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Rat liver aldehyde dehydrogenase—Immunochemical identity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inducible normal liver and 2-acetylaminofluorene inducible hepatoma isozymes*

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Interest in mammalian aldehyde dehydrogenases (EC 1.2.1.3 to 1.2.1.5, ALDH) has centered on the role of this enzyme system in ethanol metabolism[1–4]. A number of molecular forms of aldehyde dehydrogenase have been demonstrated in the mitochondrial, microsomal and cytosolic fractions of rat liver and several other mammalian tissues[5–10]. However, it is generally agreed that only certain of the liver and perhaps brain mitochondrial ALDH isozymes are responsible for the oxidation of acetaldehyde during ethanol metabolism[1–4]. To date, the role(s) of the microsomal and cytosolic aldehyde dehydrogenases remain largely undefined.

Evidence for multiple molecular forms of cytosolic aldehyde dehydrogenase comes from a variety of studies[5–14]. Among these various ALDH isozymes, Deitrich *et al.*[14, 15] have demonstrated that phenobarbital increases liver cytosolic ALDH activity 10- to 30-fold in certain genetically defined lines of rat. The increased activity is due to a phenobarbital-inducible ALDH (ϕ isozyme), which differs in a variety of properties from the basal, non-inducible ALDH[14, 15]. Recently, another cytosolic ALDH, inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (τ isozyme), has been purified from rat liver[16, 17]. The τ isozyme is physically, functionally and immunochemically distinguishable from the ϕ isozyme. In addition, Feinstein *et al.*[12, 18, 19] and Lindahl[20] have reported the presence of several cytosolic ALDH isozymes unique to hepatomas induced in several strains of rat by a variety of chemical carcinogens, including 2-acetylaminofluorene (2-AAF) and dimethylaminoazobenzene (DAB). This series of hepatoma-specific aldehyde dehydrogenases (α isozymes) has also been purified and immunochemically characterized[19].

The kinetic properties of these inducible aldehyde dehydrogenases (mM K_m values for acetaldehyde) make it unlikely that any of the three isozymes, ϕ, τ or α, plays a major role in ethanol metabolism. More probable roles for these isozymes are in the metabolism of other aldehydes, both biogenically generated and exogenously administered. Work in our laboratories has been directed toward determining the functional significance of these various inducible ALDH. To this end, we have recently become interested in determining the functional, structural and genetic relationships among the ϕ, τ and α isozymes. Here we report the results of a direct immunochemical comparison of these ALDH, each of which is induced by different conditions.

Rabbit antiserum against rat hepatoma-specific aldehyde dehydrogenase was produced as described previously[19]. Rabbit antiserum against τ isozyme was produced by a hyperimmune protocol (M. Roper *et al.*, manuscript in preparation). Ouchterlony 2-dimensional immunodiffusions were also performed as described[19]. The antiserum generated against purified α isozymes possesses two distinct antibody populations[9, 20]. One antibody population forms enzymatically active immune complexes and is hepatoma-specific, whereas the other population forms enzymatically inactive immune

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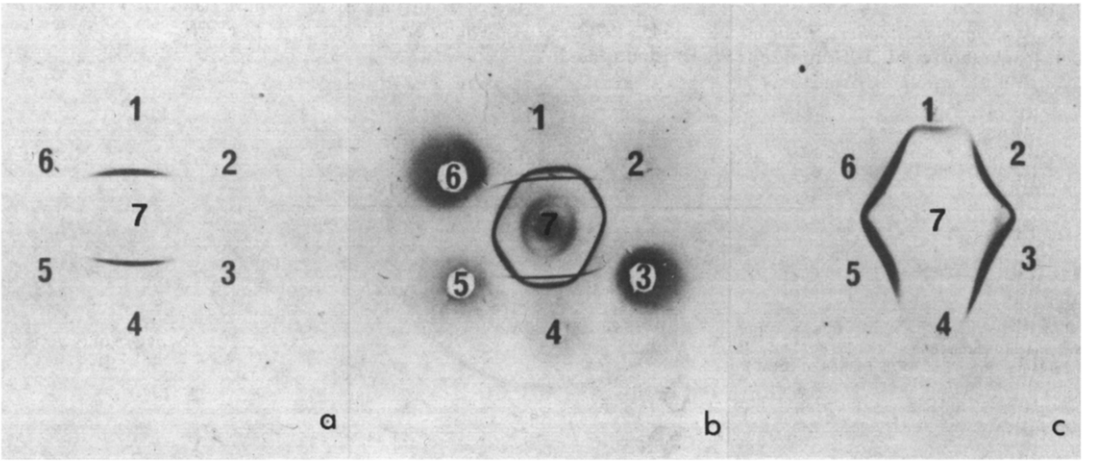
complexes with both hepatoma and normal liver preparations. The antibody population that apparently forms enzymatically inactive immune complexes is capable of removing approximately 35 per cent of the total ALDH activity from normal liver and 15 per cent of the total hepatoma ALDH activity. The hepatoma-specific antibody population removes 85 per cent of the total hepatoma ALDH activity and is without effect on normal liver ALDH activity (R. Lindahl, *Biochim. biophys. Acta*, in press, 1978). Rabbit antiserum against the τ isozyme appears to be monospecific by Ouchterlony immunodiffusion, producing a single enzymatically active immune complex with both purified τ isozyme and TCDD-induced normal liver preparations (M. Roper *et al.*, manuscript in preparation).

Anti-α isozyme serum reacts with TCDD-induced al-



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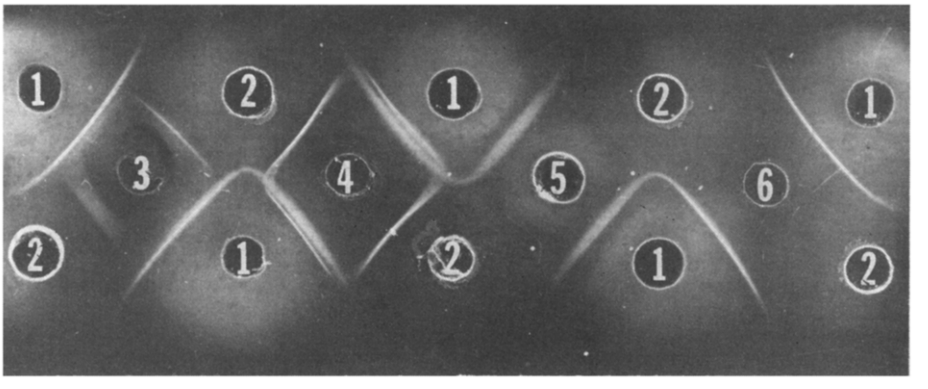

B

Fig. 1. Reaction of rat liver aldehyde dehydrogenases with various anti-aldehyde dehydrogenase sera. All tissues were prepared as 20% homogenate supernatant fractions and the immune reactions were performed as described. (A) Reaction of TCDD-inducible aldehyde dehydrogenase with various antisera. In section a (left), 1,4: TCDD-induced rat liver preparation; 2,5: phenobarbital-induced rat liver preparation; 3,6: non-induced normal liver preparation; 7: anti-α isozyme serum. The slide was stained for enzyme activity with propionaldehyde and NAD^+. In section b (middle), the key for the numbers is the same as in section a, but the slide was stained for protein with 0.1% Amido Black. In section c (right), 1,4: anti-ϕ isozyme serum; 2,5: anti-τ isozyme serum; 3,6: anti-α isozyme serum; 7: TCDD-induced normal liver preparation. The slide was stained for enzyme activity as in section a. The weak reaction of anti-ϕ isozyme serum with TCDD-induced normal liver preparations is due to a minor non-τ isozyme activity present in non-induced normal liver. Purified τ isozyme generates only the enzymatically active precipitin line when tested against anti-α isozyme serum when stained for either enzyme activity or protein (not shown). (B) Reaction of hepatoma-specific aldehyde dehydrogenase with various antisera. Key: 1: anti-α isozyme serum; 2: anti-τ isozyme serum; 3: purified α isozymes; 4: AAF-induced hepatoma preparation; 5: normal liver preparation; and 6: preparation of normal liver material which produces the enzymatically inactive precipitin line with anti-α isozyme serum. The slide was stained for protein with 0.1% Ponceau S.

dehyde dehydrogenase either in a crude preparation or after purification (Fig. 1A). Of the two precipitin lines formed with crude preparations, only one is due to the presence of the τ isozyme. This precipitin line is continuous with the single enzymatically active precipitin line formed with anti-τ isozyme serum as antibody source against the τ isozyme (Fig. 1A).

Anti-τ isozyme serum forms a single precipitin line when either pure α isozymes or a crude hepatoma preparation is used as antigen (Fig. 1B). This precipitin line is continuous with the enzymatically active, hepatoma-specific precipitin line formed when anti-α isozyme serum is used as antibody source against α isozyme preparations (Fig. 1B). Anti-τ isozyme serum does not react with Sprague–Dawley normal liver preparations nor with a number of preparations of normal liver material that forms the enzymatically inactive precipitin line with anti-α isozyme serum (Fig. 1B). Neither anti-τ nor anti-α isozyme serum reacts with the ϕ isozyme. These results indicate that the τ isozyme and one or more of the α isozymes are evidently immunochemically identical. However, neither the τ nor α isozymes are related to the ϕ isozyme immunochemically.

The aldehyde dehydrogenase activity of a hepatoma induced by 2-AAF is generally 2- to 3-fold greater than the ALDH activity of normal Sprague–Dawley liver and is due to a strictly cytosolic set of dimeric isozymes found only in hepatomas[18–19]. The molecular weight of the functional α isozymes is 105,000 by G-200 gel filtration, with a subunit molecular weight of 53,000 determined by SDS-gel electrophoresis[19]. The α isozymes possess activity with a broad spectrum of aliphatic and aromatic aldehyde substrates and can utilize NAD^+ and $NADP^+$ equally well as coenzyme. K_m values for both substrate and coenzyme are in the millimolar range. The α isozymes have broad pH optima and are very heat labile above 40°. The α isozymes do not appear during the pre- or post-natal ontogeny of Sprague–Dawley ALDH nor are they found in regenerating liver after partial hepatectomy[20].

After TCDD administration τ isozyme activity is approximately 100-fold greater than normal Long–Evans liver ALDH activity[16, 17]. By Bio-Gel P-200 gel filtration, the τ isozyme has a molecular weight of 72,000 and the ϕ isozyme a molecular weight of 122,000. The τ isozyme prefers small aliphatic aldehyde substrates but can utilize NAD^+ and $NADP^+$ equally well as coenzyme. The ϕ isozyme oxidizes phenylacetaldehyde well but the τ does not[16, 17]. The τ isozyme possesses a broad pH optimum and is heat labile in the absence of NAD^+.

The induction of the τ isozyme by TCDD requires only a single small (75 μg/kg) i.p. injection[17]. The induction follows a long time-course with a broad peak of τ isozyme activity at 10 to 14 days after exposure. The activity remains high for up to 10 days after the peak response has been attained[17]. The time-course of α isozyme appearance is only now being studied in detail. Preliminary results indicate that following chronic, low level 2-AAF exposure[21], the α isozymes do not appear until just before a tumor is histologically identifiable, generally 90–120 days post-exposure. This correlates well with the finding that a single acute 2-AAF exposure is not sufficient to alter the liver aldehyde dehydrogenase phenotype (R. A. Deitrich *et al.*, manuscript in preparation). In addition, animals carrying palpable hepatic tumors for several weeks show the same high α isozyme activity as animals sacrificed just after a tumor is first detectable. Thus, it appears that the appearance of the α isozymes marks a permanent change in ALDH phenotype.

A number of agents including DDT, Mirex, β-naphthoflavone, and polychlorinated biphenyls (Aroclor 1254), induce one, or the other, or both of the ϕ and τ isozymes as determined by kinetic and immunological means (R. A. Deitrich *et al.*, manuscript in preparation).

In summary, rabbit antiserum directed against a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-inducible normal liver aldehyde dehydrogenase (ALDH) reacts with complete identity with a series of tumor-specific aldehyde dehydrogenases found in hepatomas generated in rats by 2-acetylaminofluorene (2-AAF). A hepatoma-specific antibody population directed against the hepatoma-specific ALDH reacts with complete identity with the normal liver TCDD-inducible ALDH. Neither antisera reacts with a phenobarbital-inducible rat liver aldehyde dehydrogenase.

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