IDENTIFICATION OF A DISTINCTIVE MOLECULAR FORM OF ALCOHOL DEHYDROGENASE IN HUMAN LIVERS WITH HIGH ACTIVITY[1]

Ting-Kai Li and Leslie J. Magnes

Departments of Medicine and Biochemistry
Indiana University School of Medicine
Indianapolis, Indiana 46202

Received January 6, 1975

Summary

Human liver alcohol dehydrogenase activity and isoenzyme pattern have been compared in specimens from different sources. Specific enzymatic activity was significantly higher in surgical biopsy samples than in autopsy specimens. Furthermore, tissues from individuals who died suddenly from trauma exhibited significantly higher activities than those obtained following disease-related deaths. All livers with high specific activity revealed on starch gel electrophoresis, in addition to the usual isoenzymes, a prominent, previously unrecognized, activity band. This molecular form, more anodic than the others at pH 8.6 and 7.7, was separated by isoelectric focusing. It exhibited kinetic properties characteristic of the alcohol dehydrogenase isoenzymes.

It has been apparent ever since alcohol dehydrogenase (ADH) from human liver was first studied (1) that the enzyme exhibits multiple molecular forms. This heterogeneity, identified most clearly on starch gel electrophoresis as a complex set of isoenzymes, develops perinatally (2, 3) and is present in the livers of adults to variable and different extents (3-8). In some livers, as many as 7-9 activity bands are observed. Past studies have also shown that the enzyme is a dimeric molecule (9) and that at least some of the isoenzymes can arise from the random association of polypeptide chains (8, 10). However, if this process is the basis of formation of all the isoenzymes, there must exist more than 2 gene loci, each coding for a structurally different chain. Indeed, Harris and coworkers have recently presented convincing evidence that the variations in the ADH isoenzyme pattern of human liver can be accounted for on the basis of 3 separate gene loci, genetic polymorphism at 2 of the loci, and individual differences in phenotypic expression (6, 7, 10, 11).

[1]Supported by the U.S. Public Health Service (NO1-AM-3-2209).

Another complexity in the study of human liver ADH has been the finding that the amount of enzyme activity differs widely from liver to liver (4, 12). The cause of this variation is unknown. However, since most studies have employed autopsy specimens, the effect of events surrounding the time of death should be considered. This communication compares the activities and isoenzyme patterns of liver biopsy specimens with those of postmortem samples derived from different sources. The presence of a previously uncharacterized, electrophoretically distinct, molecular form of ADH in livers with high specific activity is described.

Materials & Methods

Autopsy liver specimens were received within 12 hours of death. Biopsy specimens were obtained with informed consent as part of the liver biopsy sample routinely taken in the surgical staging procedure for Hodgkin's disease. All samples were immediately stored at -20°C. Portions of the liver specimens were thawed, homogenized in 5 volumes of 0.1 M phosphate buffer, pH 7.4, 0°C and clarified by centrifugation at 100,000 x g for 60 min. Enzymatic activities were measured spectrophotometrically (13) with ethanol and other alcohols as substrate at pH 10.5 and 8.2. Protein concentrations were determined by the method of Lowry et al (14). Specific enzymatic activities are expressed as μmoles NADH formed/min/mg soluble protein.

Isoenzymes of ADH were separated by electrophoresis at pH 7.7 and 8.6 in 10.4% starch gels (Electrostarch Co., Madison, WI). The conditions described by Smith et al (6, 7) were employed. Samples applied to the same gel were brought to the same activity per unit volume by dilution or ultrafiltration. ADH activity in the gel was detected with ethanol or n-amyl alcohol as substrate (7).

The enzyme was purified by $(NH_4)_2SO_4$ fractionation and chromatography on DEAE-cellulose as described by Pietruszko et al (8). Recovery of 70-80% of the total activity and all of the isoenzyme bands originally present in the homogenate was attained. After dialysis against 1% glycine, isoelectric focusing was performed on the purified enzyme in an 110 ml electrofocusing column (LKB Produkter, AB, Sweden).

Results and Discussion

The specific ADH activities of 6 biopsy and 35 autopsy liver specimens were measured (Table I). Twenty-two autopsy specimens were obtained from apparently healthy individuals who died suddenly from physical trauma (group B) and 13 from hospitalized patients who had succumbed to acute and chronic illnesses (group C). Thirty-nine livers exhibited the usual pH optimum at pH 10.5 but 2 specimens in group B, showed an "atypical" (15) pH-rate profile. The mean specific activity of group A was significantly ($P<0.02$) higher than

TABLE I. Comparison of the specific ADH activities of biopsy and autopsy liver specimens.

Source	Number of Samples	Specific Enzymatic Activity[1] (μmoles NADH formed/min/mg soluble protein)	
		Range	Mean ± SD
A. Biopsy	6	0.104 - 0.053	0.082 ± 0.020
B. Autopsy, sudden traumatic deaths	22	0.098 - 0.016	0.057 ± 0.020[2]
C. Autopsy, disease-related deaths	13	0.035 - 0.014	0.022 ± 0.007

[1]Measured at pH 10.5 with ethanol as substrate.

[2]Expressed as ΔA_{340}/min/g. tissue, these results are in good agreement with those of Azevedo et al (16).

that of group B, which in turn was significantly ($P<0.001$) higher than that of group C. These results, therefore, confirm the recent report of Azevedo et al (16) that the health of the individual before death can profoundly alter ADH activity in autopsy specimens. However, the wide range of specific activities seen in group B and its lowered mean activity when compared with group A suggest that the time interval and the environmental conditions between death and autopsy can also alter ADH activity. In support of this conclusion, incubation of a liver with high initial activity at room temperature produced a decrease in activity of 10% in 5 hours and of 25% in 17 hours. By contrast, storage of the livers at -20°C for more than 4 months resulted in the loss of only 10-15% of activity. The biopsy specimens were obtained from individuals with Hodgkin's disease, although the samples themselves were devoid of tumor cells. This circumstance together with the small number of samples studied preclude any conclusions about the degree of individual variation of liver ADH activity that is truly present in the healthy man.

Starch gel electrophoresis was performed at pH 7.7 and 8.6 on all the

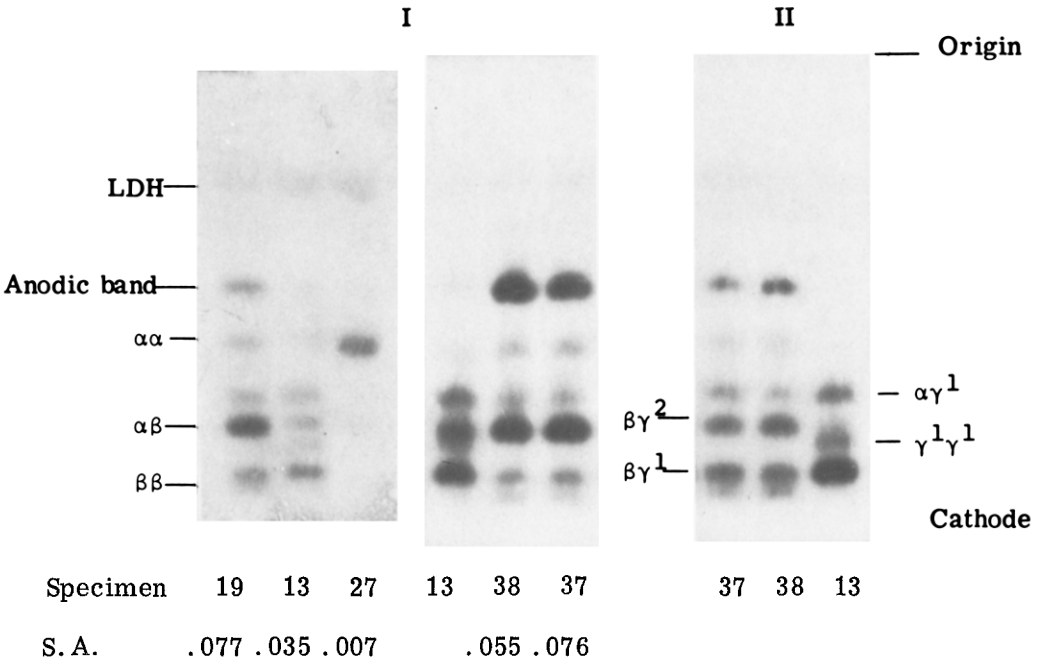
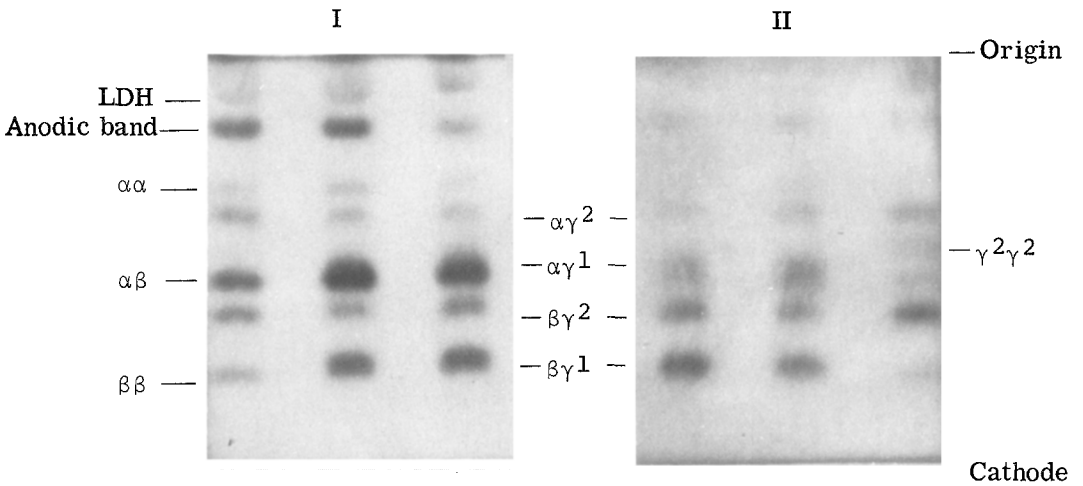


Fig. 1 Starch gel electrophoresis of human liver alcohol dehydrogenase. Conditions: pH 7.7; 7 V/cm for 19 h. at 4°C. Enzymatic activity in the gels was detected with either ethanol (I) or n-amyl alcohol (II) as substrate. Specimens 13 and 19 were obtained at autopsy from individuals who died suddenly from trauma. Specimen 27 was liver from the autopsy of a 3½ lb. premature infant who died 1 day after birth. Specimens 37 and 38 were biopsy samples. Specimen 13 is phenotype ADH_3 1 and specimens 19, 37 and 38 are ADH_3 2-1. S.A. is specific enzymatic activity of the samples expressed as μmoles NADH formed/min/mg soluble protein.

samples and the ADH isoenzyme patterns were compared with the specific enzymatic activities. Representative results are shown in Figures 1 and 2. The nomenclature of Smith et al (7) was employed for the identification of the isoenzymes The liver from a premature newborn which contained predominantly the αα isoenzyme served as a marker. Activity staining with n-amyl alcohol facilitated the detection of the isoenzymes which contained the γ chains (7). All of the isoenzymes characterized by Smith et al (6, 7) were readily identified, thus confirming their studies. However, it was of considerable interest that the specimens with high specific enzymatic activity consistently exhibited another, prominent, activity band which was more anodic than the αα isoenzyme. This molecular form (labeled anodic band in Figures 1 & 2) was present in all the



Specimen	54	53	39	39	53	54
S.A.	.077	.082	.057			

Fig. 2 Starch gel electrophoresis of human liver alcohol dehydrogenase. Conditions: pH 8.6; 7 V/cm for 18 h. at 4°C. Enzymatic activity was detected with either ethanol (I) or n-amyl alcohol (II) as substrate. All specimens were obtained at autopsy from individuals who died suddenly from trauma. Specimens 39 and 53 are phenotype ADH_3 2-1 and specimen 54 is ADH_3 2. S.A. is specific enzymatic activity.

biopsy specimens and most of the group B specimens. It was absent or only faintly visualized in the group C specimens. At pH 7.7, the band was clearly separated from the lactate dehydrogenase isoenzymes, identified by staining with lactate as substrate.

It should be noted that an activity band similar to the one here described had been demonstrated previously by Pikkarainen and Raiha (2) in a study which employed a biopsy liver specimen. Presumedly because this molecular form of the enzyme is more labile than the others *in vivo*, its presence escaped further detection in subsequent work which have utilized principally livers from deceased, hospitalized patients. However, it did not appear to be unusually labile *in vitro* at low temperatures. Storage of the supernate of homogenized livers for 3-4 days at 4°C did not result in preferential loss or deterioration of this band nor was it destroyed during purification by $(NH_4)_2SO_4$ fractionation and chromatography on DEAE-cellulose.

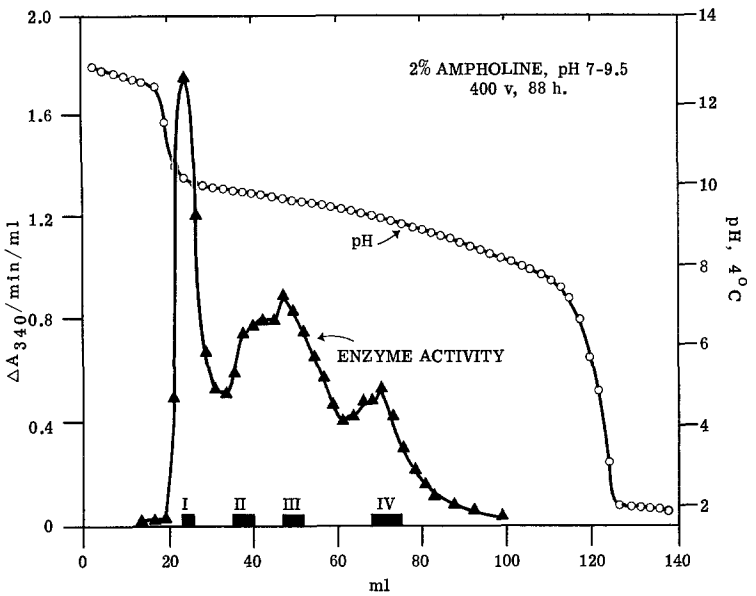


Fig. 3 Isoelectric focusing of human liver alcohol dehydrogenase. A portion of a liver with high specific ADH activity (0.080 µmoles NADH/min/mg soluble protein, phenotype ADH_3 2-1) was homogenized, purified by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography, dialyzed against 1% glycine and applied to the electrofocusing column. After 88 h, the enzymatic activity and pH of the effluent fractions (2.5 ml) were measured. Fractions within the zones I, II, III, and IV were combined and analyzed by starch gel electrophoresis.

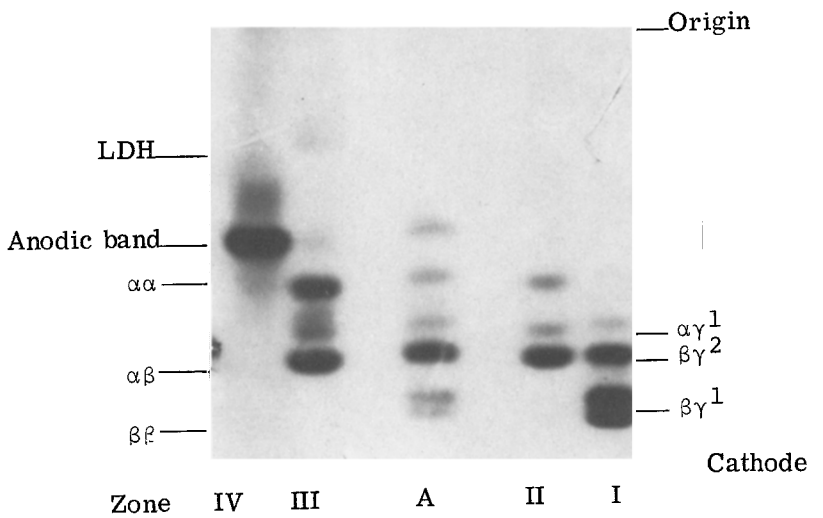


Fig. 4 Starch gel electrophoresis of purified human liver alcohol dehydrogenase. Conditions: pH 7.7; 7 V/cm for 19 h. at 4°C. The enzyme solution before electrofocusing (A) is compared with selected, combined fractions (zones I, II, III and IV; Figure 3) after electrofocusing. The gel was developed with ethanol as substrate.

Separation of the anodic band from the other isoenzymes was accomplished by isoelectric focusing. Representative results are shown in Figures 3 & 4. Under the conditions employed, its pI was 9.2. The following kinetic properties of the band (zone IV, Figures 3 & 4) were determined: Km for NAD^+, 0.05 mM (pH 10.5, 20 mM ethanol); Km for ethanol, 5.3 mM (pH 10.5, 2 mM NAD^+); pH optimum for ethanol oxidation, 10.5; substrate specificity, ethanol>butanol> n-amyl alcohol>>isopropanol>>methanol; negligible activity with $NADP^+$ as coenzyme.

The relationship of this molecular form of ADH to the others previously characterized is currently unknown and will require detailed examination. It is clear, however, that in future studies of human liver ADH, attention should be given to the nature and the handling of the specimens. Such considerations are likely to be pertinent also in the study of other enzymes in tissues of human origin.

References

1. VonWartburg, J.-P., Bethune, J. L., and Vallee, B. L. (1964) Biochemistry 3, 1775-1782.
2. Pikkarainen, P. and Raiha, N. C. R. (1969) Nature 222, 563-564.
3. Murray, R. F. and Motulsky, A. G. (1971) Science 171, 71-73.
4. Blair, A. H. and Vallee, B. L. (1966) Biochemistry 5, 2026-2034.
5. Schenker, T. M., Teeple, L. J., and vonWartburg, J.-P. (1971) Eur. J. Biochem. 24, 271-279.
6. Smith, M., Hopkinson, D. A., and Harris, H. (1971) Ann. Hum. Genet. 34, 251-271.
7. Smith, M., Hopkinson, D. A., and Harris, H. (1972) Ann. Hum. Genet. 35, 243-253.
8. Pietruszko, R., Theorell, H., and deZalenski, C. (1972) Arch. Biochem. Biophys. 153, 279-293.
9. Jornvall, H. and Pietruszko, R. (1972) Eur. J. Biochem. 25, 283-290.
10. Smith, M., Hopkinson, D. A., and Harris, H. (1973) Ann. Hum. Genet. 36, 401-414.
11. Smith, M., Hopkinson, D. A., and Harris, H. (1973) Ann. Hum. Genet. 37, 49-67.
12. Murray, R. F. and Price, P. H. (1972) Ann. N.Y. Acad. Sci. 197, 68-72.
13. Li, T.-K. and Theorell, H. (1969) Acta Chem. Scand. 23, 892-902.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
15. VonWartburg, J.-P., Papenberg, J., and Aebi, H. (1965) Can. J. Biochem. 43, 889-898.
16. Azevedo, E., Smith, M., Hopkinson, D. A., and Harris, H. (1974) Ann. Hum. Genet. 38, 31-37.