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HUMAN ALDEHYDE DEHYDROGENASE: CATALYTIC ACTIVITY IN ORIENTAL LIVER

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SUMMARY-- Population genetics followed by purification suggested that "null" mutation in the mitochondrial E2 isozyme of human aldehyde dehydrogenase (EC 1.2.1.3) occurred in the Oriental individuals who are sensitive to alcohol. This report demonstrates that the Oriental E2, thought to be a "null" mutant, is catalytically active and except for maximal velocity and isoelectric point, identical with Caucasian E2 isozyme. The data presented are not inconsistent with mutation but preclude active site of the enzyme as the point at which alteration has occurred; they are, however, inconsistent with "null" mutation.

It is known that Oriental populations exhibit facial flushing after the ingestion of small quantities of alcohol. Recent studies employing electrophoresis and isoelectric focussing have demonstrated that a different aldehyde dehydrogenase phenotype (termed "unusual"), apparently devoid of the mitochondrial low Km E2 isozyme, is present in individuals showing such alcohol sensitivity (1,2,3). A protein with molecular properties similar to those of the E2 isozyme, but inactive, has been isolated recently from Oriental livers possessing the "unusual" aldehyde dehydrogenase phenotype (4,5). These findings combined with the fact that metabolism of ethanol-derived acetaldehyde occurs in the mitochondria of the rat (6,7) appear to support a postulate that Oriental alcohol sensitivity is due to a "null" mutation at the mitochondrial low Km E2 isozyme locus. In the present paper we report that this "null mutant" of Oriental liver (5) is catalytically active, which requires a modification of the view that Oriental sensitivity to alcohol results from the absence of E2 isozyme activity.

MATERIALS AND METHODS

MATERIALS

NAD, glycolaldehyde and PIPES were from Sigma, disulfiram was from Ayerst. Propionaldehyde and acetaldehyde were redistilled before use. 5'-AMP-Sepha-

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rose 4B, CM-Sephadex C-50 and DEAE-Sephadex A-50 were from Pharmacia Fine Chemicals. All other chemicals were reagent grade. Antibodies to homogeneous E1 and E2 isozymes from Caucasian livers were developed in the rabbit by Grand Island Biological Company. The Caucasian livers were freshly frozen autopsy specimens obtained from local hospitals, the Oriental liver was from the University of California at San Francisco, mailed to us by overnight shipment in dry ice and kept frozen at -70°C until used.

METHODS

Enzyme purification was done under nitrogen by the procedure previously described (8,9,10) and used routinely in our laboratory, but only 50 g of liver was used each time instead of the usual 600 g. The DEAE-Sephadex A-50 and CM-Sephadex C-50 columns were scaled down to 2.6 x 20 cm and 5.1 x 10 cm, respectively. The 5'-AMP column was 50% of the size used for 600 g of Caucasian liver (2.5 x 11 cm). During purification on columns the protein was followed by 280 nm absorption. The protein concentration in bulked samples was also determined by the Lowry procedure (11) and by the Coomassie Blue procedure (12).

Dehydrogenase activity was measured in 3 ml total volume of nitrogen-saturated sodium pyrophosphate buffer, pH 9.0, containing 1 mM EDTA, 0.5 mM NAD, and 1 mM propionaldehyde using a Varian 635 recording spectrophotometer in cuvettes of 1 cm light path at 25°C and 340 nm employing an extinction coefficient of 6.22 mM^{-1} cm^{-1} for NADH.

Esterase activity was measured by following conversion of p-nitrophenylacetate (0.13 mM) to p-nitrophenol at 400 nm in 3 ml volume at 25°C and 1 cm light path in 0.1 M sodium phosphate buffer, pH 7.0. An extinction coefficient of 9.4 mM^{-1} cm^{-1} was used.

Isoelectric focusing was done on Agarose plates composed of 1% w/v Agarose, 10% w/v sorbitol and 0.075% v/v of Pharmalyte (pH 3.5-10.0, Pharmacia Fine Chemicals) for 20 h at 110 V for a gel size of 114 x 225 mm. The enzyme activity was visualized after incubation of the gel in 0.1M Tris/HCl buffer, pH 8.6, containing 1 mM NAD, 13 mM propionaldehyde, Nitroblue Tetrazolium (10 mg/30 ml of buffer) and Phenazine Methosulfate (1 mg/30 ml buffer). Protein staining was done by the Coomassie Blue procedure suggested by Pharmacia Fine Chemicals.

Molecular weight determination of the intact protein was done using a polyacrylamide gradient gel PAA4/30 from Pharmacia Fine Chemicals employing conditions suggested by the manufacturer. For determination of the molecular weight of subunits a procedure of Laemmli (13) was employed.

RESULTS

Before using the Oriental liver for enzyme purification it was established by isoelectric focussing (1,2,3) that aldehyde dehydrogenase phenotype was "unusual". Fifty g samples of Oriental and Caucasian livers were used for two separate purifications from each liver. There were no differences observed in the elution profiles of CM and DEAE Sephadex columns, only the elution profiles of 5'AMP Sepharose 4B columns were different (Figure 1). The E2 isozyme was eluted first in both cases, but that from Oriental liver was more difficult to elute (Figure 1, bottom). Although from both livers (see fractions 3-18 top and 3-21 bottom), the non-bound protein exhibited high esterase activity (not associated with aldehyde dehydrogenase), that from the Oriental

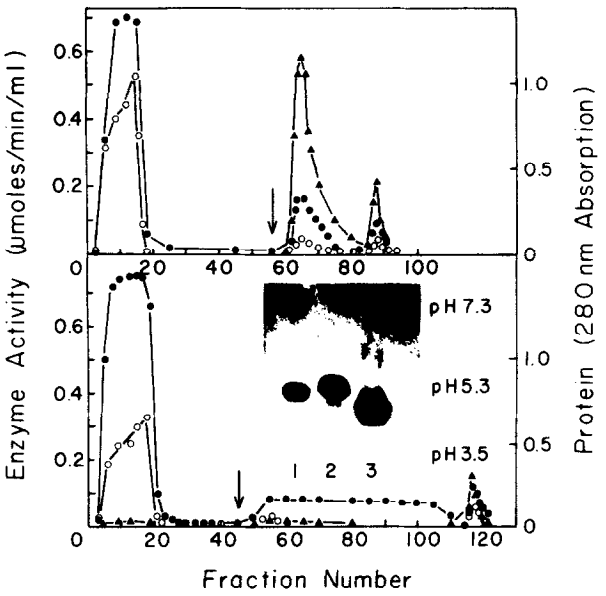

FIGURE 1. Elution profiles of E1 and E2 isozymes of 5'AMP Sepharose 4B column and identification of the isozymes by isoelectric focussing. Preparation from Caucasian liver (top); preparation from Oriental liver (bottom); isoelectric focussing gel (inserted, bottom). Absorption at 280nm ●—●; esterase activity ○—○; dehydrogenase activity ▲—▲. Arrows indicate points at which buffer was changed from pH 6.0 to pH 8.0 to elute E2; to elute E1 0.5mM NADH was added to pH 8.0 buffer. Chanel 1 of isoelectric focussing gel - Oriental E2 (from bulked and concentrated fractions 53-105, bottom); chanel 2 - Caucasian E1 (fractions 85-90, top); chanel 3 - Caucasian E2 (fractions 62-75, top). The E1 isozyme from Oriental liver (not shown on this gel) focusses in an identical way to Caucasian E1 (see Table I). The isozymes are visualized by enzyme activity staining (see Materials and Methods).

liver also contained a small amount of dehydrogenase activity (fractions 5-20 in Figure 1, bottom). After the passage through another large 5'-AMP column, the non-bound protein was now devoid of aldehyde dehydrogenase activity and showed no cross-reactivity with either anti-E1 or anti-E2 antibody. The active enzyme that attached to the second 5'-AMP column was found to be identical to that attaching to the first 5'-AMP column.

When purification yields from Oriental liver were compared with those from Caucasian liver it was found that yield of E1 isozyme was similar from both livers. The yield of E2 isozyme, however, was considerably less from Oriental than Caucasian liver (ca. 20% of that from Caucasian liver). Since both purifications were from the same Oriental liver we are unable at present to comment whether this is general or individual variation. Properties of Oriental E1 and E2 isozymes are compared with those of Caucasian E1 and E2

TABLE I. PROPERTIES OF ORIENTAL AND CAUCASIAN E1 AND E2 ISOZYMES OF HUMAN ALDEHYDE DEHYDROGENASE

PROPERTIES	E1 isozyme		E2 isozyme	
	Oriental	Caucasian	Oriental	Caucasian
Km for acetaldehyde(1) (uM)	260	280	2.5	2.9
Km for glycolaldehyde(1) (uM)	245	250	58	58
Km for NAD(2) (uM)	---	---	153	166
Dehydrogenase activity(3) umoles/mg/min	---	---	0.25	1.7
Esterase activity(3) umoles/mg/min	---	---	0.04	0.13
Effect of Mg^{2+}(4) (166 uM)	inhibition	inhibition	activation	activation
Inhibition by disulfiram (40 uM)	total	total	partial	partial
Electrophoretic(5) mobility	Same as Caucasian E1	Same as Oriental E1	superimposes with E1	more anodal than Caucasian E1
Molecular weight	240,000	240,000	225,000	225,000
Subunit molecular weight	---	---	54,000	54,000

1 - 0.1 M pyrophosphate, pH 9.0; 1 mM EDTA; 1.5mM NAD; glycolaldehyde 0.04-0.3 mM for E2; 0.04-0.6 mM for E1; acetaldehyde 0.11-1.76 for E1; 0.001-0.017 mM for E2.
2 - 0.1M pyrophosphate, pH 9.0; 1 mM EDTA; 1 mM glycolaldehyde; NAD 0.05-0.2M.
3 - See Materials and Methods.
4 - 20 mM PIPES buffer, pH 7.0; 0.01 mM EDTA; 0.13 mM p-nitrophenylacetate.
5 - Also see Figure 1.

isozymes in Table I. Electrophoretic properties of Oriental E2 are also shown in the insert to Figure 1. It can be seen that molecular and catalytic properties of Oriental E1 and Caucasian E1 are identical. Those of Oriental and Caucasian E2 (with the exception of electrophoretic mobility and specific activity) are also identical, thus making it easy to identify the isozymes. As shown in Table I, the dehydrogenase activity of Oriental E2 is ca 15% and esterase activity is ca 30% of the Caucasian E2. At this stage of purification with the use of excess 5'-AMP the Oriental E2 is not homogeneous, but ca 50% pure (by visual comparison of gels developed for activity and for protein), suggesting that the actual specific activity is higher (ca twice as high as shown in Table I).

We have also investigated substrate specificity by employing the following substrates at 500 uM concentration: glyceraldehyde, valeraldehyde, m-nitrobenzaldehyde, indolacetaldehyde and phenylacetaldehyde. The activities were

130, 130, 130, 167 and 230%, respectively of that with propionaldehyde at the same concentration. Since, it had been claimed (5) that E2 from Orientals no longer binds NAD or NADH, the effect of NAD, NADH and Mg^{++} on esterase activity of the Oriental E2 was also investigated. Both coenzymes and Mg^{++} were found to stimulate the esterase activity in the way analogous to that found with the Caucasian E2 (14).

DISCUSSION

The E2 isozyme isolated from Oriental liver during this investigation appears to possess similar molecular and catalytic characteristics (with the exception of isoelectric point and maximal velocity) to those of the Caucasian E2 isozyme (Table I). An inactive protein recently isolated from Oriental liver and reported to be a "null" mutant of the E2 isozyme (5) has similar isoelectric point and molecular properties to the enzyme isolated during this investigation. The reason why our protein is catalytically active probably lies in the fact that our purification is carried out under anaerobic conditions. There is, however, inconsistency with regard to the 5'-AMP column; the protein previously described did not bind to the 5'-AMP column. It should be noted, however, that our Oriental E2 isozyme does not bind strongly to 5'-AMP, as evidenced by the presence of an activity peak in the column wash which is not present in the column wash from Caucasian liver (compare top and bottom of Figure 1). Also note that a significantly larger amount of 5'-AMP, relative to sample size, was used by us. The Oriental and Caucasian E2 isozymes also appear to bind coenzyme similarly, as evidenced by the esterase stimulation data (see above) and by the similar values obtained for the coenzyme Michaelis constants from dehydrogenase kinetics.

Since the protein isolated previously (5) was catalytically inactive and did not bind to 5'-AMP it has been suggested that mutation resulting in structural alteration of the coenzyme-binding site probably occurred. Our results are not inconsistent with mutation but preclude significant alterations in the coenzyme and substrate binding areas, since Michaelis constants for substrate and coenzyme remain unchanged, only maximal velocity if affected. The maximal velocity

of the Oriental E2 isozyme is estimated by us to be ca 20-30% of the Caucasian E2 isozyme. When the Caucasian E2 isozyme was subjected in our laboratory to chemical modification with various reagents it was not possible to diminish its activity below residual 20% without destroying its tertiary structure in the process. By chemical modification with iodoacetamide (15), activity of the Caucasian E2 is diminished to 20% of initial, but Michaelis constants for substrates and coenzyme remain unchanged. There is therefore a possibility that in Oriental E2, alteration has occurred at the site of chemical modification by iodoacetamide, which might represent a modifier rather than the active site of this enzyme (15).

Since the Oriental liver employed in this investigation exhibited the "unusual" aldehyde dehydrogenase phenotype, our results also suggest that Oriental alcohol sensitivity cannot be a consequence of the absence of E2 isozyme activity. The mitochondrial E2 isozyme is present in Oriental individuals, but its visualization by electrophoresis and isoelectric focussing is difficult since it superimposes with the cytoplasmic E1 isozyme.

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