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Review Article

Biochemical properties and biological functions of the enzyme rhodanese in domestic animals

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Summary

The enzyme rhodanese (thiosulfate: cyanide sulfurtransferase) is a ubiquitous enzyme and its activity is present in all living organisms. Many functions including cyanide detoxification, formation of iron-sulfur centers and participation in energy metabolism have been attributed to this enzyme. The enzyme catalyzes the transfer of a sulfur atom from sulfane containing compounds (such as thiosulfate) to thiophilic anions (such as cyanide). The sulfhydryl group of cysteine-247 in the molecule of rhodanese participates in a double displacement of sulfur transfer mechanism. In this review attempt will be made to summarize the latest information available on the molecular properties and the pattern of distribution of rhodanese in different tissues of domestic animals and to combine these different lines of research to arrive at a plausible explanation regarding the biological function of this important enzyme in living organisms.

Key words: Rhodanese, Active site, Distribution, Domestic animals, Cyanide detoxification

Introduction

In 1933 Lang observed for the first time that rabbits could be protected from the lethal effects of cyanide by injecting thiosulfate. Acting on this information, he prepared an aqueous extract of liver and demonstrated it to catalyze the following reaction:

$S_2O_3^{2-} + CN^- \longrightarrow SCN^- + SO_3^{2-}$

thereby converting toxic CN^- to non-toxic thiocyanate (SCN^-). The enzyme (thiosulfate: cyanide sulfurtransferase (E.C.2.8.1.1)) was named rhodanese by Lang (1933). This name with ese instead of ase (in accordance with enzyme nomenclature) has persisted in the literature. This enzyme was later crystallized from bovine liver (Sorbo, 1953).

This review undertakes on examination of recent literature on rhodanese with particular emphasis on its distribution in tissues of domestic animals. From this information, most of which has been accumulated from the experiments performed in the laboratory of the authors of this review during the past 20 years and the wealth of data available on the active site of this enzyme, a coherent picture suggesting the involvement of rhodanese in cyanide detoxification in living organisms will be presented. Several excellent reviews on rhodanese up to 1973 are available (Roy and Trudinger, 1970; Westley, 1973).

Cyanide

Cyanide (CN^-) is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues of animals (Egekeza and Oehme, 1980; Ballantyne, 1987; McMahon and Birnbaum, 1990; Okalie and Osagie, 2000; ACGIH, 2001; Okfar *et al*., 2002; Sausa *et al*., 2002). This toxicant is widespread in the environment. Many naturally occurring substances as well as industrial products contain cyanide (Egekeza and Oehme, 1980; Chiwona-Karltun *et al*., 2000; Li *et al*., 2000; DECOS, 2002). More than 2000 species of plants are known to contain cyanogenic glycosides which can readily liberate cyanide (Culter and Conn, 1981; Vannesland *et al*., 1982; Majek, 1992;

JECFA, 1993).

It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Burrows and Way, 1977; Keeler *et al*., 1978; Calabrese, 1983). In addition, CN^- is formed as a primary product of the biotransformation of several aliphatic nitriles which are used in the manufacture of synthetic fibers, resins, plastics, pharmaceuticals and vitamins (Frankenberg and Sorbo, 1973; Willhite and Smith, 1981; Silver *et al*., 1982; Isom and Way, 1984; Wiemeyer *et al*., 1986; Dahl and Warsuzewski, 1989; Vick, 1991; Majek, 1992; ATSDR, 1997; Hillman, 2003; Boron and Baud, 2005). The general population may be exposed to cyanide from ambient air, drinking-water and food. There is a rough inverse relationship between sensitivity to hydrogen cyanide and body size, dogs being a notable exception (Barcroft, 1931). Severe dyspnoea has been observed in dogs exposed to 170-740 mg hydrogen cyanide/m^3 for 2-12 min. Pulmonary oedema was found in some dogs at necropsy (Haymaker *et al*., 1952).

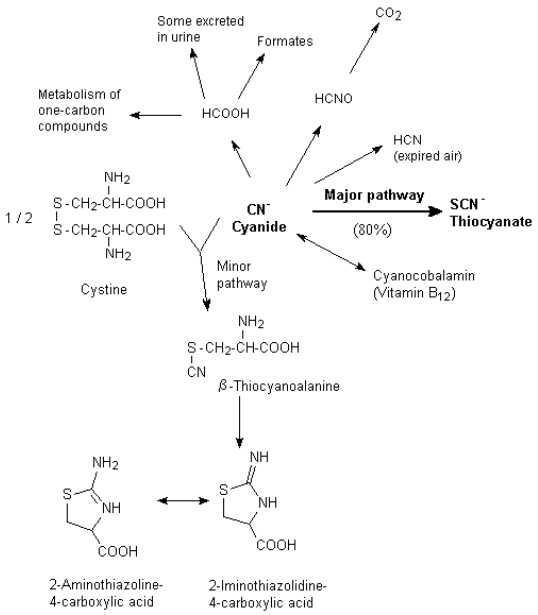

Fig. 1: Basic processes involved in the metabolism of cyanide (McMahon and Birnbaum, 1990; ATSDR, 1997)

Thus, the potential risk of oral or inhalation exposure to cyanide may be very great. Because of widespread natural occurrence of CN^-, it is not surprising that several mechanisms are operative for CN^- detoxification in vivo (Fig. 1) (McMahon and Birnbaum, 1990; ATSDR, 1997). It is believed that the primary detoxification reaction is thiocyanate (SCN^-) formation catalyzed by rhodanese. The classic antidotal combination of sodium nitrite and sodium thiosulfate has been used against cyanide toxicity for near 80 years without change. The therapy is aimed at: (1) formation of methemoglobin by sodium nitrite, which competes with cytochrome oxidase for the binding of CN^- and (2) providing a sulfur donor (thiosulfate) for rhodanese.

Taken together the above discussion suggests a fundamental role for rhodanese in cyanide metabolism. But is it the whole story?

Rhodanese

A. General

In the light of modern knowledge, the specific reactivity of rhodanese can be described as follows: free rhodanese molecule accepts sulfur atom from sulfur containing anions; sulfur-substituted rhodanese then reacts to transfer sulfur atom to strongly thiophilic anions (hence rhodanese is in fact a sulfurtransferase). This reaction can be summarized as shown in Fig. 2, where sulfur compound is thiosulfate ($S_2O_3^{2-}$) and thiophilic anion is cyanide. Many other compounds have been reported to act as sulfur donor and acceptor (Westley, 1973). A number of other sulfur transferases

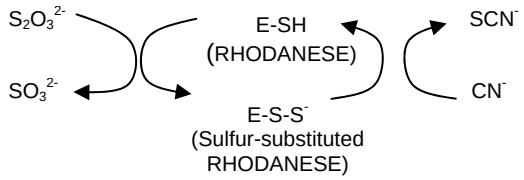

Fig. 2: Rhodanese-catalyzed reaction

can also metabolize cyanide and albumin, which carries elemental sulfur in the body in the sulfane form, can assist in the catalysis of cyanide to thiocyanate as well (Sylvester *et al*., 1982; Westley *et al*., 1983). Cyanide and thiocyanate can also be metabolized by several minor routes, including the combination of cyanide with hydroxy-

cobalamin (vitamin $B_{12}a$) to yield cyanocobalamin (vitamin B_{12}) (Boxer and Rickards, 1952) and the non-enzymatic combination of cyanide with cystine, forming 2-iminothiazoline-4-carboxylic acid, which appears to be excreted without further change (Rieders, 1971) (Fig. 1).

B. Assaying rhodanese activity

Many different methods are available to detect and assay rhodanese. The most commonly used assay is based on the colorimetric determination of ferric thiocyanate formed when enzymatically produced thiocyanate reacts with ferric nitrate in the presence of formaldehyde (Sorbo, 1953; Aminlari *et al*., 2002). Several other methods are available which use different aspects of the enzyme reactivity, including a polarographic procedure, colorimetric determination of sulfite or visualization of precipitation on polyacrylamide gel after electrophoresis (Westley, 1973). A method based on the reduction of tetrazolium for determination of rhodanese activity has been reported (Canella *et al*., 1984). Singleton and Smith (1988) reported on an improved assay for rhodanese in *thiobacillus spp*. based on inclusion of a separate boiled control in the conventional rhodanese assay which corrects for the non-biological contribution to thiocyanate formation. Currently, a new enzyme-coupled assay is being developed in our laboratory which utilizes lacto-peroxidase to oxidize thiocyanate with concomitant reduction of hydrogen peroxide. The remaining, unreacted hydrogenperoxide can be reduced by peroxidase in the presence of a chromogenic substrate (unpublished data).

C. Purification

Most of the studies performed on rhodanese have been on bovine liver rhodanese which can be easily purified from this source. Sorbo (1953) accomplished purification to crystallinity. Purification steps involved basic lead acetate ammonium sulfate fractionation at low and high pH's and acetone fractionation. Advancement in purification by Horowitz and DeToma (1970) has led to a high yield procedure routinely purifying 600-800 mg crystalline rhodanese (half of the total amount originally present in the liver homogenate) from 10 lb (4500 g) bovine liver. This provides sufficient material for further studies on the mechanism of action and other properties of this enzyme. Kurzban and Horowitz (1991) reported on purification of rhodanese by low pH chromatography.

D. Molecular structure and mechanism of the reaction

Amino acid composition and the covalent and three-dimensional structure of bovine liver rhodanese has been determined (Sorbo, 1963; Russell *et al*., 1978; Ploegman *et al*., 1978a; Saidu, 2004). The molecule consists of a single polypeptide chain of 293 residues. It is folded into an ellipsoid of 60 °A × 50 °A × 40 °A dimensions with two domains which are of remarkably similar structure and are related by a pseudo 2-fold axis. It appears that the similarity in conformation of domains is virtually not reflected in the amino acid sequence. (Ploegman *et al*., 1978b; Ploegman *et al*., 1979). The properties of the active site has been studied (Wang and Volini, 1968; Weng *et al*., 1978). The active site is made of a pocket which contains the essential sulfhydryl group of cysteine-247, two positive charges furnished by lysine-249 and arginine-186 and several hydrophobic residues lining up and forming one side of the wall of the active site. The extra sulfur atom is bound in persulfide linkage to the essential S^{γ} atom of the essential cysteine-247. (Horowitz and Westley, 1970; Gliubich *et al*., 1996).

On the basis of several kinetic, thermodynamic, isotope exchange, polarographic, X-ray crystallographic and other studies, a well defined picture of the catalytic mechanism for rhodanese has emerged (Green and Westley, 1961; Westley and Nakamato, 1962; Schwelsinger and Westley, 1974; Ploegman *et al*., 1979; Horowitz and Crisamagna, 1983; Chaw *et al*., 1985; Aird and Horowitz, 1988; Aminlari, 1995; Gliubich *et al*., 1996; Ansuri and Horowitz, 2000; Aminlari and Zohrabi, 2003). The positively charged

residues arginine-186 and lysine-249 attract the negatively charged sulfur donor molecule (e. g. thiosulfate). The orientation of the substrate in the active site is such that it is positioned to the proximity of the active site sulfhydryl group. The interaction of positive charges at the active site with oxygen of the substrate has a considerable polarizing effect on the S-S bond of the substrate (Figs. 3a and b). This will decrease the negative charge on the outer sulfur atom and would facilitate the nucleophilic attack by S atom of cysteine-247 (Fig. 3c). The next step in the catalytic process is the breaking of the S-S bond in the substrate and the formation of S-S bond in the rhodanese-sulfur complex (Figs. 3d and e). The product, sulfite, leaves the active site. The extra sulfur atom in the rhodanese-sulfur complex can be removed by several sulfur acceptors such as cyanide, sulfonates and thiol groups (Westley, 1973; Sorbo, 1975). The reaction mechanism appears to follow a double displacement mechanism with the formation of an intermediate enzyme-sulfur complex ($E\text{-}SS^-$):

$E\text{-}S^- + S\text{-}SO_3^{2-} \longleftrightarrow E\text{-}S\text{-}S^- + SO_3^{2-}$

$E\text{-}S\text{-}S^- + CN^- \longleftrightarrow E\text{-}S^- + SCN^-$

By application of fluorescent probes, it has been shown that rhodanese experiences some degree of conformational change when it cycles between the sulfur-free and sulfur-bound forms (Wasyleewski and Horowitz, 1982; Koloczek and Vanderkoohi, 1987; Horowitz and Crisamagna, 1988). Reaction of the active site sulfhydryl group apparently triggers a conformational change leading to increased protein flexibility and increased exposure of hydrophobic surface. More recent studies have explored the active site properties of rhodanese by employing different disulfides (Aminlari, 1995). The results showed that the -SH groups of rhodanese have a higher affinity for the disulfides containing positive charge (e. g. cystamine and cystine) than uncharged or negatively charged disulfides, suggesting presence of anionic groups in or in the vicinity of the active site which facilitate binding of cationic disulfides. These results were further taken to imply that these cationic disulfides might indeed be the true physiological substrates of rhodanese, thereby defining a novel biological role for rhodanese (Aminlari and Zohrabi, 2003) (see below).

E. Biological distribution

Since the initial work on rhodanese (Lang, 1933), prompted originally by the earlier finding of cyanide detoxification in vivo, an enormous variety of biological sources has been explored for this activity. In general, every source that has been examined closely has been reported at some time or other to have at least some rhodanese activity. The enzyme appears to

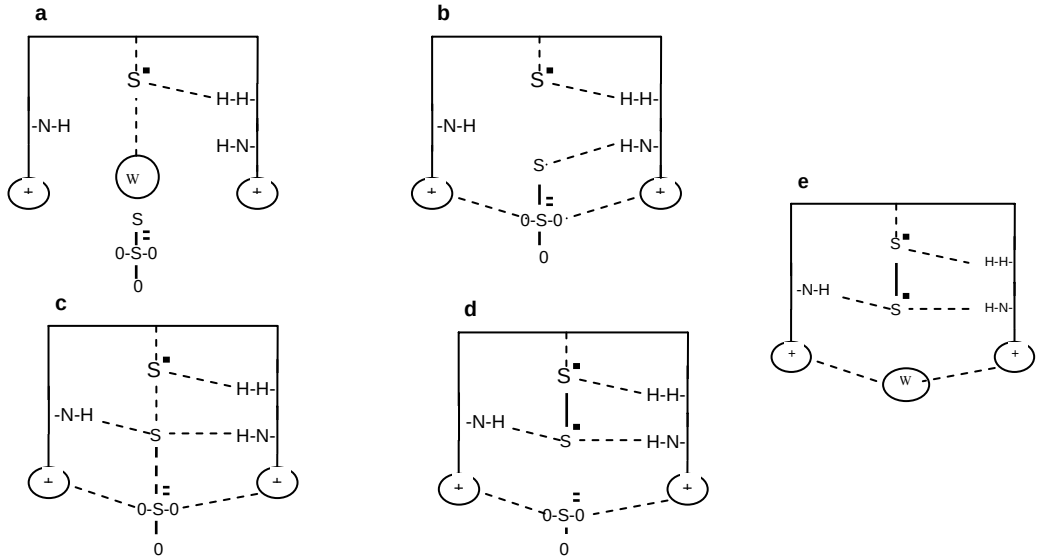

Fig. 3: Schematic presentation of the catalysis of sulfur transfer by rhodanese (from Ploegman *et al.*, 1979)

be ubiquitous, present in all living organisms, from bacteria to man (Alexander and Volini, 1967; Westley, 1973; Wood, 1975; Vandenberg and Berk, 1980; Mimori *et al*., 1984; Drawbaugh and Marrs, 1987; Sylvester and Sander, 1990; Lewis *et al*., 1992a; Aita *et al*., 1997; Hatzfeld and Saito, 2000; Aminlari *et al*., 2002; Nazifi *et al*., 2003). However, there is a significant degree of controversy with regard to the pattern of distribution of rhodanese in living organisms (Westley, 1973). Part of this discrepancy is probably the result of real biological variability, much of it, however, may be related instead to the extreme sensitivity of the thiocyanate-based assay methods and the ubiquitous occurrence of non-specific catalysis for the thiosulfate-cyanide reaction (Sorbo, 1957; Westley and Heyes, 1971). In any event, experienced investigators in this field have learned to regard with a great deal of caution all reports of the minor content in any new source.

Although extensive reports on the distribution of rhodanese in microorganisms, plants and animals is available (see for example the review of Westley, 1973), this review is confined to the pattern of distribution of rhodanese in domestic animals. The original work by Lang (1933) and many subsequent studies have fundamentally confirmed that in most domestic animals, liver, followed by kidney are the richest source of rhodanese. However, our finding since late 1980s have revealed that other organs of animals contain even higher rhodanese activity than liver (Table 1) (Aminlari *et al*., 1989; Animlari and Gilanpour, 1991; Aminlari and Shahbazi, 1994; Aminlari, *et al*., 1994; Aminlari *et al*., 1997; Gilanpour *et al*., 1997; Aminlari *et al*., 2000; Al-Qarawi *et al*., 2001; Aminlari *et al*., 2002; Karimi and Aminlari, 2003; Karimi *et al*., 2003; Nazifi *et al*., 2003). In all species studied rhodanese is present, albeit in different amounts. However, the pattern of distribution in different tissues is extensively different and appears to be species specific. In ruminants, liver is the richest source of this enzyme. In cattle, pig, horse and donkey, liver is followed by kidney in the level of

Table 1: Rhodanese activity (Units/mg protein) in different tissues of adult domestic animals

Tissues	Cattle	Sheep	Goat	Camel	Horse	Donkey	Pig	Dog
Liver	4.95	5.21	2.90	1.7	1.7	1.5	0.56	0.18
Rumen	0.90	4.25	4.16	0.81	-	-	-	-
Kidney	3.45	3.81	ND	0.08	1.40	1.47	ND	0.06
Cortex	ND	1.7	0.94	ND	ND	ND	0.44	ND
Medulla	ND	0.24	0.24	ND	ND	ND	0.14	ND
Brain	0.36	0.48	0.47	0.13	0.15	0.17	ND	0.06
Lung	0.13	0.25	0.36	0.24	0.03	0.05	0.04	0.02
Heart								
Ventricle	0.13	0.15	0.34	0.1	0.12	0.12	0.12	0.05
Atrium	0.12	0.13	0.38	0.03	0.11	0.07	0.07	0.03
Omasum	1.29	4.45	2.99	-	-	-	-	-
Reticulum	0.8	2.20	3.31	0.05	-	-	-	-
Skeletal Muscle	0.02	0.04	0.08	0.05	0.15	0.12	0.08	0.04
Abomasum								
Fundus	0.06	0.1	0.35	0.04	-	-	-	-
Pylore	0.04	0.02	0.31	0.01	-	-	-	-
Lymph Node	0.02	0.07	0.07	0.02	ND	ND	ND	ND
Spleen	0.01	0.02	0.04	0.01	ND	ND	0.02	ND
Duodenum	0.04	0.1	ND	0.01	0.11	0.07	ND	0.05
Ileum	0.04	0.05	ND	0.02	0.06	0.08	ND	0.04
Jejunum	0.06	0.1	ND	0.02	0.06	0.08	ND	0.04
Colon	0.08	0.11	0.03	0.06	0.06	ND	ND	0.2
Cecum	0.07	0.09	ND	0.02	0.04	0.06	ND	0.16
Rectum	0.03	0.12	ND	0.08	0.06	0.05	ND	0.13

ND: Not determined; (from Aminlari *et al*., 1989; Aminlari *et al*., 2002; Nazifi *et al*., 2003)

rhodanese. In sheep the order is liver and rumen and kidney, in camel liver and rumen, in goat rumen and liver and in dog brain followed by liver. The rhodanese activity is significantly higher in the most tissues of sheep than the other animals (Aminlari and Gilanpour, 1991; Aminlari *et al*., 2000; Nazifi *et al*., 2003) (Table 1).

Table 2 shows the activity of rhodanese in the epithelial and muscular layers of different regions of digestive system. The epithelium of rumen, omasum and reticulum of cattle, sheep and goat have exceptionally high rhodanese activity which far exceeds those of liver and kidney. Only rumen epithelium of camel shows some rhodanese activity, the level of which is only 4% of that found in rumen epithelium of sheep. The terminal sections of the digestive tract of sheep, goat and cattle have rhodanese activity greater than the other species (Aminlari and Gilanpour, 1991; Aminlari *et al*., 2002; Nazifi *et al*., 2003). In equine, some parts of the stomach and duodenum

Table 2: Rhodanese activity (Units/mg protein) in different parts of the digestive systems of adult domestic animals

Tissues	Cattle	Sheep	Goat	Camel	Horse	Donkey	Dog
Muscular layer							
Stomach							
Rumen	0.05	0.09	0.12	0.01	-	-	-
Omasum	0.05	0.09	0.11	0.03	-	-	-
Reticulum	0.04	0.08	0.18	0.01	-	-	-
Abomasum							
Fundus	0.04	0.08	0.21	0.01	-	-	-
Pylore	0.03	0.07	0.08	0.01	-	-	-
Cardiac region	-	-	-	-	-	-	0.05
Pyloric region	-	-	-	-	-	-	0.04
Glandular region	-	-	-	-	0.04	0.05	-
Preventricular	-	-	-	-	0.04	0.05	-
Duodenum	0.03	0.02	ND	0.01	0.05	0.04	0.03
Ileum	0.06	0.05	ND	0.02	0.05	0.04	0.03
Jejunum	0.05	0.05	ND	0.02	0.04	0.07	0.03
Colon	0.03	0.05	ND	0.02	0.04	0.03	0.06
Cecum	0.04	0.06	ND	0.01	0.04	0.04	0.05
Rectum	0.05	0.05	ND	0.01	0.05	0.03	0.05
Epithelial layer							
Stomach							
Rumen	0.28	16.3	8.9	10.3	-	-	-
Omasum	-	7.65	5.8	6.1	-	-	-
Reticulum	0.07	6.13	3.7	4.9	-	-	-
Abomasum					-	-	-
Fundus	0.07	0.09	0.51	0.04	-	-	-
Pylore	0.02	0.09	0.31	0.04	-	-	-
Cardiac region	-	-	-	-	-	-	0.05
Pyloric region	-	-	-	-	-	-	0.07
Glandular region	-	-	-	-	0.17	0.11	-
Preventricular	-	-	-	0.08	0.10	0.07	-
Duodenum	0.03	0.11	ND	0.09	0.17	0.14	0.04
Ileum	0.02	0.09	ND	0.10	0.07	0.05	0.04
Jejunum	0.04	0.14	ND	0.08	0.08	0.05	0.05
Colon	0.04	0.15	ND	0.10	0.06	0.05	0.10
Cecum	0.02	0.09	ND	0.09	0.04	0.04	0.13
Rectum	0.02	0.10	ND		0.04	0.06	0.11

ND: Not determined; (from Aminlari *et al*., 1989; Nazifi *et al*., 2003)

have significantly higher levels of the enzyme than the other parts. The epithelial layers of colon, cecum and rectum in dog have higher levels of rhodanese than the other parts (Table 2).

Studies on the pattern of distribution of rhodanese in different parts of the respiratory systems of sheep and dog revealed existence of high rhodanese activity in the nasal cavity in dog and in lower respiratory tract and lungs in sheep (Aminlari *et al*., 1994) (Table 3). These activities are however significantly lower than those found in liver and kidney. The rhodanese activity is also present in different sections of the urogenital systems of sheep and cattle (Aminlari *et al*., 2002; Karimi and Aminlari, 2003; Karimi *et al*., 2003). Kidney cortex, followed by medulla and prostate in males and ureter and oviduct in females are the richest source of rhodanese in these systems. The rhodanese activity in all parts of urogenital system increases with the age of embryo and reaches the highest value in adult animals (Khashayar, 1995; Aminlari *et al*., 2000) (Table 4).

Table 3: Rhodanese activity (Units/mg protein) in different parts of the respiratory system of dog and sheep

	Dog		Sheep	
Tissues	Whole tissue	Mucosa	Whole tissue	Mucosa
Vestibule	0.05	0.07	0.04	0.05
Respiratory of nasal cavity	0.08	0.15	0.07	0.07
Olfactory of nasal cavity	0.14	0.20	0.06	0.06
Nasopharynx	0.10	0.09	0.04	0.04
Common pharynxs	0.06	0.06	0.02	0.03
Larynx trachea	0.06	0.06	0.13	0.18
Carnial	0.05	0.06	0.13	0.18
Middle	0.05	0.05	0.13	0.18
Caudal	0.05	0.05	0.12	0.18
Bronchiole lung left lobe	0.03	0.03	0.11	0.17
Carnial	0.02		0.12	
Caudal right lobe	0.03		0.13	
Carnial	0.03		0.12	
Caudal	0.03		0.12	

(from Aminlari *et al*., 1994)

Rhodanese activity was studied in thymus, spleen, bone marrow and lymph nodes of sheep and rabbit (Gilanpour *et al*., 1997). In all sections studied, rabbit showed to have more rhodanese activity than sheep. In both species the highest activity was present in bone marrow.

Rhodanese distribution in nervous system of animals has been studied. The activity was widely distributed in all areas of nervous tissues of human and rat (Mimori *et al*., 1984). In rat, the olfactory bulb showed the highest rhodanese activity and significant activity was also found in thalamus, septum, hippocampus and dorsal part of the mid brain, while activity was low in various parts of cerebral cortex. The rhodanese distribution in post-mortem human brain was essentially the same as rat. In sheep the highest activity was found in medulla oblongata and the lowest level was in the spinal cord sacral white matter (Khameneh Bagheri, 1993).

Distribution of rhodanese in avian species is interesting. In domestic fowl, the highest activity was found in kidney, approximately twice than that in liver (OH *et al*., 1977). However, Aminlari and Shahbazi (1994) and Aminlari *et al*., (1997) showed that the level of rhodanese in chicken is age dependent such that during embryonic stage of development liver is the major source of rhodanese but in adults the proventriculus is the richest source. Furthermore, the level of activity is almost twice in the submucosal layer of proventriculus than that in the liver (0.59 and 0.31 units /mg protein, respectively).

Data on pattern of distribution of rhodanese in human tissue is scant. Jarbak and Westley (1974) studied human liver rhodanese and showed that it differed from bovine liver rhodanese in kinetic behaviour and UV absorption properties. Genetic polymorphism of rhodanese in human erythrocytes has been reported (Scott and Wright, 1980). Whitehouse *et al*., (1988) demonstrated that red blood cell and tissue rhodanese are determined by separate genes, but more than one locus may be involved in synthesis of heterogeneous tissue iso-enzymes.

Conflicting results regarding the changes in the level of liver rhodanese in patients with Leber optic atrophy is found in the literature (Cagiaunt *et al*., 1981; Nikoskelarinen, 1984). Human rhodanese from normal gastric mucosa was studied and

Table 4: Rhodanese activity (Units/mg protein) in different parts of the urogenital systems of male and female embryo and adult sheep

Tissues	Age of embryo (months)						
	2.5	3.0	3.5	4.0	4.5	5	Adult
Vesicular glands	0.032	0.034	0.018	0.050	0.044	0.056	0.020
Cowpers glands	0.022	0.026	0.026	0.028	0.040	0.046	0.080
Prostate gland	0.022	0.022	0.026	0.036	0.030	0.070	0.160
Epididymis	0.034	0.022	0.020	0.032	0.020	0.020	0.060
Testis	0.0400	0.048	0.030	0.042	0.052	0.072	0.050
Ductus deferens	0.018	0.017	0.018	0.018	0.024	0.034	0.020
Penis	0.020	0.020	0.014	0.022	0.052	0.030	0.030
Urinary Bladder							
Male	0.010	0.010	0.018	0.080	0.018	0.020	0.090
Female	0.034	0.032	0.040	0.026	0.022	0.035	0.090
Ureter							
Male	0.024	0.026	0.022	0.040	0.032	0.054	0.110
Female	0.024	0.034	0.028	0.034	0.016	0.037	0.110
Kidney Medulla							
Male	0.122	0.120	0.098	0.126	0.074	0.138	0.240
Female	0.066	0.098	0.048	0.108	0.062	0.194	0.240
Kidney cortex							
Male	0.142	0.220	0.180	0.230	0.170	0.300	1.700
Female	0.180	0.160	0.19	0.136	0.187	0.220	1.18
Urethra							
Male	0.020	0.034	0.032	0.038	0.030	0.034	0.050
Female	0.030	0.028	0.032	0.036	0.020	0.036	0.020
Ovary	0.060	0.050	0.068	0.068	0.042	0.048	0.050
Oviduct	0.046	0.034	0.094	0.116	0.116	0.222	0.190
Uterus	0.034	0.034	0.042	0.044	0.090	0.040	0.050
Cervix	0.038	0.050	0.047	0.058	0.047	0.078	0.070
Vagina	0.03	0.020	0.062	0.046	0.046	0.070	0.010

(from Aminlari *et al*., 2000)

found to be kinetically different from that of gastric adenocarcinoma (Malliopoulou *et al*., 1989). Our preliminary studies on the samples taken from humans 2-12 hrs post-mortem have showed that kidney and liver, in decreasing order are the richest sources of rhodanese (unpublished data).

F. Genetics

The gene encoding rhodanese in *Azetobacter vinelandii* has been cloned and shown to have sequence similarity to prokaryote rhodanese but not to eukaryote rhodanese (Colnaghi *et al*., 1996). A cDNA for rat liver rhodanese was cloned and sequenced and characterized (Weiland and Dooley, 1991). It was shown that rat liver rhodanese has 91% homology with bovine rhodanese. Furthermore, it was indicated that rhodanese is highly conserved at the DNA level among rodents, primates and other vertebrates.

A recombinant bovine rhodanese was produced in *E. coli* (Miller *et al*., 1992). While electrophoretic, proteolytic susceptibility, intrinsic fluorescence or specific enzyme activity of the recombinant rhodanese was identical to native rhodanese purified from bovine liver, evidence was provided to indicate that immunologically, these two forms of rhodanese have different conformation. These studies are useful in studying structure-function relationship of proteins (Merill *et al*., 1992). Aita *et al*., (1997) cloned a human rhodanese cDNA and showed that human rhodanese gene was 90% identical to rodents and bovine rhodanese, while only 70% homologous to avian rhodanese.

G. Biological functions

More than 70 years after the discovery of rhodanese by Lang (1933), despite extensive biochemical and physiological studies, its true biological function is still an enigma. As showed above, rhodanese

activity is ubiquitous in nature, suggesting an important physiological role. Besides cyanide detoxification, rhodanese is believed to perform other functions, including formation of iron-sulfur center in proteins (Pagani *et al*., 1982; Cerletti, 1986; Bonomi *et al*., 1997), participation in energy metabolism (Ogata *et al*., 1989; Ogata and Volini, 1990) and functioning as a thioredoxin oxides (Nandi *et al*., 2000). Among all speculative roles, cyanide metabolizing function has been the subject of extensive investigation during last several decades (Lang, 1933; Himwich and Saunders, 1948; Koj and Frendo, 1962; Dudeck *et al*., 1980; Cerletti, 1986; Aminlari and Gilanpour, 1991; Lewis *et al*., 1992b; Aminlari and Zohrabi, 2003).

It has been suggested that the level of rhodanese in different tissues of animals is correlated with the level of exposure to cyanide (Calabrese, 1983; Lewis *et al*., 1992a; Aminlari *et al*., 2002). As discussed earlier, in the most animals studied, liver is the richest source of rhodanese, therefore, a heavy cyanide metabolizing function is performed by this organ. However, the extensive variation in the distribution of rhodanese in different tissues of animals, which in some cases exceed that of liver, indicates that other organs are also involved in cyanide detoxification in a species-specific manner. Thus, in ruminants and birds the epithelial layer of different parts of the digestive system contains higher activity of rhodanese than liver. 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). Epithelium of stomach in these animals is the first tissue that is in direct contact with the released cyanide, hence, there is a greater activity of rhodanese in these tissues compared with others. Any cyanide escaping this tissue will further be metabolized by hepatic rhodanese. In camel, only rumen epithelium has some rhodanese activity, the level of which is about 5% that of sheep liver (Aminlari and Gilanpour, 1991). In horse, pig and carnivorous animals little cyanide detoxification occurs in the stomach and the absorbed cyanide is detoxified by rhodanese in the liver. Rhodanese activity in the epithelial layer of different parts of the stomach of equines has much less significant levels than that found in ruminants. The low pH of the stomach in monogastric animals probably does not provide a suitable environment for rhodanese. The cyanide that might be produced in the stomach of these animals due to the action of acid on cyanogenic plants is absorbed in the intestine and part of it is probably metabolized by hepatic rhodanese (Aminlari and Gilanpour, 1991). The presence of rhodanese activity in the epithelium of some parts of the large intestine of animals might be due to detoxification of cyanide produced by microbial activities in the large intestine (Aminlari and Gilanpour, 1991).

In dog, a high rhodanese activity is localized in the epithelial layer of different regions of nasal cavity. (Aminlari *et al*., 1994). As a carnivorous animal, dog is not exposed to cyanogenic glycosides, hence, has low rhodanese activity in liver. Rather, the localized rhodanese in nasal cavity metabolizes inhaled cyanide gas. These observations can lead to the conclusion that rhodanese functions in cyanide metabolism in those tissues which are apparently in direct contact with ingested or inhaled cyanide. However, the pattern of distribution of rhodanese found in animals studied and the in vivo (Aminlari and Zohrabi, 2003) as well as in vitro (Aminlari, 1995) studies performed in this laboratory and others (Cerletti, 1986; Ogata and Volini, 1986; Ogata *et al*., 1989*;* Ogata and Volini, 1990; Polo *et al*., 1992) suggest that other biochemical functions might be attributed to rhodanese that are more important than mere cyanide detoxification and that the latter function might be a simple coincidence. As will be discussed below, data on rhodanese content of different tissues, particularly of urogenital system of animals might be related to two aspects of rhodanese function.

The quantities of cyanide present or produced in tissues of animals, or the degree to which these tissues are exposed to cyanide is inconsistent with the large amount of rhodanese activities observed in some tissues (such as kidney cortex) (Ogata *et al*., 1989; Aminlari *et al*., 2000; Aminlari *et al*., 2002). In recent years, evidence has

been accumulated which indicate that the sulfur transferred by rhodanese activates enzyme of oxidative metabolism, such as NADH dehydrogenase (Pagani and Galante, 1983) and succinate dehydrogenase (Bonomi *et al*., 1997). With these enzymes as substrates, rhodanese functions as protein sulfurase. All of these effects arise because of rhodanese interactions with non-heme iron-sulfur center in catalytic proteins (Cerletti, 1986). Based on these studies, it has been suggested that the reversible sulfur transfer to and from rhodanese is an important feature of the role of this enzyme played in energy metabolism. The observation that bovine mitochondrial rhodanese is a phosphoprotein led Ogata *et al*., (1989) to propose a model in which transduction and amplification of cellular signals is altered by covalent phosphorylation of rhodanese. In this model, rhodanese participates in controlling the rate of respiratory chain and ATP production by the reversible sulfuration of key iron-sulfur centers.

The rhodanese activity of the urogenital system of sheep increases with the age of fetus and in adult animals rhodanese activity is significantly higher than those at fetal ages. In addition, highest rhodanese activity is observed in kidney cortex followed by kidney medulla. Based on above discussion, the pattern of rhodanese distribution in urogenital system of animals can be regarded from two stand points. First, it has been reported that in human, more than 25% of cardiac output is allocated to kidney cortex, which is far more than kidney medulla (4-5 ml/min/g tissue vs 0.03-0.2 ml/min/g) (Ganong, 1985). Furthermore, the flow of blood is from cortex to medulla. These facts might explain the pattern of rhodanese distribution in the urinary system. It is speculated that since the kidney cortex is the first site which is exposed to cyanide delivered by blood, a higher rhodanese activity is present in this region. Any cyanide escaping this region is further metabolized by rhodanese activity in medulla and other parts. As fetus grows older, the supply of blood to all parts is increased hence, a higher rhodanese activity is maintained. Second, in most mammals and birds, rhodanese is essentially a mitochondrial enzyme (Dudeck *et al*., 1980). The proximal convoluted tubules which are mainly located in kidney cortex contain many mitochondria (Weather *et al*., 1990). Alternatively, a transient increase in rhodanese activity in certain organs at certain developmental stages and different distribution in each tissue suggest that rhodanese might play a special metabolic role other than cyanide detoxification. Further studies are needed to confirm and ascribe either of these roles to rhodanese activity observed in this study. Furthermore, we have shown that it is possible to block the reaction of rhodanese using disulfides which have a high affinity for rhodanese active site (Aminlari, 1995) and this reaction in vivo results in increased lethality in mice exposed to cyanide (Fig. 4, Aminlari and Zohrabi, 2003). These results further prove the major physiological function of rhodanese in cyanide detoxification. Drawbaugh and Marrs (1987) studied the relationship between rhodanese activity and sensitivity to cyanide toxicity in few species of animals. Based on their study and previous studies these authors concluded that dog is the most sensitive species to cyanide toxicity due to low level of rhodanese in its liver.

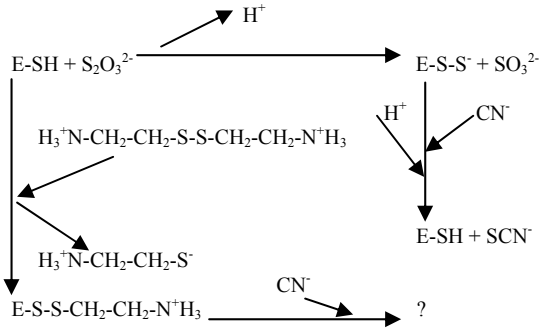

Fig. 4: Proposed mechanism for the reaction of rhodanese (E-SH) with cationic disulfide (cystamine) and its effect on cyanide detoxification (from Aminlari and Zohrabi, 2003)

Taken together, data on the distribution as well as molecular studies on rhodanese suggest the existence of a correlation between rhodanese content and sensitivity to cyanide toxicity. The level of sensitivity might, therefore, be expected to be in decreasing order: sheep, cattle, camel, horse, pig and dog. In spite of extensive studies on

the molecular properties of rhodanese from human heart and liver (Jarbak and Westley, 1974; Wood, 1975), little is known about the pattern of distribution of rhodanese in human tissues. This information will be necessary to assess human sensitivity to cyanide as compared to other animals. In view of above discussions, it is interesting to follow several report in which attempt has been made to antagonize cyanide intoxication by applying rhodanese (Frankenberg, 1980) and rhodanese encapsulation in mouse carrier erythrocytes (Leung *et al*., 1991a; Leung *et al*., 1991b; Cannon *et al*., 1994). Provision of a reduced environment for rhodanese might be necessary to treat cyanide toxicity in animals (Aminlari and Zohrabi, 2003). Intra-peritoneal administration of rhodanese along with sodium thiosulfates reduced mortality of mice exposed to injected potassium cyanide (Najarsadeghi, 1997). Further research is needed to evaluate the efficacy of enzyme therapy by rhodanese in cyanide toxicity.

Conclusions

Rhodanese is a single chain polypeptide widely distributed in nature. It is involved in a double displacement mechanism of enzymatic sulfur transfer to cyanide. The pattern of distribution in different tissues of animals suggests that rhodanese performs a cyanide detoxification function. Further research is required to substantiate this function or shed light on other functions of this interesting enzyme.

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