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PURIFICATION AND PROPERTIES OF TWO FORMS OF HUMAN α-L-FUCOSIDASE

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Summary

High (100 000) and low (50 000) molecular weight forms of α-L-fucosidase (α-fucosidase I and II) were purified to apparent homogeneity from human spleen, liver brain and kidney on the basis of differential affinity for ε-aminocaproyl fucosamine-agarose bead columns. α-Fucosidase I (the "bound" form) consisted of two 50 000 dalton monomers; however, both forms can aggregate to tetramer and hexamer forms. Most previous studies on α-fucosidase have been carried out on this form of human α-fucosidase although the bound and unbound forms of the enzyme were present in equal amounts in human spleen. The bound (100 000) form is a sialoglycoprotein whereas the unbound (50 000) form, is a neutral mannose-rich glycoprotein. Other differences with respect to amino acid composition, pH optimum, electrophoretic mobility, K_m, thermal stability, and natural substrate specificities were observed. All preparations hydrolysed 4-methylumbelliferyl-α-L-fucoside, fucosyllactose, and lacto-*N*-fucopentaose I, but the unbound fraction (α-fucosidase II) preferentially hydrolysed lacto-*N*-fucopentaose II. The unbound (mannose-rich) α-fucosidase II was taken up by human skin fibroblasts with higher affinity (5% per 2 h per $2 \cdot 10^5$ cells) than the bound (sialo-) α-fucosidase I (<1% per $2 \cdot 10^5$ cells). Uptake was inhibited by other lysosomal hydrolases, fetal calf serum, mannose 6-phosphate and phosphomannans and to a lesser extent by heparins. Our studies suggest that α-fucosidase I is not a simple dimer of α-fucosidase II and represents a less-biologically active form of the enzyme.

Abbreviation: 4MeUmb, 4-methylumbelliferyl; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

Introduction

Human α-fucosidase has been isolated and characterized from human liver [1—3], brain [4], and placenta [5]. Various techniques have been used to separate different forms of α-fucosidase, such as Sephadex G-200 gel filtration, DEAE column chromatography, isoelectric focussing, and fucosamine affinity column chromatography [5,6]. Robinson and Thorpe [1] reported that human liver contained a macromolecular form (α-fucosidase I, which was excluded from Sephadex G-200 and adsorbed on DEAE-cellulose at pH 6.5) and a low molecular weight form (α-fucosidase II, which did not bind to DEAE-cellulose). However, the differences between the two forms were not investigated further, apart from observations that form II was heat labile, and that the two forms could be interconverted, suggesting a tetramer-monomer relationship [6]. There have been few studies on the natural substrate specificity of any of these forms or isoenzymes. Wiederschain and Rosenfeld [2] used fucose-containing oligasaccharides from human milk, to investigate the substrate specificity of pig kidney α-fucosidases and concluded that this α-fucosidase had a broad substrate specificity. This has been confirmed both by our laboratory [7] and by Thorpe and Oates [8], although there are major practical difficulties in reliably measuring the amount of liberated L-fucose. In this investigation, we have determined the specificity of human spleen α-fucosidase toward a variety of ^{3}H-labeled natural substrates. The highest specific activity was found in a mannose-rich glycoprotein form (α-fucosidase II) of molecular weight approximately 50 000. The relationship of this 50 000 dalton form to the previously described 50 000 dalton α-fucosidase monomer, the 100 000 dalton sialoglycoprotein dimer (α-fucosidase I) and the previously reported 200 000 and 300 000 dalton forms of α-fucosidase is discussed.

Materials and Methods

Enzyme purification. 300 g of fresh surgically-removed human spleen was homogenized in 400 ml of 0.01 M Na_2HPO_4 buffer, pH 6.8, in a Waring blender for 3 pulses of 1 min duration. The homogenate was then centrifuged at 12 000 rev./min for 30 min and the residue discarded. α-Fucosidase was precipitated by 30—60% ammonium sulfate. The precipitate was resuspended, and then dialyzed against 0.01 M Na_2HPO_4 buffer, pH 6.8, for 24 h at 4° C.

Affinity column chromatography. 45 ml of the dialyzed enzyme was applied to a 1.8 × 20 cm affinity column (ε-amino-caproyl fucosamine-agarose, purchased from Miles-Yeda Ltd.) previously equilibrated with 0.01 M Na_2HPO_4 buffer, pH 6.8 [3]. As soon as the protein peak had been eluted with the equilibration buffer, the total α-fucosidase fraction was eluted with 5 mM fucose in the same buffer. The enzyme was concentrated by reverse dialysis against saturated $(NH_4)_2SO_4$, and the precipitate collected, resuspended, dialyzed in phosphate buffer and reapplied to a second affinity column as described above. The second affinity column was eluted with 0.01 M Na_2HPO_4 buffer, pH 6.8, until no further α-fucosidase activity was detected (unbound α-fucosidase II) and the bound α-fucosidase fraction (α-fucosidase I) was then eluted with 5 mM fucose. Final purification was achieved by fractionation on a column

(2.5 × 150 cm) of Sephadex G-200 and reapplication to an affinity column. Enzyme activity was monitored with appropriate 4-methylumbelliferyl (4MeUmb) glycoside derivatives. Homogeneity of the two preparations was also assessed by carrying out gel electrophoresis under a number of denaturing and non-denaturing conditions.

Enzyme activity. Synthetic 4-methylumbelliferyl-α-fucoside (4 MeUMb-α-L-fucoside) (obtained from Research Products International, Elk Grove Village, IL) was used as a 0.5 mM solution in 0.1 M citrate-phosphate buffer, pH 5.0, and the liberated product was determined fluorometrically [8]. Enzyme activity was defined as that amount which will hydrolyze 1 nmol of synthetic substrate per min. Milk oligosaccharides (2′-fucosyllactose, lacto-*N*-fucopentaose I, II and III) were generously donated by Dr. V. Ginsburg, NIH. These substrates were labelled in the reducing end group (glucose moiety) by NaB^3H_4 reduction in aqueous solution [9] and their hydrolysis products were separated by paper chromatography in the solvent system ethyl acetate/pyridine/H_2O in the ratio (12 : 5 : 4, v/v) [10]. For quantitative determination, the paper strips were cut at appropriate places and the radioactivity determined by liquid scintillation counting.

Enzyme studies. Molecular weights were estimated by applying the enzyme (in 0.1 M Na_2HPO_4 buffer, pH 6.8) to a column (1 × 96 cm) of Sephadex G-200 pre-calibrated with proteins of known molecular weight. The elution profile of the two enzyme forms was determined enzymatically with 4MeUmb-α-L-fucoside [8]. The bound and unbound enzymes were subjected to 10% SDS polyacrylamide slab gel electrophoresis (in either the untreated or 2-mercapto-ethanol-treated form) and the protein bands were detected by Coomassie blue staining. Parallel (non-SDS) gels were impregnated with 4MeUmb-α-L-fucoside in buffer and incubated at 37°C to localize α-fucosidase activity. Molecular weights were estimated by comparison of migration rates on Sephadex G-200 with those of proteins of known subunit size.

Enzyme preparations were subjected to cellulose acetate (Cellogel) strip electrophoresis in 0.02 M Tris-HCl buffer, pH 8.0. Following electrophoresis for 3 h, the strips were incubated at 37°C with moist filter paper pads impregnated with 4MeUmb-α-L-fucoside for 30 min and the fluorescence developed by exposure to sodium carbonate buffer (pH 10.7).

Labelling of α-fucosidase with ^{125}I-iodine. α-L-Fucosidase (10 μg) was labelled with 0.9 mCi of ^{125}I-iodine in the presence of Chloramine T (0.7 μg) in 50 μl Na_2HPO_4 buffer (pH 7.4) for 3 min. The reaction was stopped by the addition of sodium metabisulfate (1 μg) and bovine serum albumin (0.1 mg). ^{125}I-labelled enzyme was immediately repurified on Sephadex G-25 and an aliquot subjected to acrylamide gel electrophoresis to confirm that the majority of the ^{125}I-label co-migrated with α-L-fucosidase.

α-Fucosidase uptake by human skin fibroblasts. Control fibroblast cell strains and fucosidosis strain GM292 (a fucosidosis Type II strain supplied by the Human Mutant Cell Repository, Camden, NJ) were cultured as monolayers in 60 mm Petri dishes in minimal essential media supplemented with 10% fetal calf serum and 10% calf serum [8]. For enzyme uptake studies, confluent α-fucosidase deficient (fucosidosis) cells in 1 ml of medium 199 containing Hepes buffer and glutamine, were incubated with α-fucosidase in a 5% CO_2/

95% O_2 atmosphere at 37°C. Uptake of α-fucosidase was monitored at hourly intervals up to 4 h by homogenizing the cells and determining the level of hydrolysis of 4MeUmb-α-L-fucoside as described above. Cells for parallel ^{125}I-labelled α-fucosidase binding and uptake studies were plated in 8 chamber fixed gasket plastic slides (Lab-Tek Inc.) and incubated in 0.2 ml of medium 199 for up to 2 h. Cells were harvested, washed by co-centrifugation with phthalate oil, and the radioactivity in the washed pellet was determined with a Gamma counter.

Results

The two sequential applications of human spleen extracts of fucosamine-agarose affinity columns resulted in at least a 1000-fold purification of α-fucosidase. With the first column, total α-L-fucosidase activity was eluted with 5 mM fucose immediately following the protein peak. On the second affinity column (Fig. 1), buffer elution was continued after the protein peak had been eluted and we observed a peak of homogeneous α-fucosidase activity which we designated the 'unbound' enzyme or α-fucosidase II. It can be seen that the 'unbound' enzyme has a much greater affinity towards lacto-*N*-fucopentaose II (containing fucose in an α1-4 linkage to GlcNAc) than the 'bound' enzyme

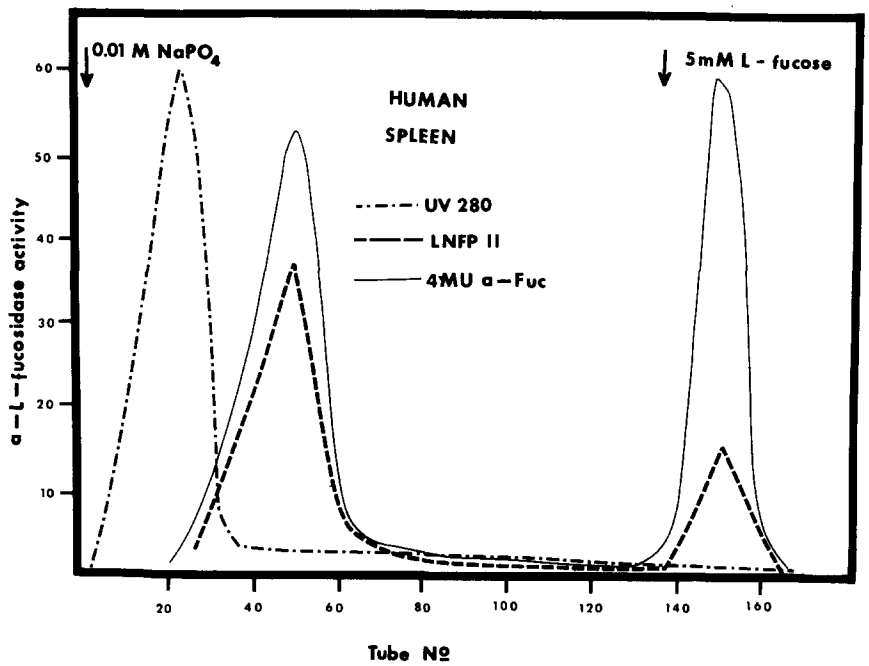


Fig. 1. Affinity column chromatography fractionation of human spleen α-L-fucosidase. The ε-amino caproyl fucosamine-agarose column (1.8 × 22 cm) was both equilibrated and eluted with 0.01 M Na_2HPO_4 buffer, pH 6.8. 28 000 Units of enzyme were applied to the column. The 'unbound' fraction was eluted in tubes 25 to 65 with the 0.01 M phosphate buffer and the 'bound' fraction was eluted with 5 mM L-fucose in the same buffer (tubes 130—165). Fractions (1.7 ml/tube) were assayed for α-fucosidase activity with the 4MeUmb(4MU) substrate. Lacto-*N*-fucopentaose II (LNFP II) activity was monitored by a radioactive assay in which the ^3H-labelled product was separated by paper chromatography as described in the text.

fraction which was subsequently eluted with 5 mM fucose. All preparations were then subjected to Sephadex G-200 to remove the last traces of other lysosomal hydrolases; verified by the absence of hydrolytic activity towards a number if 4-methylumbelliferyl glycosides. Further evidence for homogeneity and purity was based on the fact that both SDS and non-SDS gel electrophoresis of unlabelled and ^{125}I-labelled enzyme gave a single band at pH 6.0, 6.8 and 8.0.

The cellogel electrophoresis pattern for the two enzyme preparations is shown in Fig. 2. The bound enzyme moved towards the anode and the unbound stayed at the origin under these conditions (pH 8.0 buffer). This mobility was unaffected by pretreatment with *Clostridium perfringens* neuraminidase and may not, therefore, be related to the fact that the 'bound' form is a sialoglycoprotein. Gas-liquid chromatographic analysis showed the 'bound' (α-fucosidase I) form to contain mannose, galactose, GlcNAc and sialic acid (as reported previously for human spleen and placenta [8]) whereas the 'unbound' (α-fucosidase II) form contained only mannose and GlcNAc (Table I). Differences in amino acid composition were also observed (Table I), but phosphate levels were not determined.

A minimum molecular weight of 50 000 was determined by SDS-polyacrylamide gel electrophoresis as shown in Fig. 3. Both 'bound' and 'unbound' enzyme preparations showed a similar molecular weight in the region of 50 000 and both preparations appeared free from other protein contamination. Enzyme activity was correlated directly with the regions stained with Coomassie blue. However, gel filtration on Sephadex G-200 (1 × 96 cm) in 0.1 M Na_2HPO_4 buffer, pH 6.8, calibrated with chymotrypsinogen, ovalbumin, bovine serum albumin, IgG and Blue Dextran 200 000 gave a value of approximately 100 000 for the 'bound' enzyme and 50 000 for the 'unbound' enzyme, suggesting that under our conditions of G-200 elution (pH 6.8 and low salt) the bound enzyme behaves as a dimer.

The degree of hydrolysis of the ^{3}H-labelled oligosaccharide was monitored by a paper chromatographic modification of the method of Ogata-Arakawa et

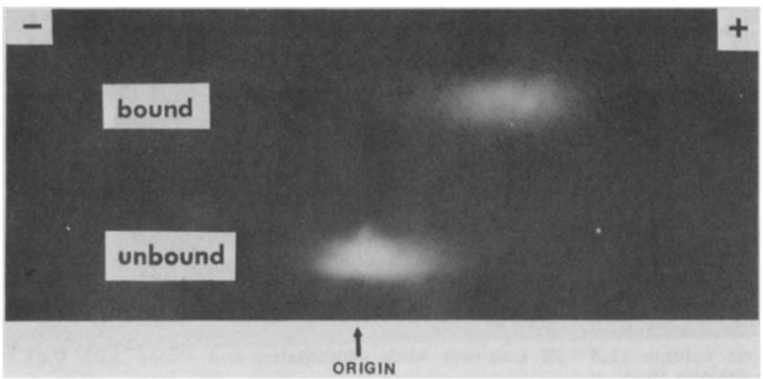


Fig. 2. Cellogel electrophoresis of α-fucosidase I (bound) and II (unbound) in 0.02 M Tris-HCl buffer, pH 8.0, at 2.5 mA for 3 h. Enzyme activity was measured by sandwiching the strip between two plastic sheets in the presence of 0.5 M 4MeUmb-α-L-fucoside and incubating at 37°C for 30 min in 0.1 M citrate-phosphate buffer, pH 5.0. Fluorescence was enhanced by adding 0.5 M Na_2CO_3 buffer, pH 10.7.

TABLE I

AMINO ACID AND CARBOHYDRATE COMPOSITION OF HUMAN SPLEEN α-FUCOSIDASE I (BOUND FORM) AND α-FUCOSIDASE II (UNBOUND FORM)

Figures in the first part of the table presented as molar ratios. The figures in the bottom part are presented in mol%.

Component	α-Fucosidase I	α-Fucosidase II
Mannose	3.0	6.0
Galactose	3.0	<0.5
GlcNAc	4.0	2.0
Sialic acid	2.5	<0.5
Gly	14.8	11.0
Ser	12.8	10.4
Glu	12.2	12.5
Asp	8.2	9.1
Ala	6.6	7.3
Leu	5.3	7.2
Thr	4.6	4.8
Val	3.9	5.0
Pro	3.7	4.6
Lys	3.7	4.4
Phe	3.2	3.5
Ile	2.8	3.0
Tyr	2.2	2.3
His	2.0	2.1
Arg	1.3	2.5
Met	0.3	0.3

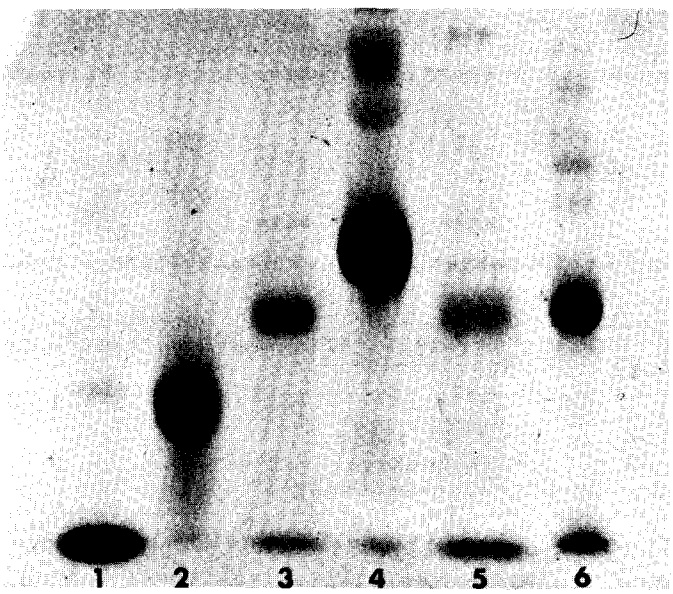


Fig. 3. SDS 10% polyacrylamide gel electrophoresis of dissociated human spleen α-L-fucosidases. Samples were treated with 1% SDS and 1% mercaptoethanol at 100°C for 5 min before applying to the gel and protein bands were stained with Coomassie blue. Lane 1, egg lysozyme (11 000 daltons); Lane 2, ovalbumin (43 000 daltons); Lane 3, unbound human spleen α-L-fucosidase II; Lane 4, bovine serum albumin (67 000 daltons); Lane 5, bound human spleen α-L-fucosidase I; Lane 6, human gamma globin (50 000 and 25 000 daltons).

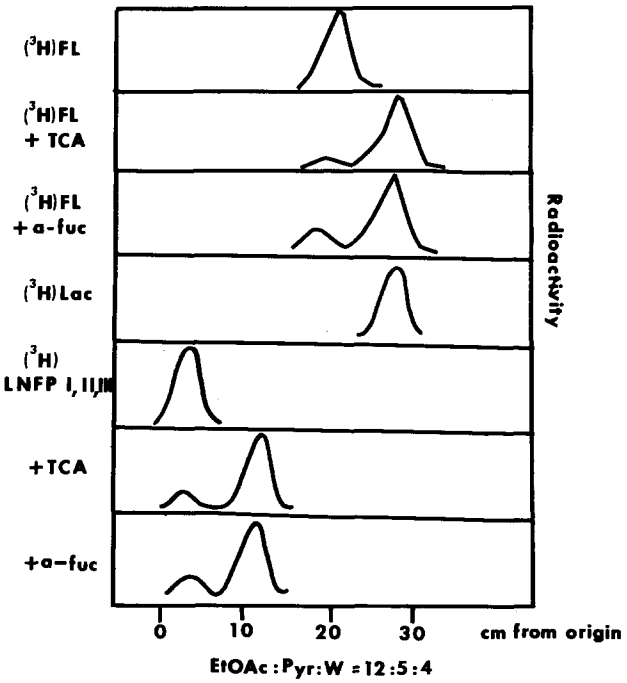


Fig. 4. Paper chromatographic separation of ^{3}H-labelled fuco-oligosaccharide hydrolysis products. Each incubation contained approx. 2 nmol of substrate (200 000 cpm) in 50 μl of pH 5.0 buffer as described in the text. Migration of hydrolysis products was compared with that of a fuco-oligosaccharide obtained by hydrolyzing with 0.5 M aqueous trichloroacetic acid for 2 h. The paper was developed in ethyl acetate/pyridine/H_2O (12 : 5 : 4, v/v). Abbreviations used: [^{3}H]FL, fucosyllactose; TCA, trichloroacetic acid; α-fuc, α-fucosidase; [^{3}H]Lac, [^{3}H]lactose; LNFP I, lacto-*N*-fucopentaose, Fuc α(1-2)Gal linkage; LNFP II, lacto-*N*-fucopentaose, Fuc α(1-4)GlcNAc linkage; LNFP III, lacto-*N*-fucopentaose; Fuc α(1-3)GlcNAc linkage. Radioactivity in the paper strips was determined by liquid scintillation counting in ACS prepared cocktail.

TABLE II

SUBSTRATE SPECIFICITY OF BOUND AND UNBOUND FORMS OF HUMAN SPLEEN α-FUCOSIDASE

Hydrolysis of ^{3}H-labelled substrate (e.g., lacto-*N*-fucopentaoses (LNFP)) ($2 \cdot 10^{5}$ cpm) was carried out in 0.1 M citrate-phosphate buffer pH 5.0 (50 μl) in the presence of 10 units each enzyme and 30 mM of D-galactono-1,4-lactone and incubated at 37°C for 1 h.

Substrate	% Hydrolysis	
	Bound (α-fucosidase I)	Unbound (α-fucosidase II)
Fucosyllactose	100	100
LNFP I	50	54
LNFP II	16	45
LNFP III	0	0
Fuc α (1-6) GlcNAc **	40 *	60 *

* 16 h incubation.

** Fuc α (1-6) GlcNAc was isolated from cultured patient skin fibroblasts which have been labeled with [^{3}H]fucose (5 μCi/plate) for 24 h.

TABLE III

COMPARISON OF MICHAELIS CONSTANTS (K_m) OF BOUND AND UNBOUND FORMS OF α-L-FUCOSIDASE

Hydrolysis of 4-methylumbelliferyl-α-L-fucoside (4MeUmb-α-Fuc) was carried out in 60 μl of 0.1 M citrate-phosphate buffer, pH 5.0. Hydrolysis of lacto-*N*-fucopentoase II (LNFP II) was carried out in 80 μl of the same buffer in the presence of 30 mM of D-galactono-1,-4-lactone.

Substrate	Bound (α-fucosidase I)	Unbound (α-fucosidase II)
4 MeUmb-α-Fuc	125 μM	83 μM
LNFP II	50 nmol	25 nmol

al. [10] (Fig. 4). Fucosyllactose migrated approximately 30 cm in 2 days, whereas the lacto-*N*-fucopentaoses migrated only about 10 cm in 3 days; all were well-resolved from L-fucose.

The extent of hydrolysis of fucose-containing milk oligosaccharides by the two enzyme preparations under comparable conditions is shown in Table II. The unbound enzyme (α-fucosidase II) hydrolyzed lacto-*N*-fucopentaose II and Fuc α(1—6)GlcNAc more efficiently than the bound form, although both hydrolyzed 2'-fucosyllactose and lacto-*N*-fucopentaose I. Neither of the spleen preparations hydrolysed lacto-*N*-fucopentaose III to any appreciable extent, whereas, similar preparations from human liver were active towards all substrates tested.

The Michaelis constant (K_m) was determined for the hydrolysis of lacto-*N*-fucopentaose II and 4-MeUmb-α-L-fucoside by both the bound and unbound enzyme and the results are given in Table III. K_m values using 4MeUmb-α-L-fucoside as substrate were 125 mM for the bound and 63 mM for the unbound forms; K_m values using lacto-*N*-fucopentaose II were 50 nM for the bound and 25 nM for the unbound, i.e. K_m was twice as high for the bound (α-fucosi-

TABLE IV

SUBSTRATE SPECIFICITY OF α-FUCOSIDASES FROM DIFFERENT HUMAN TISSUES

Hydrolysis was carried out in 0.1 M citrate-phosphate buffer, pH 5.0, in the presence of D-galactono-1,4-lactone (2 nmol). 200 000 cpm of each substrated and 2—3 units of each enzyme were present in the 50 μl of incubation mixture. Spleen, kidney and liver contained equal amounts of the 'bound' (α-fucosidase I) and 'unbound' (α-fucosidase II) forms of the enzyme whereas preparations isolated from brain and urine contained predominantly α-fucosidase I (80—90%). Figures are presented as percentage hydrolysis per 16 h.

Tissue		Fucosyl-lactose	Lacto-*N*-fucopentaose		
			I	II	III
Spleen	Bound	100	75	40	0
	Unbound	100	60	50	0
Kidney	Bound	100	90	60	20
	Unbound	100	90	60	30
Liver	Bound	100	90	60	70
	Unbound	100	80	30	5
Brain	Bound	100	60	40	15
Urine	Bound	100	0	40	0

dase I) enzyme as for the unbound enzyme. The pH optima were 5.3 (α-fucosidase I) and 5.0 (α-fucosidase II). α-Fucosidase I was more thermolabile (50% loss in activity after 40 min at 50°C) than α-fucosidase II (20% loss under the same conditions; 50% loss after 70 min).

We also examined the extent of hydrolysis of natural substrates by bound and unbound α-L-fucosidase preparations from a number of tissues as shown in Table IV. All preparations hydrolyzed 2'-fucosyllactose and lacto-*N*-fucopentaose I, with the exception of enzyme purified from urine, which did not hydrolyze lacto-*N*-fucopentaose I. Lacto-*N*-fucopentaose II was hydrolyzed by all tissues to varying degrees, but a considerable variation in the ability of enzyme preparations to hydrolyze lacto-*N*-fucopentaose III was observed. Liver α-L-fucosidase was most effective in hydrolyzing lacto-*N*-fucopentaose III, particularly the bound enzyme fraction, with some activity in kidney and brain preparations, but none in spleen, urine or human fibroblast preparations.

The unbound (α-fucosidase II) form of the enzyme was taken up by cultured skin fibroblasts from patients with fucosidosis by a high affinity mechanism which appears similar to that first described by Jourdian and Sly [12,13]. Thus, uptake was inhibited by mannose, mannose 6-phosphate and phosphomannans (Table V), although much higher concentrations (25 mM) were required for 50% inhibition than previously reported for β-galactosidase and β-glucuronidase. Certain glycosaminoglycans such as heparin were also inhibi-

TABLE V

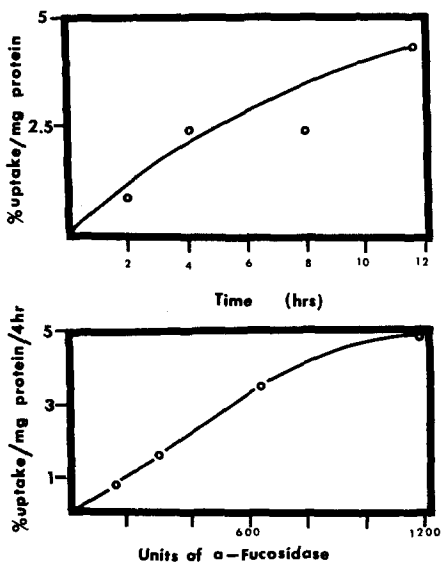
EFFECT OF DIFFERENT KNOWN INHIBITORS OF MACROMOLECULE HIGH AFFINITY UPTAKE OF ^{125}I-LABELLED α-L-FUCOSIDASE II BY CULTURED HUMAN SKIN FIBROBLASTS

Results are the average of binding studies on three different cell samples ($2 \cdot 10^5$ cells per 0.2 ml medium per well) using 5 ng ($1 \cdot 10^5$ cpm) of ^{125}I-labelled α-fucosidase II and the appropriate inhibitor. No inhibition was observed with D-mannose, L-fucose, α-methyl-D-mannoside, D-glucose 1-phosphate or D-glucose 6-phosphate at concentrations up to 25 mM. For comparison, 25 mM D-mannose 6-phosphate gave 50% inhibition of uptake.

Inhibitor	Concentration (mg/ml)	Percent inhibition
Mannose 6-phosphate	1.9	0
	3.8	14
	7.6	20
	15.2	34
	30.4	50
Yeast mannan	0.2	30
	0.5	40
	1.0	50
	2.0	52
	4.0	60
Heparin (hog mucosal)	0.5	20
	1.0	40
	2.0	52
	4.0	56
β-Glucuronidase (bovine liver)	0.06	16
	0.25	28
	1.25	50
	2.50	80
	5.00	80

tory and bovine β-glucuronidase was a potent competitor. All uptake studies had to be carried out in the absence of fetal calf serum and calf serum since both were potent inhibitors of uptake; it is not known, if the competition is due to serum glycoproteins or other receptor-mediated uptake ligands such as low density lipoprotein.

Studies on uptake of unlabelled α-fucosidase II by fucosidosis fibroblasts was linear over a period of 8 h (Fig. 5, upper graph) and was also linear up to 600 units of added enzyme (Fig. 5, lower graph). Actual uptake was of the order of 2% per 4 h for a plate of cells containing 200 μg of protein. We have previously shown [14] that the enzyme is taken up into pre-existing lysosomes and can affect the catabolism of the stored Fuc-GlcNAc-Asn-glycopeptide lysosomal storage material in fucosidosis fibroblasts. A comparison of the rate of uptake of ^{125}I-labelled human spleen α-fucosidase I and α-fucosidase II is shown in Fig. 6. The high affinity uptake of α-fucosidase II was saturable at 5 μg of unlabelled enzyme/200 000 cells and was inhibitable by phosphomannan, mannose 6-phosphate and β-glucuronidase. The low affinity uptake of α-fucosidase I was also inhibitable to a certain extent by phosphomannan (Fig. 6).



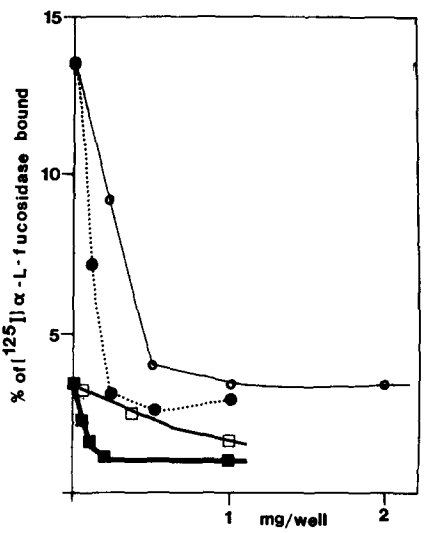


Fig. 5. High affinity uptake of α-L-fucosidase II by human skin fibroblasts (upper part). Uptake by a human skin fibroblast monolayer culture containing about 200 μg of the protein and 400 units of enzyme per 60 mm Petri dish. Experimental details are given in the text. (Lower part) A monolayer fucosidosis fibroblast culture incubated with different concentrations of α-L-fucosidase II for 4 h.

Fig. 6. Comparison of rate of high affinity uptake of ^{125}I-labelled α-L-fucosidase I (bound) and II (unbound) by normal human cultured skin fibroblasts and its inhibition by phosphomannan and β-glucuronidase. Each assay contains $2 \cdot 10^5$ cells and 5 ng of $1 \cdot 10^5$ cpm of ^{125}I-labelled α-fucosidase. Results are the average of binding assays on three different cell samples; bound (□, ■); unbound (○, ●); phosphomannan (○———○, □———□); β-glucuronidase (●- - - - -●, ■———■).

Discussion

The molecular weight of human α-fucosidase isolated from liver, spleen, placenta, and brain [1—5] has been variously estimated at between 150 000 and 200 000 with a subunit size of 50 000. Turner et al. [15] reported several active forms of α-fucosidase with different molecular sizes in the range of 49 000 to 305 000, depending on the pH of the buffer used to elute the gel filtration column. SDS-polyacrylamide gel electrophoresis of his preparations revealed two bands of molecular weight 53 000 and 25 000; however, it is not clear if either or both possessed enzymic activity since they were identified by protein stains only. On the basis of these observations, it was suggested that subunits can associate to generate different molecular size forms of α-fucosidase. This is in general agreement with Alhadeff et al. [3] who found that human liver α-fucosidase had a molecular weight of 200 000 and contained only one electrophoretically separable subunit, with a molecular weight of 50 000, and with Thorpe and Robinson [6] who reported that the material recovered from fucosamine affinity columns was exclusively the high molecular weight α-fucosidase I form whether or not forms I or II (50 000) were applied to the Sephadex column. Further, the enzyme eluted from the affinity column was found to associate into the α-fucosidase I (high molecular weight form) in 5 mM sodium acetate buffer, pH 5.0, containing 100 mM NaCl but disassociate into the α-fucosidase II (low molecular weight form) in 200 mM sodium phosphate buffer, pH 6.8. It was therefore suggested that liver enzyme is a tetramer (α-fucosidase I) of four similar subunits (α-fucosidase II). Recently, Alhadeff and Andrews-Smith [16] have presented evidence for the existence of at least two distinct sub-units in α-fucosidase I based on slight molecular weight differences and this observation is to some extent confirmed by our studies. However, it should be noted that all these studies with the possible exception of Robinson and Thorpe used enzyme purified by fucosamine affinity column method (bound enzyme or α-fucosidase I) and synthetic substrates such as 4MeUmb-α-L-fucoside.

We have separated two forms of α-fucosidase by differential affinity column chromatography. Normal human spleen contains approximately equal amounts of the two forms. The differences in electrophoresis patterns may indicate the difference in sialic acid residues as suggested by other investigators [17,18], although we could not confirm this directly. Previous studies in this laboratory [8] indicated that α-L-fucosidase is a typical sialoglycoprotein, containing mannose, galactose, *N*-acetylglucosamine, and sialic acid, plus an enigmatic quantity of glucose. This bound enzyme (α-fucosidase I) which has an apparent molecular weight of 100 000 at pH 6.8 consists of two 50 000 dalton subunits as evidenced by SDS-polyacrylamide gel electrophoresis and is taken up by cells with relatively low affinity. The unbound enzyme (α-fucosidase II) has a molecular weight of approximately 50 000 and SDS gel electrophoresis of the reduced protein is also consistent with a molecular weight in the region of 50 000. Since the oligosaccharide units with α-fucosidase II are of the $(Man)_5(GlcNAc)_2$ type and the amino acid composition is significantly different from that of α- fucosidase I, the preponderance of evidence indicates that the 50 000 'bound' enzyme subunit and the 50 000 'unbound' enzyme are non-

identical. The conclusion is further supported by differences in the affinity of the two forms for fucosamine-Agarose matrices, the differences in charge, slight pH optima differences, and a significant difference in substrate specificity, with the unbound enzyme having a much lower K_m for all substrates and a much higher affinity for the hydrolysis of Fucα(1-4) and α(1-6)GlcNAc linkages. However, at this time we cannot say unequivocally that there is more than one form of human α-L-fucosidase since it is possible that the 100 000 form (α-fucosidase I) consists of an active 50 000 dalton subunit or A chain (identical to the unbound enzyme α-fucosidase II) and a non-indentical and much less enzymically active 50 000 dalton subunit or B chain. Higher molecular weight forms of the enzyme could contain different proportions of these A and B chains. Obviously, this problem can only be resolved by preparing antibodies to the A (unbound) and AB (bound) forms of human spleen α-fucosidase and investigating the degree of cross-reactivity. Analogous situations exist in other lysosomal hydrolases such as *N*-acetyl-β-D-hexosaminidase, which has been claimed to exist as either an $\alpha_3\beta_3$ hexamer [19], or an $\alpha_2\beta_2$ tetramer [20] and arylsulfatase A, which may or may not possess two identical subunits of molecular weight approximately 50 000 [21].

The mechanism of receptor-mediated high affinity uptake of the unbound (α-fucosidase I) enzyme is not completely clear at this time although it appears to conform basically to the criteria for mannose 6-phosphate recognition defined by Kaplan et al. [22] for β-glucuronidase and subsequently for α-L-iduronidase by Neufeld et al. [23] and other lysosomal hydrolases by von Figura et al. [24]. However, we have observed some differences. Thus, the high affinity uptake was inhibited by calf serum, but was only partially inhibited by 0.5 mM mannose 6-phosphate. This suggests that the actual receptor-mediated uptake mechanism might be different than that described for β-glucuronidase [22] or α-L-iduronidase [23] and a more detailed investigation is currently in progress. An investigation of the biological role of these two forms of α-L-fucosidase with their different carbohydrate composition and different size may shed light on the mechanism of lysosomal hydrolase activation and complex carbohydrate degradation in mammalian cells.

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