A COMPARATIVE STUDY OF RHODANESE ACTIVITY IN SOME LIZARD SPECIES

EMMANUEL N. UGOCHUKWU, NGOZI P. OKOLIE* and BENJAMIN O. IZOKUN-ETIOBHIO Department of Biochemistry, University of Benin, P.M.B 1154, Benin City, Nigeria

(Received 18 April 1990)

Abstract—1. Rhodanese activity has been determined in some organs of three species of lizards, namely: rainbow lizard, wall gecko and skink.

2. While the activity was significantly higher in the livers of all the species compared to that in the kidney, small intestine and the brain, there were significant differences in the enzyme in the livers of the different species. The activity was highest in the liver of the rainbow lizard followed by that of wall gecko and then skink.

3. The order of activity of the enzyme in the organs of the rainbow lizard was liver > kidney > small intestine > brain.

4. The high level of rhodanese in lizard tissues suggests that the enzyme may be involved in roles other than cyanide detoxification.

INTRODUCTION

Rhodanese (thiosulphate: cyanide sulphur transferase EC 2.8.1.1) catalyses the formation of thiocyanate from thiosulphate or colloidal sulphur and free cyanide (Westley, 1973; Sorbo, 1975). The enzyme has been established to occur in some plants (Chow and Boey, 1972); fungi (Oi, 1973); actinomycetes (Yamamoto and Oi, 1977) and in various bacteria (Tabita *et al.*, 1969). It has also been detected in all phyla of the animal kingdom (Westley, 1981).

The primary function of rhodanese is generally believed to be cyanide detoxification (Oke, 1973; Cerletti, 1986). It has also been shown that the enzyme can detoxify the highly neurotoxic sulphide produced from cysteine by cystathionase (Szczepkowski and Wood, 1967) and reactivate ferredoxin from apoferridoxin (Tomati *et al.*, 1974; Cerletti, 1986).

The present paper deals with a comparative study of rhodanese activity in organs of some lizard species.

MATERIALS AND METHODS

Chemicals

Analytical grade chemicals were used. Ferric nitrate, sodium thiosulphate and potassium cyanide were products of E. Merck (Darmstadt, F.R.G.) All other chemicals used were purchased from British Drug Houses (BDH) Chemicals Ltd, Dorset, U.K.

Animals

The full names of the lizard species used are shown in Table 1. Adult rainbow lizards, wall geckos and skinks were caught alive in their natural habitats; in the open fields, homes and in moist, wooded areas, respectively.

Preparation of homogenates

Ten lizards of each species were killed by decapitation. The brain, liver, kidney and small intestine were quickly dissected out. Blood on the organs was washed off with ice-cold 0.9% saline (w/v). They were frozen at -15° C for analysis.

Ten percent homogenate (w/v) of all the organs were prepared in cold 0.05 M potassium phosphate buffer (containing 2 drops of Triton X-100) pH 7.0, using a Teflon homogenizer. Triton X-100 was added to solubilise rhodanese which is mainly a mitochondrial enzyme (Volini and Alexander, 1981). The homogenates were centrifuged at 4°C for 15 min at 700 g. The supernatant fractions were stored at 4°C until subsequent enzyme assays.

Enzyme assay

The rhodanese activity was measured by the method described by Sorbo (1953). The intensity of the reddishbrown colour produced was estimated spectrophotometrically at 460 nm using a pye-Unicam SP 1800 u.v. spectrophotometer. The enzyme activity was expressed as micromoles of thiocyanate formed/min g fresh tissue.

RESULTS AND DISCUSSION

Rhodanese activities in some organs of three species of lizard are summarised in Table 1. All organs investigated showed enzyme activity. Liver rhodanese activity was significantly higher in Agama agama than in Hemidactylus brookeri or Eumeces brevilineatus. Similarly, small intestine and brain rhodanese activities in A. agama were significantly higher than the activities in corresponding organs of H. brookeri and E. brevilineatus.

The order of activity of the enzyme in the tissues of A. agama was liver > kidney > small intestine > brain. This result is similar to those obtained by Lang (1933) and Sorbo (1953) who reported highest rhodanese activity in mammalian liver. Moreover, Izokun-Etiobhio and Ugochukwu (1984) have shown that the rhodanese activity in the liver of the albino rat is three times that in the kidney. The reason for the presence of high levels of this enzyme in various organs of the lizard species examined is not certain. However, the primary function of rhodanese is thought to be in detoxification of ingested cyanide

^{*}To whom all correspondence should be addressed.

Table 1. Rhodanese activity (µmol ScN/min g) tissue in organs of some lizard species*

Species	Common name	Tissues			
		Liver	Kidney	Small intestine	Brain
Agama agama	Rainbow lizard	$2.7300 \pm 0.01 \dagger$	1.0711 ± 0.022	0.6200 ± 0.028	0.3850 ± 0.01
Hemidactylus brookeri	Wall gecko	$1.2513 \pm 0.03 \ddagger$	ND	0.44 ± 0.01	0.21 ± 0.01
Eumeces brevilineatus	Skink	0.6130 ± 0.02 §	ND	0.22 ± 0.01	0.1200 ± 0.01

*Results are expressed as the mean \pm SEM of determinations on ten animals per species.

 $\{ \xi \}$: Values bearing different superscripts differ significantly (P < 0.05) using analysis of variance.

ND: Not determined.

(Oke, 1973; Cerletti, 1986). A careful study of the feeding habits of these lizards (Okolie, 1989, unpublished) showed that although lizards are mostly insectivorous (Smith, 1946), the rainbow lizard also feeds on cassava, melon seeds, tomato fruits, beans, red pepper and palm fruits. Beans and cassava are known to contain cyanogens/cyanide (Butler, 1965; Nartey, 1968; Cooke, 1978; Vanderborght, 1979; Okolie and Ugochukwu, 1989). Thus the presence of high levels of rhodanese in A. agama may partly be due to its role in the detoxification of ingested cyanide. On the other hand, rhodanese activity in skinks and geckos is not easily explained on the basis of their feeding habits. Thus, in these species, the enzyme may be involved in roles separate from cyanide detoxification. The other roles which have been proposed for the enzyme include involvement in the disposal of the highly neurotoxic cysteine sulphide (Szczepkowski and Wood, 1967) and/or formation of iron-sulphur clusters in iron-sulphur proteins (Finazzi-Agro et al., 1971; Tomati et al., 1972). It may not be unreasonable to suggest that the activity pattern of this enzyme in the various tissues of the lizards has some relevance to the variability of extent of these roles in the different tissues.

REFERENCES

- Butler G. W. (1965) The distribution cyanoglucoside linamarin and lotaustralin in higher plants. *Phytochemistry* 4, 127-131.
- Cerletti P. (1986) Seeking a better job for under-employed enzyme: rhodanese *TIBS* **11**, 369–372.
- Chew M. Y. and Boey C. G. (1972) Rhodanese in tapioca leaf. *Phytochemistry* 11, 167-169.
- Cooke R. D. (1978) An enzymatic assay for the total cyanide content of cassava (Manihot esculenta Crantz). J. Sci. Food Agric. 29, 345–352.
- Finazzi-Agro A., Cannella C., Graziani T. and Cavallini D. (1971) A possible role for rhodanese: the formation of labile sulphur from thiosulphate. *FEBS Lett.* 16, 172–174.

- Izokun-Etiobhio B. O. and Ugochukwu E. N. (1984) Effect of incorporating various levels of cassava (*Manihot esculenta Crantz*) cyanogenic glucosides in diets fed to albino rats on liver and kidney rhodanese activity. *Nutr. Rep. Int.* 29, 1475–1481.
- Jansz E. R. and Pieris N. (1978) Studies on some local legumes—11. Cyanogenic glucosides. J. natn Sci. Coun. Sri Lanka 6, 1-9.
- Lang K. (1933) Thiocyanate formation in the animal body. Biochem. Z. 259, 243-256.
- Nartey F. (1968) Studies on cassava, Manihot utillissima Pohl I. Cyanogenesis. The biosynthesis of linamarin and lotaustralin in etiolated seedlings. Phytochemistry 7, 1307–1312.
- Oke O. L. (1973) The mode of cyanide detoxification. In *Chronic Cassava Toxicity*, Nestel B. and MacIntyre R., (eds), pp. 97-i04. I.D.R.C., OlOe, Ottawa, Canada.
- Okolie N. P. and Ugochukwu E. N. (1989) Cyanide contents of some Nigerian legumes and effect of simple processing. *Food Chem.* **32**, 209–217.
- Oi S. (1973) Purification and some properties of *Trametes sanguinea* rhodanese. Agric. Biol. Chem. 37, 629-635.
- Sorbo B. H. (1953) Crystalline bovine rhodanese and its properties. Acta chem. scand. 7, 1129-1136.
- Sorbo B. (1975) Metabolic Pathways, Greenberg D. M., (ed.) (3rd Edn), Vol. 7, pp. 433–456. Academic Press, New York.
- Smith H. M. (1946) Handbook of Lizards (1st Edn), p. 34. Comstock, New York.
- Szczepkowski T. W. and Wood J. L. (1967) The cystathionase-rhodanese system. *Biochim. biophys. Acta* 139, 469-478.
- Tabita R., Silver M. and Lundgreen D. G. (1969) The rhodanese enzyme of *Ferrobacillus ferroxidans* (*Thio-bacillus ferroxidans*). Can. J. Biochem. 47, 1141–1145.
- Tomati U., Matarese R. and Federici G. (1974) Ferredoxin activation by rhodanese. *Phytochemistry* 13, 1703–1706.
- Vanderborght T. (1979) Le dosage de l'acide cyanhydrique chez Phaseolus lutatus L. Annls Gemb. 85, 29-41.

Westley J. (1973) Rhodanese. Adv. Enzymol. 39, 327-366. Westley J. (1981) Meth. Enzymol. 77, 285-291.

Yamamoto T. and Oi S. (1977) A streptomyces sp. effective for conversion of cyanide into thiocyanate. J. Ferment. Tech. 55, 560-569.