Solubilization and characterization of pellet-associated human brain α -L-fucosidase activity

Robert L. HOPFER and Jack A. ALHADEFF*

Department of Chemistry, Lehigh University, Bethlehem, PA 18015, U.S.A.

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The pellet-associated portion of human brain α -L-fucosidase (which represents approx. 20% of the homogenate activity) was solubilized with 0.5% (w/v) Triton X-100, characterized with regard to several properties and compared with the corresponding properties of the soluble supernatant-fluid enzyme in an attempt to find a second α -L-fucosidase in human brain. The solubilized and soluble α -L-fucosidase activities exhibited complete stability after storage at 2–4°C for up to 29 days, comparable thermostability after preincubation at 50°C, comparable apparent K_m values (0.07–0.08 mM) for 4-methylumbelliferyl α -L-fucopyranoside, comparable hydrophobicity, comparable isoelectric-focusing profiles (six major forms, with pI values between 4.5 and 5.8) and comparable immunoprecipitation curves (with the IgG fraction of antisera prepared against human liver α -L-fucosidase). Differences in three properties were found between solubilized and soluble α -L-fucosidase activities: the solubilized activity was less stable to storage at –20°C, had a 0.5-pH-unit neutral shift in its pH optimum (6.0) and had smaller M_r forms after gel filtration on Sephadex G-200. The overall results indicate that the pellet-associated and soluble portions of human brain α -L-fucosidase are quite similar in most of their properties. Thus there is still no compelling evidence for the existence of a second mammalian α -L-fucosidase.

The lysosomal hydrolase α -L-fucosidase (α -L-fucoside fucosylhydrolase, EC 3.2.1.51) is widely distributed in Nature (reviewed in Alhadeff & O'Brien, 1977) and is involved in the degradation of fucose-containing glycoconjugates. The importance of α -L-fucosidase in human biochemistry is evidenced by deficiency of its activity leading to the genetic disease fucosidosis, which involves storage of fucoglycoconjugates in visceral tissues and brain (Van Hoof, 1973). This disease is characterized by neurological involvement, including progressive mental and motor retardation, decorticate rigidity and progressive spasticity (Durand *et al.*, 1969). Natural substrates of α -L-fucosidase include both hydrophilic (e.g. oligosaccharides, glycopeptides) and hydrophobic (glycolipids) compounds with fucose in various α -linkages to galactose, *N*-acetylgalactosamine and *N*-acetylglucosamine (Flowers, 1981). The existence of such diverse substrates suggests that more than one mammalian α -L-fucosidase exists.

Numerous studies have been done on soluble α -L-fucosidases derived from various mammalian and human tissues (Alhadeff & O'Brien, 1977), but few studies have been done on the brain enzyme (see the Results and discussion section). Studies of brain α -L-fucosidase are particularly important because of the relatively high concentration of fucoglycoconjugates found in brain tissue (Gurd, 1979). The presence of such glycolipids as the fucogangliosides (Ando, 1983; Ghidoni *et al.*, 1976) in brain suggests that a membrane-bound α -L-fucosidase may exist which degrades these lipophilic substrates.

In a previous investigation, human brain homogenates were fractionated and the soluble portion (approx. 70% of the recovered activity) of α -L-fucosidase was purified, characterized and found to be very similar kinetically, structurally and immunochemically to human liver α -L-fucosidase (Alhadeff & Janowsky, 1977). In the present investigation the pellet-associated α -L-fucosidase activity derived from human brain homogenates has been solubilized, characterized and its proper-

* To whom correspondence should be addressed.

ties compared with those of the soluble portion of brain α -L-fucosidase. Although some differences were found, the results of our studies indicate that the pellet-associated and soluble portions of human brain α -L-fucosidase are quite similar in their properties. To our knowledge, this is the first report that characterizes the pellet-associated α -L-fucosidase activity of mammalian brain.

Materials and methods

General

All procedures 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 liver were obtained at autopsy from individuals whose tissues appeared normal upon gross pathological examination and were stored frozen (–20°C) until used.

Enzyme assay

α -L-Fucosidase activity was assayed with 4-methylumbelliferyl α -L-fucopyranoside (Koch-Light, Laboratories, Colnbrook, Bucks., U.K.) essentially as previously reported (Alhadeff & O'Brien, 1977). Enzyme samples (20 μ l) were made up to 50 μ l with 0.25M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 5.7, containing 0.02% NaN_3 . Reactions were initiated with 100 μ l of 1.0mM-4-methylumbelliferyl fucopyranoside in 0.25M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 5.7, incubated at 37°C for 30 min and terminated with 1.0 ml of 0.085M-glycine/carbonate buffer, pH 9.8. Enzyme assays were always performed under conditions where α -L-fucosidase activity was linear with the amount of protein (18–38 μ g) and time of incubation (5–30 min). Fluorescence was read on a Turner model 111 fluorometer. A unit of activity is defined as the amount of enzyme that hydrolyses 1 nmol of substrate/min at 37°C.

Tissue preparation

Samples (1.0–5.0 g) of normal human brain and liver tissue were homogenized (1:5, w/v) with 10mM- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 5.5 (phosphate buffer), containing 0.02% NaN_3 in a Potter-Elvehjem glass/Teflon tissue grinder, with four 30 s passes of the rotating pestle separated by 20 s cooling intervals in an ice bath.

Fractionation studies

Human brain and liver homogenates prepared as described above were centrifuged at 48000g for 20 min. The pellet resulting from centrifugation of the brain homogenate was washed twice by resuspension in phosphate buffer, followed by re-

centrifugation at 48000g for 20 min. Supernatant fluids, washes and resuspended pellets were assayed for α -L-fucosidase activity. The twice-washed brain pellets were used as the tissue source for solubilizing pellet-associated α -L-fucosidase activity.

Solubilization studies

Various agents, including Triton X-100, Nonidet P-40, sodium deoxycholate, sodium taurocholate (all from Sigma Chemical Co.) and NaCl were employed in preliminary attempts to solubilize pellet-associated human brain α -L-fucosidase activity. Twice-washed pellets were resuspended with two passes of the Potter-Elvehjem homogenizer in phosphate buffer containing various concentrations (0–5.8%, w/v) of the solubilizing agent and centrifuged at 48000g for 20 min. The resulting pellet was re-extracted with solubilizing agent as above and the supernatant fluids were assayed for α -L-fucosidase activity. In one experiment the Triton X-100 (0.5%, w/v) brain extract was centrifuged at 100000g for 2 h in a Beckman L-5 ultracentrifuge and the resultant supernatant fluid was assayed for α -L-fucosidase activity (to determine if the enzyme had been completely solubilized). The inhibitory or stimulatory effect of Triton X-100 (0–2%, w/v) on α -L-fucosidase activity was determined on human brain homogenate, supernatant fluid and resuspended pellet preparations.

Characterization studies

Stability, kinetic, phase-separation, gel-filtration and immunochemical studies were performed on α -L-fucosidase activity from three human brain preparations: (1) Triton X-100 (0.5%) extracts of twice-washed pellets (Triton X-100-solubilized activity); (2) supernatant fluid prepared after homogenization in phosphate buffer (soluble supernatant-fluid activity); (3) supernatant fluid prepared as in (2) made 0.5% in Triton X-100 (soluble supernatant-fluid activity plus Triton X-100).

The stability of α -L-fucosidase activity after storage at 2–4°C and –20°C for various lengths of time (0–29 days) was determined.

The pH-activity curves were determined in 0.25M 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 the actual pH values of duplicate tubes were recorded. Assays were done in duplicate at 37°C for 30 min.

Apparent Michaelis constants (K_m values) were determined from Lineweaver–Burk (1934) double-reciprocal plots, with 4-methylumbelliferyl α -L-fucopyranoside at various final concentrations (0.007–0.5 mM) in 0.25M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$

buffer, pH 5.7. Assays were done in duplicate for 5 min at 37°C.

Thermostability curves were determined with equivalent amounts of α -L-fucosidase activity (0.03 unit/tube) adjusted to the same protein concentration by the addition of 0.3 mg of exogenous human serum albumin to each tube. After preincubations in duplicate at 50°C for up to 180 min, substrate was added and the samples were assayed for α -L-fucosidase activity for 30 min at 37°C.

Phase-separation studies in Triton X-114 (Sigma Chemical Co.) were performed by the modified procedure of Bordier (1981), as follows: preparations of α -L-fucosidase (1.1–1.6 units) were adjusted to a final Triton X-114 concentration of 1% (w/v) and a final volume of 1.0 ml with phosphate buffer, layered on top of a 0.3 ml cushion of 6.0% (w/v) sucrose in phosphate buffer containing 0.06% Triton X-114, and incubated at 37°C for 3 min until clouding of the solution occurred. This solution was centrifuged at 1000g at room temperature for 3 min, the top aqueous layer was removed, adjusted to 1.0% Triton X-114 and re-applied on the original sucrose cushion (containing the detergent phase). This was reincubated at 37°C for 3 min, re-centrifuged as above and the separated aqueous and detergent phases were assayed for α -L-fucosidase activity. Preliminary experiments indicated that $\geq 94\%$ of the added α -L-fucosidase activity was recovered in the two phases and that 1% Triton X-114 had no inhibitory or stimulatory effect on either soluble or Triton X-100-solubilized α -L-fucosidase activity.

Gel-filtration studies were performed on preparations of brain α -L-fucosidase (1.7–8.1 units) and human liver supernatant-fluid α -L-fucosidase (4.6 units) on a column (1.0 cm $\times$ 80 cm) of Sephadex G-200 (Pharmacia) calibrated with 3 mg of the following standard proteins (from Schwarz–Mann Biochemicals): sperm-whale myoglobin (M_r 17000), ovalbumin (M_r 45000) and human γ -globulin (M_r 160000). The void volume of the column was determined to be 18.0 ml with 2 mg of Blue Dextran (M_r 2000000; Sigma Chemical Co.). All samples were applied in 0.5–1.0 ml of phosphate buffer, and the column was eluted with phosphate buffer at approx. 6 ml/h. The elution volumes were determined by multiplying the average volume per fraction (0.5 ml) by the fraction number of maximal A_{660} (for Blue Dextran), of maximal A_{280} (for the standard proteins) or of maximal enzymic activity (for the α -L-fucosidase preparations). The results of gel filtration were plotted as logarithm of M_r versus elution volume.

Immunoprecipitation was performed with the IgG fraction of antiserum made against purified human liver α -L-fucosidase (Andrews-Smith & Alhadeff, 1982). Equivalent amounts of α -L-

fucosidase activity (0.1 unit/tube) and 0.6 mg of human serum albumin (in 0.1 M-phosphate buffer, pH 5.7) were added to 400 μ l Microfuge tubes (Cole Scientific, Calabasas, CA, U.S.A.). Appropriate amounts of the IgG fraction were added to give 5×10^3 – 5×10^6 -fold final dilutions and the total volume of each microfuge tube was adjusted to 200 μ l with 0.1 M-phosphate buffer, pH 5.7. The contents of the tubes were thoroughly vortex-mixed, incubated at 2–4°C for 17 h, centrifuged at 48000g for 30 min, and duplicate 20 μ l samples of the resulting supernatant fluids were carefully removed and assayed for α -L-fucosidase activity.

Isoelectric focusing

This was performed on soluble supernatant-fluid α -L-fucosidase activity (11 units) and on Triton X-100-solubilized α -L-fucosidase activity (5 units) with an LKB 8100 (110 ml) Ampholine column essentially as previously described (Alhadeff & Janowsky, 1977). Ampholytes (pH 5.8; 2%, v/v) (LKB Produkter, Stockholm, Sweden) and Servalytes (pH 5–8; 2%, v/v) (Serva Fine Biochemicals, Garden City Park, NY, U.S.A.) were used in a 0–67% (w/v) sucrose gradient. Electrofocusing was conducted at starting currents of 3.0–7.0 mA (and 400–600 V) for 65 h, after which 0.5 ml fractions were collected. The pH value of each fraction was determined at 2–4°C with a Beckman Digital pH-meter, and 50 μ l samples of each fraction with a pH value between 3.0 and 10.0 were assayed at 37°C for α -L-fucosidase activity. Recoveries of activity after focusing were $\geq 52\%$ of that applied to the column.

Results and discussion

The few studies directly concerned with mammalian brain α -L-fucosidase have been done on the soluble supernatant-fluid enzyme, which represents 70–80% of the homogenate activity (Alam & Balasubramanian, 1978; Alhadeff & Janowsky, 1977; Dawson & Tsay, 1977; Quarles & Brady, 1970), or on Triton X-100 extracts of whole brain tissue (Bosmann & Hemsworth, 1971). In the present investigation the pellet-associated portion of human brain α -L-fucosidase has been solubilized and characterized for the first time. Preliminary fractionation studies on five human brains indicated that $68.8 \pm 2.3\%$ (mean $\pm$ S.D.) of the recovered activity after homogenization and centrifugation was found in the supernatant fluid. After two buffer washes, the pellet retained $19.0 \pm 2.7\%$ of the recovered activity. This pellet fraction was used in subsequent experiments as the tissue source for solubilizing pellet-associated α -L-fucosidase activity.

Several agents at various concentrations were used in attempts to solubilize pellet-associated α -L-fucosidase activity. Triton X-100, Nonidet P-40 and sodium deoxycholate appeared to have similar solubilizing effects, with 0.5% (w/v) Triton X-100 giving maximal recovery of α -L-fucosidase activity (2.4 times that of the resuspended pellet activity). Sodium taurocholate was effective only at high concentrations (1.0–2.0%, w/v) and NaCl was ineffective even at a concentration of 1.0M. Triton X-100 at 0.5% was used in all subsequent experiments to solubilize pellet-associated α -L-fucosidase activity. Ultracentrifugation of such Triton X-100 extracts (at 100000g for 2h) resulted in supernatant fluids containing $\geq 95\%$ of the recovered α -L-fucosidase activity, suggesting that the enzyme had been truly solubilized.

An investigation of the stimulatory and/or inhibitory effects of various concentrations of Triton X-100 on homogenate, supernatant-fluid and resuspended pellet preparations of brain α -L-fucosidase was undertaken in an attempt to explain the high recoveries of enzymic activity obtained when extracting resuspended pellets with this non-ionic detergent. Slight stimulation ($\leq 20\%$) of homogenate activity, slight inhibition ($\leq 15\%$) of supernatant-fluid activity and 1.5–2.0-fold stimulation of resuspended pellet activity was found at 0.25–1.0% Triton X-100 concentrations. However, the 1.5-fold stimulatory effect found at 0.5% Triton X-100 cannot fully account for the

240% recovery of α -L-fucosidase activity obtained when pellets are extracted with 0.5% Triton X-100. This suggests that cryptic enzyme is being released during solubilization.

Studies were done on the comparative properties of Triton X-100-solubilized and soluble supernatant-fluid brain α -L-fucosidase activities. In addition, the effects of Triton X-100 on the properties of soluble α -L-fucosidase were investigated to determine if any differences in the properties of this enzyme and the Triton X-100-solubilized enzyme might be due to the presence of this non-ionic detergent. Investigations on the stability of enzymic activity after storage for up to 29 days indicated that all three preparations were very stable at 2–4°C and essentially retained full activity (Table 1). However, at –20°C the Triton X-100-solubilized α -L-fucosidase activity was quite unstable, losing approx. 50% of its activity after 2–29 days, whereas the soluble supernatant-fluid α -L-fucosidase essentially retained full activity during this time period. The fact that the solubilized preparation lost approximately half of its α -L-fucosidase activity by the first 2 days and essentially lost no more activity at later time points suggests that the loss of activity is due to a portion of the solubilized α -L-fucosidase being labile to freeze-thawing. This instability of α -L-fucosidase activity in the solubilized preparation is not due to the presence of 0.5% Triton X-100, since soluble supernatant fluid stored at –20°C in the

Table 1. *Summary of properties of Triton X-100-solubilized, soluble supernatant-fluid and soluble supernatant-fluid plus Triton X-100 α -L-fucosidase activities*

Property	0.5% Triton X-100-solubilized α -L-fucosidase	Soluble supernatant-fluid α -L-fucosidase	Soluble supernatant-fluid α -L-fucosidase plus 0.5% Triton X-100
Percentage of initial activity after storage for 29 days at 2–4°C	95%	106%	101%
Percentage of initial activity after storage for 29 days at –20°C	45%	96%	99%
pH optimum	6.0	5.5	5.5
Apparent K_m value for 4-methylumbelliferyl α -L-fucopyranoside (mean $\pm$ S.D. for three trials)	0.07 $\pm$ 0.01 mM	0.08 $\pm$ 0.01 mM	0.07 $\pm$ 0.01 mM
Percentage of initial activity after preincubation for 3h at 50°C	28%	69%	23%
Percentage of initial activity found in Triton X-114 after phase separation (Bordier, 1981) (mean $\pm$ S.D. for three trials)	7.4 $\pm$ 1.4%	3.0 $\pm$ 0.2%	6.5 $\pm$ 1.1%
M_r of forms after gel filtration on Sephadex G-200 (mean $\pm$ S.D. for three trials)	27400 $\pm$ 3900 43800 $\pm$ 3200 93800 $\pm$ 6700	57600 $\pm$ 1300 82900 $\pm$ 4700 116000 $\pm$ 2300	47000 $\pm$ 2400 76400 $\pm$ 2000 109000 $\pm$ 6000
Maximum immunoprecipitation of enzymic activity	91%	91%	91%
Isoelectric-focusing profile	Presence of similar relative amounts of six major forms with pI values between 4.5 and 5.8		

presence of the same concentration of Triton X-100 retained full enzymic activity (99%) after 29 days (Table 1).

Representative pH-activity curves of Triton X-100-solubilized and soluble supernatant-fluid α -L-fucosidase activities are depicted in Figs. 1(a) and 1(b) respectively. The shapes of the curves are similarly broad, but the Triton X-100-solubilized activity has a more neutral pH optimum (6.0) than the soluble supernatant-fluid activity (5.5). Both curves have secondary optima, suggested by shoulders 1 pH unit more neutral than their primary optima. The pH-activity curve of soluble supernatant-fluid α -L-fucosidase in the presence of 0.5% Triton X-100 was identical with that of the soluble supernatant-fluid activity depicted in Fig. 1(b). Our pH optimum (5.5) for the soluble activity is very similar to that (5.2–5.5) previously reported for soluble human brain α -L-fucosidase (Dawson & Tsay, 1977).

Apparent Michaelis constants (K_m values) for 4-methylumbelliferyl α -L-fucopyranoside were deter-

mined for the three brain α -L-fucosidase preparations, and on three separate occasions Lineweaver-Burk double-reciprocal plots gave straight lines with comparable K_m values (0.07–0.08 mM) (see Table 1). Our K_m value is approximately one-fifth of that (0.36 mM) previously reported for soluble human brain α -L-fucosidase (Dawson & Tsay, 1977).

Thermostability studies of α -L-fucosidase activity from the three preparations after preincubation at 50°C for various amounts of time indicated that the Triton X-100-solubilized α -L-fucosidase activity is considerably less thermostable than the soluble supernatant-fluid activity, with approx. 70% and 30% activity losses, respectively, after 3 h preincubation. However, addition of 0.5% Triton X-100 to the soluble supernatant-fluid enzyme preparation resulted in a thermostability curve similar to that of the Triton X-100-solubilized activity, suggesting that the apparent decreased thermostability of the solubilized α -L-fucosidase is due to the presence of Triton X-100.

To investigate the nature of the association of the solubilized α -L-fucosidase with the membranes from which it was released, phase-separation studies with Triton X-114 were used to assess the relative hydrophobicity of the solubilized and soluble supernatant-fluid brain α -L-fucosidases. The results of three separate experiments indicated that an increased amount ($7.4 \pm 1.4\%$; mean $\pm$ s.d.) of the added solubilized enzyme was found in the hydrophobic detergent phase as compared with the amount ($3.0 \pm 0.2\%$) found in this phase for the soluble enzyme. However, addition of 0.5% Triton X-100 to the soluble enzyme preparation increased the amount of enzymic activity found in the detergent phase to $6.5 \pm 1.1\%$. These results suggest that the solubilized enzyme is at most only slightly more hydrophobic than the soluble enzyme, and this, in turn, suggests that the solubilized α -L-fucosidase is not an integral membrane protein (Bordier, 1981).

Since several previous studies have shown that human α -L-fucosidases exist in multiple M_r forms separable by gel filtration (Robinson & Thorpe, 1973; Wiederschain *et al.*, 1973; Chester *et al.*, 1977), gel filtration on Sephadex G-200 was used to investigate if these forms are present in the soluble and Triton X-100-solubilized brain α -L-fucosidase preparations. The results of three separate experiments indicated that soluble supernatant-fluid brain α -L-fucosidase consists of roughly comparable amounts of three enzymic forms, with approximate M_r values (means $\pm$ s.d.) of 57600 ± 1300 , 82900 ± 4700 and 116000 ± 2300 , whereas the solubilized brain α -L-fucosidase consists of roughly comparable amounts of three forms with approximate M_r values of 27400 ± 3900 , 43800 ± 3200 and

Fig. 1. pH-activity curves of 0.5% Triton X-100-solubilized (a) and soluble supernatant-fluid (b) human brain α -L-fucosidase activities

See the Materials and methods section for details.

93800 $\pm$ 6700. These gel-filtration differences are not due to the presence of Triton X-100 in the solubilized preparation of α -L-fucosidase, since gel filtration of soluble α -L-fucosidase in the presence of 0.5% Triton X-100 gave results similar to those for soluble α -L-fucosidase (see Table 1). It is possible that the different pattern of M_r forms of soluble and solubilized brain α -L-fucosidases accounts for their observed pH-optimum differences (Fig. 1), since previous studies have shown that the forms of α -L-fucosidase separable by gel filtration have different pH-activity curves (Robinson & Thorpe, 1973; Wiederschain *et al.*, 1973). Not only were the gel-filtration profiles of the soluble and solubilized brain α -L-fucosidases different from each other, but both were different from the profile obtained for human liver supernatant-fluid α -L-fucosidase, which gave roughly equal amounts of three major forms with approximate M_r values of 63800, 117000 and 216000, possibly corresponding to monomeric, dimeric and tetrameric aggregated forms of the enzyme respectively.

Immunochemical studies of Triton X-100-solubilized and soluble supernatant-fluid brain α -L-fucosidases with the antibody previously prepared against purified human liver α -L-fucosidase resulted in comparable immunoprecipitation curves (Fig. 2) when the percentage of initial supernatant-fluid activity remaining is plotted versus antibody dilution. For both enzyme preparations, maximum immunoprecipitation (approx. 90%) occurred on two separate occasions at the highest antibody concentration. The addition of 0.5% Triton X-100 to the soluble supernatant fluid did not change the α -L-fucosidase immunoprecipitation curve (curve not shown). Our finding that human brain α -L-fucosidase is antigenically very similar to the

Fig. 2. Immunoprecipitation curves of 0.5% Triton X-100-solubilized (●) and soluble supernatant-fluid (○) human brain α -L-fucosidase activities

See the Materials and methods section for details.

human liver enzyme is unlike the finding by Alam & Balasubramanian (1978) that α -L-fucosidase from monkey brain has only partial immunological identity with the enzyme from non-neural monkey tissues.

The isoelectric-focusing profiles of Triton X-100-solubilized and soluble supernatant-fluid α -L-fucosidase activity are shown in Figs. 3(a) and 3(b) respectively. These profiles are similar with regard to the number, interpeak distances and relative amounts of the six isoelectric forms present. The small shift (0.2 pI unit) between the two profiles may be due to the presence of Triton X-100 in the solubilized preparation. However, this is unlikely, since the small shift is within the limits (0.2–0.3 pI unit) of the technique as evidenced by repeated focusing of the same enzyme preparation. The brain α -L-fucosidase isoelectric-focusing profiles are very similar to those previously found for α -L-fucosidase from human liver, placenta and cultured fibroblasts (Alhadeff & O'Brien, 1977).

The Triton X-100-solubilized brain α -L-fucosidase activity was very similar to, if not identical with, the soluble supernatant-fluid brain activity for most properties investigated: stability after storage at 2–4°C, apparent K_m values for the 4-methylumbelliferyl substrate, hydrophobicity, isoelectric-focusing profile and ability to be recognized and immunoprecipitated by the IgG fraction of antiserum prepared against purified human liver α -L-fucosidase (see summary of properties in Table 1). On the other hand, differences in three

Fig. 3. Isoelectric-focusing profiles of 0.5% Triton X-100-solubilized (a) and soluble supernatant-fluid (b) human brain α -L-fucosidase activities

See the Materials and methods section for details.

properties were found: the solubilized activity was less stable to storage at -20°C (presumably owing to greater lability to freeze-thawing), had a 0.5-pH-unit neutral shift in its pH optimum, and the solubilized activity had smaller M_r forms after gel filtration.

The overall results of our studies indicate that the pellet-associated and soluble portions of human brain α -L-fucosidase activities are quite similar in most of their properties. Thus there is still no compelling evidence for the existence of a second mammalian α -L-fucosidase that is significantly different from the well-characterized soluble enzyme. However, the results of our study and most previous studies must be interpreted with caution, since they have been done primarily, if not exclusively, with synthetic substrates, and an α -L-fucosidase with little or no activity towards these substrates but with activity towards natural substrate(s) (e.g. fucoglycolipids) could have been overlooked. The possibility of detecting such an α -L-fucosidase is presently limited because of lack of ready availability of the appropriate fucoglyco-conjugate substrates.

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References

- Alam, T. & Balasubramanian, A. S. (1978) *Biochim. Biophys. Acta* **524**, 373–384
- Alhadeff, J. A. & Janowsky, A. J. (1977) *J. Neurochem.* **28**, 423–427
- Alhadeff, J. A. & O'Brien, J. S. (1977) in *Practical Enzymology of the Sphingolipidoses* (Glew, R. H. & Peters, S. P., eds.), pp. 247–281, Alan R. Liss, New York
- Ando, S. (1983) *Neurochem. Int.* **5**, 507–537
- Andrews-Smith, G. & Alhadeff, J. A. (1982) *Biochim. Biophys. Acta* **715**, 90–96
- Bordier, C. (1981) *J. Biol. Chem.* **256**, 1604–1607
- Bosmann, H. B. & Hemsworth, B. A. (1971) *Biochim. Biophys. Acta* **242**, 152–171
- Chester, M. A., Hultberg, B. & Sjoblad, S. (1977) *Biochim. Biophys. Acta* **485**, 147–155
- Dawson, G. & Tsay, G. (1977) *Arch. Biochem. Biophys.* **184**, 12–23
- Durand, P., Borrone, C. & Della Cella, G. (1969) *J. Pediatr.* **75**, 665–674
- Flowers, H. M. (1981) *Adv. Carbohydr. Chem. Biochem.* **39**, 279–345
- Ghidoni, R., Sonnino, S., Tettamanti, G., Wiegandt, H. & Zambotti, V. (1976) *J. Neurochem.* **27**, 511–515
- Gurd, J. W. (1979) *Biochim. Biophys. Acta* **555**, 221–229
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Quarles, R. H. & Brady, R. O. (1970) *J. Neurochem.* **17**, 801–807
- Robinson, D. & Thorpe, R. (1973) *Clin. Chim. Acta* **47**, 403–407
- Van Hoof, F. (1973) in *Lysosomes and Storage Diseases* (Hers, H. G. & Van Hoof, F., eds.), pp. 277–290, Academic Press, New York
- Wiederschain, G. Y., Kolibaba, L. G. & Rosenfeld, E. L. (1973) *Clin. Chim. Acta* **46**, 305–310