Human Liver $\alpha$-L-Fucosidase

PURIFICATION, CHARACTERIZATION, AND IMMUNOCHEMICAL STUDIES*

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SUMMARY

Human liver $\alpha$-L-fucosidase has been purified 6300-fold to apparent homogeneity with 66% yield by a two-step affinity chromatographic procedure utilizing agarose $\epsilon$-aminocaproyl-fucosamine. Isoelectric focusing revealed that all six isoelectric forms of the enzyme were purified. Polyacrylamide gel electrophoresis of the purified $\alpha$-L-fucosidase demonstrated the presence of six bands of protein which all contained fucosidase activity. The purified enzyme preparation was found to contain only trace amounts of seven glycosidases. Quantitative amino acid analysis was performed on the purified fucosidase. Preliminary carbohydrate analysis indicated that only about 1% of the molecule is carbohydrate. Gel filtration on Sepharose 4B indicated an approximate molecular weight for the enzyme of 175,000 ± 18,000. High speed sedimentation equilibrium yielded a molecular weight of 230,000 ± 10,000. Sodium dodecyl sulfate polyacrylamide gels indicated the presence of a single subunit of molecular weight 50,100 ± 2,500. The enzyme had a pH optimum of 4.6 with a suggested second optimum of 6.5. Apparent Michaelis constants and maximal velocities were determined on the purified enzyme with respect to the 4-methylumbelliferyl $\alpha$-L-fucoside and the $\beta$-nitrophenyl substrates and were found to be 0.22 mm and 14.1 $\mu$mol/mg of protein/min and 0.43 mm and 19.6 $\mu$mol/mg of protein/min, respectively. Several salts had little or no effect on fucosidase activity although $Ag^+$ and $Hg^{+2}$ completely inactivated the enzyme. Antibodies made against the purified fucosidase were found to be monospecific against crude human liver supernatant fluids and the pure antigen. No cross-reacting material was detected in the crude liver supernatant fluid from a patient who died with fucosidosis.

$\alpha$-L-Fucosidase is an important enzyme not only because its activity is deficient in the neurovisceral storage disease fucosidosis (1, 2) but also because the enzyme is involved in the metabolism of several biologically active molecules containing L-fucose (3-8). A highly purified preparation of $\alpha$-L-fucosidase free of contaminating glycosidases would be useful for structural studies on the many oligosaccharides, glycolipids, and glycoproteins which contain L-fucose and for studying the nature of the molecular defect in fucosidosis.

$\alpha$-L-Fucosidase has been purified by conventional methods from several mammalian sources (9-13). To date, no one has reported a purification of a mammalian $\alpha$-L-fucosidase which is free of contaminating glycosidases. Carlsen and Pierce (9) have purified $\alpha$-L-fucosidase from rat epididymis but their preparation contained N-acetylglucosaminidase as a 3.8% (by activity) contaminant. Previously we reported a one-step affinity chromatographic procedure for the partial purification of human placental $\alpha$-L-fucosidase (14). This fucosidase preparation contained hexosaminidase as a 3.7% contaminant, by activity.

In the present communication we describe an improved affinity chromatographic purification procedure for $\alpha$-L-fucosidase and properties and immunochemical studies on the apparently homogeneous enzyme from human liver.

EXPERIMENTAL PROCEDURES

General Protein was determined by the Lowry method (15) using bovine serum albumin as standard. All procedures were carried out at 0-4° unless otherwise stated. Enzyme solutions were concentrated by ultrafiltration using Amicon concentrators with UM-10 Diaflo membranes at 50 to 70 p.s.i. Agarose-$\epsilon$-aminocaproyl-fucosamine (lot numbers AF-5, 7, 10, and 11) was purchased from Miles-Yeda Ltd.

Enzyme Assays—$\alpha$-L-Fucosidase activity was assayed either by using $p$-nitrophenyl-$\alpha$-L-fucopyranoside (Sigma) or 4-methylumbelliferyl-$\alpha$-L-fucopyranoside (Koch-Light, Ltd.) as previously reported (16, 17). Enzyme assays were always performed under conditions where enzyme activity was linear with the amount of protein and time of incubation. Absorbances were read on a Gilford 2400-S spectrophotometer.

Polyacrylamide Gels—Polyacrylamide gel electrophoresis in 5% gels was performed as previously reported (14) by a modification of the general method of Davis (18). The presence of subunits was determined in 10% polyacrylamide gels (0.6 x 10 cm) containing 0.1% sodium dodecyl sulfate according to the general method of Laemml (19). Forty micrograms each of bovine serum albumin, ovalbumin, $\alpha$-chymotrypsinogen, and cytochrome c (all from Sigma) were used for molecular weight determination. Cytochrome c was also subjected to electrophoresis in the enzyme samples (20 $\mu$g/gel) as an internal standard. Two samples of $\alpha$-L-fucosidase (30 $\mu$g and 70 $\mu$g) were subjected to electrophoresis after treating them with 1% sodium dodecyl sulfate, 5% $\beta$-mercapto-

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ethanol, and 8 M urea in 62.5 mM Tris-HCl, pH 6.8, in a boiling water bath for 5 min. Electrophoresin was at 2 mA/tube for 6 hours at room temperature. The gels were stained with 0.2% Coomassie blue in 10% acetic acid (v/v) and 50% methanol (v/v). The gels were destained in a transverse destainer for 6 hours in 10% (v/v) acetic acid.

**Isoelectric Focusing**—Isoelectric focusing was performed according to the method of Haghani (20) using an LKB 5010 (110 ml isoelectric focusing apparatus. Two per cent ampholines (pH 5 to 8) were used in a gradient of 0 to 6% (w/v) sucrose. The pH of each fraction was measured at 0–2°C with a temperature was maintained at 0–2°C with a circulating water bath 520 to 530 volts. Electrofocusing was conducted for 67 to 68 hours at room temperature. The gels were stained with 0.2% Coomassie blue in 10% acetic acid. The pH of each fraction was measured at 0–2°C with a circulating water bath 520 to 530 volts. Electrofocusing was conducted for 67 to 68 hours at room temperature. The gels were stained with 0.2% Coomassie blue in 10% acetic acid.

**Kinetic Studies**—Apparent Michaelis constants (Km values) and maximal velocities (Vmax) were determined for the purified enzyme graphically by the Lineweaver-Burk method (21) using both the 4-methylumbelliferyl and the p-nitrophenyl substrates. For these kinetic studies, 0.4 µg of the purified α-L-fucosidase was incubated in substrates of varying concentrations containing human serum albumin (10 mg/ml), in 100 mM citrate-sodium citrate buffer (pH 5.0). All samples were incubated in duplicate for 5 min at 37°C. In one experiment with the p-nitrophenyl substrate, 0.3 mM L-fucose was present in the incubation medium of the varying substrate concentrations to determine its mode of inhibition of α-L-fucosidase.

**Hyaluronate Assays**—Various salts at concentrations ranging from 0.25 to 29 mM (Na+, K+, Ca2+, Mg2+, Mn2+, Zn2+, Fe2+, Hg2+, Ag+, Fe2+, and Cu2+), 0.2 to 1.2 mM dithiothreitol, and 0.12 to 1.2 mM ethylenedinitrilotetraacetic acid (EDTA) were incubated with 0.2 µg of the purified fucosidase to determine their effect on α-L-fucosidase activity. Incubations were carried out in duplicate at 37°C for 6 min.

**pH Optimum**—The pH optimum of the purified α-L-fucosidase as determined from 0.4 µg of enzyme protein (in 10 µl of 100 mg of human albumin/ml of H2O) was added to 90 µl of 100 mM buffers (citrate-sodium citrate for pH values 3.1 to 5.8; KH2PO4-Na2HPO4 for pH values 6.0 to 8.2) of varying pH values containing human albumin (10 mg/ml). The reaction was initiated with 200 µl of p-nitrophenyl-[L fucoside (3 mM) in buffers of varying pH values containing human albumin (10 mg/ml). The reaction was initiated with 200 µl of p-nitrophenyl-L-fucoside (3 mM) in buffers of varying pH values containing human albumin (10 mg/ml). The reaction was initiated with 200 µl of p-nitrophenyl-L-fucoside (3 mM) in buffers of varying pH values containing human albumin (10 mg/ml). The reaction was initiated with 200 µl of p-nitrophenyl-L-fucoside (3 mM) in buffers of varying pH values containing human albumin (10 mg/ml). 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Preparation of Supernatants for Immunochemical Studies—A normal human liver supernatant (1/4, w/v) was prepared as previously described (17) and concentrated 6-fold by ultrafiltration. A liver supernatant (1/4, w/v) from a patient who died with fucosidosis (17) was prepared and concentrated 11-fold by ultrafiltration. These concentrated supernatant fluids were used for the double immunodiffusion experiments.

Purification of Fucosidase

Tissue Extraction—Portions of human livers (1000 g total, wet weight) from five autopsyed individuals which appeared normal on gross pathological examination were washed with distilled water, cut into small pieces, and homogenized in a Waring Blender in 4 liters of buffer containing 10 mM NaH2PO4 (pH 5.5) and NaN3 (0.02%, w/v) for 1 min at low speed and 1 min at high speed. The crude homogenate was centrifuged for 30 min at 23,500 X g and the supernatant fluid was filtered through cheesecloth. The supernatant fluid contained 94% of the protein present in the crude homogenate. The supernatant fluid (4.2 liters) was dialyzed against 10 mM NaH2PO4 (pH 5.5) containing NaN3 (0.02%, w/v) for 3 days using 33 liters/change. The dialyzed supernatant fluid was centrifuged for 30 min at 23,500 X g, filtered through cheesecloth, and then used for affinity chromatography.

Affinity Chromatography—A portion of the supernatant fluid (900 ml, 42,000 units, 10.0 g of protein) was applied to a column (2.8 X 35 cm) of agarose-e-aminocaproyl-fucosamine (affinity column I) at a rate of 50 ml/hour at room temperature. The column was subsequently washed with approximately 12 liters of 10 mM NaH2PO4 (pH 5.5) containing 0.02% NaN3 (w/v) until the absorbance at 280 nm was 0.002. The column was then eluted sequentially at a rate of 100 ml/hour with 10 mM NaH2PO4 (pH 5.5), 0.02% in NaN3 (w/v) containing either 50 mM L-fucose (Sigma) or 0.25 M NaCl or 7 M urea (Fig. 1). Initially, 10-ml fractions were collected and 1.5-ml fractions were collected when eluting with L-fucose. After assaying for α-L-fucosidase activity, fractions 1380 to 1600 were combined, concentrated, and assayed for α-L-fucosidase in the presence of human albumin (3 mg/ml). The fractions of highest specific activity (Fractions 50 to 64) were combined, concentrated, and assayed for contaminating glycosidases by previously described methods (16, 27-29). Polyacrylamide gels were carried out using the concentrated Fractions 50 to 64 from affinity column II. These gels were either stained for protein with Coomassie blue or stained for activity for 20 min at 0° with 1 mM 4-methylumbelliferyl-α-L-fucopyranoside (Koch-Light, Ltd.) and then visualized with an ultraviolet lamp.

RESULTS

Purification of α-L-Fucosidase

The elution profile of α-L-fucosidase from affinity column I is depicted in Fig. 1. Eighty per cent of the enzyme was eluted with 10 mM NaH2PO4 (pH 5.5) containing 0.02% NaN3 (w/v) and 50 mM L-fucose yielding a 3980-fold purification. As in the purification of placental α-L-fucosidase (14), 2 to 3% of α-L-fucosidase was eluted with the buffer containing 0.25 M NaCl.

The 3980-fold purified α-L-fucosidase was passed through a smaller column (affinity column II) of agarose-e-aminocaproyl-fucosamine (Fig. 2). The capacity of this affinity column for the partially purified fucosidase was approximately 5 times as great as that for the α-L-fucosidase in the crude supernatant fluid. Two per cent of the enzyme did not bind to the column and eluted near the front of the column. Eighty-three per cent of α-L-fucosidase was eluted with 10 mM NaH2PO4 (pH 5.5) containing 0.02% NaN3 (w/v) and 50 mM L-fucose. In the activity profile from this column, it appears that only a small amount of fucosidase activity was eluted by L-fucose, but this is due to the inhibition of α-L-fucosidase by the 100 mM L-fucose (14).

Table I summarizes the data on the affinity chromatographic purification of human liver α-L-fucosidase. The two-step procedure results in a 6300-fold purification with a 66% yield.
FIG. 2. Affinity chromatography of partially purified human liver α-L-fucosidase using a column (1 × 27 cm) of agaroose-α-aminocaproyl-fucosamine (affinity column II). The column was eluted as described under “Experimental Procedures” and the arrows indicate where the buffers were applied. Protein (absorbance at 280 nm) is indicated by •—• and enzymatic activity (absorbance at 420 nm) by O—O. Aliquots of 50 μl were assayed for 15 min at 37°C.

TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (nmol/mg protein/min)</th>
<th>Purification factor</th>
<th>Over-all recovery of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23,500 × g supernatant which was put on affinity column I</td>
<td>10,900</td>
<td>42,000</td>
<td>3.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractions 1380 to 1600 from affinity column I (see Fig. 1) (80% recovery)</td>
<td>2.20</td>
<td>33,600</td>
<td>15,300</td>
<td>3,980</td>
<td>80</td>
</tr>
<tr>
<td>Fractions 50 to 64 from affinity column II (see Fig. 2) (83% recovery*)</td>
<td>0.75</td>
<td>18,100</td>
<td>24,200</td>
<td>6,300</td>
<td>66</td>
</tr>
</tbody>
</table>

* A unit of activity is the hydrolysis of 1 nmol of substrate/min at 37°C.

A Recovery of activity put on affinity column II.

FIG. 3. Polyacrylamide gel electrophoresis of human liver α-L-fucosidase. Electrophoresis was for 3 hours at 1.6 ma/tube (300 volts) in 0.5 × 9.0 cm, 5% acrylamide gels. Gel buffer was Tris (375 mM)-HCl (60 mM), pH 8.9; running buffer was Tris (50 mm)-glycine (384 mM), pH 8.2. A, concentrated postaffinity column I fractions 1380 to 1600 (33 μg of enzyme protein) stained with Coomassie blue. B, concentrated postaffinity column II fractions 50 to 64 (33 μg of enzyme protein) stained with Coomassie blue.

**Purity of α-L-Fucosidase**

Polyacrylamide Gels—Polyacrylamide gels of the postaffinity column II sample (Fig. 3B) demonstrated the presence of only the group of six bands of protein. Incubation of a duplicate gel with the fluorescent substrate 4-methylumbelliferyl-α-L-fucopyranoside indicated that all six bands of protein appear to be associated with α-L-fucosidase activity.

Sodium Dodecyl Sulfate-Polyacrylamide Gels—Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the postaffinity column II sample (Fig. 4A and B; 30 μg and 70 μg of α-L-fucosidase, respectively) indicated the presence of a single subunit. Standard proteins were subjected to electrophoresis (Fig. 4C) on a sodium dodecyl sulfate gel and the molecular weight of the subunit was graphically determined to be 50,100 ± 2,500 (Fig. 5).

Assay of α-L-Fucosidase for Contaminating Glycosidases—Table II indicates that the 6300-fold purified α-L-fucosidase preparation had only trace amounts of other glycosidase activities. Whereas in our partial purification of human placental α-L-fucosidase (15) hexosaminidase was a large contaminant (3.7%), it has been reduced to a trace contaminant (0.007%) in the human liver α-L-fucosidase preparation.

Behavior of Purified α-L-Fucosidase on Sepharose 4B—Fig. 6 depicts the elution profile of the purified α-L-fucosidase during gel filtration on Sepharose 4B. Only one protein peak can be seen and this peak coincides with the one peak of fucosidase activity. Approximately 90% of the fucosidase eluted from the Sepharose 4B column over several fractions of constant specific activity.

Immunochemical Criteria of Purity—The purified α-L-fucosidase can be seen migrating faster toward the anode than α-L-fucosidase. Polyacrylamide gels of the postaffinity column II sample (Fig. 3B) demonstrated the presence of only the group of six bands of protein. Incubation of a duplicate gel with the fluorescent substrate 4-methylumbelliferyl-α-L-fucopyranoside indicated that all six bands of protein appear to be associated with α-L-fucosidase activity.
Table II

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Substrate</th>
<th>Per cent of α-L-fucosidase activity</th>
<th>Reference for assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-L-Fucosidase</td>
<td>p-Nitrophenyl α-L-fucoside</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>β-D-Fucosidase</td>
<td>p-Nitrophenyl β-D-fucoside</td>
<td>0.007</td>
<td>16</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>4-Methylumbelliferyl α-D-galactopyranoside</td>
<td>0.002</td>
<td>27</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>4-Methylumbelliferyl β-D-galactopyranoside</td>
<td>0.0007</td>
<td>28</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>4-Methylumbelliferyl β-D-glucuronide</td>
<td>0.001</td>
<td>27</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>4-Methylumbelliferyl α-D-glucopyranoside</td>
<td>0.001</td>
<td>29</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>4-Methylumbelliferyl α-D-mannopyranoside</td>
<td>0.001</td>
<td>28</td>
</tr>
<tr>
<td>Hexosaminidase</td>
<td>4-Methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside</td>
<td>0.007</td>
<td>27</td>
</tr>
</tbody>
</table>

Fig. 4. Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate and stained with Coomassie blue. See "Experimental Procedures" for details. A, purified α-L-fucosidase (30 μg of enzyme protein) treated for the determination of subunits (19). B, purified α-L-fucosidase (70 μg of enzyme protein) treated for the determination of subunits (19). C, protein standards (bovine serum albumin, ovalbumin, α-chymotrypsinogen, and cytochrome c—40 μg of each) run for molecular weight determination of α-L-fucosidase subunit.

Fig. 5. Mobilities of α-L-fucosidase treated for dissociation of subunits (19) and molecular weight standards on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. See "Experimental Procedures" for details.

dase when injected into rabbits caused the production of monospecific antibodies against α-L-fucosidase. This can be seen in Fig. 7 which depicts the double immunodiffusion (Ouchterlony) plate of rabbit anti-α-L-fucosidase antiserum (IgG fraction) against purified α-L-fucosidase (Wells A and B) and normal human liver supernatant (Wells C and D).

Characterization of Purified α-L-Fucosidase—Fig. 8 depicts the results of isoelectric focusing on both crude (A) and highly purified (B) human liver α-L-fucosidase. As previously reported (17), six isoelectric forms are present in the crude human liver supernatant fluid (Fig. 8A). The same isoelectric forms appear to be present, and in approximately the same proportion, in the highly purified α-L-fucosidase (Fig. 8B).

Using the purified α-L-fucosidase, apparent Kₐ values and Vₐₘₐₓ values were determined graphically with respect to the 4-methylumbelliferyl and p-nitrophenyl substrates. They were found to be 0.22 mM and 14.1 μmol/mg of protein/min and 0.43 mM and 19.6 μmol/mg of protein/min, respectively. Fig. 9 demonstrates that 1-fucose is a competitive inhibitor of α-L-fucosidase and suggests a probable mechanism by which the agaroose-ε-aminocaproyl-fucosamine resin binds the enzyme.

Mn²⁺ (5 mM) stimulated fucosidase activity 22% and Zn²⁺ had no effect on enzymatic activity at concentrations of 0.25 to 5 mM. Hg²⁺ and Ag⁺ (0.1 to 0.2 mM) completely inactivated enzymatic activity and dithiothreitol was mildly stimulatory (13%) at a concentration of 0.2 mM. Dithiothreitol (1.1 mM) completely reverses the inhibition of α-L-fucosidase activity by Ag⁺ and Hg²⁺.

Quantitative amino acid analysis was performed on the puri-
Amino acid composition of purified α-L-fucosidase

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues per 230,000 molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic</td>
<td>210</td>
</tr>
<tr>
<td>Threonine</td>
<td>102</td>
</tr>
<tr>
<td>Serine</td>
<td>138</td>
</tr>
<tr>
<td>Glutamic*</td>
<td>178</td>
</tr>
<tr>
<td>Proline</td>
<td>172</td>
</tr>
<tr>
<td>Cysteine</td>
<td>8</td>
</tr>
<tr>
<td>Glycine</td>
<td>149</td>
</tr>
<tr>
<td>Alanine</td>
<td>94</td>
</tr>
<tr>
<td>Valineb</td>
<td>84</td>
</tr>
<tr>
<td>Methionine</td>
<td>21</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>54</td>
</tr>
<tr>
<td>Leucine</td>
<td>107</td>
</tr>
<tr>
<td>Tyrosinec</td>
<td>75</td>
</tr>
<tr>
<td>Phenylyalanine</td>
<td>120</td>
</tr>
<tr>
<td>Histidine</td>
<td>56</td>
</tr>
<tr>
<td>Lysine</td>
<td>114</td>
</tr>
<tr>
<td>Arginine</td>
<td>55</td>
</tr>
</tbody>
</table>

* No attempt was made to measure proportions which might occur as the amides.

b Calculated for 72-hour hydrolysis.

c Calculated by extrapolation to zero time.

The purified α-L-fucosidase hydrolyzed both 2'-fucosyllactose and lacto-N-fucopentaose I as is shown by the thin layer chromatogram in Fig. 12. From a gross inspection of the thin layer plate it appears that the enzyme was more active on 2'-fucosyllactose (Lane 5) than on lacto-N-fucopentaose I (Lane 6).

Stability studies performed on the purified fucosidase indicated that the enzyme lost no activity for at least 163 days when stored at 0-4°C in 10 mM Na2HPO4 (pH 5.5) containing 0.02% (w/v) NaN3 and 100 mM L-fucose. The inhibitor L-fucose which was used to stabilize the purified enzyme was either dialyzed away or diluted to insignificant amounts when kinetic studies were performed on the purified enzyme. The purified enzyme was very thermolabile and gradually lost activity after 6 min at 37°C. This is in marked contrast to the thermostability of fucosidase in the crude state, where 4 hour incubations at 37°C did not decrease enzymatic activity.

Antibodies were made against the purified fucosidase and the IgG fraction was partially purified and concentrated by DEAE-chromatography. This IgG fraction, as well as the antiserum,
purified α-L-fucosidase and several standard proteins on a column (0.5 × 91 cm) of Sepharose 4B.

Fig. 11. Molecular weight determination by gel filtration of purified α-L-fucosidase and several standard proteins on a column (0.5 × 91 cm) of Sepharose 4B.

Fig. 12. Thin layer chromatogram showing products of reaction after incubation of purified liver α-L-fucosidase with 2'-fucosyllactose and lacto-N-fucopentaose I. See "Experimental Procedures" for details. Lanes 1 and 7, α-L-fucose standard; lane 2, tissue (enzyme) blank; lane 3, 62 μg of enzyme protein + 1.6 mg of 2'-fucosyllactose; lane 4, 2'-fucosyllactose substrate blank; lane 5, 62 μg of enzyme protein + 1.6 mg of lacto-N-fucopentaose I; lane 6, lacto-N-fucopentaose I substrate blank.

was found to contain monospecific antibodies against the pure antigen (Fig. 7, A and B) and against a concentrated crude human liver supernatant (Fig. 7, C and D). Cross-reacting material was not found against a concentrated liver supernatant fluid from a patient who had fucosidosis (Fig. 7, E and F). No precipitin bands were seen when controls were run against preimmunization rabbit antisera.

DISCUSSION

In this paper we have described a two-step affinity chromatographic procedure for the purification of human liver α-L-fucosidase to apparent homogeneity. Several criteria were used to assess purity including polyacrylamide gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, assay of the purified fucosidase for containing glycosidases, behavior of the purified α-L-fucosidase on Sepharose 4B, and the production of monospecific antibodies against the purified fucosidase. To our knowledge this is the first report of a mammalian α-L-fucosidase purified to apparent homogeneity. Carlsen and Pierce (9) have described a procedure for obtaining highly purified rat epididymal α-L-fucosidase, but their preparation contained N-acetylglucosaminidase as a 3.8% (by activity) contaminant. Our preparation of α-L-fucosidase has only trace amounts (Table II) of other glycosidase activities. This suggests that our preparation should be useful for structural studies of glycoproteins, glycolipids, and oligosaccharides which contain L-fucose (5-8).

Isoelectric focusing indicated that all six forms of α-L-fucosidase (17) were purified by the affinity chromatographic procedure. This illustrates the usefulness of the purification procedure for isolating all forms of the enzyme. The structural relationship of the six forms of α-L-fucosidase is not known, but the activity of at least two of the forms appears to be dependent on the presence of sialic acid residue(s) (17). Robinson and Thorpe (31) and Wiederschain et al. (13) have demonstrated the presence of two forms of α-L-fucosidase (designated I and II) in human liver by gel filtration on Sephadex G-200. The relationship of these two forms of α-L-fucosidase to the six isoelectric forms we have found is unclear and is currently under investigation.

The purified fucosidase was characterized kinetically. The $K_m$ values of the purified fucosidase (0.22 to 0.43 mM) which we obtained were about the same as those obtained for the p-nitrophenyl substrate by Levy and McAllan (12) (0.21 mM) for rat epididymal α-L-fucosidase and by Wiederschain and Rosenfeld (32) (0.4 mM) for pig kidney α-L-fucosidase. Our pH optimum curve is similar to that obtained earlier for α-L-fucosidase in crude human liver supernatant fluid with the 4-methylumbelliferyl substrate (17). However, for the crude enzyme the pH curve was shifted approximately 0.8 pH unit toward a more neutral pH optimum (5.4). These results are similar to the shape of the curve and the pH 5 value obtained by Robinson and Thorpe (31) for human liver fucosidase. Human liver α-L-fucosidase was found to hydrolyze the two fucose-containing oligosaccharides 2'-fucosyllactose and lacto-N-fucopentaose I. Several preparations of α-L-fucosidase have been found effective in hydrolyzing 2'-fucosyllactose and lacto-N-fucopentaose I (3, 4, 33).

The molecular weight of the purified fucosidase was determined to be approximately 175,000 by gel filtration and 230,000 by sedimentation equilibrium. Since proteins partition on gel filtration according to their Stokes radii (34) and since glycoproteins are known to give anomalous molecular weights on gel filtration (30), the molecular weight obtained by sedimentation equilibrium (230,000) is probably most accurate. Our results are similar to the value (210,000 to 220,000) found for rat epididymal α-L-fucosidase by Carlsen and Pierce (9). Treatment of the purified fucosidase with 1% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 8 M urea followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (19) indicated the presence of a single subunit with 50,100 molecular weight. Our results, which are different from those of Carlsen and Pierce (9) who found two nonidentical subunits (46,700 and 65,700) for rat epididymal α-L-fucosidase, suggest that human liver α-L-fucosidase is a tetramer of four identical subunits.

Several salts, dithiothreitol, and EDTA were tested for their effect on pure human liver α-L-fucosidase activity. Zn$^{2+}$ had no
effect on enzymatic activity unlike the large inhibition (90%) of rat epididymal α-L-fucosidase found with 5 mM Zn²⁺ by Carlsen and Pierce (9). Hg²⁺ and Ag⁺ were found to completely inactivate fucosidase activity. This inactivation was prevented in the presence of dithiothreitol. These results suggest that α-L-fucosidase contains a sulfhydryl group which is important for enzymatic activity.

The suggested presence of sulfhydryl-containing amino acids in fucosidase was confirmed by amino acid analysis revealing the presence of 8 cysteine residues. The amino acid composition of human liver α-L-fucosidase (Table III) very closely resembles that for rat epididymal α-L-fucosidase (9). Our preliminary carbohydrate analysis indicates that only a very small portion (approximately 1%) of human liver fucosidase is carbohydrate. This also is similar to the finding of Carlsen and Pierce (9) that rat epididymal α-L-fucosidase contained 0.6% carbohydrate. Large amounts of pure enzyme will be necessary to determine the complete carbohydrate composition of human liver α-L-fucosidase.

Immunochromal studies were performed with antibodies (IgG fraction) made against α-L-fucosidase. Single precipitin lines were found in double immunodiffusion experiments when anti-α-L-fucosidase antibody was run against either crude liver supernatant fluids or pure antigen (α-L-fucosidase). No precipitin lines were found against a concentrated liver supernatant (IgG fraction) made against α-L-fucosidase. Single precipitin lines were found against a patient who died with fucosidosis. At least three possible explanations might account for this latter observation. The patient’s liver may not be synthesizing any fucosidase (a regulatory defect), may be making small amounts of α-L-fucosidase below the limits of detection by double immunodiffusion techniques, or may be synthesizing enzyme protein which is antigenically different from normal human liver fucosidase. Experiments using highly sensitive radioimmunochemical techniques will be carried out to distinguish between the above explanations and to characterize further the nature of the biochemical defect in fucosidosis.

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