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Effects of Temperature, pH and Some Monoatomic Sulphur Compounds on Rhodanese from Sheep Liver

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Abstract
The ubiquitous enzyme rhodanese (cyanide: thiosulphate sulphur transferase E.C.2.8.1.1.) in sheep liver was studied. The effects of temperature, pH and some monoatomic sulphur compound were investigated. Results revealed that the temperature and pH optima were 30^{O}C and 8.5 respectively. Cysteine, dithio oxiamide, glutathione and 2-mercaptoethanol were not able to substitute for thiocyanate in rhodanese assay; these monoatomic sulphur compounds never served as sulphur donors in sheep liver rhodanese catalyzed reaction of cyanide detoxification.

INTRODUCTION

Many plants and plant products, used as food in tropical countries contain cyanogenic glycosides. Upon hydrolysis these cyanogenic glycosides yield cyanide, which is a potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the mitochondrial electron transport chain.

The enzyme rhodanese (cyanide: thiosulphate sulphur transferase E.C.2.8.1.1.). is a ubiquitous enzyme that is known to be responsible for biotransformation of cyanide to thiocyanate.[1] Researchers has shown that rhodanase is found in a wide variety of plants and animals. It is believed that rhodanese is involved in cyanide detoxification.[2,3] However there is some evidence that rhodanese may be involved in other functions, including formation of iron sulfur centers[4] and participation in energy metabolism.[5]

Rhodanese is a sulphur transferase that catalyses, *in vitro*, the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors. The enzyme is multifunctional *in vivo*. In general, it is believed that cyanide detoxification is the major function of rhodanese.[6] This function is more prominent in mammals where highly toxic cyanide is converted to a less toxic thiocyanate and is excreted through the kidney.[7]

Research has shown that pH optimum for rhodanase from sheep liver falls in the range of the enzyme was 7.5 - 9 and its optimum temperature was vary widely depending on the source. Femi and Rapheal[8] reported that the rhodanese from fruit bat liver had pH optimum was 9.0 and the optimum temperature was 35^{O}C. From kinetic studies of guinea pig liver rhodanese, Anosike and Jack[9] recorded a pH optimum of 7.5 and temperature optimum of 20^{O}C. The optimum temperature was 20^{O}C while the optimum pH was 7.5.

OBJECTIVE OF STUDY

Owing to the fact that rhodanese is widely distributed in nature this work is aimed at isolating, partiallt purifying and studying some behavior of rhodanese from the liver of sheep.

MATERIALS AND METHODS

The liver of a sheep was collected from the slaughter at Rainbow, Port Harcourt, Nigeria.
Bovine serum albumin used for protein estimation and glutathione used to study the effect of monoatomic sulphur compounds were products of Sigma, England. 2-mercaptoethanol was a product of Riedel Hannover and Hopkin, England. Hydrochloric acid was a product of Anglia chemicals. disodium hydrogen phosphate and sodium dihydrogen phosphate used to prepare phosphate buffer and ferric nitrate used to prepare Sorbo reagent were all products of BDH, England.
All chemicals used were of analytical grade. Distilled water was used for preparing solutions all through this project.

METHOD

ISOLATION AND PARTIAL PURIFICATION OF RHODANASE FROM SHEEP LIVER

The method of Horowit and De Tomas[10] was followed for the isolation and partial purification of rhodanese from sheep liver with slight modifications. The extraction and partial purification was carried out in four steps as described below.

STEP I: BUFFER EXTRACTION

The liver isolated from a sheep at the field (abattoir) was immediately stored in the freezer for use. Portion of the liver (50g) was homogenized in 120ml of 0.0lM glycine-acetate buffer of pH 5.0 using a kenitone blender.
The homogenate was stirred mechanically for 30 minutes while the tissue in its container was placed in ice bath. The homogenate was then centrifuged at 14,000g using zentrifugen refrigerated centrifuge.

STEP II: AMMONIUM SULPHATE PRECIPITATION AND ACID TREATMENT

To the supernatant from step I, ammonium sulphate crystals were added to attain a concentration of 0.4M, after which it was stirred in the cold for 30 minutes. The pH was then adjusted to 5.88 by the addition of cold 0.1M HCI. After stirring the supernatant so treated for 30 minutes in the cold, it was centrifuged at 4,000g for 5 minutes. The precipitate was dissolved in 10^{-3}M Tris-HCI buffer of pH 7.6. The resulting suspension was stirred in the cold for 30minutes and then centrifuged at 4,000g for 5 minutes.

STEP III: AMMONIUM SULPHATE PRECIPITATION

Ammonium sulphate crystals were added to the supernatant from step II above, so that it contained 0.4M ammonium sulphate. This supernatant was stirred in the cold for 30 minutes and then centrifuged at 4,000g for 5 minutes.

STEP IV: ENZYME PROTEIN PRECIPITATION

To the supernatant from step III, ammonium sulphate crystals were added to raise the concentration to 2.5M. It was then stirred in the cold for 30 minutes and centrifuged at 10,000g for 5 minutes. The precipitate was dissolved in 10^{-3}M Tris-HCl buffer of pH 7.6, to get the enzyme in solution. This was centrifuged at 4,000g for 5 minutes to remove any undissolved protein. The resulting supernatant was used as the source of the enzyme for subsequent studies.

METHOD OF ASSAY OF SHEEP LIVER RHODANESE ACTIVITY.

For routine assay of enzyme activity, the method used is described below:
The reaction mixture of 6ml contained,
i. 1.5ml of 0.1M phosphate buffer pH 8.2
ii. 0.5ml of 0.1M thiosulphate solution
iii. 0.5ml of enzyme extract
iv. 0.5ml of 0.1M potassium cyanide
v. 0.25ml of 35% formaldehyde
vi. 2.75ml of Sorbo reagent
A mixture of i, ii and iii was incubated for 10 minutes at 30^{O}C then 0.5ml of 0.1M potassium cyanide was added. After leaving the mixture for 10 minutes on the bench (at room temperature), 0.25ml of 35% formaldehyde was added to stop the reaction. Finally 2.75ml of Sorbo reagent was added to develop the colour of the thiocyanate formed from the reaction mixture. The addition of Sorbo reagent was to enable the thiocyanate formed to be estimated spetrophotometrically at 460nm.

PROTEIN ESTIMATION

Protein was estimated spectrophotometrically at each isolation and purification step using the method of Lowry *et al.*[11] using bovine serum albumin (B.S.A) as standard.

STANDARD CALIBRATION CURVE FOR THIOCYANATE

100µM solution of potassium thiocyanate was used as standard. Dilutions were made to concentrations ranging from 1.67µM, 6.67µM. The solutions were treated and absorbance read at 460nm and a calibration curve was prepared for thiocyanate estimation.

EFFECT OF TEMPERATURE ON SHEEP LIVER RHODANASE

The study of the effect of temperature on sheep liver rhodanese catalyzed reaction was studied using the temperature range: 15^{O}C to 56^{O}C. In each test temperature two test tubes were used; one serving as blank in which the enzyme extract was not added. Since the formation of thiocyanate from cyanide is also heat catalyzed the duplication at each test temperature is meant to account for the thiocyanate formed through thermal catalysis.

EFFECT OF pH ON SHEEP LIVER RHODANESE

The effect of pH on sheep liver rhodanese enzyme preparation was determined using 0.01M Tris-HCI (pH5.0-7.0) and 0.01M sodium phosphate (pH7.5-9.0) as buffer systems.
For each test pH, 1.5ml of each type of buffer was used, in which the following was added: 0.5ml of enzyme extract, 0.5ml of 0.1M potassium thiosulphate solution. After incubating these for 10 minutes, 0.5ml of 0.1M potassium cyanide solution was added. It was left at room temperature for 10 minutes then 0.25ml of 35% formaldehyde was used to stop the reaction. 2.75ml of Sorbo reagent was added and absorbance was read at 460nm. The effect of pH was studied in the direction of thiocyanate formation.

EFFECT OF OTHER SULPHYDRYL COMPOUNDS ON SHEEP LIVER RHODANESE CATALYSIS

The effect of some sulphydryl compounds on sheep liver rhodanese was studied using dithio oxamide, cysteine, 2-mercaptoethanol and glutathione at the concentration of 0.1M. This was done to compare the sulphur donating

property of thiosulphate and the above named monoatomic sulphur compounds on sheep liver rhodanese catalyzed reaction. This experiment was to find out whether these compounds could replace thiosulphate in rhodanese assay.
In the assay procedure thiosulphate was replaced with each of the monoatomic sulphur compounds.

RESULTS AND DISCUSSION

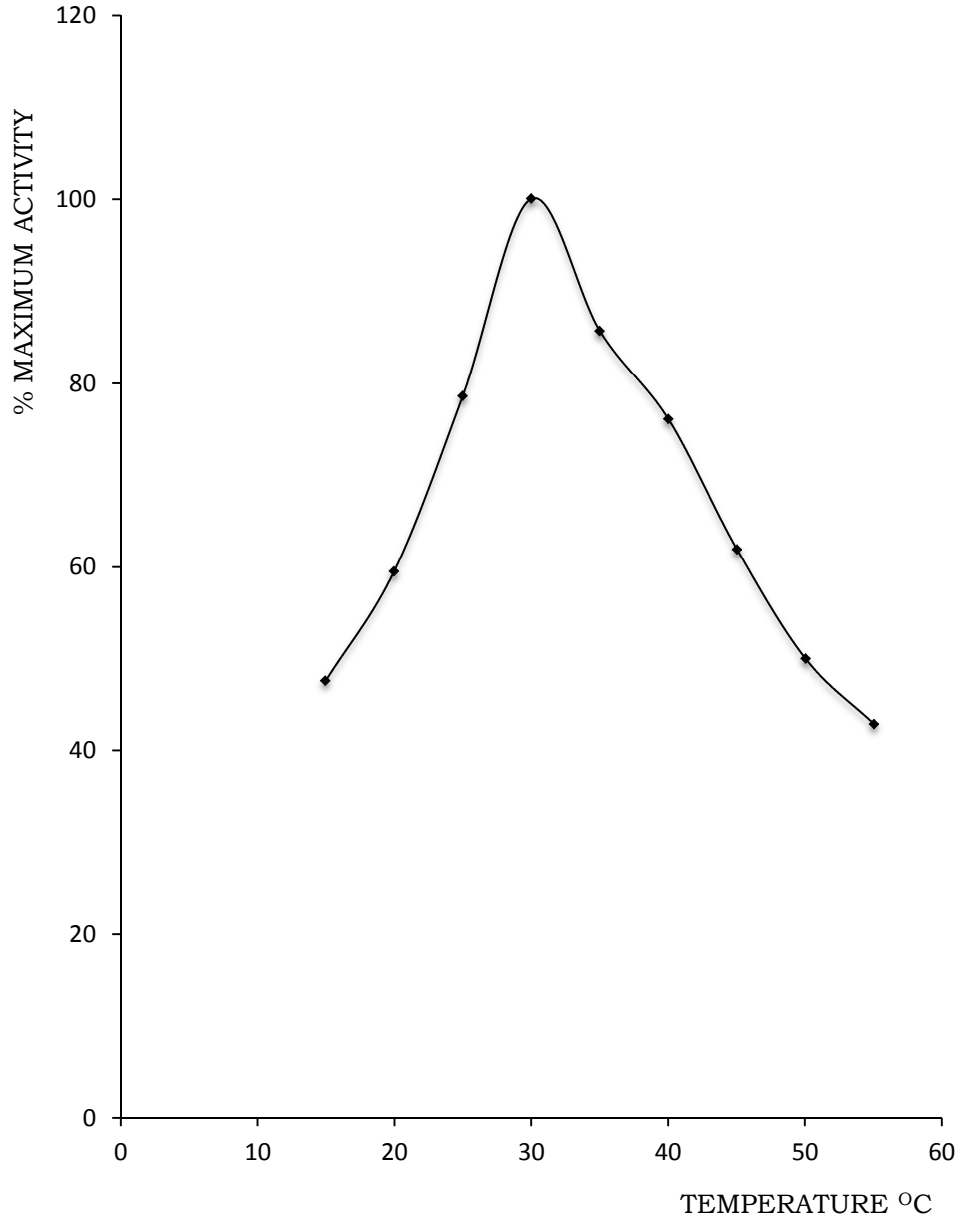

Fig.9: Effect of temperature on sheep liver rhodanese-catalysed reaction. A plot of percentage maximum activity versus temperature (^{O}C). Assay was done with 8.33mM of sodium thiosulphate and 8.33mM of potassium cyanide.

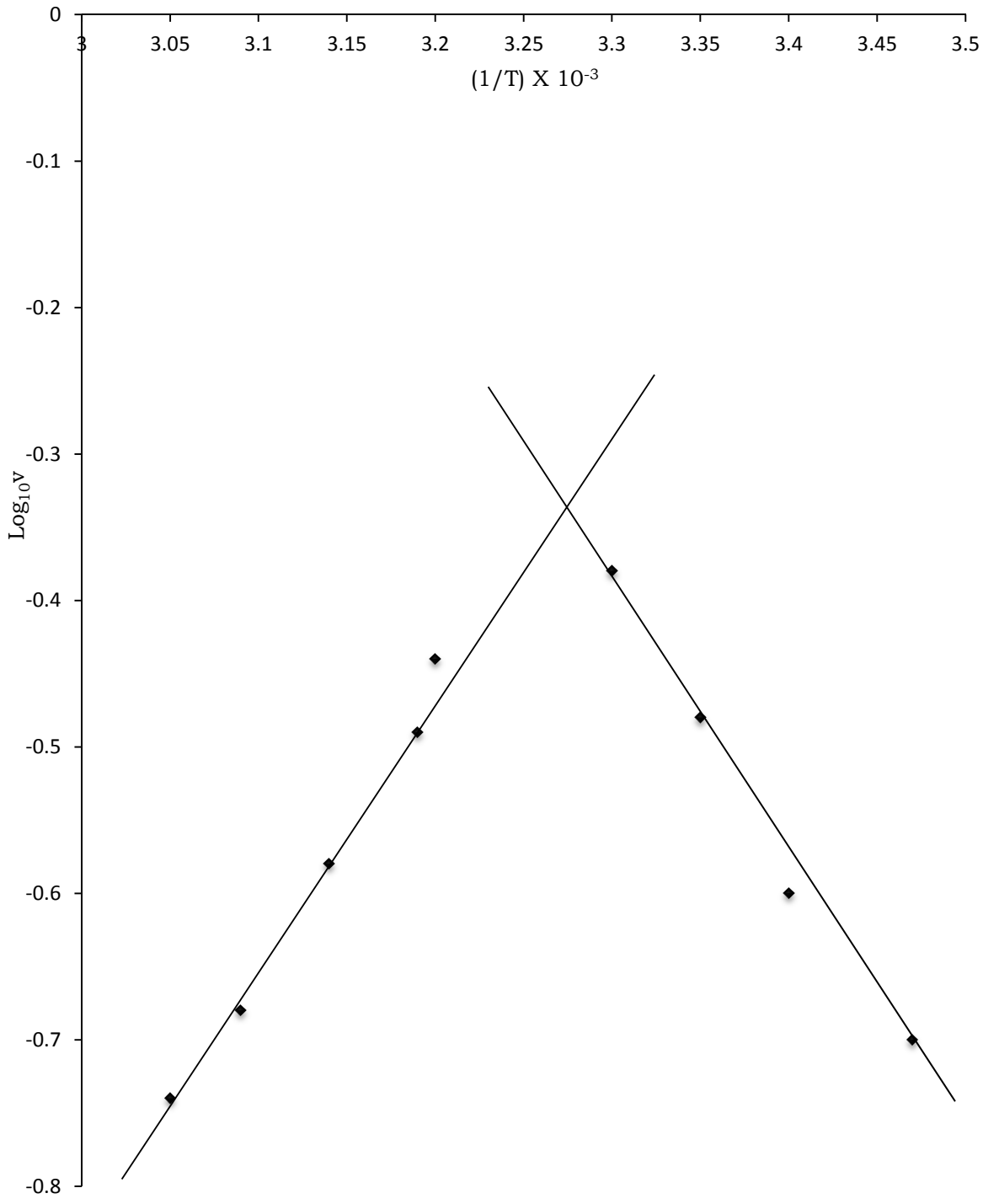

Fig. 10. Arrhenius plot: A graph of $\log_{10}$v versus reciprocal of absolute trmperature (10^{-3}) for determination of activation (Ea)and deactivation (-Ea) energies

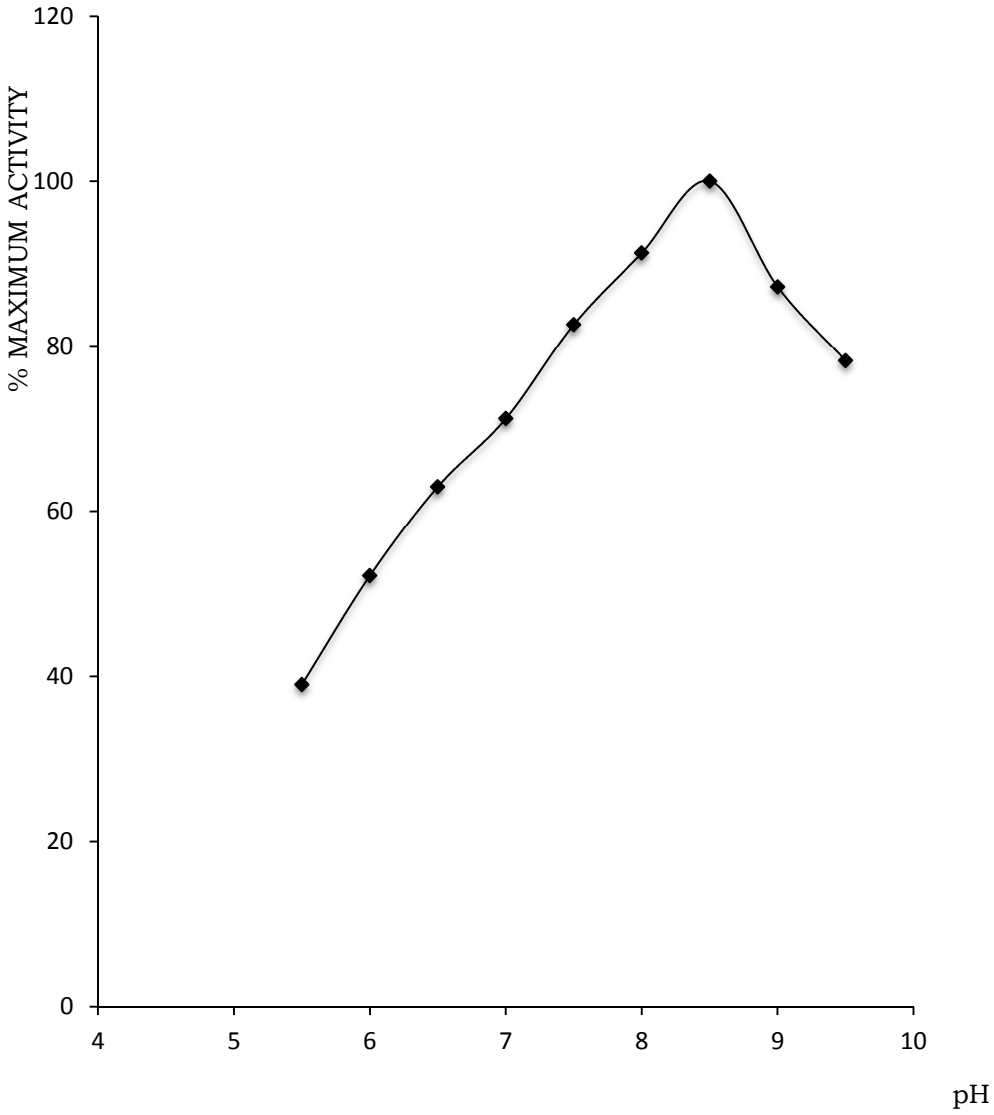

Fig. 11: Effect of pH on sheep liver rhodanese-catalysed reaction. A plot of percentage maximum activity versus pH. Assay was done with 8.33mM of sodium thiosulphate and 8.33mM of potassium cyanide, with 1.5ml of each type of buffer.

The rhodanese is present in the liver of a sheep. The partially purified preparation of rhodanase from the liver of a sheep in this work was purified 12.5 fold with 13.3% activity recovery had a specific activity of 8.81 mole min^{-1} mg^{-1} protein.

Temperature optimum of sheep liver rhodanese was found to be $30^{O}C$. This value falls within the range of $25^{O}C$ – $35^{O}C$ reported by Sorbo[12] for bovine liver rhodanese. Other investigators working with rhodanese from animal sources such as Tabita *et al.* [13] with *Ferrobacillus ferroxicans* rhodanese, Anosike and Jack[9] with guinea pig liver rhodanese, and Femi and Rapheal[8] with fruit bat liver rhodanese recorded temperature optimum values of $25^{O}C$, $20^{O}C$ and $35^{O}C$ respectively.

On the other hand those investigators that worked on rhodanese from plant sources recorded higher temperature optimum. For instance Tomati *et al.*[14] with cabbage, and Boey *et al.*[15] with tapioca leaf rhodanese recorded temperature optimum ranges of $57^{O}C$ - $59^{O}C$ and $50^{O}C$ - $55^{O}C$ respectively. It can be deduced that rhodanese from plant sources are more thermostable than those from animal sources. With the Arrhenius plot (fig 2), it was deduced that the activation energy (Ea) of sheep liver rhodanese was 8.28 $KJmole^{-1}$ while the deactivation energy (-Ea) was 12.88KJ $mole^{-1}$.

The optimum pH of sheep liver rhodanese was 8.5, which is in conformity with the pH optimum ranges of 7.5 - 9.0 and 8.0 - 9.0 reported for rhodanese from *Ferrobacillus ferroxidans* and from bovine liver by Tabita *et al* [13] and by Sorbo [12] respectively. Other investigators such as Silver and Kelly[16] and Boey *et al* [15] reported higher pH ranges of 10.2 - 10.4 and 10.2 - 11.0 for rhodanese from *Thiobacillus* A_2 and tapioca leaf respectively. Femi and Rapheal[8] reported pH optimum of 9.0 for rhodanese from fruit bat liver.

The attempt to use some monoatomic sulphur compounds namely: cysteine, dithio oxiamide, glutathione and 2-mercaptoethanol to substitute for thiocyanate in rhodanese assay failed, for these monoatomic sulphur compounds never served as sulphur donors in sheep liver rhodanese catalyzed reaction of cyanide detoxification. This finding is in conformity with the findings of Tabita *et al.*[13] and Anosike and Jack.[9] The reason for this observation could be that the enzyme is not able to form the important intermediate of rhodanese-sulfide ion complex with the monoatomic sulphur compounds used.

CONCLUSION

Rhodanese, a ubiquitous enzyme found in most living organism is present in the liver of a sheep. A partially purified preparation of the enzyme was characterized as found in the result section of this work. The temperature and pH optima as well as the molecular weight of rhodanese all fall within the accepted ranges.

REFERENCES

1. Westley, J.(1973). Rhodanese. Adv. Enzymol. 39:327-368.
2. Lang, K. (1933). Die rhodanbildung in tierporper. Biochem. Z.259:243- 256.
3. Koj, A. and Frendo, J. (1962): Activity of cysteine desulfurhydrase and rhodanese nin animal tiessue.'' Acta. Biochem.pol. 9, 373-379.
4. Hasan and Mahmoud (2008). Comparison of rhodanese distribution in different tissues of Japanese quail, partridge and pigeon. Clin Pathol (18), 217-220
5. Ogata, K., and M. volini (1990). Mitochondrial rhodanese: membrane- bound and complexed activity. J. Biol. Chem. 265:8087-8093.
6. Smith J. D. G, ploegman J. H, Pierrot M, Kalk K. H, Jansonius J. N, Drenth J. (1974). The structure of Rhodanese at 4 A Resolution: the conformation of the polypeptide chain. Lsraeli j12(1/2): 287- 304.
7. Keith A, Procell L. R, Kirby S. D, Baskin SI (1989). The Inactivation of Rhodanese by Nitrite and Inhibition by Other Anions in vitro. J. Biochem. Toxicol. 4(spring):29-34.
8. . Femi K. A. and Rapheal E. O. (2003). Presence of rhodanese in the cytosolic fraction of fruit bat (Eidolon helvum) liver. J. Biochem and Mol. Biol. (37) 275-281.
9. Anosike E.O. and Jack A. (1982), A Comparison Of Some Biochemical Properties of Liver Tiosulphate Sulphurtransferase From Guinea Pig & Albino Rat. Ind. J. Biochem. and Biophysics. (19): 13-16
10. Horowitz P. and De Tomas (1970). Improved preparation of bovine liver rhodanese. J. Biol. Chem. 245 (5): 981-986.
11. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951). Estimation of Protein. J. Biol. Chem. **193,** 265-275
12. Sorbo B. H. (1953). Crystalline bovine liver rhodanese and its properties. Acta. Scand. **7**, 1129-1136
13. Tabita R., Silver M. and Lungren D. C. (1969). The rhodanese enzyme of *Ferrobacillus ferroxidans.* Can. J. Biochem. **47** 1141-1142.
14. Tomati U., Federici G. and Cannella C. (1974). Ferredoxin oxidation by cabbage leaf rhodanese. Phytochemistry (OXF), 13 (9): 1703-1706.
15. Boey C. C., Yeoh H. H. and Chew M. Y. (1976). Purification of tapioca leaf rhodanese. Phytochemistry (OXF), 15 (9): 1343-1344.

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