L-Fucose Kinase*  

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**Purification to Apparent Homogeneity and Properties of Pig Kidney L-Fucose Kinase**

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L-Fucokinase was purified to apparent homogeneity from pig kidney cytosol. The molecular mass of the enzyme on a gel filtration column was 440 kDa, whereas on SDS gels a single protein band of 110 kDa was observed. This 110-kDa protein was labeled in a concentration-dependent manner by azido-[32P]ATP, and labeling was inhibited by cold ATP. The 110-kDa protein was subjected to endo-Lys-C digestion, and several peptides were sequenced. These showed very little similarity to other known protein sequences. The enzyme phosphorylated L-fucose using ATP to form β-L-fucose-1-P. Of many sugars tested, the only other sugar phosphorylated by the purified enzyme was D-arabinose, at about 10% the rate of L-fucose. Many of the properties of the enzyme were determined and are described in this paper. This enzyme is part of a salvage pathway for reutilization of L-fucose and is also a valuable biochemical tool to prepare activated L-fucose derivatives for fucosylation reactions.

6-Deoxy-t-galactose (t-fucose) is an important sugar in animal cells since it is involved in various recognition reactions of glycoproteins and glycolipids (1). Thus, oligosaccharides that have α-1,2-linked t-fucose are precursors for blood group A and B antigens (2). In the Lewis blood group antigens, Galβ1,3(Fucα1,4)GlcNAc-R, and Fucα1,2Galβ1,3(Fucα1,4)GlcNAc-R are determinants for Lewisα and Lewisβ blood group antigens. In addition, fucosylated and sialylated oligosaccharides have been found to be the recognition molecules for the E- and P-selectins, two members of the selectin family of cell adhesion molecules (3). These selectins and their fucosylated (and sialylated) ligands are important in inflammation and in the recognition of leukocytes for endothelial cells (4).

The primary pathway for the formation of t-fucose in procarcayotic and eucaryotic cells is from D-mannose via an internal oxidation reduction and then epimerization of GDP-D-mannose to produce GDP-t-fucose (5–8). However, studies in rats showed that radiolabeled L-fucose could be incorporated into glycoproteins (9, 10), suggesting an alternate route for activation of L-fucose. An L-fucokinase that synthesizes β-L-fucose-1-phosphate (11) and a GDP-L-fucose pyrophosphorylase (12) were partially purified from pig liver. However, the fucokinase preparation had rather broad substrate specificity with regard to sugar and nucleoside triphosphate, probably because of contaminating enzymes such as hexokinase in the partially purified fraction. In the present report, we describe the purification to apparent homogeneity of the pig kidney fucokinase. This enzyme preparation was very specific for t-fucose, and the only other sugar that could be phosphorylated, at about 10% of the rate with t-fucose, was D-arabinose. The fucokinase is also quite specific for ATP as the phosphate donor. This enzyme should be valuable for the synthesis of large amounts of l-fucose-1-P, as well as for the formation of radiolabeled fucose-1-P.

**EXPERIMENTAL PROCEDURES**

**Materials**

L-[3H]Fucose (52 Ci/mmol) and other radioactive sugars were purchased from American Radiolabeled Chemicals, Inc., or New England Nuclear Co. L-fucose-1-P, non-radioactive sugars, and nucleoside diphosphate sugars were obtained from Sigma Chemical Co. Various adsorbents were obtained from the following sources: DE-52 from Whatman Chemical Ltd., hydroxyapatite from Bio-Rad, omega-aminohexyl-Agarose and Sephacryl S-300-HR from Sigma Chemical Co. The following materials were obtained from Bio-Rad: Sodium dodecylsulfate (SDS), Acrylamide, Bisacrylamide, Comassie blue, Protein assay reagent. All other chemicals were from reliable chemical sources, and were of the best grade available.

**Assay of Fucokinase Activity**

Fucokinase activity was assayed by measuring the production of L-fucose-1-P from t-[3H]fucose and ATP. The incubation mixtures contained the following components (final concentrations) in a total volume of 150 μl: 0.1 mM t-fucose, 5 mM ATP, 5 mM MgSO₄, 65 mM Tris-HCl buffer, pH 8.0, and various amounts of enzyme at the different stages of purification. Incubations were terminated by heating the reaction mixtures in a boiling water bath for 1 min. The incubation mixtures were then applied to columns of DE-52 contained in Pasteur pipettes, and the columns were washed with at least 5 column volumes of 10 mM (NH₄)HCO₃ to remove the unbound material. The [3H]fucose-1-P was then eluted with 500 mM (NH₄)HCO₃. Aliquots of the eluates were assayed for their radioactive content by subjecting a portion to scintillation counting.

**Purification of the Fucokinase**

**Preparation of Cytosolic Fraction**—Pig kidneys were obtained from a local slaughterhouse and were transported to the laboratory on ice. The fresh kidneys were defatted and cut into pieces that were washed with cold distilled water. Each kidney piece was homogenized in a Waring blender in 2 volumes of Buffer A (30 mM Tris-HCl, pH 7.8, containing 10% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM β-mercaptoethanol). The homogenates were centrifuged at 12,000 × g for 30 min in a Beckman J-21 centrifuge. The supernatant liquid was removed and filtered through six layers of cheesecloth. The filtered supernatant liquid was then further centrifuged at 100,000 × g for 45 min. The clarified supernatant liquid was used as the starting material for purification since the fucokinase activity was much lower after freezing the tissue.

**DE-52 Column Chromatography**—A 5 × 16 cm column of DE-52 was prepared, and the column was equilibrated with Buffer A. The super-

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The abbreviations used are: t-fucose, 6-deoxy-L-galactose; PAGE, polyacrylamide gel electrophoresis; MES, 4-morpholinolmethanesulfonic acid.
natant liquid from the ultracentrifugation of 400 g of kidney (i.e., about 500 ml of supernatant liquid) was applied to the column, and the column was washed well with Buffer A and then with 800 ml of 0.1 M KCl. The fucokinase was eluted with 1000 ml of a 0.1–0.5 M gradient of KCl. Nine-ml fractions were collected, and every other fraction was assayed for activity and for protein. Active fractions were pooled and brought to 60% saturation by the addition of solid ammonium sulfate. After standing on ice for 15 min, the precipitate was isolated by cen-
trifugation and dissolved in Buffer A containing 1 M ammonium sulfate.

Hydrophobic Chromatography on Macro-Prep Methyl HIC Support—The dissolved ammonium sulfate fraction was applied to a 2.5 × 20 cm column of Macro-Prep Methyl HIC Support (Bio-Rad) that had been equilibrated with Buffer A containing ammonium sulfate. The column was then washed with the equilibration buffer. The kinase was eluted from the column with a linear gradient of 1–0 M ammonium sulfate in Buffer A. Active fractions were pooled and concentrated to about 30 ml on an Amicon filtration apparatus using a PM 30 membrane. The concentrated enzyme was then dialyzed against Buffer B (30 mM HEPES buffer, pH 7.6, containing 1 mM β-mercaptoethanol and 10% glycerol).

Chromatography on Hydroxylapatite—The dialyzed enzyme was loaded onto a 2.5 × 10 cm column of hydroxylapatite that had been equilibrated with Buffer B. The column was washed with the same buffer and then eluted with a 0–50 mM linear gradient of KH2PO4 in Buffer B. Under these conditions, the enzyme did not bind to the resin as tightly as other proteins in the preparation. Thus, about 60–70% of the enzyme emerged from the column at 15–25 mM KH2PO4. Some of the enzyme did remain on the column and could be eluted with the bulk of the protein at about 50 mM KH2PO4 in Buffer B.

Gel Filtration Chromatography—Active fractions, eluted from the hydroxylapatite column, were pooled and concentrated to about 2 ml on the Amicon apparatus. The concentrated enzyme preparation was ap-
plied to a 1.5 × 95 cm column of Sephacryl S-300 that had been equilibrated with Buffer C (25 mM HEPES buffer, pH 7.1, containing 1 mM β-mercaptoethanol and 10% glycerol). Four-ml fractions were collected and assayed for fucokinase activity. Active fractions were pooled and concentrated to a small volume.

Chromatography on Aminohexyl Agarose—The concentrated enzyme fraction from Sephacryl was applied to a 1.5 × 10 cm column of amin-
oxyethyl agarose, which had been equilibrated with Buffer C. The column was washed with 300 ml NaCl in Buffer C, and the kinase was eluted with 160 ml of a linear gradient of 300–700 mM NaCl in Buffer C. Fractions containing the active enzyme were pooled and the NaCl was removed by filtration on an Amicon apparatus and stored at −80 °C until used for various experiments. The most purified enzyme preparation gave a single protein band of 110 kDa on SDS gels but was found to still be contaminated with α-mannosidase activity (see "Results"), which also had a molecular mass of 110 kDa on SDS-PAGE. Thus, fractions from the aminohexyl-agarose column were assayed for fucoki-
rase and α-mannosidase, and fractions containing fucokinase activity were incubated with the N-32P]ATP probe and examined by SDS-
PAGE and autoradiography to identify the fucokinase.

Polyacrylamide (Native) Gel Electrophoresis
Preparative polyacrylamide gel electrophoresis was done at 4 °C in tubes containing 7% acrylamide and 10% glycerol as described by Laemmli (13) and using Tris buffer. The pH of the stacking gel was 6.7 and that of the resolving gel was 8.9. The samples of fucokinase were made up to 10% with respect to sucrose and contained bromophenol blue. During electrophoresis, the current was maintained at 3 mA/gel, and the temperature was kept at 4 °C. Two samples were run in parallel. One gel was stained with Coomassie Blue to detect proteins while the other gel was cut into 0.25 cm pieces, and the enzyme was eluted by overnight diffusion at 4 °C into Buffer A. The various elutions were then assayed for enzymatic activity.

On native gels, the fucokinase (molecular mass of 440 kDa, see lower band in Fig. 4A) was separated from the α-mannosidase (see upper band in Fig. 4A). To show that both of these bands were composed of 110-kDa subunits, the native gel was removed from the tube and laid on its side on top of an SDS slab gel and polymerized to that gel. Standard proteins were also combined in a native gel and added to the top of the slab. The proteins in the native gels were then subject to SDS-PAGE (as seen in Fig. 4B).

Photoaffinity Labeling of the Fucokinase with 8-Azido-32P]ATP
Enzyme, at various stages of purity, was mixed with 8-azido-
ATP32P] in buffer and allowed to incubate for 20 s in an ice bath. 8-Azido-32P]ATP was prepared as described previously (14). After incubation, the reaction mixture was exposed to short wave UV light for about 90 s to activate the azido group, and the protein was subjected to SDS-gel electrophoresis to separate the proteins. The gels were dried and exposed to film to locate the radioactive bands and were also stained with Coomassie Blue to locate the various proteins. The specificity of the labeling was determined by examining the effect of various concentrations of unlabeled ATP or other nucleotides on the labeling of the protein by N-32P]ATP. Various controls were also run, such as one in which exposure to UV was omitted.

Characterization of the Product
The radioactive sugar phosphate produced in the reaction was iso-
lated by ion exchange chromatography from large scale incubations of [3H]fucose and ATP with purified enzyme. The radiolabeled peak that eluted from DE-52 with a gradient of 0–250 mM (NH4)2HCO3 was lyoph-
ilized several times to remove the bicarbonate and was then sub-
jected to hydrolysis in various concentrations of HCl to determine the location of the phosphate group. Sugar-1-phosphates are quite sensitive to mild acid hydrolysis (0.05 N), and the phosphate group is lost fairly rapidly, whereas phosphate residues on other hydroxy groups are quite stable to these conditions. In addition, the product was analyzed by proton NMR to determine the location and anomeric configuration of the phosphate group. Three hundred MHz proton NMR and 31P-de-
coupled (GARP) NMR on the sample of L-fucose-1-P were performed on a Bruker ARX300 NMR. Data were acquired in D2O at pH 6.0. Other Methods
Protein was measured by the method of Bradford (15) using bovine serum albumin as the standard. The molecular weight of the native fucokinase was determined by gel filtration on Sephacryl S-300 and that of the subunit by SDS-gel electrophoresis. A number of molecular weight standards were run including: thyroglobulin (M, 669,000), apo-
ferritin (M, 443,000), β-amylase (M, 200,000), alcohol dehydrogenase (M, 150,000), bovine serum albumin (M, 66,000), and cytochrome c (M, 12,000).

RESULTS
Purification of the Fucokinase—The pig kidney fucokinase was purified about 5000-fold with a recovery of activity of about 21% using the methods described under "Experimental Proce-
dures." Fig. 1 shows two of the key steps in the purification procedure, i.e., chromatography on hydroxylapatite (panel A) and chromatography on an aminohexyl agarose column (panel B). The hydroxylapatite step gave about a 10-fold purification, whereas chromatography on aminohexyl-agarose gave better than a 6-fold purification. Table I presents a summary of the purification procedure showing the changes in specific activity at each step and the recovery of activity. Based on gel filtration, the native enzyme emerged from the column in the same area as apoferritin and had an estimated molecular mass of about 440 kDa.

The purified enzyme was subjected to SDS gel electrophore-
sis as shown in Fig. 2. The initial crude extract showed a number of protein bands (lane 2) while the most purified prep-
aration (lane 7) gave one major protein band with a molecular mass of about 110 kDa. That this band was indeed the fucokinase was demonstrated by the fact that it was specifi-
cally labeled by the photoprobe, azido-[32P]ATP. Thus, as seen in Fig. 3, incubation of enzyme with N-32P]ATP gave a single labeled band at the 110-kDa region (lane b), but no labeled protein band was seen in the absence of exposure to UV light (lane 1). The labeling was shown to be specific since it was inhibited in a dose-dependent manner by the addition of increasing amounts of unlabeled ATP (i.e., 0.5, 0.6, 0.9, and 1.2 mM) to the incubation mixtures (lanes 2–6). On the other hand, GTP at 0.6 and 1.2 mM (lanes 7 and 8) or ITP at 0.6 or 1.2 mM (lanes 9 and 10) were considerably less effective in inhibiting the reaction. Lanes 11–13 of Fig. 3 show the results of another experiment designed to determine whether 8-N3-32P]GTP could label the fucokinase. Lane 12 shows that incubation with this probe did not give rise to labeled protein, whereas incuba-
tion with N₃-[³²P]ATP did result in labeling of the 110-kDa protein (lane 13). These experiments indicate that the fucokinase is quite specific for ATP.

In addition, when various fractions from the aminohexyl-agarose column (Fig. 1B, fractions 48–56) were incubated with the N₃-[³²P]ATP probe, maximum labeling of the 110-kDa band was coincident with maximum fucokinase activity, i.e., fractions 48, 50, and 52 (data not shown). These data provide convincing evidence that the 110-kDa band is the fucokinase.

The 110-kDa protein was cut from the gel and sent to Harvard Microsystems for amino acid sequencing. One peptide, obtained by endo-Lys-C digestion, was sequenced, and a BLAST search indicated significant homology to α-mannosidase. The purified enzyme preparation was found to have strong fucokinase activity but also had readily detectable α-mannosidase activity. Although much of the α-mannosidase activity was removed on the aminohexyl-agarose column, some activity still emerged with the fucokinase in fractions 46–56 (Fig. 1B). This enzyme preparation gave a single sharp band at 110 kDa on SDS gels as shown in Fig. 2, lane 7.

The fucokinase could be separated from the α-mannosidase by native gel electrophoresis. Thus as seen in Fig. 4A, gel electrophoresis of the enzyme preparation from the aminohexyl-agarose column on native gels gave two protein bands, one with an estimated molecular mass of 440 kDa and a slower migrating band (Fig. 4A). The gel was sliced into 2.5-mm sections, and the proteins were eluted into buffer and assayed for activity. The fucokinase activity was only associated with the lower band while α-mannosidase was found in the upper, slower moving band. When this native gel with the two bands was then subjected to SDS-PAGE in a second dimension (Fig.

TABLE I

| Purification step         | Protein | Total activity of protein | Specific activity | -Fold | Yield |
|---------------------------|---------|--------------------------|-------------------|------|-------|
| Crude Extract             | 22000.0 | 5620                     | 0.255             | 100  | 100   |
| DE-cellulose              | 1000.0  | 5560                     | 5.56              | 21.8 | 98.9  |
| Macro-Prep Methyl HIC     | 232.5   | 4050                     | 17.42             | 68.3 | 72.1  |
| Hydroxyl-apatite          | 13.2    | 2446                     | 185.30            | 726.7| 43.5  |
| Sephacryl S-300           | 11.25   | 2497                     | 221.96            | 870.4| 44.4  |
| Aminohexyl-agarose        | 0.95    | 1210                     | 1273.88           | 4994.8| 21.5  |

a Units are nmol of fucose-1-P produced in 1 minute.
The purified enzyme was incubated for 20 s with azido-[32P]ATP, and the mixture was exposed to UV light for 90 s. The reaction was stopped by adding loading buffer and the mixture being subjected to SDS-PAGE. Radioactive bands were detected by exposure to film. Lanes are as follows: lane 1, probe but no UV; lane 2, probe + enzyme + UV; lanes 3–6, probe + enzyme + 0.3, 0.6, 0.9, and 1.2 mM ATP + exposure to UV light; lanes 7 and 8, 0.6 and 1.2 mM GTP + probe + exposure to UV light; and lanes 9 and 10, 0.6 and 1.2 mM ITP + probe + exposure to UV light. In lanes 11 and 12, the enzyme was incubated with N$_2$-[32P]GTP and then exposed to UV light (lane 12) or not exposed to UV light (lane 11). Lane 13 is a control of enzyme + N$_2$-[32P]ATP + exposure to UV light.

The 110-kDa subunit isolated from the 440-kDa protein (Fig. 4B), both the 440-kDa protein and the slower moving protein gave a single protein band of 110 kDa. These data indicate that both the fucokinase and the α-mannosidase are composed of 110-kDa subunits, but the native enzymes are quite different in size or charge.

The 110-kDa subunit isolated from the 440-kDa protein (Fig. 4B) was subjected to endo-Lys-C digestion, peptide isolation, and amino acid sequencing of several of the well-separated peptides. The amino acid sequences of three peptides were as follows: peptide 1, VDFSGGWSDTPPLAYE; peptide 2, (T)(G)-IRDWDLDWPDTCTP/T)ER; and peptide 3, LSHEQLQPC-LDR. These sequences do not show significant homology to any known sequences in the BLAST search.

Properties of the Fucokinase—The purified enzyme was studied to determine its substrate specificity, as well as various other properties of the enzymatic reaction. The enzyme showed a typical pH profile from 5.5 to 8.0 using MES and HEPES buffers, with a sharp pH optimum at about 8.0 in HEPES buffer. However, the pH curve on the alkaline side, between 8.0 and 9.0 in Tris buffer, did not show a sharp optimum. The activity in Tris buffer, pH 8.0, was about 80% of that in HEPES buffer, pH 8.0 (data not shown).

The enzyme had an absolute requirement for a divalent cation for activity. As shown in Fig. 5, Mg$^{2+}$ gave the best stimulation, with optimum activity being seen at 3 mM concentration. Fe$^{2+}$ also stimulated the enzyme to nearly the same degree as Mg$^{2+}$, but in this case, optimum activity occurred at about 10 mM. Co$^{2+}$ and Mn$^{2+}$ were also stimulatory, with maximum activity occurring at about 3–5 mM, but the maximum activity was only about one-fourth to one-third of that observed with Mg$^{2+}$. A variety of other metal ions were tested and found to be inactive, including Ca$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, Mo$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$. However, when Cu$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$ were added at 1 mM concentrations to incubations containing Mg$^{2+}$, they completely inhibited activity. The specificity of the kinase for various sugar substrates was examined in two different ways. In the first set of experiments, various radiola-
beled sugars were tested as phosphate acceptors for the purified enzyme using the ion-exchange chromatography method for assay of activity. Table II shows the results of this experiment. It can be seen that, of all the sugars tested, L-fucose was by far the best substrate and was readily phosphorylated. D-Arabinose, which has the same configuration at carbons 1 through 4 as L-fucose, was also a reasonable substrate for phosphorylation and was about 10% as effective as L-fucose. On the other hand, all of the other sugars were ineffective as phosphate acceptors.

Although it was possible to get a reasonable assessment of the sugar specificity of the kinase from the above experiment, it was not possible to test sugars such as D-fucose since these sugars are not available in radioactive form. Thus, these unla-
beled sugars were tested for their ability to inhibit the phosphorylation of L-[3H]fucose. The rationale for this experiment is that a sugar that inhibits the phosphorylation of L-fucose would probably compete with L-fucose for the phosphorylation (i.e. active) site. Table III shows the results. As expected, unla-
beled L-fucose was a reasonable inhibitor of the activity, and unla-
beled D-arabinose also inhibited although considerably less so than the L-fucose. Interestingly, D-fucose and L-rhamnose were ineffective as inhibitors, as was 2-deoxyglucose or other sugars. These data indicate that the configuration of the sugar at carbons 1 through 4 must be in the L-galactose configuration to be a substrate or inhibitor.

The specificity of the nucleoside triphosphate was also ex-
amined by testing the ability of a variety of nucleotides to serve as phosphate donors in the phosphorylation of L-[3H]fucose. Table IV demonstrates that the kinase is very specific for ATP as the phosphate donor and shows less than 2% activity with any other nucleoside triphosphate. In addition, no activity is observed with any nucleoside diphosphates or monophosphates.

The effect of concentration of the substrates, L-fucose and ATP, on the velocity of the reaction was determined. The $K_m$ for L-fucose was determined at saturating concentrations of the other substrates, i.e. 5 mM ATP and 5 mM Mg$_{2+}$. The data were plotted by the method of Lineweaver and Burke, and the $K_m$ for L-fucose was determined to be 27 μM (data not shown). A similar experiment was performed with ATP. In this case, the reactions were done in the presence of 5 mM Mg$_{2+}$ and 100 μM L-fucose. The $K_m$ for ATP was estimated to be 600 μM (data not shown).

### Table II
**Sugar specificity of purified fucokinase**

| Sugar added | Specific activity (nmol/mg protein) |
|-------------|-----------------------------------|
| L-Fucose    | 11.8                              |
| D-Arabinose | 1.1                               |
| D-Glucose   | 0.3                               |
| D-Ribose    | 0                                 |
| D-Mannose   | 0                                 |
| D-Galactose | 0                                 |
| D-Xylose    | 0                                 |

### Table III
**Inhibition of fucose phosphorylation by various monosaccharides**

Unlabeled monosaccharides were added at 500 μM concentrations. Radiolabeled L-fucose as the substrate was present at 100 μM.

| Monosaccharides | Relative activity |
|-----------------|------------------|
| None            | 100              |
| L-Fucose        | 23               |
| D-Fucose        | 102              |
| D-Arabinose     | 80               |
| D-Mannose       | 100              |
| D-Galactose     | 100              |
| D-Xylose        | 102              |
| D-Ribose        | 96               |
| 2-Deoxy-d-glucose | 92             |

The kinase was found to be inhibited by the final product of this alternate pathway, GDP-L-fucose. The data in Fig. 6 shows the effect of adding increasing amounts of GDP-L-fucose to reaction mixtures containing fucokinase, L-fucose, and ATP. It can be seen that the amount of inhibition of fucokinase increased with increasing amounts of GDP-L-fucose, and the $K_i$ for GDP-L-fucose was estimated to be about 10 μM. Other GDP-linked sugars, such as GDP-D-mannose and GDP-D-glucose, did not inhibit the fucokinase, nor did GDP-L-fucose (data not shown).

**Tissue Distribution**—To determine whether the fucokinase was present in other tissues besides kidney, crude cytosolic extracts were prepared from various porcine tissues, and each of these extracts was incubated with L-[3H]fucose, Mg$_{2+}$, and ATP for various times. The amount of label that bound to DE-52 and the specific activity of each extract are presented in Table V. It can be seen that L-fucokinase activity was present in many different tissues, and in fact, lung and kidney were the tissues with the highest specific activity for this enzyme. Aorta and brain also had reasonably high activity, whereas pancreas, heart, and spleen were the lowest. Crude extracts prepared...
from cultured MDCK cells and HT-29 cells were also assayed, but no detectable fucokinase activity was found in those extracts.

Identification of the Product—The product of the reaction was isolated from large scale incubations of L-fucose with ATP and active enzyme and was purified by ion-exchange chromatography and by paper chromatography. The radioactive fucose product eluted from the DE-52 column in the same position as authentic sugar-1-phosphates, such as glucose-1-P or GlcNAc-1-P. The product was subjected to mild acid hydrolysis in 0.05 N HCl at 100 °C. Aliquots of the hydrolysis mixture were withdrawn at various times after the initiation of heating, and each aliquot was passed through a column of DE-52. The wash and salt elution of the column were subjected to scintillation counting to determine the rate of hydrolysis. The phosphorylated sugar completely lost its charge (and no longer bound to DE-52) within the first 3 min of hydrolysis (data not shown). These data provide convincing evidence that the phosphate group is attached to the anomeric carbon of the sugar.

The sugar-1-P produced in the reaction was subjected to 300 mHz NMR as an aid in the structural characterization as well as to determine the anomeric configuration of the phosphate group. The radioactive fucose product eluted from the DE-52 column in the same position as authentic sugar-1-phosphates, such as glucose-1-P or GlcNAc-1-P. The product was subjected to mild acid hydrolysis in 0.05 N HCl at 100 °C. Aliquots of the hydrolysis mixture were withdrawn at various times after the initiation of heating, and each aliquot was passed through a column of DE-52. The wash and salt elution of the column were subjected to scintillation counting to determine the rate of hydrolysis. The phosphorylated sugar completely lost its charge (and no longer bound to DE-52) within the first 3 min of hydrolysis (data not shown). These data provide convincing evidence that the phosphate group is attached to the anomeric carbon of the sugar.

The sugar-1-P produced in the reaction was subjected to 300 mHz NMR as an aid in the structural characterization as well as to determine the anomeric configuration of the phosphate group. Panel A of Fig. 7 shows 300 mHz NMR proton detection for the anomeric proton region of L-fucose-1-phosphate, and panel B shows the 31P-decoupled spectrum. The J₁,₂ spin-coupling is 4 Hz for the anomeric proton, consistent with a β-L-fucose-1-phosphate configuration.

**DISCUSSION**

L-Fucose is an important component of many animal glycolipids and glycoproteins, and turnover of these polymers in the lysosomes must lead to the formation of free L-fucose (9). Thus, it is not surprising to find that certain tissues, especially liver and kidney, contain a specific kinase that can phosphorylate L-fucose to form L-fucose-1-phosphate. In fact, early labeling studies in rats indicated that various tissues are capable of utilizing free L-fucose as a precursor of the L-fucose in glycoproteins (9, 10), suggesting a pathway to reutilize L-fucose.

The presence of the enzyme L-fucokinase was first demonstrated by Ishihara et al. (11) who partially purified this enzyme from pig liver. However, the initial purification of this enzyme was only about 70-fold, and the enzyme preparation still had considerable activity for phosphorylating D-glucose, D-ribose, and L-rhamnose. Thus, that enzyme fraction still probably contained hexokinase, ribokinase, and other enzymatic activities. Interestingly enough, the kinase was also purified about 3500-fold from pig liver by Yurchenko and Atkinson (16). Their enzyme preparation also phosphorylated D-glucose, D-galactose, and D-mannose at about the same rate or better than it phosphorylated L-fucose. These data suggest...
either that their kinase had a very broad specificity for sugar or that the preparation was still contaminated with hexokinase and galactokinase. Unfortunately, those authors did not examine the nature of the products formed from glucose and mannose to determine whether the phosphate group was in the 1 or 6 position since that would readily have shown whether the reaction was catalyzed by hexokinase. Otherwise, it is difficult to envision how a sugar kinase could catalyze a reaction with four different sugars, all having a different stereochemistry at carbons 2 through 4.

On the other hand, the L-fucokinase described in the present report shows very strong specificity for sugars having the L-galactose configuration at carbons 2 through 4. Thus, only L-fucose and D-arabinose were active as phosphate acceptors although d-arabinose was only about 10% as effective as L-fucose. In addition, the enzyme only utilized ATP as the phosphate donor in contrast to the enzyme reported by Ishihara et al. (11), which could also use CTP, UTP, and GTP as phosphate donors. Thus, it is not clear whether the enzyme reported here is a new enzyme or whether it is the same protein as described earlier but has been purified much more extensively to apparent homogeneity. We sent the purified enzyme to Harvard Microsystems for amino acid sequencing and obtained the sequences of three peptides. These sequences do not show any more than 40% homology to known sequences by the BLAST search. Thus, our protein is clearly not very closely related to other reported kinases.

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