A Radioisotopic Method for Fructose-1-phosphate Aldolase Assay That Facilitates Diagnosis of Hereditary Fructose Intolerance

Yoon S. Shin, Vinco Moro, Hanni Doliwa, and Wolf Endres

A sensitive new method in which α-[U-14C]fructose-1-phosphate is used for fructose-bisphosphate aldolase (EC 2.1.2.13) assay is described. The radioactive fructose-1-phosphate compound was prepared from [U-14C]fructose by use of partly purified fructokinase (EC 2.7.1.4). With this method we measured normal values for aldolase in human liver (2.4–10.0 mmol/min per mg of protein), kidney (3.6–3.8), and intestine (4.2–10.0) as well as Km values for fructose-1-phosphate (~ 1.0–2.2 mmol/L). In patients with hereditary fructose intolerance the aldolase activity in liver and intestine was <10% of normal values. The Lineweaver-Burk plots for data from patients with hereditary fructose intolerance were hyperbolic, indicating a structural alteration in the enzyme.

Additional Keyphrases: reference interval · heritable disorders of metabolism

Hereditary fructose intolerance (HFI),¹ an autosomal recessive trait, characterized by diarrhea, vomiting, and hypoglycemia after ingestion of fructose, is associated with a deficiency of fructose-bisphosphate aldolase (EC 2.1.2.13) (I, 2). The aldolase is present in the form of three genetically distinct isoenzymes: muscle type A, liver type B, and brain type C (3). Only aldolase B catalyzes metabolism of fructose-1-phosphate (F1P). A deficiency of aldolase B was demonstrated in liver (2), intestine (4), and kidney (5) of patients with HFI. The biochemical basis of the disorder was a mutant enzyme with altered characteristics (6–9). The homozygosity can be diagnosed by determining the aldolase activity on two substrates: F1P and fructose-1,6-bisphosphate (FBP). Spectrophotometry involving NADH-dependent glyceraldehyde dehydrogenase (EC 1.1.1.8) (I, 10, 11), however, requires a relatively large quantity of tissue sample and is not always specific. For some uncertain cases other diagnostic procedures (8, 12, 13) were necessary to establish the diagnosis.

Our purpose was to develop a more sensitive and specific method for aldolase B assay, to facilitate diagnosis of the disorder. The simple radioisotopic method described here is very specific and much more sensitive than the former methods. The activity of aldolase B and its kinetic characteristics were determined by use of the method for samples of liver and intestine from patients with HFI.

Materials and Methods

Preparation of Tissue Extracts

Fresh tissue samples, obtained at autopsy or by biopsy, were homogenized in 100 volumes of triethanolamine buffer (50 mmol/L, pH 7.4) per volume of tissue, briefly sonicated, and centrifuged. The supernates were used for the assay and the enzyme activity was stable to dialysis and storage at −20°C for at least six months.

Enzyme Assay

Synthesis of [14C]fructose-1-phosphate: [U-14C]fructose-1-phosphate was prepared from [U-14C]fructose (Amersham Buchler, Braunschweig, F.R.G.) with catalysis by fructokinase (EC 2.7.1.4) partly purified from rat liver (14). The reaction mixture contained 150 μmol of potassium phosphate buffer (pH 7.6), 30 μmol of MgCl2, 10 μmol of ATP, 1 μmol of [14C]fructose (250 μCi), and 0.1 U of fructokinase, all in a total volume of 5 mL. After a 1-h incubation at 37°C the reaction was stopped by heating at 95°C for 3 min, and the mixture was diluted to 10 mL with distilled water. The product was purified on a 0.9 × 15 cm diethylaminomethyl (DEAE)–cellulose column with a gradient of 0.01 to 0.3 mol/L triethanolamine buffer, pH 7.4. The yield was approximately 30%.

Radioisotopic method for F1P aldolase assay: For this assay, the mixture contained 3 μmol of triethanolamine buffer (pH 7.4), 0.02 μCi of [14C]F1P, and appropriate amounts of F1P (10–100 nmol), all in a total volume of 50 μL. The reaction was initiated by adding 30 μL of the tissue extract containing 1–10 μg of protein. After a 1-h incubation the reaction was terminated by heating at 95°C for 3 min.

The product, glyceraldehyde, was separated from F1P and dihydroxyacetone phosphate on a mini DEAE–cellulose column as described previously for galactokinase assay (15) and pyridoxal-5’-PO4 assay (16) except that the glyceraldehyde was eluted with water instead of dilute HCl. We examined the reaction of nonspecific phosphatase, which may contaminate the water fractions with fructose, by looking for fructose in these fractions, but could detect none. Blanks consisted of extracts in which the protein had been denatured before being added to the reaction mixture. Protein was determined with a modification (17) of the procedure of Lowry et al. (18); 1 μg of protein could be determined. The aldolase activity was expressed as nanomoles of glyceraldehyde formed per minute per milligram of protein.

Spectrophotometry. This was according to the procedure of Gitzelmann (10).

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¹ Nonstandard abbreviations: HFI, hereditary fructose intolerance; F1P, fructose-1-phosphate; FBP, fructose-1,6-bisphosphate

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Results

Figures 1 and 2 illustrate the dependency of the aldolase reaction on the duration of incubation and the protein concentration. Radioactivity of the product was linearly related to incubation time for up to 30 min. Reaction velocity was linearly related to protein concentration up to 7 μg in the case of liver, 12 μg in the case of fetal liver, and 40 μg in the case of liver from HFI patients.

Table 1 summarizes the activity of this aldolase in various human tissues and in hepatic and intestinal tissues from patients with HFI, as measured by two different methods. By the present method the activity in HFI patients was approximately 5–8% of normal values, whereas the activity by spectrophotometry varied considerably.

In two special cases suspected of HFI (Table 1), the values for the aldolase activity and the ratio FBP/F1P by the spectrophotometric method were somewhat below the normal reference interval. With the radioisotopic method, however, we found both the aldolase activity and \( K_m \) values to be normal, and indeed both cases were eventually found to be clinically normal. On the other hand, HFI liver 2, for which the spectrophotometric method at first yielded a relatively high value for the aldolase activity (3 units) as well as a low activity ratio, was confirmed to be HFI by the enzyme assay with the radioisotopic method and by the clinical symptoms. Repetition of the assay with the spectrophotometric procedure produced quite different values (Table 1). Study of the kinetic characteristics shows that the residual activities in liver and intestine were ascribable to the mutant enzyme, for which the relationship between reaction velocity and substrate concentration differed from the normal (Table 1 and Figure 3). Lineweaver–Burk plots

![Graph](image1)

**Fig. 1.** Aldolase reaction in a liver extract (autopsy specimen from a 10-year-old boy) as related to incubation interval

Final concentration of F1P: 2.5 mmol/L. 4 μg of protein used for the assay

![Graph](image2)

**Fig. 2.** Relationship of the aldolase reaction to the protein concentration

Conditions same as in Fig. 1. , adult liver (41 yr, , autopsy); , fetal liver (18 wk, ); , HFI liver (2 mo., , biopsy)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Activity (μmol/L)</th>
<th>( K_m ) (μmol/L)</th>
<th>Activity (μmol/L)</th>
<th>Ratio (FBP/F1P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>4.46 ± 2.17</td>
<td>1.50 ± 0.35</td>
<td>7.6 ± 2.4</td>
<td>2.4–10.0</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>8.5</td>
<td>1.5</td>
<td>2.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>4</td>
<td>2.10 ± 0.46</td>
<td>2.3 ± 0.22</td>
<td>(1.6–2.8)</td>
<td>(2.1–2.6)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>3.6, 3.8</td>
<td>1.4, 1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>5</td>
<td>6.24 ± 2.08</td>
<td>1.48 ± 0.37</td>
<td>(4.2–10.0)</td>
<td>(3.7–15.7)</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>HFI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Liver 1</td>
<td></td>
<td>0.33</td>
<td>50</td>
<td>0.26</td>
<td>17.9</td>
</tr>
<tr>
<td>Liver 2</td>
<td></td>
<td>0.36</td>
<td>50</td>
<td>3.0</td>
<td>0.75</td>
</tr>
<tr>
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<td></td>
<td>0.34</td>
<td>50</td>
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<td></td>
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<td>Liver 4</td>
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<td>0.32</td>
<td>50</td>
<td>0.07</td>
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<tr>
<td>Liver 5</td>
<td></td>
<td>0.13</td>
<td>50</td>
<td>0</td>
<td></td>
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<tr>
<td>Intestine 1</td>
<td>0.50</td>
<td>50</td>
<td>1.84</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD in nanomol/min per milligram of protein. Values in parentheses are ranges. The final concentration of F1P was 0.33 mmol/L for the radioisotopic method, 30 mmol/L for the spectrophotometric method (FBP, 5 mmol/L). All tissue samples were taken at the age of 1 month to 41 yr, fetal liver at 16–20 wk. *Uncertain cases. *Hyperbolic forms of Lineweaver–Burk plots. *Assay repeated after confirming the diagnosis with the radioisotopic method. The F1P aldolase activity was 0.2 U and the ratio 2.8 by the spectrophotometric method.

![Graph](image3)

**Fig. 3.** Lineweaver–Burk plots of fructose-bisphosphate aldolase activity for fructose-1-phosphate (mmol/L) in human tissues

, adult liver (41 yr, , autopsy); , liver (3 mo., , biopsy); , fetal liver (18 wk, ); , HFI liver (2 mo., , biopsy); , intestine (20 yr, , biopsy); , intestine from a HFI patient (2 mo., , biopsy)
for $K_m$ values in the patients gave nonlinear hyperbolic curves, indicating a substrate activation effect (19), in contrast to the case of the normal (apparent $K_m$ 1.5 mmol/L). As shown in Figure 4, therefore, the difference in enzyme activity between the normal subjects and the homozygotes for HFI was more pronounced when a lower concentration of F1P was used in the assay. When a high concentration of F1P such as 10 mmol/L was used, the values for normal livers were as high as 60 U/g of protein, and in HFI as high as 15 U/g.

**Discussion**

The present procedure is relatively simple and reproducible. Radioactive fructose-1-phosphate is easily prepared from inexpensive [U-14C]fructose. Furthermore, relatively small amounts of biopsy sample are required as compared with the spectrophotometric method. Multiple assays can be performed when the partly automated mini-column chromatographic procedure (15, 16) is applied. In some cases the activity values with the spectrophotometric method exceeded those with the radioisotopic method, possibly owing to the higher substrate concentration used in the former method, or to its low accuracy. Increases in the Michaelis constants for aldolase B as well as other findings of structural alteration of the enzyme in HFI have been reported (6–9). We have also observed an extremely low affinity to F1P in the patients with HFI under normal physiological conditions. In addition, aldolase B in HFI yielded a hyperbolic Lineweaver–Burk plot for F1P, suggesting a substrate-activation effect (19). As seen in Figures 3 and 4, the difference in the enzyme activity between normal and HFI is more distinctive with lower concentrations of F1P. Therefore we recommend use of a F1P concentration of approximately 0.5 mmol/L in the assay if only single measurements are to be done. In some obscure cases, such as a few cases shown in Table 1 and others (8, 20), determination of $K_m$ values for F1P will help elucidate the diagnosis. Recently, Cox et al. (9) reported a kinetic alteration of aldolase B in the intestinal mucosa among heterozygotes for HFI. With the sensitive radioisotopic method it would be simpler to establish the genotypes of HFI, using a relatively small biopsy sample from the intestinal mucosa. In the preliminary study (20) we reported aldolase activities in fibroblasts of various genotypes. As was true for leukocytes, there was no significant difference in the fibroblast enzyme activity between the genotypes. However, the activity ratio (FBP/ F1P) was much lower (~3) than that of aldolase A or C (~10), being in the range of that for fetal liver or hepatoma cells (20–23). These observations led us to speculate that fast-growing and young populations of cells may contain proportionately more aldolase A, and aldolase B may be produced as they mature. It would be interesting to investigate this enzyme in cultured fibroblasts at various stages of growth, to explore the feasibility of using cells for the diagnosis of this aberration. Such a study is in progress.

**References**


