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Liver Alcohol Dehydrogenase and Aldehyde Dehydrogenase in the Japanese: Isozyme Variation and Its Possible Role in Alcohol Intoxication

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SUMMARY

Forty autopsy livers from Japanese individuals were studied concerning alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) isozymes using electrophoretic and enzyme assay methods. A remarkably high frequency (85%) was found for the atypical ADH phenotype. The gene frequencies of $ADH_2{}^2$ and $ADH_3{}^2$ were .625 and .05, respectively.

The usual ALDH phenotype showed two major isozyme bands, a faster migrating (low K_m for acetaldehyde) and a slower migrating isozyme (high K_m for acetaldehyde). Fifty-two percent of the specimens had an unusual phenotype of ALDH, which showed only the slower migrating isozyme. The usual phenotype was inhibited about 20%–30% by disulfiram and the unusual type up to 90%. Such a high incidence in the Japanese of the unusual phenotype, which lacks in the low K_m isozyme, suggests that the initial intoxicating symptoms after alcohol drinking in these subjects might be due to delayed oxidation of acetaldehyde rather than its higher-than-normal production by typical or atypical ADH.

INTRODUCTION

An atypical form of liver ADH (E.C.1.1.1.1) with severalfold higher activity and a pH optimum at 8.8 instead of 10.5 has been described by von Wartburg et al. [1]. The

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atypical ADH also differs in other properties, such as substrate specificity and sensitivity toward certain inhibitors [1, 2]. According to the genetic model proposed by Smith et al. [3–5] using starch gel electrophoresis, the atypical enzyme is controlled by the ADH_2 locus. Genetic polymorphism has also been shown at the ADH_3 locus [5, 6]. Based on quantitative enzyme assays (pH-activity ratio), a very high frequency (about 90%) of the atypical form of ADH_2 was reported in the Japanese population [7]. Subsequently, Stamatoyannopoulos et al. [8] studied ADH_2 polymorphism in Japanese liver autopsy specimens with quantitative and electrophoretic methods and reported a frequency of 85% atypical types. However, in their electrophoretic system, atypical ADH_2^2 homozygotes and ADH_2^1/ADH_2^2 heterozygotes were indistinguishable. Moreover, no data concerning the polymorphism of the ADH_3 locus were presented.

In contrast to the work on ADH isozymes, little is known about isozyme composition and variation of liver ALDH (E.C.1.2.1.3). Two major isozymes of ALDH have been characterized in human liver [3]. Earlier, Stamatoyannopoulos et al. [8] found no polymorphism in the two isozymes of ALDH in Japanese liver samples. We have recently developed improved methods for the separation of ADH and ALDH isozymes in human tissues [9, 10]. Using these techniques, we have analyzed 40 post-mortem liver specimens from Japanese individuals.

In this paper, frequencies of different alleles of ADH_2 and ADH_3 in the Japanese are presented. The phenotypes of the ADH_2 locus could be clearly identified either as a homodimer (ADH_2 2) or as a heterodimer (ADH_2 2-1). Evidence is also given that ALDH is polymorphic in the Japanese, and the frequencies of usual and unusual types are reported. Additional data on the pH-activity relationship for ADH and ALDH are presented, and possible implications of variant forms of both enzymes in alcohol sensitivity are discussed.

MATERIALS AND METHODS

Liver specimens from 40 Japanese individuals were collected from autopsies carried out routinely within 72 hrs after death at the Departments of Legal Medicine, Universities of Tsukuba and Tokyo, Japan. Liver homogenates were prepared as described earlier [9]. Assays for ADH activity were performed according to Smith et al. [3]. Different buffer systems from pH 6 to 12 were used to determine the optimum pH range for the normal and atypical types. Assays for ALDH activity were carried out as described [10]. The activities of ADH and ALDH are expressed as change in optical density due to the reduction of NAD at 340 nm/min per gram wet tissue. Blanks were run without ethanol and acetaldehyde.

To separate and identify ADH isozymes, high voltage starch gel electrophoresis and specific staining methods were employed as described earlier [9]. For electrophoresis of ADH and ALDH, Biotestgel starch (Biotest-Serum-Institut GmbH, Frankfurt, West Germany) was used. The Connaught starch as well as electro starch gave relatively poor resolution.

For the separation of isozymes of ALDH, either of the two following buffers were used: 0.1 M Tris/0.1 M maleic acid, 0.01 M EDTA, pH adjusted to 7.6 with NaOH or 0.2 M phosphate buffer, pH 7.2. Twenty times diluted buffer was used as the gel buffer. Electrophoresis was carried out for 5 hrs at 9V/cm. A specific staining method for ALDH isozymes was used as described previously [10]. Preparative isoelectric focusing (IEF) in a column (ampholyte, pH 3.5–10) was employed to isolate isozymes (enzyme 1 and enzyme 2) of ALDH from liver extracts. For enzyme kinetic studies, the assay conditions of Greenfield and Pietruszko [11] were used. The K_m values were determined from Lineweaver-Burk plots. Ten μl of saturated disulfiram dissolved in methanol was added into 25 ml assay solution (final concentration of

disulfiram, 20 μM), containing either 20 μM or 330 μM acetaldehyde for inhibition studies. Inhibition of isozymes was tested after electrophoretic separation by adding 50 μl of disulfiram solution into 25 ml of the staining solution.

RESULTS

Alcohol Dehydrogenase

In figure 1, a typical pattern on starch gel showing three different phenotypes for the ADH_2 locus and two different phenotypes for the ADH_3 locus is given. Comparing Japanese and Caucasian livers with atypical ADH (phenotype ADH_2 2-1), the electrophoretic pattern was found to be identical.

In table 1, the gene frequencies of $ADH_2{}^2$ and $ADH_3{}^2$ alleles are given. Out of 40 livers, 85% had the atypical ADH and 15% the typical. Figure 2 shows the enzyme activity profile of three phenotypes, ADH_2 1, ADH_2 2-1, and ADH_2 2, at different pH values. The atypical hetero- and homozygotes showed a pH optimum at 8.8, while the typical type (ADH_2 1) showed a pH optimum at 10.3. Atypical homozygotes and heterozygotes (ADH_2 locus), distinguished on the basis of electrophoresis, were compared for enzyme activity/gram fresh liver tissue. As seen in figure 2, the homozygotes had a little higher activity than the heterozygotes.

Aldehyde Dehydrogenase

Recently, Greenfield and Pietruszko [11] have characterized two isozymes of ALDH from human liver. The slow migrating isozyme, enzyme 1, has a high K_m for acetaldehyde and a low K_m for NAD. It is inhibited by disulfiram. The faster migrating isozyme, enzyme 2, has a low K_m for acetaldehyde and a higher K_m for NAD and is not inhibited by disulfiram.

In this study, we found two different types of ALDH in Japanese liver extracts. As

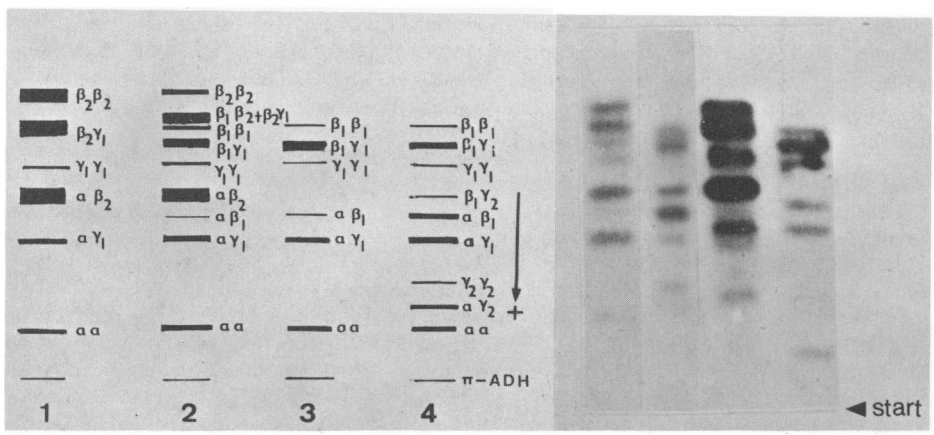

FIG. 1.—*Left*, Liver ADH isozyme patterns obtained by prolonged high voltage starch gel electrophoresis. *1*, ADH_2 2, ADH_3 1; *2*, ADH_2 2-1, ADH_3 1; *3*, ADH_2 1, ADH_3 1; *4*, ADH_2 1, ADH_3 2-1. *Right*, ADH isozyme patterns observed in Japanese liver samples. From *left* to *right*: ADH_2 2-1, ADH_3 1; ADH_2 1, ADH_3 2-1; ADH_2 2, ADH_3 1; ADH_2 1, ADH_3 1.

TABLE 1

GENE FREQUENCIES OF ADH_2 AND ADH_3 LOCI

GENE LOCI	*ADH_2* LOCUS			*ADH_3* LOCUS		
Phenotypes	ADH_2 1	ADH_2 2-1	ADH_2 2	ADH_3 1	ADH_3 2-1	ADH_3 2
Observed no.	6	18	16	36	4	0
%	15	45	40	90	10	0
Expected no.	5.62	18.75	15.62	36.1	3.8	0.1
Gene frequencies	$ADH_2^1 = .375$; $ADH_2^2 = .625$			$ADH_3^1 = .950$; $ADH_3^2 = .050$		
	$\chi^2 = .065$; df = 1; $P > .75$			$\chi^2 = .11$; df = 1; $P > .75$		

shown in figure 3, only 48% of the liver specimens had two isozyme bands (enzyme 1 and 2), and 52% had only the slow migrating band (enzyme 1).

The pH optima for usual and unusual types were determined at high concentration of acetaldehyde (330 μM). As shown in figure 4*a*, the pH values were approximately 9.7 and 9.0, respectively. At low concentration of acetaldehyde (20 μM), the total activity of the unusual type was lower than that of the usual type, as shown in figure 4*b*.

DISCUSSION

Using starch gel electrophoresis, Smith et al. [5] have shown that human liver ADH consists of three separate gene loci, *ADH_1*, *ADH_2*, and *ADH_3*, which control the three structurally different polypeptide subunits, α, β, and γ, respectively. Since *ADH_2* and *ADH_3* are polymorphic, the corresponding polypeptide chains are β_1, β_2 and γ_1, γ_2, respectively.

Electrophoretic analysis of Japanese liver extracts showed a clear distinction between homodimers and heterodimers of normal and variant β-subunits, namely, $\beta_1\beta_1$, $\beta_1\beta_2$, and $\beta_2\beta_2$. Similarly homo- and heterodimers of γ_1 and γ_2 subunits due to *ADH_3* polymorphism were also detected. Thus, our electrophoretic study enabled us to determine the phenotypes for the *ADH_2* and *ADH_3* locus in each liver specimen. These frequencies are in agreement with the earlier findings of Stamatoyannopoulos et al. [8] in Japanese individuals. Hitherto, no data on the frequency of the *ADH_3^2* gene have been reported in the Japanese. In comparison to a frequency of .37 in Europeans [5, 9], a value of .05 was found in the Japanese.

A pH optimum of 10.3 was found for the typical ADH. This is particularly interesting, as other authors [5, 7, 8] have mostly used a buffer of pH 10.8–11.0 for pH-activity determinations. We have always readjusted the final pH of assay solutions after addition of NAD. This may explain the apparent difference in the pH optimum.

Based on electrophoretic and kinetic characteristics, two different isozymes of human liver ALDH have been reported [3]. In previous studies [10, 12], we also found two major isozymes in autopsy liver extracts from Germans. The Japanese livers analyzed in the present investigation showed two electrophoretically distinct isozymes. In 52% of the specimens, the faster migrating isozyme was absent. No such polymorphism was observed in 68 liver specimens from German individuals [10].

Stamatoyannopoulos et al. [8] failed to detect any electrophoretic variation in ALDH isozymes in their study of Japanese livers. In this paper, the isozyme set showing both

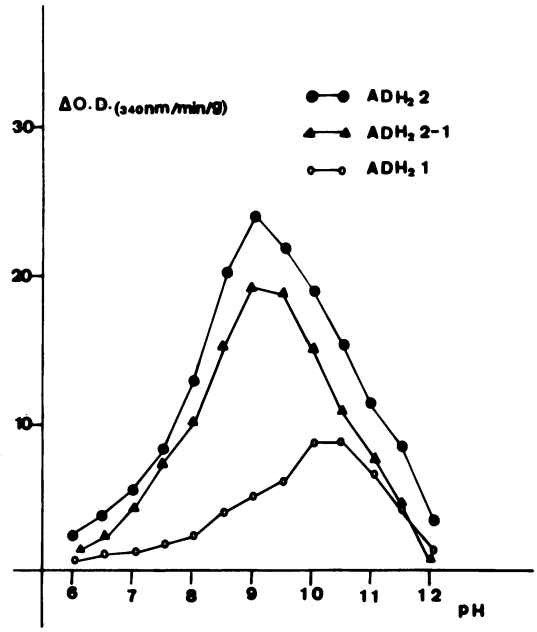

FIG. 2. —Activity curves (mean values from four different samples) at different pH for ADH_2 2, ADH_2 2-1, and ADH_2 1 phenotypes.

enzyme 1 and 2 bands will be called the "usual type," and the isozyme showing only enzyme 1 band, the "unusual type." The two enzyme bands purified partially by IEF were found to differ in substrate affinity for acetaldehyde and inhibition with disulfiram. The slower migrating isozyme has a low affinity for acetaldehyde at pH 9.5 (K_m value, approximately 0.1–0.4 mM) and the faster migrating isozyme, a high affinity (K_m value, approximately 0.01–0.02 mM). Only 20%–30% of the total activity in the usual type's crude extracts was inhibited by disulfiram, while in the unusual type, up to 90% was inhibited. In starch gel electrophoresis, the enzyme 1

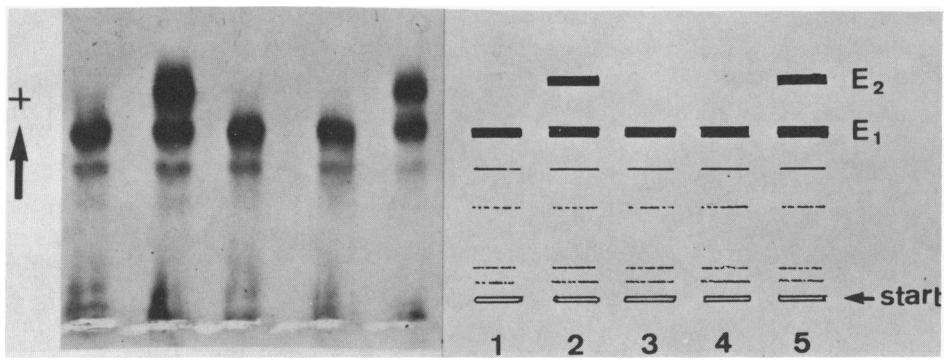

FIG. 3. —Liver ALDH isozyme patterns obtained by starch gel electrophoresis at pH 7.6 in Japanese (*1*–*4*) and European liver samples (*5*). *1*, *3*, and *4* correspond to unusual type; *2* and *5* correspond to usual type. Other minor bands also showed ALDH activity.

band was predominantly inhibited when disulfiram was included in the staining mixture.

We presume that the slow migrating enzyme band in our electrophoretic system is identical with the high K_m enzyme 1 described by Greenfield and Pietruszko [11]. We have isolated and purified both enzyme 1 and enzyme 2 from Japanese and German

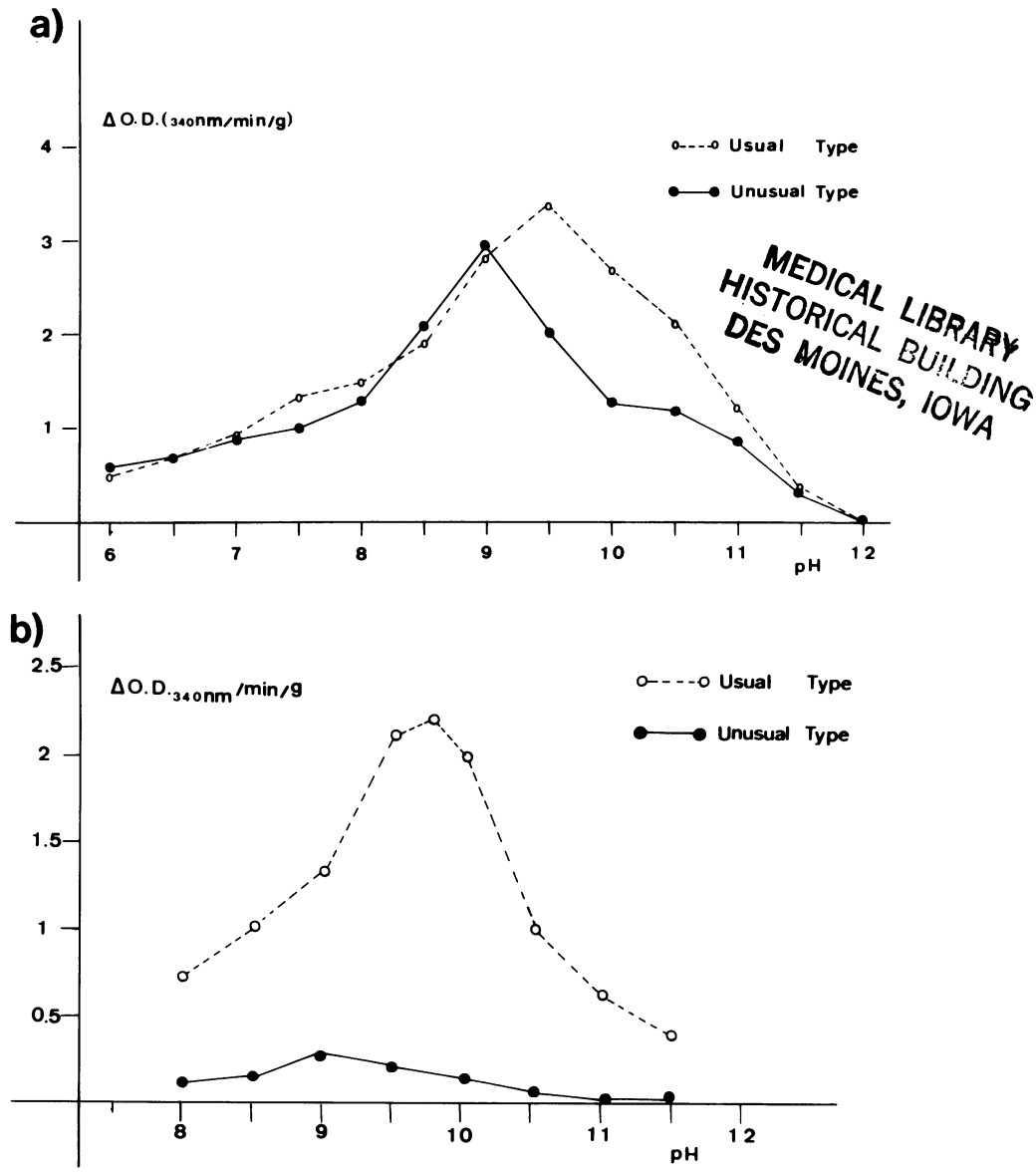

FIG. 4.—*a*, Activity curves (mean values from eight different samples) at different pH for usual and unusual ALDH types at high concentration of acetaldehyde (330 μM); *b*, activity curves at different pH for usual and unusual ALDH types at low concentration of acetaldehyde (20 μM).

livers. Subcellular distribution, as well as detailed kinetic data regarding isozymes, will be published elsewhere.

The atypical ADH, which is highly active at physiological pH [1], has been speculated to be responsible for the phenomenon of alcohol sensitivity in the Japanese by producing higher-than-normal acetaldehyde concentrations in response to ethanol [8]. Indeed, several toxicological effects of alcohol drinking have been attributed to acetaldehyde [13, 14]. Normally, most of the acetaldehyde formed in the liver is immediately further oxidized, but any change in ALDH isozymes might result in an altered concentration of blood acetaldehyde [15].

On the basis of our findings, we suggest that the initial intoxication after alcohol intake in the Japanese might be due to the absence of enzyme 2 (high affinity for acetaldehyde), as was indicated in more than 50% of the liver specimens. A major consequence of this may be that subjects possessing the unusual enzyme would be exposed to elevated blood acetaldehyde concentrations until its further oxidation by enzyme 1, which has a low affinity for acetaldehyde. Thus, delayed oxidation of acetaldehyde, rather than its higher-than-normal production following alcohol ingestion, may be responsible for the marked initial sensitivity to alcohol in the Japanese and other Mongoloids [16]. However, this hypothesis remains speculative until more evidence is gathered by measuring the alcohol and acetaldehyde degradation rate in subjects who possess either atypical ADH or unusual ALDH or both at the same time. We are currently trying to screen both normal subjects and chronic alcoholics for their ADH and ALDH phenotype using skin biopsies and cultured fibroblasts [12]. We also detected ALDH isozymes in human hair roots and in blood lymphocytes (unpublished results). The choice of subjects with atypical enzymes for alcohol metabolism may help in elucidation of differences in sensitivity to alcohol in various ethnic groups.

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