ZINC CONTENT AND SPECIFIC ACTIVITY OF HORSE LIVER ALCOHOL DEHYDROGENASE ISOENZYMES*

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SUMMARY

Zinc analyses and specific activity determinations on the two major electrophoretic bands of horse liver alcohol dehydrogenase revealed no significant differences between them or between the isolated bands and the starting material.

It is now established that horse liver alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1) is heterogeneous on starch gel or cellulose acetate electrophoresis (1-3). This heterogeneity now appears to be a consequence of the existence of isoenzymes, due to the formation of hybrids of two different sub-units (4,5). Of the 5 bands showing alcohol dehydrogenase activity, one band, the most cathodally moving band ("Band 1", according to Pietruszko (2)), also possesses steroid dehydrogenase activity. However, this band forms at most about 1% of the total protein in commercially prepared horse liver alcohol dehydrogenase.

Vallee and coworkers (6), who find a somewhat lower amount of zinc in their preparations of horse liver alcohol dehydrogenase than has been reported by Akeson (7) and from our laboratory (8), has proposed that the non-integral number of zinc atoms he finds is due to a different amount of zinc bound to the various subunits of the enzyme, resulting in differing

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amounts of zinc bound to the isoenzymes, thus leading to non-integral values when a mixture of all of the isoenzymes is analyzed. Isolation of the isoenzymes, followed by zinc analysis of each one would provide evidence for the validity of this proposal, and forms the substance of this report.

MATERIALS AND METHODS

Horse liver alcohol dehydrogenase, lots No. 6277431 and 6397231, and NAD^+ were obtained from Boehringer Mannheim Corporation, San Francisco, California; starch was obtained from Connaught Medical Research Laboratories, Toronto, Canada; all other reagents and chemicals were obtained from Sigma Chemical Co., St. Louis, Missouri.

The enzyme was dialyzed, assayed and its zinc content determined as previously described (8). Starch gel electrophoresis was carried out in 0.005 M PO_4 buffer, pH 7.5 (tank buffer: 0.075 M PO_4, pH 7.5, 62 gms of starch/500 ml 0.005 M PO_4, pH 7.5 was used to form the gel). Samples were placed on filter paper strips and inserted in slots cut in the gel. Runs were done at 4° at approximately 9 V/cm, for 15-18 hours. Band 1 moved 9-10 cm and band 3 (the major band) moved 5-6 cm from the origin. One half of the gel strip was stained for activity using the phenazine methosulfate-nitro blue tetrazolium method as described in "Methods in Enzymology", VI, 968 (1963). The protein bands on the unstained halves were cut out, homogenized with a glass rod at 4° in a minimum volume of buffer, filtered on a Buchner funnel, then filtered through a 0.22 μ Millipore filter, and assayed for zinc, activity, and protein. The protein method used for the starting material (absorbance at 280 nm) could not be used in the gel extracts due to the presence of soluble starch, which gave a high absorbance at 280 nm, so the method of Lowry (9) was used.

RESULTS AND DISCUSSION

Soluble starch was detected in the clear gel extracts by the iodine test. This starch interfered with both the protein determination and the

zinc analysis. Although reasonably low values were obtained when blank areas of the gel were extracted, the presence of starch enhanced the absorbancy of zinc standards, and depressed the reading of protein standards, as shown in Fig. 1. These effects complicated the analyses, but when the corrections were made, the correct specific activity and zinc content of

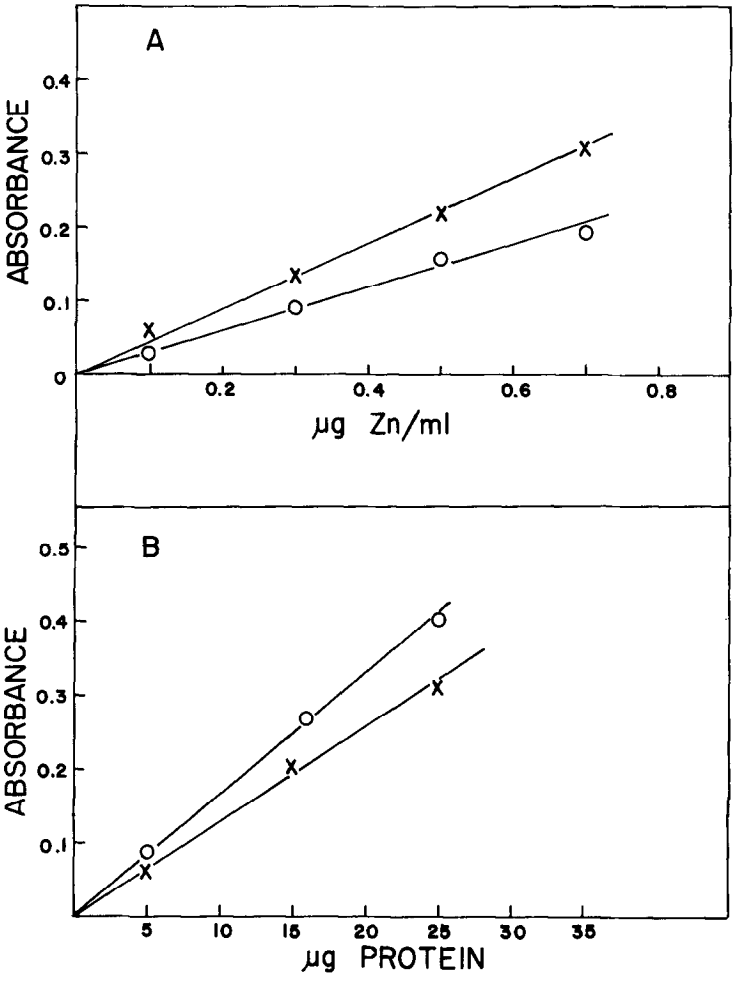

Fig. 1. A. The effect of starch on the zinc analysis. Zinc is determined by atomic absorption spectrophotometry at 213.8 nm. O, zinc standard in 0.005M phosphate buffer, pH 7.5; X, zinc standard in the buffer extract from 0.5 gm. of starch gel.

B. The effect of starch on the Lowry protein analysis. The absorbance is determined in 1 cm cells at 750 nm. O, enzyme in buffer only; X, enzyme in the buffer extract from 0.5 gm. of starch gel.

the starting enzyme added to a blank gel homogenate was obtained. The starch had no effect on the enzymatic activity.

In Table I is summarized our data on the two major electrophoretic bands - bands 3 and 4 which accounted for over 90% of the total protein (approximately 70-75% in band 3 and 15-20% in band 4).

Analysis of the data of Table I indicates that there are no statistically significant differences between the specific activities and zinc contents of band 3 and band 4. Since both bands contain nearly 4 gm. atoms of zinc/mole enzyme and account for 90% of the total protein, even if the other 3 bands contained no zinc, the average value for all bands together would be 3.5-3.6 gm. atoms zinc/mole. This represents a low estimate, since at least 2 zinc atoms appear essential for activity, and all bands possess alcohol dehydrogenase activity. If the remaining 10% of protein has 2 gm. atoms zinc/mole, then the average value for the total would be 3.7-3.8 gm. atoms/mole, a value somewhat higher than that reported by Vallee (6). It should also be noted that Theorell (3) obtained 4 gm. atoms zinc/mole for band 1, which he isolated by free boundary electrophoresis.

In conclusion, we feel the weight of evidence obtained so far indicates no significant differences in the zinc content and specific activity of the two major electrophoretic bands of horse liver alcohol dehydrogenase.

TABLE I

Specific Activity and Zinc Content of Electrophoretic Bands from Horse Liver Alcohol Dehydrogenase

Band No.	Specific Activity*	Gram-Atoms Zn/Mole
3	15.5 ± 0.6 S. D.	3.9 ± 0.6 S.D.
4	17.1 ± 1.8 S. D.	3.9 ± 1.2 S.D.

*ΔOD_{340} nm/mg enzyme/min at 25°, data from 6 consecutive electrophoresis runs. The starting material assayed 15.9 ± 0.7 S.D. for specific activity, and 3.9 ± 0.1 gm. atoms Zn/mole (n = 5).

Because of the low protein/gel ratio in bands 2 and 5, we have not yet succeeded in obtaining sufficiently accurate zinc values for these bands. Good data from these bands will hopefully resolve the question of zinc heterogeneity in horse liver alcohol dehydrogenase isoenzymes.

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