Bulletin of SAN Vol. 26 (2005): 494 - 498

COMPARATIVE RHODANESE ACTIVITY IN LIVER AND BREAST MUSCLE OF SOME AVIAN SPECIES

SAIDU, Y.; YUSUF, A.A.; BILBIS, LS. AND SAHABI, D.M.
Biochemistry Department, Usmanu Danfodiyo University, Sokoto Nigeria

ABSTRACT

This work compares rhodanese activity profile in liver and skeletal muscle of some selected avian species. The enzyme activity was estimated colorimetrically by reacting thiocyanate released from enzymatic hydrolysis with Fe^{3+} forming a red coloured complex. The rhodanese activity in the liver of duck, dove, pigeon, domestic fowl and guinea fowl were 57.90 ± 6.66, 17.491 ± 8.38, 16.848 ± 9.40, 13.744 ± 3.21 and 9.785 ± 1.24 μmol/min/mg protein. The activity of the enzyme in the skeletal muscles of the birds were 4.611 ± 1.45, 6.672 ± 0.98, 5.783 ± 0.69, 5.160 ± 0.73 and 4.359 ± 1.11 μmol/min/mg protein respectively. The result indicated that the ratio of rhodanese activity of muscle to liver appears to be higher in the birds engaged in active muscular activity. The findings suggest that the enzyme, in addition to cyanide detoxification may also play an important role in energy metabolism in these birds.

Keywords: rhodanese; liver; breast-muscle; avian-species.

INTRODUCTION

The physiological role of rhodanese (E.C.2.8.1.1. cyanide: thiosulphate sulphur transferase) in animal tissues and perhaps in plants is controversial; particularly its function in the detoxification of acute cyanide exposure (Delvin *et al,* 1989; Sylvester and Sander, 1990). Rhodanese is a sulphur transferase that catalyses, *in vitro* the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors. *In vivo* the enzyme is, however multifunctional (Smith and Urbanska, 1986).

$$S_2O_3^{2-} + CN^- \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{2-}$$

It is generally believed that the major function of rhodanese is cyanide detoxification (Smith and Urbanska, 1986; Buzaleh *et al,* 1990). This function is more prominent in mammals where highly cytotoxic cyanide is converted to a less toxic thiosulphate and excreted through the kidney (Cagianut *et al,* 1984; Keith *et al*, 1989; Bourdoux *et al,* 1980). In plants, a close relationship exists between rhodanese activity and cyanogenesis, which suggest that the enzyme provides a mechanism for cyanide detoxification in cyanogenic plants (Smith and Urbanska, 1986). The enzyme is also implicated in energy metabolism (Keith and Volini, 1987; Ogata and Volini, 1990).

Investigation on the pattern of distribution of enzymes in cells, tissues and species may assist in locating certain biochemical processes that are unique to these biological entities and unraveled some of the roles of the enzymes. This work aimed at studying the tissue distribution of rhodanese with particular interest in liver and breast muscles of different avian species.

MATERIALS AND METHODS

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

Animals

Three animals of each avian specie (pigeon, dove, guinea fowl, domestic fowl and duck) were purchased from Sokoto Central Market.

Preparation of Tissue Homogenate

The animals were sacrificed by decapitation and dissected immediately. Both the liver and skeletal muscles of each of the animal were excised and washed immediately with ice-cold normal saline. Ten grams of each of the tissue were separately ground into small pieces using laboratory pestle and mortar. The ground tissue was then homogenized in a 100ml 0.1mol/l phosphate buffer, pH 7.3, containing 3 drops of triton X-100 per 100ml buffer, using a Teflon homogenizer. The homogenized tissues were centrifuged at 3000rpm for 10minutes using a bench centrifuge. The supernatants were carefully decanted into small labelled plastic bottles, additional 3 drops of triton-X 100 was added per 100ml of the supernatant to solubilized the enzyme from the mitochondria (Ugochukwu *et al.,* 1991) and stored at -20°C until required.

Assay of Rhodanese Activity

Rhodanese activity was assayed at 460 nm according to the method described by Sorbo (1955). It is based on the colorimetric determination of thiocyanate formed in the reaction:

$$CN^- + S_2O_3^{2-} \xrightarrow{\text{Rhodanese}} SCN^- + SO_3^{2-}$$

Protein content of the extracts was estimated colorimetrically at 540 nm by Biuret method (Cheesbrough, 1991).

RESULTS AND DISCUSSION

Rhodanese activity, expressed as micromole thiocyanate produced per minute per mg protein (μmol SCN min^{-1}.mg $protein^{-1}$), in the liver and breast muscles of some avian species is presented in Table 1 below.

Table 1: Rhodanese Activity of the Liver and Breast Muscles of Some Avian Species

	ACTIVITY(μmol/min/mg protein)*	
Avian Specie	Liver	Breast Muscle
DOVE	17.49±8.38	6.67±0.98**
PIGEON	16.85±9.4	5.78±0.69**
DOMESTIC FOWL	13.74±3.21	5.16±0.73**
GUINEA FOWL	9.79±1.24	4.36±1.11**
DUCK	57.9±6.66	4.61±1.45**

* Results are mean ±standard deviation

** results differ significantly ($P<0.05$) from the liver enzyme activity

The activity of the enzyme in the liver of the avian species studied were in the order duck, dove, pigeon, domestic fowl, guinea fowl. In the breast muscle, however, the activity is

in the order dove, pigeon, domestic fowl, duck, guinea fowl. The activity of the enzyme in the breast muscle and liver of each of the avian species differs significantly ($P<0.05$). There was however, no significant difference ($P>0.05$) in the activity of rhodanese among the breast muscles of these birds.

Rhodanese has been reported in organs and tissues of many organisms where it occurs significantly as a liver mitochondrial bound enzyme (Ogata and Volini, 1990). In the current work, the activity of the enzyme in all the species studied was highest in the liver. In a survey of rhodanese activity in tissues of domestic fowl, sparrow, pigeons and duck, however, Oh *et al.* (1977) reported that the activity of the enzyme was highest in the kidney of these species. Delvin *et al.* (1989) also reported that the activity of the enzyme was highest in rat liver compared to the skeletal muscles. These observations may not be unconnected to the role of liver in the detoxification of xenobiotics, cyanide inclusive.

The activity of the enzyme in the species studied was highest in duck liver. This may not be unconnected with its feeding habit as compared to other species. It was reported earlier that rhodanese activity in tissues of many animal species is affected by the type of diet they feed on. Those species that encountered cyanogenic glycosides in their diets are more likely to have higher rhodanese actity in their tissues (Izokun-Etiobhio and Ugochukwu, 1984; Ugochukwu *et al..,* 1991)

The percentage rhodanese activity of breast muscles to the liver enzyme activity of each specie is shown in Table 2. The activity of rhodanese in muscles relative to the liver enzyme is highest in guinea fowl and dove.

Table 2: Percentage Rhodanese Activity of the Breast Muscle Relative to the Liver in Some Avian Species

SPECIE	PERCENT
DOVE	38.15
PIGEON	34.32
DOMESTIC FOWL	37.54
GUINEA FOWL	44.55
DUCK	7.96

The distribution of rhodanese in some organisms is not restricted to those species that encounter exogenous cyanide through feeding on cyanogenic plants alone (Beesley *et al.*, 1985). This is an indication that cyanide detoxification may not be the only role of this enzyme in these organisms. In insects it was proposed that the enzyme might be involved in a more important role of sulphur transfer for protein synthesis.

Rhodanese in its phosphorylated and dephosphorylated forms has been reported to function as a converter enzyme that interact with mitochondrial membrane bound iron–sulphur centers of the mitochondrial electron transport chain where it modulates the rate of respiration (Ogata and Volini, 1990). There is an indication of a possible role of rhodanese in providing labile sulphide necessary for the synthesis of ferredoxin in the chloroplast of spinach, parsley, cabbage, and red turnips (Tomati, 1972). It also catalyses the formation of iron-sulphur centers in *E. coli*, and a physiological role of the enzyme in aerobic metabolism in this organism was suggested (Keith and Volini, 1987). Rhodanese was also reported to reconstitute spinach ferredoxin (Pagani *et al.*, 1984). It also restored partially, the activity of NADH dehydogenase (Pagani and Galante, 1983). It was also fund to increase the activity of malate dehydrogenase (Agro *et al,* 1976). Restoration of Mg^{2+}ATP and chelator inactivated

nitrogenase of *Klebsiella pneumoniae* has been reported (Pagani *et al.,* 1987). These observations stress the fact that the physiological role of rhodanese is not restricted to cyanide detoxification

In the current work, the appreciable activity of the enzyme recorded in the breast muscles of these birds, especially those engaged in strenuous muscular activity during flights, indicates that the physiological role of this enzyme in the breast muscle may not be restricted to cyanide detoxification alone. It could be possible that the enzyme may also play a vital role in energy metabolism in these birds.

References:

Agro A.F., Mavelli I., Cannella C. and Federici (1976). Activation of Porcine Heart Mitochondrial Malate Dehydrogenase by Zero Valence Sulfur and Rhodanese; *Biochem. Biophys. Res. Commun.*; **68(2)**:553-560

Beesley, S.G., Compton S.G. and Jones D.A. (1985). Rhodanese in Insects; *J. Chem. Ecol.*; **11(1)**: 45-50

Bourdoux, P., Manita, M., Hanson A. and Ermans A.M.(1980)."Cassava toxicity: the role of linamarin" In *Role of Cassava in the Etiology of endemic goite and cretinism* (A.M. Ermans, N.M. Mbulamoko, F. Delange and R. Ahluwalia – Editors) IDRC; Ottawa, Canada; 133-152

Buzaleh A.M.; Vazquez E.S. and Battle A.M.D. (1990). The Effect of Cyanide Intoxication on Hepatic Rhodanese Kinetics; *Gen. Pharmacol;* **21(2)**: 219 - 222

Cagianut, B., Schnebli, H.P., Rhyner, K. and Furrer, J. (1984). Decreased Rhodanese in Lebers' Hereditory Optic Atrophy; *Klin Wochenschr.*; **62 (18)**: 850-854

Cheesbrough, M. (1991). Medical laboratory manual for tropical countries vol. 1(2nd edition) ELSB, Cambridge; 465-567.

Delvin D.J., Mills J.W and Smith R.P. (1989). Histochemical Localization of Rhodanese Activity in Rat Liver and Skeletal Muscles; *Toxico.l Appl. Pharmacol.* **97:** 247 –255

Izokun-Etiobhio, E.O. and Ugochukwu, E.N. (1984). Effect of Incorporating Various levels of Cassava Cyanogenic Glycosides in Diets Fed to Albino rats on Liver and Kidney Rhodanese Activity; *Nutr. Rep. Int.* **29;** 1475 - 1481

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

Keith, A., Procell, L.R., Kirby, S.D., and Baskin S.I. (1989).The Inactivation of Rhodanese by Nitrite and Inhibition by Other Anions *in vitro; J. Biochem. Toxicol*; **4(spring):** 29 –34

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

Oh, S.Y., Jalaludin, S., Davies R.H. and Sykes A.H. (1977). The Activity of Avian Rhodanese; *Br. Poult Sci*; **18(4)**; 385 -390

Pagani S. and Galante Y.M. (1983). Interaction of Rhodanese with Mitochondrial NADH Dehydrogenase; *Biochim. Biophys. ACTA*; **742(2)**: 278-284

Pagani S., Bonomi, F. and Cerletti P.(1984). Enzymic Synthesis of the Iron-Sulfur Cluster of Spinach Ferredoxin; *Eur. J. Biochem*; **142(2):** 361-366

Pagani S., Marie E., and Eady R.R. (1987). Nitrogenase of *Klebsiella pneumoniae; Biochem. J.*; **244(2):** 485-488

Smith J. and Urbanska K.M. (1986). Rhodanese Activity in *Lotus corniculatus* sensu-lato; *J. Nat. Histol*; **20(6):**1467 –1476

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

Sylvester D.M. and Sander C. (1990). Immunohistochemical Localization of Rhodanese; *Histochem. J.*; **22(4)**: 197-200

Tomati U. (1972). Rhodanese Activity in Chloroplast; *Physiol Plant;* **4(2)**; 193 -196

Ugochukwu,E.N., Okolie, N.P. and Izokun-Etiodhip E.O (1991). A comparative Study of Rhodanese Activity in Some Lizard Species; *Comp. Biochem Physiol;* **98c**;**(2/3):** 275 –276.