Purification and Characterization of α -L-Fucosidase from the Liver of a Fucosidosis Patient

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α -L-Fucosidase was partially purified from the liver of a fucosidosis patient by column chromatography either on agarose- ϵ -aminohexanoylfucosamine or on concanavalin A-Sepharose, despite no apparent enzymic activity in the crude liver supernatant. Mixing studies indicated that the liver of the fucosidosis patient did not lack activators or contain inhibitors of α -L-fucosidase activity. The partially purified α -L-fucosidase from the liver of the fucosidosis patient exhibits a 4–5-fold-increased Michaelis constant for the 4-methylumbelliferyl substrate (700–750 μ M) and a greatly decreased thermostability at 55°C compared with the normal liver enzyme. The pH-activity curve is similar to that for the normal enzyme between pH 5 and 8, but quite dissimilar in the acid region (pH 3.0–4.5): below pH 4.5 the α -L-fucosidase shows no activity, whereas the normal enzyme retains considerable activity ($\geq 50\%$ of maximal activity). Isoelectric focusing of the α -L-fucosidase revealed one major form with pI 5.8 and other possible minor forms. No cross-reacting material was detected when the α -L-fucosidase was run in double-immunodiffusion experiments against the immunoglobulin-G fraction of anti-(α -L-fucosidase) antibodies, but the enzyme was immunoprecipitated by this immunoglobulin-G fraction. For at least the fucosidosis patient being studied here, all the data suggest retention of a thermolabile portion of normal α -L-fucosidase (with characteristic Michaelis constant and pH-activity curve) or production of a kinetically altered α -L-fucosidase with decreased catalytic activity but antigenic similarity to the normal enzyme.

Little is known about the precise nature of the molecular defect in the neurovisceral storage disease fucosidosis (Durand *et al.*, 1966) other than that the activity of the lysosomal hydrolase α -L-fucosidase (EC 3.2.1.51) is absent or grossly deficient (Van Hoof & Hers, 1968). There are at least five possibilities to explain the deficiency of α -L-fucosidase activity in fucosidosis: (a) α -L-fucosidase is normal, but its activity is deficient owing to the lack of activators or presence of inhibitors; (b) α -L-fucosidase is not synthesized, or is synthesized in smaller amounts, secondary to a regulatory defect; (c) α -L-fucosidase is synthesized normally, but is rapidly degraded; (d) only a selective portion of normal α -L-fucosidase is retained in fucosidosis; (e) α -L-fucosidase is synthesized as a structurally altered mutant with decreased catalytic activity.

In order to decide between the above possibilities, α -L-fucosidase from the liver of a fucosidosis patient

has been partially purified by affinity chromatography on agarose- ϵ -aminohexanoylfucosamine and by chromatography on the lectin column concanavalin A-Sepharose. Kinetic and immunochemical characterization of this enzyme gives evidence that, at least for the fucosidosis patient studied here, either a selective thermolabile portion of α -L-fucosidase is being retained or an altered α -L-fucosidase is being produced with decreased catalytic activity but antigenic similarity to the normal enzyme.

Experimental

General

All procedures were carried out at 0–4°C unless otherwise stated. Agarose- ϵ -aminohexanoylfucosamine (Miles-Yeda, Elkhart, IN, U.S.A.) and concanavalin A-Sepharose 4B (Pharmacia) were used in the partial purification of α -L-fucosidase from the liver of a fucosidosis patient. α -L-Fuco-

Abbreviation used: IgG, immunoglobulin G.

sidase was assayed at pH 5.0 by using either 3 mM-*p*-nitrophenyl α -L-fucopyranoside (Sigma Chemical Co., St. Louis, MO, U.S.A.) or 1 mM-4-methylumbelliferyl α -L-fucopyranoside (Koch-Light, Colnbrook, Bucks., U.K.) as previously described (Alhadeff & O'Brien, 1977). A unit of α -L-fucosidase activity is defined as the amount of enzyme necessary to hydrolyse 1 nmol of substrate/min at 37°C (this corresponds to a milliunit of the International Enzyme Unit System). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Enzyme solutions were concentrated by vacuum dialysis in a collodion-bag apparatus with 25 000-mol.wt.-retention collodion bags (Schleicher and Schuell, Keene, NH, U.S.A.) or by ultrafiltration using Amicon (Amicon Corp., Lexington, MA, U.S.A.) concentrators with UM-10 Diaflo membranes at 350–480 kPa (50–70 lb/in²).

Tissues

Human livers were obtained at autopsy from a patient who died with the typical clinical and biochemical picture of fucosidosis (Alhadeff & O'Brien, 1977) and from controls whose livers appeared normal on gross pathological examination. These livers were stored frozen (–20°C) until used. Immediately before use, control liver tissue was homogenized in a Potter–Elvehjem apparatus in 4 vol. (w/v) of 10 mM-NaH₂PO₄, pH 5.6, containing 0.02% (w/v) NaN₃. The homogenates were centrifuged for 30 min at 16 300 g and the resulting supernatants were used for characterizing crude α -L-fucosidase. Purified human liver α -L-fucosidases from previous studies (Alhadeff & Freeze, 1977; Alhadeff *et al.*, 1978b) were also used for the comparative kinetic, mixing and immunochemical studies. The procedures that involved human tissues were approved by the Committee on Investigations/Activities Involving Human Subjects of the School of Medicine, University of California, San Diego.

Purification of α -L-fucosidase from the liver of a fucosidosis patient

Liver (100 g wet wt.) from a fucosidosis patient was cut into small pieces and homogenized in 400 ml of ice-cold 10 mM-NaH₂PO₄, pH 5.6, containing 0.02% NaN₃ in a cold Waring blender for three 30 s intervals at high speed. A sample of the homogenate was assayed for α -L-fucosidase activity and the remainder centrifuged for 30 min at 16 300 g. The resulting supernatant was assayed for α -L-fucosidase activity, and a portion (1 ml) was kept for the mixing studies (see below). The remaining supernatant (380 ml) was divided into two equal portions and applied separately at 2–4°C to columns of the

affinity resin agarose- ϵ -aminohexanoylfucosamine (1 cm $\times$ 52 cm) and concanavalin A-Sepharose (1 cm $\times$ 54.5 cm). The affinity column was washed with 35 column vol. of 10 mM-NaH₂PO₄, pH 5.6, containing 0.02% NaN₃ and eluted sequentially at room temperature with (a) 100 mM-L-fucose (Sigma) and (b) 100 mM-L-fucose + 0.25 M-NaCl in the phosphate buffer as previously described (Alhadeff & Freeze, 1977). The latter eluting buffer (b) was included to increase the yield, since previous studies have shown that a small percentage of the α -L-fucosidase comes off the affinity column at higher ionic strength (Alhadeff *et al.*, 1975). This salt eluate has lower specific activity than, but properties comparable with, the eluate obtained by using only L-fucose (a). The two eluates, 150 ml and 90 ml for (a) and (b) respectively, were concentrated by vacuum dialysis to 300 and 500 μ l respectively. These two fractions were assayed for α -L-fucosidase activity, and thermostability curves were obtained for the activity from eluate (a) and a Michaelis constant (K_m) was determined for the activity from eluate (b).

The concanavalin A-Sepharose column was washed with 21 column vol. of 10 mM-NaH₂PO₄, pH 5.6, containing 0.02% NaN₃ and the column was eluted at room temperature with 530 ml of the above phosphate buffer containing 0.75 M- α -methyl mannoside (Sigma; grade II). The eluate was concentrated to 1.5 ml by ultrafiltration and vacuum dialysis, assayed for α -L-fucosidase activity, and this activity was used for pH-optimum, thermostability, K_m and immunoprecipitation studies.

Mixing studies

Various amounts (10–30 μ l) of liver supernatant from a fucosidosis patient were mixed with either crude α -L-fucosidase from a normal-liver supernatant or purified α -L-fucosidase from a normal liver, adjusted to a total constant volume (50 μ l) with 10 mM-NaH₂PO₄, pH 5.6, and assayed in duplicate with the 4-methylumbelliferyl substrate for 30 min at 37°C.

Kinetic studies

Apparent Michaelis constants (K_m values) were determined (for the partially purified α -L-fucosidase and for purified and crude normal-liver α -L-fucosidase) graphically by the Lineweaver–Burk (1934) double-reciprocal method by using the 4-methylumbelliferyl substrate. Samples of enzyme (which had been dialysed against phosphate buffer to remove L-fucose) were incubated with various concentrations (0.006–0.66 mM) of substrate in 0.1 M-citric acid/sodium citrate buffer, pH 5.0, in duplicate for 30 min. Thermostability studies were performed on partially purified α -L-fucosidase from

fucosidosis- and normal-liver supernatant with the 4-methylumbelliferyl substrate. Enzyme samples (adjusted to the same protein concentration) were preincubated at 55°C for up to 60 min, the substrate was added, and the samples were assayed in duplicate at 37°C for 30 min. The pH-activity profiles were determined on partially purified α -L-fucosidases from fucosidosis- and normal-liver supernatant with the 4-methylumbelliferyl substrate in 0.1M buffers of various pH values (citric acid/sodium citrate for pH 3.1–5.8; $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ for pH 6.0–8.1).

Isoelectric focusing

Liver (17g) from a fucosidosis patient was homogenized in 68 ml of 10mM- NaH_2PO_4 , pH 5.6, containing 0.02% NaN_3 , centrifuged for 30 min at 16300g, and the resulting supernatant was concentrated 4-fold by ultrafiltration to 16 ml. This concentrated supernatant, which displayed no apparent α -L-fucosidase activity, and normal-liver supernatant α -L-fucosidase (0.2 ml and 11 units), were subjected to isoelectric focusing in a 110 ml column as previously described (Alhadeff *et al.*, 1975, 1978b); 1% ampholytes (pH 5–8, LKB Produkter) were used in a gradient of 0–67% (w/v) sucrose. Electrofocusing was conducted at a starting current of 2.3–2.5 mA and 400 V for 55–88 h, after which 0.3 ml fractions were collected. The pH values of the fractions were determined and 50 μ l portions of each fraction were assayed for 30 min with the 4-methylumbelliferyl substrate. Fluorescence was read on a Turner Fluorometer. The α -L-fucosidase activities from the liver of the fucosidosis patient were read at 3–4 times the sensitivity for the normal liver enzyme.

Immunochemical studies

The IgG fraction of antibodies made against purified human liver α -L-fucosidase in a previous

study (Alhadeff *et al.*, 1975) was used for the immunochemical studies. Comparable amounts of α -L-fucosidase activity from fucosidosis and normal livers were separately preincubated with various amounts (0–70 μ l) of anti-(liver α -L-fucosidase) IgG in 400 μ l conical plastic centrifuge tubes (Cole Scientific, Calabasas, CA, U.S.A.) for 20 h at 2–4°C. The preincubated samples were centrifuged at 48000g for 30 min and the resulting supernatants were assayed in duplicate for α -L-fucosidase activity. The IgG fraction (8 μ l) was also used in double-immunodiffusion experiments on Hyland Immunoplates (Travenol Laboratories, Costa Mesa, CA, U.S.A.) against a concentrated sample (96 μ l) of the fucosidosis-liver α -L-fucosidase which had been partially purified by affinity chromatography. The plates were spotted with samples, placed at 0–4°C and studied by visual inspection after 2–3 days.

Results

Repeated assay of the liver from a fucosidosis patient revealed no α -L-fucosidase activity with either the 4-methylumbelliferyl or the *p*-nitrophenyl substrates. Nevertheless, crude supernatant from the patient's liver was applied independently to columns of agarose- ϵ -aminohehexanoylfucosamine and concanavalin A-Sepharose. Both columns were washed extensively with phosphate buffer and eluted batchwise with 100 mM-L-fucose or 0.75 M- α -methyl mannoside respectively. Small amounts of α -L-fucosidase activity (toward both substrates) were found in the concentrated eluates from both columns, 0.2 and 0.8 unit for the affinity and concanavalin A-Sepharose columns respectively. Mixing studies were employed to determine the effect of various amounts of fucosidosis-liver supernatant on the activity of crude and purified normal-liver α -L-fucosidase (Table 1). No evidence was found for

Table 1. *Effect of various amounts of fucosidosis-liver supernatant on crude and purified normal-liver α -L-fucosidase activity*

Full details are given in the Experimental section and discussed in the first paragraph of the Results section.

Fucosidosis-liver supernatant (μ l)	Normal-liver supernatant (μ l)	Purified liver α - L-fucosidase (200-fold dilution) (μ l)	α -L-Fucosidase activity (unit)
0	10	—	0.50
10	10	—	0.52
20	10	—	0.49
30	10	—	0.46
0	—	5	0.54
10	—	5	0.51
20	—	5	0.50
30	—	5	0.50
30	—	—	0.00

lack of activators or the presence of inhibitors in the patient's tissue, even after addition of a large excess (3-fold) of fucosidosis-liver to normal-liver supernatant.

Fig. 1 depicts the pH-activity curves for normal and concanavalin A-Sepharose-purified α -L-fucosidase from the fucosidosis patient's liver. Although both curves are biphasic and quite similar from pH 5.0 to 8.1, they are quite dissimilar in the acid region (pH 3.0–4.5). Whereas the patient's α -L-fucosidase has no activity below pH 4.5, the normal enzyme has considerable activity ($\geq 50\%$ of maximal activity) between pH 3.0 and 4.5. The pH curve

Fig. 1. pH-activity curves of α -L-fucosidase from normal (O) and fucosidosis (●) liver
See the Experimental section for details.

for normal α -L-fucosidase, depicted in Fig. 1, was typical of the three normal livers studied.

Kinetic and immunochemical studies were performed on both preparations of partially purified α -L-fucosidase from the fucosidosis patient. Apparent K_m values for the 4-methylumbelliferyl substrate were determined for crude and purified normal-liver α -L-fucosidase as well as for the fucosidosis-liver enzyme. The Lineweaver-Burk double-reciprocal plots yielded straight lines, giving the apparent K_m values reported in Table 2, which also includes the K_m values from previous studies in our laboratory. The α -L-fucosidase from the fucosidosis patient's liver exhibits an apparent K_m (700–750 μ M) 4–5 times the average values found for purified and crude normal-liver enzymes. Furthermore, this K_m is considerably larger than the highest value (220 μ M) in the range of K_m values found for normal-liver α -L-fucosidases. A control experiment (K_m determined in the presence of 0.75 M- α -methyl mannoside) indicated that the high K_m value was not due to the presence of α -methyl mannoside in the preparation.

Thermostability studies were done on the concanavalin A-Sepharose-purified α -L-fucosidase and on enzyme from three normal control livers under conditions of equivalent total protein concentration. Preincubations were at 55°C for up to 60 min. Fig. 2 indicates that the α -L-fucosidase from the patient's liver is extremely thermolabile, losing 100% of its activity after 10 min preincubation, compared with minimal losses (10–15%) for the three normal-liver α -L-fucosidases. The normal enzyme retains approx. 80% of its initial activity even after 60 min of

Table 2. Michaelis constants of α -L-fucosidase from normal and fucosidosis livers for 4-methylumbelliferyl α -L-fucopyranoside

Full details are explained in the Experimental section and discussed in the third paragraph of the Results section.

Source of α -L-fucosidase	K_m (μ M)	Reference
(I) Controls (normal livers)		
Crude:		
1	140	Present study
2	210	Present study
3	120	Alhadeff <i>et al.</i> (1978a)
4	85	Alhadeff <i>et al.</i> (1974)
Average $\pm$ mean deviation	139 $\pm$ 37	
Purified:		
1	210	Present study
2	58	Present study
3	220	Alhadeff <i>et al.</i> (1975)
Average $\pm$ mean deviation	163 $\pm$ 70	
(II) Fucosidosis liver		
Prepared by affinity column	750 $\pm$ 150*	—
Prepared by concanavalin A-Sepharose column	700 $\pm$ 100*	—

* Average $\pm$ mean deviation of two determinations.

preincubation. The affinity-column-purified α -L-fucosidase (with fucose as eluent) gave a thermostability curve indistinguishable from that depicted in Fig. 2 (for the concanavalin A-Sepharose-purified enzyme).

Fig. 2. Thermostability curves after preincubation at 55°C for α -L-fucosidase from normal and fucosidosis liver

See the Experimental section for details. Open symbols represent three normal-liver α -L-fucosidases, and closed circles represent fucosidosis-liver α -L-fucosidase.

Fig. 3. Isoelectric-focusing profile of human α -L-fucosidase

(a) Normal human liver (11 units of α -L-fucosidase activity); (b) fucosidosis liver (16 ml of 4-fold-concentrated liver supernatant). See the Experimental section for details.

Isoelectric-focusing profiles of normal- and fucosidosis-liver α -L-fucosidase are depicted in Figs. 3(a) and 3(b) respectively. The profile for normal-liver enzyme is typical of many other livers previously studied, with multiple peaks (six or seven), having isoelectric points between pH 5 and 7 (Alhadeff *et al.*, 1975, 1978b). Despite no apparent α -L-fucosidase activity in the crude concentrated supernatant, the profile for the fucosidosis-liver enzyme revealed one major peak of activity with pI 5.8, probably comparable with the peak of pI 5.9 in the normal profile. The fucosidosis-liver enzyme profile also appears to have other minor forms, but their significance is unknown, owing to the higher background readings obtained when using the high sensitivity necessary to detect these small amounts of enzymic activity (approx. 9–10% of that used in the normal profile).

The IgG fraction of antiserum made against purified normal-liver α -L-fucosidase was used in double-immunodiffusion experiments against the partially purified α -L-fucosidase from fucosidosis liver, but no cross-reacting material was found. However, when normal- and fucosidosis-liver α -L-fucosidases were preincubated separately with various amounts of anti-(liver α -L-fucosidase) IgG, both enzymes gave comparable curves, with near-quantitative immunoprecipitation of activity (approx. 90–95%).

Discussion

In the present investigation α -L-fucosidase has been partially purified from liver of a fucosidosis patient. Since no enzymic activity was demonstrable in the crude supernatant, it is difficult to determine the amount of purification resulting from the chromatographic procedures. Mixing studies indicated that this lack of enzymic activity in the crude supernatant was not due to lack of activators or the presence of inhibitors in the liver from the fucosidosis patient, a finding similar to that found for many other fucosidosis patients' tissues (Troost *et al.*, 1976; Patel & Zeman, 1976; DiMatteo *et al.*, 1976; Beratis *et al.*, 1977). Previous studies on an unusual case of fucosidosis suggested that that particular patient's liver may have contained inhibitors to 4-methylumbelliferyl and not *p*-nitrophenyl α -L-fucosidase activity (Alhadeff *et al.*, 1978a).

The purification procedures employed were based on the assumptions that an abnormal α -L-fucosidase, if present, would (1) be recognized by an affinity column for the normal enzyme, possibly with decreased affinity, and/or (2) be a mannose-containing glycoprotein like the normal enzyme (Alhadeff & Freeze, 1977) and be recognized by the lectin concanavalin A insolubilized on polymeric Sepharose. Both assumptions appear to have been

warranted, since both column-chromatographic procedures resulted in the purification of small amounts of α -L-fucosidase activity. The small amount of activity recovered from the affinity column suggests that the altered enzyme may have less affinity for the fucosamine ligand on the affinity resin than does the normal enzyme. The increased K_m for 4-methylumbelliferyl α -L-fucopyranoside of the partially purified enzyme supports this idea. Previous studies in our laboratory on the crude residual liver α -L-fucosidase activity of two other fucosidosis patients found near-normal K_m values for the 4-methylumbelliferyl substrate (Alhadeff *et al.*, 1974, 1978a). However, at least two laboratories have reported finding α -L-fucosidases with increased K_m values in crude tissues from fucosidosis patients. Troost *et al.* (1976) found 7–10- and 14-fold-increased K_m values for the *p*-nitrophenyl substrate in leucocytes and sera, respectively, of fucosidosis patients. DiMatteo *et al.* (1976) found 3–4-fold-increased K_m values (910–1000 μ M) for the 4-methylumbelliferyl substrate in sera from two fucosidosis patients.

The liver α -L-fucosidase from the fucosidosis patient exhibits decreased thermostability, a property previously reported for α -L-fucosidase in sera (DiMatteo *et al.*, 1976; Patel & Zeman, 1976) and cultured fibroblasts (DiMatteo *et al.*, 1976) from fucosidosis patients. It is noteworthy that the α -L-fucosidase from all three normal control livers (see Fig. 2) loses 10–20% of its activity in the first 10–15 min of preincubation at 55°C and then is thermostable for at least 60 min preincubation. This finding, and the fact that the fucosidosis patient's α -L-fucosidase loses 100% of its activity in the first 10 min of preincubation, suggests that a particular thermolabile molecular species of α -L-fucosidase, one of a number normally present, is being retained in this patient's liver.

Previous studies have reported both normal (Beratis *et al.*, 1977) and abnormal (Beratis & Hirschhorn, 1978) α -L-fucosidase pH-activity curves for various tissues from fucosidosis patients. In a previous study, little or no residual α -L-fucosidase activity was found between pH 3.0 and 4.0 in the liver of a fucosidosis patient (Alhadeff *et al.*, 1974). In the present study, the partially purified α -L-fucosidase displayed no activity in the acid region (pH 3.0–4.5) of the pH curve, compared with considerable activity ($\geq 50\%$ of maximal activity) found in this region for the normal enzyme in the present and previous studies (Alhadeff *et al.*, 1974, 1975, 1978b).

The significance of the above kinetic findings is not fully understood, but the retention of a selective thermolabile molecular species of α -L-fucosidase in fucosidosis, with a characteristic K_m and pH-activity curve, is suggested. This speculation is

reasonable in view of our previous finding that the separated isoenzymes of purified human liver α -L-fucosidase exhibit small K_m differences and rather large differences in the percentage of maximal enzymic activity at various pH values (Alhadeff *et al.*, 1978b). The properties (increased K_m and decreased enzymic activity at acid pH values) previously found for the most neutral form (I) of normal liver α -L-fucosidase (Alhadeff *et al.*, 1978b) are similar to the kinetic properties of the fucosidosis patient's α -L-fucosidase being studied here. It is also possible that retention of only the low-molecular-weight form of α -L-fucosidase (Fuc II) in the liver from the fucosidosis patient accounts for our findings, since other investigators have shown that Fuc II enzyme exhibits decreased thermostability and has less activity at acidic pH values when compared with the high-molecular-weight form (Fuc I) (Robinson & Thorpe, 1973; Wood, 1976). Thorpe & Robinson (1975) have shown that enzymes Fuc I and Fuc II are composed of the same electrophoretic forms and probably result from different states of aggregation of the same group of isoenzymes.

The finding of one major isoelectric peak of activity for the fucosidosis patient's α -L-fucosidase also suggests the retention of a selective molecular species of the enzyme in fucosidosis. Our findings are similar to those of DiMatteo *et al.* (1976), who have reported a single major peak of α -L-fucosidase activity in the sera of two fucosidosis patients. Because of the decreased thermostability of the patients' α -L-fucosidase, they have suggested that the enzyme could be structurally altered in fucosidosis so that its ability to form the thermostable higher-molecular-weight aggregates is impaired.

The variable results reported in the literature for K_m values, pH-activity curves and thermostability of α -L-fucosidase in different fucosidosis patients and in different tissues are confusing, but may be due to differential expression of the genetic defect in different tissues and/or to the well-documented heterogeneity displayed in this disease (Schafer *et al.*, 1971; Patel *et al.*, 1972; Gatti *et al.*, 1973; Kousseff *et al.*, 1973; Alhadeff & O'Brien, 1977; Alhadeff *et al.*, 1978a).

Many previous studies have failed to detect cross-reacting material to antibodies prepared against normal α -L-fucosidase in plasma (MacPhee & Logan, 1977), fibroblasts and serum (Thorpe & Robinson, 1978) and liver (Alhadeff *et al.*, 1975) from fucosidosis patients. A previous study has shown that the kinetically normal residual α -L-fucosidase activity in the liver from an atypical fucosidosis patient is immunoprecipitable with the IgG fraction of antibodies made against normal-liver α -L-fucosidase (Alhadeff *et al.*, 1978a). In the present study the partially purified kinetically altered

α -L-fucosidase was also immunoprecipitable, but no cross-reacting material was detected in the less-sensitive double-immunodiffusion experiment. More sensitive and quantitative radioimmunochemical techniques are required to determine more precisely how much α -L-fucosidase enzyme protein is being produced in fucosidosis.

To our knowledge this is the first reported partial purification and characterization of liver α -L-fucosidase from a fucosidosis patient. The presence of a kinetically altered α -L-fucosidase which cross-reacts with antibodies prepared against the normal enzyme suggests that either (a) a selective, thermolabile molecular species of α -L-fucosidase (with characteristic K_m and pH-activity curve) is being retained or (b) a kinetically altered enzyme is being produced in fucosidosis. For the patient being studied here, the former explanation seems most likely to account for the near-absence of α -L-fucosidase activity.

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