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# A GENUINE ORGAN SPECIFIC ALCOHOL DEHYDROGENASE FROM HAMSTER TESTES: ISOLATION, CHARACTERIZATION AND DEVELOPMENTAL CHANGES

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ABSTRACT: Testes of golden hamsters (Mesocricetus auratus) contain a unique form of alcohol dehydrogenase. The enzyme purified to homogeneity is a dimer composed of two identical 41000 dalton subunits and was inhibited by 4-methylpyrazole and 1,10-phenanthroline. The enzyme prefers primary over secondary alcohol substrates and oxidizes vitamin A (retinol) most efficiently. Its activity in the testis increases during the prepubertal development and such increase is concomitant with, but preceding slightly, the increase in testicular weight and plasma testosterone. These results may implicate the involvement of this enzyme in the development of male hamsters' reproductive system. 

INTRODUCTION: The golden hamster (Mesocricetus auratus), in contrast to most outbred strains of laboratory rodents, prefers to drink ethanol under free choice situations (1-4). Sex differences in ethanol preference and sensitivity in golden hamsters have also been reported (5). These species and sex differences correspond very well to the species and sex-differences in the activities of liver alcohol dehydrogenase (ADH), ethanol metabolism in isolated hepatocytes and blood ethanol elimination rates (2-5). In an attempt to characterize the alcohol metabolizing enzymes in golden hamsters, a testis specific form of ADH was discovered.

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Abbreviations: ADH, alcohol dehydrogenase; CapGapp-, 4-(3-(N-(6-aminocaproyl)amino)propyl)pyrazole-; $NaDodSO_4$, sodium dodecyl sulfate; OP, 1,10-phenanthroline; 4-MP, 4-methylpyrazole.

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Organ specific ADH isozymes have been reported in many species including man (6,7), mouse (8) and rat (9). Studies on the catalytic properties of organ specific isozymes in relation to the specialized metabolic function(s) of the particular organs have provided insights to the understanding of the functional role(s) these isozymes played in their specific organs and to the understanding of the physiological function(s) of ADH isozymes in general. The hamster testis ADH reported here is unique not only because it is the only form of ADH found in the testes but also that it is not found in other tissues. This report describes the isolation, characterization of this testis ADH and the changes in its activity during prepubertal development of the golden hamsters.

MATERIALS AND METHODS: Golden hamsters (Mesocricetus auratus) were bred in our animal colony from animals originally obtained from Simonson laboratories, Gilroy, California and were maintained from birth in a long photoperiod, 14 h light:10 h darkness. The animals had free access to food and water. Adult male golden hamsters (70-90 days old) were killed by decapitation and testes were removed and frozen immediately in liquid $N_2$ and stored at -70 $^{o}$C. The frozen testes were used within one month.

Fresh or frozen and thawed testes were homogenized in 50 mM sodium phosphate buffer, pH 7.5 containing 1 mM $NAD^+$. The homogenate was centrifuged at 38,000 g for one hour and the supernatant was loaded onto a CapGapp-Sepharose column (2x10 cm). The column was washed and ADH was eluted according to the procedure of Lange & Vallee (10).

Starch gel electrophoresis was performed according to the method of Bosron et al. (11). Electrophoresis in NaDodSO$_4$ polyacrylamide gel was carried out by the procedure of Laemmli and Favre (12). Urea gel electrophoresis was carried out by the system developed by Keung et al. (13). Protein concentration was determined by the method of Lowry et al. (14) using bovine serum albumin as standard. ADH activity was determined spectrophotometrically as the increase in absorbance at 340 nm due to the reduction of $NAD^+$ in buffers specified in the text. One unit of activity is defined as the amount of enzyme that catalyses the formation of 1 $\mu$mole NADH per min at 25 $^{o}$C.

All alcohol substrates and other reagents used were of analytical grade and used without further purification.

RESULTS AND DISCUSSION: Electrophoresis of adult hamster testis extracts on starch gel revealed only one detectable ADH activity band (ADH-4)(Fig. 1). The electrophoretic pattern of the hamster liver extract containing seven bands is also shown for comparison. While ADH-3, ADH-5, ADH-6, ADH-7 and ADH-8 were stained with ethanol as substrate, ADH-1 and ADH-2 could be visualized only when long chain alcohols, e.g. 1-octanol, were used. ADH-4 was the only ADH detected in the testis, independent

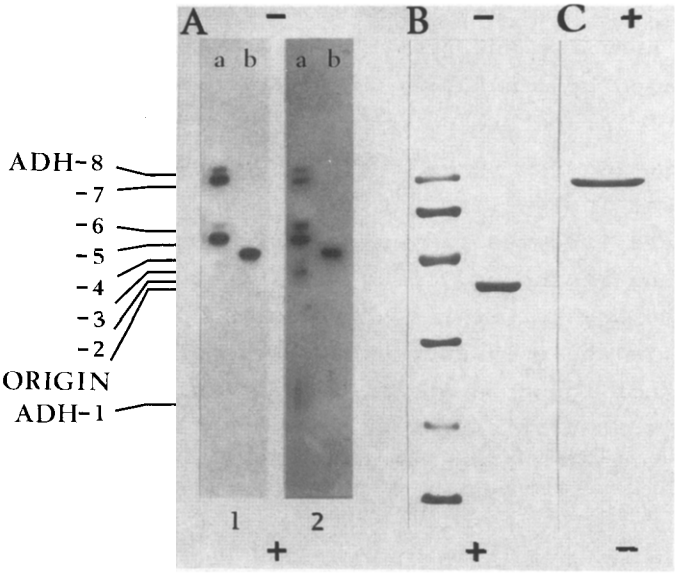


Fig. 1 (A) Starch gel electrophoretic patterns of (a) hamster liver and (b) hamster testis ADH. Gels were stained for ADH activity using (1) ethanol and (2) 1-octanol as substrates. (B) $NaDodSO_4$ gel electrophoresis of purified hamster testis ADH and a standard mixture (Pharmacia) composed of phosphorylase b (Mr. 94,000), bovine serum albumin (Mr. 66,000), ovalbumin (Mr. 45,000), carbonic anhydrase (Mr. 29,000), soybean trypsin inhibitor (Mr. 20,100) and alpha-lactalbumin (Mr. 14,400). (C) Urea gel electrophoresis of hamster testis ADH.

of the staining substrate. None of the other tissues studied including liver, kidney, brain, lung, heart, cecum, spleen, intestine, ovary and stomach, showed any detectable ADH-4 activity (result not shown).

The testis ADH was purified to homogeneity by affinity chromatography on a CapGapp-Sepharose column and the result of the purification procedure is shown in Table 1. The enzyme was purified in satisfactory yield (60-70 %) by this single step procedure which was accomplished in two days. From 60 g of testes, about 18 units of pure ADH-4 was obtained.

Chromatography of the purified testis ADH on a molecular sieving HPLC column yielded a single protein peak which co-eluted with human liver ADH (not shown) indicating that the hamster testis ADH, like the human ADHs (15,16), has a molecular weight around 80,000. $NaDodSO_4$ gel electrophoresis of ADH-4 revealed a single protein band corresponding to a subunit molecular weight of about 41,000 (Fig. 1). Electrophoresis of the urea dissociated ADH-4 on polyacrylamide gels containing 7 M urea also showed a single protein band (Fig. 1). These results not only establish the homogeneity of the enzyme preparation but also

Table 1: Purification of Hamster Testis ADH[a]

| Purification step | Total Activity[b] (unit) | Total Protein (mg) | Specific Activity (U/mg) | Yield (%) | Purification Fold |
|---|---|---|---|---|---|
| 38,000 g supernatant | 27 | 1080 | 0.025 | 100 | 1 |
| CapGapp-Sepharose | 18 | 4.6 | 4.0 | 67 | 160 |

[a] From 34 testes, about 60 g.

[b] Activity was determined at pH 7.5 in 0.1 M sodium phosphate buffer containing 2.4 mM $NAD^+$ and 40 mM ethanol.

suggest that ADH-4 is a dimeric enzyme composed of two identical subunits.

Like the human class I ADHs (15,16), hamster testis ADH is extremely sensitive to 4-methylpyrazole and OP inhibition. While 4-methylpyrazole inhibits the enyzme in a competitive fashion ($K_i$=0.5 $\mu$M), OP is a mixed type inhibitor ($K_i$=0.13 mM)(Fig. 2).

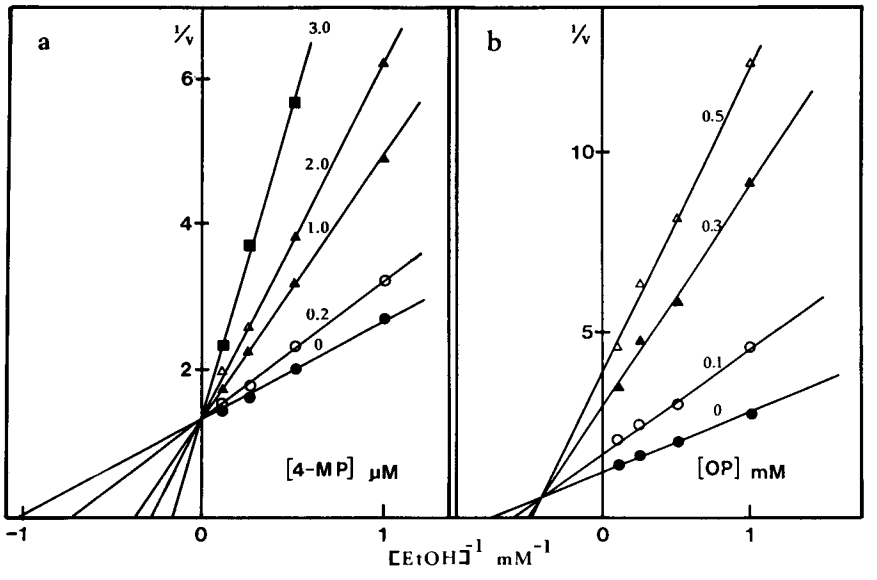


Fig. 2 Lineweaver-Burk plots of (a) 4-MP and (b) OP inhibition of hamster testis ADH catalysed ethanol oxidation. Initial velocities were determined at pH 7.5 in 0.1 M sodium phosphate containing 2.4 mM $NAD^+$ and varied inhibitor and ethanol concentrations.

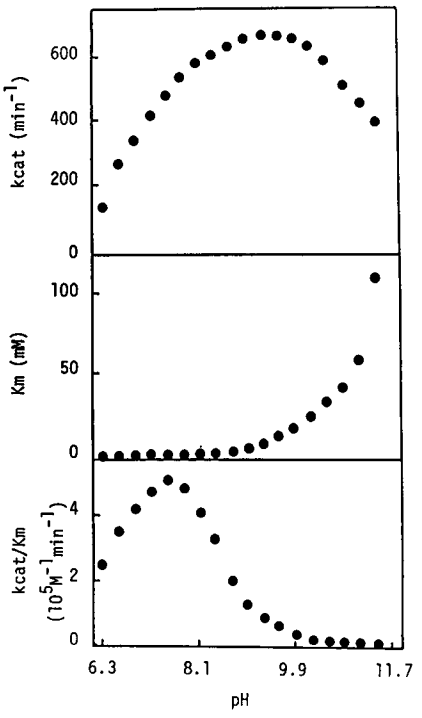


Fig. 3 pH-(a) $k_{cat}$, (b) $K_m$, and (c) $k_{cat}/K_m$ profiles for hamster testis ADH catalysed ethanol oxidation. Initial velocities were determined in 0.1 M glycine-NaOH (pH 11.4 to pH 9.3), sodium pyrophosphate (pH 9.3 to pH 7.5) or sodium phosphate (pH 7.5 to 6.3) buffers.

The effect of pH on the kinetic properties of ADH-4 was studied using ethanol as substrate and the pH-$K_m$, pH-$k_{cat}$, and pH-$k_{cat}/K_m$ profiles are shown in Fig. 3. The value of $k_{cat}$ is optimal at a pH between 9 and 10 whereas the $K_m$ value decreases logarithmically from 0.11 M at pH 11.4 to 0.52 mM at pH 6.3. The $k_{cat}/K_m$ is maximal at pH 7.5.

The steady state kinetics of ADH-4 was studied at pH 7.5 using a series of primary and secondary alcohol as substrates. The kinetic parameters are shown in Table 2. With the exception of methanol, the enzyme oxidizes all the primary alcohols very efficiently. Both $K_m$ and $k_{cat}$ values decrease as the chain length of the substrate increases. The $k_{cat}/K_m$ values indicate that alcohols with longer chain length are better substrates.

At pH 7.5, 12-hydroxydodecanoate is virtually not oxidized by the testis ADH. However, it is an effective competitive inhibitor of ethanol oxidation with a $K_i$=1 $\mu$M. At pH 10 however, this substrate is oxidized very efficiently. Secondary alcohols are also oxidized by the testis ADH but with much higher $K_m$'s and as a consequence, the $k_{cat}/K_m$ values determined for secondary

Table 2: Kinetic parameters for hamster testis ADH[a]

| Substrate | $K_m$ ($\mu$M) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($M^{-1}$ $min^{-1}$) |
|---|---|---|---|
| ethanol | 960 | 480 | $5.0 \times 10^5$ |
| 1-butanol | 440 | 450 | $1.0 \times 10^6$ |
| 1-hexanol | 69 | 182 | $2.6 \times 10^6$ |
| 12-hydroxy-dodecanoate[b] | 50 | 146 | $2.9 \times 10^6$ |
| all-trans-retinol[c] | 20 | 78 | $3.9 \times 10^6$ |
| benzyl alcohol | 410 | 82 | $2.0 \times 10^5$ |
| 2-butanol | 250000 | 285 | $1.1 \times 10^3$ |
| cyclohexanol | 31000 | 122 | $3.9 \times 10^3$ |
| $NAD^+$[d] | 57 | 465 | $8.2 \times 10^6$ |
| $NADP^+$(2.4 mM) | no activity | | |

[a] Activity was determined at pH 7.5 in 0.1 M sodium phosphate containing 2.4 mM $NAD^+$ and various concentrations of alcohol substrates. Kinetic parameters were estimated by Lineweaver-Burk plots.

[b] Determined at pH 10 in 0.1 M glycine-NaOH buffer.

[c] Retinol oxidation was studied at pH 7.5 in 0.1 M sodium phosphate containing 0.02% Tween 80 (22).

[d] Kinetic parameters for $NAD^+$ were estimated at saturating ethanol concentration, 40 mM.

alcohols are about 1000 fold lower than those for the primary alcohols.

The coenzyme requirement for the testis ADH, using ethanol as substrate, indicates that the enzyme requires $NAD^+$ for activity. $NADP^+$, at a concentration of 2.4 mM yielded no activity.

It is of interest to note that vitamin A (retinol) is oxidized most efficiently by the testis ADH. It is well established that vitamin A is essential for developement and growth(17-19). Vitamin A is ingested as the relatively inactive retinol and is oxidized to the more active retinoic acid or its metabolites in vivo (19-20). The fact that hamster testis ADH oxidizes retinol so efficiently suggests that this enzyme may play an important role in the development of the golden hamsters. To further substantiate this hypothesis, a study on the changes in ADH activity in the golden hamster testes has been carried out and the result is shown in Fig. 4. During prepubertal development in the golden hamster, there is major age-related changes in the testicular ADH activity. Since throughout the prepubertal

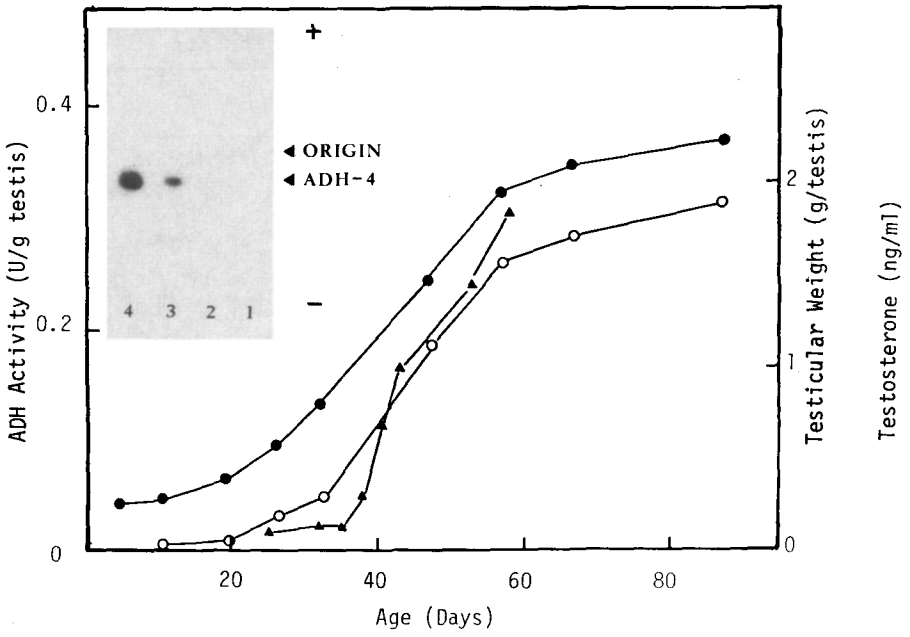


Fig. 4 Changes in testis ADH activity ( ● ), testicular weight ( O ) and plasma testosterone level ( ▲ ) during the development of golden hamsters. Testes from hamsters of different ages were homogenized and centrifuged for 1 h at 38,000 g. ADH activities in the supernatants were determined at pH 7.5 in 0.1 M sodium phosphate containing 2.4 mM $NAD^+$ and 40 mM ethanol. Background activities, determined in the presence of 1 mM 4-MP, were subtracted from data. Plasma testosterone data were taken from reference 20. Insert: starch gel electrophoretogram of testis extracts from hamsters of different ages: (1) 12 days, (2) 25 days, (3) 41 days and (4) 62 days. Extracts from equal amount (40 $\mu$g) of testis were applied to each sample well.

development, no ADH isozyme other than ADH-4 was detected in the testes (Fig. 4 insert), the observed changes in testicular ADH activity reflects the changes in the level of ADH-4. Between 20 and 55 days of age, there is greater than 10 fold increase in testicular ADH activity. Such increase is in general parallel to, but preceding slightly, the increase in testicular weight and plasma testosterone concentrations reported previously (21). These results implicate the involvement of testis ADH in the development of the male hamsters' reproductive system. Further investigation on this subject is in progress.

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