Hydrolysis of fucosyl-G_{M1} ganglioside by purified pellet-associated human brain and human liver α -L-fucosidases without activator proteins or detergents

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Pellet-associated human brain α -L-fucosidase was solubilized with 0.5% (w/v) Triton X-100 and purified by affinity chromatography on agarose–6-aminohexanoyl-fucosamine resin. The procedure resulted in a 290 000-fold purification, a 58% yield and a final specific activity of 11 500 nmol/min per mg of protein. Isoelectric focusing indicated that all six major isoforms (with pI values between 4.1 and 5.3) present in crude brain pellet preparations were purified by the affinity technique. SDS/PAGE indicated the presence of one subunit (54 kDa) and a minor protein band at 67 kDa, which presumably is a contaminant since it was not immunoreactive on Western blotting. The pH optimum of the brain enzyme and its apparent K_m for the synthetic substrate 4-methylumbelliferyl α -L-fucopyranoside were 5.5 and 0.07 mM respectively. Pellet-associated human brain and liver α -L-fucosidases were both capable of hydrolysing fucosyl-G_{M1} ganglioside without activator proteins or detergents. Linear hydrolysis rates were found only for short incubation times (1–5 min). Optimal enzymic activity at 37 °C was found at pH 3.4 for both α -L-fucosidases, with no activity at pH values above 4.0.

INTRODUCTION

Lysosomal glycosidases are involved in the hydrolysis of a diverse array of hydrophilic and hydrophobic glycoconjugates. Hydrolysis of the more hydrophobic substrates (i.e. glycolipids) sometimes requires the presence of activator proteins and/or detergents (Sandhoff, 1984). α -L-Fucosidase (EC 3.2.1.51) is a widely distributed glycosidase that is involved in the hydrolysis of numerous fucoglycoconjugates, including oligosaccharides, glycopeptides, glycoproteins and glycolipids. These compounds contain fucose in various α -glycosidic linkages, primarily to galactose [α -(1→2), α -(1→3) and α -(1→6)] and *N*-acetylglucosamine [α -(1→3), α -(1→4) and α -(1→6)] (Flowers, 1981). The existence of so many different linkages in both hydrophilic and hydrophobic substrates suggests that more than one mammalian α -L-fucosidase exists. The importance of α -L-fucosidase in mammalian biochemistry is evidenced by deficiency of its activity leading to the genetic disease fucosidosis in humans (Van Hoof, 1973) and in English springer spaniels (Hartley *et al.*, 1982).

Brain tissues contain relatively high concentrations of fucoglycoconjugates (Gurd, 1979), including the fucogangliosides (Ghidoni *et al.*, 1976; Ando, 1983). The presence of these lipophilic compounds suggests that brain may contain a membrane-bound α -L-fucosidase involved in their degradation. In a previous investigation (Hopfer & Alhadeff, 1985), a pellet-associated α -L-fucosidase from human brain was solubilized, characterized with the use of the synthetic substrate 4-methylumbelliferyl α -L-fucopyranoside and found to be quite similar to soluble human brain α -L-fucosidase (Alhadeff & Janow-

sky, 1977). Since only crude enzyme preparations and synthetic substrate were employed in our previous study on pellet-associated brain α -L-fucosidase (Hopfer & Alhadeff, 1985), an α -L-fucosidase active only on natural substrates could have been overlooked.

In the present investigation, solubilized pellet-associated human brain α -L-fucosidase was purified for the first time by the use of affinity chromatography (Alhadeff *et al.*, 1975). The highly purified brain fucosidase was characterized and compared with purified human liver α -L-fucosidase with regard to subunit composition, isoelectric-focusing pattern and ability to hydrolyse fucosyl-G_{M1} ganglioside. Both purified fucosidases were able to hydrolyse fucosyl-G_{M1} ganglioside in the absence of activator proteins and detergents, with optimal activity at pH 3.4 and no activity at pH values above 4. This is the first report of the interactions of human fucosidase with a fucoganglioside substrate.

MATERIALS AND METHODS

General

All procedures involving enzymes were carried out at 2–4 °C unless otherwise specified. Protein was determined by the method of Lowry *et al.* (1951), with human serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO, U.S.A.) as standard. Human adult brains and livers were obtained at autopsy from individuals whose tissues appeared normal upon gross pathological examination and were stored at –20 °C until used. The procedures that involved human tissues were approved by an institutional Committee on Investigations/Activities Involving Human Subjects.

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Extraction and purification of pellet-associated human brain α -L-fucosidase

Normal human brain tissue (729 g) was cut into small pieces, rinsed with 10 mM- $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer, pH 5.5, containing 0.02% NaN_3 (phosphate buffer) and homogenized (1:5, w/v) with phosphate buffer in a Waring blender for 30 s at low speed followed by 30 s at high speed. The homogenate was centrifuged at 25 500 g for 45 min, and the resulting pellet was washed twice by resuspension in phosphate buffer with two 30 s passes of a Potter-Elvehjem rotating pestle separated by 20 s cooling intervals. Resuspension was followed by re-centrifugation at 25 500 g for 45 min. Solubilization of human brain pellet-associated α -L-fucosidase was carried out as previously described (Hopfer & Alhadeff, 1985). The twice-washed pellet was resuspended in phosphate buffer containing 0.5% (w/v) Triton X-100, homogenized by use of the Potter-Elvehjem tissue grinder as described above and centrifuged at 25 000 g for 45 min. The resulting pellet was re-extracted with 0.5% Triton X-100 as above, and the supernatant fluids from the two extractions were assayed for α -L-fucosidase activity, combined and used for purification purposes.

Affinity-chromatographic purification of pellet-associated brain α -L-fucosidase activity was accomplished as described by Alhadeff *et al.* (1975). A column (1.7 cm $\times$ 15 cm) of agarose-6-aminohexanoyl-fucosamine resin was equilibrated with phosphate buffer containing 0.5% Triton X-100. The combined supernatant fluid preparation (1870 ml) from the Triton X-100 extractions containing solubilized α -L-fucosidase activity was applied to the column at a flow rate of 0.5 ml/min, and the column was washed by elution with approx. 880 ml of phosphate buffer containing 0.5% Triton X-100. In order to remove Triton X-100 from the preparation, the column was finally washed with approx. 500 ml of phosphate buffer. When the absorbance (0.002) at 280 nm of the buffer being applied to the column was equal to that emerging from the column, washing was stopped and α -L-fucosidase was eluted from the column by means of 50 mM-L-fucose (Sigma Chemical Co.) in phosphate buffer (Alhadeff *et al.*, 1975). Fractions (approx. 3.7 ml) were collected and assayed for α -L-fucosidase activity with *p*-nitrophenyl α -L-fucopyranoside as previously described (Alhadeff *et al.*, 1975), and the fractions (8–32) containing α -L-fucosidase activity were pooled and concentrated in a 50 ml Amicon ultrafiltration apparatus with a YM-10 membrane (Amicon, Danvers, MA, U.S.A.). Final concentration of the preparation (to 285 μ l, 0.8 mg of protein/ml) was accomplished by using Centricon 10 micro-concentrators (Amicon). Purified pellet-associated human brain α -L-fucosidase was stored in a 400 μ l micro-centrifuge tube (Cole-Parmer Instrument Co, Chicago, IL, U.S.A.) at 2–4 °C.

Purification of human liver α -L-fucosidase

α -L-Fucosidase from human liver was purified to apparent homogeneity (as assessed by SDS/PAGE) by affinity chromatography on agarose-6-aminohexanoyl-fucosamine resin as described previously (Alhadeff *et al.*, 1975).

Preparation of fucosyl- G_{M1} and G_{M1} gangliosides

G_{M1} and fucosyl- G_{M1} gangliosides were extracted and

purified from bovine brain and pig brain respectively as described by Tettamanti *et al.* (1973). Identification, structural analysis and purity were assessed as described by Sonnino *et al.* (1978). The final purity of both gangliosides was over 99%, as determined by t.l.c. with increasing amounts of gangliosides followed by densitometric scanning of the plate.

Fluorimetric and fucosyl- G_{M1} substrate assays

Characterization studies on purified pellet-associated brain α -L-fucosidase were accomplished with 1 mM-4-methylumbelliferyl α -L-fucopyranoside (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) according to the assay method previously reported (Alhadeff & O'Brien, 1977). One unit of activity is the amount of enzyme needed to hydrolyse 1 nmol of 4-methylumbelliferyl fucoside/min at 37 °C.

Fucosyl- G_{M1} ganglioside [2.3–82.8 μ g (1.4–49 nmol); 9.2 μ g (5.4 nmol) routinely] was put into 250 μ l micro-centrifuge tubes (Cole-Parmer), freeze-dried and adjusted to 10 μ l with 100 mM-citric acid/sodium phosphate buffer of appropriate pH (2.6–6.2, 3.4 optimum). The concentrations of fucosyl- G_{M1} and G_{M1} gangliosides in distilled deionized water were calculated on the basis of bound sialic acid determined by the resorcinol method of Jourdan *et al.* (1971) against a standard curve of *N*-acetylneuraminic acid (Kantoishi Pharmaceuticals, Tokyo, Japan). The fucosyl- G_{M1} ganglioside solution was left at room temperature overnight to assure formation of stable micelles (Masserini *et al.*, 1985). L-Fucose, residual from the purification, was removed by dialysing α -L-fucosidase (25–250 μ l) in a collodion bag (2 ml capacity; Schleicher and Schuell, Keene, NH, U.S.A.) against a total of 4 litres of 10 mM-sodium phosphate buffer for 4 h immediately before assay. α -L-Fucosidase (60–180 units) was added to each reaction mixture, and enough 10 mM-phosphate buffer was added so that the total volume of each reaction mixture was 18 μ l. The reaction mixtures were incubated at 37 °C for 1–120 min (5 min routinely). Reactions were terminated by freezing in liquid N_2 , the frozen mixtures were freeze-dried and the products were subjected to high-performance t.l.c. analysis.

The amount of G_{M1} ganglioside produced from the hydrolysis of fucosyl- G_{M1} ganglioside by human brain and liver α -L-fucosidases was quantified by a t.l.c. method similar to that described by Ando *et al.* (1978). Each freeze-dried reaction mixture received 15 μ l of methanol and was vortex-mixed vigorously. The entire 15 μ l extract was applied to the pre-adsorbent area of a 10 cm $\times$ 20 cm Whatman LHP-K high-performance t.l.c. plate coated with a 200 μ m layer of silica (Whatman, Clifton, NJ, U.S.A.). Each reaction vial was rinsed with 10 μ l of methanol, and the rinse was also applied to the t.l.c. plate. Each reaction mixture was streaked over a 4–5 mm segment at the origin. Before application of the sample, the plates were pre-run in methylene dichloride/methanol (1:1, v/v) and then activated at 100 °C for 1 h. After application of the sample the plates were developed in chloroform/methanol/0.02% (w/v) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (11:8:2, by vol.) until the solvent was 5 mm from the top of the plate (approx. 25 min). Plates were dried under a stream of warm air for 10 min, and gangliosides were detected with a fine mist of Ehrlich reagent (Sigma Chemical Co.) (Partridge, 1948). A clean glass plate (10 cm $\times$ 20 cm) was quickly placed over the silica of the

sprayed plate and was secured with metal clamps to avoid evaporation of the Ehrlich reagent solvent (acidified methanol). When the plate was heated at 100 °C for 20 min, gangliosides appeared as purple bands. G_{M1} ganglioside typically ran 2–3 mm ahead of the fucosyl- G_{M1} ganglioside on the high-performance t.l.c. plates.

High-performance t.l.c. plates were scanned with an LKB Ultrosan XL laser densitometer, which scans with a single beam of monochromatic light at 633 nm. Lanes were routinely scanned starting 5 mm below the lowest point of the fucosyl- G_{M1} ganglioside band to 5 mm above the highest point of the G_{M1} ganglioside band. Bands that co-migrated with authentic G_{M1} ganglioside were quantified by constructing a standard curve of known amounts of authentic G_{M1} ganglioside versus area units (Chigorno *et al.*, 1982). Preliminary studies indicated that the linear range of detection of authentic G_{M1} ganglioside was 0.2–10 μ g (results not shown).

Isoelectric focusing

Isoelectric focusing of purified pellet-associated brain α -L-fucosidase was accomplished as previously described for crude preparations of pellet-associated human brain α -L-fucosidase (Hopfer & Alhadeff, 1985). α -L-Fucosidase activity (6.4 units) was loaded on an LKB 8100 (110 ml) Ampholine column with Ampholytes (pH 5–8; 2%, v/v) (LKB Produkter, Stockholm, Sweden) in a 0–67% (w/v) sucrose gradient. Electrofocusing was conducted at a starting current of 4.5 mA (and 400 V) for 87.5 h, after which 0.5 ml fractions were collected. The pH value of each fraction was determined, and 100 μ l samples of each fraction with a pH value between 3.8 and 8.2 were assayed for α -L-fucosidase activity with 4-methylumbelliferyl fucoside as substrate.

Characterization studies

The stability of purified pellet-associated brain α -L-fucosidase activity at 2–4 °C was determined by comparing the activity after purification with the activity after storage for 27 days.

The pH-activity curve of purified pellet-associated human brain α -L-fucosidase activity (0.3 unit) was determined with 1 mM-4-methylumbelliferyl fucoside in 0.25 M buffers of various pH values (citric acid/sodium citrate for pH 2.8–6.4; $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ for pH 6.6–8.0) and including human serum albumin (1.1 mg/ml, final concentration) to stabilize the purified enzyme. The actual pH values of duplicate blanks were recorded and assays were done in duplicate at 37 °C for 10 min.

The apparent Michaelis constant (K_m value) was determined for purified pellet-associated brain α -L-fucosidase with 4-methylumbelliferyl fucoside at various final concentrations (0.007–0.5 mM) in 0.25 M- $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer, pH 5.5, from Lineweaver–Burk (1934) double-reciprocal plots. Assays were done in duplicate for 5 min at 37 °C.

The pH-activity curves of purified human liver α -L-fucosidase (180 units) and purified pellet-associated brain α -L-fucosidase (60 units) were determined for fucosyl- G_{M1} ganglioside [9.2 μ g (5.4 nmol)/assay] as described above in 0.1 M-citrate/sodium phosphate buffers at various pH values (2.6–6.2). The assays were run at 37 °C for 5 min (liver) or 6 min (brain).

SDS/PAGE

Slab PAGE was used, according to the method of

Laemmli (1970), to determine the purity and subunit composition of purified pellet-associated human brain α -L-fucosidase and purified liver α -L-fucosidase. Each enzyme (7 μ g) was incubated at 100 °C for 5 min in the presence of 1.5% (w/v) SDS, 3% (v/v) 2-mercaptoethanol, 93.7 mM-Tris/HCl buffer, pH 6.8, 7.5% (v/v) glycerol and 0.0009% Bromophenol Blue. The protein samples were loaded on 4% polyacrylamide stacking gels with 12% polyacrylamide separating gels, each containing 0.1% SDS. Electrophoresis was at 100 V for 10 h at 4 °C. The gels were stained with 0.1% (w/v) Coomassie Blue R-250 (Bio-Rad Laboratories, Richmond, CA, U.S.A.) prepared in methanol/acetic acid/water (2:3:35, by vol.) and destained with methanol/acetic acid/water (2:3:35 and 4:1:5, by vol.). Bovine serum albumin (67 kDa), γ -globulin (20 and 55 kDa) and ovalbumin (45 kDa) (4–8 μ g; all purchased from Schwarz–Mann, Cambridge, MA, U.S.A.) were used as standards for determination of molecular mass. The relative intensities of the bands were determined with an LKB Ultrosan XL scanning laser densitometer.

Western blotting of human brain pellet-associated α -L-fucosidase

After SDS/PAGE performed as described above, the gel was allowed to equilibrate for 30 min in 25 mM-Tris/192 mM-glycine in 20% (v/v) methanol, pH 8.3. The protein was transferred on to 0.2 μ m-pore-size nitrocellulose (Schleicher and Schuell) at 100 V for 60 min with a Bio-Rad Mini Trans-Blot electrophoretic transfer cell according to the method described in the instruction manual accompanying the unit. The portion of the nitrocellulose containing 0.12 μ g of pellet-associated brain α -L-fucosidase was stained for 5 min with 0.1% (w/v) Amido Black (Allied Chemicals, Morristown, NJ, U.S.A.) in propan-2-ol/acetic acid/water (5:2:13, by vol.) followed by destaining with propan-2-ol/acetic acid/water (5:2:13, by vol.). The remainder of the nitrocellulose, containing 0.06 μ g of human brain α -L-fucosidase, was incubated with 1.0% (w/v) BSA in 10 mM-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl and 0.05% Tween 20 (Tris buffer) for 12 h. The nitrocellulose was incubated with a 1:10⁴ dilution of purified goat anti-(human liver α -L-fucosidase) antibody (Andrews-Smith & Alhadeff, 1982) in the Tris buffer for 30 min. The samples were subsequently washed three times in the Tris buffer (10 min/wash) followed by a 30 min incubation with a 1:5000 dilution of rabbit anti-(goat IgG) antibody coupled to alkaline phosphatase (Jackson Immunoresearch, West Grove, PA, U.S.A.). After three more 10 min washes in the Tris buffer, the nitrocellulose was placed into developing buffer (3.3 mg of Nitro Blue Tetrazolium and 1.7 mg of 5-bromo-4-chloroindol-3-yl phosphate in 10 ml of 0.1 M-Tris/HCl buffer, pH 9.5, containing 0.1 M-NaCl and 5 mM-MgCl₂) for 5 min. The colour development was stopped by immersing the nitrocellulose in water.

RESULTS

Table 1 summarizes the affinity-chromatographic purification of pellet-associated human brain α -L-fucosidase. The resuspended pellet (in the absence of Triton X-100) contained 23% of the total α -L-fucosidase activity found in the homogenate, similar to what was previously found in preliminary fractionation studies (Hopfer & Alhadeff,

Table 1. Purification of pellet-associated human brain α -L-fucosidase

See the Materials and methods section for details.

Fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg of protein)	Purification factor (fold)	Yield (%)
Resuspended pellet (before addition of Triton X-100)	87 500	3616	0.04	1	100
Triton X-100-solubilized enzyme	*	4572	*	*	126
Post-affinity-column eluate	0.23	2656	11 500	289 000	58†

* Not determined owing to Triton X-100 interference with protein determination.
† Yield calculated with respect to the total activity applied to the affinity column.

1985). Solubilization of α -L-fucosidase from the pellet with two 0.5% Triton X-100 extractions resulted in 126% recovery of α -L-fucosidase activity. The single-column affinity-chromatographic purification procedure resulted in a 290 000-fold purification and a 58% yield relative to the total activity applied to the column. The final preparation of purified pellet-associated brain α -L-fucosidase had a specific activity of 11 500 nmol/min per mg of protein. After storage of a sample at 2–4 °C for 27 days the activity was decreased to 80% of the original activity.

The isoelectric-focusing profile of purified pellet-associated brain α -L-fucosidase (Fig. 1) indicated the presence of at least six isoforms occurring between pI 4.1 and 5.3, with most activity associated with the isoforms at pI values of 4.4, 4.6, 4.9 and 5.2.

The affinity-chromatographic purification of human liver α -L-fucosidase resulted in a final specific activity of 19 200 nmol/min per mg of protein and a 75% yield, similar to results obtained for purifications of human liver α -L-fucosidase by the same method (Argade *et al.*, 1988).

SDS/PAGE of pellet-associated human brain α -L-fucosidase (Fig. 2, lane 2) indicated the presence of a major protein band and a minor protein band of higher molecular mass. Standard proteins were subjected to electrophoresis (Fig. 2, lane 1) on the gel and the approximate molecular masses of the protein bands in

Fig. 1. Isoelectric-focusing profile of purified pellet-associated human brain α -L-fucosidase (6.4 units) with 2% pH 5–8 Ampholytes

See the Materials and methods section for details.

Fig. 2. SDS/PAGE on a slab gel of purified pellet-associated human brain α -L-fucosidase

Lane 1, protein standards: A, BSA (67 kDa); B, γ -globulin heavy chain (55 kDa); C, ovalbumin (45 kDa). Lane 2, pellet-associated human brain α -L-fucosidase (7 μ g). Lane 3, human liver α -L-fucosidase (7 μ g). See the Materials and methods section for details.

lane 2 were determined graphically to be 54 and 67 kDa. The minor protein band with a molecular mass of 67 kDa in the brain fucosidase preparation comprised approx. 23% of the total protein in lane 2, as quantified by scanning laser densitometry. SDS/PAGE of the purified human liver α -L-fucosidase indicated the presence of two protein bands (Fig. 2, lane 3) with apparent molecular masses of 51 and 56 kDa.

Western blotting of purified pellet-associated human brain α -L-fucosidase [with polyclonal antibody against human liver α -L-fucosidase, which cross-reacts with pellet-associated brain α -L-fucosidase (Hopfer & Alhadeff, 1985)] indicated that only the 54 kDa band was immunoreactive (Fig. 3).

Hydrolysis of fucosyl- G_{M1} ganglioside by purified liver α -L-fucosidase (150 units, 7.5 μ g of protein) was investigated over a wide range of incubation times at 37 °C and found to be approximately linear from 1 to 5 min. Longer incubation times led to non-linear rates of hydrolysis, levelling off at approx. 30% hydrolysis of fucosyl- G_{M1} .

Fig. 3. Western blot of purified pellet-associated human brain α -L-fucosidase (0.12 μ g) with a 1:10⁴ dilution of the IgG fraction of polyclonal antibodies against human liver α -L-fucosidase (Andrews-Smith & Alhadeff, 1982)

See the Materials and methods section for details.

Fig. 4. pH-activity curves of purified pellet-associated human brain α -L-fucosidase for fucosyl- G_{M1} ganglioside (5.4 nmol/assay) (Δ) (60 units/assay) and 4-methylumbelliferyl fucoside (150 nmol/assay) (O) (0.3 units/assay)

See the Materials and methods section for details.

ganglioside for 30–120 min. Similar results were found for the time course of hydrolysis of fucosyl- G_{M1} ganglioside by purified pellet-associated human brain α -L-fucosidase.

Fig. 4 depicts the pH-activity curves of purified pellet-associated human brain α -L-fucosidase for fucosyl- G_{M1} ganglioside and 4-methylumbelliferyl fucoside. The former curve indicates a narrow acidic pH range (approx. 3.0–4.0) over which pellet-associated α -L-fucosidase is active towards micellar fucosyl- G_{M1} ganglioside with optimal activity at pH 3.4, which is identical with the pH of optimal activity of human liver α -L-fucosidase for fucosyl- G_{M1} ganglioside (Fig. 5). The low pH (3.4) of optimal activity of both brain and liver fucosidases for fucosyl- G_{M1} ganglioside is in sharp contrast with the optimal pH (5.5) of human brain pellet-associated α -L-fucosidase activity for 4-methylumbelliferyl fucoside (Fig. 4). There is essentially no overlap in the pH-activity profiles for fucosyl- G_{M1} ganglioside and 4-methyl-

Fig. 5. pH-activity curve of purified human liver α -L-fucosidase (180 units/assay) for fucosyl- G_{M1} ganglioside (5.4 nmol/assay)

See the Materials and methods section for details.

umbelliferyl fucoside. A K_m value for the 4-methylumbelliferyl fucoside substrate was determined on the purified pellet-associated brain α -L-fucosidase preparation. The Lineweaver–Burk double-reciprocal plot yielded a straight line with an apparent K_m of 0.07 mM.

DISCUSSION

In the present investigation pellet-associated human brain α -L-fucosidase was purified for the first time and partially characterized with respect to a synthetic substrate (4-methylumbelliferyl fucoside) and a natural glycolipid substrate (fucosyl- G_{M1} ganglioside). Isoelectric focusing of the purified brain α -L-fucosidase gave an isoform pattern (number of peaks, relative amounts and pI values) very similar to that obtained for crude preparations of pellet-associated brain α -L-fucosidase (Hopfer & Alhadeff, 1985), indicating that all isoforms were purified by the affinity-chromatographic procedure. The purification procedure resulted in a final specific activity (11 500 units/mg of protein) about 1.5 times the specific activity (7500 units/mg of protein) previously found when soluble human brain α -L-fucosidase was purified by the same affinity-chromatographic procedure (Alhadeff & Janowsky, 1977). SDS/PAGE indicated that the pellet-associated brain α -L-fucosidase appears to contain a single subunit of 54 kDa, which is close to the value (51 kDa) previously found for soluble human brain α -L-fucosidase (Alhadeff & Janowsky, 1977). Western blotting indicated that the minor 67 kDa band in the purified brain fucosidase is probably a contaminating protein, since it was not immunoreactive.

The pellet-associated brain and soluble human liver α -L-fucosidases were both able to hydrolyse fucosyl- G_{M1} ganglioside in the absence of activator proteins and detergents. Preliminary analysis indicated that the kinetics of hydrolysis of fucosyl- G_{M1} ganglioside are quite complex (results not shown) and beyond the scope of the present investigation. Both α -L-fucosidases exhibited pH optima of 3.4 for fucosyl- G_{M1} ganglioside, which is the same as that reported for the hydrolysis of fucosyl- G_{M1} ganglioside by α -L-fucosidase from *Octopus hepatopancreas* (Masserini *et al.*, 1985). This pH of optimal activity is considerably lower than the optimal pH (5.5) found for hydrolysis of 4-methylumbelliferyl fucoside by pellet-associated brain α -L-fucosidase, which

is similar to that (pH 5.2–5.5) previously reported for soluble human brain α -L-fucosidase (Dawson & Tsay, 1977) and somewhat lower than the value (pH 6.0) found for crude pellet-associated brain α -L-fucosidase (Hopfer & Alhadeff, 1985). The low-pH requirement for activity of α -L-fucosidase towards fucosyl- G_{M1} ganglioside may be due to formation of an enzyme–ganglioside complex that then serves as substrate for excess α -L-fucosidase (Masserini *et al.*, 1985). The apparent K_m of the purified pellet-associated brain α -L-fucosidase for 4-methylumbelliferyl fucoside was 0.07 mM, comparable with the values (0.07–0.08 mM) reported earlier for crude preparations of soluble and pellet-associated human brain α -L-fucosidases (Hopfer & Alhadeff, 1985).

The overall results of the present study, and our previous studies on human brain (Alhadeff & Janowsky, 1977; Hopfer & Alhadeff, 1985) and human liver (Alhadeff *et al.*, 1975; Alhadeff & Andrews-Smith, 1979) α -L-fucosidases, indicate that pellet-associated human brain α -L-fucosidase is very similar to the soluble α -L-fucosidases even in its interactions with the natural ganglioside substrate fucosyl- G_{M1} ganglioside. Despite the presence of a wide range of α -fucosyl linkages in diverse classes of fucoglycoconjugates and despite the presence of an unusual pellet-associated α -L-fucosidase in brain, there is still no compelling evidence for an α -L-fucosidase that is distinct and functionally different from soluble forms of the enzyme.

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