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ORIGINAL ARTICLE

Comparison of rhodanese distribution in different tissues of Japanese quail, partridge, and pigeon

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Abstract Rhodanese (thiosulfate: cyanide sulfurtransferase, EC. 2.8.1.1) is a ubiquitous enzyme present in all living organisms, from bacteria to humans, and plays a central role in cyanide detoxification. The purpose of this investigation was to determine and compare the pattern of distribution of rhodanese in different tissues of adult Japanese quail, partridge, and pigeon. In the three species studied, rhodanese was present in all tissues albeit in different amounts. The highest activity of rhodanese in Japanese quail was observed in kidney and proventriculus followed by liver and heart. In partridge, the highest activity of the enzyme was observed in heart, kidney, and proventriculus. In pigeon, the highest activity of the enzyme was observed in liver, kidney, and heart. Inter-species differences were seen in the level of enzyme in different tissues. The results of this study may indicate the involvement of rhodanese in cyanide detoxification in tissues that have greater potential to be exposed to higher levels of cyanide and also involvement of rhodanese in functions other than cyanide detoxification in other tissues.

Keywords Rhodanese · Cyanide detoxification · Japanese quail · Partridge · Pigeon

Introduction

Cyanide is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues (Egekeze and Oehme 1980). This toxicant is widespread in the environment. Many naturally occurring substances as well as industrial products contain cyanide (Egekeze and Oehme 1980). More than 2,000 species of plants are known to contain cyanogenic glycosides (Vennesland et al. 1982). It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Keeler et al. 1978). Many studies report the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants (Wiemeyer et al. 1986).

The enzyme rhodanese (EC. 2.8.1.1., thiosulfate: cyanide sulfurtransferase) is a ubiquitous enzyme that is known to be responsible for biotransformation of cyanide to thiocyanate (Westley 1973). It is believed that rhodanese is involved in cyanide detoxification (Lang 1933; Himwich and Saunders 1948; Koj and Frendo 1962; Wood 1975; Drawbaugh and Marrs 1987; Aminlari et al. 2000). However, there is some evidence that the enzyme may be involved in other functions, including formation of iron sulfur centers (Cerletti 1986), participation in energy metabolism (Ogata et al. 1989; Bonomi et al. 1997), and function as a thioredoxin oxidase (Nandi et al. 2000).

The presence of rhodanese has been detected in many tissues of animals (Dudeck et al. 1980; Westley 1981; Drawbaugh and Marrs 1987; Aminlari and Gilanpour 1991; Aminlari and Shahbazi 1994; Aminlari et al. 1994, 2000, 2002; Al-qarawi et al. 2001; Agbola et al. 2006), but to our knowledge there is no information about the activity of rhodanese in Japanese quail and partridge tissues and little information about tissue distribution of this enzyme in pigeon. The aim of this investigation was to determine and compare the pattern of distribution of rhodanese in different tissues of adult Japanese quail, partridge, and pigeon. The results of this study will be

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discussed in terms of possible role of rhodanese in cyanide detoxification in these tissues.

Materials and methods

Healthy adult Japanese quails (*Coturnix japonica*), partridges (*Alectoris chukar*), and pigeons (*Columbia domesticus*) were bought from a local market at Shiraz, Iran. Birds were killed and then dissected. Samples of each tissue were separated, stripped from fat, washed a few times with physiological saline, and then blotted. Tissue extracts were prepared by freezing the samples in liquid nitrogen, homogenizing with a hand homogenizer, and suspending the homogenates in 0.025 M sodium phosphate, pH 7.2. The suspensions were centrifuged for 15 min at 4,000 ×*g* and supernatants were used as the source of enzyme. Rhodanese was assayed by the modified method of Sorbo (1953). Reaction mixture contained 16.8 mM sodium thiosulfate, 40 mM glycine buffer, pH 9.2, 167 mM KCN, and 50 μl enzyme solution in a final volume of 4.0 ml. The reaction was carried out for 15 min at 37°C and stopped by adding 0.5 ml 38% formaldehyde. In control tubes, formaldehyde was added prior to the addition of enzyme solution. Concentration of thiocyanate was determined as follows (Sorbo 1953): Samples were mixed with 1 ml ferric nitrate solution containing 0.025 g $Fe(NO_3)_3{\cdot}9H_2O$ in 0.74 ml water and 0.26 ml concentrated nitric acid. After centrifuging the mixture to remove the interfering turbidity, the absorbencies were measured at 460 nm against a blank containing all reagents except that 50 μl water was used instead of enzyme solution. Concentration of thiocyanate formed was obtained from a standard curve produced by treating solutions containing different concentrations of thiocyanate as described above. In this study, pH 9.2 was used instead of 7.4 (which is routinely used) to decrease the turbidity of solutions after addition of ferric nitrate. No significant difference was observed when purified rhodanese was assayed at pH 7.4 or 9.2 (Aminlari 1995). The unit of enzyme activity was defined as micromole of thiocyanate formed per min at pH 9.2 and 37°C. Rhodanese activity is reported as specific activity (U/mg protein). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. The results are reported in terms as units per milligram protein. All data were statistically analyzed by ANOVA (SPSS/PC$^+$) software and the mean comparison was carried out using DMRT ($P<0.05$).

Results

The specific activity of rhodanese in crude extracts from Japanese quails, partridges, and pigeons is shown in Table 1. In the three species studied, rhodanese is present in all tissues albeit in different amounts. Activities of rhodanese in different tissues of Japanese quail, partridge, and pigeon range 0.017–0.212, 0.033–0.312, and 0.059–0.301, respectively. The highest activity of rhodanese in Japanese quail is observed in kidney and proventriculus followed by liver and heart while the lowest activity is present in trachea. In partridge, the highest activity is present in heart, kidney,

Table 1 Mean (±SD) of rhodanese activity (enzyme U/mg protein) in tissue homogenates from Japanese quail, partridge, and pigeon ($n=5$ for each tissue studied)

Tissue	Japanese quail	Partridge	Pigeon
Liver	$0.13\pm0.021^{bc,x}$	$0.200\pm0.042^{bc,y}$	$0.301\pm0.053^{a,z}$
Kidney	0.212 ± 0.031^{a}	$0.277\pm0.079^{a,b}$	$0.288\pm0.025^{a,b}$
Proventriculus	$0.170\pm0.060^{ab,x}$	$0.266\pm0.043^{ab,y}$	$0.190\pm0.035^{bcd,xy}$
Heart	$0.068\pm0.048^{e,x}$	$0.312\pm0.046^{a,y}$	$0.234\pm0.070^{abc,y}$
Esophagus	$0.060\pm0.044^{d,x}$	$0.156\pm0.037^{c,y}$	$0.115\pm0.026^{df,xy}$
Cecum	$0.059\pm0.017^{d,x}$	$0.154\pm0.030^{c,y}$	*
Gizzard	$0.057\pm0.025^{d,x}$	$0.147\pm0.037^{c,y}$	$0.091\pm0.048^{df,xy}$
Muscle	0.055 ± 0.022^{d}	0.111 ± 0.073^{ce}	0.141 ± 0.073^{cdf}
Large intestine	$0.055\pm0.055^{d,x}$	$0.139\pm0.062^{ce,xy}$	$0.156\pm0.043^{cdf,y}$
Brain	$0.046\pm0.011^{d,xy}$	$0.033\pm0.011^{de,x}$	$0.091\pm0.048^{df,y}$
Crop	$0.044\pm0.011^{d,x}$	$0.111\pm0.060^{ce,xy}$	$0.129\pm0.037^{df,y}$
Duodenum	$0.035\pm0.011^{d,x}$	$0.148\pm0.061^{c,y}$	$0.087\pm0.043^{ef,xy}$
Pancreas	0.030 ± 0.014^{d}	0.096 ± 0.068^{ce}	0.096 ± 0.040^{df}
Lung	$0.025\pm0.01^{d,x}$	$0.035\pm0.011^{de,y}$	$0.082\pm0.045^{ef,xy}$
Spleen	0.020 ± 0.013^{d}	$0.037\pm0.012^{d,e}$	$0.059\pm0.029^{e,f}$
Trachea	$0.017\pm0.008^{d,x}$	$0.039\pm0.012^{de,xy}$	$0.074\pm0.041^{ef,y}$

[a–f] Mean±SD in each column with no common superscript differ significantly ($P<0.05$). [x–z] Mean±SD in each row with no common superscript differ significantly ($P<0.05$). *Cecum in pigeon is vestigial and was not separated

and proventriculus and the lowest is seen in brain. In pigeon, the highest activity of the enzyme is observed in liver, kidney, and heart and lowest in spleen. Inter-species differences are observed in the level of enzyme in different tissues. Specific activity of rhodanese is lowest in most tissues of Japanese quail in comparison with two other species.

Discussion

Rhodanese activity is ubiquitous in nature (Westley 1973; Wood 1975; Drawbaugh and Marrs 1987) suggesting an important physiological role. It has been suggested that the level of rhodanese in different tissues of animals is correlated with the level of exposure to cyanide (Lewis et al. 1992; Aminlari et al. 2000). The potential risk of cyanide toxicity to man and animals is great. It is believed that the primary function of rhodanese is cyanide detoxification (Cerletti 1986; Aminlari et al. 1994, 1997).

The pattern of distribution of rhodanese in different animals appears to be highly species and tissue specific. In most animals studied, the liver appears to be the richest source of rhodanese (Dudeck et al. 1980; Drawbaugh and Marrs 1987; Westley 1973). However, in avian species, some controversy exists in the literature. For instance, it has been reported that renal activity of rhodanese in chicken is approximately twice that of the hepatic activity (Castella Bertran 1952; Oh et al. 1977). This result is different from findings of others that, in chicken, rhodanese activity in liver is greater than kidney (Aminlari and Shahbazi 1994; Al-qarawi et al. 2001; Agbola et al. 2006). In the present study, no significant difference was seen between liver and kidney rhodanese activity in pigeon. This is in agreement with the results of Oh et al. (1977) but different from findings of Agbola et al. (2006) and Al-qarawi et al. (2001) who reported that, in pigeon, renal activity of rhodanese significantly exceeds that of liver. In Japanese quail, rhodanese activity in kidney is significantly higher than that of liver but in partridge no significant difference is seen between renal and hepatic activities of the enzyme. High hepatic activity of rhodanese in these three species reflects the importance of the liver as the major site of cyanide detoxification (Drawbaugh and Marrs 1987; Aminlari and Gilanpour 1991; Al-qarawi et al. 2001). Considering the mass of liver, this organ contains a large amount of rhodanese, and probably is more involved in cyanide toxicity than other organs. High activity of rhodanese in kidney may be related to the role of this organ in eliminating the metabolites through urine. On the other hand, it is possible that there has been adaptation in the poultry kidney which is related to some other function of rhodanese (Oh et al. 1977). The discrepancies in literatures mentioned above may be due to differences in procedures used for enzyme assay, strain, type of feed consumed, or other unknown factors.

In partridge and pigeon, high activity of rhodanese is also seen in heart. This is consistent with the previous results documented in chicken (Oh et al. 1977; Aminlari and Shahbazi 1994) and in pigeon (Agbola et al. 2006). In view of accumulating evidence that rhodanese is involved in the regulation of mitochondrial energy metabolism through phosphorylation–dephosphorylation of rhodanese molecule (Ogata and Volini 1990), high rhodanese activity in the heart might reflect the energy demands of this organ that is provided mostly by aerobic pathways.

The distribution of rhodanese activity in the digestive systems of birds studied here is similar to the previous results reported (Oh et al. 1977; Aminlari and Shahbazi 1994). The highest activity of the rhodanese in digestive system is observed in proventriculus. A higher rhodanese activity in the proventriculus than in other parts of the digestive tract is not unexpected because this organ is the first section of the digestive tract in which feed is digested. In the proventriculus or glandular stomach, feedstuff is digested through the secretion of pepsin and hydrochloric acid (Duke 1986). The acidic conditions might result in spontaneous hydrolysis of cyanogenic glycosides and liberation of cyanide, which is easily absorbed (Conn 1978). Presence of high rhodanese activity in the proventriculus of poultry, particularly in sub-mucosal layers (Aminlari and Shahbazi, 1994; Aminlari et al, 1997), probably ensures cyanide detoxification before it reaches general circulation. As shown in Table 1, significant levels of rhodanese activities are also seen in different parts of the intestine. It has been reported that rhodanese in the large intestine is the principal enzyme involved in H_2S detoxification which is produced normally in the intestine (Picton et al. 2002). Therefore, this enzyme may have an important role in detoxifying hydrogen sulfide in the large intestine of birds in addition to its cyanide detoxification effect.

The lower activity of rhodanese in most organs of Japanese quail in comparison with two other species may be due to different management systems under which these birds are kept. Japanese quails are kept on semi-intensive or intensive management systems, while birds such as partridge or pigeon usually kept on free-range management systems and find their own foods which consist of leaves, grass, seeds, fruits, etc., probability of exposure to cyanogenic glycosides in free-range systems is more and may be related to higher rhodanese activity in birds reared under these conditions.

In summary, this study showed a widespread distribution of rhodanese activity in hepatic and extrahepatic tissues of adult Japanese quail, partridge, and pigeon. The presence of rhodanese in various extrahepatic tissues suggests that this

enzyme may be functional in many physiological activities in these species. Future studies are needed to clarify the involvement of rhodanese in various physiological processes and pathophysiological conditions in these species.

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