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TISSUE DISTRIBUTION OF THE MOLYBDENUM HYDROXYLASES, ALDEHYDE OXIDASE AND XANTHINE OXIDASE, IN NAJDI SHEEP

(With 3 Tables)

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التوزيع النسيجي للموليبدينوم هيدروكسيليزز (الدهايد أكسيداز والزانثين أكسيداز) في الضأن النجدي

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تم تعيين نشاطية الموليبدينوم هيدروكسيليزز (الدهايد أكسيداز والزانثين أكسيداز) المعلقة في وسط من كبريتات الأمونيوم والمستخلصة من عينات الكبد والكلى والرئة والأمعاء الدقيقة والطحال والقلب. ولقد وجد أن مستويات الدهايد أكسيداز في كل حالة نسيجية أعلى من الزانثين أكسيداز وأن أعلى نشاطية للأنزيم الأول كانت في الكبد وكذلك قدرت مستويات عالية منه في الرئة. وكان الكبد هو النسيج الوحيد ضمن الأنسجة المختبرة الذي أظهر نشاط لأنزيم الزانثين أكسيداز وذلك باستخدام الزانثين كمادة يعمل عليها الإنزيم. ولم يكن هناك وجود لنشاطية إنزيم الدهايد أكسيداز وكذلك إنزيم الزانثين أكسيداز في الأمعاء الدقيقة أو الطحال أو القلب. كما تم تعيين قيَم ثابت ميخائيل km و Vmax الحد الأعلى لمعدل النشاط الانزيمي على الثلازين و٣-ميثايل أيزوكوينولين وفينانثريدين والتي يعمل عليها إنزيم الدهايد أكسيداز الكبدي كما وجد أن الميناديون يعمل كمثبط تنافسي لإنزيم الدهايد أكسيداز الكبدي وكذلك تم حساب ثوابت ذلك المثبط لنشاط الإنزيم.

SUMMARY

The Activity of the molybdenum hydroxylases, aldehyde oxidase and xanthine oxidase were determined in ammonium sulfate fractions prepared from sheep liver, kidney, lung, small intestines, spleen and heart. In all examined tissues, aldehyde oxidase levels were higher than those of xanthine oxidase; the activity of the former enzyme was the highest in the liver, also present in the lung. Xanthine oxidase activity was not detected in all tissues except the liver. No aldehyde oxidase or xanthine oxidase activity was detected in the small intestine, spleen or

heart. Both Km and Vmax values were determined for phthalazine, 3-methylisoquinoline, phenanthridine and xanthine with hepatic aldehyde oxidase and xanthine oxidase. By using three substrates [phthalazine, 3-methylisoquinoline, phenanthridine] for aldehyde oxidase. Menadione was found to be a competitive inhibitor of this enzyme and the inhibitor constants were calculated.

Key words: *Aldehyde oxidase - Xanthine oxidase - Molybdenum hydroxylases – Kinetic constants – Sheep.*

INTRODUCTION

Both molybdenum hydroxylases, aldehyde oxidase (EC 1.2.3.1) and xanthine oxidase (EC 1.2.3.2) are broadly distributed in nature, being found in species as different as sea anemone and human (Wurzinger & Hartenstein 1974; Krenitsky *et al.* 1974; Beedham 1987; Beedham *et al.* 1987 and Al-Tayib 2000). As early as 1979, two detailed studies had compared the occurrence of the two enzymes in 79 species and 8 animal phyla (Wurzinger & Hartenstein 1974; Krenitsky *et al.* 1974). The variations in the levels of aldehyde oxidase of species appear to be more marked than those of xanthine oxidase.

Commonly, herbivores contain a higher specific activity of aldehyde oxidase than xanthine oxidase. Although rabbit liver appear to be the richest source of the former enzyme (Krenitsky *et al.* 1974). Xanthine oxidase was established abundantly in mammalian intestine (Al-Khalidi and Chaglassian, 1969). and in bovine milk (Bruder *et al.* 1982). Aldehyde oxidase activity in guinea pig kidney is approximately 50% of liver, besides, the intestine, lung and spleen that contain lower activity (Beedham, Bruce and Rance, 1987). 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 (Nichols, & Dixon 1969; Krenitsky *et al.* 1974; Holmes 1978; Furnival *et al.* 1983 and Beedham *et al.* 1987).

On the other hand, xanthine oxidase was identified in different organs including the kidney, lung, spleen, muscle and heart of mouse and rabbit (Holmes 1978; Chen & Chiou 1982; Sasaki *et al.* 1983 and Schoutsen *et al.* 1984).

Due to lack of information of aldehyde and xanthine oxidase distribution in sheep tissues, this work has been designed in order to recognize the tissue distribution and the kinetics of these enzymes in sheep.

MATERIALS and METHODS

Chemicals

Phthalazine and phenanthridine were supplied by the Aldrich Chemicals Company (Gullingham, U.K.), 3-methylisoquinoline from ICN Pharmaceuticals Inc. (K&K) (Irvine, CA). Xanthine, Menadione and Hydralazine were purchased from Sigma Chemical Company (St. Louis, Mo, USA).

Preparation of partially purified molybdenum hydroxylases

Partially purified aldehyde oxidase was prepared from male Najdi sheep, aged 6-8 months. The animals were killed by cervical dislocation as part of daily routine at 9:00 a.m. in Al-Taif Slaughter House, Saudi Arabia. Fresh tissues were collected from liver, kidney, lung, heart, spleen and small intestine, stored in a deep freeze at -80°C, for one week. The enzyme was prepared as described by Johnson *et al.* (1987) and Al-Tayib (1999).

Molybdenum Hydroxylases Assays

The activity of both aldehyde oxidase and xanthine oxidase was determined at 37°C in 67mM phosphate buffer pH7 using spectrophotometric methods. The specific activity of aldehyde oxidase was determined using three substrates (phthalazine, 3-methylisoquinoline and phenanthridine) as described by Johnson *et al.* (1983). The oxidation rate of either phthalazine (1mM) or 3-methylisoquinoline (1mM) was evaluated at 420 nm by following potassium ferricyanide reduction, while that of phenanthridine (0.05 mM) was monitored by following the increase in absorbance at 322 nm. The specific activity of xanthine oxidase was determined using xanthine (0.05 mM) at 295 nm as reported previously by Beedham *et al.* 1989, and Johnson *et al.* 1987.

Protein Determination

All protein determinations were performed with the biuret method (Gornall *et al.* 1948) using bovine serum albumin as standard.

Determination of Michaelis – Menten Constants and Inhibitor constants.

The initial velocity V, corresponding to a range of substrate concentration [S], was determined the Michaelis-Menten constant, Km and maximum velocity Vmax, were calculated from the double reciprocal plot (line weaver Burk plot) of 1/v versus 1/[S].

In the present study Menadione was used as an inhibitor. Menadione (0.06M) was dissolved in 25ml absolute ethanol and series

of standard solutions were prepared by diluting the master solution with 67mM phosphate buffer pH7. The effect of an inhibitor upon the rate of oxidation was measured spectrophotometrically by recording the decrease in oxidation rate in the presence of a single concentration of the inhibitor. The double reciprocal plots of velocity [V] against substrate concentration [S] of both non-inhibited and inhibited data used when the inhibitor constant (Ki) was Calculated.

RESULTS

The obtained results are summarized in Tables 1-3.

DISCUSSION

Molybdenum hydroxylases are broadly distributed in nature and earlier works (Krenitsky *et al.* 1974; Stubley *et al.* 1979; Seely *et al.* 1984; Beedham *et al.* 1987 and Chritchley *et al.* 1992) 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 wide work on the two enzymes has previously been carried out using sheep tissues. Employing phthalazine as substrate, the greatest specific activity of aldehyde oxidase appeared in liver (0.0288 μ mol/min/mg protein), followed by lung ($p<0.0005$) while kidney ($p<0.0005$) enzyme showed the lowest activity (Table 1).

This result is in agreement with previous works which showed that liver give maximum specific activity of aldehyde oxidase in other species (Krenitsky *et al.* 1974 and Beedham *et al.* 1987). In addition, there were no activities in other sheep tissues (small intestine, spleen and heart) when phthalazine was used. The enzyme activity in sheep kidney was 21% of that in the liver, whereas in guinea pig the activity in the kidney was about 34% (Beedham *et al.* 1987).

With 3-methylisoquinoline as a substrate, the specific activity of hepatic aldehyde oxidase was nearly the same to that of the lung.

Using phenanthridine as a substrate, the maximum specific activity of aldehyde oxidase occurred in liver (0.0179 μmol/min/mg protein), followed by lung ($p<0.005$) while kidney ($p<0.0005$) enzyme exhibited the minimum activity. Sheep aldehyde oxidase extracted from small intestine, spleen and heart failed to react with any of the three substrates (phthalazine, 3-methylisoquinoline, phenanthridine), unlike the case with the enzymes of rabbit, guinea pig or the lizard Uromastyx Microlepis (Johnson *et al.* 1983; Beedham *et al.* 1987; Beedham *et al.*

1989 and Al-Tayib 2000). This confirms the species variations of aldehyde oxidase (Beedham *et al.* 1987). Furthermore, by using phthalazine and phenanthridine, the activity of sheep lung aldehyde oxidase was about 62% of that in liver, while the activity of lung enzyme was nearly equal to that of liver when 3-methylisoquinoline was used as a substrate. The variations in the enzyme activity with these three substrates could indicate – the presence of different isoenzymes for aldehyde oxidase in sheep tissues.

The specific activity of the related enzyme, xanthine oxidase, was determined using xanthine as a substrate. Table (1) shows that the enzymatic oxidation of xanthine was apparent in liver only, while no enzyme activity was detected in other examined sheep tissue.

Table (2) shows the kinetic measurements of hepatic aldehyde oxidase and xanthine oxidase activities for phthalazine, 3-methylisoquinoline, phenanthridine and xanthine. Km and Ks values of phthalazine and phenanthridine were of the same order, whereas km value of 3-methylisoquinoline was four times higher and low substrate efficiency (Ks).

The low Km value shows that phthalazine can be an excellent substrate for sheep hepatic aldehyde oxidase. Previously Km values of 4.1×10^{-5M} and 5×10^{-5M} have been calculated with hepatic aldehyde oxidase from man and guinea pig with phthalazine (Beedham *et al.* 1987 and Beedham *et al.* 1995) which are very similar to the value reported in the present study.

Sheep liver xanthine oxidase had low Km value for xanthine and low Vmax. However, the related low Km suggests that xanthine binds to the active side but is only slowly oxidized.

Menadione is a potent inhibitor of aldehyde oxidase (Rajagopalan and Handler 1964) and in this study the inhibitor constants, Ki values, for menadione were determined with hepatic aldehyde oxidase using phthalazine, 3-methylisoquinoline and phenanthridine as substrate for this enzyme. The results sumarised in Table (3) shows that the lowest Ki of Menadione was achieved with phthalazine, while with 3-methylisoquinoline the Ki was about 4 times higher.

Moreover, in each case inhibition was found to be competitive. Further investigation is required to separate the sheep isoenzymes, since aldehyde oxidase isoenzymes have been identified in different species (Holmes 1978; Ohkubo *et al.* 1983; Critchley *et al.* 1992 and Beedham *et al.* 1995).

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Table 1: Distribution of molybdenum hydroxylases in various male sheep tissues using different substrates (means ± SD; n=6).

Tissue	Specific activity (μ mol/min/mg protein) of aldehyde oxidase and xanthine oxidase			
	Phthalazine	3-Methylisoquinoline	Phenanthridine	Xanthine
Liver	0.0288±0.0062	0.0034±0.0002	0.0179±0.0029	0.0008±0.0002
Kidney	0.0061±0.0009*	0.0013±0.0002*	0.0034±0.0003*	0
Lung	0.016±0.0012*	0.0032±0.0003	0.012±0.0015$^{\Theta}$	0
Small intestine	0	0	0	0
Spleen	0	0	0	0
Heart	0	0	0	0

As compared with liver, *p< 0.0005;
Θ p<0.005 (student's – t-test)

Table 2: Kinetic constants for azanaphthelenzs with hepatic sheep aldehyde oxidase and xanthine oxidase (means of three determinations)

Compound	K_m (M)	V max (μ mol/min/mg protein)	K_s
Phthalazine	$5.6x10^{-5}$	0.0285	0.5089
3-Methylisoquinoline	$1.7x10^{-4}$	0.0049	0.0288
Phenanthridine	$7.9x10^{-5}$	0.0235	0.2975
Xanthine	$3.1x10^{-5}$	0.0017	0.0548

Table 3: Inhibitor constant (Ki) values for menadione with hepatic aldehyde oxidase using three substrates

Substrate	Ki values (M) at 37°c in 0.067M phosphate buffer
Phthalazine	$2.8x10^{-8}$
3-Methylisoquinoline	$1.02x10^{-7}$
Phenanthridine	$9.5x10^{-8}$