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Structural Mutation in a Major Human Aldehyde Dehydrogenase Gene Results in Loss of Enzyme Activity

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SUMMARY

Most Caucasians have two major liver aldehyde dehydrogenase isozymes, $ALDH_1$ and $ALDH_2$, while approximately 50% of Orientals have only $ALDH_1$ isozyme, missing the $ALDH_2$ isozyme. A remarkably higher frequency of acute alcohol intoxication among Orientals than among Caucasians could be related to the absence of the $ALDH_2$ isozyme, which has a low apparent K_m for acetaldehyde. Examination of liver extracts by two-dimensional crossed immunoelectrophoresis revealed that an atypical Japanese liver, which had no $ALDH_2$ isozyme, contained an enzymatically inactive but immunologically cross-reactive material corresponding to $ALDH_2$, beside the active $ALDH_1$ isozyme. Therefore, the absence of $ALDH_2$ isozyme in atypical Orientals is not due to regulatory mutation, gene deletion, or nonsense mutation, but must be due to a structural mutation in a gene for the *$ALDH_2$* locus, resulting in synthesis of enzymatically inactive abnormal protein.

INTRODUCTION

Virtually all Caucasians have two major liver aldehyde dehydrogenases (aldehyde: NAD oxidoreductase [ALDH], E.C.1.2.1.3). $ALDH_1$, an isozyme with slower anodal electrophoretic mobility at neutral to basic pH, has a low K_m for NAD and a high apparent K_m for acetaldehyde, and $ALDH_2$, an isozyme with faster anodal electrophoretic mobility, has a high K_m for NAD and a low apparent K_m for acetaldehyde [1, 2]. Judging from enzymatic characteristics of the isozymes of animal liver, human $ALDH_1$ is cytosolic and $ALDH_2$ is presumably of mito-

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chondrial origin [3, 4]. Approximately 50% of Orientals (Japanese, Chinese, and Asiatic Indians) have only one major isozyme, $ALDH_1$, missing the $ALDH_2$ isozyme [2, 5, 6]. A remarkably higher frequency of acute alcohol intoxication was observed in Orientals than in Caucasians (i.e., more than 80% vs. only about 5% [7, 8]). Goedde et al. proposed that an absence of the $ALDH_2$ isozyme with high affinity to acetaldehyde might be related to the high incidence of alcohol sensitivity among the Oriental-type atypical subjects [2]. On the other hand, Stamatoyannopoulos et al. suggested that the racial difference in acute alcohol intoxication could be due to rapid acetaldehyde formation by the highly active atypical alcohol dehydrogenase isozyme (alcohol:NAD oxidoreductase, E.C.1.1.1.1), which is common (about 90%) in Orientals [9]. Functional and structural differences between the usual and atypical alcohol dehydrogenases were recently disclosed in our laboratory [10].

Greenfield and Pietruszko reported that the mol. wt. of $ALDH_1$ and $ALDH_2$ are 245,000 and 225,000, respectively, and both enzymes are tetramers [1]. However, the subunit structure (i.e., whether each consists of a single type subunit or shares a common subunit) has not yet been fully elucidated. The genetic background of the absence of $ALDH_2$ isozyme in atypical Orientals is unknown. We report here the existence of enzymatically inactive but immunologically cross-reactive material corresponding to $ALDH_2$ in an atypical Japanese liver that does not have the active $ALDH_2$ isozyme.

MATERIALS AND METHODS

Human Liver

Autopsy livers from Caucasians and Japanese were stored at −65°C. The phenotype of aldehyde dehydrogenase was determined by starch gel electrophoresis as described [11]. We used usual and homozygous atypical livers for our study.

Preparation of Enzymes and Antibody

$ALDH_1$ and $ALDH_2$ were purified to homogeneity from a usual Caucasian liver. The purification procedures include: (1) chromatography with carboxymethyl Sephadex, (2) chromatography with DEAE-Sephadex, (3) affinity chromatography with 5′-AMP-Sepharose 4B, and (4) separation of $ALDH_1$ and $ALDH_2$ with DEAE-cellulose. The method was similar to that previously reported [1]. $ALDH_1$ was also purified to homogeneity from an atypical Japanese liver that had no $ALDH_2$ isozyme. Antibodies against $ALDH_1$ and $ALDH_2$ were produced in rabbits by immunizing the animals with the purified enzyme. An aliquot of antigen (0.26 mg of $ALDH_1$ or $ALDH_2$ dissolved in 0.5 ml of 0.15 M NaCl) was mixed with an equal volume of complete Freund's adjuvant and subcutaneously injected once a week. A week after the fourth injection, blood was collected and the antibody (Ig-G fraction) salted out with 2 M $(NH_4)_2SO_4$ and dissolved in 0.07 M acetate buffer, pH 5.0, in a volume about four times less than the original volume of the antiserum.

Crossed Immunoelectrophoresis

For the first dimension, electrophoresis was carried out in 12% starch gel (Electrostarch, Madison, Wis.) using one of the following buffer systems: sodium phosphate, 0.01 M, pH 6.5 and pH 7.2; Tris-malate, 5 mM, pH 7.6, containing 0.5 mM EDTA and 1 mM KCl; and Tris-Cl, 0.015 M, pH 8.6. For the second dimension, electroimmunodiffusion was carried

out in 1% agarose gels containing antibodies against $ALDH_1$ or $ALDH_2$ using veronal buffer system at pH 8.6 [12].

RESULTS AND DISCUSSION

Antibody against $ALDH_1$ precipitated and neutralized not only $ALDH_1$ but also $ALDH_2$. Antibody against $ALDH_2$ also reacted with both $ALDH_1$ and $ALDH_2$. Thus, $ALDH_1$ and $ALDH_2$ are immunologically homologous.

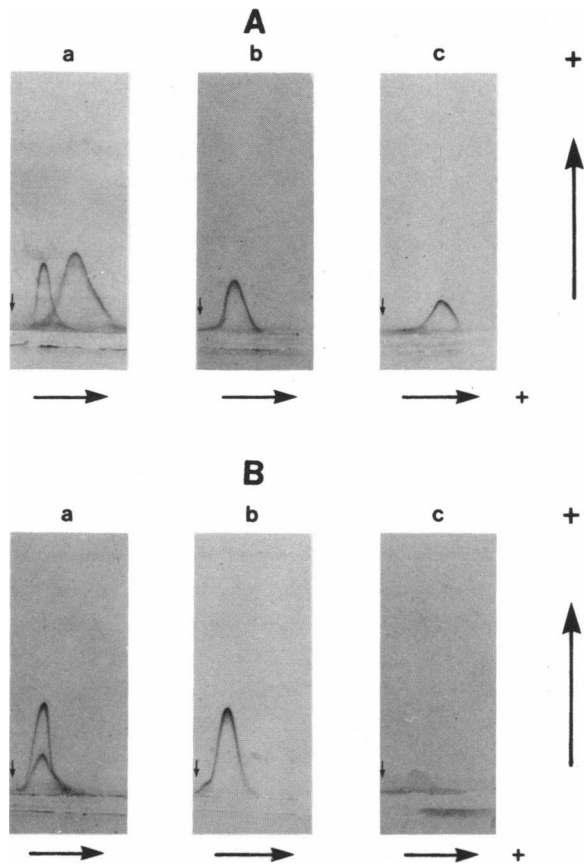

FIG. 1.—Crossed immunoelectrophoresis of aldehyde dehydrogenase. First dimension: Starch-gel electrophoresis using sodium phosphate buffer, 0.01 M, pH 7.2; 9 V/cm, 4 hrs, 4°C. Second dimension: Immunoelectrophoresis in agarose gel (1%, w/v) containing antibody against $ALDH_2$ (0.1%, v/v), using veronal buffer system at pH 8.6; 4 V/cm, 16 hrs, 4°C. Precipitin patterns were stained by Coomassie Brilliant Blue R. ↓ indicates sample origin. Source of enzyme preparation: *A*, Usual Caucasian liver—*a*, partially purified extract (at the step of DEAE-Sephadex); *b*, purified, homogeneous $ALDH_1$; *c*, purified, homogeneous $ALDH_2$; *B*, Atypical Japanese liver—*a*, partially purified extract (at the step of DEAE-Sephadex); *b*, enzymatically active fraction eluted from affinity column; *c*, unbound, enzymatically inactive fraction from affinity column. The unbound, enzymatically inactive fraction of usual Caucasian liver preparation from the affinity column did not show any immunologically active component.

Precipitin patterns in two-dimensional crossed immunoelectrophoresis indicated that partially purified preparation (at the step of DEAE Sephadex) of the Caucasian liver contained two antigenic components that corresponded to $ALDH_1$ and $ALDH_2$ (fig. 1*A-a*). The partially purified preparation (at the step of DEAE-Sephadex) of the atypical Japanese liver contained two distinctive immunologically active components that comigrate in starch gel electrophoresis but are clearly distinguishable in electroimmunodiffusion (fig. 1*B-a*). When the partially purified preparation of Japanese liver extract was treated with 5'-AMP Sepharose 4B, washed with 30 mM sodium phosphate, pH 6.0, containing 1 mM EDTA and 0.1% 2'-mercaptoethanol, and extracted with 100 mM sodium phosphate, pH 8.0, containing 1 mM EDTA and 0.1% 2'-mercaptoethanol, the extracted enzyme fraction contained only one immunologically active component (fig. 1*B-b*). The unbound eluate from the affinity column, which had no aldehyde dehydrogenase activity, also contained an immunologically active component, as demonstrated in a precipitin peak in crossed immunoelectrophoresis (fig. 1*B-c*). These results unequivocally indicate that the atypical Japanese liver contains an enzymatically inactive but immunologically cross-reactive material that is homologous to $ALDH_2$ (CRM-$ALDH_2$) beside the enzymatically active $ALDH_1$ isozyme. $ALDH_1$ and CRM-$ALDH_2$ always comigrated in starch gel electrophoresis at pH 6.5, 7.2, 7.6, and 8.6.

The absence of the $ALDH_2$ isozyme in about 50% of Orientals [5, 6] is not due to regulatory mutation, gene deletion, or nonsense mutation, but must be due to a structural mutation in a gene for the $ALDH_2$ locus, resulting in formation of an enzymatically inactive, more basic protein. It would be interesting to investigate an exact amino acid substitution that caused the total loss of aldehyde dehydrogenase activity in the mutant $ALDH_2$ protein.

Since the usual structural gene $ALDH_2^1$ and the atypical gene $ALDH_2^2$ are most likely codominantly expressed, the estimated frequency of usual $ALDH_2^1$ = .10, heterozygous $ALDH_2^1/ALDH_2^2$ = .40, and homozygous atypical $ALDH_2^2/ALDH_2^2$ = .50 in Orientals. Thus far, it was impossible to distinguish heterozygous $ALDH_2^1/ALDH_2^2$ from homozygous usual $ALDH_2^1/ALDH_2^1$, since both are expected to exhibit the two major isozyme components in starch gel electrophoresis and in isoelectric focusing. Examination of the presence or absence of CRM component by the crossed immunoelectrophoresis, as described in this paper, will allow us to distinguish the heterozygous from the usual homozygous and experimentally prove the above estimation of genotypes of $ALDH_2$ locus.

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