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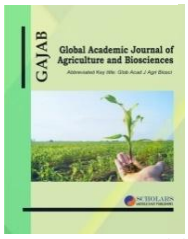

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Original Research Article

Studies on some Physiochemical Properties of Crude Extracts of Rhodanese from Liver and Kidney of an Adult Ram

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Abstract: The enzyme rhodanese (thiosulfate: cyanide sulfur transferase, E.C. 2.8.1.1) is a ubiquitous enzyme known to be responsible for the biotransformation of toxic cyanide to less toxic thiocyanate using thiosulphate as the donor substrate, the aim of this research was to compare the kinetic properties of rhodanese extracted from the liver and kidney of a ram. The enzyme was assayed by measuring the amount of thiocyanate formed per minute. Rhodanese extracted from ram liver had Km values of 12.5mM±0.04 and 50mM±0.43 for $Na_2S_2O_3$ and KCN respectively, while ram kidney rhodanese had Km values of 2.27mM±0.01 and 1.79mM±0.10 for $Na_2S_2O_3$ and KCN respectively.. Rhodanese extracted from both the liver and kidney of ram had optimum pH and temperature as 8.5 and 30°C respectively. Rhodanese from both organs are inhibited by lead and mercury. The lower km of liver rhodanese for thiosulphate suggests that the ram liver rhodanese is more functional in transferring sulfur atom cyanide to form thiocianate.

Keywords: Cyanide, detoxification, ram, liver, kidney, rhodanese.

INTRODUCTION

Cyanide is a potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the mitochondrial electron transport chain. Cyanide poisoning occurs when a living organism is exposed to compounds that produce cyanide ions in aqueous solution (Peter et al., 2013). Many plants and plant products used as food in tropical countries contain cyanogenic glycosides. Upon hydrolysis, these compounds yield cyanide, a sugar and a ketone or aldehyde. It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Burrows and Way, 1977; Calabrese, 1983).

Thus, the potential risk of oral or nasal exposure to cyanide may be very great. Because of widespread natural occurrence of CN, it is not surprising that several mechanisms are operative for cyanide detoxification in vivo (McMahon and Birnbaum, 1990). It is believed that the primary detoxification reaction is thiocyanate formation catalyzed by rhodanese.

The enzyme rhodanese (thiosulfate: cyanide sulfur transfarase, E.C. 2.8.1.1) is a ubiquitous enzyme and its activity is present in all living organisms (Aminlari and Vaseghi, 2006). It is known to be responsible for the biotransformation of cyanide to thiocyanate using thiosulphate as the donor substrate (Okonji et al., 2015). Many functions including cyanide detoxification, formation of iron-sulfur centers and participation in energy metabolism have been attributed to this enzyme (Rapheal, et al., 2015).

The sheep feeds generally on foliage plants (elephant grass, cassava, southern gamba, etc) which have been shown to contain cyanogenic glycosides and on ingestion release cyanide.

It is known that rhodanese plays a Central role in cyanide detoxification; hence the aim of the present study is to compare some biochemical characteristics of rhodanese extracted from the liver and kidney of a ram.

MATERIALS AND METHODS

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

Sample collection

The liver and kidney of the sheep implicated in the study were excised from an adult ram and stored in a refrigerator at -4^{o}C until required.

Preparation of tissue extract

Tissue extracts were prepared by homogenizing 10g (w/v) of each tissue in 3 volume of homogenization buffer (phosphate buffer, pH 8.2). The suspensions were centrifuged for 20 min at 4,000 rpm in a refrigerated centrifuge (Model universal 320R). The supernatant were 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, the protein absorbance was interpolated from a standard protein curve. Rhodanese was assayed by the method of Agboola and Okonji (2004) with slight modifications. The reaction mixture contained 10mM sodium thiosulphate, 10mM potassium cyanide, 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^{0}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 $Fe(NO_3)_{3,}9H_2O$ 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 micromoles thiocyanate formed per minute at 37^{0}C and pH 8.2

Determination of kinetic constant

The kinetic constants (K_m and V_{max}) were determined by varying the concentrations of one substrate at the fixed concentration of the other. The varied concentrations were 2mM to 10mM at fixed concentrations of 10mM.

Kinetic parameters were estimated from the lineweaver-Burk plots (Lineweaver and Burk, 1934).

Effect of temperature

Rhodanese was assayed at temperatures between 20^{0}C to 80^{0}C. The assay mixture was first incubated at the test temperature for ten (10) minutes before adding the enzyme which had been equilibrated at the same temperature.

Effect of pH

The effect of pH on rhodanese extracted from ram liver and kidney were determined using 50mM citrate buffer (pH 4-6.5), 10mM potassium phosphate buffer (pH 7.0-8.5) and 50mM borate buffer (pH 9-11).

Effect of Metal ions on Rhodanese Activity

The effect of Metal ions on liver and kidney rhodanese was investigated. The tested salts were $BaCl_2$, $CoCl_2$, $PbCl_2$, $MnCl_2$, $ZnCl_2$, $HgCl_2$ and $NiCl_2$ at 1 mM and 10 mM in assay mixture.

RESULTS AND DISCUSSION

In this study, the effect of temperature, pH and substrate concentration were investigated on crude extract of rhodanese from ram liver and kidney. The mean±standard deviations of the parameters investigated are presented in tables 1, 2 and 3.

The results in tables 1 revealed that the km values for cyanide and thiosulphate for ram kidney rhodanese were 1.79mM±0.10 and 2.23mM±0.01 respectively, while in table 2 km values for cyanide and thiosulphate for ram liver rhodanese were 50.0mM±0.43 and 12.5mM±0.04 respectively.

Ram kidney rhodanese catalyzed reaction indicated higher affinity for potassium cyanide than for sodium thiosulphate, while liver rhodanese showed higher affinity for thioslphate. Other investigations carried out by other researchers showed that rhodanese has higher affinity for both substrates depending on the tissue it was extracted from. Okonji et al., (2010) reported K_m values of 7.0mM and 5.3mM for KCN and $Na_2S_2O_3$ respectively for the rhodanese from the soldier termites, which had higher affinity from sodium thiosulphate than cyanide. Also Akinsiku et al., (2010) reported 35.40mM and 18.60mM for KCN and $Na_2S_2O_3$ indicating higher affinity for sodium thiosulphate than for KCN. Watanabe et al., (1985) with an apparent K_m of 35mM and 13.5mM for potassium cyanide and sodium thiosulphate respectively for rhodanese in Euglena gracilis. Keith and Volini, (1987) reported apparent K_m values of 78mM and 17mM for potassium cyanide and sodium thiosulphate respectively for rhodanese from Escherichia coli. Hossein and Reza (2011) reported apparent K_m values of 36.81mM and 19.84mM for potassium cyanide and sodium thiosulphate respectively for rhodanese from rainbow trout liver.

The result reported by these investigators indicated that rhodanese had higher affinity for thiosulphate. However, some researchers had reported higher affinity for cyanide. For example, Anosike and Jack, (1982) reported km values 14.2mM and 31.5mM for cyanide and thiosulphate respectively for rat kidney rhodanese. Agboola and Okonji (2004) also reported Km values of 13.5 mM and 19.15 mM for cyanide and thiosulphate respectively for rhodanese from fruit bat liver. The kinetic parameters from this work are similar to those reported by other researchers.

Table-1: Kinetic Parameters of Kidney Rhodanese

	Km (mM)	Vmax (mole/min)	Temp Optimum (^{0}C)	pH Optimum
KCN	1.79±0.01	0.0011±0.0001	30.0±1.1	8.5±1.1
$Na_2S_2O_2$	2.23±0.01	0.0014±0.0001		

Values are reported as mean±SD

Table-2: Kinetic Parameters of Liver Rhodanese

	Km (mM)	Vmax (mole/min)	Temp (mole/min) (^{0}C)	pH Optimum
KCN	50.0±0.42	0.0050±0.002	30.0±5.1	8.5±2.0
$Na_2S_2O_2$	12.5±0.04	0.0013±0.0001		

Values are reported as mean±SD

Table-3: Effects of Metal ions on Kidney and Liver Rhodanese Activity

	Enzyme Activity (%)			
	Kidney		Liver	
Ion	1mM	10mM	1mM	10mM
Hg^{2+}	90.28±4.01^a	40.23±3.91^b	90.28±4.01^a	50.06±3.32^b
Mn^{2+}	99.01±7.20^a	87.41±0.57^a	99.01±7.20^a	87.32±3.19^a
Ba^{2+}	97.63±10.17^a	89.05±9.00^a	97.63±10.17^a	91.39±11.65^a
Pb^{2+}	81.96±5.50^a	21.42±2.90^b	81.96±5.50^a	38.08±11.04^b
Ni^{2+}	98.35±5.32^a	94.09±8.05^a	98.35±5.32^a	93.22±10.00^a
Co^{2+}	97.32±4.28^a	97.42±9.58^a	97.32±4.28^a	98.63±1.17^a
Zn^{2+}	90.40±10.19^a	85.97±2.86^a	90.40±10.19^a	83.90±9.20^a

Values are reported as mean±SD. Means with same superscript letters are not statistically significant at 95% confidence limit.

Various optimum temperatures have been reported for the enzymes from different sources. The optimum temperature for ram rhodanese liver and kidney was 30^{0}C. Optimum temperature range of 25^{0}C - 35^{0}C was reported by Sorbo (1953) for bovine liver rhodanese. Optimum temperature of 30^{0}C have been reported for rhodanese from different sources. Tayefi-Nasrabadi and Rahmani, (2011) and Wodu, (2015) reported 30^{0}C for rhodanese from Alcoligene sp DN25 and sheep liver respectively. Higher temperature values have been reported for rhodanese from other sources. Ezzi et al,. (2003) obtained a wide temperature optimum of 35-55^{0}C for the rhodanese enzyme in different stains of Trichoderma. Much higher optimum temperatures have been reported by Tomati et al., (1974) for cabbage rhodanese (57^{0}C -59^{0}C) and Okonji et al., (2017) for P. brazzeama root rhodanese (55-60^{0}C). Published results of optimum temperatures of rhodanese from different sources shows that enzyme from plant sources seem to be higher than those from animal sources.

The enzyme rhodanese from different sources have been shown to displays maximum activity at a wide pH range –acidic, neutral and alkaline. The optimum pH of 8.5 was obtained for both the liver and kidney rhodanese of ram fall within the range of 8.0-9.0 reported by Sorbo, (1953) as optimum pH for rhodanese from bovine liver. Optimum pH of 8.5 was reported by Wodu, (2015) for sheep liver rhodanese, while Okonji et al., (2010) reported optimum pH of 8.0 for soldier termite. Acidic optimum pH value of 6.0, reported for giant freshwater prawn rhodanese by Okonj et al., (2008) and optimum pH of 6.5 reported by Akinsiku et al., (2010) for African catfish liver rhodanese. Thus, the level for the buffering of the physiological system of ram liver and kidney rhodanese occurs within alkaline pH range of 7.0-9.0, where pH 8.5 appeared to be the optimum pH for the enzyme extracted form both sources. The optimum pH range of 8.0-11.0 which encompasses the optimum pH 8.5 found in this study have been reported for different organisms by Jarabak and Westley, (1974), Anosike and Ugochukwu, (1981) and Lee et al., (1995) for human liver rhodanese,

cassava leaves and mouse liver rhodanese respectively.

$BaCl_2$, $NiCl_2$, $MnCl_2$, $CoCl_2$, and $ZnCl_2$ did not show significant effect on both kidney and liver rhodanese activity at the concentrations studied. Similar results have also been reported by Fagbohunka et al., (2004), Okonji et al., (2011) and Okonji et al., (2015) for the rhodanese isolated from the hepatopancreas of giant African snail and liver of Mudskipper and hepatopancreas of garden snail respectively. $HgCl_2$ and $PbCl_2$ significantly inhibited the enzyme from both sources at 10mM concentration (Table 3). Similar inhibition by Hg^{2+} and Pb^{2+} was reported by Tayefifi-Nasrabadi and Rahmani (2012) for rhodanese isolated from Rainbow trout liver. Agboola and Okonji (2004) reported the inhibition of rhodanese from fruit bat liver by Hg^{2+}. Okonji et al., (2010) also reported inhibition of soldier termite rhodanese by Hg^{2+}, Zn^{2+} and Mg^{2+}. The inhibition of metal ions on enzyme activity has been reported by several researchers. The observed inhibition of liver and kidney rhodanese activity by Pb^{2+} and Hg^{2+} suggests that exposure of a ram to these metals may reduce the cyanide detoxifying ability of the animal. Inhibition of B. cereus rhodanese by Hg^{2+} and Na^{+} was reported by Itakorode et al., (2019) likely because the reaction of these metal ions with the active site residues of the enzyme may prompt the change in the functional structure of the enzyme.

CONCLUSION

The optimum pH and temperature as well as the K_m and V_{max} all fall within the accepted ranges. The properties investigated in the present work suggest that rhodanese present in the liver and kidney of ram have the same temperature and pH optima. Both liver and kidney rhodanese have higher affinity for cyanide than thiosulfate. This finding suggests that the enzyme in both organs studied may among other physiological functions be active in the detoxification of cyanide which is necessary for the survival of the animal in its environment.

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