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# PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION OF ALDEHYDE DEHYDROGENASE FROM 2-ACETYLAMINOFLUORENE-INDUCED RAT HEPATOMAS

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## Summary

1. A series of aldehyde dehydrogenase isozymes (aldehyde:NAD (P)$^+$ oxidoreductase, EC 1.2.1.5), has been purified from hepatomas ** induced in Sprague-Dawley rats by 2-acetylaminofluorene.

2. The functional hepatoma-specific aldehyde dehydrogenase isozymes exist as 105 000-dalton dimers composed of two subunits of 53 000 daltons. Isoelectric points of the purified isozymes are 6.9—7.2.

3. Antiserum to these purified hepatoma-specific aldehyde dehydrogenases has been produced and the immunological relationships of these isozymes to their normal liver counterpart have been studied. Results of Ouchterlony double diffusions, agar-gel immunoelectrophoresis and polyacrylamide gel and agar immunoelectrophoresis indicate that anti-hepatoma aldehyde dehydrogenase antiserum cross-reacts with normal liver aldehyde dehydrogenase.

## Introduction

Aldehyde dehydrogenase (aldehyde:NAD (P)$^+$oxidoreductase, EC 1.2.1.5.) in hepatomas induced in Sprague-Dawley rats by 2-acetylaminofluorine (2-AAF) exists as three major and several minor isozymes. In normal liver, only one aldehyde dehydrogenase isozyme is detectable by polyacrylamide gel elec-

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** We are aware of the recent workshop at the National Cancer Institute (Cancer Res. (1975) 35, 3214) at which the term "hepatoma" was disapproved. However, for convenience, and with no histological connotation implied, we should like to use the term in this paper to refer to the wide spectrum of liver tumors examined.

Abbreviations used in this paper: 2-AAF, 2-acetylaminofluorine; SDS, sodium dodecyl sulfate.

trophoresis [1—2]. These hepatoma aldehyde dehydrogenase possess activity with a broad spectrum of aldehyde substrates and can use either NAD or NADP as coenzyme. The normal enzyme can use only NAD and possesses a much narrower substrate specificity. The hepatoma aldehyde dehydrogenases are much more stable than their normal liver counterpart to changes in pH, increasing heat and increasing urea concentration [2]. The enzymes from the two sources also differ in several other biochemical properties [1—2].

All 2-AAF-induced hepatomas examined to date show the same pattern of new aldehyde dehydrogenase isozymes and the same biochemical differences from normal liver aldehyde dehydrogenase. These similarities indicate that the new isozymes are not the result of a somatic mutation. Rather, these isozymes are the result of either the derepression of a normally repressed gene(s) or the post-translational modification of otherwise normal enzyme species. This paper describes the purification of aldehyde dehydrogenase from 2-AAF-induced hepatomas and the immunochemical properties of both tumor and normal liver aldehyde dehydrogenase. Despite numerous attempts, the extreme temperature and pH instability of Sprague-Dawley normal liver aldehyde dehydrogenase [2] has prevented its purification.

## Materials and Methods

Male Sprague-Dawley rats were used. Hepatomas, induced as described by Peraino et al. [3], were obtained from rats killed by cervical dislocation. The tumors were excised, rinsed free of blood, cut into small pieces, immediately frozen in a solid $CO_2$/acetone bath, and stored at $-70°C$ until needed.

Aldehyde dehydrogenase activity was determined at 25°C by following the reduction of $NAD^+$ to NADH at 340 nm during the oxidation of benzaldehyde, basically as described by Racker [4]. The reaction mixture contained 1.0 ml of 0.1 M sodium phosphate buffer at pH 8.5, 1.0 ml of 1.0% aqueous NAD or NADP solution, 0.25 ml of a saturated benzaldehyde solution, 10—200 µl of enzyme and water to 3.0 ml. Enzyme was added last and the increase in absorbance was read at 1 min intervals for 4 min. All appropriate controls for 'nothing dehydrogenase' and non-enzymatic changes in optical density were run. Activities were based on protein content as determined by the method of Lowry et al. [5].

Polyacrylamide gel electrophoresis was performed using the Canalco (Rockville, Maryland, U.S.A.) apparatus and reagents, except where specified. Gels were stained for aldehyde dehydrogenase by the tetrazolium method, using 40 mg of nitroblue tetrazolium, 85 mg NAD or 98 mg NADP, 2.0 mg phenazine methosulfate and 100 µl of substrate in 50 ml of 0.06 M phosphate buffer, pH 7.5. Control gels were stained without added substrate to test for 'nothing dehydrogenase' or without coenzyme to test for aldehyde oxidase. Some gels were stained for protein with 0.1% Amido Black.

Analytical isoelectric focusing in polyacrylamide gel slabs was done using the LKB (Bromma, Sweden) apparatus and methods as described [6].

For purification, 100 g of pooled frozen hepatoma was used. All steps were carried out at 0—4°C. After thawing, the hepatoma was homogenized for 1 min in a Waring Blendor in 2 vols. of 0.06 M phosphate buffer, pH 7.5. The homo-

genate was then sonicated in 50-ml aliquots for 3 min at approximately 50 W power input using a Branson sonicator. The sonicated material was then centrifuged at 40 000 × *g* for 30 min in a Sorvall RC2-B centrifuge. The supernatant was poured through S & S 410 filter paper to remove lipid. To the filtered supernatant, solid ammonium sulfate was added to 30% of saturation and the solution allowed to stand for 2 h. This solution was centrifuged at 40 000 × *g* for 30 min and the precipitate discarded. Solid ammonium sulfate was then added to the supernatant to 70% saturation and allowed to stand for 18 h. The solution was then centrifuged and the supernatant discarded. After dissolving the precipitate in a minimal amount of 0.015 M phosphate buffer, pH 7.5, the resulting solution was dialysed for 24 h against five 20-vol. changes of the same buffer.

The dialysate was clarified by centrifugation at 40 000 × *g* for 15 min and this supernatant was applied to a 5 × 70-cm DEAE-Sephadex column equilibrated with 0.015 M phosphate buffer, pH 7.5. After washing with 2 vols. of this buffer, a linear phosphate buffer gradient from 15 to 150 mM was applied and 5.0 ml fractions collected at a flow rate of 20 ml/h. Fractions containing aldehyde dehydrogenase activity capable of utilizing NADP as coenzyme to reduce nitroblue tetrazolium to its insoluble formazan in a test tube and behaving as hepatoma-specific aldehyde dehydrogenase on polyacrylamide gel electrophoresis were pooled and concentrated by ammonium sulfate precipitation. The precipitate was dissolved in a minimal amount of 0.06 M phosphate buffer, pH 7.5, and dialysed against several changes of the same buffer for 24 h.

The resulting material was subjected to isoelectric focusing using the LKB 8100 preparative isoelectric focusing apparatus. Focusing in the pH range 5.0—8.0 in a sucrose gradient was carried out for 72 h. The column was then drained by collecting 1.0-ml fractions. Those fractions possessing NADP-specific aldehyde dehydrogenase activity in assay and on polyacrylamide gel electrophoresis were pooled and concentrated as described above.

Molecular weights of the functional aldehyde dehydrogenase isozymes were determined by G-200 gel-filtration. A 2.5 × 90-cm column was equilibrated with 0.06 M phosphate buffer, pH 7.5. The flow rate was 10 ml/h and 2.0-ml fractions were collected. All proteins were used at a concentration of 5.0 mg/ml and 2.0-ml samples were applied to the column. Subunit molecular weights were determined by SDS-polyacrylamide gel electrophoresis using 10% gels as described by Weber and Osborn [7]. In some experiments 10 M urea/0.1 M $\beta$-mercaptoethanol replaced the SDS. All proteins for these molecular weight determinations were used at a concentration of 1.0 mg/ml.

Antiserum to NAD(P)-aldehyde dehydrogenase that had been purified from rat hepatomas was produced by subcutaneous injection of 1.0 ml of purified aldehyde dehydrogenase (2.0 mg/ml emulsified in 1.0 ml of Freund's complete adjuvant). Six weeks later a freshly prepared emulsion of 0.5 ml of purified aldehyde dehydrogenase (2.0 mg/ml) in 0.5 ml of Freund's complete adjuvant was again subcutaneously injected into each rabbit. Rabbits were bled from the ear vein 7 days after the second injection and the serum and cells separated by allowing clot formation for 18 h with subsequent centrifugation at 5000 × *g* for 15 min.

Ouchterlony double diffusions and immunoelectrophoresis were performed

according to the method of Crowle [8]. For the immune reaction, slides were incubated in a humid environment at 37°C for 24 h and stained for protein with 0.1% Ponceau S in 10% glacial acetic acid. On some slides, enzyme active antibody-antigen complexes were detected by staining for enzyme activity as described above for polyacrylamide gels.

## Results

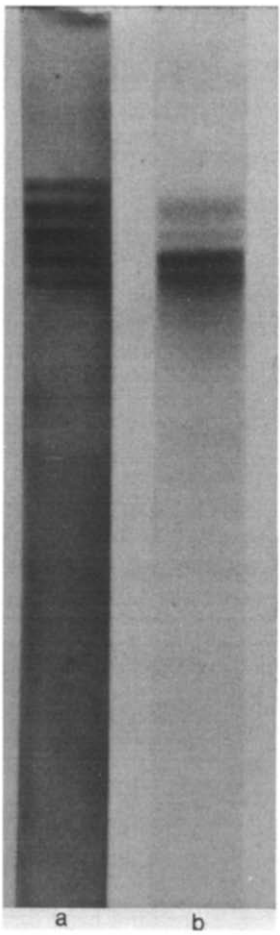
The results of a typical hepatoma aldehyde dehydrogenase purification are presented in Figs. 1 and 2 and in Table I. Ammonium sulfate fractionation, followed by DEAE-Sephadex and gel-filtration chromatography and preparative isoelectric focusing provides a 30-fold purification of hepatoma aldehyde dehydrogenase. Approximately 30—40 mg of purified material has been obtainable from 100 g of tumor.

Analytical polyacrylamide gels stained either for enzyme activity, using NAD or NADP as coenzyme, or for protein with Amido Black (Fig. 1) reveal that this 30-fold purified material consists of 2—3 major and several minor isozymes. An identical pattern is obtained in analytical isoelectric focusing at pH values of 6.9—7.2; in good agreement with the single peak obtained in column isoelectric focusing (Fig. 2b). As the molecular nature and subunit composition of the hepatoma aldehyde dehydrogenases was unknown, this 30-fold purified material, homogenous in that all detectable protein is aldehyde dehydrogenase, represented the state of purity needed to undertake such studies. The purified isozymes remain stable for up to six months in ammonium sulfate at 4°C without apparent decrease in activity or change in isoenzyme distribution.

The molecular weight of the functional isozymes determined by gel-filtration through G-200 Sephadex is 105 000 (mean for four determinations) (Fig. 3). All of the isozymes elute from the column as a single peak of activity, indicating all are of the same molecular weight. Treatment of the purified aldehyde dehydrogenases with 1.0% SDS and 1.0% β-mercaptoethanol overnight at 37°C followed by electrophoresis in 1.0% SDS produces a single band at a molecular weight of 53 000 for five determinations (Fig. 4). Electrophoresis in 8 M urea/ 0.1 M β-mercaptoethanol following treatment of the isozymes with 10 M 0.1 M β-mercaptoethanol at 37°C for 18 h also produces a single band at a molecular weight of 55 000.

Analytical polyacrylamide gel electrophoresis and analytical isoelectric focusing indicate that although the products of this purification scheme are a series of aldehyde dehydrogenase isozymes when stained for enzyme activity, all detectable protein corresponds to an enzyme activity. That the hepatoma aldehyde dehydrogenase preparations obtained by this procedure are homogenous in that all detectable protein is aldehyde dehydrogenase is also indicated by the molecular weight determinations. A single peak emerges from the gel-filtration column and the subunit molecular weight determinations reveal only a single protein species at a molecular weight of 53 000—55 000, again with no contaminating species detectable. Thus, by a variety of criteria, this purification procedure yields a preparation of hepatoma-specific aldehyde dehydrogenase free of contaminating protein species.

The immunochemical reactivity of aldehyde dehydrogenase from both



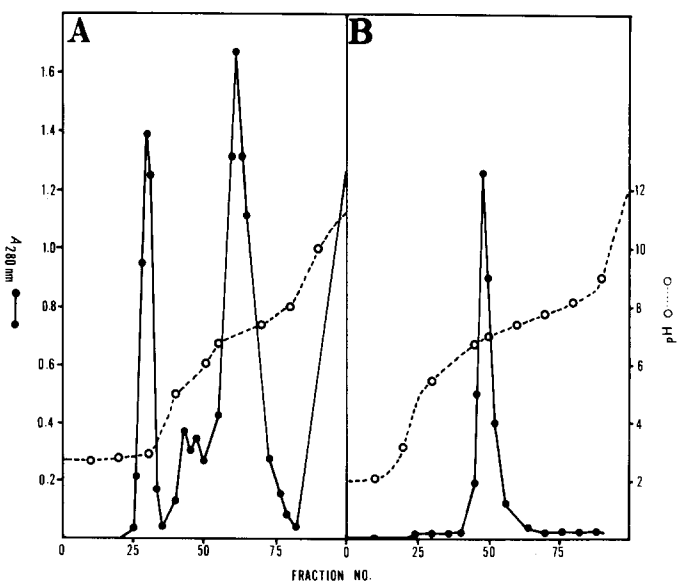




Fig. 1. Polyacrylamide gel electrophoresis of purified aldehyde dehydrogenase. Samples are 20-μl aliquots of the pooled concentrated material (Fractions 55—72) following preparative isoelectric focusing (see Fig. 2. and Table I). a, stained for enzyme activity using benzaldehyde and NAD; b, stained for protein with 0.1% Amido Black. Identical patterns are obtained using benzaldehyde and NADP.

Fig. 2. Isoelectric focusing of rat hepatoma aldehyde dehydrogenase. A, preparative isoelectric focusing of partially purified hepatoma aldehyde dehydrogenase (440-ml column, pH range 5.0—8.0, 2.0 ml fractions collected). Fractions 55—72, p*I* 6.8—7.2, possessed the greatest NAD(P)-specific aldehyde dehydrogenase activity as judged by assay and polyacrylamide gel electrophoresis. These fractions were pooled, dialysed and concentrated to 5 mg protein/ml. B, Subsequent isoelectric focusing of an aliquot (5 mg) of the pooled concentrated aldehyde dehydrogenase obtained in A (110-ml column, pH range 5.0—8.0, 1.0-ml fractions collected). A single symmetrical peak is obtained at a p*I* of 6.9—7.1.

normal liver and hepatoma has been studied by both Ouchterlony double diffusion and agar gel immunoelectrophoresis. Two precipitin lines are detectable by double diffusion or immunoelectrophoresis when either pure hepatoma aldehyde dehydrogenase or crude hepatoma homogenates are being used as antigen (Figs. 5—7). One of the precipitin lines, formed only with these hepatoma antigens, is composed of enzymatically active antibody-antigen complexes that can use either NAD or NADP as coenzyme. The second precipitin line is en-

TABLE I

PURIFICATION SCHEME FOR ALDEHYDE DEHYDROGENASE FROM 2-AAF-INDUCED RAT HEPATOMAS

| Purification step | Total Protein (mg) | Total aldehyde dehydrogenase (units) | Specific activity (units/mg protein) | Purification (fold) |
|---|---|---|---|---|
| Initial sonicate | 22 020 | 3 013 000 | 136 | — |
| 30—70% $(NH_4)_2SO_4$ | 10 060 | 2 675 000 | 268 | 1.9 |
| DEAE-Sephadex | 660 | 555 000 | 841 | 6.1 |
| Gel-filtration, Sephadex G-200 | 420 | 412 000 | 981 | 7.2 |
| Isoelectric focusing, pH 5—8 | 27 | 110 000 | 4074 | 30.5 |

zymatically inactive. It is formed not only with purified hepatoma aldehyde dehydrogenase or hepatoma homogenates, but it is the only line formed with normal liver homogenates as antigen source (Figs. 5 and 6). Although enzymatically inactive, the second precipitin line corresponds to functional enzymatic species as judged by immunoelectrophoresis in agar gels (Fig. 7) or by

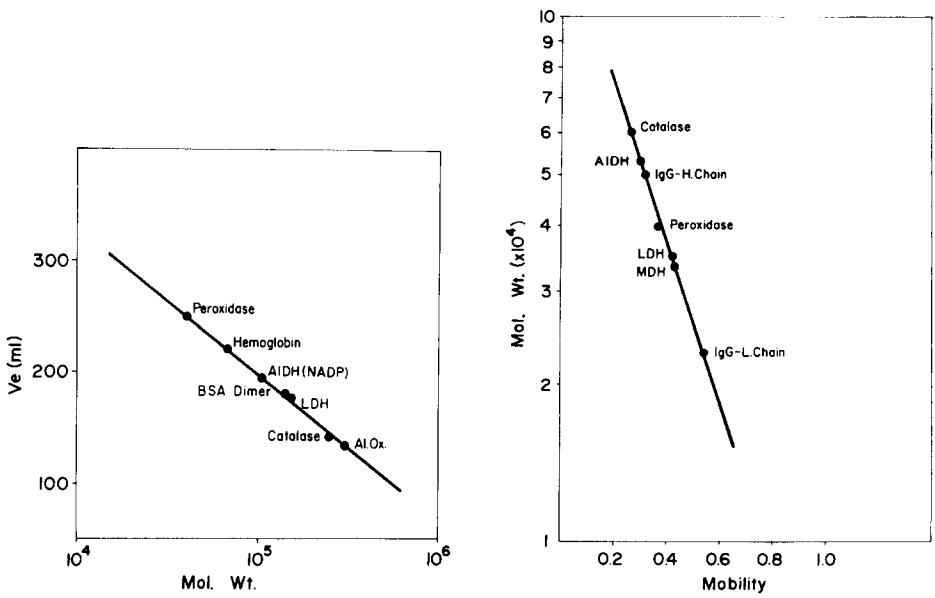



Fig. 3. Molecular weight determination of rat hepatoma aldehyde dehydrogenase (AlDH(NADP)) by G-200 gel-filtration. Elution volume ($V_e$) is plotted against the logarithm of the molecular weight of the indicated proteins: rat liver aldehyde oxidase (Al.Ox.), 300 000; murine catalase, 250 000; bovine lactate dehydrogenase (LDH), 140 000; bovine serum albumin (BSA), 134 000; rat hemoglobin, 67 000; horseradish peroxidase, 40 000.

Fig. 4. Subunit molecular weight determination of rat hepatoma aldehyde dehydrogenase by SDS gel electrophoresis. Logarithm of subunit molecular weight is plotted against protein mobility relative to a Bromophenol Blue standard.Molecular weights are: murine catalase, 60 000; human IgG heavy (H.) chain, 50 000; horseradish peroxidase, 40 000; bovine lactate dehydrogenase (LDH), 35 000; bovine malate dehydrogenase (MDH), 32 000; human IgG light (L.) chain, 23 000.

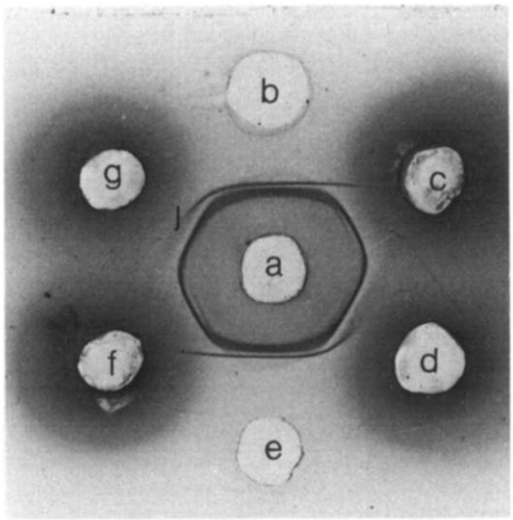



Fig. 5. Immunochemical reactivity of anti-hepatoma aldehyde dehydrogenase against various antigens. a, antiserum; b and e, antibody-inducing antigen, pure hepatoma aldehyde dehydrogenase 2 mg/ml; c and f 50% Sprague-Dawley rat liver homogenate; d and g, 20% rat hepatoma homogenate. Staining for protein was with 0.1% Ponceau S. All wells contain 20 $\mu$l of sample.

polyacrylamide gel electrophoresis followed by immunodiffision in agar. This enzymatically inactive precipitin line is also the only line formed when other rat tissues are used as antigen source. In these cases, the precipitin line is very weak and is formed nearer the antigen well than when liver or hepatoma are

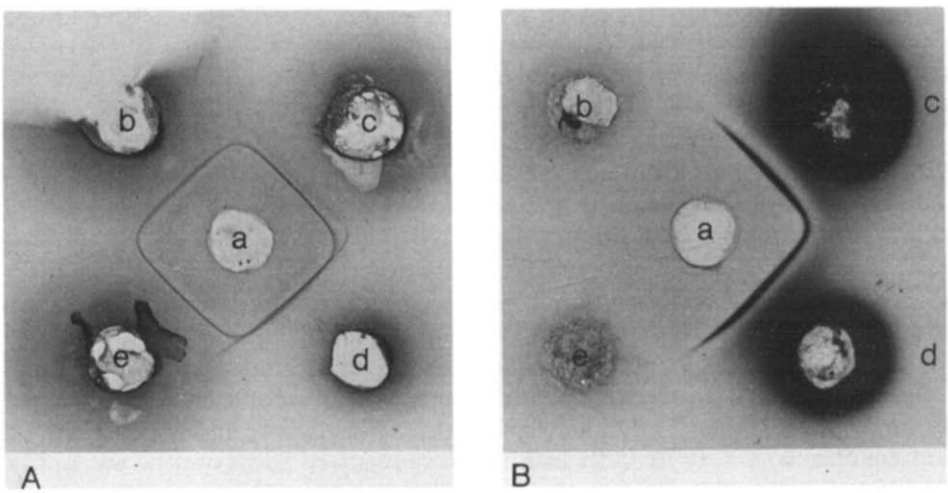



Fig. 6. A, immunochemical reactivity of various antigens with antihepatoma aldehyde dehydrogenase antiserum. a, antiserum; b, 50% normal female Sprague-Dawley liver homogenate; c, 20% hepatoma homogenate; d, pure hepatoma 2 mg/ml; e, 50% normal male Sprague-Dawley liver homogenate. Staining for protein was with 0.1% Ponceau S. All wells contain 20 $\mu$l of sample. B, same as in A but stained for enzyme activity with benzaldehyde and NAD. Identical results are obtained with benzaldehyde and NADP.

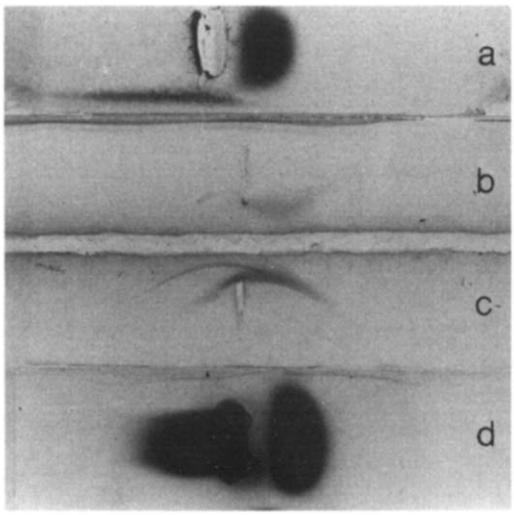



Fig. 7. Immunoelectrophoresis of normal liver and hepatoma aldehyde dehydrogenase. a, 20% normal liver homogenate stained for enzyme activity with benzaldehyde and NAD after electrophoresis; b, 20% normal liver homogenate after electrophoresis and 18 h incubation with anti-hepatoma aldehyde dehydrogenase antiserum, stained with 0.1% Ponceau S; c, 20% hepatoma homogenate after electrophoresis and 18 h incubation with anti-hepatoma aldehyde dehydrogenase antiserum, stained as in b; d, 20% hepatoma homogenate stained for enzyme activity with benzaldehyde and NAD after electrophoresis.

used. This observation indicates very low antigen concentrations in the tissues examined.

## Discussion

Aldehyde dehydrogenases have been isolated previously from the liver of several mammalian species and their properties described. Most studies of rat liver aldehyde dehydrogenase report more than one molecular species possessing aldehyde dehydrogenase activity. Shum and Blair [9] found two aldehyde dehydrogenases in rat liver supernatant, both with molecular weights near 180 000. Marjanen [10] also described two aldehyde dehydrogenases in rat liver, a soluble form of molecular weight 110 000 and mitochondrial form of molecular weight 180 000. Deitrich [11] described the properties of two aldehyde dehydrogenases isolated from the mitochondrial and supernatant fractions of rat liver. The mitochondrial form was of higher molecular weight than the soluble form. Siew et al. [12] have isolated several aldehyde dehydrogenases from rat liver with activity being found in the soluble, microsomal and mitochondrial fractions. They have isolated two enzymes from mitochondria, a high-molecular-weight (275 000) intermembrane space form and a low-molecular-weight (67 000) intermatrix enzyme. We are aware of no reports in which it has been conclusively demonstrated that mammalian aldehyde dehydrogenase is capable of utilizing NADP as a coenzyme.

We have shown that the isozymic forms of aldehyde dehydrogenase isolated from 2-AAF-induced hepatomas in Sprague-Dawley rats exist exclusively in the liver cytosol [2]. The molecular weight of 105 000 for the functional species is

in good agreement with the molecular weights reported for normal liver soluble aldehyde dehydrogenase. The subunit molecular weight of 53 000 is in close agreement with the subunit molecular weights reported by Feldman and Weiner [13] for horse liver aldehyde dehydrogenase and by Clark and Jakoby [14] for yeast aldehyde dehydrogenase. That the functional species are dimeric and exist in multiple molecular forms indicates that the subunits are not identical. Moreover, as the studies of subunit molecular weight indicate only a single species of subunit, they must differ primarily in charge.

The isoelectric point of the hepatoma-specific aldehyde dehydrogenases is in close agreement with the p*I* of 7.0 reported for a phenobarbital-inducible aldehyde dehydrogenase isolated by Koivula and Koivusalo [15] from the cytosol of Wistar rat liver. This phenobarbital-induced aldehyde dehydrogenase has a molecular weight of 150 000, considerably in excess of that of tumor-specific aldehyde dehydrogenases described here.

As phenobarbital is a component in the tumor-inducing diet [3], we have considered that our hepatoma-specific aldehyde dehydrogenases are the result of phenobarbital induction. However, we have demonstrated that the rats used in the tumor-inducing study were all non-reactors for phenobarbital induction of aldehyde dehydrogenase (2, Table I). In addition, phenobarbital induction of aldehyde dehydrogenase in Long-Evans rats, which possess a phenobarbital-inducible aldehyde dehydrogenase, indicates that all the induced activity is in all respects identical with normal liver aldehyde dehydrogenase [2]. The relationship, if any, between hepatoma-specific aldehyde dehydrogenase and the aldehyde dehydrogenase induced by phenobarbital will require a direct comparison of their biochemical and physical properties.

Although the isoelectric point of the normal liver aldehyde dehydrogenase is lower (pI 6.2—6.4) than that of hepatoma aldehyde dehydrogenase (pI 6.9—7.2) by almost a full pH unit, they have identical mobilities on polyacrylamide gel electrophoresis [2]. It the enzymes from the two sources were of identical molecular weight, the normal liver enzyme should migrate much more anodically than the hepatoma isozymes. The fact that the two enzymes have the same mobility indicates the normal enzyme must be larger than the hepatoma isozymes. This size difference would cause its mobility to diminish such that the normal liver enzyme would have the same mobility as the hepatoma aldehyde dehydrogenases. In support of this, particulate normal liver aldehyde dehydrogenase is generally of molecular weight in excess of 200 000 [10—14] and we have demonstrated that the majority of the aldehyde dehydrogenase in normal Sprague-Dawley liver is found in particulate fractions [2]. Thus the hepatoma-specific isozymes are, by all criteria employed, distinctly different in physical and chemical properties from normal liver aldehyde dehydrogenase.

As reported previously [2], a small amount of aldehyde dehydrogenase with properties of the normal liver enzyme is found in hepatomas. In the purification of hepatoma aldehyde dehydrogenase, polyacrylamide gel electrophoresis and the use of NADP in the purification monitoring assays at each step assure that only those fractions containing hepatoma-specific aldehyde dehydrogenases are retained. Moreover, the difference in isoelectric point of the enzymes from the two sources allows the use of preparative isoelectric focusing as the final purification step. This technique ensures the removal of any normal liver

aldehyde dehydrogenase from the final hepatoma preparations. That the final preparations are hepatoma-specific aldehyde dehydrogenase with no detectable contaminating species has been demonstrated by both analytical polyacrylamide gel electrophoresis and analytical isoelectric focusing.

Even though the hepatoma-specific aldehyde dehydrogenases appear to be distinctly different enzymatic species, they exhibit partial immunological cross-reactivity with normal liver aldehyde dehydrogenase. The enzymatically active precipitin line found only in hepatomas must represent a population of antibodies that are specific for hepatoma aldehyde dehydrogenase. Likewise, the enzymatically inactive precipitin line that is the only line formed with normal liver as antigen must represent the normal liver aldehyde dehydrogenase. This interpretation is confirmed by immunoabsorption experiments in which all the aldehyde dehydrogenase activity can be removed from normal liver homogenates by previous absorption with anti-hepatoma aldehyde dehydrogenase antibodies.

That the enzymatically inactive precipitin line is also found in hepatoma homogenates could be explained as the formation of antibody-antigen complexes with the normal liver aldehyde dehydrogenase component found in hepatomas. Immunoabsorption experiments using hepatoma homogenates confirm that this is a plausible interpretation. However, the formation of the normal liver precipitin line when purified hepatoma aldehyde dehydrogenase, possessing only the physical and chemical properties of the hepatoma-specific aldehyde dehydrogenases is used as antigen, indicates that such an interpretation is greatly oversimplified.

The purified hepatoma-specific aldehyde dehydrogenases used to elicit antibody production, although homogeneous in that all detectable protein is hepatoma-specific aldehyde dehydrogenase, cause the production of an antiserum that possesses antibodies to at least two antigenic sites. Although this may be attributed to the presence of several isozymes in the antigen preparation, the ability of the antibodies produced to crossreact with normal liver aldehyde dehydrogenase indicates that the hepatoma specific aldehyde dehydrogenases are not as distinctly different from the normal liver enzymes as the biochemical data indicate.

The exact nature of the immunological cross-reactivity is a present unknown, but three possibilities are consistent with all the available data.

1. The hepatoma isozymes and the normal liver enzyme share a common subunit. If the hepatoma aldehyde dehydrogenases are the result of derepression of one or more genes for aldehyde dehydrogenase normally repressed in liver, the product(s) of which can form hybrid dimers with subunits of the normally occurring liver aldehyde dehydrogenase enzyme, several isozymes would be generated, each consisting of a newly synthesized monomer and a normal liver aldehyde dehydrogenase monomer.

2. The hepatoma isozymes are the result of post-translational modification(s) of otherwise normal aldehyde dehydrogenase subunits. Changes such as the addition or loss of amino and/or carboxyl groups, acetylation, phosphorylation, or methylation would not only effect the electrophoretic mobility and perhaps the ability of the monomers to polymerize, but could alter antigenic properties of the monomers. Alteration of the antigenic properties could result in the ap-

pearance of new antigenic sites while still not affecting existing ones.

3. The hepatoma isozymes are the result of both the derepression of a normally repressed aldehyde dehydrogenase gene as well as post-translational modification of an otherwise normal aldehyde dehydrogenase monomer. This unique subunit could form dimeric molecules with a series of modified normal liver aldehyde dehydrogenase monomers to produce a series of isozymes with properties identical to those exhibited by hepatoma aldehyde dehydrogenases.

As noted previously, the extreme temperature- and pH-instability of Sprague-Dawley normal liver aldehyde dehydrogenase [2] have prevented its purification despite numerous attempts. Until a suitably pure preparation of Sprague-Dawley normal liver aldehyde dehydrogenase can be obtained, the exact nature of the relationship of the hepatoma aldehyde dehydrogenases with their normal liver counterpart will remain unclarified.

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