THE "ISOMERASE" ACTIVITY OF HORSE LIVER ALCOHOL DEHYDROGENASE

Robert H. McKay and Priscilla B. Searl

Department of Biochemistry and Biophysics, School of Medicine

University of Hawaii, Honolulu, Hawaii 96822

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Van Eys, (1961) and later Snyder and coworkers in a series of papers (Vogel, et al., 1963; Snyder, et al., 1963 and 1965) published evidence for a triose phosphate isomerase activity of horse liver alcohol dehydrogenase (LADH) (E.C.1.1.1.1. alcohol:NAD oxidoreductase, equine liver). Recently, Snyder and Lee (1966) succeeded in separating an isomerase from crude LADH preparations by a chromatographic procedure using crude CM-cellulose. They observed that present commercially available preparations of LADH do not contain isomerase.

We have instituted studies in this laboratory on the role of zinc in LADH (Oppenheimer, et al., 1967), and also had investigated the role of zinc in the "isomerase" of LADH prior to the publication of Snyder's latest paper (Snyder and Lee, 1966). The results of that paper have prompted us to summarize our findings at this time, since we obtained somewhat different results.

We also do not find isomerase activity in commercial LADH when glyceraldehyde-3-phosphate (GAP) is used as the substrate and is coupled to the α-glycerol phosphate dehydrogenase reaction. However, we do find an increase in material yielding formaldehyde after periodate oxidation, following incubation of GAP with commercial LADH and NAD--e.g. using the method of Bailey, (1959) which both Snyder and Van Eys had used to make their initial observations. Van Eys (1961) observed both a color increase using the method of Bailey, and a reaction with α-glycerol phosphate dehyrogenase when GAP

was incubated with LADH. However, his LADH preparation was probably impure. There is no clear evidence that Snyder and Lee (1966) performed this crucial experiment with their purified LADH.

Materials and Methods

LADH (Lot #6156427, 64704, 6096127), and α-glycerol phosphate dehydrogenase (rabbit muscle) (Lot #6056508) were from Boehringer Mannheim Corp. Triosephosphate isomerase (rabbit muscle) (Lot # 76B-0920), glyceraldehyde phoshpate dehydrogenase (rabbit muscle) (Lot #104B-1231), dl-glyceraldehyde-3-phosphate diethylacetal, monobarium salt, and dihydroxyacetone phosphate dimethylketal, di-monocyclohexylamine salt were from Sigma Chemical Co. NAD Was obtained from Calbiochem, and $NADH_2$ from P-L Biochemicals, Inc.

LADH was dialyzed, assayed, and its zinc content determined as previously described (Oppenheimer, et al., 1967) except the enzyme was dialyzed against phosphate buffer, since tris buffer is cleaved by periodate and contributes a large blank to the Bailey colorimetric assay. d-GAP concentration was determined enzymatically using glyceraldehyde phosphate dehydrogenase by the method supplied by Sigma with the GAP. Dihydroxyacetone phosphate (DHAP) concentration was determined using α-glycerol phosphate dehydrogenase by a modification of the method of Beisenherz, et.al.(1955). Tris buffer was used, the amount of enzyme was doubled to 2 μg, and an amount of DHAP (approximately 0.1 μ mole) was added so that the absorbancy change was approximately 0.2 (1 cm cell, 25°, 340 mμ, 3.0 ml total volume). Isomerase activity was measured according to Beisenherz (1955), except that tris buffer was used, the optical path length was 1 cm, and the absorbancy was measured at 340 mμ. Application of the Bailey procedure to the measurement of isomerase activity was exactly as described by Snyder, et al., (1965).

Results and Discussion

Figure 1 shows the standard curves obtained with various compounds using the Bailey procedure. DHAP, dihydroxyacetone, 1,2-propanediol, and dl-glycer-

aldehyde all yield a color equivalent to that expected for the release of 1 mole of formaldehyde following periodate oxidation. dl-GAP, however, yields a considerably reduced, although significant, color, presumably due to the uncleaved aldehyde, since cleavage by periodate would yield formic acid and glycolic or 2-phosphoglycolic acid. These data illustrate why it would be impossible to determine by this procedure alone the amount of dihydroxyacetone phosphate present in a mixture also containing GAP or any of the other compounds shown in Figure 1, unless some independent method of calibrating the color reaction is used.

The increased color detected by the Bailey method when LADH is incubated with dl-GAP, and NAD, is not found in the absence of NAD or enzyme, is not consistently dependent upon LADH concentration, and shows a rather high standard deviation. The deviations in the presence of 1,2-propanediol alone are considerable. These points are summarized in Table I, which includes all of our data for 3 different lots of LADH. The difference in the mean of GAP in the presence of LADH and NAD over that for GAP alone is 40%. It

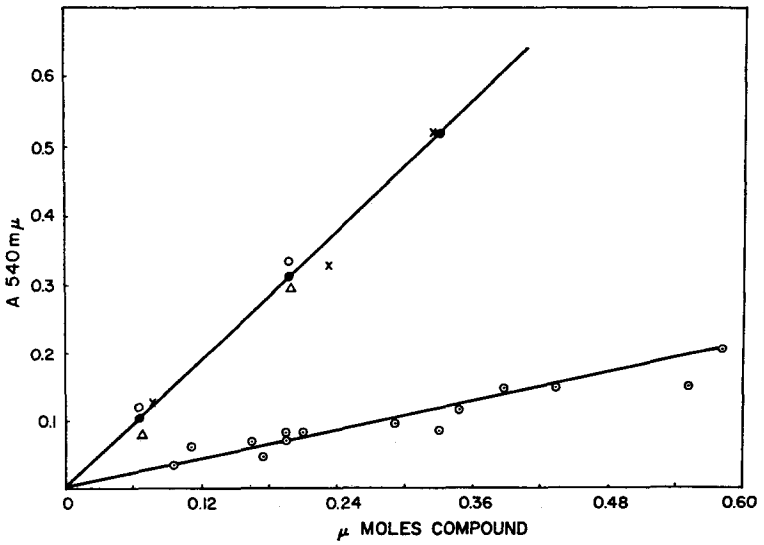

FIGURE 1. Absorbance (540 mμ, 1cm) versus amount of compound in assay cuvette (Bailey colorimetric procedure). ●——●, 1,2-Propanediol; X——X, Dihydroxyacetone phosphate; O——O, Dihydroxyacetone; △——△, Glyceraldehyde; ⊙——⊙, dl-Glyceraldehyde-3-phosphate.

can be seen that varying the enzyme concentration had no statistically significant effect, since the coefficient of variation is still less than that for GAP alone.

TABLE I

Color Yield of the Phenylhydrazine-ferricyanide Complex After Incubation of the Indicated Compound (s) for 15 min. at 37° Prior to Assay*

Compound	No. of Expts.	Absorbance** 540 mμ (Mean ± S.D.)	Coefficient of Variation %
1,2-propanediol***	15	0.282 ± 0.029	10
dl-Glyceraldehyde-3-phosphate#	34	0.118 ± 0.024	20
dl-Glyceraldehyde-3-phosphate + LADH + NAD##	24	0.165 ± 0.021	12

*Method of Bailey as adapted by Snyder, (1965)
**1 cm light path
***15 μg (0.198 μ mole)
#88.5 μg (0.520 μ mole)
##88.5 μg dl-Glyceraldehyde-3-phosphate + 0.9-1.8 μg LADH + 1.5 μg NAD

Aliquots of the same incubation mixture, containing GAP, NAD, and LADH, which gave a color increase in the Bailey assay were then assayed enzymatically for the presence of any DHAP formed at the end of 15 minutes incubation at 37°. In no case was any DHAP detected, even when aliquots containing 4.5 μg LADH and 2.4 μ mole dl-GAP to start with were taken (3 times the amount used in the Bailey assay). The enzymic method could easily have detected 0.005 μ moles of DHAP, and according to the color increase of the Bailey assay, (assuming all of the color increase to be due to the formation of DHAP), there should have been between 0.2 and 0.6 μ moles DHAP in the aliquots tested enzymatically.

As a further test, LADH, up to 90 μg was tested in the isomerase assay of Beisenherz, and no activity was found. Under these conditions, 0.03 μg of muscle triose phosphate isomerase converted approximately 0.06 μ mole d-GAP/min

It was also found that the GAP (at least the d-isomer) was unstable during the 15 min. incubation at 37°. The concentration decreased about 25% as determined by glyceraldhyde-3-phosphate dehydrogenase. The same decrease of d-GAP was found in the mixture containing LADH and NAD. This result suggests that the color increase seen by the Bailey assay may be due to a reaction with GAP decomposition products, rather than to any formation of DHAP.

In conclusion, we find no evidence of isomerase activity in LADH, using GAP as the substrate, under conditions where a color increase is observed by the Bailey method. We would urge caution in using relatively unspecific and unstable color reactions of this type to detect products formed enzymatically, especially when more specific and sensitive enzymatic methods are readily available.

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