World Journal of Chemistry 4 (1): 45-51, 2009
ISSN 1817-3128

Some Biochemical Studies on the Aldehyde Oxidase and Xanthine Oxidase of Agamid Lizard Uromastyx Microlepis (Dhubb)

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Abstract: The present study investigated the activity levels of both aldehyde oxidase and xanthine oxidase, in ammonium sulfate fractions prepared from several dhubb, Uromastyx microlepis, tissues (liver, lung, heart, kidney, stomach, duodenum, ileum, leg muscle, adipose tissue, tail muscle and testis). The biochemical tests indicated that the highest aldehyde oxidase activity observed in tail muscle and the lowest enzyme activity showed in heart. No aldehyde oxidase activity was detected in any of the tissues when phenanthridine was used as a substrate. Of all the tissues, only kidney showed xanthine oxidase activity. The optimal conditions of the activities of both molybdenum hydroxylases were recorded.

Key word: Distribution, Aldehyde oxidase, Xanthine oxidase, Molybdenum hydroxylases, Uromastyx microlepis, Optimum conditions

INTRODUCTION

Aldehyde oxidase (EC1.2.3.1) and xanthine oxidase (EC1.2.3.2) are related to group of enzymes known as the molybdenum – containing hydroxylases [1-5]. In addition to the microsomal monoxygenases, aldehyde oxidase and xanthine oxidase, play very important role in the biotransformation of drugs and xenobiotics [6- 9]. These enzymes are widely distributed throughout the animal kingdom from primitive species like the sea anemone, molluscs, crustaceans, insects, birds, reptiles and mammals such as sheep [10-14]. Very little studies on molybdenum hydroxylases distribution in desert organisms were conducted, consequently *Uromastyx microlepis* or dhubb as it is named in Saudi Arabia, was chosen to give information on the distribution of molybdenum hydroxylases in this reptile.

MATERIALS AND METHODS

Chemicals: Phthalazine and phenanthridine were purchased from Aldrich Chemical Company (Gillingham, U.K). Xanthine was purchased from Sigma Chemical Company (UK).

Preparation of Partially Purified Molybdenum Hydroxylases: Forty mature male dhubbs (644-750 g) were bought from the animal market in Riyadh and used in these studies. The animals were killed by cervical dislocation and the following tissues were collected: liver, lung, heart, kidney, stomach, duodenum, ileum, leg muscle, adipose tissue, tail muscle and testis.These collected tissues were frozen by liquid nitrogen and stored at -80°C for one week. Partially purified aldehyde oxidase was prepared from tissue homogenate, as stated by Johnson *et al*. [15] as follows: each tissue was weighed and transferred to a beaker containing potassium chloride solution (1.15 w/v) containing 10^{-4}M EDTA, two to three times by volume. The tissue was chopped finely by scissors; aliquots were transferred to a glass homogenising tube and homogenised using a Janke & Kunkel homogeniser. The homogenate was heated at 50-55°C for 10 minutes, followed by immediate cooling in ice to 10°C and then centrifuged for 45 minutes at 15,000 xg at 4°C using a Heraeus Christ 20-3 (LABSCO) centrifuge. The supernatant was filtered through glass wool into a measuring cylinder and the volume of the clear solution was noted. Solid ammonium sulphate was added to 50% saturation (35.4 g/100 ml at 4°C) with stirring using a magnetic stirrer on an ice bath. When precipitation was complete (15 minutes), the suspension was centrifuged at 6000 xg for 20 minutes at 4°C. The supernatant was discarded and the precipitate was rinsed with distilled water and dissolved in a minimum of 10^{-4}M EDTA solution (approximately 3-5 ml). The partially purified enzyme was kept in a deep freezer at -80C° and used when required.

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Enzymes Assays: The activity of both aldehyde oxidase and xanthine oxidase has been determined spectrophotometrically by a Varian UV/VIS spectrophotometer, fitted with a thermostatically controlled cell holder maintained at 37°C. The specific activity of aldehyde oxidase was defined using two substrates (Phthalazine and phenanthridine) as described by Johnson *et al*. [16]. The oxidation rate of phthalazine (1×10^{-3}M) was also monitored at 420 nm by following potassium ferricyanide reduction, while that of phenanthridine (5×10^{-5} M) was detected by following the increase in absorbance at 322 nm. The specific activity of xanthine oxidase was evaluated using xanthine ($1x10^{-4}$ M) at 295 nm as reported previously by Johnson *et al*. [15] and Stubley and stell [17]. Protein concentration was measured by the Biuret method.

Determination of Optimum Conditions: Since the rate of enzyme activity may be changed according to the reaction conditions, so all factors (pH, temperature, substrate concentration and volume of enzyme) that affect the activity of molybdenum hydroxylases have been investigated. In all of these determinations, the final volume in cuvette was 3 ml and Varian series 634 spectrophotometer fitted with MGW Lauda C12 water bath was used.

Volume of Enzyme Added: The activities of aldehyde oxidase and xanthine oxidase were measured at different enzyme volumes (50,100,150 and 200 µl). The other factors of the assay: pH, temperature and substrate concentration have been fixed in all samples as follows: pH =7, temperature = 37°C, substrate concentrations = 1×10^{-3}M for Phthalazine, 5×10^{-5}M for xanthine.

pH of Buffer: The activities of aldehyde oxidase and xanthine oxidase were measured at different pH values 5.5, 6.0, 7, 7.5, 8.0 and 8.5 using phosphate buffer (6.7×10^{-2} M). The assay was carried out at constant substrate (1×10^{-3}M for Phthalazine and 5×10^{-5}M for xanthine) and enzyme volume (100 µl) at 37°C.

Temperature: The activities of aldehyde oxidase and xanthine oxidase were measured at different temperature values 25, 37, 45, 55, 65,75,82 and 85°C.

Substrate Concentration: Serial dilution of stock substrates (6×10^{-2} M stock phthalazine and xanthine solutions) were prepared giving different substrate concentrations covering the range from 1×10^{-5} to 5×10^{-3}M.

RESULTS AND DISCUSSION

Optimal Conditions of Molybdenum Hydroxylases

Molybdenum Hydroxylases Activity at Different Enzyme Volumes: Phthalazine (1×10^{-3}M) and xanthine (5×10^{-5}M) were used as substrates for measuring the activity of aldehyde oxidase and xanthine oxidase respectively using different enzymes volumes as illustrated in Fig. 1 and 2. The previous figures show that the greatest activity of hepatic aldehyde oxidase with Phthalazine was at 100 µl of enzyme while the renal xanthine oxidase activity was highest at 150 µl of enzyme.

Molybdenum Hydroxylases Activity at Different pH Values: Measurement of the reaction rate at different pH values indicated that hepatic aldehyde oxidase with phthalazine (1×10^{-3}M) showed its optimum pH at 7 as shown in Fig. 3. Renal xanthine oxidase revealed its optimum pH at 7.5 (Fig. 4) using xanthine (5×10^{-5}M) as substrate.

Molybdenum Hydroxylases Activity at Different Temperatures: The rate of most chemical reactions, catalyzed or not, increases as phthalazine increases. However, thermal denaturation of the enzyme protein with increasing temperature will decrease the effective concentration of an enzyme and thus decline the reaction rate. The rate of reaction of hepatic aldehyde oxidase with phthalazine raised gradually with increasing temperature from 25°C up to 55°C, then the activity of enzyme decreased as shown in Fig. 5. Unusual result was obtained from renal xanthine oxidase with xanthine, in which the activity of enzyme raised with increasing temperature up to 82°C, then acute decreasing happened (Fig. 6).

Molybdenum Hydroxylases Activity at Different Substrate Concentrations: The activities of hepatic aldehyde oxidase and renal xanthine oxidase were measured at different substrate concentrations ranging from 10^{-5}M to$5x10^{-3}$M. It was found that both enzymes gave typical hyperbolic curves (Fig. 7 and 8). However the concentrations of phthalazine and xanthine were $1x10^{-3}$M and $1x10^{-4}$M respectively which gave maximum velocities.

Many animal species such as guinea pigs, hamsters and Uromastyx microlepis showed daily rhythmic variations in hepatic aldehyde oxidase and xanthine oxidase [18-20], in addition multiple forms were reported

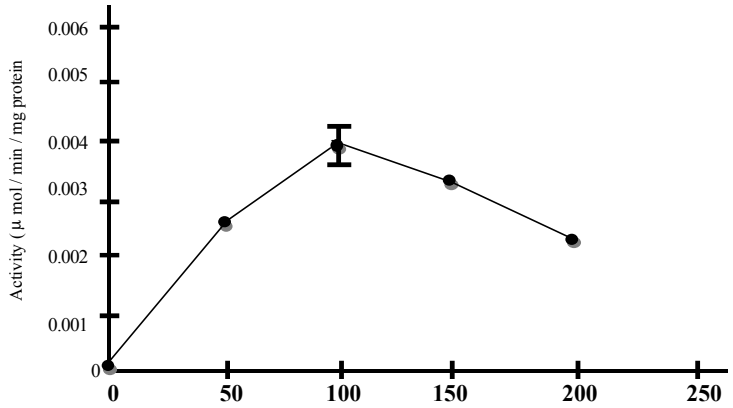

Fig. 1: Activity of hepatic aldehyde oxidase at different enzyme volumes (µ 1). Enzyme activity was measured at 37°C, pH7 and expressed as µ mol phthalazine consumed/ min / mg protein. Each point represent the mean ± SE of three animals

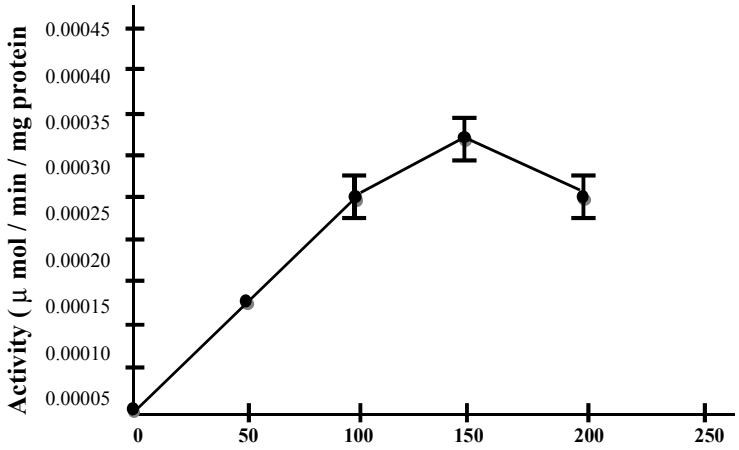

Fig. 2: Activity of renal xanthine oxidase at different enzyme volumes (µ1) Enzyme activity was measured at 37°C, pH7 and expressed as µ mol xanthine consumed / min / mg protein. Each point represents the mean ± SE of three animals

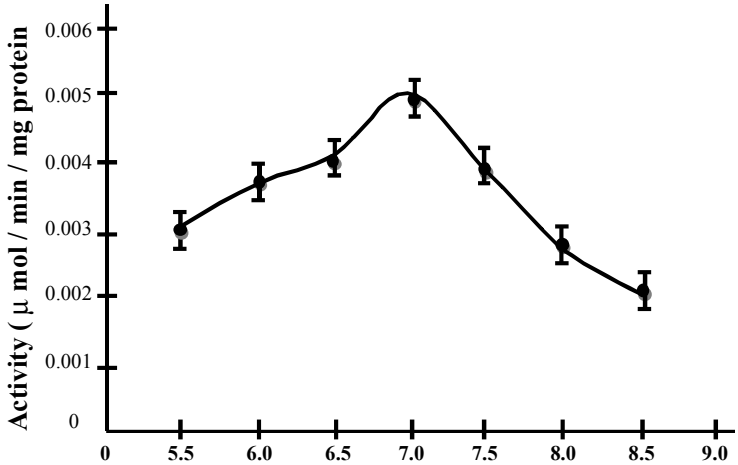

Fig. 3: Effect of different pH values on activity of hepatic aldehyde oxidase Enzyme activity was measured at 37 ° C, pH7 and expressed as µ mol phthalazine consumed/ min / mg protein. Each point represent the mean ± SE of three animals

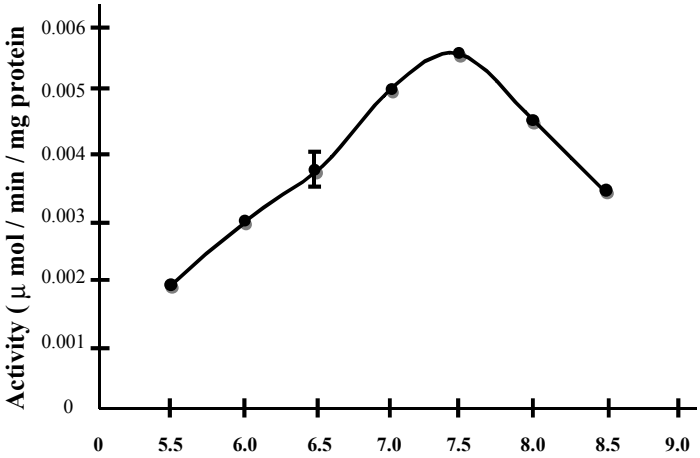

Fig. 4: Effect of different pH values on activity of renal xanthine oxidase Enzyme activity was measured at 37 ° C, pH7 and expressed as μ mol xanthine consumed / min / mg protein. Each point represents the mean ± SE of three animals

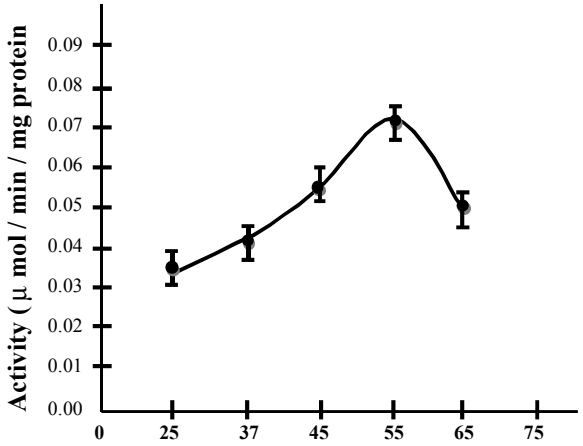

Fig. 5: Effect of the temperature on activity of hepatic aldehyde oxidase Enzyme activity was measured at 37 ° C, pH7 and expressed as μ mol phthalazine consumed/ min / mg protein. Each point represent the mean ± SE of three animals

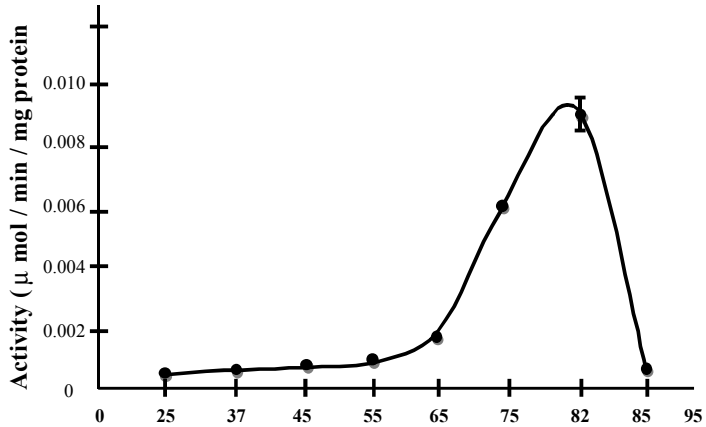

Fig. 6: Effect of the temperature on activity of renal oxidase Enzyme activity was measured at 37 ° C, pH7 and expressed as μ mol xanthine consumed / min / mg protein. Each point represents the mean ± SE of three animals

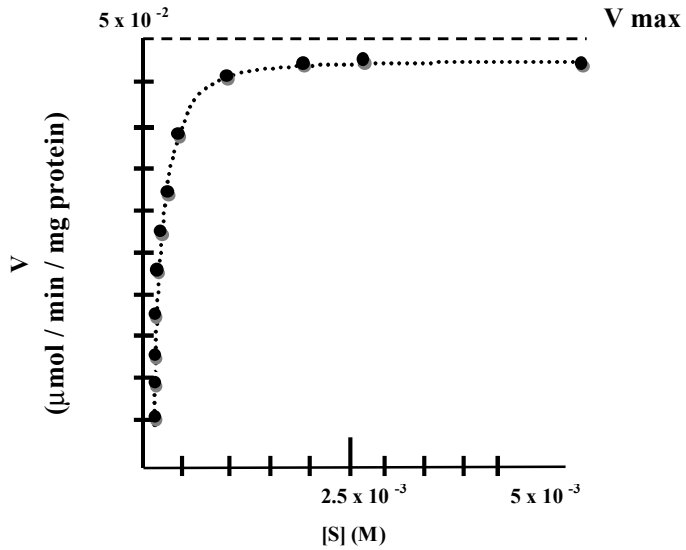

Fig. 7: Effect of different phthalazine concentrations (M) on the activity of hepatic aldehyde oxidase Enzyme activity was measured at 37°C, pH7 and expressed as µ mol phthalazine consumed/ min / mg protein. Each point represent the mean ± SE of three animals

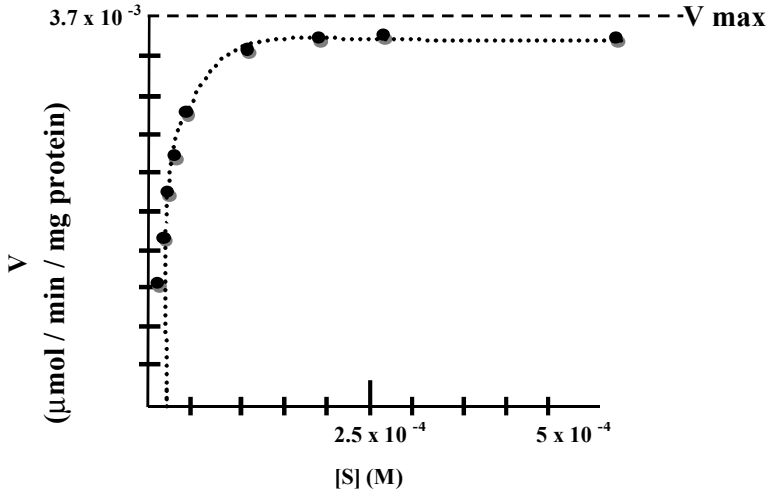

Fig. 8: Effect of different xanthine concentrations (M) on the activity of renal xanthine oxidase Enzyme activity was measured at 37°C, pH7 and expressed as µ mol phthalazine consumed/ min / mg protein. Each point represent the mean ± SE of three animals

for aldehyde oxidase [21-23], whilst only a single form of xanthine oxidase was reported [24-26]. Regarding comparison with other species specific conditions, animals were killed at 9 a.m. and the enzymes activity were measured at 37°C in 0.067 M Sodium / Potassium phosphate buffer pH 7 using phthalazine (1×10^{-3}M), phenanthridine (5×10^{-5} M) and xanthine ($1x10^{-4}$ M).

Therefore the previous specific conditions were used for measuring molybdenum hydroxylases activities in different Dhubb tissues (Tables 1 and 2) and two substrates were employed to determine the specific activity of aldehyde oxidase.

The tissue distribution of aldehyde oxidase using phthalazine (Table 1) showed that tail muscle gave the highest enzyme activity whereas the lowest enzyme activity was that of heart. In contrast, the study undertaken by Beedham *et al*. [27] declared that guinea pig liver gave the greatest aldehyde oxidase activity.

Table 1: Distribution of aldehyde oxidase in various dubbs tissues using phthalazine and Phenanthridine as substrates

	Specific activity * (µmol/min/mg protein) (n=5)	
Tissue	Phthalazine	Phenanthridine
Liver	0.049±0.0026	0.000
Lung	0.078± 0.0067	0.000
Heart	0.026±0.0036	0.000
Kidney	0.064± 0.0170	0.000
Stomach	0.033±0.0051	0.000
Duodenum	0.058±0.0032	0.000
Ileum	0.049±0.0030	0.000
Leg muscle	0.082± 0.0180	0.000
Adipose tissue	0.056±0.0021	0.000
Tail muscle	0.138 ± 0.035	0.000
Testis	0.054±0.0190	0.000

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

Table 2: Distribution of xanthine oxidase in various Dhubbs tissues using xanthine as a substrate

Tissue	Specific activity * (µmol/min/mg protein) (n=5)
Liver	0.000
Lung	0.000
Heart	0.000
Kidney	0.004± 0.007
Stomach	0.000
Duodenum	0.000
Ileum	0.000
Leg muscle	0.000
Adipose tissue	0.000
Tail muscle	0.000
Testis	0.000

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

However, these results confirm the findings of other investigators that aldehyde oxidase alters markedly among species [28, 29]. Human aldehyde oxidase activity from the study of Krenitsky *et al*. [10] found that the liver did not show the highest enzyme activity and this is in agreement with dhubb results.

It has been assumed that the observed species differences are primarily a function of dietary intake[30]. Aldehyde oxidase failed to react with phenanthridine (see Table 1), unlike the case with the enzymes of rabbit or guinea pig [16, 18].Thus, this result has given indication to the presence of multiform of aldehyde oxidase in different species [22, 23]. The other molybdenum hydroxylase, xanthine oxidase, was found only in kidney of dhubb while no enzyme activity was detected in other examined reptile tissues (Table2). This result is different from other species such as man, guinea pig, cat, dog, sheep and cow, in which xanthine oxidase was found in many tissues of them [10,27,31]. In addition, studies on the tissue distribution of xanthine oxidase using xanthine as a substrate showed that this enzyme, unlike aldehyde oxidase, was found in highest concentrations in the mammary gland and small intestine in most species [10,26,27].

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