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Biochemical Genetic Analysis of Human and Rodent Aldehyde Dehydrogenase (ALDH)

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HOPKINSON, D. A., I. SANTISTEBAN, S. POVEY AND M. SMITH. *Biochemical genetic analysis of human and rodent aldehyde dehydrogenase (ALDH)*. ALCOHOL 2(1) 73–78, 1985.—ALDH isozymes have been characterized in terms of substrate and coenzyme specificity, heat stability, tissue distribution and electrophoretic properties. The activity of the isozymes has also been examined in rodent-human somatic cell hybrids in order to map the structural genes to specific chromosomes and to study the control of gene expression. One isozyme, designated ALDH3, which is very active against benzaldehyde, was found to show variable expression in hybrids made between rat hepatoma cells and human fibroblasts or fetal liver. Segregation analysis of these hybrids indicates that the structural locus for human ALDH3 may be on chromosome 17. The expression of rodent ALDH3 in these hybrids was extremely variable and not correlated with the appearance of the human enzyme. In hybrids expressing human and rodent ALDH3 no heteromeric isozymes were observed. The human “cytosolic” ALDH1 and “mitochondrial” ALDH2 isozymes did not appear to be expressed in any of the somatic cell hybrids examined.

Aldehyde dehydrogenase   Biochemical genetic analysis   ALDH isozymes

ALDEHYDE dehydrogenase (ALDH) has a major role in the degradation of alcohols by catalysing the oxidation of aldehydes to acids. Multiple forms of the enzyme have been identified in several different species and the isozymes may be differentiated on the basis of their kinetic properties, sub-cellular tissue distribution and various molecular characteristics [7].

At least six different human ALDH isozymes have been identified [1] by gel electrophoresis and isoelectric focussing and they fall into four different groups, referred to in this report as ALDH1, 2, 3 and 4. ALDH1 and ALDH2 correspond to the “cytosolic” and “mitochondrial” isozymes first described by Greenfield and Pietruszko and designated the E1 and E2 isozymes respectively [2]. The same numerical designation has been used by Yoshida *et al.* [10] but the nomenclature used by others, notably the Hamburg group [1] is ALDH I and ALDH II for the “mitochondrial” and “cytosolic” forms respectively. ALDH3 and ALDH4 in this report correspond to the isozymes previously designated ALDH III and ALDH IV [1].

Genetically determined deficiency of ALDH has been identified in Oriental populations and associated with a marked alcohol-induced flush response in individuals homozygous for the defect [4]. This deficiency is confined exclusively to the ALDH2 components and thus it seems likely that different groups of ALDH isozymes are determined by independent structural genes. This notion is also supported by a previous report of possible genetic variation in the ALDH isozymes of human stomach, designated ALDH3, which was independent of the ALDH2 polymorphism [9]. This paper reports further studies on the genetic determination of the human ALDH isozymes and attempts

to map the genes using the technique of somatic cell hybridization.

METHOD

Adult tissues were from routine autopsies. Human fetal tissues were obtained from abortions carried out at 16–24 weeks gestation. The tissues were stored at -20°C . Tissue extracts were prepared in an equal volume of 0.1% (v/v) Triton X100 in water or in the gel buffer used for electrophoresis. The homogenates were centrifuged at $15000g$ for 15 min at 4°C . Rat tissues, obtained from a laboratory colony of Wistar rats, were treated in the same way as human material. The preparation of the cultured cell extracts, including the somatic cell hybrids, was carried out as described by Kielty *et al.* [5]. The parental lines for the hybrids were the rat hepatoma lines Faza 967 or Fu 5 and human fibroblasts or fetal liver cells.

Two buffer systems were used for horizontal starch gel electrophoresis, a Tris/HCl buffer at pH 8.6 (gel 0.025 M and bridge 0.3 M Tris/HCl) and a TEMK buffer at pH 7.6 (5 mM Tris, 0.5 mM EDTA, 5 mM Maleic anhydride and 0.5 mM KCl gel buffer and 10 fold greater concentration for the bridge buffer). In either case the starch gels contained 4 mM NAD. Electrophoresis was carried out for 18 hr at 7 v/cm with cooling. Isoelectric focussing was performed in 7.5% polyacrylamide gels ($25 \times 12 \times 0.05$ cm) using 1% LKB Ampholine pH 3.5–10.0 and 2% pH 5.0–8.0. The focussing was carried out in the Pharmacia FBE 3000 apparatus at 4°C for 5 hr at $1000 \text{ v} < 2 \text{ mA}$. The gels were stained for ALDH activity with an agar overlay containing propionaldehyde or benzaldehyde as the substrate [5].

TABLE 1
PROPERTIES OF HUMAN ALDH ISOZYMES

ALDH Isozymes	Substrate Preference				Tissue distribution		Heat Stability
		NAD	NADP	Sepharose Blue Affinity	-Adult	-Fetus	
(anode +) ALDH2	Broad Aliphatic (propionaldehyde)	+	-	-	Mitochondria (liver, kidney, heart)	-	Moderate
ALDH1	Heterogeneous (propionaldehyde)	+	+	+	Heterogenous	++	High
ALDH3	Broad aromatic (benzaldehyde)	+	+	+++	Lung, gut	-	Very low
ALDH4 (cathode -)	Propionaldehyde	+	-	-	Liver, kidney	-	Low

FIG. 1. ALDH isozymes in human lung, gut and kidney (kid) tissue homogenates after starch gel electrophoresis at pH 7.4. Gel stained with benzaldehyde as substrate to demonstrate the ALDH3 isozymes in stomach and lung. The more anodal weak isozyme, common to all tissues, is ALDH1. The two specimens derived from the same individual, No. 5046, exhibit a variant 3 banded ALDH 3 pattern compared to the usual pattern shown by individuals 5048 and 5045.

The ALDH3 isozymes of human stomach were partially purified using a two step procedure which involved ion exchange (CM Sephadex) and affinity (Sephadex AMP) chromatography. Full details are given in [8]. The final preparation, shown to be free of other human ALDH isozymes but contaminated by other small proteins devoid of ALDH activity, was used to immunise a rabbit by subcutaneous injection of c. 2 mg protein in complete Freund's adjuvant at 3

sites. The immunisation was repeated after 3 weeks and the antisera obtained was used without purification for the immunoprecipitation experiments.

RESULTS

Human Tissue ALDH Isozymes

Four different sets of human ALDH isozymes were iden-

FIG. 2. ALDH isozymes in human and rat liver and stomach tissue homogenates after starch gel electrophoresis at pH 8.6. Left side of gel stained with benzaldehyde, right side with propionaldehyde. Note the 3 unlabelled bands in human liver correspond to the ALDH1 ("cytosolic") isozymes and under these conditions of electrophoresis have similar mobilities to components in the rat tissues and also the human stomach ALDH3.

tified by starch gel electrophoresis in human tissues. The most anodal set, designated ALDH2, appeared to be identical to the "mitochondrial" E2 isozymes [2] or ALDH I [1] since it was found with highest activity in liver, kidney and heart tissue extracts and was absent in about 50% of the liver homogenates prepared from a population sample ($n=66$) of autopsy specimens collected in Hong Kong. The next most anodal set of isozymes, designated ALDH1, appeared to correspond to the "cytosolic" E1 isozymes [2] or ALDH II [1] since they occurred in every human tissue examined including erythrocytes. ALDH3 was restricted to stomach and lung and ALDH4 was only found in liver and kidney extracts. The four sets of isozymes were also demonstrably different in their substrate and coenzyme specificity and heat stabilities, as indicated in Table 1, and our findings in these respects agree very closely with those previously reported [1,3].

Analysis of a range of fetal tissues (liver, kidney, heart, skeletal muscle, gonad, stomach, lung) from pregnancies terminated between 10 to 20 weeks gestation revealed no fetal specific isozymes and very low or absent activity of the ALDH2, 3, 4 isozymes. Only the "cytosolic," ALDH1,

components were found to be regularly expressed in the fetal tissue homogenates.

Genetic Variation

Genetic variation manifesting as total deficiency of ALDH2 but normal activity of the other ALDH isozymes was identified by starch gel electrophoresis in a small population sample ($n=66$) of liver specimens collected in Hong Kong. This variant which was found to occur with a frequency of 54%, is presumably identical to that previously reported in Oriental populations [4] and recently characterised by immunological analysis [10]. No variants of this type were identified on electrophoretic screening of liver specimens ($n>200$) from N. Europeans and indeed the ALDH1 and ALDH4 isozymes were found to be invariant.

An electrophoretic variant of ALDH3 was found, however, with a frequency of about 4% in N. European individuals ($n=180$). This was identified in stomach or lung homogenates using benzaldehyde as the substrate (Fig. 1). In some cases it was possible to test lung and stomach from the same individual and on each occasion the ALDH3 isozyme

FIG. 3. ALDH isozymes in homogenates of rat and human (hum) stomach and rat $\times$ human hybrid (hyb) cells, after starch gel electrophoresis at pH 8.6. Gel stained with benzaldehyde. Left portion heated at 55°C for 5 minutes prior to staining to demonstrate labile human ALDH3 in stomach and in the hybrid which is expressing human and rat ALDH3.

patterns were identical. Also, repeated tests on fresh homogenates of the same tissue gave reproducible results and the variant pattern was not altered by treatment with mercaptoethanol (Fig. 1). The three banded variant pattern indicates that ALDH3 is a dimeric protein; the two outer bands representing homodimeric molecules and the middle band the heterodimer characteristic of an individual heterozygous at the ALDH3 locus.

Comparison of Human and Rat ALDH Isozymes

Attempts to map the human ALDH genes were made using human $\times$ rodent somatic cell hybrids. The non-human parent was a differentiated rat liver cell line (Faza 967 or Fu 5) which expresses several liver specific enzymes including ALDH [6].

Preliminary electrophoretic analysis of rat tissues revealed complex ALDH isozyme patterns and using the characteristics listed in Table 1 the probably homologues of human ALDH2 and ALDH3 were identified in the rat. The rat ALDH2, viz the "mitochondrial" form of rat ALDH, was most active in the liver tissue homogenates, using

propionaldehyde as the substrate, and was the most acidic isozyme on gel electrophoresis (Fig. 2) and on isoelectric focussing. It was however only slightly more anodal than human ALDH2 and showed overlapping electrophoretic mobility. In contrast the rat ALDH3 was clearly resolved from human ALDH3 by electrophoresis (Fig. 2) but showed the same substrate and coenzyme specificity and tissue distribution as the human (Table 1). Also, both rat and human ALDH3 isozymes were selectively immunoprecipitated by a rabbit anti-human ALDH3 antiserum. The two species were found to differ however in the heat stability of ALDH3 and this characteristic was used to verify the presence of human ALDH3 in the rat $\times$ human somatic cell hybrids. As shown in Fig. 3, human ALDH3 is relatively less stable than rodent ALDH3 when heated for a few minutes at 55°C.

Rat homologues of human ALDH1 and ALDH4 were not identified with certainty. Several rat ALDH isozymes were demonstrable in liver (Fig. 2) and other tissues, which were optimally active with propionaldehyde as the substrate and probably correspond with human ALDH1, viz the "cytosolic" form of the enzyme. However it was not possi-

FIG. 4. ALDH3 isozymes in homogenates of rat and human stomach, rat hepatoma cell line (Faza) and rat $\times$ human hybrids. Starch gel electrophoresis pH 8.6, substrate benzaldehyde. One hybrid is positive only for human ALDH3, the other is expressing both rat and human ALDH3. Note the absence of an intermediate heteromeric isozyme in the latter.

ble to establish whether the rat homologue of human ALDH4 was also concealed in this group of isozymes and since in any case they were overlapping the human ALDH2 isozymes in their electrophoretic mobilities it was decided not to pursue their analysis in the somatic hybrids.

Gene Mapping

Thirteen independent somatic cell hybrids, constructed using Faza 967 or Fu 5 as the non human parent, were examined. The expression of human ALDH2 was generally rather weak and variable and difficult to distinguish from rat ALDH2 and it was not possible to make an accurate assessment of the segregation of this locus in the hybrid panel. The human ALDH3, in contrast, was often very strongly expressed in the hybrids and was easily distinguished from the other isozymes by its electrophoretic mobility (Fig. 4), heat stability (Fig. 3) and immunological specificity. The segregation data (summarised in Table 2) indicate that human *ALDH3* is located on chromosome 17 and detailed analysis of the chromosome content and genetic markers in subclones of the primary hybrids confirmed this assignment [8].

DISCUSSION

The multiple forms of human ALDH have been divided into 4 sets on the basis of several biochemical genetic criteria and our findings are in general agreement with the subdivision previously proposed [1]. We have confirmed that the "cytosolic" (ALDH1) and "mitochondrial" (ALDH2) isozymes are expressed in most human tissues and that ALDH3, most active in stomach and lung and ALDH4, only active in liver and kidney, occur in relatively few tissues. Examination of a broad range of human fetal tissues revealed no evidence of fetal specific ALDH isozymes and in general the isozyme patterns shown by material from the second trimester are simpler than in adults since only ALDH1 is expressed with high activity.

The previously reported [4] genetic polymorphism of ALDH2 in Oriental populations was confirmed and probable genetic variants of ALDH3, resembling those described by Teng [9] were identified in the European population. No variations of the ALDH1 and ALDH4 isozymes were encountered. The simplest interpretation of these biochemical and genetical data is that the 4 sets of human ALDH are

TABLE 2
SEGREGATION OF HUMAN ALDH3 IN 13 INDEPENDENT HYBRIDS

Human Chromosome	Marker/ALDH3			
	+/+	-/-	+/-	-/+
1	3	5	1	4
2	2	5	1	3
3	4	0	5	3
4	6	2	4	1
5	4	3	3	2
6	4	2	3	3
7	3	2	1	1
8	4	3	2	1
9	4	4	0	3
10	5	3	3	2
11	3	3	2	4
12	4	2	3	2
13	4	1	2	2
14	3	0	3	1
15	5	2	4	2
16	6	1	5	1
17	6	6	0	0
18	5	4	2	2
19	4	4	2	2
20	3	2	4	2
21	3	5	1	3
22	2	1	2	1
X	6	0	6	0

The human chromosome content of the hybrids was usually assessed by isozyme analysis and in some cases by use of specific DNA probes and/or detailed karyotypes. Not every hybrid was tested for each chromosome marker.

determined by 4 independent structural genes. Immunological data also support this view since the ALDH1, ALDH2 and ALDH3 isozymes are distinguishable from each other by specific antibodies, though there is some cross reactivity between ALDH1 and ALDH2.

Further data on the genetic determination of human ALDH should come from attempts to map the putative genes. We have taken a first step in this direction by mapping *ALDH3* to human chromosome 17 but unfortunately were not able to map the other loci using a somatic cell hybrid technique which depends on active isozyme expression. Gene cloning and direct DNA analysis will probably be necessary to confirm the assignment of ALDH3 and to map the other loci.

Another approach is that of comparative biochemical genetics. We were able to identify the rat homologue of human ALDH3 with a fair degree of certainty on the basis of its biochemical and immunological characteristics and a similar isozyme, very active with benzaldehyde as substrate and expressed in the stomach, has been found in the mouse (R. S. Holmes personal communication) and is also presumably homologous. However there are some disconcerting features of this comparative analysis. For example it seems likely that human ALDH3 is a dimeric molecule, judging by the 3 banded variant isozyme patterns shown in Fig. 2 and the estimated native molecular size (c. 80,000) which is about half that of the total size of the tetrameric ALDH1 and ALDH2 isozymes [7]. However the expected heterodimeric hybrid ALDH3 isozyme was not demonstrable in those Faza × human somatic cell hybrids which expressed both human and rat ALDH3. Presumably there are structural differences between the rat and human enzymes which prevent the hybridisation or the hybrid is dissociated during the electrophoretic separation or the homology is not nearly as close as we believe at present.

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