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Tissue Distribution of Molybdenum Hydroxylases, Aldehyde Oxidase and Xanthine Oxidase, in Male and Female Camels

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Abstract: The specific activities of aldehyde oxidase and xanthine oxidase were assessed in ammonium sulfate fractions of male and female camel, tissues (stomach, lung, liver, kidney, heart, hump, duodenum, jejunum, ileum, large intestine, rumen,,, leg muscle, pancreas, spleen, testis, uterus, milk). It has been found that the maximum aldehyde oxidase activity existed mainly in stomach and the minimum enzyme activity was in pancreas using phthalazine and 3-Methylisoquinoline.There was significant difference ($P< 0.025$) in the activity of lung aldehyde oxidase between male and female camels.Only liver showed aldehyde oxidase activity when phenanthridine was used as substrate with highly significant difference ($P< 0.025$) in the activity of this enzyme between male and female camels was noticed. On the other hand, the xanthine oxidase activity could not detected in any of the tissues. However, xanthine oxidase was established in milk. Both K_m and V_{max} values were determined for phthalazine, 3-Methylisoquinoline and phenanthridine with hepatic aldehyde oxidase. It would be concluded that the tissue distribution of molybdenum hydroxylases varied according to the camel tissue. Other variations in tissue distribution of molybdenum hydroxylases in relation to different animals species are presented and discussed.

Key words: Tissue distribution · Aldehyde oxidase · Xanthine oxidase · Camel · Molybdenum hydroxylases · Kinetic constants

INTRODUCTION

Both molybdenum hydroxylases, aldehyde oxidase (EC1.2.3.1) and xanthine oxidase (EC1.2.3.2) are widely distributed in nature, being found in species as various as sea anemone and mammals such as sheep and human [1-5]. As early as 1974, two detailed studies had compared the occurrence of two enzymes in 79 species [5] and 8 animal phyla [4]. Generally herbivores contain a higher specific activity of aldehyde oxidase than xanthine oxidase [4]. Although rabbit liver appeared to be the richest source of the former enzyme [4], xanthine oxidase was found abundantly in mammalian intestine [4,6] and in bovine milk [7]. Aldehyde oxidase activity in guinea pig kidney was nearly 50 % of liver, beside the intestine, lung and spleen contain lower activity [3,4,8]. In addition, the enzyme was also found in other tissues such as brain, stomach, heart, placenta,skeletal muscle and pancreas of laboratory animals and man in very low levels [4,9,10]. Furthermore, xanthine oxidase was identified in different organs including the kidney, lung, spleen, muscle and heart of mouse and rabbit [11-14].

Due to lack of information of aldehyde oxidase and xanthine oxidase in camel tissues (Camelus dromedaries), the present work was conducted to study the tissue distribution and the kinetics of these enzymes in the Arabian camel.

MATERIALS AND METHODS

Chemicals: Phthalazine and phenanthridine were supplied by the Aldrich Chemical Company (Gillingham, U.K).3-Methylisoquinoline was obtained from ICN Pharmaceuticals Inc. (K&K) (Irvine, CA), Xanthine was purchased from Sigma Chemical Company (UK).

Enzyme Preparation: For the biochemical investigation partially purified aldehyde oxidase was prepared from male and female one –humped Arabian Camels (Camelus dromedaries). The animals were killed by cervical dislocation as part of daily routine at 7 a.m. in Jeddah Slaughter House, Saudi Arabia and the following tissues were collected: Liver, kidney, hump, stomach, lung, heart, duodenum, ileum, jejunum, large intestine,

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rumen,pancreas, spleen, testis, uterus and leg muscle. These collected tissues were frozen by liquid nitrogen and stored at -80°C for one week. Partly purified aldehyde oxidase was prepared as described by Johnson *et al*. [15] with some modifications. The tissues were ground in liquid nitrogen. To the resulting powder (26.5 g) ice –cold 1.15% KCl solution (50 ml) containing 10^{-4}M EDTA was added and the suspension was homogenized using a polytron homogenizer, then the homogenate was placed in a flask and heated on steam-bath at 50-55°C for 15 minutes. Thereafter the flask was immediately cooled in an ice bath until the temperature dropped to 10°C and the suspension was centrifuged at 20,000 g for 30 minutes at 4 °C.The resulting supernatant was filtered through glass wool and the volume of the clear solution was noted.Different concentrations of solid ammonium sulphate ranging from 20 -70 % saturation were added to the supernatant with stirring for 15 minutes at 4 °C, to find out the higher enzyme activity. Finally the precipitate from 50 % saturation fraction (35.4 g / 100 ml) of the maximum activity was collected by centrifugation at 3,000 g for 15 minutes at 4 °C, rinsed gently with distilled water and dissolved in 10^{-4}M EDTA solution (5 ml). The enzyme was stored in a deep-freeze at -80°C until used.

Molybdenum Hydroxylases Assays: The activity of molybdenum hydroxylase was determined spectrophotometrically by a Varian UV/VIS spectrophotometer, at 37°C in 67mM phosphate buffer pH7.

Using three substrates (Phthalazine, 3-methylisoquinoline and phenanthridine) as described by Johnson *et al*. [16] the specific activity of aldehyde oxidase was measured. The oxidation rate of either phthalazine (3×10^{-5} M) or 3-methylisoquinoline (2×10^{-5} M) was monitored at 420 nm by following potassium ferricyanide reduction, while that of phenanthridine (3×10^{-5} M) was estimated by following the increase in absorbance at 322 nm. The specific activity of xanthine oxidase was evaluated using xanthine(3×10^{-5} M) at 295 nm as reported previously by Johnson *et al*. [15] and Stubley and stell [17].

Protein Determination: All protein determinations were performed with the biuret method using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Molybdenum hydroxylases are widely distributed in nature and previous work [4,18-20] showed that both aldehyde oxidase and xanthine oxidase were found in human and guinea pig, whereas in rabbit and rat only one of them is predominant. However,no detailed work on the two enzymes was carried out with camel. The tissue distribution data of male and female camel aldehyde oxidase using phthalazine and 3-methylisoquinoline are illustrated in Tables 1 and 2. It has appeared that

Table 1: Distribution of aldehyde oxidase in various male and female camel tissues using phthalazine as a substrate

	Specific activity * (μmol/min/mg protein)		
Tissue	Male (n=10)	Female (n=6)	P<**
Stomach	0.0104 ± 0.0020	0.0098±0.0009	N.S.
Lung	0.0099± 0.0023	0.0082±0.0007	0.025
Ileum	0.0097± 0.0033	0.0078±0.0012	N.S.
Large intestine	0.0096± 0.0025	0.0077±0.0029	N.S.
Liver	0.0091±0.0022	0.0055±0.0016	N.S.
Hump	0.0090±0.0008	0.0064± 0.0010	N.S
Rumen	0.0076±0.0044	0.0067±0.0010	N.S.
Jejunum	0.0063±0.0027	0.0060±0.0027	N.S.
Duodenum	0.0048±0.0019	0.0039±0.0007	N.S.
Kidney	0.0041±0.0017	0.0035±0.0014	N.S.
Heart	0.0035±0.0012	0.0032±0.0004	N.S.
Leg muscle	0.0034±0.0010	0.0031±0.0015	N.S.
Spleen	0.0027±0.0009	0.0024±0.0005	N.S.
Pancreas	0.0019±0.0009	0.0016±0.0002	N.S.
Uterus		0.0017±0.0003	
Milk		0.0014±0.0001	
Testis	0.0011±0.0005		

*The values are given as means±SD for the number of animals examined (n)
**The statistical significance of differences between male and female values are obtained using a two- tailed Student's t-test. N.S.: Not significant.

Table 2: Distribution of aldehyde oxidase in various male and female camel tissues using 3- methylisoquinoline as a substrate

	Specific activity * (μmol/min/mg protein)		
Tissue	Male (n=10)	Female (n=6)	P<**
Stomach	0.128 ± 0.0027	0.0117±0.0013	N.S.
Lung	0.0127± 0.0031	0.0106±0.0003	0.025
Ileum	0.0107± 0.0031	0.0091±0.0019	N.S.
Large intestine	0.0104± 0.0025	0.0089±0.0026	N.S.
Liver	0.0028±0.0003	0.0024±0.0003	N.S.
Hump	0.0126±0.0013	0.0093± 0.0016	N.S
Rumen	0.0088±0.0046	0.0083±0.0012	N.S.
Jejunum	0.0072±0.0027	0.0071±0.0032	N.S.
Duodenum	0.0057±0.0021	0.0050±0.0009	N.S.
Kidney	0.0050±0.0016	0.0040±0.0007	N.S.
Heart	0.0045±0.0017	0.0034±0.0006	N.S.
Leg muscle	0.0027±0.0010	0.0025±0.0009	N.S.
Spleen	0.0032±0.0010	0.0026±0.0005	N.S.
Pancreas	0.0027±0.0011	0.0023±0.0005	N.S.
Uterus		0.0023±0.0003	
Milk		0.0019±0.0001	
Testis	0.0016±0.0006		

*The values are given as means±SD for the number of animals examined (n)

Table 3: Distribution of aldehyde oxidase in various male and female camel tissues using phenanthridine as a substrate

	Specific activity * (µmol/min/mg protein)		
Tissue	Male (n=10)	Female (n=6)	P<**
Stomach	0.0000	0.0000	
Lung	0.0000	0.0000	
Ileum	0.0000	0.0000	
Large intestine	0.0000	0.0000	
Liver	0.0056±0.0016	0.0034±0.0017	0.025
Hump	0.0000	0.0000	
Rumen	0.0000	0.0000	
Jejunum	0.0000	0.0000	
Duodenum	0.0000	0.0000	
Kidney	0.0000	0.0000	
Heart	0.0000	0.0000	
Leg muscle	0.0000	0.0000	
Spleen	0.0000	0.0000	
Pancreas	0.0000	0.0000	
Uterus	0.0000	0.0000	
Milk	0.0000	0.0000	
Testis	0.0000	0.0000	

Table 4: Distribution of xanthine oxidase in various male and female camel tissues using xanthine as a substrate

	Specific activity * (µmol/min/mg protein)	
Tissue	Male (n=10)	Female (n=6)
Stomach	0.00000	0.00000
Lung	0.0000	0.0000
Ileum	0.0000	0.0000
Large intestine	0.0000	0.0000
Liver	0.0000	0.0000
Hump	0.0000	0.0000
Rumen	0.0000	0.0000
Jejunum	0.0000	0.0000
Duodenum	0.0000	0.0000
Kidney	0.0000	0.0000
Heart	0.0000	0.0000
Leg muscle	0.0000	0.0000
Spleen	0.0000	0.0000
Pancreas	0.0000	0.0000
Uterus		0.0000
Milk		0.0008±0.00005
Testis	0.0000	

the maximum specific activity of aldehyde oxidase recorded in stomach followed by lung while pancreas showed the minimum activity. These results are in contrast with previous studies which revealed that liver gave the highest specific activity of aldehyde oxidase in other species [3,4]. Human aldehyde oxidase activity from the study of Krenitsky *et al*. [4] found that the liver did not show the highest enzyme activity and this is in agreement with camel results. In addition, the present results confirm the findings of other workers that aldehyde oxidase varies markedly among species [21,22]. However, with phenanthridine as substrate, aldehyde oxidase activity was apparent only in the liver (Table 3). Thus, this result could be indicate the presence of different isoenzymes in camel tissues,since aldehyde oxidase isoenzymes have been identified in different species [12,23].

Only a single form of xanthine oxidase was reported [19,24,25], therefore, one substrate (xanthine) was used to determine the specific activity of xanthine oxidase in different tissues, Table 4 declares that the enzymatic oxidation of xanthine was not detected in any of the tested tissues. Moreover, xanthine reacted with xanthine oxidase extracted from milk.This result is in agreement to some extent with recorded by Mura *et al.* [26] who investigated xanthine oxidase in camel liver only. Also, the present study revealed that there is a different data from other species such as man, guinea pig, cat, dog, sheep and cow, in which xanthine oxidase was found in many tissues of them [3,4,6]. In addition, studies on the tissue distribution of xanthine oxidase using xanthine as a substrate showed that this enzyme was found in highest concetrations in the mammary gland and small intestine in most species [3,4,25]. Results recorded inTable 5 show the kinetic measurements of male hepatic aldehyde oxidase activity for Phthalazine, 3-methylisoquinoline and phenanthridine. k_m and k_s values of 3-methylisoquinoline and phenanthridine were of same order, whereas the k_m value of phthalazine was ten times higher. These results indicate that the first two compounds are excellent substrates hepatic aldehyde oxidase. Further investigation is needed to separate the camel aldehyde oxidase isoenzymes definitely.

Table 5: Kinetic constants obtained for azanaphthelens with hepatic camel aldehyde oxidase (means of three determinations)

Compound	$K_{m\,(M)}$	V_{max} (µmol/min/mg)	K_S
Phthalazine	1.5×10^{-4}	0.0120	0.08
3-Methylisoquinoline	1.4×10^{-5}	0.0070	0.5
phenanthridine	7.3×10^{-5}	0.0337	0.462

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