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KINETIC STUDIES OF RHODANESE IN SOME TISSUES OF SOKOTO RED GOAT

SAIDU, Y.; ALAWIYE, G.A.; BILBIS, L.S. AND ABBAS, A.Y.
Biochemistry Department, Usmanu Danfodiyo University, Sokoto Nigeria

ABSTRACT

Cyanide:thiosulphate sulphur transferase (rhodanese) activity was assayed spectrophotometrically in some tissues of Sokoto red goat. The liver enzyme was subjected to further kinetic studies. Other tissues studied were kidney, heart and lungs. The order of the activity of the enzyme in these tissues was liver (4.13μmol $SCNmin^{-1}g^{-1}$ fresh tissue) > Kidney (2.3 μmol $SCNmin^{-1}g^{-1}$ fresh tissue) >heart (1.4 μmol $SCNmin^{-1}g^{-1}$ fresh tissue) > lungs (1.10 μmol $SCNmin^{-1}g^{-1}$ fresh tissue). Kinetic studies of the liver enzyme indicated an optimum pH and temperature of 8.0 and 40 °C respectively. The activation energy was 17.9 KJ/mol. Preincubation of the enzyme extract in cyanide inhibited its activity while preincubation in thiosulphate enhances its activity. These results provide an insight regarding physiological role and mechanism of action of rhodanese in the tissues of Sokoto red goat.

Keywords: Kinetics, rhodanese, tissues, Sokoto-red-goat

INTRODUCTION

Goats are small ruminant animals that feed on low quality food, particularly fibrous vegetation. They are thus, a convenient way of converting poor quality foods into desirable high quality animal proteins.

Many plants and plant products used as food and animal feeds in tropical countries contains cyanogenic glycosides. These plants include cassava, linseed, beans and peas, which are known to contain the cyanogenic glycoside, linamarin coexisting with lotaustralin. Millet, sorghum, tropical grass and maize are sources of dhurin. Amygdalin is found in plums, cherries, pears, apple and apricots. These compounds are also present in plants such as rice, unripe sugar cane, several species of nuts and certain species of yam (Osuntokun, 1981; Oke, 1979). Most of these plants and their products/byproducts are staple food/feedstuffs in the tropics. Upon hydrolysis these compounds yield cyanide, a sugar and a ketone or aldehyde. Cyanide, one of the products of cyanogenic glycosides hydrolysis is a potent cytotoxic agent that acts by inhibiting cytochrome oxidase of the mitochondrial electron transport chain. When ingested, cyanide activates the body's own mechanisms of detoxification, resulting in the transformation of cyanide into a less toxic compound, thiocyanate.

The principal detoxification pathway of cyanide is that catalysed by the liver mitochondrial enzyme, rhodanese (Cyanide: Thiosulphate Sulphur Transferase; E.C.2.8.1.1), which converts cyanide into thiocyanate, by sulphuration, which is then excreted through the kidney. In addition to cyanide detoxification, a number of other physiological roles have been proposed for the enzyme. Prominent among them is its role in modulation of energy metabolism through activation by sulphuration of several enzymes involved in oxidative metabolism.

Rhodanese is widely distributed in both plants and animal species. Its activity has been demonstrated in rats and guinea pig livers (Anosike and Jack, 1982; Baskin and Kirby, 1990), different organs of sheep and cattle (Aminlari *et al.,* 1989) and various tissues of domestic fowl (Saidu *et al,* 2000).

This work was aimed at comparing rhodanese activity in tissues of Sokoto Red Goat and evaluating some kinetic parameters of the liver enzyme.

Materials And Methods

All the chemicals and reagents used in this work were of analytical grade.

Source of Tissues

Three sets of each of the tissues were collected from early morning sacrificed animals at the Sokoto Central Abattoir. The tissues collected were liver, kidney, lungs and heart muscle. They were identified at the abattoir by the Butchers, immediately kept in a cold box and transported to the Biochemistry Laboratory, Usmanu Danfodiyo University, Sokoto. The tissues were authenticated by a veterinary anatomist in the faculty of veterinary medicine the same university. They were then washed with ice-cold normal saline ready for homogenization.

Preparation of Tissue homogenate

A 10%(w/v) tissue homogenate of each of the tissues in 0.1M phosphate buffer, pH 7.3 was prepared according to the method of Ugochukwu *et al.* (1991). The supernatant was collected and stored in a refrigerator at -20°C, in small labelled plastic bottles until required. The extracts were used for the kinetic studies of the rhodanese within 72 hours of preparation.

Assay of Rhodanese

Rhodanese activity was measured by following the formation of thiocyanate which was subsequently reacted with ferric ion to form a red coloured-complex. This complex was then measured spectrophotometrically at 460nm according to the method of Sorbo (1955). The liver extract was used to study some of the kinetic parameters of rhodanese. The parameters were:

1. **Effect of pH on the Activity of Rhodanese:**

Phosphate-citrate buffer, pH 3 -8 and glycine buffer, pH 9.1 – 11.6 were used to prepare 10 assay mixtures of different pH values. The activity of rhodanese at different pH values was measured using the method of Sorbo (1955).

2. **Effect of Temperature on Rhodanese Activity:**

Assay mixtures were prepared, incubated at different temperatures ranging from 20 - 70 °C and the activity of rhodanese measured.

3. **Effect of Preincubation of the Extract in the Substrates on the Activity of Rhodanese:**

One milliliter of the liver extract was incubated in 2 ml of 0.125 M $Na_2S_2O_3$ and at time intervals of 1, 2, 5, 10, 15, 20 and 30 minutes, 0.3 ml of the preincubated extract was transferred into labelled test tubes containg 0.1ml each of 0.25M KCN and 0.20M of KH_2PO_4. The mixture was allowed to stand at 30°C for five minutes and the activity of rhodanese estimated as explained above Sorbo (1955).

RESULTS AND DISCUSSION

Rhodanese activity is expressed as micromole thiocyanide formed per minute per gram fresh tissue (μmol SCN $min^{-1}g^{-1}$).

Rhodanese activity in the tissues of Sokoto red goat is presented in fig 1. The results show that the activity was highest in the liver and lowest in the lungs. The high rhodanese activity observed in the liver may be related to its role in xenobiotics detoxification. Ogata

and Volini (1990) reported that liver is the main organ for cyanide detoxification. The current result is consistent with earlier reports (Saidu *et al.*, 2000; Ugochukwu *et al.*, 1991; Aminlari *et al.*, 1989). In addition to cyanide detoxification, rhodanese in the tissues of Sokoto red goat may be involved in other roles. Other functions proposed for rhodanese include its role in energy metabolism (Keith and Volini, 1987; Ogata and Volini, 1990)

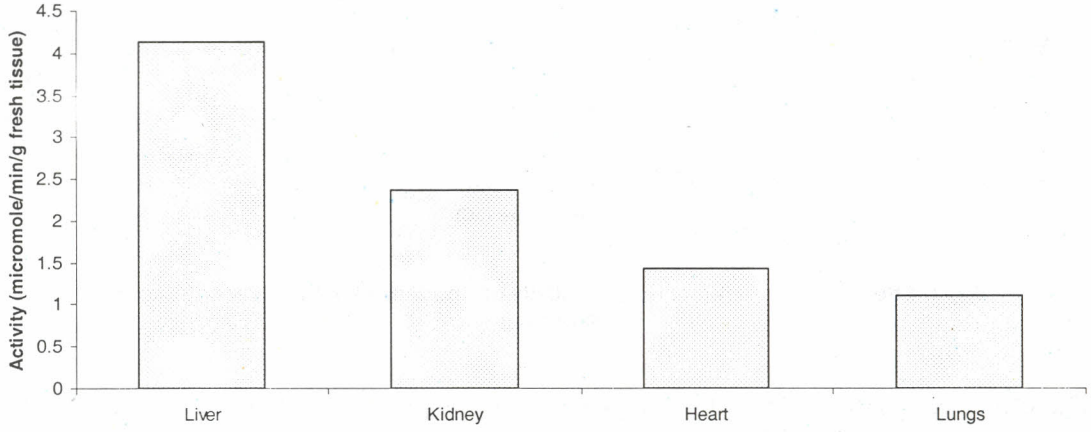

Fig 1: Rhodanese Activity in Some Tissues of Sokoto Red Goat

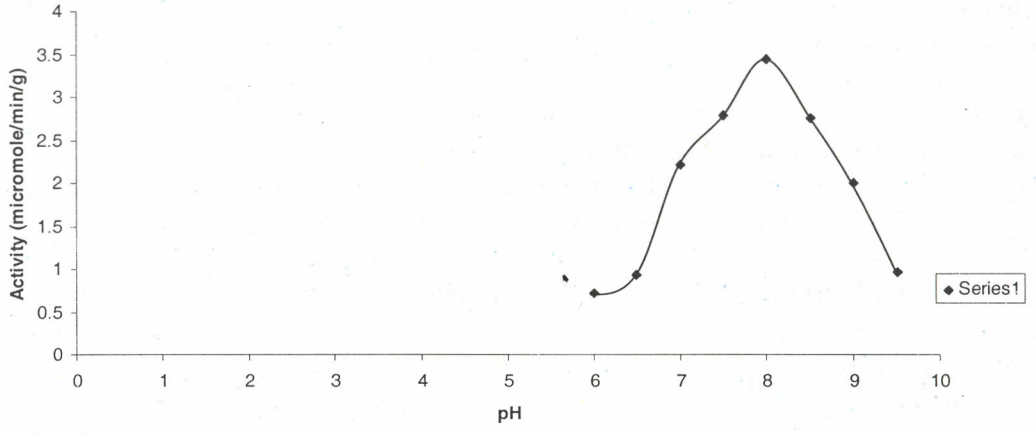

Fig 2: Effect of pH on the Activity of Rhodanese in the Liver of Sokoto Red Goat

The effect of pH on the activity of rhodanese is shown in fig 2. The result indicated that the liver enzyme has an optimum pH of 8.0. Enzymes posses ionizable groups in the catalytic sites in particular, which dictate the three dimensional structure of the sites in particular and the entire molecule in general. The ability of enzymes to perform their statutory role depends on the precise orientation of these groups. pH of the medium affects significantly this orientation. The pH reported in the current work is close to 8.5 reported by Sorbo (1955) for the beef liver rhodanese. A higher value of 10.2 – 10.4 was reported for rhodanese from *Thiobacillus* A_2 (Marvin and Kelly, 1976).

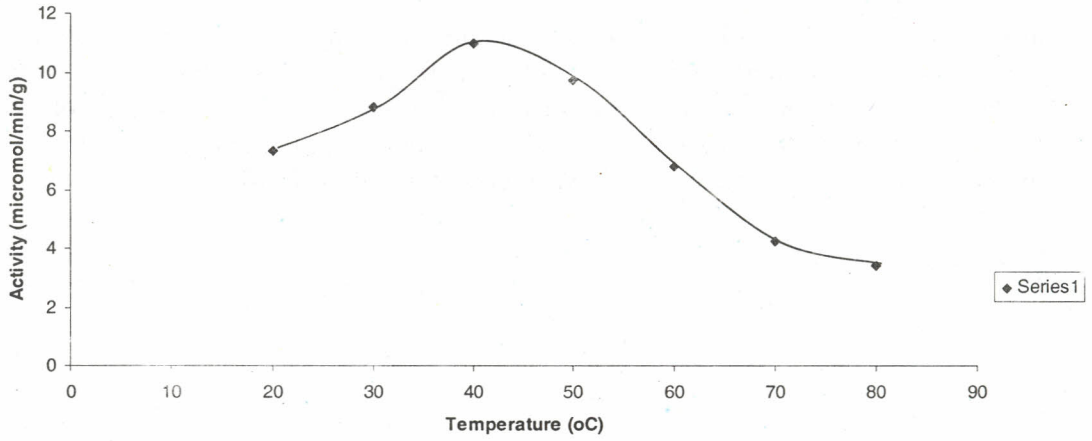

Fig 3: Effect of Temoperature on the Activity of Rhodanese in the Liver of Sokoto Red Goat

The effect of temperature on the enzyme activity is presented in fig 3. The result indicated a temperature optimum of 40°C. Enzymes are proteins, whose three-dimensional structures are stabilized by weak forces such as hydrogen bonds, electrostatic interaction, vander waal forces and hydrophobic interactions. These forces of attraction, because of their weak nature could be broken/disrupted when enzymes are *heated-up,* resulting in the disruption of the three dimensional structure of the enzyme. The effect is a decrease in the enzyme activity at temperatures above the optimum temperature.

Fig 4 represents the plot of $\log_{10}V$ against the reciprocal of the corresponding absolute temperature (T) for the reaction catalysed by Sokoto red goat liver rhodanese. The slope of the right hand side of the curve was used for the calculation of the activation energy (Ea) of the reaction catalysed by the enzyme, According to the Arrhenius equation, the slope (m) of the curve is related to the activation energy (Ea) by the equation:

$M = - Ea/2.303R$ (Where R is the universal gas constant, which is $8.314 Jmol^{-1}$.)

In the current study the activation energy of the enzyme is 17.9KJ/mol. Enzymes bring about increase in rate of reaction by providing an alternative pathway for the reaction. This they do by reducing the activation energy. Sorbo (1955) reported 7.8Kcal/mol for beef liver enzme.

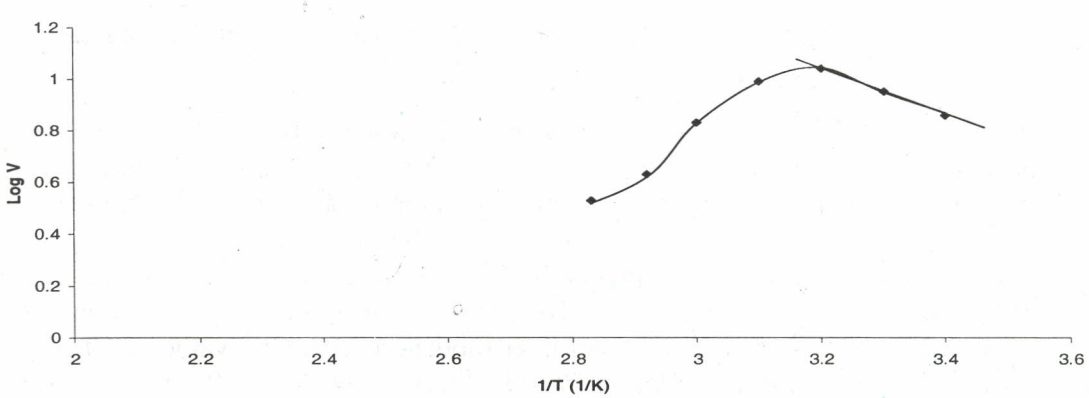

Fig 4: Plot of LogV against Reciprocal of Absolute Temperature

Preincubating the enzyme extract in the two substrates of rhodanese affects the activity of the enzyme differently as shown in fig 5 below. Incubating the extract in cyanide decreases the activity of the enzyme with incubation time; while in the case of thiosulphate the activity increases with incubation time. This result indicates that in the catalysis of sulphuration of cyanide by rhodanese which is through ping-pong mechanism (Keith and Volini, 1987; Pagani *et al,* 1993), thiosulphate is the first substrate that binds to the surface of the enzyme. It is also an indication that cyanide is an inhibitor of the enzyme in the absence of the second substrate. This is consistent with the earlier report of Sorbo (1955) which shows that sulfite and cyanide inhibit rhodanese in the absence of thiosulphate.

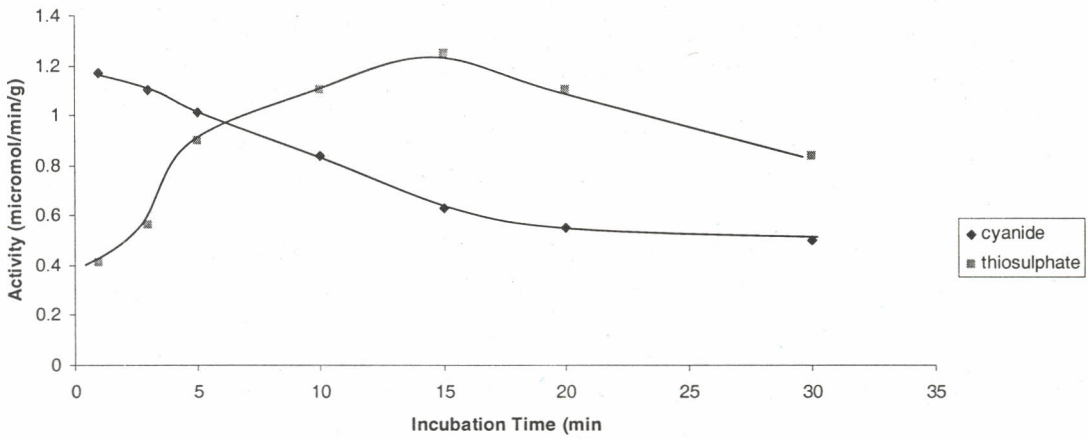

Fig 5: Effect of Preincubation of Liver Extract in the enzyme substrates on the Activity of Rhodanese

The results obtained in the current study may reflect some of the physiological properties of the enzyme in Sokoto red goat.

REFERENCES

Aminlari, M.; Hassan, G.; Hadayatolah, T. and Taleb, V. (1989). Comparative Studies on the Distribution of Rhodanese and □ mercaptopyruvate sulpfurtransferase in different organs of Sheep (*Ovis aries)* and Cattle (*Bos taurus)*; *Comp Biochem Physiol Comp Pharmacol Toxicol;* **92 (2)**: 259 -262.

Anosike E.O. and Jack, A. (1982. A comparison of some Biochemical properties of liver thiosulphate sulphurtransferase fom guinea pig (*Lepus caniculus)* and albino rats (*Mus musculus); Ind. J. Biochem Biophys* **19(1):** 13 -16.

Baskin, S.I. and Kirby, S.D. (1990). The Effect of Sodium Tetrathionate on Cyanide Conversion to Thiocyanate by Enzymatic and Non-enzymatic Mechanism; *J. Appl Toxicol.*; **10(5)**: 379-382.

Keith, A. and Volini, M. (1987). Properties of *E. coli* Rhodanese; *J.Biol. Chem.*; **262 (14)**: 6595-6604.

Marvin, S. and Kelly, D.P. (1976). Rhodanese from *Thiobacillus A2:* Catalysis of Reactions of Thisulphate with Dihydrolipoate and Dihydrolipoamide; *J. Gen Microbiol*; **97(2)**; 277 -284.

Ogata, K. and Volini, M. (1990). Mitochondrial Rhodanese: Membrane Bound and Complex Activity; *J. Biol. Chem.* **265 (14)**: 8087-8093.

Oke, O.L. (1979). Some Aspect of the Role of Cyanogenic Glycosides in Nutrition; *Wld. Rev. Nutr. Diet.*; **33:** 70 -103.

Osuntokun, B.O. (1981). Cassava Diet, Chronic Cyanide Intoxication and Neurophathy in Nigerian Africans; *Wld. Rev. Nutr. Diet.*; **36:** 141 –173.

Pagani S.; Sessa, G.; Sessa, F. and Colnaghi, R. (1993). Properties of *Azotobacter vinelandii* Rhodanese; *Biochem Mol Biol Int*; **29(4)**: 595 -604.

Saidu, Y.; Temple V.J.; Wasagu R.S.U. and Sahabi, D.M (2000). Rhodanese activity profile in some tissue of developing domestic fowl (*Gallus domesticus)* fed 20% cassava supplemented poultry feeds; *Nig J Biochem Mol Biol;* **15(1)**: 21 -23.

Sorbo, B.H. (1955). 'Rhodanese' in *Methods of Enzymology* (SidneyP.L. and Kaplan N.O. editors); Vol. II: Academic press; New York, San Francisco, London; 334-337.

Ugochukwu E.N.; Okolie, N.P. and Izokun-Etiobhio B.O. (1991). A comparative study of rhodanese activity in some liver species; *Comp Biochem Physiol;* **98c (2/3):** 275 -276.