daltons.

The double reciprocal plot of the change in KCN concentrations of fixed $Na_2S_2O_3$ is shown in Figure 5a, while that of varying concentrations of $Na_2S_2O_3$ at fixed KCN concentration is shown in Figure 5b. Table 3 shows the results of the kinetic parameters. The influences of pH on the rate of enzyme activity are shown in Figure 6. There were steady increases in enzyme activities until pH 6.5.

The effect of temperature on the activity of rhodanese

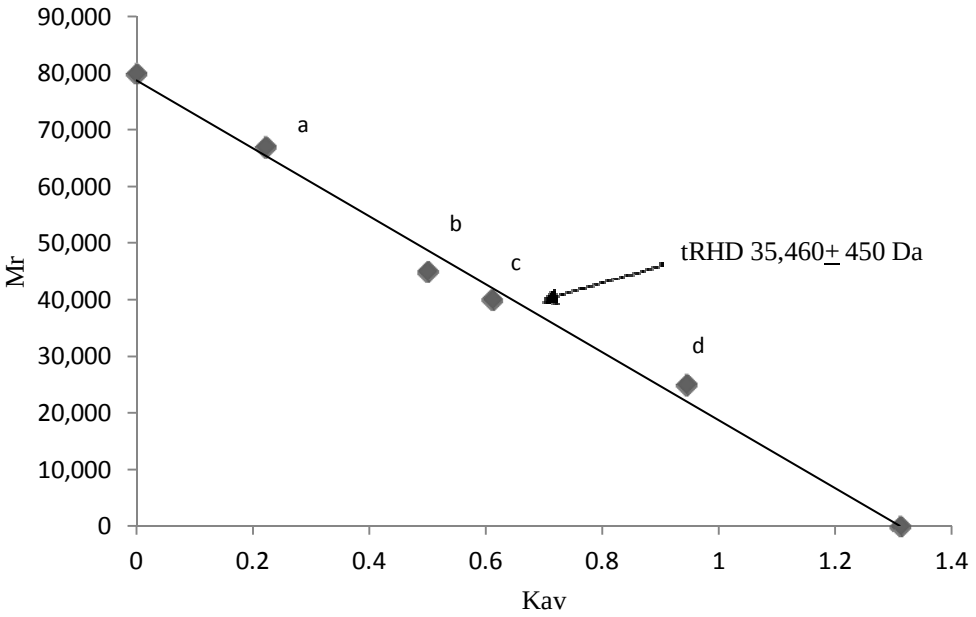

Figure 3. Calibration curve for molecular weight determination by gel filtration on sephacyl S-400. 3 ml of standard proteins was applied to the column (1.5 × 100 cm) and eluted with 10 mM phosphate buffer, pH 7.0 at a flow rate of 17 ml/h. Blue dextran (2 mg/ml) elution was used to determine the void volume. The standard proteins are (**a**) bovine serum albumin (66 kDa), (**b**) ovine albumin (45 kDa), (**c**) peroxidase (40 kDa), (**d**) chymotrypsinogen (25 kDa) and (**e**) β-lactoglobulin (20.1 kDa). The arrow indicates the position of the rhodanese enzyme (tRHD).

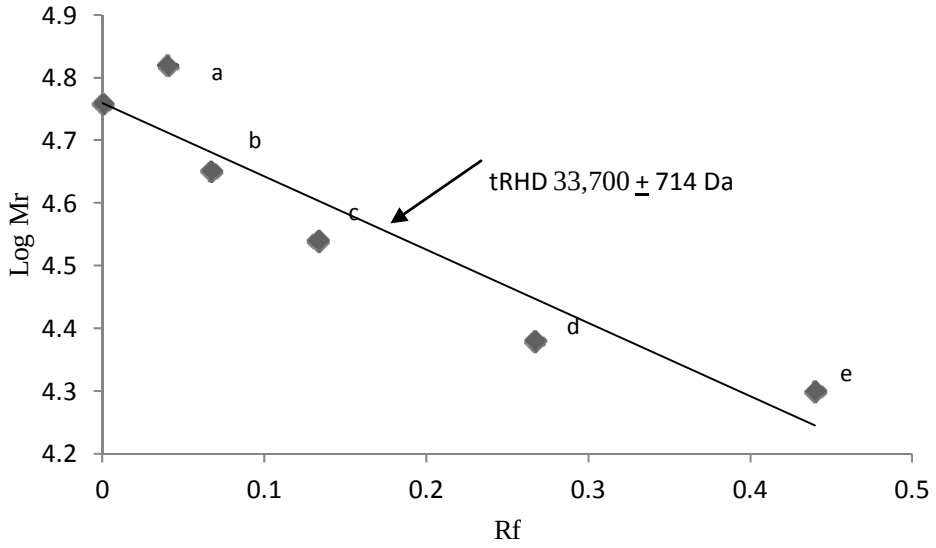

Figure 4. Calibration Curve for sub unit molecular weight determination on SDS-PAGE. The subunit molecular weight was determined by interpolation. The standard proteins are (**a**) bovine serum albumin (66 kDa), (**b**) ovine albumin (45 kDa), (**c**) peroxidase (40 kDa), (**d**) chymotrypsinogen (25 kDa) and (**e**) β-lactoglobulin (20.1 kDa). The arrow indicates the position of the rhodanese enzyme (tRHD).

from the tilapia liver is shown in Figure 7. Figure 8 shows the heat stability of the enzyme from the tilapia liver. The result of the effect of various chloride salts on the activity of catfish liver rhodanese (Table 4) showed that $MgCl_2$, KCl, NH_4Cl, $MnCl_2$ and $CaCl_2$ had little or no effect on the activity of the enzyme. Both concentrations of $AlCl_3$ have

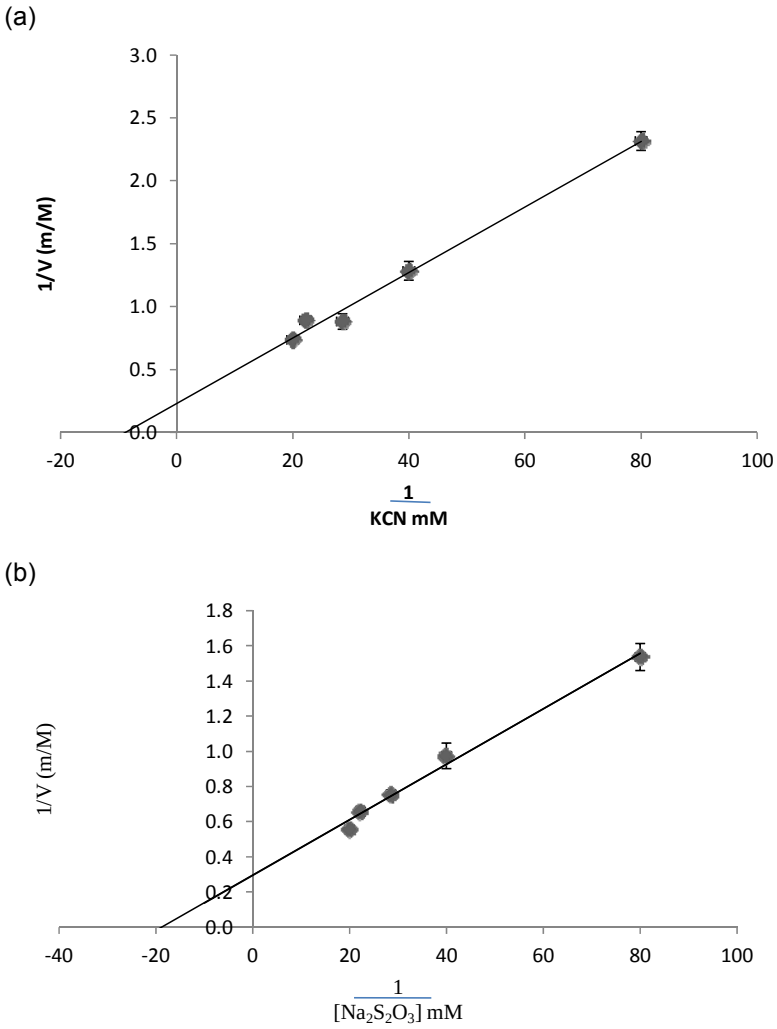

Figure 5. Lineweaver-Burk plot for the determination of K_m and $V_{max.}$ **(a)** The concentration of $Na_2S_2O_3$ was varied between 12.5 mM and 50 mM at fixed concentration of 50 mM KCN while the concentration of KCN was determined at fixed concentrations of 50 mM $Na_2S_2O_3$. **(b)**. The reaction mixture contained 25 mM borate buffer pH 9 and 0.1 ml of enzyme in a total reaction volume of 3.0 ml at room temperature.

Table 3. Kinetics Parameters of KCN and Na_2SO_4 as Substrates of Rhodanese from Tilapia Fish liver.

Parameter	KCN	$Na_2S_2O_3$
K_m (mM)	0.1240 ± 0.0201	0.0516 ± 0.0097
V_{max} (RU/ml)	4.395 ± 0.3800	3.4208 ± 0.3690

The above kinetic parameters K_m and V_{max} were determined from the double reciprocal plots and were a mean of triplicate determinations

the inhibition effect on the enzyme.

DISCUSSION

Tilapia (*Oreochromis niloticus*) is more tolerant than most commonly freshwater fish to high salinity, high water temperature, low dissolved oxygen, and high ammonia concentrations (Popma and Masser, 1999). Their habitants such as lakes, streams, rivers are constantly subject to contaminations from various sources such as effluents from manufacturing companies (Bruton, 1979). Cyanides are present in many industrial wastewaters,

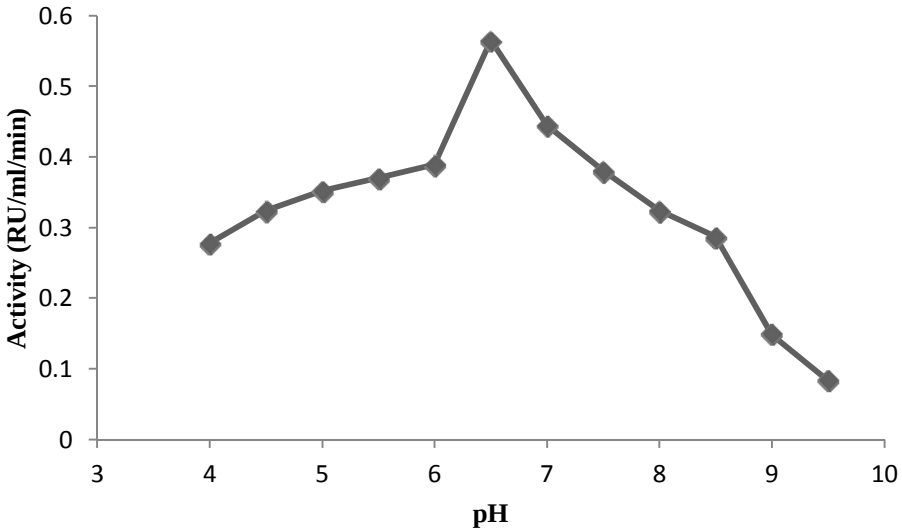

Figure 6. Effect of pH on the tilapia liver rhodanese. The assay mixture contained the appropriate buffers: 50 mM citrate buffer (pH 4.0-6.5), 10 mM phosphate buffer (pH 7.0-8.5), 0.2 ml of 0.25 KCN, 0.2 ml of 0.25 mm of $Na_2S_2O_3$ and 0.1 ml of enzyme inn final reaction volume of 3.0 ml.

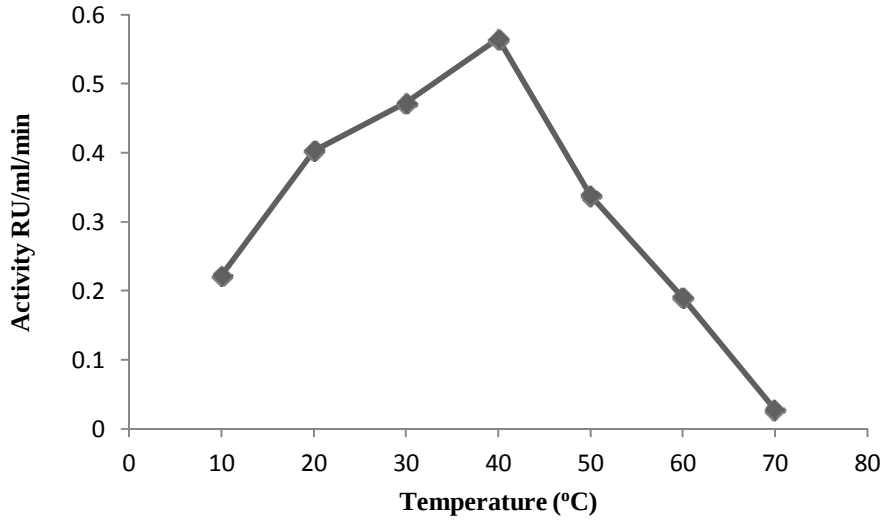

Figure 7. Activity-temperature profile showing optimum temperature of rhodanese. The assay mixture was first incubated at the incubated temperature for 10 min before initiating the reaction by the addition of an aliquot of the enzyme that had been previously equilibrated at same temperature.

especially effluents from iron and steel processing plants, petroleum refineries, and metal-plating plants, and constitute a hazard to aquatic ecosystems in certain waste-receiving waters (Eisler and Wiemeyer, 2004).

Study on feeding behaviour and nutrition requirements of *O. niloticus* by Popma and Masser (1999) shown that Tilapia ingest a wide variety of natural food organisms, including plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, larval fish, detritus, insects, even small fish fry and decomposing organic matter. With heavy supplemental feeding, natural food organisms typically account for 30 to 50% of tilapia growth. Most of these food items contain cyanogenic glycosides which upon hydrolysis release cyanide or hydrocyanic acid (HCN).

The Asejire Lake, the source of tilapia for this study also received cyanide from cassava processing activities around the lake. Rhodanese and 3-mercaptopyruvate sulphur transferase represent the chief enzymes of cyanide detoxification (Nagahara et al., 1999; Aminlari et

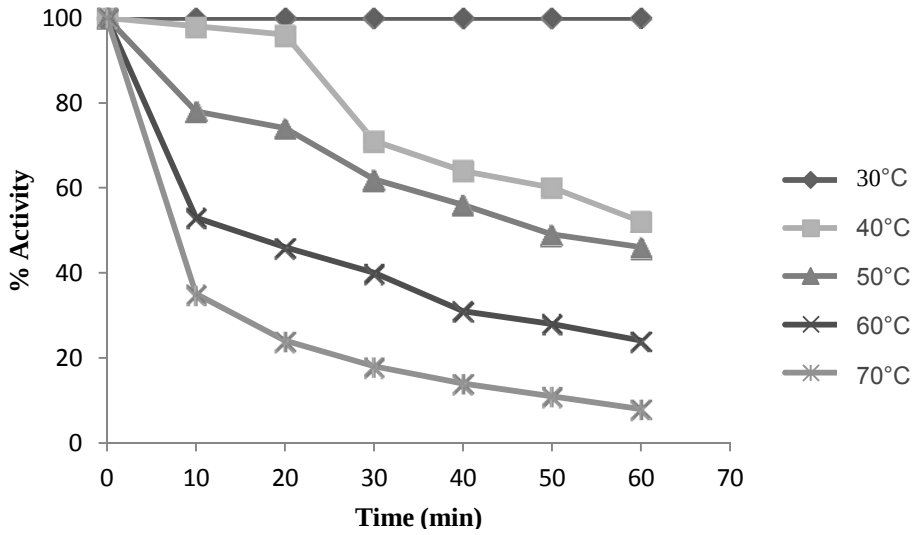

Figure 8. Effect of temperature on heat stability of tilapia rhodanese. 0.6 ml of enzyme was incubated at temperature ranges indicated above and aliquots of 0.1 ml was withdrawn at respective temperatures and assay was carried out for residual enzyme activity in 50 mM borate buffer. Percent residual activity is compared to the activity of the native enzyme at 30°C.

Table 4. Effect of chloride salts on tilapia liver rhodanese.

Parameter	Percentage enzyme residual activity	
Salt	0.5 mM	1.0 mM
None	100	100
$MgCl_2$	92 ± 6.14	90 ± 3.25
KCl	91 ± 2.07	89 ± 3.32
NH_4Cl	84 ± 1.25	79 ± 2.20
$MnCl_2$	83 ± 2.01	86 ± 3.25
$CaCl_2$	74 ±2.98	77 ± 2.82
$AlCl_3$	67 ± 5.45	67 ± 0.19

Enzyme assay were carried out as described in methods with each assay mixture containing the salts at final concentration of 0.5 and 1.0 mM. The values are a mean of at least triplicate determinations

al., 2002).

The purification procedures involved ion-exchange chromatography on CM-Sephadex C-50 and gel filtration on Sephadex G-75. Our initial attempts to use ammonium suphate fractionation resulted into a great lost of enzyme activities hence we omitted the purification process.

The specific activity of rhodanese from the liver of tilapia was found to be 56.98 RU/mg of protein. Sorbo (1953) and Himwich and Saunders (1948) obtained 256 RU/mg and 131 RU/mg, respectively for bovine liver rhodanese. Lee et al. (1995) obtained 1,070 RU/mg of protein for purified mouse liver rhodanese. Agboola and Okonji (2004) obtained 136.6 RU/mg protein for fruit bat liver rhodanese, Akinsiku et al. (2010) obtained 73 and 72 RU/mg for cRHD I and cRHD II, respectively for catfish liver rhodanese. Tayefi-Nasrabadi and Rahmani (2012) obtained 0.206 RU/mg protein for rhodanese from liver Rainbow Trout (*Oncorhynchus mykiss*).

The apparent relative molecular weight of 35,460 Da compares very well with that reported for the enzyme from other sources. It is in line with the reports that rhodanese has molecular weight value approximately between 33000 and 37000 daltons (Nagahara et al., 1999). The subunit molecular weight was 33,700±714 daltons. This result suggests that the enzyme is a monomeric protein. While Jarabak and Westley (1974) and Lee et al. (1995) showed that purified mouse and human liver rhodaneses are monomeric proteins, other workers have shown that they are dimers of two identical subunits of molecular weights 18,000-19,000 daltons (Blumenthal and Heinrikson, 1971).

The apparent K_m values, KCN and $Na_2S_2O_3$, were 0.1240 and 0.0516 mM, respectively. These values are lower than those of rainbow trout (KCN = 36.81mM; Na_2SO_4 = 19.84 mM) (Tayefi-Nasrabadi and Rahmani, 2012), african catfish liver (KCN = 25.40 mM; Na_2SO_4 = 18.60 mM) (Akinsiku et al., 2010), fruit bat liver (KCN = 13.36 mM; Na_2SO_4 = 19.15 mM) (Agboola and Okonji, 2004), mouse liver (KCN = 12.50 mM; Na_2SO_4 = 8.30 mM) (Lee et al., 1995), bovine liver (KCN = 19.0 mM; Na_2SO_4 = 6.7 mM) (Sorbo, 1953), and human liver (KCN = 9.50 mM; Na_2SO_4 = 4.50 mM) (Jarabak and Westley, 1974), indicating that the affinity of tilapia enzyme for these substrates is more than that of the other enzymes and that it would catalyze the detoxification reaction with high efficiency. As reported by Agboola and Okonji (2004) less effective enzymatic system may be due to a

lower exposure to cyanide and high affinity in some mammalian animals is due to continue exposure through their diet. This high affinity of tilapia rhodanese is supported by feeding and nutrition requirements of the fish that include wide range of natural foods which are cyanogenic (Popma and Masser, 1999). Madalla (2008) reported that (*Oreochromis niloticus* L) survived on diet which containing 40% and 10% of hydrogen cyanide from cassava leaf meal and cassava root meal respectively. It should be noted that cyanide detoxification is a secondary benefit of rhodanese, which has a number of major physiological roles, including the supply of sulphide for the formation of iron-sulphur centres for the electron transport chain (Cerletti, 1986), participation in energy metabolism (Ogata et al., 1989), and function as a thioredoxin oxidase (Nandi et al., 2000).

An optimum temperature of 40°C was obtained for tilapia liver rhodanese. This result is in good agreement with the results reported for rhodaneses from different sources. Sorbo (1953) reported an optimum temperature of 50°C for bovine liver rhodanese. Ezzi et al. (2003) obtained a wide temperature optimum of 35-55°C for rhodanese enzyme in all different *Trichoderma* strains. Agboola and Okonji (2004) reported 35°C for the rhodanese in the cytosolic fraction of fruit bat liver. Also, Akinsiku et al. (2010) reported 40°C for the rhodanese in the liver of catfish (*C. gariepinus*) from Asejire Lake, same source of tilapia for this study. Tayefi-Nasrabadi and Rahmani (2012) reported 25°C for rhodanese from the liver of rainbow trout. Akinsiku et al. (2010) posited that with the level of pollution in this water, there will be various metabolic activities going on in it which likely results in the release of heat and the ambient temperature of 40°C which might have conditioned the enzyme to function at higher temperature. The heat stability experiment showed that the enzyme was stable up to 60°C for about 30 min which means that the enzyme is thermostable.

An optimum pH of 6.5 was obtained for rhodanese from the liver of the tilapia from Asejire Lake. Same pH value was obtained by Akinsiku et al. (2010) for the rhodanese of catfish from the same lake. They posited that the value is as a result of the acidity of the water. Pollutants in water include wide spectrum of chemicals and pathogens which sometimes alters the acidity, conductivity and temperature of the water. The water sample collected from Asejire Lake showed a pH of 5.7, 6.3 and 6.5 at different points of the Lake (Lameed and Obadara, 2006).

Studies on effect of chloride salts on the enzyme showed that the activity of the enzyme was not affected by $MgCl_2$, KCl, NH_4Cl, $MnCl_2$ and $CaCl_2$ while $AlCl_3$ inhibited the enzyme greatly. Metal ions showing inhibitions are those that have strong affinity for cysteinyl and histidyl side chains of proteins (Ulmer and Vallee, 1972). The properties of rhodanese from the liver of tilapia are similar to those from other animal sources and might properly function in the same manner.

Conflict of interests

The author(s) have not declared any conflict of interests.

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