PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 605-607 (1974)

Aldolase in Cultivated Human Fibroblasts (38156)

BARBARA K. BURTON, CLARAMMA M. CHACKO, AND HENRY L. NADLER
(Introduced by G. P. Youmans)

Department of Pediatrics, Northwestern University, Division of Genetics, Children's Memorial Hospital, Chicago, Illinois

Biochemical and immunologic studies have demonstrated the existence of at least 3 distinct isozymes of aldolase in mammalian tissues (1). The two major isozymes, A and B, exhibit activity with both fructose 1,6-diphosphate (FDP) and fructose-1-phosphate (F1P) but their activities can be distinguished in crude tissue extracts by (1) substrate specificity, the FDP/F1P activity ratios being approximately equal to 15–50 and 1 for aldolases A and B respectively (1), (2) their catalytic properties, such as Km's and pH optima, and (3) inhibition with specific antibodies (2). The distribution of aldolases A and B in various tissues has been defined by the utilization of these criteria in combination with electrophoretic techniques (3). Aldolase A is the only isozyme detectable in muscle, heart and spleen tissue; in these tissues, the FDP/F1P activity ratio approximates 50. In liver and kidney, on the other hand, the predominant form is aldolase B although small amounts of adlolase A can also be detected. The FDP/F1P activity ratios of mammalian liver and kidney extracts are approximately 1 and 2 respectively. Because of its distribution and substrate specificity, aldolase A has ambiguously been referred to as muscle aldolase or FDP aldolase. Aldolase B has conversely been referred to as liver aldolase or F1P aldolase, even though it exhibits equal activity with F1P and FDP. Aldolase C has thus far been detected only in mammalian brain extracts (1) and in fetal liver (4–6).

Hereditary fructose intolerance is an inherited metabolic disorder associated with a deficiency of aldolase activity in human liver (7–9). The residual activity toward F1P in this disease is 1%–5% of normal, while the activity toward FDP is 10%–20% of normal. As a result, the residual FDP/F1P activity ratio, which is normally 1, is approximately 6 in this disorder. Measurements of the residual activity have demonstrated the similarity of the residual enzyme to normal aldolase A. Thus, it has been theorized that the deficient enzyme in hereditary fructose intolerance is actually aldolase B, while aldolase A is present and unchanged. This hypothesis is supported by the demonstration of a protein with the immunologic properties of aldolase B but with essentially no enzymatic activity in the liver of patients with this disorder (4, 10). Additional evidence is provided by the demonstration that in renal cortical tissue, where the primary form of aldolase present is aldolase B (3), activity toward F1P is essentially undetectable while activity toward FDP is markedly decreased in patients with hereditary fructose intolerance (11).

The properties of aldolase in cultivated human fibroblasts have not previously been described. If significant levels of aldolase B could be detected in these cells, the definitive diagnosis of hereditary fructose intolerance, previously made only by liver biopsy, might be made on the basis of a deficiency of enzyme activity in the cultivated fibroblasts.

This study provides evidence that the predominant enzyme present in cultivated human fibroblasts is aldolase A. The FDP/F1P activity ratio, Km's and pH optima of the aldolase in crude fibroblast extracts are comparable to those previously described for aldolase A. No differences in activity or catalytic properties could be detected between the aldolase present in normal human fibroblasts and in fibroblasts from a patient with hereditary fructose intolerance.

Materials and Methods. Fibroblasts were

Copyright © 1974 by the Society for Experimental Biology and Medicine *Printed in U.S.A.*
All rights reserved.

TABLE I. Aldolase Activity in Cultivated Fibroblast Preparations.

	Specific Activity [a]				Ratio
	F1P		FDP		
	(Mean)	(Range)	(Mean)	(Range)	(FDP/F1P)
Controls (10)	2.0	1.1–2.9	28.0	19.0–42.0	14/1
Hereditary fructose intolerance	1.9	1.6–2.1	30.0	21.0–38.0	15/1

[a] μmoles of FDP or F1P hydrolyzed/min/mg of protein.

cultivated from skin biopsies obtained from normal controls and from a patient with hereditary fructose intolerance as previously described (12). Cells were grown in Minimum Essential Medium supplemented with 15% fetal calf serum and were incubated at 37° in an atmosphere of 5% CO_2. At the time of our studies, all cells had undergone 5–15 passages in tissue culture.

Cells to be harvested were washed three times with normal saline and removed from the flasks with a chemically defined medium containing 0.1% trypsin. The suspended cells were centrifuged and washed twice with normal saline. The cell pellet was resuspended in dilute triethanolamine buffer, pH 7.6, and the cells were disrupted by repeated freezing and thawing. The homogenate was spun at 25,000 *g* for 20 min and the supernatant used in the studies reported.

D-fructose-1-phosphate, D-fructose 1,6-diphosphate, DPNH, α-glycerophosphate dehydrogenase, and triosephosphate isomerase were obtained from Sigma Chemical Corporation. Aldolase activity was determined using a modification of the spectrophotometric method of Bruns and Bergmeyer (13). Protein content was determined using the method of Lowry *et al.* (14).

Results. The activity of aldolase in crude fibroblast preparations from 10 normal controls and 1 patient with hereditary fructose intolerance is illustrated in Table I. The specific activity toward both substrates, F1P and FDP, was similar in control and patient fibroblasts. Furthermore, the FDP/F1P activity ratio is essentially identical in these cell lines and is comparable to that reported in tissues in which the predominant isozyme present is aldolase A (1).

The Km's and pH optima of the aldolase present in fibroblast extracts are presented in Table II and are compared with the values for pure aldolases A and B derived from the literature. In control and patient fibroblasts, the Km's for F1P and FDP are similar and are comparable to those previously reported for pure aldolase A. They are markedly dissimilar to those reported for aldolase B.

In both normal and patient fibroblasts, the aldolase present exhibits a broad pH optimum with FDP, the specific activity being unchanged between pH 7 and 9. The pH optimum with F1P is more sharply defined, being

TABLE II. Catalytic Properties of Aldolases.

	Km [a]		pH Optimum	
	(F1P)	(FDP)	(F1P)	(FDP)
Control fibroblasts	1.15×10^{-2}	5.7×10^{-5}	7.0–7.5	7–9
Hereditary fructose intolerance fibroblasts	0.92×10^{-2}	5.3×10^{-5}	7.0	7–9
Aldolase A (2, 15)	1.2×10^{-2}	6.0×10^{-6}	7.0	7–9
Aldolase B (2, 15)	8.5×10^{-4}	1.8×10^{-6}	7.6	7.7

[a] *M*.

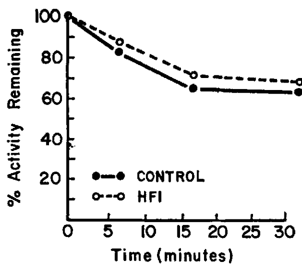

FIG. 1. Thermal stability of aldolase using F1P as the substrate and assayed at 45°. (HFI refers to the patient with hereditary fructose intolerance).

7.0–7.5 in control fibroblasts and 7.0 in patient fibroblasts. These findings are all comparable to those previously reported for aldolase A and are dissimilar to those reported for aldolase B which has a sharp pH optimum with FDP at 7.7.

The thermal stability of aldolase in control and patient fibroblasts at 45° is illustrated in Figs. 1 and 2. The remaining activity with FDP and F1P was similar in control and patient fibroblasts at 5, 15, and 30 min.

Discussion. These data strongly suggest that the predominant form of aldolase present in cultivated human fibroblasts is aldolase A. The specific activities, FDP/F1P activity ratio and catalytic properties of aldolase are similar in fibroblasts from controls and from a patient with hereditary fructose intolerance and are comparable to those of pure aldolase A. Thus, the diagnosis of hereditary fructose intolerance, dependent on the demonstration of a deficiency of aldolase B activity, cannot be made by the analysis of cultivated fibroblasts.

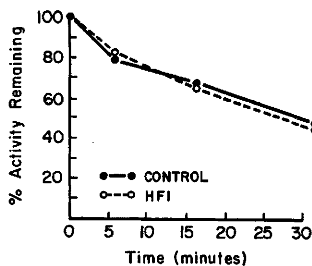

FIG. 2. Thermal stability of aldolase using FDP as the substrate and assayed at 45°. (HFI refers to the patient with hereditary fructose intolerance).

Summary. This study presents evidence that the predominant enzyme present in cultivated human fibroblasts is aldolase A. The FDP/F1P activity ratio, K_m and pH optima of the aldolase in crude fibroblast extracts are comparable to those previously described for aldolase A. No differences in activity or catalytic properties could be detected between the aldolase present in normal human fibroblasts and in fibroblasts from a patient with hereditary fructose intolerance.

These studies were supported by grants from The National Institutes of Health HD 00036 and HD 04252 and The National Foundation-March of Dimes. Henry L. Nadler is the Irene Heinz Given and John La Porte Given Research Professor of Pediatrics.

1. Penhoet, E., Rajkumar, T., and Rutter, W. J., Proc. Nat. Acad. Sci. **56**, 1275 (1966).

2. Rutter, W. J., Woodfin, B. M., and Blostein, R. E., Acta Chem. Scand. **17**, S 226 (1963).

3. Blostein, R., and Rutter, W. J., J. Biol. Chem. **238**, 3280 (1963).

4. Schapira, F., Nordmann, Y., and Gregori, C., *in* Symposium on Clinical and Metabolic Aspects of Fructose. Acta Med. Scand. Supp. **542**, 77 (1972).

5. Schapira, F., Hatzfeld, A., and Reuber, M. D., Cancer Res. **31**, 1224 (1971).

6. Schapira, F., and Nordmann, Y., Clin. Chim. Acta **26**, 189 (1969).

7. Hers, H. G., and Joassin, G., Enzymol. Biol. Clin. **1**, 4 (1961).

8. Froesch, E. R., Wolf, H. P., Baitsch, H., Prader, A., and Labhart, A., Amer. J. Med. **34**, 151 (1963).

9. Nikkila, E. A., Somersalo, O., Pitkanen, E., and Perheentupa, J., Metabolism **11**, 727 (1962).

10. Nordmann, Y., Schapira, F., and Dreyfus, J. C., Biochem. Biophys. Res. Commun. **31**, 884 (1968).

11. Morris, R. C., Ueki, I., Loh, D., Eanes, R. Z., and McLin, P., Nature **214**, 920 (1967).

12. Nadler, H. L., Monteleone, P. L., Inouye, T., and Hsia, D. Y-Y., Nature **213**, 1261 (1967).

13. Bruns, F. H., and Bergmeyer, H. U., *in* "Methods of Enzymatic Analysis", (H. U. Bergmeyer, ed.), pp. 724–728, Academic Press, New York, (1965).

14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

15. Rutter, W. J., Fed. Proc. **19**, 1248 (1964).

Received Feb. 7, 1974. P.S.E.B.M., 1974, Vol. 146.