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Physicochemical properties of rhodanese from the kidney of cane rat (*Thryonomys swinderianus)*

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We report the properties of partially purified rhodanese, a cyanide detoxifying enzyme isolated from the kidney of the greater cane rat commonly known as grasscutter (*Thryonomys swinderianus*). *T. swinderianus* kidney rhodanese had a specific activity of 1.43 µmole/min/mg protein, a fold of 2.31 and a 55% recovery. The apparent K_m values of 0.408 and 0.316 mM were obtained for both sodium thiosulphate ($Na_2S_2O_3$) and potassium cyanide (KCN) respectively. Substrate specificity study showed that *T. swinderianus* rhodanese can use other substrates. The enzyme showed its maximum activity at pH 8.0 and 50°C. The assay for the effect of metal ions showed that the enzyme is not affected or inhibited by metal ions such as Na^+, Fe^{3+} but inhibited by Hg^{2+}. The continued existence of *T. swinderianus* after consumption of food crops with high cyanogenic glycosides suggests that the animal has a functional cyanide detoxification mechanism.

Keywords: Rhodanese, cyanide, kinetics, detoxification, cane rat, grasscutter.

INTRODUCTION

Due to its inhibitory effect on cellular respiration, cyanide is considered as a toxic compound for aerobic organisms. Various sources of cyanide reported include photo-synthetic bacteria, algae, fungi, plants and foods (such as beans, almonds and cashew nuts, etc) and even in the animal kingdom (ATSDR, 1997; EPA, 2003). Its presence has been reported in the roots of tubers such as cassava and potato grown in tropical countries. Cyanides are very reactive poisons that can be deadly and may be released into the environment through industrial usage or vehicle exhaust (ATSDR, 2006). Some plant species contain cyanogen glycosides which upon ingestion release hydrogen cyanide, a poisonous gas (Conn, 2008). Plants, such as beans, cassava, and peas used as food in tropical countries has been reported to contain cyanogenic glycosides. Cassava and peas are

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known to contain linamarin coexisting with lotaustralin (Uyoh et al., 2007).

Rhodanese (EC 2.8.1.1) is a ubiquitous enzyme and is widely distributed in plants (Nagahara et al., 1999; Aminlari et al., 2000). It is a detoxification enzyme that involves in the conversion cyanide (a toxic compound) to thiocyanate a less toxic compound (Agboola and Okonji, 2004; Lee et al., 1995). Various studies have reported the presence of rhodanese in the cytosol and other organelles (Nagahara et al., 1999; Steiner et al., 2018; Itakorode et al., 2019). Its detoxification potential has been reported in the liver tissues of diverse animals (Lee et al., 1995; Akinsiku et al., 2009). The highest concentration of rhodanese has been reported to be found in the liver of mammals, though, significant amounts can also reside in the kidney and other tissues (Cipollone and Visca, 2007). In spite of its widely studied detoxification role, research had made known that the enzyme also involved in other physiological activities such as energy metabolism and iron sulphur centres formation (Ogata and Volini 1990; Aussignargues et al., 2012). Grasscutter (*Thryonomys swinderianus*) feeds generally on plant debris, cassava and grasses which have been demonstrated to contain a high content of cyanogenic glycosides and on consumption release cyanide, a poisonous compound (Conn, 2008). This study, therefore, attempts to characterize rhodanese from *T. swinderianus* kidney for the purpose of biochemical comparison and to further understand the cyanide detoxification mechanism of the animal.

MATERIALS AND METHODS

The animal, grasscutter (*T. swinderianus*) was obtained from hunters in Abagboro village in Ile-Ife, Osun state, Nigeria. It was transported to the laboratory within 10 min of its slaughter. It was then dissected to remove the kidney.

Extraction of intracellular rhodanese

The kidney was rinsed properly with distilled water to remove blood stains and weighed. 20 g of the kidney tissue was cut into pieces and then homogenized in Tris buffer, (100 mM, pH 7.2) with a warring blender. The homogenate was filtered and then centrifuged at 10, 000 rpm at 4°C for 30 min. The supernatant obtained was checked for rhodanese activity and protein content.

Enzyme assay

Modified method of Lee et al., (1995) was used to determine rhodanese activity. Briefly, the assay mixture consists of 50 mM (borate buffer pH 9.4), 200 mM $Na_2S_2O_3$, 200 mM KCN, and 100 µl of the enzyme in 1 ml solution. The mixture was incubated with 15% formaldehyde (0.5 ml) for 1 min, followed by the addition of an appropriate volume of Sorbo reagent (Sorbo, 1951). The absorbance was then read at 460 nm using a visible spectrophotometer. One rhodanese unit (RU) was taken as the amount of the enzyme, which under the given conditions produced an optical density reading of 1.08 at 460 nm per min. Protein content was determined using the Bradford (1976) method.

Purification procedure

The crude enzyme was subjected to 70% ammonium sulphate precipitation and left overnight at 4°C. After centrifugation for 30 min, the precipitate obtained was re-suspended in a small amount of 0.1 M Tris buffer (pH 7.2) and dialyzed. The dialysate was assayed for rhodanese activity and protein concentration. 5 ml of the dialysate was applied to pretreated CM-Sephadex c-50 column equilibrated with 0.1 M Tris-HCl buffer (pH 7.2). Unbound protein was removed with 0.1 M Tris-HCl buffer, pH 7.2, followed by step-wise elution with 0.5 M NaCl in the same buffer. At the rate of 20 ml per hour, fractions of 2 ml were collected and monitored for protein and rhodanese activity. The active fractions from the column were then pooled.

Characterization procedure

Kinetic parameters determination

The kinetic parameters (Km and Vmax) of the enzyme were determined using potassium cyanide and sodium thiosulphate as substrates. This was done by varying concentrations of each substrate between 10 and 60 mM at a constant concentration of the other substrate. The Lineweaver-Burk double reciprocal plot was used to determine the kinetic parameters (Lineweaver and Burk, 1934).

Substrate specificity

The ability of the enzyme to use different sulphur compounds was investigated using copper sulphate, ammonium sulphate, ammonium persulphate, 2-mercaptoethanol, Sodium sulphite, and sodium sulphate decahydrate. The activity was determined as described above and expressed as a percentage activity of the enzyme using sodium thiosulphate as the control.

Effect of pH and temperature and metal ions on rhodanese activity

To check the effect of pH on enzyme activity, the enzyme was assayed using buffers of different pH: Citrate buffer (50 mM, pH 3-5), phosphate buffer (50 mM, pH 6-7), 50 mM Tris-HCl buffer (pH 8) and borate buffer (50 mM, pH 9-10). To study the effect of temperature, the enzyme was assayed at temperatures between 30 and 100°C. Influence of metals such as $HgCl_2$, NaCl, $FeCl_3$ and $MnCl_2$ was investigated at 0.1, 0.2 and 0.3 mM concentrations. The reaction mixture without the salt served as the control with 100% activity.

RESULTS

Purification of rhodanese

T. swinderianus kidney rhodanese had a specific activity of 1.43 µmol/mg protein, a fold of 2.31 and a yield of 55%. Table 1 summarized the result of the purification of *T. swinderianus* kidney rhodanese. The elution profiles after CM Sephadex c-50 ion-exchange chromatography is shown in Figure 1.

Table 1. Summary of purification of *T. swinderianus* kidney rhodanese.

Purification steps	Total protein (mg)	Total activity (µmole/min)	Specific activity (µmole/min/mg)	Purification fold	% yield
Crude sample	523.7	316.2	0.62	1	100
70% Ammonium Sulphate precipitation	221.3	196.7	0.89	1.44	62.2
Ion exchange chromatography	122.3	174.3	1.43	2.31	55

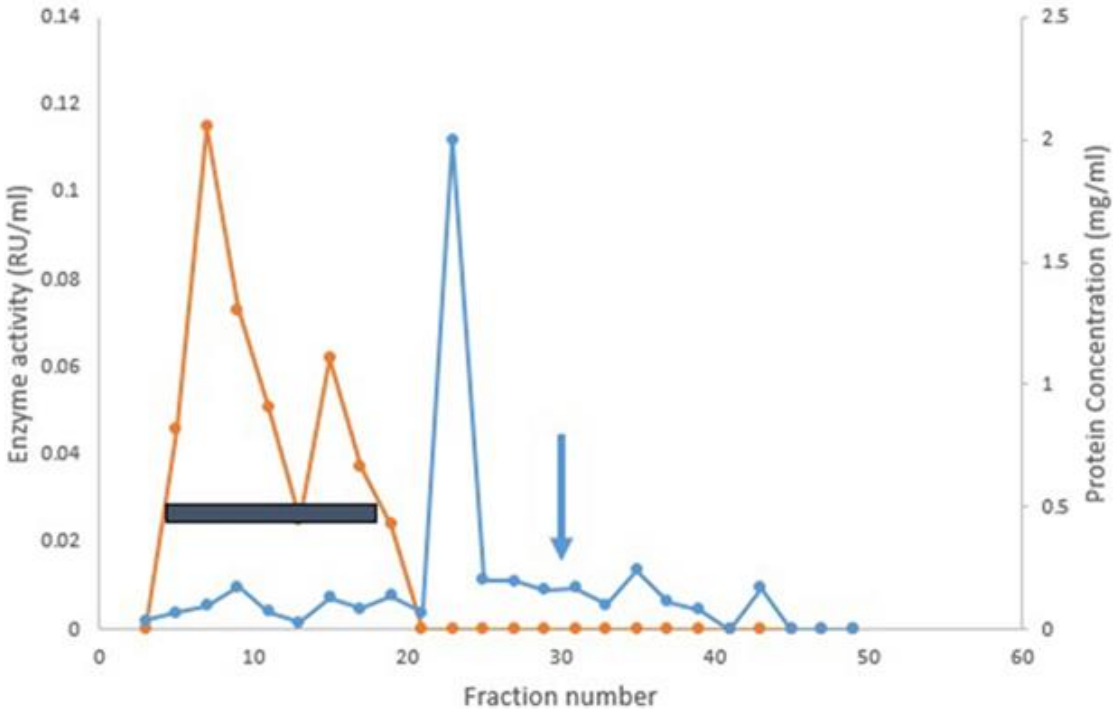

Figure 1. Elution profile of *T. swinderianus* kidney rhodanese on CM-Sephadex c-50 ion exchange chromatography.

Enzyme at 460 nm

Protein at 595 nm

Pooled fractions.

Kinetic parameters

The Line weaver-Burk plots for a fixed concentration of thiosulphate and KCN are shown in Figures 2 and 3 respectively. KCN and $Na_2S_2O_3$ had K_m values of 0.316 and 0.408 mM respectively while their V_{max} was 10.92 and 12.03 RU respectively.

Effect of pH and temperature and metal ions on rhodanese activity

The optimum pH for the activity of *T. swinderianus* kidney rhodanese was found to be 8.0 (Figure 4), while the optimum temperature for the activity of the enzyme was found to be 50°C (Figure 5). Metal ions such as Na^+, Fe^{3+} had little or no inhibitory effect on *T. swinderianus* rhodanese while Hg^{2+} had an inhibitory effect (Table 2).

DISCUSSION

Rhodanese has been reported in many organisms from bacteria to animals (Agboola and okonji, 2004; Aladesanmi et al., 2009; Itakorode et al., 2019). In this study, we report the presence of rhodanese, a cyanide

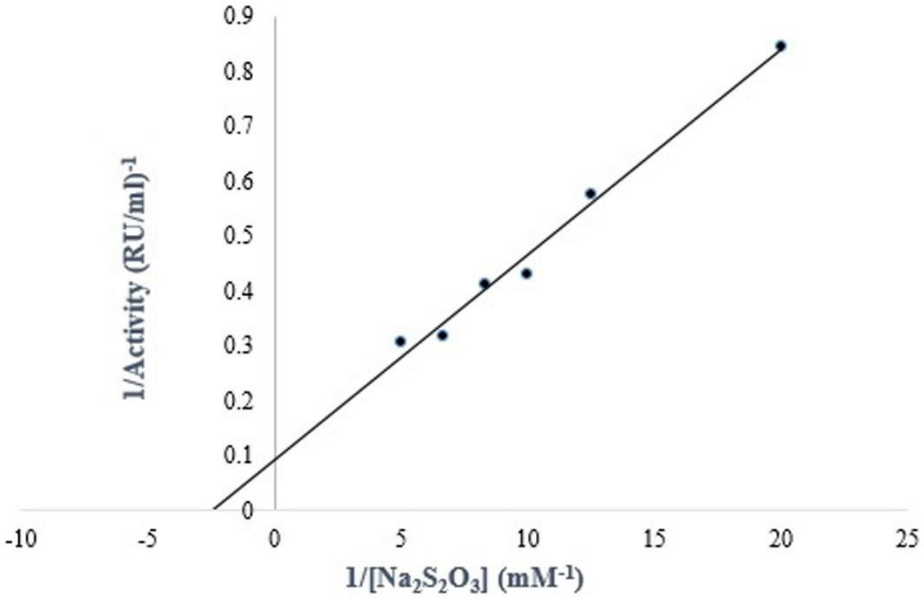

Figure 2. Lineweaver-Burk Plot for varying concentration of sodium thiosulphate between 10 mM and 60 mM and a fixed concentration KCN.

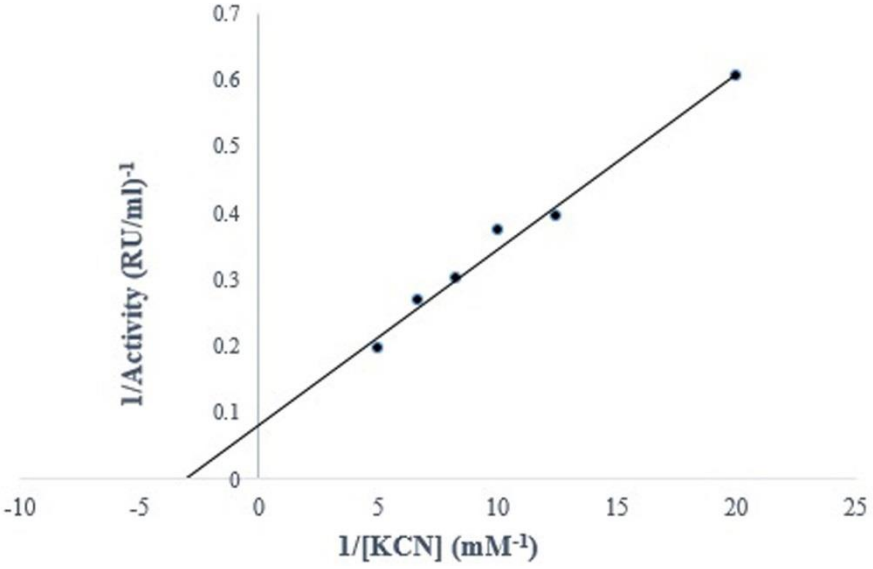

Figure 3. Lineweaver-Burk plot for varying concentration of potassium cyanide between 10 mM and 60 mM and a fixed concentration of $Na_2S_2O_3$.

detoxifying enzyme in the kidney of grasscutter (*T. swinderianus*). *T. swinderianus* feeds on cyanogenic plants such as green maize, sorghum, grasses with fairly high fibre content, cassava, etc. *T. swinderianus* kidney rhodanese was isolated and partially purified using 70% ammonium sulphate precipitation, and ion exchange chromatography on CM-Sephadex c-50. The enzyme had a specific activity of 1.43 µmole/min/mg of protein and 55% recovery. Different specific activity values have been reported for rhodanese from various sources (Aladesanmi et al., 2009; Okonji et al., 2010b). A value of 1.076 µmole/min/mg was reported for mouse liver rhodanese (Lee et al. 1995). Okonji et al., (2010b) also obtained a value of 8.4 µmole/min/mg for rhodanese from

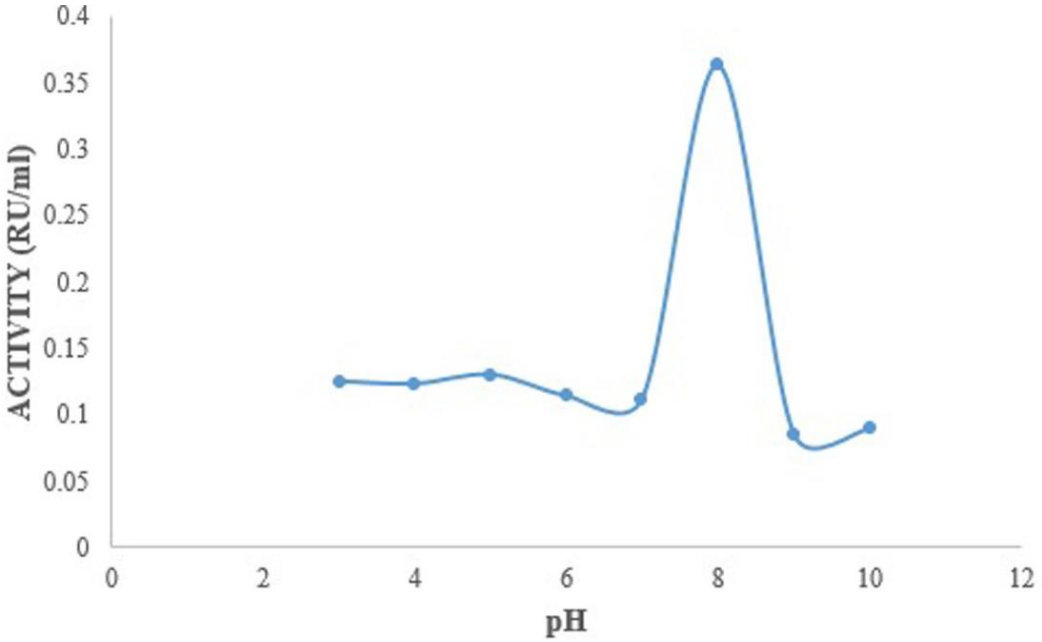

Figure 4. Effect of pH on *T. swinderianus* kidney rhodanese activity.

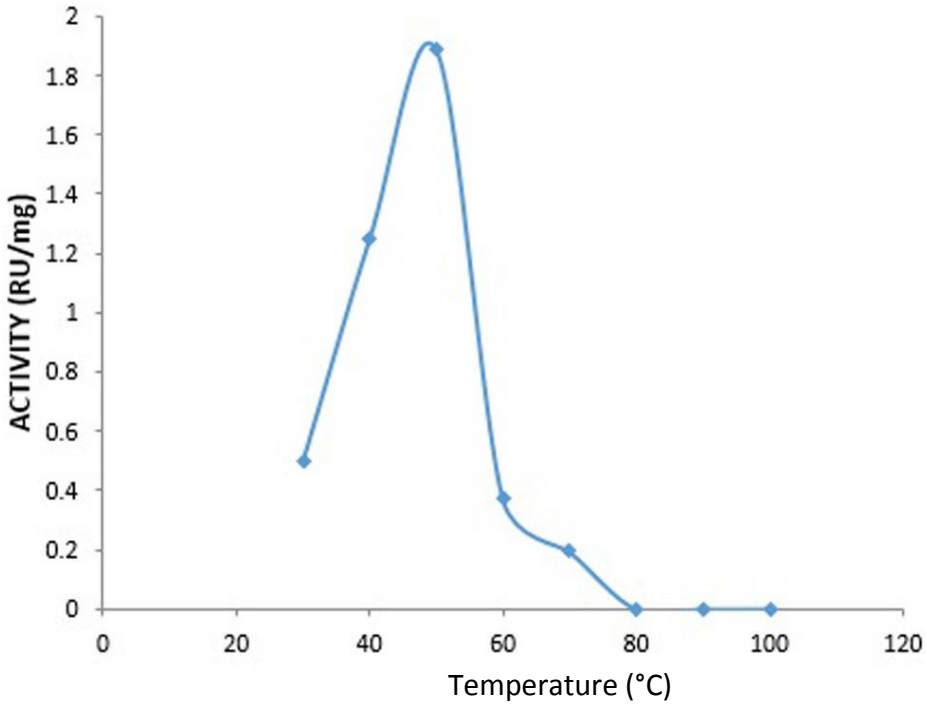

Figure 5. Effect of temperature on *T. swinderianus* kidney rhodanese activity.

the liver of mudskipper. *T. swinderianus* kidney rhodanese has low specific activity. This may be due to the source of the enzyme. In most animals studied, the liver is the richest source of rhodanese (Westley, 1973; Dudeck et al., 1980; Drawbaugh and Marrs, 1987), and this could indicate a heavy cyanide metabolizing function performed by this organ.

The kinetic parameters (K_m and V_{max}) of *T. swinderianus* rhodanese were obtained from the double reciprocal plot of Lineweaver-Burk. The K_m values of

Table 2. Effect of metal ions on *T. swinderianus* kidney rhodanese.

Metal	% relative activity		
	0.1 mM	0.2 mM	0.3 mM
$HgCl_2$	80.00 ± 0.00	40.00 ± 0.00	10.00 ± 0.00
NaCl	98.01 ± 1.91	100.00 ± 0.00	100.00 ± 0.00
$FeCl_3$	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
$MnCl_2$	52.97 ± 4.98	65.78 ± 0.95	76.00 ± 0.00

0.316 and 0.408 mM were obtained for both potassium cyanide (KCN) and sodium thiosulphate ($Na_2S_2O_3$) respectively. These results are similar but however, lower than those reported from other sources: bovine liver (Sorbo, 1953b: 19.0 and 6.7 mM for KCN and $Na_2S_2O_3$ respectively), human liver (Jarabak and Westley, 1974: 9.5 and 4.5 mM for KCN and $Na_2S_2O_3$ respectively), mouse liver (Lee et al., 1995: 12.5 and 8.3 mM for KCN and $Na_2S_2O_3$ respectively. The result from the K_m values indicates that *T. swinderianus* kidney rhodanese has a preference for thiosulphate. This may suggest a possible efficient cyanide detoxification catalytic mechanism which is important for the survival of the animal. The affinity of the enzyme for thiosulphate would enhance the biotransformation of the toxic cyanide into less toxic thiocyanate.

The results for substrate specificity study showed that *T. swinderianus* can use other sulphur containing compounds for its detoxification activity apart from thiosulphate, which corresponds with the studies reported by other researchers. Sulphur has been reported to play a prominent role in the cyanide detoxification mechanism rhodanese (Westley, 1981; Okonji et al., 2011). Wodu (2015) investigated the effect of some sulphydryl compounds such as dithiooxamide, cysteine, 2-mercaptoethanol and glutathione on sheep liver rhodanese. These monoatomic sulphur compounds never served as sulphur donors in sheep liver rhodanese as compared to the sulphur donating property of thiosulphate. In this work, the enzyme showed maximum activity with other sulphur containing compounds. This could suggest an efficient detoxification mechanism of the enzyme even in the presence of other sulphur containing compounds other than thiosulphate.

In this study, maximum enzyme activity was observed at pH 8.0. This value compares to the range reported for other sources (Aladesanmi et al., 2009; Hossein and Reza, 2011). Aladesanmi et al. (2009) obtained an optimum pH of 8.5 for rhodanese from the liver of land tortoise. Optimum pH as low as 6.0 and 6.5 was reported for giant freshwater prawn (*M. rosenbergii*) hepatopancreas and catfish liver rhodanese respectively (Akinsiku et al., 2009). The optimum high pH obtained may be due to the habitat of the animal as rhodanese obtained from an aquatic organism tend to show a relatively lower optimum pH. The optimum temperature of the enzyme was found to be 50°C. The optimum temperature of 50 and 55°C were reported for bovine liver and Trichoderma strain rhodanese respectively (Sorbo, 1953b; Ezzi et al., 2003).

Okonji et al., (2010b) obtained an optimum temperature of 55°C for soldier termite rhodanese. The optimum temperature of 50°C for *T. swinderianus* rhodanese indicates that the enzyme could endure a harsh condition. This optimum temperature could be an adaptive feature which sustains its survival in the kidney of *T. swinderianus* found in the tropical regions. Also, the effect of metals showed that metal ions such as Na^+, Fe^{3+} had no inhibitory effect on *T. swinderianus* rhodanese activity while Hg^{2+} had an inhibitory effect on the enzyme activity. Okonji et al., (2010b) reported the inhibition of soldier termite rhodanese by Hg^{2+} and Mn^{2+}. The absence of inhibitory effect of metal ions such as Na^+, Fe^{3+} observed in this study may be due to constant exposure of the animal to these metal ions from inorganic fertilizers, pesticides and other compounds used in the cultivation of food crops on which the animal feed. Also, the inhibition of Hg^{2+} on the enzyme activity may be due to interactions of the metal ion with the sulphydryl groups at the active site of the enzyme, thereby causing a conformational change in the structure of the enzyme.

Conclusion

In conclusion, the study was able to establish the presence of rhodanese in *T. swinderianus* kidney and the enzyme showed similar physicochemical properties to rhodaneses obtained from other sources. The continuing existence of the animal after consumption of cyanogenic plants suggest that the animal has a means of detoxifying cyanide and our report has shown that rhodanese is one of them.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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