PROPERTIES OF OCTANOL DEHYDROGENASE FROM DROSOPHILA

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1. Introduction

As many as ten different alcohol dehydrogenase isozymes have been detected upon electrophoresis of total Drosophila homogenates in agar gels [1]. Genetic and preliminary biochemical evidence indicated that the complexity of the pattern is due to the presence of two different enzyme systems which are independently polymorphic at their respective gene loci; seven of the isozymes are termed alcohol dehydrogenase (ADH), and three are termed octanol dehydrogenase (ODH) [2]. ADH has been purified and partially characterized [3–6]. The present paper describes the partial purification of ODH and the determination of its substrate specificity. ODH has a marked preference for long-chain primary alcohols in contrast to ADH, which favors secondary alcohols. A molecular weight of approx. 110,000 is reported for ODH, as compared to values between 44,000 [3] and 60,000 [6] for ADH.

2. Materials and methods

Since ADH and ODH partially overlap in their substrate specificities [1], an ADH-negative strain of Drosophila (w; Adh^{nl} [7]) was used. The organisms were grown in mass cultures on standard Drosophila food [8]. They were either processed immediately after collection or kept frozen at −20° for up to 6 months.

ODH was routinely assayed in 50 mM sodium carbonate–bicarbonate, pH 10.2 containing 2 mM NAD^+ and 150 mM n-butanol. Other buffers and substrates are indicated under Results. The reaction was followed at 340 nm in a Gilford 2400 recording spectrophotometer at 25°. Initial velocities were measured, and activities expressed as International Units. Protein concentration was determined according to [9] or [10], using BSA as a standard.

The molecular weight of ODH was estimated by the gel-filtration method of Andrews [11] using a 1.6 × 82 cm column of Sephadex G-150 equilibrated in 10 mM sodium phosphate, pH 7.5, 1 mM EDTA, 2% (v/v of a saturated solution) phenylthiourea (PTU, to prevent the formation of melanin by tyrosinase). Yeast ADH (M.W. 148,000), pig heart lactate dehydrogenase (M.W. 109,000), horse liver ADH (M.W. 78,000), pig heart isocitrate dehydrogenase (M.W. 61,000) and egg white lysozyme (M.W. 13,930) were used as molecular weight standards for calibrating the column.

Electrophoresis was done either in agar gels [1] or on strips of cellulosepolyacetate [12]. ODH activity was detected as described earlier for ADH [1] varying only the pH (Tris-HCl, pH 8.5) and the type of alcohol used as substrate. For staining the Sepharose strips, this mixture was solidified into a gel by adding 0.5% Agar noble, and the staining gels used as overlays. For protein staining, the cellulose acetate strips were

Table 1
Partial purification of ODH.

Step	Total protein (mg)	Total activity (IU)	Specific activity (IU/mg)	Yield (%)
Crude extract	1528.2	11.8	0.008	100
Ammonium sulfate (60–80%)	325.3	10.1	0.031	85.9
DEAE-Sephadex A-50	15.4	5.4	0.350	45.9
Hydroxylapatite	3.1	1.8	0.570	15.5

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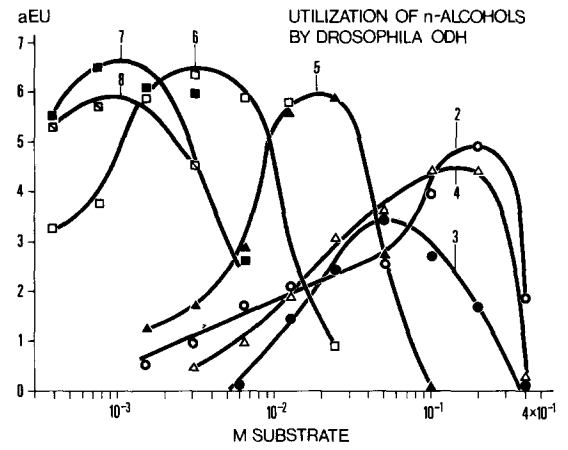

Fig. 1. Substrate specificity of Drosophila ODH. Initial velocities in crude homogenates were determined spectrophotometrically as described in Methods, using various primary alcohols differing in chain length (numbers). Arbitrary enzyme units are used on the ordinate to show relative activities.

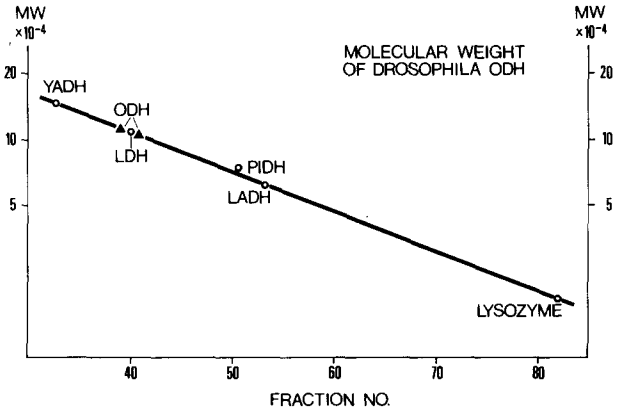

Fig. 2. Gel filtration of Drosophila ODH (▲) and various marker enzymes (○). YADH, yeast alcohol dehydrogenase; LDH, pig heart lactate dehydrogenase; LADH, horse liver ADH; PIDH, pig heart isocitrate dehydrogenase.

fixed in 5% TCA for 10 min, stained in 0.5% Coomassie Brilliant-Blue (20 min) and destained in 5% acetic acid. A typical zymogram obtained on agar gels using n-octanol as the substrate has been published elsewhere ([2], fig. 1).

3. Results

Qualitatively, ODH activity was detected on agar gels with the following substrates (Merck): ethanol, n-propanol, n-butanol, n-pentanol, n-hexanol, n-heptanol, n-octanol, benzyl alcohol, farnesol (synthetic stereoisomer mixture, kindly provided by Dr. J.P. McCormick). No activity was detected using methanol, iso-propanol, 2-butanol, 3-butanol, iso-butanol, 2-pentanol, 3-pentanol, 2-hexanol, 3-hexanol, 2-heptanol, 3-heptanol, 2-octanol, cyclohexanol.

ODH activity was also determined spectrophotometrically using various alcohols (Merck) at concentrations ranging from 4×10^{-4} M to 0.4 M. Fig. 1 shows that ODH exhibits a strong preference for long-chain alcohols. Notice also that in general, the longer the carbon chain is, the lower are the substrate concentrations at which substrate inhibition begins to be observed. Because of its good solubility in water as well as its broad activity maximum, n-butanol was adopted as substrate for all routine assays. Using it in 50 mM sodium phosphate and sodium carbonate buffers, the pH optimum for the spectrophotometric assay was determined to be 10.2.

Fig. 2 shows the elution position of ODH relative to the marker enzymes. Two separate determinations gave molecular weights of 106,000 and 112,000. The molecular weight is therefore estimated to be 109,000.

Table 1 summarizes the partial purification of ODH. All operations were carried out at 4°. Centrifugations were for 1 hr at 30,000 *g*. Fifty g of flies were homogenized in 250 ml of 0.1 M Tris-HCl, pH 8.3, 1 mM EDTA, 1 mM dithiothreitol (DTT), and 2% (v/v) of a saturated solution of PTU using a Sorvall Omnimixer at top speed for 3 X 15 sec. After centrifugation the supernatant was fractionated with ammonium sulfate allowing 3 hr for equilibration between centrifugations. The 60–80% precipitate was resuspended and dialysed exhaustively against 0.1 M Tris-HCl, pH 8.3, 1 mM EDTA, 2% PTU, 0.5 mM DTT. The dialysate was applied to 3.3 X 31.5 cm column of Sephadex A-50 equilibrated in the same buffer and ODH activity was eluted with a linear 0–0.5 M NaCl gradient in dialysis buffer. Fractions containing ODH activity were pooled and dialysed exhaustively against 20 mM sodium phosphate, pH 6.5, 2% PTU, 0.5 mM DTT. The dialysate was applied to a 2 X 7 cm column of hydroxylapatite equilibrated in the same buffer. The column was washed

with successive 30 ml aliquots of 60 mM, and 100 mM sodium phosphate before being eluted with 100 ml of 200 mM buffer. A recovery of 15% with a purification of almost 75-fold (more than 100-fold in the peak fraction) was obtained.

4. Discussion

ODH has a molecular weight of roughly twice that of ADH. On genetic grounds we can exclude the possibility that ODH is merely a multimer of ADH: the structural genes responsible for electrophoretic variants of the two enzyme systems are located on different chromosome [2]. It is of course possible that these genes evolved from one another by duplication, mutation, and translocation.

An obvious drawback of the purification protocol used is the low yield. This is due to the instability of the enzyme which we have not overcome thus far.

Controversy exists on the nature of ADH-isozymes in vertebrates [13, 14]. The fact that some isozymes of horse liver ADH have been reported in some laboratories but not in others is explained [13] by the observation that occasionally livers are used in which one or the other isozyme is "mostly weak". Our own demonstration of genetic polymorphisms in invertebrate alcohol dehydrogenase raises the possibility that the conflicting results discussed in [13] reflect similar polymorphisms in vertebrates.

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