Thiosulphate: cyanide sulphur transferase activity in some species of helminth parasites

H. Baghshani · M. Seyed Abadi

Received: 25 July 2012 / Accepted: 20 November 2012 / Published online: 11 December 2012
© Indian Society for Parasitology 2012

Abstract Investigation on the characteristic pattern of enzymes in various organisms may be of particular importance, as this type of information can help to understand certain physiological and biochemical processes in living organisms. Thiosulfate: cyanide sulfurtransferase (rhodanese) is a ubiquitous multifunctional enzyme, which its major function is generally believed to be in cyanide detoxification. The present study was conducted to determine the activity of this enzyme in some parasitic helminths including, cestodes (*Moniezia benedeni*, *Helicometra giardi*), trematodes (*Fasciola hepatica*, *Fasciola gigantica*, *Dicrocoelium dendriticum*) and nematodes (*Haemonchus longistipes*, *Marshallagia marshalli*). The data resultant from this study indicates that at least some rhodanese activity was present in all the seven investigated parasites, although its activity was much lower in comparison with previously reported values for some tissues of their vertebrate hosts. With respect to some degrees of cyanide insensitivity in parasitic helminths, it may be suggested that cyanide detoxification may not be a primary benefit of rhodanese in parasitic helminths and the true physiological and biochemical functions of the enzyme remains to be further defined in such organisms.

Keywords Thiosulfate: cyanide sulfurtransferase · Parasitic helminth · Cyanide detoxification

Introduction

Thiosulfate: cyanide sulfurtransferase (EC 2.8.1.1) or rhodanese is a ubiquitous enzyme that is active in all living organisms, from bacteria to *Homo sapiens* and plants (Nagahara et al. 1999; Aminlari et al. 2000; Agboola and Okonji 2004). In vitro, the enzyme catalyzes the irreversible transfer of a sulfane sulfur atom from a suitable donor (i.e., thiosulfate) to cyanide, leading to formation of less toxic sulfite and thiocyanate (Raybuck 1992).

In spite of extensive studies, the true biological role of this enzyme is still controversial. In those species of organisms in which rhodanese is present, it is thought to play a role in cyanide detoxification (Beesley et al. 1985; Raybuck 1992). The role of rhodanese in cyanide detoxification has been known for many years (Sorbo 1953) and is supported by the high concentration of this enzyme in some mammalian tissues and organs (e.g., the liver) exposed to cyanide (Sylvester and Sander 1990; Aminlari and Gilanpour 1991). In plants, a close relationship exists between rhodanese activity and cyanogenesis, which suggests that the enzyme provides a mechanism for cyanide detoxification in cyanogenic plants (Smith and Urbanska 1986). It has been proposed that the enzyme might be involved in other important roles including, formation of iron sulfur centers, participation in selenium metabolism and thiamine biosynthesis, participation in energy metabolism, and functions as a thioredoxin oxidase (Smith and Urbanska 1986; Nandi et al. 2000; Bordo and Bork 2002).

Cyanide toxicity is mainly due to its affinity for the terminal cytochrome oxidase in the mitochondrial respiratory chain (Nagahara et al. 1999). Many insects and other invertebrates are highly tolerant to cyanide exposure and rhodanese activity has often been assumed to enable herbivorous insects to feed on cyanogenic foliage with impunity (Jones 1972; Conn 1979). Parasitic helminths,

H. Baghshani (✉)
Department of Basic Sciences, School of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran
e-mail: baghshani@ferdowsi.um.ac.ir

M. Seyed Abadi
Department of Pathobiology, School of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran

in general, are much less sensitive to cyanide than mammals. Cyanide-insensitive respiration—i.e. where oxygen uptake occurs in the presence of cyanide—is characteristic of most parasitic helminths (Smyth and McManus 1989), thereby may rendering this species less susceptible to cyanide toxicity. This unique metabolic feature bring into question whether presence of rhodanese is important for cyanide detoxification in these organisms.

The pattern of distribution of rhodanese in different animals appears to be highly species and tissue specific (Aminlari et al. 2000). Also, the activity of the enzyme in a particular tissue may reflect the ability of that tissue to detoxify cyanide (Al-Qarawi et al. 2001). Presence of thiosulfate: cyanide sulfurtransferase, the main enzyme involved in cyanide detoxification, had been studied extensively amongst a wide range of organisms while far less is known for parasitic helminths, which have some degrees of cyanide tolerance. This study was conducted to determine the activity of this enzyme in some species of parasitic helminths.

Materials and methods

Parasitic helminths including, cestodes (*Moniezia benedeni*, *Helicometra giardi*), trematodes (*Fasciola hepatica*, *Fasciola gigantica*, *Dicrocoelium dendriticum*) and nematodes (*Haemonchus longistipes*, *Marshallagia marshalli*) were obtained from freshly-slaughtered animals in the Mashhad slaughter house. The worms were washed well to remove host material and saved directly in an ice box during transportation to the Lab. Crude extract was prepared by homogenization of the samples using 50 mM sodium phosphate buffer, pH 7.2. The homogenates were centrifuged for 15 min at 4,000×g and supernatants were used as the source of enzyme. Three independent determinations were made for each parasite and for species with small sizes; helminths were pooled for each enzyme assay.

The rhodanese content of the parasites was assayed based on the colorimetric determination of thiocyanate (Sorbo 1953), as described by Aminlari et al. (2000). In this case thiocyanate produced by the action of the enzyme on cyanide and thiosulphate, is reacted with ferric nitrate reagent to produce a red coloured complex which can be estimated colorimetrically at 460 nm. The unit of enzyme activity was defined as micromole of thiocyanate formed per minute under assay conditions. Rhodanese activity is reported as enzyme units/g wet weight of the parasite.

Results

The activity of thiosulfate: cyanide sulfurtransferase in crude extracts obtained from examined parasites is given in

Table 1 Thiosulfate: cyanide sulfurtransferase activity in some parasitic helminths

Helminth species	Enzyme activity (units/g wet weight)
<i>Moniezia benedeni</i>	0.194 ± 0.061
<i>Helicometra giardi</i>	0.188 ± 0.019
<i>Haemonchus longistipes</i>	0.283 ± 0.088
<i>Marshallagia marshalli</i>	0.337 ± 0.122
<i>Fasciola hepatica</i>	0.245 ± 0.028
<i>Fasciola gigantica</i>	0.296 ± 0.022
<i>Dicrocoelium dendriticum</i>	0.137 ± 0.075

Each value is the mean ± SEM of 3 independent experiments

Table 1. It appears that all parasites investigated contain the enzyme activity, although, the enzyme activities were in different levels in species of helminths.

Discussion

Investigation on the characteristic pattern of enzymes in various organisms may be of particular importance, as this type of information can help to understand certain physiological and biochemical processes in living organisms. Living organisms employ a variety of metabolic pathways when detoxifying xenobiotic compounds. Many insects and also mammals are known to have the enzyme thiosulfate: cyanide sulfurtransferase, which catalyses sulphate transfer to detoxify cyanide and is therefore a widespread mode of detoxification (Parsons and Rothschild 1964; Seigler 1991). The ability of animals to feed on cyanogenic plants should be correlated with the amounts of detoxifying enzymes that they possess (Conn 1979). It has been suggested that the level of rhodanese in different tissues of animals is correlated with the level of exposure to cyanide (Aminlari et al. 2000; Al-Qarawi et al. 2001).

The data resultant from this study indicates that at least some rhodanese activity is present in all parasitic helminths sampled. In agreement with this study, Bertran (1952) found rhodanese activity in several internal parasites including larvae of the horse botfly (*Gasterophilus intestinalis*) and suggested that it was the major mechanism for enabling them to survive in the stomach of clover-feeding horses. The enzyme activity had been observed in the present study in some parasitic helminths is very lower compared to the previously reported values (Aminlari and Gilanpour 1991; Nazifi et al. 2003) for some tissues of their hosts. Similarly, Beesley et al. (1985) assayed for rhodanese in 44 species of insects and found that it occurs at much lower levels than in vertebrates. Moreover, it has been reported that the rat liver had about ten-fold higher

rhodanese activity than the southern armyworm (*Spodoptera erldanla Cramer*), although the activity level in the rat small intestine was quite comparable to that in the southern armyworm (Long and Brattsten 1982).

Parasitic helminths exhibit greater diversity in their metabolic pathways than do the host animals that may be as adaptations to their natural habitats (Kita et al. 1997; Marr et al. 2003). Extensive evidences have shown that parasitic helminths possess a respiratory chain which differs from the mammalian system in being branched and possessing multiple terminal oxidases. Mitochondria from these organisms use unsaturated organic acids in addition to oxygen as terminal electron acceptors, and cyanide-insensitive electron-transport-associated ATP synthesis is coupled to the excretion of reduced organic acids as end-products of carbohydrate metabolism (Marr et al. 2003). This cyanide-resistant respiratory chain in parasitic helminths may cause some degrees of cyanide tolerance in these organisms and less rhodanese enzymatic activity observed in the present study may be related to cyanide insensitivity in these organisms. It has been also reported that cyanide detoxification may not be the primary role of rhodanese in insects (Beesley et al. 1985; Saidu 2004). Nevertheless, as above mentioned, all studied helminths had at least some rhodanese activity which suggests its involvement in certain biochemical processes in parasitic helminths.

Examined parasitic helminths are taxonomically diverse and inhabiting various environments, including abomasum (*H. longistipes*, *M. marshalli*), intestine (*M. benedeni*, *H. giardi*) and bile duct (*F. hepatica*, *F. gigantica*, *D. dendriticum*) of the hosts. These habitats exhibit a wide range of physicochemical characteristics. Moreover, the nutritional materials available to helminths in various habitats are different in composition. For instance, the cyanide exposure potential may be considered relatively high in gastrointestinal helminths of ruminants. In these animals, the enzymatic action of microflora of rumen and other parts of the digestive system on cyanogenic glycosides ingested through foodstuff liberates hydrogen cyanide (Wood 1975). However, as the results of the present study showed approximately similar values of rhodanese activity was observed in all sampled helminths. Therefore, habitat, type of feed and environmental conditions does not appear to influence the rhodanese activity of examined parasites.

In conclusion, thiosulphate: cyanide sulphur transferase activity was detected in all seven parasitic species examined, although its activity was much lower in comparison with previously reported values for some tissues of their vertebrate hosts. Additionally, low activities of rhodanese detected in examined helminths might be associated with their cyanide-insensitive respiration. On the other hand, it may be suggested that cyanide detoxification may not be a

primary benefit of rhodanese in parasitic helminths and the true physiological and biochemical functions of the enzyme remains to be further defined in such organisms.

Acknowledgments This work was supported by grant from Ferdowsi University of Mashhad, Mashhad, Iran.

References

- Agboola FK, Okonji RE (2004) Presence of rhodanese in the cytosolic fraction of the fruit bat (*Eidolon helvum*) liver. *J Biochem Mol Biol* 37(3):275–281
- Al-qarawi A, Mousa HM, Ali BH (2001) Tissue and intracellular distribution of rhodanese and mercaptopyruvate sulphotransferases in ruminants and birds. *Vet Res* 32:63–70
- Aminlari M, Gilanpour H (1991) Comparative studies on the distribution of rhodanese in different tissues of domestic animals. *Comp Biochem Physiol B* 99:673–677
- Aminlari M, Gholami S, Vaseghi T, Azadi A, Karimi H (2000) Distribution of rhodanese in different parts of the urogenital systems of sheep at pre- and post-natal stages. *Comp Biochem Physiol B* 27:369–374
- Beesley SG, Compton SG, Jones DA (1985) Rhodanese in insects. *J Chem Ecol* 11:45–50
- Bertran EC (1952) Rhodanese in parasites. *Ann Fac Vet Univ Madr Inst Invest Vet* 4:334–343
- Bordo D, Bork P (2002) The rhodanese/Cdc25 phosphatase superfamily. Sequence–structure–function relation. *EMBO Rep* 3:741–746
- Conn EE (1979) Cyanide and cyanogenic glycosides. In: Rosenthal A, Janzen DH (eds) *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, pp 387–412
- Jones DA (1972) Cyanogenic glycosides and their function. In: Harborne JB (ed) *Phytochemical ecology*. Academic Press, New York, pp 103–124
- Kita K, Hirawake H, Takamiya S (1997) Cytochromes in the respiratory chain of helminth mitochondria. *Int J Parasitol* 27(6):617–630
- Long KY, Brattsten LB (1982) Is rhodanese important in the detoxification of dietary cyanide in southern armyworm (*Spodoptera eridania* Cramer) larvae? *Insect Biochem* 12:367–375
- Marr JJ, Nilsen TW, Komuniecki RW (2003) *Molecular medical parasitology*, 1st edn. Academic Press, London, pp 339–358
- Nagahara N, Ito T, Minam M (1999) Mercaptopyruvate sulphurtransferase as a defence against cyanide toxication: molecular properties and mode of detoxification. *Histol Histopathol* 14: 1277–1286
- Nandi DL, Horowitz PM, Westley J (2000) Rhodanese as thioredoxin oxidase. *Int J Biochem Cell Biol* 32:465–473
- Nazifi S, Aminlari M, Alaibakhsh MA (2003) Distribution of rhodanese in tissues of goat (*Capra hircus*). *Comp Biochem Physiol B* 234:515–518
- Parsons J, Rothschild M (1964) Rhodanese in the larva and pupa of the common blue butterfly (*Polyommatus icarus* Rott.) (Lepidoptera). *Entomol Gaz* 15:58–59
- Raybuck SA (1992) Microbes and microbial enzymes for cyanide degradation. *Biodegradation* 3:3–18
- Saidu Y (2004) Physicochemical features of rhodanese: a review. *Afr J Biotechnol* 3(4):370–374
- Seigler DS (1991) Cyanide and cyanogenic glycosides. In: Rosenthal GA, Berenbaum MR (eds) *Herbivores: their interaction with secondary plant metabolites*. Academic Press, San Diego, pp 35–78

- Smith J, Urbanska KM (1986) Rhodanese activity in *Lotus corniculatus* sensu lato. J Nat Histol 20(6):1467–1476
- Smyth JD, McManus DP (1989) The physiology and biochemistry of cestodes, 1st edn. Cambridge University Press, Cambridge, pp 107–109
- Sorbo BH (1953) Crystalline rhodanese. I. Purification and physico-chemical examination. Acta Chem Scand 7:1129–1136
- Sylvester M, Sander C (1990) Immunohistochemical localization of rhodanese. Histochem J 22:197–200
- Wood JL (1975) Biochemistry of thiocyanic acid. In: Newmann AA (ed) Chemistry and biochemistry of thiocyanic acid and its derivatives. Academic Press, New York, pp 156–221