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Subcellular Distribution and Properties of Aldehyde Dehydrogenase from 2-Acetylaminofluorene-Induced Rat Hepatomas

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The subcellular distribution and properties of four aldehyde dehydrogenase isoenzymes (I–IV) identified in 2-acetylaminofluorene-induced rat hepatomas and three aldehyde dehydrogenases (I–III) identified in normal rat liver are compared. In normal liver, mitochondria (50%) and microsomal fraction (27%) possess the majority of the aldehyde dehydrogenase, with cytosol possessing little, if any, activity. Isoenzymes I–III can be identified in both fractions and differ from each other on the basis of substrate and coenzyme specificity, substrate K_m, inhibition by disulfiram and anti-(hepatoma aldehyde dehydrogenase) sera, and/or isoelectric point. Hepatomas possess considerable cytosolic aldehyde dehydrogenase (20%), in addition to mitochondrial (23%) and microsomal (35%) activity. Although isoenzymes I–III are present in tumour mitochondrial and microsomal fractions, little isoenzyme I or II is found in cytosol. Of hepatoma cytosolic aldehyde dehydrogenase activity, 50% is a hepatoma-specific isoenzyme (IV), differing in several properties from isoenzymes I–III; the remainder of the tumour cytosolic activity is due to isoenzyme III (48%). The data indicate that the tumour-specific aldehyde dehydrogenase phenotype is explainable by qualitative and quantitative changes involving primarily cytosolic and microsomal aldehyde dehydrogenase. The qualitative change requires the derepression of a gene for an aldehyde dehydrogenase expressed in normal liver only after exposure to potentially harmful xenobiotics. The quantitative change involves both an increase in activity and a change in subcellular location of a basal normal-liver aldehyde dehydrogenase isoenzyme.

Our investigations of the mechanisms underlying the expression of a series of aldehyde dehydrogenase isoenzymes [aldehyde–NAD(P)$^+$ oxidoreductase, EC 1.2.1.3 and 1.2.1.5] unique to chemically induced rat hepatomas (Lindahl & Feinstein, 1976; Lindahl, 1977, 1978; Lindahl *et al.*, 1978) indicate that events in addition to gene derepression contribute to the generation of the hepatoma-specific aldehyde dehydrogenase phenotype. The hepatoma-specific isoenzymes consistently differ in a number of physical and functional properties from normal-liver aldehyde dehydrogenase (Lindahl & Feinstein, 1976; Lindahl, 1977). Antisera generated against the hepatoma-specific aldehyde dehydrogenases possess two distinct antibody populations. Antibodies to the hepatoma-specific isoenzymes react with complete identity with a normal-liver aldehyde dehydrogenase inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, but not with a phenobarbital-inducible normal-liver isoenzyme, or with non-induced normal-liver aldehyde dehydrogenases (Lindahl *et al.*, 1978). In whole tissue homogenates, the second antibody population recognizes a basal, non-inducible normal-liver aldehyde dehydrogenase which accounts for 35% of the normal-liver aldehyde dehydrogenase activity (Lindahl, 1978).

Observations of a significant contribution by normal aldehyde dehydrogenases to the hepatoma-specific aldehyde dehydrogenase phenotype has prompted an examination of the subcellular distribution of the aldehyde dehydrogenase activity in hepatomas and normal liver to identify more specifically the nature of this contribution. This paper compares the properties of a number of aldehyde dehydrogenase isoenzymes identified in both hepatomas and normal liver and presents evidence suggesting that both gene derepression and reorganization of the normal-liver aldehyde dehydrogenase phenotype occur during the formation of the hepatoma-specific aldehyde dehydrogenase isoenzymes.

Materials and Methods

Tissue fractionation

Normal livers were obtained from adult male and female Sprague–Dawley rats killed by cervical dislocation. Livers were placed in ice-cold 60mM-sodium phosphate buffer (pH 8.5) containing 1mM-EDTA, 1mM-β-mercaptoethanol and 250mM-sucrose (buffered sucrose). Frozen pooled hepatomas induced

in male Sprague–Dawley rats by 2-acetylaminofluorene by the method of Peraino *et al.* (1971) were kindly provided by Dr. Robert N. Feinstein of Argonne National Laboratory. Occasionally, a frozen normal liver was fractionated to examine the effects of freezing. No significant changes in enzyme distribution or activities were observed.

All procedures were performed at 0–4°C. A 5g portion of normal liver or hepatoma was homogenized for 3min at 1100rev./min in 20ml of buffered sucrose in a Potter–Elvehjem tissue homogenizer. The homogenate was made to 10% (w/v) by the addition of buffered sucrose and fractionated according to the principles of de Duve *et al.* (1955) as modified below after several trial fractionations. The homogenate was centrifuged for 10min at $400g_{max.}$, the supernatant drawn off and the pellet washed with 2×25ml of buffered sucrose. The pellet was then resuspended in 10ml of 60mM-sodium phosphate buffer (pH8.5) containing 1mM-EDTA and 1mM-β-mercaptoethanol to yield the nuclear (N) fraction. The pooled supernatant, designated cytoplasmic extract (E), was centrifuged for 10min at $7500g_{max.}$, the pellet washed twice and finally resuspended in 10ml of buffer to give a mitochondrial fraction (M). The combined supernatant was centrifuged for 10min at $19000g_{max.}$, the pellet washed twice with buffered sucrose and resuspended in 10ml of buffer to give the lysosomal fraction (L). The pooled supernatant was centrifuged for 60min at $110000g_{max.}$, the surface of the pellet gently washed with buffered sucrose and resuspended in 10ml of buffer to yield the microsomal fraction (P). The final combined supernatant is considered the true cytosol (S). The fractions were routinely separated into two 5ml batches and frozen at −20°C.

Preparation of fractions

For spectrophotometric assays, fractions were thawed and adjusted to 1% with Triton X-100, incubated for 30min at 0°C and centrifuged at $48000g_{max.}$ for 30min at 4°C to remove any debris. The resulting supernatants were used for analysis. For isoelectric focusing, fractions were thawed and sonicated as described previously (Lindahl, 1977). Although less activity was released by sonication than by Triton X-100, the resolution of enzyme activities after electrofocusing was found to be superior when sonicated samples were used. No differential loss of any particular activity was noted in assay or in electrofocusing owing to sonication.

Enzyme assays

All assays were performed at 23°C unless otherwise stated. Aldehyde dehydrogenase activity was determined spectrophotometrically as described previously (Lindahl, 1977) in 60mM-sodium phosphate buffer (pH8.5) containing 1mM-EDTA and 1mM-β-mercaptoethanol. Occasionally, tetraethylthiuram disulfide (disulfiram, Antabuse) (100mM final concn.) was included to study its effect on aldehyde dehydrogenase. Disulfiram was dissolved in methanol (89mg/10ml) and 10μl of this solution was then added to 2.74ml of assay mixture containing sample, but no substrate. After a 5min preincubation, the reaction was initiated by the addition of 0.25ml of substrate. When disulfiram was to be included, β-mercaptoethanol was omitted from both the fractionation and assay buffers.

As marker enzymes for fractions M, L and P respectively, monoamine oxidase, acid phosphatase and NADPH–cytochrome *c* reductase were assayed as described by Tottmar *et al.* (1974). Lactate dehydrogenase (marker for fraction S) was determined as described in the *Worthington Enzyme Manual* (1977; Worthington Biochemical Corp., Freehold, NJ, U.S.A.). The assay mixture consisted of 0.1ml of 6.6mM-NADH and 0.1ml of 30mM-sodium pyruvate in 2.8ml of 60mM-sodium phosphate buffer (pH7.5). The reaction was started by the addition of sample and the decrease in A_{340} resulting from the oxidation of NADH was measured for 5min.

Activities are expressed either in munits (1 unit = 1μmol/min)/g of tissue or munits/mg of protein as appropriate. Protein concentrations were determined by the method of Lowry *et al.* (1951) by using bovine serum albumin as standard.

Immunoabsorption

Pooled anti-(rat hepatoma aldehyde dehydrogenase) sera produced in rabbits were employed (Lindahl & Feinstein, 1976). Immune reactions were performed as previously described (Lindahl, 1978).

Miscellaneous methods

Analytical isoelectric focusing in polyacrylamide-gel slabs was done with the LKB (Bromma, Sweden) apparatus and methods, and the gels were stained for aldehyde dehydrogenase as described by Feinstein (1976). Control gels were stained without added substrate to test for activity due to endogenous substrates or without coenzyme to test for aldehyde oxidase.

Results

The subcellular distributions of aldehyde dehydrogenase activity in hepatomas and normal liver reveal several differences (Figs. 1 and 2; Table 1). With propionaldehyde and NAD^+, normal-liver mitochondrial and microsomal fractions possess 49.5 and 26.9%, respectively, of the total aldehyde dehydrogenase activity. On the basis of marker-enzyme distributions, the majority of the nuclear-

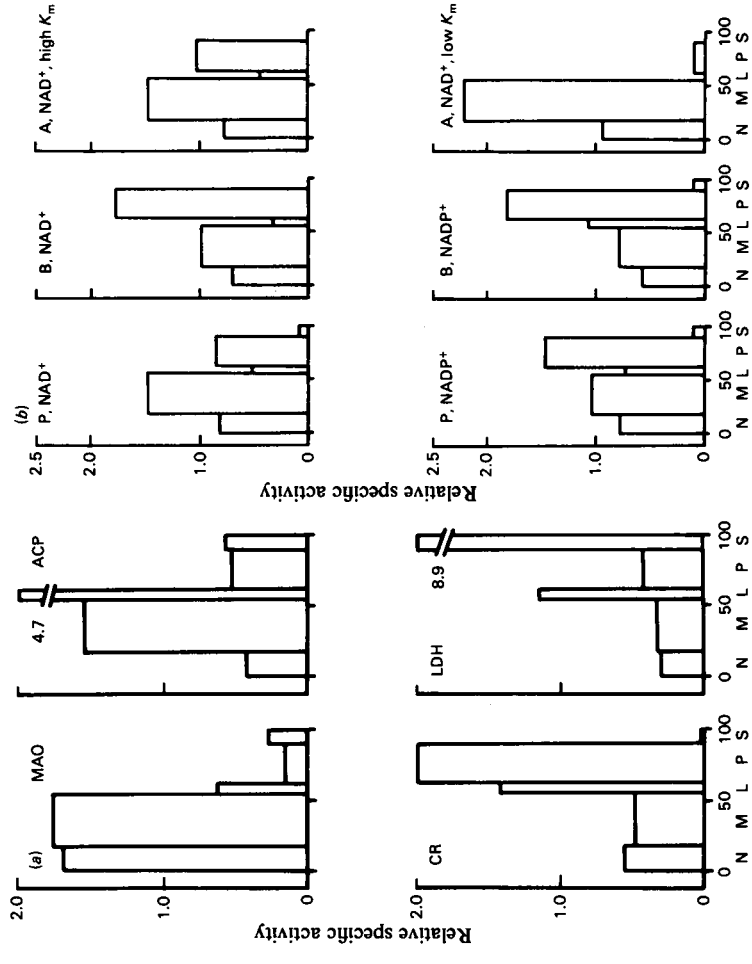

Fig. 1. *(a) Marker-enzyme-activity distributions and (b) aldehyde dehydrogenase activity in normal-liver subcellular fractions*

(*a*) Key: MAO, monoamine oxidase; ACP, acid phosphatase; CR, NADPH–cytochrome *c* reductase; LDH, lactate dehydrogenase. Relative specific activity is the ratio of percentage of activity to the percentage of protein for each fraction. (*b*) Key: P, propionaldehyde; B, benzaldehyde; A, acetaldehyde; high-K_m, activity measured with 5mM-acetaldehyde; low-K_m, activity measured with 5 μM-acetaldehyde. The subcellular fractions are defined in the text.

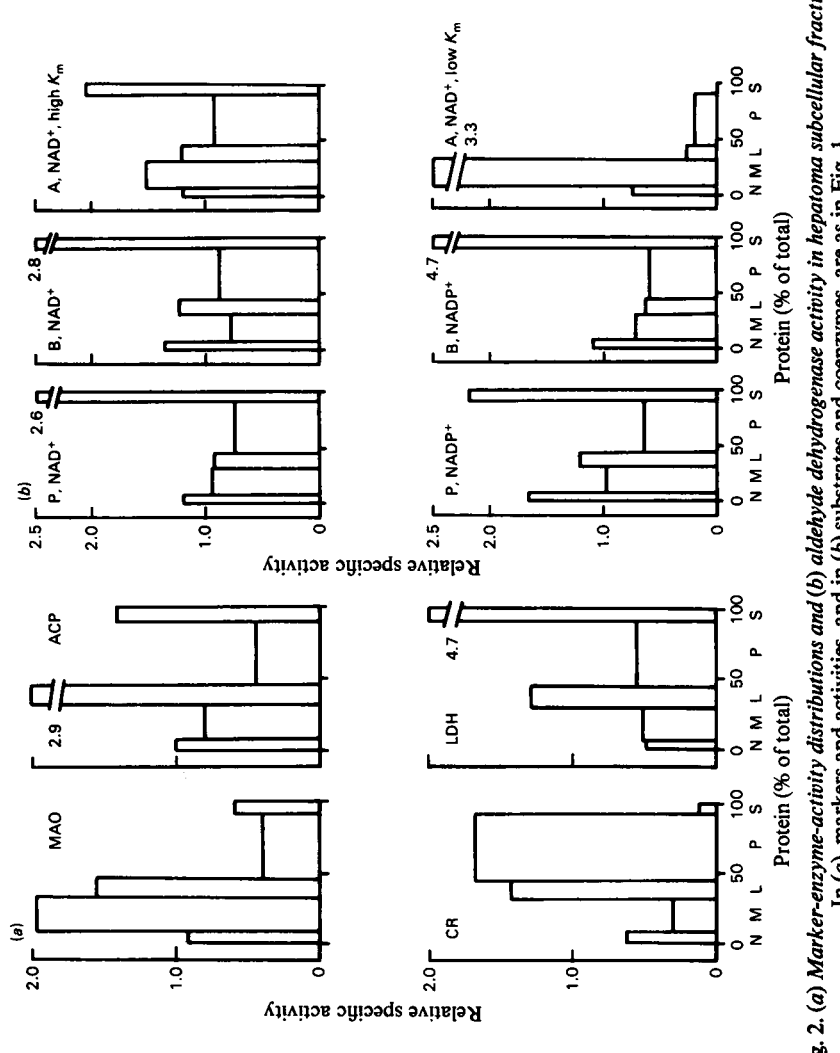

Fig. 2. *(a) Marker-enzyme-activity distributions and (b) aldehyde dehydrogenase activity in hepatoma subcellular fractions*
In *(a)*, markers and activities, and in *(b)* substrates and coenzymes, are as in Fig. 1.

fraction activity is due to contamination by mitochondria. The lysosomal and cytosolic fractions possess little NAD^+-dependent aldehyde dehydrogenase activity. Hepatoma mitochondrial and microsomal fractions possess 23.6 and 35.0%, respectively, of the total aldehyde dehydrogenase when assayed with propionaldehyde and NAD^+. The nuclear fraction accounts for 8.4% of the total, with the lysosomal and cytosolic fractions accounting for 13.2 and 19.8%, respectively, of the total hepatoma aldehyde dehydrogenase (Fig. 2 and Table 1).

With benzaldehyde and $NADP^+$, the differences in subcellular distribution are more striking (Figs. 1 and 2; Table 1). The mitochondrial and microsomal fractions account for 29.5 and 52.0% of the total normal-liver aldehyde dehydrogenase. The nuclear, lysosomal and cytosolic fractions possess about the same percentage activity as with propionaldehyde and NAD^+. In hepatomas, the cytosol accounts for 40.6% of the total aldehyde dehydrogenase when assayed with benzaldehyde and $NADP^+$. The microsomal and lysosomal fractions account for 28.6 and 11.5%, respectively, of the hepatoma activity, and the mitochondrial and nuclear fractions, 13.9 and 5.3%. Both marker-enzyme and aldehyde dehydrogenase determinations indicate that the majority of the nuclear and lysosomal activity in both tissues is due to mitochondrial and/or microsomal contamination. Therefore, subsequent studies concentrate on the properties of the mitochondrial, microsomal and cytosolic aldehyde dehydrogenases from normal liver and hepatomas.

The aldehyde dehydrogenase and marker-enzyme distributions in hepatoma fractions as well as in fractions obtained from either fresh or pooled frozen normal livers indicate that the decreased mitochondrial and increased cytosolic activity in hepatomas is not due to leakage into the cytosol of aldehyde dehydrogenase from a freeze–thaw-damaged mitochondrial or microsomal population in hepatomas. However, as fresh tumour material was not available, we cannot rule out the possibility that hepatoma mitochondria are inherently structurally labile.

Differences in aldehyde dehydrogenase substrate and coenzyme specificity exist in normal-liver and hepatoma subcellular fractions (Figs. 1 and 2; additional results not shown). Both normal-liver and hepatoma mitochondria possess high- and low-K_m aldehyde dehydrogenases (for acetaldehyde and NAD^+). In both, the total activity and ratio of high-K_m to low-K_m activities (2.0) are nearly identical. NAD^+ is the preferred coenzyme in both mitochondrial fractions for all substrates tested. With $NADP^+$, aromatic aldehydes are the preferred substrates in mitochondria from both tissues.

In microsomal fractions, very little low-K_m activity is detectable (Figs. 1 and 2). Compared with the situation in mitochondria, $NADP^+$ is a very good coenzyme with benzaldehyde and its 4-chloro and 4-nitro derivatives, as well as phenylacetaldehyde. In fact, hepatoma microsomal fraction oxidizes these aromatic aldehydes more rapidly with $NADP^+$ than with NAD^+. In normal-liver microsomal fraction, NAD^+ is the preferred coenzyme with all substrates tested, except propionaldehyde, where $NADP^+$ is preferred.

Hepatoma cytosol aldehyde dehydrogenase consists exclusively of high-K_m activity characterized by very high $NADP^+$-dependent activity with benzaldehyde and its derivatives, but not phenylacetaldehyde. With NAD^+ as coenzyme, hepatoma cytosol aldehyde dehydrogenase is much like tumour microsomal aldehyde dehydrogenase, except that cytosol possesses very little activity with acetaldehyde at any concentration.

Disulfiram significantly inhibits the NAD^+-dependent aldehyde dehydrogenase activity of the normal-liver mitochondrial fraction (Table 2). Normal-liver microsomal NAD^+-dependent aldehyde dehydrogenase activity is only slightly decreased in the presence of disulfiram. Disulfiram significantly decreases the $NADP^+$-dependent activity of

Table 1. *Aldehyde dehydrogenase activity in normal-liver and hepatoma subcellular fractions*
Activity (munits/g of tissue) is the average ± S.E.M. for the numbers of determinations indicated in parentheses.

Substrate, coenzyme	Fraction ... N	M	L	P	S	Sum of N–S
Normal liver						
Propionaldehyde, NAD^+	(10) 43.4±3.6	119.0±9.6	10.7±1.6	64.8±7.7	2.6±0.5	240.5
Propionaldehyde, $NADP^+$	(10) 38.1±4.6	65.8±6.2	9.9±1.4	85.2±7.4	1.7±0.3	200.7
Benzaldehyde, NAD^+	(3) 13.2±4.9	29.1±1.2	1.9±1.5	39.5±1.5	1.7±0.6	85.4
Benzaldehyde, $NADP^+$	(3) 8.6±2.7	17.1±2.6	1.5±0.2	30.1±3.9	0.6±0.3	57.9
Hepatoma						
Propionaldehyde, NAD^+	(8) 27.5±1.7	77.7±8.9	43.6±3.3	115.0±16.0	65.2±11.3	329.0
Propionaldehyde, $NADP^+$	(3) 28.2±1.1	53.2±1.1	40.9±6.2	106.6±2.4	34.1±2.1	262.9
Benzaldehyde, NAD^+	(4) 13.7±0.9	30.9±4.8	24.7±1.4	60.8±6.0	32.1±2.2	162.2
Benzaldehyde, $NADP^+$	(7) 26.2±3.4	68.3±17.4	56.4±15.5	140.6±47.0	199.4±71.4	490.9

hepatoma mitochondrial and cytosolic fractions (Table 2). The NAD^+-dependent activity of hepatoma microsomal fraction is only slightly decreased by disulfiram. The mitochondrial NAD^+-dependent aldehyde dehydrogenase inhibited by disulfiram in both tissues is the low-K_m activity (>90% inhibition; results not shown).

Antisera generated against the hepatoma-specific aldehyde dehydrogenases react with normal-liver mitochondrial and microsomal aldehyde dehydrogenases, as well as hepatoma mitochondrial, microsomal and cytosolic activities (Table 3). With propionaldehyde and NAD^+, antisera remove aldehyde dehydrogenase from normal-liver mitochondrial (27%) and microsomal (55%) fractions. With propionaldehyde and $NADP^+$, the antisera remove slightly more aldehyde dehydrogenase from both fractions (32 and 62%, respectively) (Table 3). The homologous antisera remove all the $NADP^+$-dependent aldehyde dehydrogenase from hepatoma mitochondria, microsomal fraction and cytosol (Table 3). With propionaldehyde and NAD^+, the

Table 2. *Effect of disulfiram on normal-liver and hepatoma aldehyde dehydrogenase activity*
Activity (munits/g of tissue) is the average ± S.E.M. for three determinations in each tissue. Percentage inhibition is that for samples incubated with disulfiram (100 μM) compared with their corresponding controls. *Activities are significantly different from their corresponding controls at at least the $P<0.05$ level of significance by a paired t test.

	Fraction ... M		P		S	
Substrate, coenzyme	Activity	Inhibition (%)	Activity	Inhibition (%)	Activity	Inhibition (%)
Normal liver						
Propionaldehyde, NAD^+						
Control	136.6±20.8		98.1±5.0		1.5±0.3	
Disulfiram	84.5±15.3*	38.2	87.9±3.3	10.4	0.3±0.3*	78.8
Propionaldehyde, $NADP^+$						
Control	72.4±7.3		108.4±2.1		2.0±0.8	
Disulfiram	26.6±9.8*	63.3	47.7±10.9*	60.6	0.2±0.2*	88.3
Hepatoma						
Propionaldehyde, NAD^+						
Control	90.5±18.5		138.5±29.7		80.8±28.9	
Disulfiram	54.6±9.5*	39.7	122.7±28.3	11.4	34.6±11.9*	57.1
Benzaldehyde, $NADP^+$						
Control	90.7±39.3		200.8±99.1		333.8±140.5	
Disulfiram	19.9±13.2*	78.0	37.2±23.5*	83.2	4.0±2.1*	98.8

Table 3. *Effect of anti-(hepatoma aldehyde dehydrogenase) sera on normal-liver and hepatoma aldehyde dehydrogenase*
Activity (munits/g of tissue) is the average ± S.E.M. for three determinations in normal liver and the average of two determinations in hepatomas. Percentage inhibition is that for absorbed samples compared with their corresponding controls. Abbreviation: N.D., not determined. *Activities are significantly different from their corresponding controls at at least the $P<0.05$ level of significance by a paired t test.

	Fraction ... M		P		S	
Substrate, coenzyme	Activity	Inhibition (%)	Activity	Inhibition (%)	Activity	Inhibition (%)
Normal liver						
Propionaldehyde, NAD^+						
Control	27.1±2.6		28.3±5.9		N.D.	
Absorbed	19.9±0.6*	26.8	12.9±2.1*	54.6		
Propionaldehyde, $NADP^+$						
Control	18.0±2.1		28.0±3.8		N.D.	
Absorbed	12.2±1.7*	32.2	10.6±4.8*	62.1		
Hepatoma						
Propionaldehyde, NAD^+						
Control	20.3		34.8		40.6	
Absorbed	8.7	57.4	1.9	94.4	1.0	97.5
Benzaldehyde, $NADP^+$						
Control	30.9		79.3		178.7	
Absorbed	0.0	100.0	0.0	100.0	0.0	100.0

antisera remove virtually all the aldehyde dehydrogenase from hepatoma microsomal fraction (94%) and cytosol (98%) (Table 3). However, under these conditions, the antisera remove only 57% of the hepatoma mitochondrial aldehyde dehydrogenases.

Polyacrylamide-gel isoelectric focusing resolves different aldehyde dehydrogenase isoenzymes in hepatoma mitochondria, microsomal fraction and cytosol, and in normal-liver mitochondria and microsomal fraction (Fig. 3). Hepatoma cytosol consists almost exclusively of two major and several minor high-pI (pH 6.8–7.0) isoenzymes characterized by their high activity with benzaldehyde and $NADP^+$. An additional very weak activity is seen with pI 5.4–5.5, and only in the presence of small aliphatic aldehydes and NAD^+. Hepatoma microsomal fractions possess only one major high-pI isoenzyme. Hepatoma mitochondrial aldehyde dehydrogenase consists of both low-pI (5.4) and high-pI (6.8–7.0) isoenzymes. The activity of the high-pI isoenzymes in both cytosol and microsomal fraction appears to be largely unaffected by disulfiram, whereas the hepatoma mitochondrial low-pI activity is very sensitive to this inhibitor.

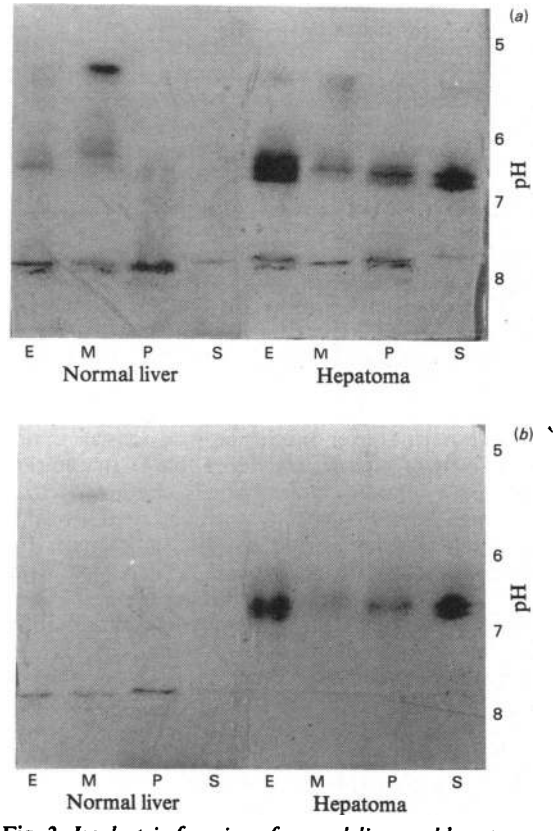

Fig. 3. *Isoelectric focusing of normal-liver and hepatoma aldehyde dehydrogenase*

(*a*) Control gel, electrofocused and incubated for 30min in 60mM-phosphate buffer, pH 7.5, before staining with propionaldehyde and NAD^+. (*b*) Disulfiram-treated gel; after electrofocusing the gel was incubated for 30min in 60mM-phosphate buffer, pH 7.5, containing 100μM-disulfiram. The gel was then stained for activity. Key: E, cytoplasmic extract; M, mitochondria; P, microsomal fraction; S, cytosol.

Normal-liver mitochondria possess both high- (pH 6.8) and low- (pH 5.4) pI isoenzymes, with the low-pI form being more active with propionaldehyde and NAD^+ (Fig. 3). Disulfiram largely eliminates the low-pI isoenzyme, without significant effect on the high-pI form. Although there is some activity very near the cathode (pI 8.5), normal-liver microsomal fraction possesses little activity at pI 5.4 or 6.8, although some activity is seen at the origin. No normal-liver isoenzyme is characterized by very high activity in the presence of benzaldehyde and $NADP^+$.

Discussion

Several studies have reported multiple aldehyde dehydrogenase activities in normal rat liver (Deitrich, 1966; Marjanen, 1972; Tottmar *et al.*, 1974; Koivula & Koivusalo, 1975). However, none have described the same number or subcellular distribution of aldehyde dehydrogenases, although most indicate at least two mitochondrial and one cytosolic form, with one microsomal activity often being described. The subcellular distribution in normal liver reported here is consistent with the observations of Tottmar *et al.* (1974), who identified two mitochondrial and one microsomal rat liver aldehyde dehydrogenase. Our results are also generally similar to those of Deitrich (1966) and Koivula & Koivusalo (1975), although both report considerable cytosolic aldehyde dehydrogenase activity.

Disulfiram is a potent inhibitor of certain rat liver aldehyde dehydrogenases. Tottmar *et al.* (1974) and Tottmar & Marchner (1976) reported that disulfiram selectively inhibits (75–100%) the low-K_m mitochondrial rat liver isoenzyme *in vivo* and *in vitro*, with little or no effect on the high-K_m mitochondrial or microsomal activity. Deitrich *et al.* (1972) reported approx. 80% inhibition of cytoplasmic rat propionaldehyde dehydrogenase activity by disulfiram. Koivula & Koivusalo (1975) described the total inhibition of a cytoplasmic phenobarbital-inducible rat liver aldehyde dehydrogenase.

On the basis of our studies, mitochondria from normal liver and hepatomas possess three aldehyde dehydrogenase isoenzymes (Table 4). Isoenzyme I is an NAD^+-specific low-K_m form (μM-K_m for acetaldehyde), with pI 5.4. Isoenzyme I is sensitive

Table 4. *Properties of aldehyde dehydrogenase isoenzymes from normal rat liver and 2-acetylaminofluorene-induced hepatomas*
Distribution values are approximate percentages (±S.E.M.) of the total aldehyde dehydrogenase activity with propionaldehyde and NAD^+ in the fraction. Percentages are based on the following: firstly the % high- versus low-K_m activity, then of the high-K_m activity; the % recognized by antisera; and finally, the % $NADP^+$-dependent inhibition by disulfiram.

Property	Isoenzyme I	Isoenzyme II	Isoenzyme III	Isoenzyme IV
Distribution (%)				
Mitochondria: Liver	34±2	39±3	27±3	0
Hepatoma	34±1	11±4	55±6	0
Microsomal fraction: Liver	3	42±2	55±4	0
Hepatoma	3	3±3	94±2	0
Cytosol: Liver	—	—	—	0
Hepatoma	1	1	48±3	50±2
K_m for acetaldehyde	μM	mM	mM	mM
Coenzyme specificity	NAD^+	$NAD(P)^+$	$NAD(P)^+$	$NAD(P)^+$
Disulfiram inhibition	With NAD^+	No	With $NADP^+$	With NAD^+ or $NADP^+$
Antisera inhibition	No	No	Yes (normal liver cross-reacting material)	Yes
pI	5.4	6.8–7.0	6.8–7.0	6.8–7.0
Substrate preference	Small aliphatic aldehydes	NAD^+ with aliphatics; $NADP^+$ with aromatics	Benzaldehyde and phenylacetaldehyde good substrates with either coenzyme	Prefers benzaldehyde and $NADP^+$; cannot use phenylacetaldehyde

to disulfiram and not recognized by anti-(hepatoma aldehyde dehydrogenase) sera. Comparison of the activities at high and low acetaldehyde concentrations indicates that isoenzyme I accounts for approx. 34% of the total mitochondrial activity in both tissues. As noted, in both normal liver and hepatomas, approx. 39% of the total NAD^+-dependent mitochondrial aldehyde dehydrogenase, including more than 90% of the low-K_m activity, is sensitive to disulfiram. This is in close agreement with the 34% of mitochondrial isoenzyme-I activity.

In the present study, two high-K_m mitochondrial aldehyde dehydrogenases, isoenzymes II and III, have been identified by their differing sensitivities to both disulfiram and anti-(hepatoma aldehyde dehydrogenase) sera. Isoenzyme II is an $NAD(P)^+$-dependent aldehyde dehydrogenase with pI 6.8–7.0, is insensitive to disulfiram, and is not recognized by antisera to the hepatoma isoenzymes. Isoenzyme III also has a pI near 6.9–7.0 and can also use NAD^+ or $NADP^+$ as coenzyme. Isoenzyme III is disulfiram-sensitive when $NADP^+$ is coenzyme. Moreover, isoenzyme III is recognized by antisera to the hepatoma isoenzymes.

From the available data, the contribution of isoenzymes II and III to the total mitochondrial aldehyde dehydrogenase activity can be estimated (Table 4). In normal liver, isoenzyme II accounts for approx. 39% of the mitochondrial activity and isoenzyme III 27%. In hepatoma mitochondria, isoenzyme II accounts for only 11% of the total activity and isoenzyme III approx. 55%. The observed decrease in total hepatoma mitochondrial aldehyde dehydrogenase is consistent with the loss of isoenzyme II. This quantitative alteration explains the increase in sensitivity of hepatoma mitochondrial aldehyde dehydrogenase to antisera and the increased $NADP^+$-dependent disulfiram sensitivity.

The microsomal aldehyde dehydrogenase activity of both normal liver and hepatomas consists almost exclusively of high-K_m activity (Table 4). In addition, hepatoma microsomal activity is characterized by very high $NADP^+$-dependent activity with benzaldehyde, its derivatives and phenylacetaldehyde. Isoenzyme-I activity is very low (3%) and most likely due to mitochondrial contamination. In both tissues, microsomal aldehyde dehydrogenase is largely disulfiram-insensitive when NAD^+ is coenzyme. This is also consistent with the observations of Tottmar *et al.* (1974) and Tottmar & Marchner (1976). However, as with the high-K_m mitochondrial activity, at least two microsomal aldehyde dehydrogenases can be identified on the basis of their sensitivities to disulfiram and antisera to the hepatoma-specific aldehyde dehydrogenases. Both tissue microsomal fractions possess isoenzymes II and III. In normal liver, isoenzyme II accounts for 42% of the total aldehyde dehydrogenase and isoenzyme III approx. 55%. In hepatoma microsomal fraction, isoenzyme II is greatly decreased, accounting for only 3% of the total activity. Isoenzyme III accounts for about 94% of the total hepatoma microsomal activity. This quantitative reorganization explains the increased disulfiram-sensitivity and

increased recognition of microsomal aldehyde dehydrogenase by antisera. The increased $NADP^+$-dependent hepatoma aldehyde dehydrogenase activity with benzaldehyde and phenylacetaldehyde is also explained by the increased isoenzyme-III activity.

In normal liver, isoenzyme III appears to be the cross-reacting material isolated and characterized previously (Lindahl, 1978). On the basis of the distribution of isoenzyme III in normal-liver mitochondrial and microsomal fractions (Tables 2 and 3) and the total normal-liver aldehyde dehydrogenase activity (Table 1), isoenzyme III accounts for approx. 33% of the total normal-liver aldehyde dehydrogenase, in close agreement with the 35% determined previously (Lindahl, 1978).

To date, studies of normal-liver mitochondrial and microsomal aldehyde dehydrogenase have reported a single low-K_m mitochondrial and a single high-K_m mitochondrial and microsomal isoenzyme (Siew *et al.*, 1976; Tottmar *et al.*, 1974; Koivula & Koivusalo, 1975). However, none of these studies has examined disulfiram sensitivity with both NAD^+ and $NADP^+$, and none has used immunochemical methods to characterize the high-K_m activities. We can occasionally resolve multiple high-pI activities in both fractions and often observe multiple activities after electrophoresis. However, the lability of the mitochondrial aldehyde dehydrogenase and the particulate nature of the high-K_m activities in both fractions (Simpson & Lindahl, 1979) have prevented their consistent resolution.

Hepatoma cytosolic aldehyde dehydrogenase activity consists exclusively of high-K_m activity characterized by high activity with benzaldehyde and its derivatives, but not phenylacetaldehyde, with $NADP^+$ as coenzyme (Table 4). As in the hepatoma microsomal fraction, virtually all (98%) of the cytosolic activity is recognized by anti-(hepatoma aldehyde dehydrogenase) sera. However, in contrast with microsomal aldehyde dehydrogenase, hepatoma cytosol is sensitive to disulfiram with NAD^+ (57% inhibition). These observations are consistent with the presence of two major hepatoma cytosolic aldehyde dehydrogenase isoenzymes. The increased disulfiram sensitivity and high activity with benzaldehyde and $NADP^+$, but not phenylacetaldehyde, is due to the presence of a new isoenzyme unique to hepatoma cytosol. This isoenzyme, IV, has many of the physical and functional properties of a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible normal-liver cytosolic isoenzyme (Deitrich *et al.*, 1977; Lindahl *et al.*, 1978). Isoenzyme IV is also like the phenobarbital-inducible normal-liver isoenzyme isolated by Koivula & Koivusalo (1975), in that it is very disulfiram-sensitive. However, we have demonstrated that the hepatoma-specific aldehyde dehydrogenases are not related to the phenobarbital-inducible normal-liver isoenzyme (Lindahl & Feinstein, 1976; Lindahl *et al.*, 1978). Our results indicate that the tetrachlorodibenzodioxin-inducible normal-liver aldehyde dehydrogenase should be very sensitive to disulfiram and should possess high activity with benzaldehyde and its derivatives with $NADP^+$ as coenzyme.

Isoenzyme IV accounts for approx. 50% of the total cytosolic aldehyde dehydrogenase, with the remaining disulfiram-insensitive activity being due to isoenzyme III. Isoenzymes I and II could account for no more than 2% of the total cytosolic activity. Although isoenzyme III is the major microsomal isoenzyme in hepatomas, the high hepatoma microsomal activity and marker-enzyme determinations suggest that the isoenzyme III in hepatoma cytosol is not due to damage to microsomal membranes caused by freezing or fractionation.

Our results indicate that both normal liver and hepatomas possess aldehyde dehydrogenase isoenzymes with similar properties in mitochondrial and microsomal fractions. In addition, hepatoma cytosol possesses an aldehyde dehydrogenase with properties not found in any normal-liver isoenzyme. The differences between normal-liver and hepatoma aldehyde dehydrogenase reported earlier in whole tissue homogenates (Feinstein & Cameron, 1972; Lindahl & Feinstein, 1976) may be explained by qualitative and quantitative changes involving primarily cytosolic and microsomal aldehyde dehydrogenase. The qualitative change requires the derepression of a gene for an aldehyde dehydrogenase expressed in normal liver only after exposure to potentially harmful xenobiotics (Lindahl *et al.*, 1978; Deitrich *et al.*, 1978). The quantitative change involves both an increase in activity and a change in subcellular location of a basal normal-liver aldehyde dehydrogenase isoenzyme.

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