Purification and Properties of Rat Liver Fructokinase*

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SUMMARY

New spectrophotometric and radiochemical assay procedures for measuring fructokinase (ATP:L-fructose 1-phosphotransferase, EC 2.7.1.3) activity are reported. This enzyme has been purified from the liver of adult rats by acid and heat treatment, ammonium sulfate fractionation, and chromatography on Sephadex G-200 and diethylaminoethyl cellulose. The enzyme preparation has a specific activity of 15 to 30 unite per mg of protein at 25° and has been purified 450-fold from an acid-treated homogenate (approximately 1,000-fold from the 100,000 × g supernatant). Purified liver fructokinase has a Km of 0.2 to 0.5 mM for fructose and 1 to 2 mM for magnesium adenosine triphosphate, and the enzyme reaction produces equimolar amounts of fructose 1-phosphate and ADP. The enzyme phosphorylated D-fructose with ATP, 2'-dATP, or 3'-dATP and also phosphorylated L-sorbose, D-xylulose, and L-galactoheptulose with ATP. It is stimulated by cysteine and other thiols, inhibited by β-chloromercuribenzamide, and has an activation energy of about 11 kcal per mole and a Q10 of 2.

Although fructose is readily phosphorylated by hexokinase (ATP:β-hexose 6-phosphotransferase, EC 2.7.1.1) (1, 2) to fructose 6-phosphate, its low activity in rat liver and its Km for fructose of 2 to 6 mM (3, 4) as compared with a Km for glucose of <0.1 mM makes it unlikely that this enzyme phosphorylates fructose in appreciable quantities under normal circumstances. Thus, fructose-1-P is probably the sole initial product of hepatic fructose utilization catalysed by fructokinase (EC 2.7.1.3) (5-8). Although numerous chemical and physiological investigations of this enzyme have been reported (2-4, 8-19), little is yet known of its properties and kinetics. In the present study we are reporting highly sensitive spectrophotometric and radiochemical procedures for assay of this enzyme, a method for its purification from rat liver, some information on its distribution in rat tissues, and some of its chemical and kinetic properties.

EXPERIMENTAL PROCEDURE

Animals—Normal male rats of the CFN strain, purchased from Carworth Farms, were maintained on a commercial stock diet and were used for these experiments when they weighed between 300 and 400 g.

Chemicals and Materials—2'-dATP was purchased from P-L Biochemicals; the sodium salts of ATP, ADP, NADP, NADH, P-enolpyruvate, and NADH, the dicyclohexylammonium salt of fructose-1-P, and suspensions of pyruvate kinase, lactate dehydrogenase, muscle aldolase, α-glycerophosphate dehydrogenase, triose-P isomerase, glucose-P isomerase, and glucose-6-P dehydrogenase from the Boehringer Mannheim Corporation; D-fructose, L-sorbose, the sodium salts of fructose-6-P and fructose-1,6-di-P, Sephadex G-200 (beads, particle size 40 to 120 μ, DEAE-cellulose (capacity 0.9 meq per g, fine mesh), Dowex 50-X8 (60 to 100 mesh), and Dowex 1-XS (200 to 400 mesh) from Sigma; Amorbrite CG-400 (100 to 200 mesh) from Mallinkrodt Chemical Works; n-tagatose from Pierce Chemical Company; and uniformly labeled 4C-fructose from Nuclear-Chicago Corporation. The authors thank the following for generous gifts: R. Suhadolnik for 3'-dATP and n-psicose; J. Hickman for n-ribulose, L-ribulose, n-xylulose, and L-erythrulose; N. K. Richtmeyer for n-mannoheptulose, n-glucoheptulose, L-glucoheptulose, L-galactoheptulose, L-galactoheptulose, and L-sorbose; and M. L. Wolf for n-mannoheptulose.

Assay Methods—Fructokinase activity was measured either spectrophotometrically or radiochemically. In the spectrophotometric assay, the formation of ADP was coupled with P-enolpyruvate, pyruvate kinase, NADH, and lactate dehydrogenase, and the reaction was followed at 340 μm with a Zeiss model PM& II spectrophotometer. Each 3-ml cuvette contained 100 μmoles of triethanolamine buffer (adjusted to pH 7.5 with 2 N HCl), 300 μmoles of KCl (17), 10 units each of pyruvate kinase and lactate dehydrogenase, 1 μmole of NADH, 10 μmoles of P-enolpyruvate (adjusted to pH 7.5 with 2 N KOH), 45 μmoles of ATP (adjusted to pH 7.5 with 2 N KOH), 60 μmoles of MgCl2, 15 μmoles of fructose, and enzyme. The reaction was measured in a water bath at 30°. The reaction was stopped after 5 min by the addition of 0.3 ml of 10% glacial acetic acid and the contents were extracted with 2 ml of ether. The aqueous phase was poured into a 10 cm square cuvette for determination of NADH at 340 μm. The activity was expressed in units per mg of protein at 25° as the number of μmoles of NADH converted per minute per mg of protein.

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1 A preliminary report of this assay was presented in connection with a nutritional study of the enzyme (9).
enzyme that will catalyze the formation of 1 pmole of ADP per min at 25°C, and the fructokinase activity was obtained by subtracting the blank activity in the absence of fructose. Under these conditions, enzyme activity was linear with time for at least 10 min and proportional to the amount of enzyme preparation added (Fig. 1a). One unit of fructokinase is defined as the amount of enzyme that will catalyze the formation of 1 pmole of ADP per min at 25°C.

Several problems were encountered when this assay procedure was applied to crude preparations. In 100,000 × g liver supernatants, we observed a very active sorbitol dehydrogenase (EC 1.1.1.14), which reduces fructose with NADH. Since fructokinase has a greater affinity for fructose (Fig. 4) than does the dehydrogenase (K_m = 100 mM) (20), a concentration of about 1 mM fructose almost saturates the former while showing only minor interference by the latter. Acid treatment, as described in the purification procedure, completely destroys sorbitol dehydrogenase activity.

In high speed liver supernatants we have observed that the addition of ATP alone causes a rapid ADP formation (presumably due either to ATPase or adenylate kinase). This high blank can be minimized by keeping the ATP at 4 mM and adding 16 mM sodium fluoride. However, this concentration of ATP does not saturate fructokinase (Fig. 4), and gives values which represent approximately 70% of the V_max. The acid and heat treatment, described in the purification procedure, completely destroy this "ATPase" activity, and thus it does not interfere with studies of the purified enzyme.

In order to assay fructokinase under conditions which were not convenient for ADP measurement, an assay procedure described by Sherman (21) and Sherman and Adler (22) for galactokinase was modified for fructokinase as follows. The reaction mixture contained 50 μmoles of triethanolamine buffer (adjusted to pH 7.5 with 2 N HCl), 100 μmoles of KCl, 3 units of pyruvate kinase, 3.5 μmoles of P-enolpyruvate (adjusted to pH 7.5 with 2 N KOH), 15 μ moles of ATP (adjusted to pH 7.5 with 2 N KOH), 20 μmoles of MgCl_2, 5 μmoles of fructose containing 0.5 μC of uniformly labeled ^14C-fructose, and enzyme preparation in a final volume of 1 ml. Incubations were carried out at 37°C, and the reactions were stopped at 5, 10, and 15-min intervals by spotting 30-μl aliquots onto Whatman DEAE-cellulose paper strips (1.5 x 23 cm). The latter were chromatographed with distilled water until the front descended about 10 cm below the origin, and was dried and cut into 4-cm sections from 2 cm above the origin to the solvent front. Radioactivity in the lowermost 4-cm section (uniformly labeled ^14C-fructose) and the other two sections (uniformly labeled ^14C-fructose-1-P) was determined radiochemically as described under "Experimental Procedure." The rates of conversion were 0.009, 0.018, and 0.027 mole per min for 0.25, 0.75, and 1.51 μg of protein (Curves 1, 2, and 3, respectively).

The conversion of fructose to fructose-1-P was corrected by subtracting a small blank activity due to a contaminant in the radioactive substrate. Only in the presence of the ATP-regenerating system (pyruvate kinase and P-enolpyruvate) and when the rate of conversion was <0.03 μmole per min was the reaction velocity linear with time and enzyme concentration (Fig. 1b). Enzyme activity is expressed in international units, namely, micromoles of fructose-1-P formed per min at 37°C.

Protein was measured by the method of Warburg and Christian (23). In the identification of fructose-1-P, carbohydrate was assayed according to the method of Dische, Shettles, and Osnos (24), as reported by Diedrich and Anderson (25). Fructose-6-P was identified spectrophotometrically by coupling with hexose-P isomerase, glucose-6-P dehydrogenase, and NADP, as described by Hohorst (26). Fructose-1-P was identified spectrophotometrically by coupling with muscle aldolase, a mixture of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Fructokinase assays with the purified enzyme. a, the change in absorbance at 340 nm was followed spectrophotometrically as described under "Experimental Procedure." The absorbance changes were 0.01, 0.03, and 0.06 per min for 0.25, 0.75, and 1.51 μg of protein (Curves 1, 2, and 3, respectively). b, the percentage of conversion of uniformly labeled ^14C-fructose to uniformly labeled ^14C-fructose-1-P was determined radiochemically as described under "Experimental Procedure." The rates of conversion were 0.009, 0.018, and 0.027 μmole per min for 0.08, 0.25, and 0.42 μg of protein (Curves 4, 5, and 6, respectively).

**Table I.** Occurrence of fructokinase in tissues of rat

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Activity (units/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult liver</td>
<td>3.12</td>
</tr>
<tr>
<td>17-Day fetal liver</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>2.80</td>
</tr>
<tr>
<td>48 hours</td>
<td>2.43</td>
</tr>
<tr>
<td>72 hours</td>
<td>2.11</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.60</td>
</tr>
<tr>
<td>Intestinal mucosa</td>
<td>0.35</td>
</tr>
<tr>
<td>Heart muscle, skeletal muscle, brain, adipose tissue, and seminal vesicles</td>
<td>0</td>
</tr>
</tbody>
</table>
solution at 4°. All of the following steps in the purification of fructose-1,6-di-P, except that it was necessary to use 50 times the reported concentration of aldolase (28, 29).

In the same concentration range no enzyme activity could be detected with the following sugars: n-sedoheptulose, n-psicose, n-sorbose, D-glucoheptulose, L-sorbose, L-galactoheptulose, and D-mannoheptulose, except that it was necessary to use 50 times the reported concentration of aldolase (28, 29).

RESULTS

Distribution of Fructokinase—The distribution of fructokinase in various rat tissues is shown in Table I. Enzyme activity was measured as described for crude extracts under “Experimental Procedure,” and in a previous publication (9). The activity in adult rat liver was 3 units per g of tissue, and, of all of the other tissues tested, it was found only in kidney and intestinal mucosa, but at much reduced levels. It was absent from fetal liver, and tissues tested, it was found only in kidney and intestinal mucosa, present in somewhat reduced activity in regenerating liver. Adult rat liver was 3 units per g of tissue, and, of all of the other tissues tested, it was found only in kidney and intestinal mucosa, present in somewhat reduced activity in regenerating liver.

Purification of Fructokinase—Male rats, weighing 300 to 400 g, were decapitated, and the livers were placed in 0.25 M mannitol solution to 40% saturation. The precipitate, which showed no fructokinase activity, was removed by centrifugation and discarded. The precipitate formed after the solution was brought to 50% saturation was dissolved in 10 ml of 10 mM triethanolamine, pH 7.3 (Fraction 3).

A Sephadex G-200 column with bed dimensions of 16 cm x 35 cm was prepared as described by Flodin (30) and washed with at least 1 liter of 10 mM triethanolamine, pH 7.3. Fraction 3 was applied to this column and protein eluted with the same triethanolamine solution at a flow rate of three ml per min. Those fractions containing fructokinase activity (Fig. 2) were pooled to give Fraction 4.

DEAE-cellulose was suspended in 10 mM triethanolamine, pH 7.3, and poured in the same way as for Sephadex to give a column with bed dimensions of 5 cm x 25 cm. No hydrostatic pressure was applied to the column either in the preparation or in the chromatography. The column was washed with 1 liter of 10 mM EDTA and then 1 liter of the triethanolamine solution. The combined fractions from Sephadex G-200 chromatography were applied to the column and protein eluted with the same triethanolamine solution containing 100 ml of the triethanolamine solution. The fructokinase was eluted with an approximately linear gradient of 10 mM triethanolamine containing 0 to 1 M potassium chloride at a flow rate of about 2 ml per min. The gradient volume was 500 ml. The enzyme was eluted between 0.1 M and 0.2 potassium chloride (Fig. 3). Those fractions containing fructokinase activity were pooled to give Fraction 5.

Solid ammonium sulfate was added to Fraction 5 to bring the solution to 40% saturation. The precipitate was removed by centrifugation and discarded. The precipitate formed after the solution was brought to 60% saturation was collected and suspended in saturated ammonium sulfate (Fraction 6). In suspension the enzyme is stable for at least 6 months at 0-4°.

The entire purification procedure requires about 48 hours. The enzyme has been purified 225- to 550-fold over the acid-treated supernatant solution and has a specific activity of 15 to 30 units per mg of protein at 25°. A representative purification is shown in Table II.

Properties of Purified Fructokinase

Substrate Specificity—The Michaelis constant for fructose was between 0.2 and 0.5 mM (Fig. 4). No substrate inhibition was observed up to a concentration of 10 mM. At concentrations from 0.5 to 25 mM only L-sorbose, L-galactoheptulose, and D-xylulose also showed activity with the enzyme preparation. The K_m and relative V_max of these substrates are given in Table III. In the same concentration range no enzyme activity could be detected with the following sugars: r-sedoheptulose, r- and L-mannoheptulose, r- and L-glucoheptulose, r-psicose, r-sorbose,
TABLE II
Purification of fructokinase from rat liver

A summary of the purification procedure for fructokinase from 130 g of rat liver is shown. Enzyme activity was measured spectrophotometrically as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total activity</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Purification factor</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 35,000 X g supernatant of acid-treated homogenate</td>
<td>100</td>
<td>196</td>
<td>4900</td>
<td>0.04</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2. Heat-treated supernatant</td>
<td>86</td>
<td>207</td>
<td>2300</td>
<td>0.09</td>
<td>2.3</td>
<td>100</td>
</tr>
<tr>
<td>3. 40-50% (NH₄)₂SO₄ precipitate</td>
<td>9</td>
<td>172</td>
<td>637</td>
<td>0.27</td>
<td>0.7</td>
<td>88</td>
</tr>
<tr>
<td>4. Combined fractions from Sephadex G-200</td>
<td>116</td>
<td>166</td>
<td>151</td>
<td>1.1</td>
<td>27</td>
<td>85</td>
</tr>
<tr>
<td>5. Combined fractions from DEAE-cellulose</td>
<td>16</td>
<td>78</td>
<td>14</td>
<td>5.4</td>
<td>135</td>
<td>39</td>
</tr>
<tr>
<td>6. 40-60% (NH₄)₂SO₄ precipitate</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>18.3</td>
<td>457</td>
<td>39</td>
</tr>
</tbody>
</table>

Enzyme activity was assayed spectrophotometrically as described in the text, except that the concentrations of ATP and MgCl₂ were varied at a molar ratio of 0.75. The velocity units are arbitrary. These experiments give $K_m$ values of 1.5 mM for MgATP, and 0.4 mM for fructose.

**Table III**
Substrate specificity of fructokinase

Enzyme activity was determined with the spectrophotometric assay. The rates of fructokinase activity toward the various substrates are compared at the maximum velocity determined by Lineweaver-Burk plots (31).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration range</th>
<th>$K_m$ (mM)</th>
<th>Relative $V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Fructose</td>
<td>0.5-25</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>0.5-25</td>
<td>1.7</td>
<td>67</td>
</tr>
<tr>
<td>L-Galactoheptulose</td>
<td>0.5-25</td>
<td>2.3</td>
<td>58</td>
</tr>
<tr>
<td>D-Xylulose</td>
<td>0.5-25</td>
<td>4.6</td>
<td>33</td>
</tr>
</tbody>
</table>

**Fig. 4.** The effect of fructose (1) and ATP (2) concentration on fructokinase activity is also shown in Fig. 4. From this Lineweaver-Burk plot the $K_m$ for MgATP was found to be 1.5 mM. In this experiment the molar ratio of ATP to MgCl₂ was maintained at 0.75 in order to ensure predominance of the MgATP complex (33). No inhibition by substrate was observed up to a concentration of 20 mM. With the use of a concentration range from 0.5 to 25 mM, only 2'-dATP and 3'-dATP (cordycepin triphosphate) also phosphorylated fructose. No enzyme activity could be detected with ADP, AMP, GTP, 2'-dGTP, CTP, 2'-dCTP, UTP, or TTP at similar concentrations. Whereas the spectrophotometric assay was used to measure the phosphorylation of fructose by ATP, the other nucleotides were tested with the radiochemical assay (34, 35).

**Product of Fructokinase Reaction**—Purified fructokinase, 0.5 unit, was added to a reaction mixture as described in the radiochemical assay, but it contained 5.0 μmoles (15 μC) of uniformly labeled 14C-fructose and 50 μmoles of P-enolpyruvate. The reaction was allowed to run the calculated time required for completion, and the mixture was added to 30 μmoles each of fructose-1-P and fructose-6-P ($F_1P$ and $F_6P$) are indicated. The fraction volume is 20 ml.

**Fig. 5.** Identification of fructose-1-P ($F_1P$) as the product of the fructokinase reaction. Radioactivity (□—□), carbohydrate (○—○), and sugar phosphates were detected as described in the text. The peaks corresponding to fructose, fructose-1-P, and fructose-6-P ($F_6P$) are indicated. The fraction volume is 20 ml.

Although we confirmed an earlier report of activity toward D-tagatose (32), a recent analysis of the commercial sample of this sugar used by us showed about 25% contamination by D-fructose.
a Dowex 1 column by Diedrich and Anderson (25). The column was eluted with an approximately linear gradient of sodium tetraborate (0.10 to 0.45 M), pH 9.3, which separates fructose-1-P and fructose-6-P (36). All the radioactivity was found in fructose-1-P and in the remaining fructose (Fig. 5). Since borate interfered with the enzymatic determinations of the sugar phosphates (by inhibiting glucose-6-P dehydrogenase and aldolase), it was necessary to remove the borate from the chromatographic eluents. This was accomplished by employing the ion exchange method of Diedrich and Anderson (25). Fructose-1-P and fructose-6-P were then identified enzymatically as described under “Experimental Procedure.”

**Stoichiometry of Fructokinase Reaction**—Different amounts of fructose (0.10, 0.20, and 0.30 μmole) were added to reaction mixtures, as described for the spectrophotometric assay, together with 0.2 unit of purified fructokinase, and the reactions were followed spectrophotometrically until completion. As shown in Table IV, 1 μmole of ADP was formed for each micromole of fructose added. In addition, from the preceding experiment in which the phosphorylated product was found to be fructose-1-P, only slightly less than 5.0 μmoles of fructose-1-P were formed from 5.0 μmoles of fructose (Table IV).

**Effect of Thiols and Thiol-blocking Reagents on Fructokinase Activity**

Enzyme activity was measured by the radiochemical assay as described in the text, except that P-enolpyruvate and pyruvate kinase were deleted from the reaction mixture.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration (mM)</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5</td>
<td>2.2</td>
</tr>
<tr>
<td>Glutathione</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>Thioglycolate</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td>Mercaptoethamine</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The most abundant source of fructokinase in the rat is liver, where the enzyme is found entirely in the cytoplasm. The enzyme activity is very low in fetal liver, in agreement with earlier reports that fructokinase is not synthesized in substantial amounts until after birth (3, 13). Also, it is not selectively synthesized by regenerating liver, and it was previously reported to be induced only slightly by long term fructose feeding (9). We have confirmed that fructokinase is present also in kidney (37) and intestinal mucosa (38), and the enzyme is also present in many well differentiated hepatomas.3 We were unable to detect any fructokinase activity in heart muscle, skeletal muscle, brain, seminal vesicle, or adipose tissue, or in several poorly differentiated hepatomas.3 Its absence from adipose tissue was already reported by Günther, Siller, and Sols (37), but was reported to be present by Gromova (39).

The specific activity of our preparation (15 to 30 units per mg of protein) is considerably greater than those reported by previous investigators (7, 6, 16-18), the highest of which was 1 to 2

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units per mg of protein at 30° (17). Although we have no evidence of the degree of purity of fructokinase, substrate specificity studies and the deletion of either pyruvate kinase or lactate dehydrogenase from the assays show that the preparation contains no pyruvate kinase, lactate dehydrogenase, sorbitol dehydrogenase, myokinase, aldolase, hexokinase, glucokinase, galactokinase, phosphofructokinase, or triokinase, all of which are present in liver cytoplasm. In addition, no evidence was obtained for heterogeneity of fructokinase on Sephadex G-200 or DEAE-cellulose chromatography.

Our $K_m$ for fructose (0.2 to 0.5 mM) is in good agreement with previously reported values of 0.1 mM by Günther et al. (37) and 0.4 mM by Parks, Ben-Gershom, and Lardy (17). Whereas our MgATP $K_m$ of 1.5 mM corresponds to those of Hers, 1 to 5 mM (19), and Günther et al. (37), 1 to 3 mM, it is about 10 times higher than that of Parks et al., 0.2 mM (17).

Although we observed no effects upon fructokinase activity of either iodoacetate or iodoacetamide, evidence for the presence of essential sulphydryl groups in the enzyme is provided by both the stimulation of enzyme activity by 5 mM cysteine, and the reversible inhibition by p-chloromercuribenzoate as reported by Ponz and Llinas (15).

We have confirmed earlier reports that fructokinase catalyzes the phosphorylation of L-sorbose (18), and also detected activity with n-xylulose and L-galactoheptulose. However, the enzyme required considerably higher concentrations of these sugars.

The structural specificity of fructokinase can be summarized as follows: an apparent requirement for the dihydroxyacetone moiety in the first 3 carbon atoms; the obvious importance of a free or potentially free carbonyl group at carbon atom 2, and a trans configuration at carbon atoms 3 and 4. Since the aldolase reaction is an obligatory step for the further metabolism of fructose-1-P in liver (4, 12), it is of interest that aldolase also shows a high degree of selectivity for the dihydroxyacetone-P moiety in the first 3 carbon atoms, requires a free or potentially free carbonyl group at carbon atom 2, and favors a trans configuration at carbon atoms 3 and 4, for which the only known exception is D-tagatose-di-P (40). The similarity in specificity may reflect a corresponding similarity in the functional groups at the active sites of the two enzymes.

Since L-galactoheptulose is a substrate of fructokinase, it is noteworthy that no reactivity was observed toward n-xylulose or L-glucogalactose which have the same steric configuration in carbon atoms 1 through 4. Evidently other structural requirements must be met.

Acknowledgments—We extend our gratitude to M. A. Günther, A. Sillero, and A. Sols for showing us their recent studies concerning fructokinase prior to publication.

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