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ORIGINAL ARTICLE

Purification and Physicochemical Properties of Rhodanese from Liver of Goat, Capra Aegagrus Hircus

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ABSTRACT

Rhodanese from the liver of domestic goat (*Capra aegagrus hircus*) was extracted, purified and the catalytic as well as physicochemical properties determined in order to gain an insight into how domestic goats are able to feed and thrive successfully on cyanogenic plants such as sorghum, millet and cassava. A domestic goat was purchased from a freehold rearing place in Ile-Ife, Osun State, Nigeria, where it was slaughtered and the liver excised. It was rinsed with normal saline (0.9 % NaCl, pH 7.4) to remove blood and other impurities. Approximately 95 g of the liver was homogenized in 3 volumes of 0.1 M acetate glycine buffer, pH 7.8 containing 1 mM ε-amino-n-caprioc acid and 10 mM sodium thiosulphate in a blender, after which it was centrifuged for 15 min at 12,000 rpm at 4℃. A specific activity of 1.55 micromole thiocyanate formed per millilitre per minute (Rhodanese Unit; RU) per mg of protein (U/mg) with a purification fold of 1.88 and 36 % yield was obtained from the rhodanese extracted from the liver of goat rhodanese after CM-Sephadex, Sephacryl S-400 and Reactive Blue 2- agarose column chromatography. An apparent molecular weight of 36 kDa was obtained on Sephacryl S-400 and a subunit molecular weight of 19 kDa was obtained from Sodium dodecyl sulphate polyacylamide gel electrophoresis (SDS-PAGE). A K_m value of 0.034±0.007 mM and V_{max} of 16.70±3.36 units/mL was obtained for $Na_2S_2O_3$ while a K_m value of 0.038±0.004 mM and V_{max} of 18.77±1.58 units/mL was obtained for KCN. An optimal pH of 8.5, 9.5 and 7.0 were obtained using citrate-phosphate-borate, Tris-HCl and citrate-phosphate buffers respectively. Goat liver rhodanese showed an optimal temperature of 40℃. All the chloride salts used which included KCl, $MgCl_2$, $CaCl_2$, $MnCl_2$, $AlCl_3$ and NH_4Cl inhibited rhodanese activity, with the highest inhibition was observed with $MnCl_2$ and least inhibition observed with KCl. Goat liver rhodanese was inhibited by all active site inhibitors, with the the highest inhibition observed with arsenic acid. It can therefore be concluded that domestic goats (Capara aegagrus hircus) are able to feed and survive on cyanogenic plants due to the presence of the cyanide detoxyfying enzyme, rhodanese, present in their liver at high activity with suitable kinetic properties.

Key words: Cyanide; Rhodanese; Goat liver

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INTRODUCTION

Cyanide is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues. This toxicant is widespread in the environment and many naturally occurring substances as well as industrial products contain cyanide[1]. More than 2,000 species of plants are known to contain cyanogenic glycosides[2]. It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals[3]. Many studies had reported the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants[4].

Living organisms detoxify cyanide when contacted from feed, water bodies and the environment due largely to a number of enzyme catalyzed reactions among which are rhodanese (thiosulphate: cyanide sulphurtransferase, EC 2.8.1.1) and 3-mercaptopyruvate sulphurtransferase (EC 2.8.1.2). These enzymes are widely distributed[5]. Of all cyanide detoxifying enzymes, rhodanese is the most important and studied mechanisms by which cyanide ion, CN-, is removed from the body by enzymatic conversion to less toxic thiocyanate, SCN, in the presence of sulphur have been reported[6,7].

Domestic goats being herbivorous animals feed majorly on plants most of which are cyanogenic. The major diet of locally reared goats in Nigeria includes cassava, sorghum and millet. Cassava contains the cyanogenic glycoside linamarin, that is hydrolysed on its marceration by the enzyme linamarase to produce a sugar molecule and acetone cyanohydrin, which is further broken down by α-hydroxynitrile lyase to yield acetone and hydrogen cyanide.

The present study aims to isolate, purify and determine the biochemical properties of rhodanase from the liver of a domestic goat, *Capra aegagrus hircus*.

MATERIALS AND METHOD

1. Chemicals

The standard proteins for SDS-PAGE were obtained from Sigma Chemical Company (St. Louis, Mo., USA). CM-Sephadex C-25 and Sephacryl S-400 was obtained from Pharmacia Fine Chemical, Uppsala, Sweden. All other reagents were of analytical grades.

2. Enzyme Extraction

The goat was slaughtered and the liver was immediately excised and kept in an ice bucket. It was 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. Ninety-five grams (95 g) of the liver was minced into smaller pieces and homogenized in three volumes of 0.1 M acetate glycine buffer, pH 7.8 containing 1 mM ε-amino-n-caprioc acid and 10 mM sodium thiosulphate (Buffer A) with a Warring Blender for about 5 min. The homogenate was filtered through a double layer of cheese cloth, centrifuged at 12, 000 rpm for 30 min at 4℃ using a HITACHI High Speed Refrigerated Centrifuge. The pellet collected after the first round of centrifugation was suspended in one volume of the same buffer and centrifuged as earlier described. The first and second supernatant was combined, the pellets were discarded and an aliquot of the supernatant was then assayed for rhodanese activity and protein concentration.

3. Enzyme Assay and Protein Concentration Determination

Rhodanese activity was assayed according to Agboola and Okonji[8]. The reaction mixture consisted of 50 mM borate buffer, at pH 9.4, 200 µL of 250 mM KCN, 200 µL of 250 mM Na2S2O3 and 20 µL of enzyme solution in a total volume of 1 ml. The mixture was incubated for 1 min at room temperature and the reaction was stopped by the addition of 500 µL of 15 % formaldehyde followed by the addition of 1.5 mL of Sorbo reagent [10.1 g $Fe(NO_3)_3$ $9H_2O$ and 20 ml of concentrated nitric acid in 100 mL of distilled water]. The absorbance was read at 460 nm. One unit of activity is represented as the Rhodanese unit (RU). One RU was taken as the amount of enzyme which under the given condition produced an optical density reading of 1.08 at 460nm per minute which is 10 µ-equivalent of thiocyanate[8]. The protein concentration was determined according to Bradford[9] using Bovine Serum Albumin (BSA) as the standard protein.

4. Protein Purification

4. 1. Ammonium Sulphate Precipitatio: The supernatant obtained from the centrifugation step was brought to 70 % ammonium sulphate saturation (43.6 g/100 mL) by the slow addition of solid ammonium sulphate. This was kept for 1 hour with occasional stirring until all the salt had dissolved completely in the supernatant. The mixture was left in the fridge for about 12 hr followed by centrifugation at 15,000 rpm for 30 min at 4℃. The supernatant was discarded and the precipitate was collected and resuspended in a small amount of Buffer A.

4. 2. Chromatography on CM-Sephadex: The dialysed extract (28 mL) was layered on the CM-Sephadex column (1.5×10 cm). Fractions of 4 ml were collected from the column at a rate of 30 ml per hour. Protein was monitored spectrophotometrically at 280nm. The fractions were also assayed for rhodanese activity. The active fractions were pooled and immediately dialysed against several changes of 50 % glycerol to store the enzyme.

4. 3. Gel filtration on Sephacryl S-400: Twenty milliliters (20 mL) of the post CM-Sephadex sample was layered on Sephacryl S-400 column (1.5×100 cm). The column was eluted with 400 ml of 10 mM phosphate buffer, pH 7.2 containing 10 mM sodium thiosulphate. Fractions of 5 mL were collected from the column at a rate of 10 mL per hour. The active fractions were pooled and immediately dialysed against several changes of 50% glycerol to store the enzyme.

4. 4. Reactive Blue 2-Agarose Affinity Chromatography: Affinity chromatography was carried out on Reactive Blue-2 Agarose column (1.5×10 cm). Two milliliters (2 mL) of the post gel filtration aliquot was layered on the column. The column was then washed with 240 mL of buffer to remove unbound protein followed by elution with a 200 mL linear gradient of 0-1.0 M KC1 in 50 mM citrate buffer, pH 5.0 containing 10 mM sodium thiosuphate. Fractions of 2 mL were collected from the column at a rate of 42 mL per hour. Protein was monitored spectrophotometrically at 280 nm. The active fractions were pooled and immediately dialysed.

5. Determination of Native and Subunit Molecular Weights

The SDS-PAGE was carried out on a 12% acrylamide solution to determine the subunit molecular weight. The standard proteins were bovine albumin (66,000), egg albumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,100) and α-lactalbumin (14,000). After electrophoresis, the gels were stained in 1% Coomasie brilliant blue R-250 in a solution containing 10 % acetic acid and 10 % methanol for about 2 h followed by destaining in the same solution. The native molecular weight was determined using gel filtration on a Sephacryl S-400 column (2.5×90 cm). The standard proteins were Bovine serum albumin (66,000), ovalbumin (45,000), peroxidase (40,000) and α-chymotrypsinogen (25,000).

6. Determination of Kinetic Parameters

The kinetic parameters (K_m and V_{max}) of the enzyme were determined by varying the concentrations of KCN between 0.01 M and 0.05 M at fixed concentration of 0.2 M $Na_2S_2O_3$. Also, the concentration of $Na_2S_2O_3$ was varied between 0.01 M and 0.05 M at fixed concentration of 0.2 M KCN. The parameters were estimated from the plots of the reciprocal of initial reaction velocity (1/V) versus reciprocal of the varied substrates 1/[S] at each fixed concentrations of the other substrate[10].

7. Effect of pH on the Enzyme Activity

The effect of pH on the goat liver rhodanese activity was performed by assaying the enzyme using different buffers at the indicated pHs: citrate-phosphate buffer (pH 3-7), 50 mM of citrate (pH 4-6), 50 mM phosphate (7-8), 0.1 M Tris- HCl buffer (pH 7-11) and 50 mM borate (pH 7-11)[8].

8. Optimum Temperature

The enzyme was assayed at temperatures between 0℃ and 70℃ to investigate the effect of temperature on the activity of the enzyme and to determine the optimum temperature of the enzyme. 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 routinely.

9. Determination of Heat Stability

The heat stability of the enzyme was determined by incubating the enzyme for 1 hr at 30℃, 40℃, 50℃, 60℃ and 70℃ respectively. 1.0 ml was withdrawn at 10 min interval and assayed for residual activity. The activity at 30℃, 40℃, 50℃, 60℃ and 70℃ was expressed as a percentage of activity of the enzyme incubated at 30℃ which was the control.

10. Effect of salt on the Enzyme Activity

The method of Lee *et al*[11] was used to study the effect of various metal ions on the activity of the goat liver rhodanese. The salts include NH_4C1, $MgCl_2$, $CaCl_2$, $MnCl_2$, $AlCl_3$ and KCl and at the final concentrations of 0.5 mM and 1.0 mM.

11. Effect of Active Site Inhibitors

The chemical nature of the active site of the enzyme was studied by including certain sulphydryl reagents as well as certain reagents that are inhibitors of thiol groups in the assay mixture. These reagents include cycloheximide, iodoacetate, N-methylmaleimide, arsenic acid, and N-iodoacetyl-N-(5-sulfo-1-naphthyl) ethylenediamine and 5, 5'-dithiobis-(2-nitrobenzoic acid). The routine assay contained 0.5 mM and 1.0 mM of the reagent.

RESULTS

The CM-Sephadex chromatographic step gave a peak of rhodanese activity after elution with 0-1 M KCl (Figure 1). Also, one peak of rhodanese activity was obtained when the post-CM-Sephadex sample was layered on Sephacryl S-400 (Figure 2). The Reactive Blue 2-Agarose Affinity column also gave a single peak of enzyme activity (Figure 3).

The pure enzyme had a specific activity of 1.55 micromole thiocyanate formed per millilitre per minute (Rhodanese Unit; RU) per mg of protein (U/mg), a purification fold of 1.88 and a percentage yield of 36. The summary of the purification of goat rhodanese is shown in Table 1. Only one band was observed after gel electrophoresis of the purified rhodanese either in the presence or absence of sodium dodecyl sulphate (Figure 4).

Gel filtration on Sephacryl S-400 column resulted in an apparent molecular weight of 36 kDa (Figure 5) while the subunit molecular weight obtained on 12% acrylamide is 20 kDa (Figure 6).

The Lineweaver-Burk plots for the determination of kinetic parameters K_m and V_{max} of goat liver rhodanese were shown in Figures 7 and 8 and the result summarized in Table 2.

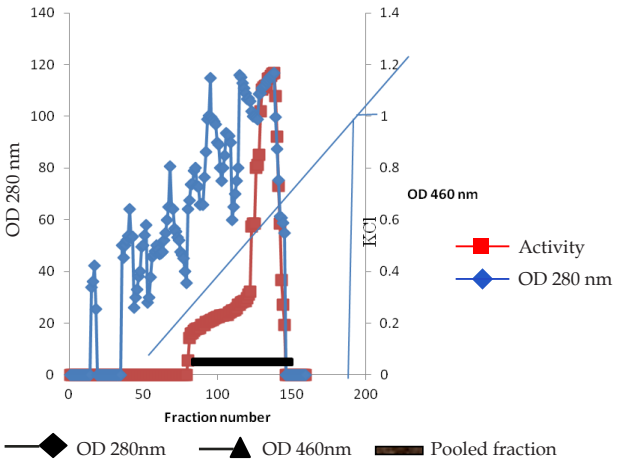

Figure 1 Ion-exchange chromatography on CM-Sephadex C-25 (2.5×40 cm) of goat liver rhodanese. A total volume of 28 mL of rhodanese crude extract was applied to the column and eluted at a flow rate of 30 mL/hr with a linear gradient of 0-1 M NaCl dissolved in the buffer. Each fraction of 5 mL was collected and the active fractions, 81-145 were combined and dialysed against several changes of 50% glycerol for 12 h.

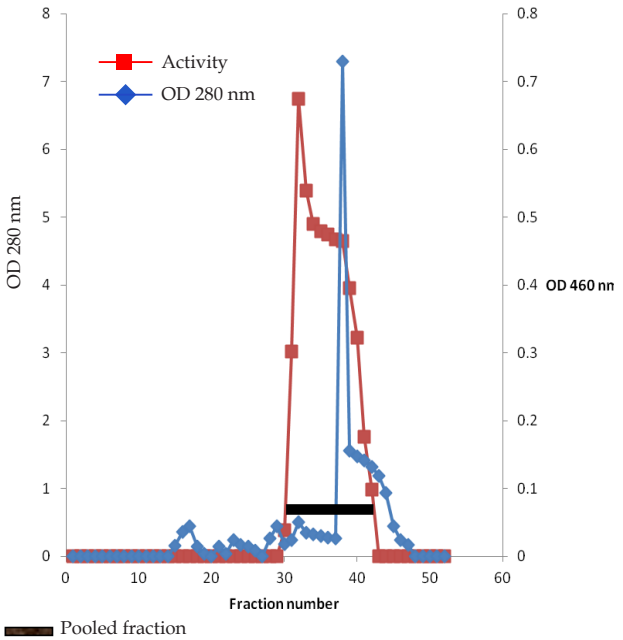

Figure 2 Gel filtration on Sephacryl S-400 (1.5×100 cm column) of goat liver rhodanese. A total volume of 20 mL of pooled and concentrated post ion exchange rhodanese was applied to the column and eluted at a flow rate of 10 mL/hr. Each fraction of 5 mL was collected and the active fractions, 30-50 were combined and dialysed against several changes of 50% glycerol for 12 hr.

The optimum pH was 8.5 (Figure 9). The optimum temperature obtained for the goat liver rhodanese was 40℃ (Figure 10).

The Arhenius plot of temperature effect on rhodanese activity at pH 7.0 is biphasic (Figure 11). The apparent activation energy values from these slopes are 7.3 kcal/mol and 72.9 kcal/mol respectively. The goat liver rhodanese was thermostable, retaining about 90 % of its activity at 40℃ after 60 min (Figure 12).

The effect of various salt ions on the activity of goat liver rhodanese was presented in Table 3 and the effect of sulphydryl reagents and other active site inhibitors was presented in Table 4.

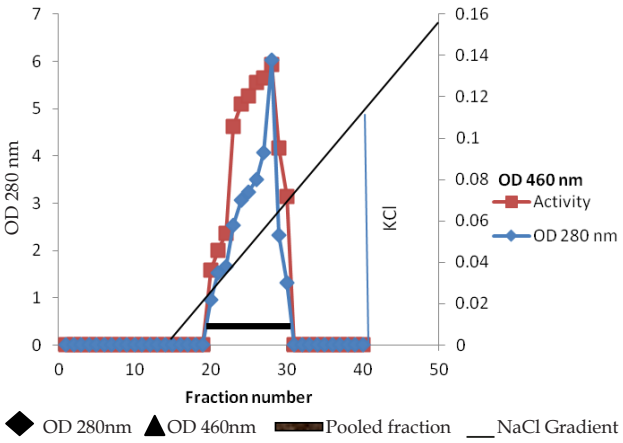

Figure 3 Affinity chromatography on Reactive Blue 2-Agarose (0.5×10 cm column) of goat liver rhodanese. A total volume of 2 mL of pooled and concentrated post gel filtration rhodanese was applied to the column and eluted at a flow rate of 42 mL/hr. Each fraction of 2 mL was collected and the active fractions, 20-30 were combined and dialysed against several changes of 50% glycerol for 12 h.

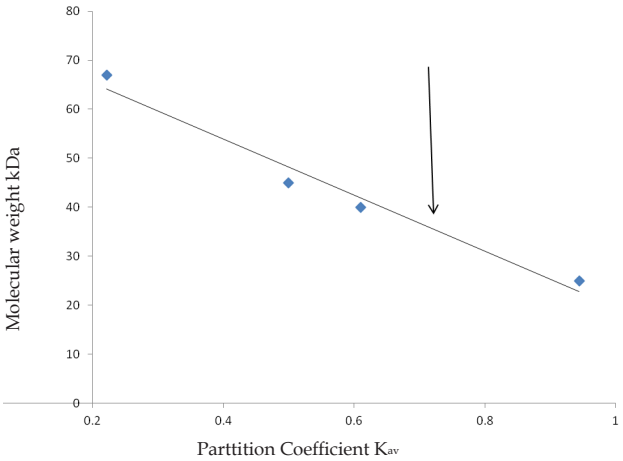

Figure 5 Native Molecular weight determination. Marker proteins were applied to a Sephacryl S-400 column (2.5×100 cm) and eluted by buffer C, pH 7.0 at a flow rate of 25 mL/hour. The void volume Vo was determined by the elution of 2 mg/mL solution of Blue Dextran under the same condition. Marker proteins used were. A = Bovine Serum Albumin: 66 kDa (7 mg/mL) B = Oval albumin: 45 kDa (7 mg/mL), Peroxidase: 40 kDa (5 mg/mL) and α-Chymotrypsinogen: 25 kDa (3 mg/mL). The position of of goat rhodanese is indicated by the arrow 36 kDa.

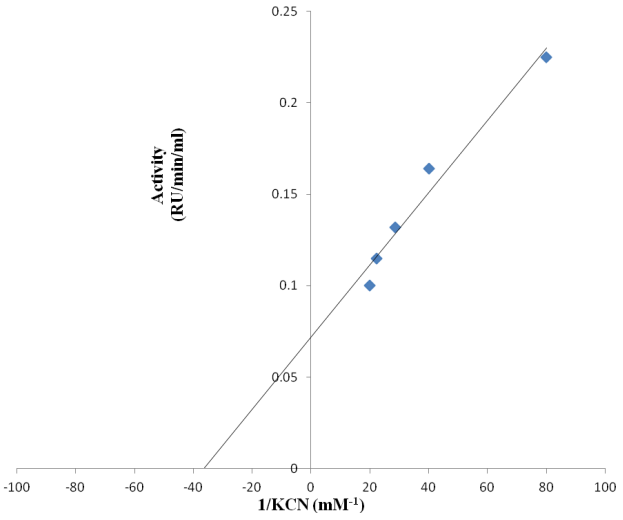

Figure 7 Lineweaver-Burk plot of 1/V against 1/[S] by varying concentrations of 250 mM KCN between 0.0125 M and 0.05 M at fixed concentrations of 0.2 M of 250 mM $Na_2S_2O_3$.

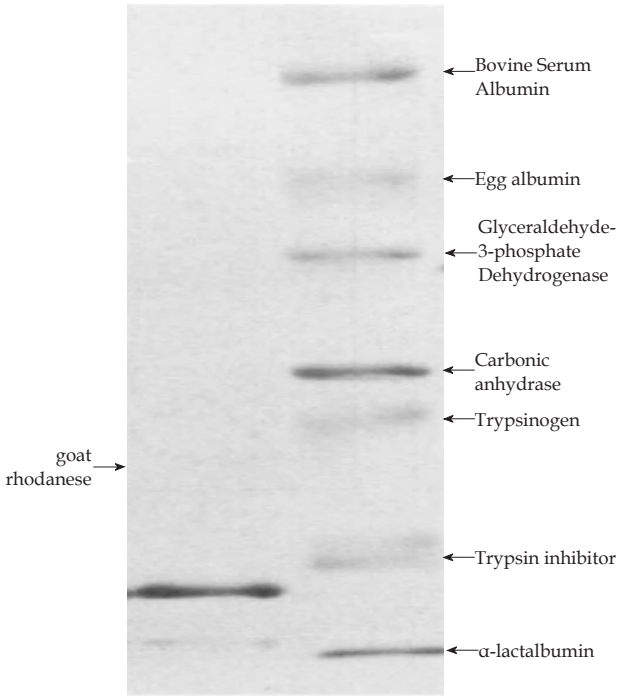

Figure 4 SDS-polyacrylamide slab gel electrophoresis of the pure enzyme. The pure enzyme is shown on lane A while marker proteins; BSA (66,000Da), Egg albumin (45,000 Da), Glyceraldehyde-3-phosphate Dehydrogenase (36,000 Da), Carbonic anhydrase (29,000), Trypsinogen (24,000 Da), Trpsin inhibitor (20,100) and α- Lactalbumin (14,000 Da) are shown on lane B.

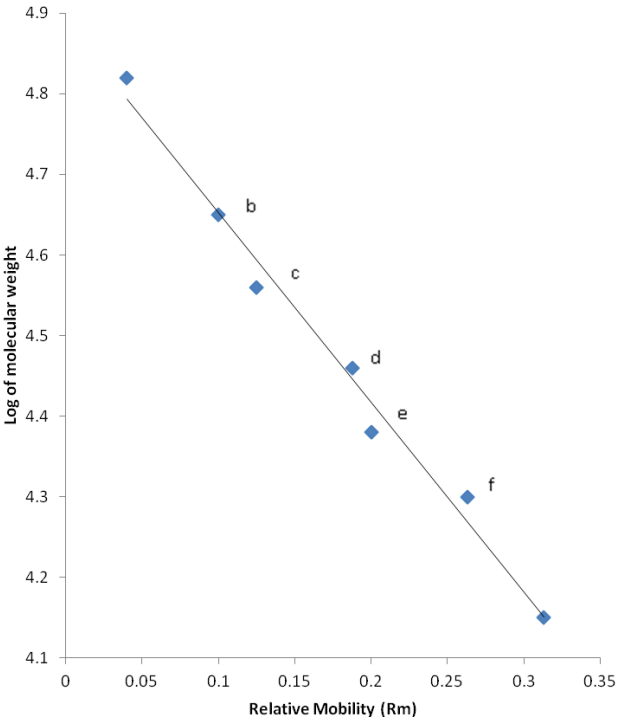

Figure 6 Subunit molecular weight determination on SDS-PAGE on 12 % acrylamide gel using the Tris glycine buffer system. The Subunit molecular weight of goat rhodanese was 19 kDa, which was obtained by extrapolation using the following standard proteins. Where a = BSA (66,000Da), b = Egg albumin (45,000 Da) c = Glyceraldehyde-3-phosphate Dehydrogenase (36,000 Da) d = carbonic anhydrase (29,000), e = Trypsinogen (24,000 Da) f = Trypsin inhibitor (20,100), g = α- Lactalbumin (14,000 Da).

Table 1 Purification Table for Goat Liver Rhodanese.

Fraction	Volume (mL)	Total Activity(U)	Total Protein(mg)	Specific Activity (U/mg protein)	% Yield	Purification fold
Crude Extract	273	49,292	59,678	0.826	100	1.00
70% Ammonium Sulphate precipitation	116	29,537	33,118	0.892	60	1.08
Ion exchange on CM-Sephadex	220	10,165	9,196	1.105	34	1.34
Gel filtration on Sephacryl S-400	80	2,770	1,923	1.44	27	1.74
Affinity Chromatography on Reactive Blue 2-AgaroseBlue	50	996	642	1.55	36	1.88

These are the various steps in the purification of goat liver as described in the methods. Rhodanese activity was assayed by the method of Agboola and Okonji[8]. One unit of activity is represented as the Rhodanese unit (RU). One RU was taken as the amount of enzyme which under the given condition, produced an optical density reading of 1.08 at 460nm per minute which is 10 µ-equivalent of thiocyanate[8,13].

Table 2 Kinetic Parameters of Goat Liver rhodanase.

	K_m (mM)	V_{max} (U/mL)
KCN	0.034± 0.007	16.70±3.36
$Na_2S_2O_3$	0.038±0.004	18.77±1.58

Table 3 Effect of Chloride Salts on Goat Liver Rhodanase.

Salt	Enzyme activity (%)	
	0.5 mM	1.0 mM
None	100	100
KCl	91.3±1.2	82.7±1.2
$AlCl_3$	70.7±1.2	69.0±1.0
$CaCl_2$	76.3±1.2	73.0±1.0
NH_4Cl	66.3±1.2	64.3±1.5
$MgCl_2$	63.0±1.0	57.7±1.2
$MnCl_2$	49.7±1.5	44.3±1.5

The salts include KCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, NH_4C1 and $AlCl_3$, at the final concentrations of 0.05 mM and 0.1 mM. Rhodanese assay was routinely carried out in the presence of these chloride salts.

Table 4 Effect of Sulphydryl Reagents and Other Active Site Inhibitors on Goat Liver Rhodanese.

Inhibitors	Enzyme activity (%)	
	0.5 mM	1.0 mM
Control	100	100
Iodoacetamide	16.3	11.3
Cycloheximide	28.0	17.3
Arsenic acid	7.70	8.0
N-methylmaleimide	22.0	28.0
N-iodoacetyl-N-(5-sulfo-1-naphthyl) ethylenediamine	10.7	9.0
5,5′-dithiobis-2-nitrobenzoic acid (DTNB)	12.3	11.7

The chemical properties of the active site on goat rhodanese was studied by including the reagents in the table in the assay mixture at concentrations 0.5 mM and 1.0 mM.

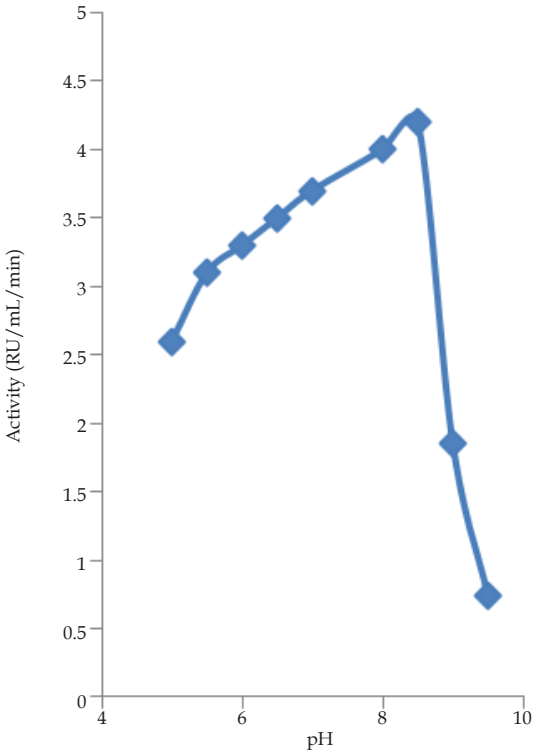

Figure 9 Effect of pH on Goat Rhodanese. The optimum pH was determined using 50 mM citrate buffer (pH 4.0-6.5) – 50 mM phosphate buffer (pH 7-8.5) – 50 mM borate buffer (pH 9-11).

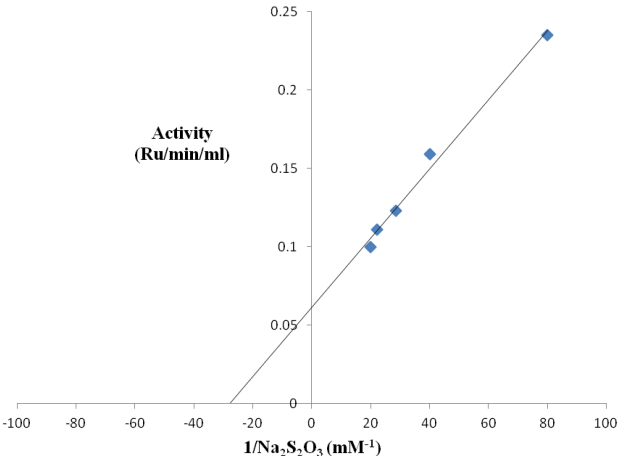

Figure 8 Lineweaver-Burk plot of 1/V against 1/[S] at fixed concentrations of 0.2 M of 250 mM KCN and varying concentrations of 250 mM $Na_2S_2O_3$ between 0.0125 M and 0.05 M.

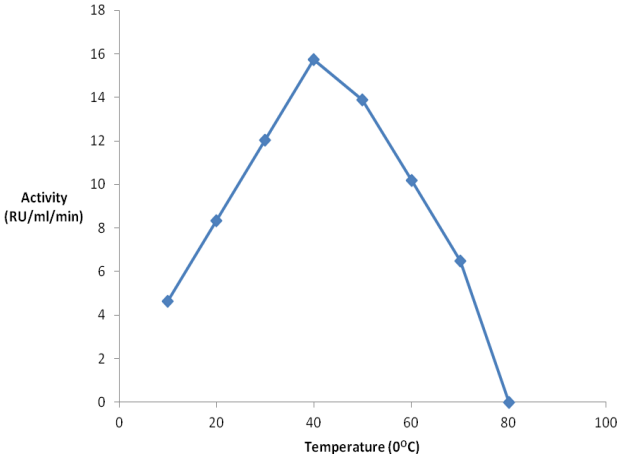

Figure 10 Effect of temperature on goat liver rhodanese. The activity-temperature profile was obtained by varying the temperature between 0 and 80℃. The assay mixture was first incubated at the indicated temperature for 10 min before initiating the reaction by the addition an aliquot of the enzyme that has been equilibrated. Percent residual activity is compared to the activity of the native enzyme at 0℃.

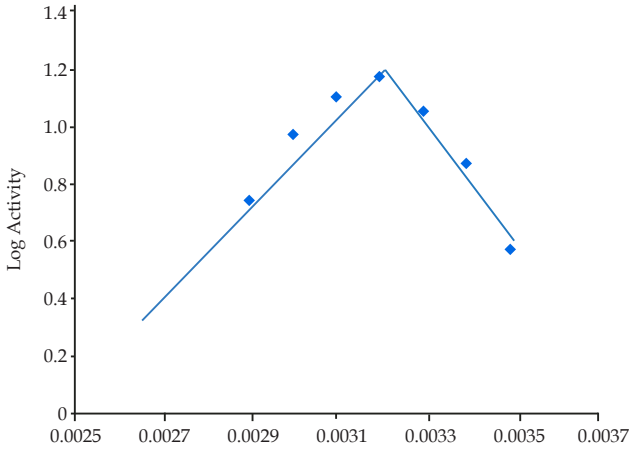

Figure 11 The Arrhenius plot of logarithm of activity against the reciprocal of incubation temperature (1/Kelvin) on the activity of the enzyme and to determine the activation energy of the enzyme.

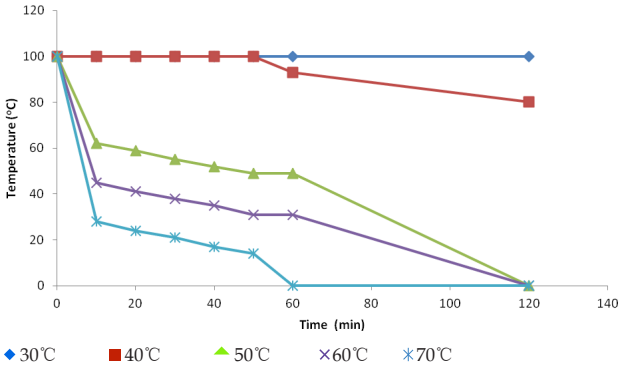

Figure 12 Heat stability of Goat Liver Rhodanese. The heat stability was determined by incubating the enzyme for 1 hr at the indicated temperature before addition into the assay mixture as described in methods. The activity at 30℃, 40℃, 50℃, 60℃ and 70℃ was expressed as a percentage of activity of the enzyme incubated at 30℃ which was the control.

DISCUSSION

In this study, the enzyme was purified to homogeneity using 70% ammonium sulphate precipitation, ion-exchange on CM-Sephadex, gel filtration on Sephacryl S-400 and affinity chromatography on Reactive Blue 2-agarose column. The purified enzyme was obtained as a well separated single band on SDS-Polyacrylamide gel electrophoresis prepared at 12% acrylamide concentration.

The specific activity obtained from the purification of goat rhodanese was 1.5 RU/mg of protein with 36 % yield and a purification fold of 1.88. Different folds of purification have been obtained from the purification of rhodanese from various sources such as a purification of 150 folds from beef liver[12], 776 folds from mouse liver[11], 49 and 48 folds from catfish rhodanese I and II[13], purification of 5.6 folds from mudskipper rhodanese[14] and purification of 1.7 folds from rainbow trout[15].

A molecular weight of 36 kDa was obtained for goat liver rhodanese on a gel filtration which is in agreement with the molecular weight range of this enzyme established to be approximately between 33,000 and 37,000 Da[16-18]. Different molecular weights have been obtained for rhodanese from various sources and they include 37.1 kDa from beef liver[12], 34.8 kDa from mouse liver[11], 34,500±707 daltons, 36 kDa from fruit bat[8] and 36,800±283 for catfish rhodanese I and II[13] which is relatively similar to the molecular weight obtained for goat rhodanese from this study. Sedimentation equilibrium studies shows that there are two forms of bovine liver rhodanese in crystalline enzyme preparations. One form dissociates to a species with a limiting molecular weight close to 19,000, while the second form is nondissociable under the same experimental conditions and exhibits a molecular weight of ~33,000. Previous evidence indicated that the bovine rhodanese of Mr ~37,000 was a dimer of similar, possibly identical, subunits with two catalytic sites per dimer[19]. In contrast to this evidence, a recent report of data from amino acid sequence and x-ray crystallographic studies has indicated that bovine liver rhodanese is a single polypeptide chain of M_r ~33,000 containing one catalytic site[20]. So a subunit molecular weight of ~20,000 Da obtained for goat liver rhodanese on a 12% acrylamide is similar to one form of bovine liver rhodanese that dissociates to a species with a limiting molecular weight close to 19,000 Da suggesting that rhodanese from goat liver is a homodimer[12].

From the study carried out on rhodanese from goat liver, the K_m for both substrates was relatively lower compared to rhodanese from other mammalian sources such as human liver[21], bovine liver[12], mouse liver[11], rat liver[18], but in close range with what was obtained for bovine liver[22], velvet monkey[23] and acinetobacter sulphane sulphur transferase[24]. The values reported for goat liver rhodanese is an indication that the enzyme has high affinity for the substrate and it would catalyse the detoxification reaction of cyanide to a harmless compound called thiocyanate more efficiently considering the level of exposure of goat to cyanogenic glycosides present in its diet majorly cassava, sorghum and millet. The high affinity of goat liver for cyanide could explain how the organism is able to survive and reproduce feeding on these cyanogenic plants.

The optimum pH values compares well with that obtained for rhodanese from other mammals such as pH 8.3 from rabbit liver rhodanese[25], pH 8.0 and 9.0 from bovine liver rhodanese[12] and pH 9.4 from mouse liver rhodanese[11].

In this study, an optimum temperature of 40℃ was obtained which compares well with the values obtained from other studies such as 50-59℃ and 38-400C for bovine liver[21] and a much lower optimum temperature of 25℃ for mouse liver[11]. These temperature values are also close to those of non-mammalian rhodanese e.g. 50-59℃ for tapioca leaf rhodanese[27], 35-55°C in Trichoderma strains[12], 35℃ for rhodanese from fruit bat liver[8], 40℃ catfish liver rhodanese[13], 25℃ for rainbow trout[15] and 500C from mudskipper[14]. The thermal stability experiment showed that goat liver rhodanese was thermostable, retaining about 90 % of its activity at 40℃ after 60 min. The enzyme had 52 %, 30 % and no activity at 50, 60 and 70℃ respectively. However, after heating the enzyme for 2 hr, the enzyme retained its full activity at 30℃, had 77 % activity at 40℃, but lost all its activity at 50, 60 and 70℃ respectively. Ploegman *et al*[20] reported that bovine liver rhodanese consists of two equally sized, similarly folded domains stabilized by extensive hydrophobic interactions. This enzyme appears sensitive to thermal inactivation, a process which apparently results from thermally induced transitions of the native structure which leads to the exposure of hydrophobic surfaces and irreversible protein association[29].

Arrhenius plot of effects of temperature on reaction rate consists of two linear segments with a break occurring at 45℃. The apparent activation energy values from these slopes are 7.3 kcal/mol and 72.9 kcal/mol respectively. The first activation energy value of 7.3 kcal/mol is closely related to the 7.5 kcal/mol reported for bovine liver rhodanese[12] and also falls within the physiological activation energy range (1 kcal- 25 kcal) for physiological processes in living organisms[30]. The second activation energy value of 72.9 kcal/mol lies within the range (40 kcal/mol-100 kcal/mol) for protein denaturation[31].

From the result obtained from the study of the effect of salts especially those of chloride on goat liver rhodanese, all the salts inhibited goat rhodanese activity although with differing levels of inhibition. According to Stokinger[30], metal ions showing inhibition of rhodanese activity are those that have strong affinity for ligands such as phosphate, cysteinyl and histidyl side chain of protein.

Inhibition of rhodanese by 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), an aromatic nitro compound is similar to what was obtained by Aminlari[33] in his study of the active site of rhodanese with disulphide reagents. DTNB is usually used for probing the active site of rhodanese because it reacts with a favourable equilibrium constant with sulphydryl groups, and especially because it generates an intensely chromophoric product, thionitrobenzoate which can be monitored spectrophotometrically. DTNB usually oxidizes rhodanese by causing a formation of an intermolecular disulphide bond between its monomers. Disruption of the conformation of the active site of rhodanese by DTNB could perhaps be responsible for the inhibition observed in this study.

The physicochemical and catalytic properties of rhodanese isolated from the liver of goat are similar to what has been obtained from other mammalian sources. The low K_m observed signifies high affinity of rhodanese for its substrates. This may support how goats are able to efficiently feed on cyanogenic foods such as yam, cassava, millet, sorghum e.t.c. all of which are high in hydrogen cyanide content without experiencing cyanide toxicity.

CONCLUSION

In conclusion, this work has established the presence of rhodanese with properties similar to that of enzymes from vertebrates, and the biophysical and other structural properties of this enzyme are required to assign roles and physiological function to the enzyme.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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