ALDOSE-1-EPIMERASE FROM HOG KIDNEY: ISOLATION AND EVIDENCE OF PURITY, CHEMICAL PROPERTIES, AND INHIBITION KINETICS

Selma L. Lapedes and Aurin M. Chase
Department of Biology, Princeton University, Princeton, New Jersey

Received May 24, 1968

Extracts from the mold, Penicillium notatum, were reported by Bentley and Neuberger (1949) to catalyze the mutarotation of D-glucose, and Keilin and Hartree (1952), extending this work, named the catalyst "mutarotase" (now listed as aldose-1-epimerase). Keston (1954) reported such an enzyme from animal sources. The function of these enzymes is unknown but a possible role in the active transport of sugars has been postulated by Keston. Involvement in the metabolic utilization of certain sugars has also been suggested.

Mutarotases have to date been reported from a bacterium (E. coli), a mold (P. notatum), higher plants, and -- in mammals -- from various organs and tissues, including erythrocytes and the lens of the eye. The enzymes have been purified to a greater or less degree from some of these sources. Comparison of their physical, chemical and kinetic properties indicates that they may differ from one another.

In the case of hog kidney mutarotase, we have recently introduced modifications in the purification procedure of Li (1965) which have yielded a preparation with a single symmetrical peak (see Figure 1, A and B) on sedimentation with the Spinco Model E ultracentrifuge. This indication of homogeneity was corroborated by disc gel electrophoresis, which showed only one band following our final purification step. This is illustrated by Tubes 1 and 2 in Figure 1. In a recent abstract (Fishman et al., 1968) one band on disc gel electrophoresis was reported for bovine kidney mutarotase, but details of the purification procedure were not given. However, the molecular weight of that enzyme, 45,000 ± 5,000, is sufficiently greater than our value for hog kidney mutarotase, 34,000, to justify the conclusion that the two enzymes are different.

Our purification procedure starts, like Li's, with homogenization of the kidney cortex with chloroform and 0.1 M Tris buffer, pH 7.2, followed by centrifugation. The adsorption and ammonium sulfate fractionation steps in Li's method have been eliminated. The supernatant (Homogenate I) was placed in a 35° water bath for two hours (all other purification steps being done at 4° C), resulting in the precipitation of some inactive protein which was removed by centrifugation. The second supernatant (Homogenate II) was then condensed, using the negative pressure dialysis tubing technique, and the condensed sample was dialyzed against two changes of 0.01 M Tris, pH 7.2. On removal from the dialysis tubing the total volume was brought, with the buffer, to one-tenth that of Homogenate II. After centrifugation the supernatant, called "Condensed Extract", is quite viscous and dark brown in color.

Chromatography on a DEAE-cellulose column, used in Li's method, was also eliminated. Instead we applied aliquots of the Condensed Extract directly to a column of Sephadex G-75 gel, previously equilibrated with 0.01 M Tris, pH 7.2. The sample was eluted from the column with the same buffer.

The effluent showed two major peaks, one with a high $O.D._{280}$ but no activity, the other very symmetrical, having a relatively low 280 mμ absorbance and containing all the eluted mutarotase activity. This result has been quite reproducible. Impurities are, however, still present at this point as evidenced by the several bands in Tubes 3 and 4 of Figure 1.

An aliquot of these pooled active fractions was concentrated and dialyzed against 0.005 M sodium phosphate buffer, pH 7.2. This sample was then applied to a column of Bio-Gel HTP hydroxylapatite suspended in the same buffer. Using a stepwise procedure, most of the activity was eluted with 0.02 M sodium phosphate buffer; the activity peak closely paralleling the $O.D._{280}$ peak.

The peak active fractions of this hydroxylapatite effluent were also subjected to disc gel electrophoresis. A single band resulted, as shown by Tubes 1 and 2 of Figure 1. Tracing with the Joyce, Loebl and Co., Ltd., recording densitometer clearly showed that only one zone was present.

A sedimentation velocity run in the Spinco Model E ultracentrifuge on a portion of the hydroxylapatite effluent showed a single sharp, symmetrical peak and gave a value for s_{20} of 2.99 S.

A value for the diffusion coefficient was determined from pictures from the same sedimentation velocity run, by the method of plotting against time the half-

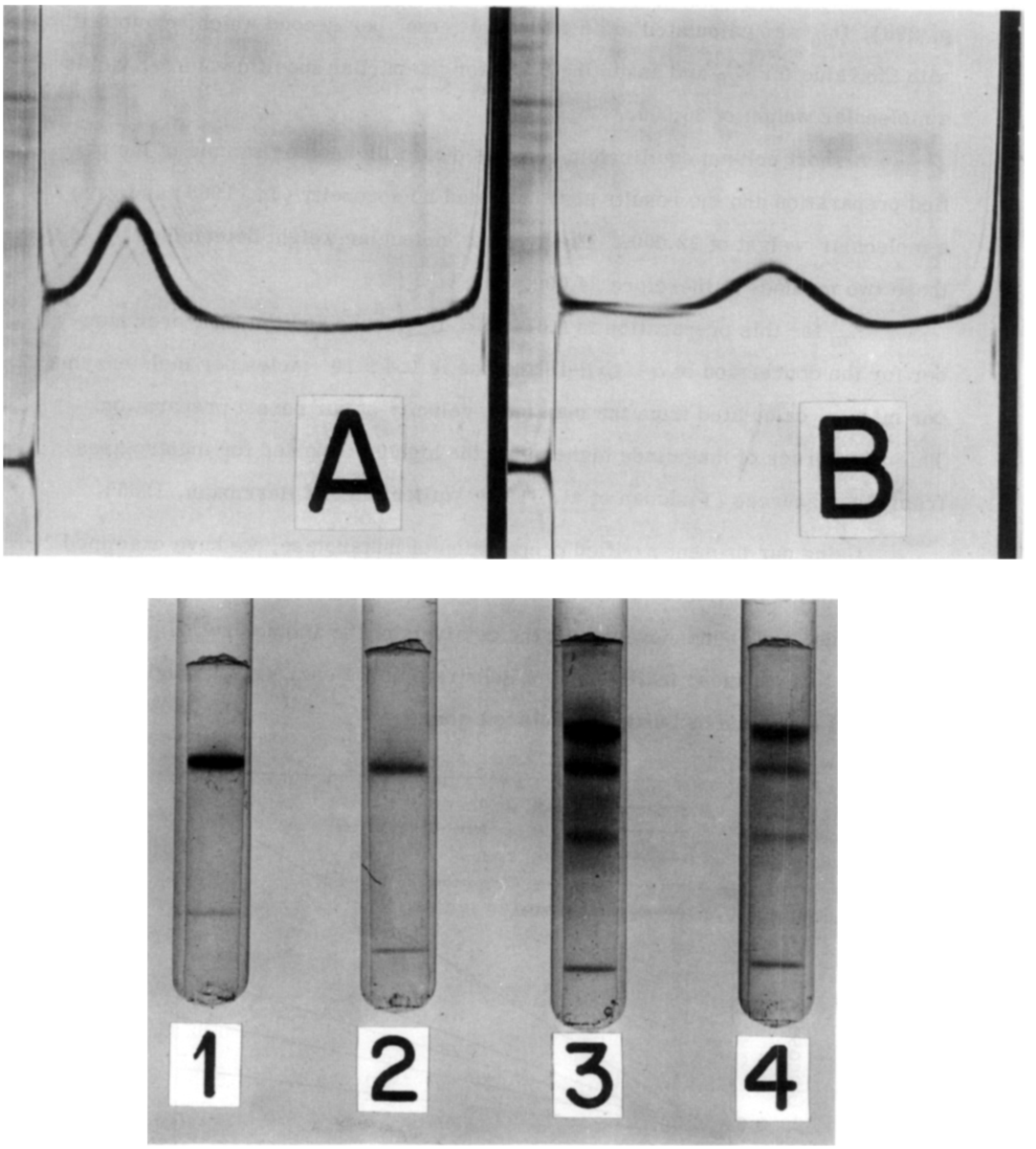

Figure 1.

Pictures A and B show schlieren patterns of a sedimentation velocity run, taken at 51 and 125 minutes, respectively, after reaching maximum speed (59,780 rpm), with a 55° bar angle. Protein concentration was 4.7 mg per ml, in 0.1 M Tris, pH 7.2.

At the right are pictures from Canalco disc electrophoresis on acrylamide gel, run 40 minutes at 4 ma per sample. Staining was with aniline blue black. Tubes 1 and 2 show two different concentrations of peak fractions of hydroxylapatite effluent. Tubes 3 and 4 are samples from the Sephadex G-75 pooled effluent. (See text.)

widths squared at various heights of the peaks (Svedberg and Pedersen, 1940, p. 298). D_{20} was calculated to be 7.28×10^{-7} cm^2 per second which, combined with the value for s_{20} and assuming 0.725 for the partial specific volume, yields a molecular weight of 36,000.

A short column equilibrium run was done with another sample of the purified preparation and the results also indicated homogeneity (Li, 1965) and gave a molecular weight of 32,000. The average molecular weight determined by these two methods is therefore 34,000.

K_m for this preparation is 0.014 M α-D-glucose and the turn-over number for the conversion of α- to β-D-glucose is 1.4×10^7 moles per mole enzyme per minute, calculated from the maximum velocity of our purest preparation. This is an order of magnitude higher than the highest reported for mutarotases from other sources (Fishman et al., 1968; Wallenfels and Herrmann, 1965).

Using our present purified preparation of mutarotase, we have examined inhibition of the enzyme by 2-deoxy-D-glucose, 2-deoxy-D-galactose, methyl-α-D-glucoside, and D-mannose during the catalysis of the anomerization of α-D-glucose. None of these inhibitors is a substrate of the enzyme, although all but the methyl glucoside can undergo mutarotation.

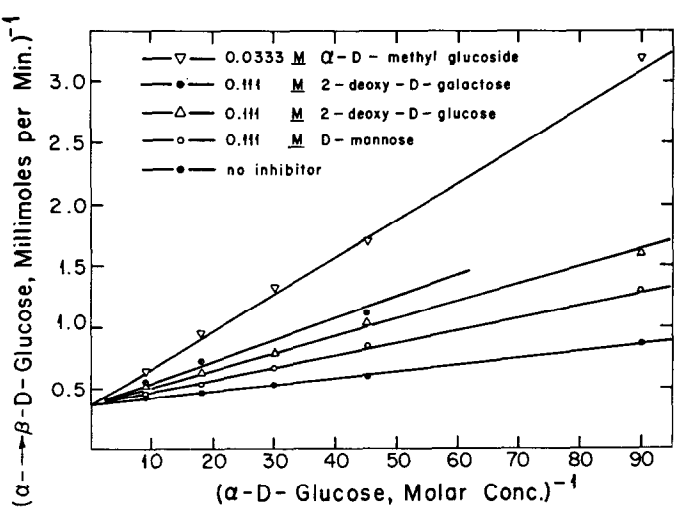

Figure 2.

Double reciprocal plot showing representative results of mutarotase inhibition in the presence of three sugar derivatives and of D-mannose, with α-D-glucose as substrate. Other experiments, not illustrated, with additional concentrations of each inhibitor (see text), gave slopes which followed the pattern of those shown and which had the same intercept when analyzed in this way.

Our object was to explore the possibility, considered earlier (Chase et al., 1967), that activating or inhibitory sites (Atkinson, 1966) might exist in the case of this enzyme. To this end we measured the activity of the enzyme over a twenty-fold range of initial concentrations of α-D-glucose and in the presence of three concentrations (0.0333 M, 0.111 M, and 0.222 M) of each of the four inhibitors named above. A constant concentration of enzyme was present in all the experiments, which were run at 26° C in Tris buffer of pH 7.2. The first order rate constant for each experiment was measured as described by Li, Chase, and Lapedes (1964), using the Zeiss Photoelectric Precision Polarimeter, 0.005°. After subtracting the rate constant for spontaneous mutarotation, the resulting net rate constant was multiplied by the concentration of α-D-glucose initially present less that remaining at equilibrium, to give the initial rate.

Figure 2 shows Lineweaver-Burk double reciprocal plots of representative results. It is quite apparent that inhibition of the enzyme occurs in the presence of each of the sugar derivatives and of D-mannose, and that the effectiveness of the inhibitors varies in the order, methylglucoside > deoxygalactose > deoxyglucose > mannose. Bailey and Pentchev (1965) used the first three of these compounds with mutarotases from rat kidney and rat intestine and reported similar results.

The competitive nature of the inhibition by these four compounds is unequivocally shown in Figure 2. From these experimental results it is clear that this enzyme exhibits simple Michaelis-Menten kinetics; at least when D-glucose is the substrate and with the four inhibitors used here.

Application of the so-called Hill equation (Atkinson, 1966),

$$\log [v/(V_{max} - v)] = n \log [S] - \log K_m ,$$

to the data from the unihibited reaction yielded a slope of unity. As might be expected from the fact that the reaction in the presence of all four inhibitors obeys the equation for simple competitive inhibition, Hill plots of the inhibited reactions also gave slopes close to unity.

Exploratory experiments, using D-galactose as substrate and the same inhibitors, were technically considerably more difficult to perform and yielded less precise data, because of the more rapid mutarotation of D-galactose and also perhaps because of the complex mutarotation of this sugar (Isbell and Pigman, 1937). However, similar results were obtained and Hill plots again gave slopes approximating unity.

It would appear that this particular enzyme system is a relatively simple one, with only one site capable of combining with D-glucose and D-galactose and these particular inhibitory compounds.

ACKNOWLEDGMENTS

This work was supported by Grants No. GB-4300 and GB-7089 from the National Science Foundation, and we also used facilities made available to the Biology Department of Princeton University by the Whitehall Foundation and by the John A. Hartford Foundation.

Analyses of the kinetic data and calculations of sedimentation constants and equilibrium molecular weights were done using computer facilities at Princeton University supported by National Science Foundation Grant No. GP-579.

We thank L. R. Hyde and Miss B. Bamman for the ultracentrifugation and Hideo Shinagawa for performing the disc gel electrophoresis.

REFERENCES

Atkinson, D. E., in Ann. Rev. Biochem., Paul D. Boyer, Ed. 35, pt. I (Annual Reviews, Inc., Palo Alto, Calif.), pp. 85-124 (1966).

Bailey, J. M., and Pentchev, P. G., Am. J. Physiol., 208, 385 (1965).

Bentley, R., and Neuberger, A., Biochem. J., 45, 584 (1949).

Chase, A. M., Lapedes, S. L., and Li, L.-K., Seventh International Congress of Biochemistry Abstracts, Tokyo, F-141, p. 784 (1967).

Fishman, P., Pentchev, P. G., Mulhern, S., and Bailey, J. M., Federation Proceedings, 27, 841 (1968).

Isbell, H. S., and Pigman, W. W., J. Research Natl. Bur. Standards, 18, 141 (1937).

Keilin, D., and Hartree, E. F., Biochem. J., 50, 341 (1952).

Keston, A. S., Science, 120, 355 (1954).

Li, L.-K., Arch. Biochem. Biophys., 110, 156 (1965).

Li, L.-K., Chase, A. M., and Lapedes, S. L., J. Cell. Comp. Physiol., 64, 283 (1964).

Svedberg, T., and Pedersen, K. O., The Ultracentrifuge (Clarendon Press, New York), p. 298 (1940).

Wallenfels, K., and Herrmann, K., Biochem. Zeitschr., 343, 294 (1965).