

The metabolism of ethanol-derived acetaldehyde by alcohol dehydrogenase (EC 1.1.1.1) and aldehyde dehydrogenase (EC 1.2.1.3) in *Drosophila melanogaster* larvae

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Both aldehyde dehydrogenase (ALDH, EC 1.2.1.3) and the aldehyde dehydrogenase activity of alcohol dehydrogenase (ADH, EC 1.1.1.1) were found to coexist in *Drosophila melanogaster* larvae. The enzymes, however, showed different inhibition patterns with respect to pyrazole, cyanamide and disulphiram. ALDH-1 and ALDH-2 isoenzymes were detected in larvae by electrophoretic methods. Nonetheless, in tracer studies *in vivo*, more than 75% of the acetaldehyde converted to acetate by the ADH ethanol-degrading pathway appeared to be also catalysed by the ADH enzyme. The larval fat body probably was the major site of this pathway.

INTRODUCTION

Because the fruit fly, *Drosophila melanogaster*, feeds on fermenting plant materials, the metabolism of alcohols by this species is of special ecological interest. *D. melanogaster* is both very tolerant to the toxic effects of the major environmental alcohol, ethanol (van Delden, 1982), and is able to use ethanol efficiently as an energy source at low concentrations (Geer *et al.*, 1985). Studies using two different experimental approaches indicate that more than 90% of the ethanol is degraded in *D. melanogaster* by a pathway initiated by alcohol dehydrogenase (ADH, EC 1.1.1.1) (Geer *et al.*, 1985; Heinstra *et al.*, 1987).

In mammals, ADH is the major enzyme catalysing the initial oxidation of ethanol (Rognstad & Grunnet, 1979), and aldehyde dehydrogenase (ALDH, EC 1.2.1.3), an enzyme present in isoenzymic forms in different subcellular fractions, is responsible for the subsequent oxidation of the acetaldehyde into acetate (Weiner, 1979*a,b*). However, there are two viewpoints with respect to the second step of the ethanol degradation pathway in *D. melanogaster*. According to one viewpoint, this reaction is catalysed by an ALDH enzyme (Lietaert *et al.*, 1982; Garcin *et al.*, 1983, 1985; David *et al.*, 1984), whereas it has also been postulated that *Drosophila* ADH has dual catalytic properties and can oxidize acetaldehyde as well as ethanol (Heinstra *et al.*, 1983; Eisses *et al.*, 1985; Geer *et al.*, 1985; Moxom *et al.*, 1985). Unlike the mammalian ADH, the ADH of *Drosophila* is not a metalloenzyme, and it differs significantly in size and amino acid sequence (Jörnvall *et al.*, 1981, 1984); consequently, the diverse properties of the ADHs are not surprising. The issue of acetaldehyde oxidation in *Drosophila* also warrants clarification because Garcin *et al.* (1985) have stated that an ALDH activity was

not coincident with the ADH enzyme activity, whereas Heinstra *et al.* (1983) and Moxom *et al.* (1985) were unable to detect a separate ALDH activity from that of the ADH enzyme.

The purpose of this investigation was to establish the relative importance of the ADH and the ALDH enzymes in the oxidation of ethanol-derived acetaldehyde in *D. melanogaster*. In this study we confirmed that an ALDH enzyme (EC 1.2.1.3) is indeed present in *Drosophila* larvae, but found that the activity of this enzyme differs from the ALDH activity of the ADH enzyme in several ways. Moreover, studies of the flux of ethanol into lipid suggested that more than 75% of the oxidation of acetaldehyde in wild-type *D. melanogaster* larvae is catalysed by ADH.

MATERIALS AND METHODS

Cyanamide, disulphiram, NAD (Grade III), pyrazole, ²H₂O, Nitro Blue Tetrazolium and phenazine methosulphate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. For the n.m.r. studies we used NAD (Grade I), pyruvate and lactate dehydrogenase (rabbit muscle) from Boehringer, Mannheim, Germany, and [2-¹³C]ethanol from MSD Isotopes, Montreal, Canada. Alcohols and aldehydes were of the highest analytical grade available. For other reagents, see Heinstra *et al.* (1983) and Geer *et al.* (1985).

Strains and dietary conditions

The Canton-S wild-type strain homozygous for the *Adh^F* allele; a wild-type strain homozygous for the *Adh^{71k}* (Heinstra *et al.*, 1987); the Tahbilk wild-type strain homozygous for the *Adh^S* (Geer *et al.*, 1988); and the Groningen wild-type strain homozygous for the *Adh^S*

Abbreviations used: ADH, alcohol dehydrogenase (EC 1.1.1.1); ALDH, aldehyde dehydrogenase (EC 1.2.1.3); LDH, lactate dehydrogenase (EC 1.1.1.27); AO, aldehyde oxidase (EC 1.2.3.1).

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(Heinstra *et al.*, 1988) were employed in different experiments. ADH-null activity strains used in the studies were *Adhⁿ² pr; cn*, *Adhⁿ⁶ pr; cn*, and *bAdhⁿ⁴* (Heinstra *et al.*, 1987; Laurie-Ahlberg & Stam, 1987). A strain of the sibling species *D. simulans* was from Malaga, Spain (Heinstra *et al.*, 1987).

Larvae were grown under axenic conditions on a defined synthetic medium (Geer *et al.*, 1985, 1988) supplemented with either 0.3% or 1% sucrose (w/v), and transferred to different test cultures as described by Geer *et al.* (1976, 1983). The test cultures contained 40–80 larvae per vial and were maintained at 22.8 °C and 50% relative humidity with a 15 h-light–9 h-dark schedule. Under these conditions, wild-type larvae pupate in about 9.5 days when fed a 1% sucrose diet. Alcohols and other test compounds were added to the diets according to Geer *et al.* (1985). Details of the experimental outline for each given test can be found in the Results section.

Enzyme assays

ADH and ALDH were assayed in whole larval homogenates by following the change of absorbance at 340 nm in the reaction mixture with a Gilford Model 222 spectrophotometer at 30 °C. The ADH activity was routinely assayed by the method of McKechnie & Geer (1984) using 100 mM-ethanol as the substrate. The activity of ALDH was assayed in a 50 mM-Tris/HCl buffer, pH 8.5/1 mM-NAD⁺ mixture with 1.8 mM-acetaldehyde as the substrate. Pyrazole (2 mM) was added to inhibit ADH activity. The pH dependence was studied using a 50 mM-sodium phosphate buffer pH 7.4, and a 50 mM-sodium pyrophosphate buffer, pH 9.6. The background activity of the ALDH assay, about 10% of total ALDH activity, was monitored during each assay. When the effects of various inhibitors were tested, the enzyme and the inhibitor were added to the reaction mixture and the substrate was added after a 2 min incubation period.

The same homogenizing buffer was used for ADH and ALDH. Ten third-instar larvae were homogenized with a Teflon-pestle homogenizer in 0.16 ml of isolation buffer consisting of 50 mM-Na₂HPO₄/NaH₂PO₄ buffer, pH 7.4, 0.24 M-sucrose, 0.5 mM-EDTA, 0.5 mM-dithiothreitol, 0.001% (w/v) phenylthiourea, and 1% (v/v) Triton X-100. The homogenate was allowed to stand for 15 min and was then centrifuged at 15000 g for 20 min at 4 °C. The supernatant was used in all experiments. All operations were conducted at 4 °C. The soluble protein content of the homogenates was assayed by the method of Bradford (1976) using bovine serum albumin as the standard. Tissues were dissected from third-instar larvae in freshly prepared *Drosophila* Ringer's solution (Butterworth *et al.*, 1965).

Lipid and electrophoretic analysis

[1,2-¹⁴C]Ethanol and [U-¹⁴C]glucose were traced into lipid analysis by the methods of Geer *et al.* (1985). Electrophoresis was done according to procedures given by Laemmli (1970) using 10% polyacrylamide slab gels. A sample (25 µl) of the larval extracts was applied to each well, and gels were electrophoresed for 2 h at 4 °C under a constant current of 11 mA per gel. The enzyme bands were revealed by staining the gels in the dark at 30 °C for 30–60 min. The staining solution contained a 50 mM-Tris/HCl buffer, pH 8.5, 1 mM-NAD⁺, 0.5 mM-Nitro Blue Tetrazolium, 1 µM-phenazine methosulphate and either 1 mM-acetaldehyde, or 1 or 2 mM-benz-

aldehyde as the substrate. Gels were washed several times with water, and then fixed in 5% acetic acid solution.

Gas chromatographic analysis

The propan-2-ol and acetone concentrations in the incubation mixture were determined by injecting samples into a Carle 211 Analytical Chromatograph equipped with a 1.83 m × 0.33 cm Carbopack B column (Supelco Co., Bellefonte, PA, U.S.A.) and a 3390A Hewlett-Packard Integrator. The separation of acetone and propan-2-ol was completed at 90 °C with N₂ serving as the carrier gas at 15 ml/min.

Nuclear magnetic resonance (n.m.r.)

Proton-¹³C-n.m.r. spectroscopy was performed in a Bruker WM 200 apparatus. ADH from different *Drosophila* strains was purified from adults as reported previously (Eisses *et al.*, 1985; Heinstra *et al.*, 1988). Purified protein was dried under vacuum evaporation at 4 °C, and brought into a small volume of ²H₂O-borate/NaOH buffer, pH 8.0. The reaction mixture during the n.m.r. studies consisted of 50 mM-[2-¹³C]ethanol, 1 mM-NAD, 20 mM-pyruvate, 1 I.U. of lactate dehydrogenase (LDH), and aliquots of enzyme in a 200 mM-²H₂O-borate/NaOH buffer, pH 8.0.

The reactions were followed at 25 °C and analysed after 96 scans (3.5 min) of the reaction tubes at 200 MHz with presaturation of the ²H₂O signal. Pyruvate and LDH were included in the reaction mixture to regenerate NAD⁺ from NADH.

Statistical analysis

Data were analysed using Student's *t* test (Snedecor & Cochran, 1980) to assess significant differences.

RESULTS

Spectrophotometric assays

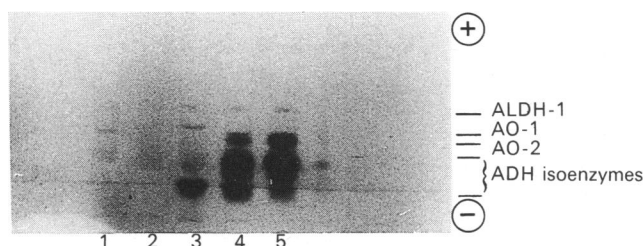
First, our major objective was to determine the existence of an ALDH enzyme separate from the ALDH activity of the ADH enzyme in *D. melanogaster* larvae. The ALDH activity of ADH has been identified and characterized by several independent investigators (Heinstra *et al.*, 1983; Eisses *et al.*, 1985; Geer *et al.*, 1985; Moxom *et al.*, 1985). The inclusion of Triton X-100 in the isolation buffer was found to be essential for the assay of ALDH, otherwise little activity was found. Presumably, ALDH must be liberated from membranes for activity analysis.

The analysis of ALDH activity in homogenates of an ADH-null activity strain (*Adhⁿ²*) is shown in Table 1. The ALDH enzyme was slightly inhibited by 2.2 mM-pyrazole, but strongly inhibited by 1 mM-cyanamide, and showed a pH-dependent inhibition with 0.145 mM-disulfiram. Neither 0.1 M-ethanol nor 1.1 M-methanol (necessary to dissolve disulfiram) affected the ALDH activity. In similar experiments with larval extracts of Canton-S wild-type larvae, no ALDH activity was found at pH 7.4, but after inclusion of 2.2 mM-pyrazole, the ALDH activity was similar to that of the ADH-null strain. In the absence of the ADH inhibitor, the NADH produced by the ALDH enzyme was apparently used by ADH to reduce the acetaldehyde. At pH 8.5, without pyrazole, about 80% of the maximum ALDH activity was recorded, and at pH 9.6 full ALDH activity was found. Cyanamide and disulfiram inhibited the ALDH

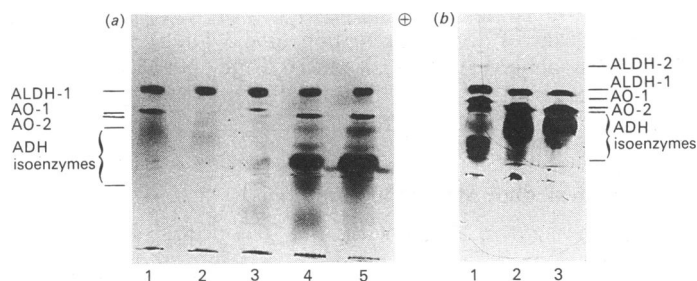
Table 1. Effects of inhibitors on ALDH and ADH in *D. melanogaster*

For the determination of ALDH activity, a larval extract from an ADH-null activity strain (*Adhⁿ²*) was used. For the determination of ADH activity, a larval extract from the Canton-S wild-type strain was used. Concentrations of the inhibitors were 2.2 mM-pyrazole, 1 mM-cyanamide and 0.145 mM-disulfiram. Activities are given in μmol of NADH produced/min per ml of extract. Standard errors were lower than 5% ($n = 3$). Relative activities are given in parentheses.

Condition	Enzyme... pH...	Activity (μmol of NADH/min per ml of extract)					
		ALDH			ADH		
		7.4	8.5	9.6	7.4	8.5	9.6
Control		0.068 (100)	0.128 (100)	0.126 (100)	0.198 (100)	0.296 (100)	0.457 (100)
+ Pyrazole		0.063 (93)	0.112 (88)	0.114 (90)	0.028 (14)	0.019 (6)	0.028 (6)
+ Cyanamide		0.005 (7)	0.013 (10)	0.030 (24)	0.174 (88)	0.221 (75)	0.359 (79)
+ Disulfiram		0.039 (57)	0.025 (20)	0.009 (7)	0.184 (93)	0.248 (84)	0.325 (71)

**Fig. 1. Polyacrylamide-gel electrophoresis of larval extracts from different *Adh*-genotypes of *Drosophila* with acetaldehyde as substrate**

Gels were stained for aldehyde dehydrogenase activity with 1 mM-acetaldehyde as the substrate. Genotypes: lane 1, *bAdh⁴*; lane 2, *Adhⁿ²*; *cinnamon*; lane 3, wild-type *D. simulans*; lane 4, *Adh^{S/S}* of *D. melanogaster*; lane 5, *Adh^{F/F}* of *D. melanogaster*.

**Fig. 2. Polyacrylamide-gel electrophoresis of larval extracts from different *Adh*-genotypes of *Drosophila* with benzaldehyde as substrate**

Gels were stained for aldehyde dehydrogenase activity with 1 mM-benzaldehyde as the substrate for 30 min (a), and 2 mM-benzaldehyde for 60 min (b). Loading gel (a) was as described in Fig. 1. Loading gel (b): lane 1, wild-type *D. simulans*; lane 2, *Adh^{S/S}* of *D. melanogaster*; lane 3, *Adh^{F/F}* of *D. melanogaster*.

shown that pyrazole and disulfiram inhibit the ALDH activity of the crude as well as the purified ADH enzyme (Heinstra *et al.*, 1983; Moxom *et al.*, 1985).

In summary, our data showed that ALDH and ADH in *Drosophila* are different proteins with different inhibition patterns.

Electrophoretic analysis

ALDH activity in larval homogenates was examined by slab-gel polyacrylamide-gel electrophoresis (Figs. 1 and 2). With 1 mM-acetaldehyde as the substrate, the ADH isoenzymes from *D. melanogaster* strains showed high activity, whereas the ADH isoenzymes from *D. simulans* exhibited low activity (Fig. 1; Heinstra *et al.*, 1983). ADH bands were absent from the lanes of extracts of the two ADH-null strains, *bAdh⁴* and *Adhⁿ²*; *cinnamon*.

Aldehyde oxidase (AO, EC 1.2.3.1) isoenzymes were identified (1) as bands that appeared in gels that were stained with reaction mixtures lacking NAD^+ , and (2) as bands which were absent in gel lanes loaded with homogenates of *Adhⁿ²*; *cin*, also an AO-negative strain. Another low-activity band that was found towards the anode in all strains with acetaldehyde as the substrate was tentatively identified as ALDH-1 (Fig. 1). The activities of the two AO isoenzymes and the ALDH-1 isoenzyme were greatly enhanced by the use of benzaldehyde as the substrate, whereas the activities of the ADH isoenzymes were diminished (Figs. 2a and 2b). After prolonged incubation, another ALDH activity band closer to the anode became visible (tentatively ALDH-2; Fig. 2b). This band was present in all larval extracts from genetically different strains. There was no electrophoretic mobility variation between the ALDH-1 and ALDH-2 bands of the different strains. Larvae fed cyanamide, an ALDH inhibitor, showed a strongly reduced ALDH-1 activity, whereas AO activity was not affected at all.

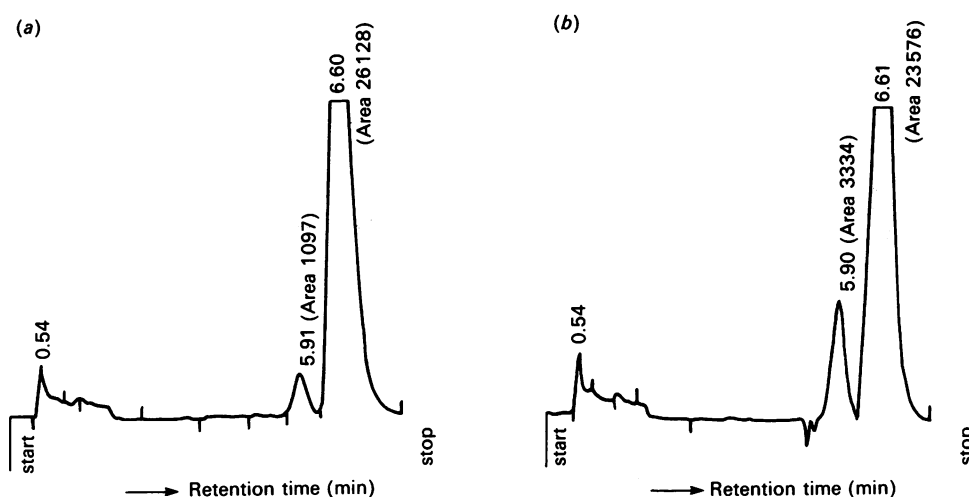
In summary, two isoenzymes of ALDH were found in addition to the ALDH activity of ADH in *Drosophila*. Our data do not allow us to determine whether one or two *Adh* loci are involved and/or whether either isoenzyme is the product of a post-translational modification of one isoenzyme. Preliminary observations in our laboratory suggest that the ALDH-1 isoenzyme is located in the mitochondrion.

of Canton-S larvae in a similar manner as with the ADH-null larvae. Pyrazole strongly inhibited ADH activity, whereas cyanamide and disulfiram were moderate inhibitors of ADH (Table 1). Previously, it has been

Table 2. Tissue distribution of activities of ADH and ALDH in *Drosophila*

Activities are expressed in μmol of NADH produced/min per ml of tissue extract (\pm s.d.). Means significantly different from either *Adh*^{F/F} intestine or fat body for ADH activity or to the means of ALDH activity in intestines are indicated by * $P < 0.05$. ($n = 3$).

Enzyme...	Tissue...	Activity (μmol of NADH/min per ml of extract)			
		ADH		ALDH	
		Intestine	Fat body	Intestine	Fat body
<i>Adh</i> ^{F/F}		0.126 \pm 0.004	0.124 \pm 0.010	0.063 \pm 0.005	0.035 \pm 0.003*
<i>Adh</i> ^{S/S}		0.054 \pm 0.003*	0.060 \pm 0.007*	0.054 \pm 0.008	0.025 \pm 0.003*
<i>Adh</i> ^{simulans}		0.056 \pm 0.005*	0.059 \pm 0.007*	0.053 \pm 0.007	0.037 \pm 0.010

**Fig. 3. Gas chromatographic analysis of ADH-mediated formation of acetone from tissues incubated with propan-2-ol at 22 °C**

Intestines (with Malpighian tubules) and fat bodies were isolated from five Canton-S third-instar larvae, pooled separately in 10 μl of Ringer solution, and then 25 μl propan-2-ol was added to give a final substrate concentration of 3.7 mM. After 15 min intervals, 5 μl of the incubation mixture was analysed by means of g.l.c. Peaks of acetone (retention time 5.9 min) and of propan-2-ol (retention time 6.6 min) are shown after a 60 min incubation period. Acetone produced from intestines (a) corresponds to 155 μM and from fat bodies (b) to 475 μM .

Tissue distribution

The expression of both the overall ALDH enzyme and the ADH enzyme was determined in tissues from third-instar larvae. Maroni & Stamey (1983) have reported that ADH activity in the intestine and the fat body represents about 90% of the total ADH activity present in larvae homozygous for *Adh*^F. Consequently, expressions of ALDH and ADH were examined only in intestines and fat bodies.

The activities of the ADH at the tissue level were similar in intestines and in fat bodies of the three *Adh*-genotypes (Table 2). On the other hand, the ALDH activity was about 2-fold greater in the intestine than in the fat body. The ratio of ADH/ALDH activities in the intestines of larvae homozygous for *Adh*^F is 2, for the other *Adh*-genotypes it is 1. This ratio in fat bodies ranges from about 4 for larvae homozygous for *Adh*^F to about 1.5 for *D. simulans* (Table 2). Apparently, the expression of the *Aldh* gene(s) in tissues is different from that of the *Adh* gene.

Aspects of alcohol metabolism *in vitro*

The one-step oxidation of the secondary alcohol, propan-2-ol, into the end product, acetone, allowed an assessment of alcohol metabolism *in vivo* (Heinstra *et al.*, 1986, 1987). A similar approach can be used to assess aspects of alcohol metabolism at the tissue level *in vitro*. Intestines and fat bodies from five larvae were pooled and their ability to convert propan-2-ol into acetone *in vitro* was analysed by gas chromatographic methods. Acetone was formed in a linear relationship over a 60 min period; the fat bodies produced three times more acetone than intestines (Fig. 3), suggesting that the fat bodies represent the major site of alcohol degradation.

Proton-¹³C-n.m.r. studies of ethanol degradation *in vitro*

To assess the individual ability of purified ADHs from *Drosophila* to convert ethanol into acetaldehyde and then into acetate, proton-n.m.r. spectroscopy was applied (Fig. 4). Both the ADH-S and the ADH-71k alloenzymes from *D. melanogaster* were capable of forming acet-

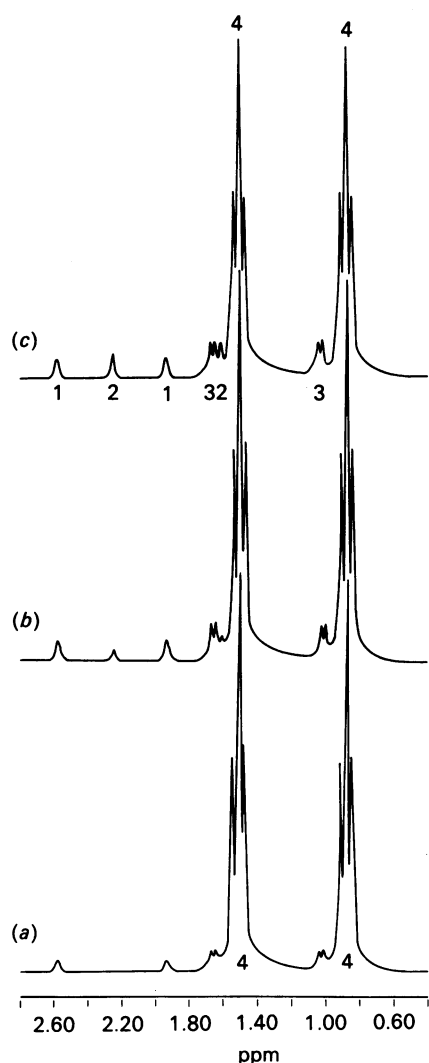


Fig. 4. Proton-¹³C-n.m.r. pictures from [2-¹³C]ethanol catabolism catalysed by three purified ADHs from *Drosophila*

Pictures show the formation of acetaldehyde (1), acetate (2), hydrated-aldehyde (3) from ethanol (4) degradation by the ADH from *D. simulans* (a), by the ADH-71k variant (b), and by the ADH-S variant (c) from *D. melanogaster*.

aldehyde (and its hydrated -diol- form) and acetate. On the other hand, the ADH from *D. simulans* showed lower activity towards ethanol, producing lower levels of acetaldehyde/diol- aldehyde, but no detectable acetate.

Aspects of ethanol metabolism *in vivo*

The differential expression of both aldehyde-dehydrogenating enzymes, and their coexistence, raises the critical question as to which of the two enzymes (or both) has the primary responsibility for the oxidation of the ethanol-derived acetaldehyde *in vivo*. The ethanol- and acetaldehyde-oxidizing properties of *Drosophila* ADH apparently reside on the same active site (Heinstra *et al.*, 1983; Geer *et al.*, 1985; Moxom *et al.*, 1985). Consequently, it would not be possible to inhibit the acetaldehyde-oxidizing reaction of the ADH without affecting the ethanol-oxidizing reaction. Therefore, emphasis was placed on the inhibition of the activity of ALDH by cyanamide *in vivo*. Disulfiram was not used because it must be dissolved in methanol. Methanol is not a substrate for *Drosophila* ADH, but it is a competitive inhibitor (Winberg *et al.*, 1982).

Pre-feeding mid third-instar larvae with 62.5 or 125 μ M-cyanamide for 24 h resulted in a complete inhibition of the ALDH activity (Table 3). ADH activity was also decreased 20–25% by cyanamide (Table 3). After the initial exposure of larvae to cyanamide, larvae were transferred to either [U-¹⁴C]glucose or [1,2-¹⁴C]ethanol for a 17 h pulse-label into lipid. Cyanamide was not included in the latter diets because it caused some adverse effects on larval survival during the first part of the experimentation. ALDH and ADH activities were assayed in the test larvae at the beginning and end of the pulse-labelling period. The incorporation of label into lipid from the two substrates was used as an indication of the flux through the glucose- and ethanol-degrading pathways into lipid. Theoretically, the glucose-to-lipid pathway should be independent of the activities of ADH and ALDH. Conversely, the ethanol-to-lipid pathway should require one or both activities, depending on the degree of involvement of the enzymes in the acetaldehyde-to-acetate conversion.

On average, flux from ethanol into lipid was decreased by cyanamide by about 20%, whereas ADH-null activity larvae incorporated only 10% of the level observed in

Table 3. ALDH and ADH activities from extracts of *D. melanogaster* larvae pre-fed with cyanamide, before and after feeding with ethanol

Canton-S wild-type larvae were reared on standard medium (1% sucrose) until 4 days old. Then they were transferred to 1% sucrose medium with either 62.5 μ M or 125.0 μ M-cyanamide. After 24 h, all the test groups were transferred to 0.3% sucrose medium containing 7.7 μ Ci of [1,2-¹⁴C]ethanol (0.2 M total ethanol) per 100 ml of medium. Control and ADH-null activity (*Adh^{tn6}*) larvae were without inhibitor, etc. Larvae were maintained on the ethanol medium for 17 h. Activities are given in nmol of NADH produced/min per mg of protein. Means \pm s.d. are given for six determinations. Means significantly different from corresponding control values are indicated by * $P < 0.05$. N.D., not determined.

Strain/diet	Enzyme...	Activity (nmol of NADH/min per mg of protein)			
		ALDH		ADH	
		Before	After flux	Before	After flux
<i>Adh^{F/F}</i> /control		63.3 \pm 6.7	82.0 \pm 10.8	103.2 \pm 9.1	108.7 \pm 31.3
<i>Adh^{F/F}</i> /low cyanamide		0.8 \pm 0.9*	20.7 \pm 3.2*	70.3 \pm 15.2	86.5 \pm 10.5
<i>Adh^{F/F}</i> /high cyanamide		0.1 \pm 1.8*	11.3 \pm 3.4*	79.7 \pm 12.4	85.2 \pm 12.9
<i>Adh^{tn6}</i> /control		N.D.	145.1 \pm 15.6	N.D.	2.6 \pm 1.9*

Table 4. Incorporation of label from ethanol or glucose into lipid in *D. melanogaster* larvae

Four-day-old third-instar larvae were transferred to 1% sucrose medium alone (control) or 1% sucrose medium with cyanamide. After 24 h on the intervening diet, larvae were transferred to 0.3% sucrose medium with 7.7 μCi of [1,2- ^{14}C]ethanol (0.2 M total ethanol) per 100 ml of medium or with 12.8 μCi of [U- ^{14}C]glucose per 100 ml of medium. Larvae were maintained on the latter media for 17 h before analysis. The number of independent replicates is given in parentheses. The results are expressed as nmol of ethanol or glucose incorporated into lipid/mg of larval protein (\pm s.d.). N.D., not determined.

Strain/intervening diet	Substrate...	Incorporation into lipid (nmol/mg of protein)	
		[1,2- ^{14}C]ethanol ($n = 6$)	[U- ^{14}C]glucose ($n = 5$)
<i>Adh^{F1F}</i> /control		3795 \pm 596	221 \pm 28
<i>Adh^{F1F}</i> /62.5 μM -cyanamide		3163 \pm 330	N.D.
<i>Adh^{F1F}</i> /125.0 μM -cyanamide		3077 \pm 262	205 \pm 35
<i>Adh^{f^{m6}}</i> /control		477 \pm 56	201 \pm 40

wild-type larvae (Table 4). This corresponded to the inhibition of ADH activity rather than the inhibition of ALDH activity. ALDH activity was still inhibited by 70–90% after the pulse-label period, whereas ADH activity was inhibited by 20–25% (Table 3). The *Adh^{f^{m6}}* strain was characterized by a high ALDH activity compared to the other test strains. This activity level was similar before the ethanol pulse experiments (results not shown), suggesting a genetic cause. The flux from glucose into lipid in the test strains was hardly affected by cyanamide (Table 4), suggesting that cyanamide did not exert a general effect on carbohydrate metabolism. The slight restoration of ALDH activity during the pulse period was not ethanol-dependent, because similar results were found after transfer of the larvae to the standard medium.

DISCUSSION

In the current study we have determined that an ALDH enzyme and the ALDH activity of ADH coexist in the larvae of *D. melanogaster*, and we have evaluated the relative contribution of each enzyme to the conversion of acetaldehyde into acetate *in vivo*. Our present attempts by proton- ^{13}C -n.m.r. methods to assess the capacities of ADH alloenzymes to form acetaldehyde and acetate *in vitro* were mixed. Nonetheless, the ADH-S and ADH-71k alloenzymes were found to form these products. Because equilibria of the reactions of the ethanol-degrading pathway may be pulled far to the right *in vivo* (Middleton & Kacser, 1983; Geer *et al.*, 1985; Heinstra *et al.*, 1987) and this is not the case with the proton-n.m.r. reactions *in vitro*, the relative contributions of the ALDH and ADH enzymes to acetaldehyde-to-acetate conversion were monitored by flux methods *in vivo*. By following the incorporation of [^{14}C]ethanol into lipid, the relative importance of the two activities to the ethanol-degrading pathway was estimated.

Although cyanamide inhibited the activity of ALDH by more than 90% at the highest concentration employed in the current investigation, the flux from ethanol to lipid declined by only 20%. This matched the cyanamide- (or a derivative, DeMaster *et al.*, 1984) induced decrease in the ADH reaction with ethanol as the substrate. If ALDH represents the major activity for acetaldehyde-to-acetate conversion, an accumulation of acetaldehyde would be expected in larvae exposed to ethanol after

cyanamide treatment (DeMaster *et al.*, 1984). We have found by gas chromatographic analysis that larvae exposed to this dietary regime accumulate relatively high levels of intracellular ethanol without accumulating acetaldehyde. Also, in the present study the tissue pattern of alcohol degradation as determined by g.l.c. also matched the tissue pattern for ADH more closely than that of ALDH. Compared to the gut, the larval fat body was observed to be the major location of alcohol degradation *in situ*, and the ratios of the activities of *D. melanogaster* ADH alloenzymes to the ALDH activity in the fat body were greater than in the gut. Moreover, ADH activity with acetaldehyde *in vitro* as the substrate ranges from 15 to 40% of that of ethanol oxidation (Heinstra *et al.*, 1983; Geer *et al.*, 1985; Moxom *et al.*, 1985; Eisses, 1989). Collectively, these observations suggest that the ALDH activity of the ADH enzyme is the major catalyst of the acetaldehyde-to-acetate reaction *in vivo* in *D. melanogaster*.

If the ALDH enzyme does not play a major role in ethanol degradation in *D. melanogaster*, its other function(s) are not immediately obvious. However, there are many aldehydes in intermediary metabolism. It perhaps is noteworthy that wild-type larvae fed cyanamide develop into adult flies as phenocopies of the mutant 'cripple' [see Lindsley & Grell (1968) for a full description of this mutant]. Further studies will be directed to see whether this gene is associated with ALDH and if the gene has important functions in the normal development of insect legs.

Why the ALDH activity of ADH, and not the ALDH enzyme, catalyses the conversion of most of the acetaldehyde to acetate in *D. melanogaster* can only be speculated upon, but the manner in which aldehydes are hydrated may be a factor in determining this metabolic phenomenon. In aqueous solutions, 60–75% of the acetaldehyde is present in its hydrated diol-form (Bodley & Blair, 1971; Brooks *et al.*, 1985; this study). Mammalian ALDHs have a strong preference for the unhydrated forms of aldehydes, e.g. benzaldehyde, which is completely unhydrated (Bodley & Blair, 1971). *Drosophila* ADH was observed in the current investigation to have a higher reactivity with acetaldehyde than with benzaldehyde after electrophoresis. On the other hand, *Drosophila* ALDH exhibited a higher reactivity with benzaldehyde than with acetaldehyde. These traits of *Drosophila* ALDH and ADH may explain in part why acetaldehyde (actually its diol-form) (see also

Eisses, 1989), is metabolized to a greater extent by ADH than by ALDH.

At first glance, the major involvement of ADH rather than ALDH in the acetaldehyde-to-acetate reaction in *D. melanogaster* seems a radical departure from other animal metabolic systems. Nevertheless, the properties of ALDH and ADH in *D. melanogaster* differ in many aspects, e.g. inhibition patterns, electrophoretic mobilities of their isoenzymes, and in tissue distribution. Furthermore, mammalian and *D. melanogaster* forms of the ADH enzyme differ markedly. *Drosophila* ADH is a smaller enzyme than its mammalian counterpart and it is not a metalloenzyme (Jörnvall *et al.*, 1981, 1984). Moreover, *Drosophila* ADH is inhibited by cyanamide (or a derivative) which is not the case for mammalian ADHs (Marchner & Tottmar, 1976; Svanas & Weiner, 1985).

A technical point previously obscured the ALDH enzyme from study in our and other laboratories (Heinstra *et al.*, 1983). A strong detergent such as Triton X-100 must be used in the homogenization buffer to liberate the membrane-bound enzyme.

In conclusion, although an ALDH enzyme and an ADH enzyme with ALDH activity coexist in *D. melanogaster*, based upon several independent considerations, the acetaldehyde-to-acetate conversion in the ethanol-degrading pathway *in vivo* appears to be largely mediated as a second-half reaction of ADH.

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