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Biochimica et Biophysica Acta 1270 (1995) 7–11

BBA Biochimica et Biophysica Acta

# Change of isoforms' spectra of α-L-fucosidase from human skin fibroblasts in intracellular storage of nonhydrolyzable substances

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Received 1 February 1994; revised 7 July 1994; accepted 15 August 1994

**Abstract**

The effect of exogenous and endogenous products storage in lysosomes on the activity and multiple forms of α-L-fucosidase from human skin fibroblasts was investigated. It was shown that sucrose load, modelling intralysosomal accumulation of nonhydrolyzable products, causes certain changes in secretion level of α-L-fucosidase and multiple forms' spectra of the intracellular and secreted enzymes. These changes were different for the enzyme from embryonal and postnatal normal fibroblasts. Some changes of secreted α-L-fucosidase isoforms' spectra were found in fibroblasts from a patient with Fabry's disease, characterized by the intralysosomal storage of di- and trihexosylceramides. The alterations of isoforms' profiles in Fabry fibroblasts at the early and late accumulation stages were similar to those in sucrose-loaded embryonal and postnatal normal fibroblasts, respectively. It is proposed that intralysosomal accumulation of nonhydrolyzable compounds influences the α-L-fucosidase posttranslational processing.

*Keywords:* α-L-Fucosidase; Lysosomal storage disease; Isoform spectrum; Intracellular storage; Fibroblast; (Human skin)

## 1. Introduction

At the present time more than 40 hereditary lysosomal storage diseases are described. Most of these disorders are due to a deficiency of one or another glycosidase and subsequent accumulation of nonhydrolyzable carbohydrate-containing compounds in lysosomes. Earlier it was shown that intralysosomal accumulation of nonhydrolyzable endo- and exogenous compounds causing hypertrophy of the lysosomal compartment, leads to selective intracellular activation of acid glycosidases, unrelated to the primary genetic defect, and also to changes in their secretion level and in some properties of secreted enzymes [1–3]. However, the nature of the quantitative and qualitative changes in glycosidases secretion, as well as the questions on identity of enzymes synthesized in the normal state and under the extreme conditions for cells were not clarified. Intracellular lysosomal and secreted glycosidases differ in their carbohydrate chain structure [4], which largely determines the functioning of these enzymes [5,6] and their intracellular sorting – directed transport to the lysosomes and secretion from the cells [7].

To clarify the qualitative differences between the intracellular and secreted forms of glycosidases in response to accumulation of nonhydrolyzable products in cells, we have centered our attention on α-L-fucosidase, a ubiquitous lysosomal hydrolase involved in the degradation of fucose-containing glycoconjugates [8]. α-L-Fucosidase is a glycoprotein, consisting of multiple molecular forms, which are related, at least in part, by sialic acid residues and demonstrate different spectra in human tissues and blood serum [9,10]. Furthermore, the cDNA for human α-L-fucosidase is known to have been cloned; it is a monomer with four potential glycosylation sites [11].

The purpose of the present investigation was to compare multiple forms of the intracellular and secreted α-L-fucosidases of human skin fibroblasts in the normal, after sucrose load and in the typical lysosomal storage disease, Fabry's disease [12], which is caused by a hereditary deficiency of α-D-galactosidase activity and characterized by accumulation in cell lysosomes of di- and trihexosylceramides as a main storage galactose-containing products. Our preliminary results were partly published elsewhere [13].

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*SSDI* 0925-4439(94)00062-X

## 2. Materials and methods

### 2.1. Cell cultures

Human skin fibroblasts (normal embryonal and postnatal, and pathological lines) were obtained from the Cell Bank of Medical Genetic Centre (Moscow). The cells were grown in 75 $cm^2$ culture flasks in Eagle's minimum essential medium containing 5% fetal bovine serum, 0.03% glutamine, 50 units $ml^{-1}$ penicillum (all supplied by Serva, Germany). After trypsinization fibroblasts were plated at a density $5 \cdot 10^5$ cells per vessel, grown at 37°C to about confluence ($(3–5) \cdot 10^6$ cells). Since intensive accumulation of nonhydrolyzable compounds in affected tissues begins only upon termination of cell proliferation, confluent monolayers of the pathological cells were studied at 3 (stage I, early), 10 (stage II, medium) and 17 (stage III, late) days. Normal cells were maintained for 6 days in the same culture medium with 0.04 M sucrose. Then the growth medium was replaced by serum-free medium in all vessels, with control, sucrose-loaded and pathological cells. After 24 h this medium was decanted, centrifuged ($3000 \times g$), concentrated 20–30-times using an Amicon ultrafiltration cell with a Diaflo Membrane PM-30 at 4°C. Cells were harvested by trypsinization, washed, pelleted and stored frozen at −20°C until analysed. Cell number was determined by directly counting aliquots of trypsinized cells in a hemocytometer. Before enzyme assay the cell pellets were subjected to 5 cycles of freeze-thawing, homogenization in distilled water and centrifugation at $7000 \times g$.

### 2.2. Enzyme assay

The α-L-fucosidase activity was judged by the amount of free 4-methylumbelliferone released in the splitting of 4-methylumbelliferyl-α-L-fucoside. The incubation sample (final volume 0.1 ml) contained 5 μl of cell extract or concentrated medium and 95 μl of a substrate (final concentration, 1 mM) dissolved in 0.1 M acetate buffer pH 4.5. The activity of enzyme secreted in culture medium was determined in the presence of bovine serum albumin (final concentration, 1 mg/ml). The reaction was stopped by adding 2 ml of 0.4 M glycine-NaOH buffer, pH 10.5. Fluorescence was measured at the excitation and emission wavelengths 365 nm and 450 nm, respectively. One unit of activity was defined as the amount of the enzyme which hydrolysed 1 nmol of substrate per h per $10^6$ cells.

### 2.3. Isoelectric focusing

Isoelectric focusing of the enzyme preparations was carried out on PAG plates (LKB, Sweden) over a pH range of 4.0–6.5 according to the Instruction LKB 'Ampholine PAG Plates' and using 0.1 M glutamic acid in 0.5 M $H_3PO_4$ and 0.1 M β-alanine as the anode- and cathode-electrode solutions, respectively.

The samples from cell extract and culture medium contained 100–200 and 300–500 μg of protein, respectively. For the identification of α-L-fucosidase activity gel strips with focused samples were cut into pieces of $0.25 \times 1.0$ cm in the direction from the anode to the cathode, placed into 0.2 ml of 1 mM 4-methylumbelliferyl-α-L-fucoside prepared in 0.1 M acetate buffer, pH 4.5 and incubated for 18–24 h at 37°C. The enzymatic reaction was stopped by adding 2 ml of 0.4 M glycine-NaOH buffer, pH 10.5. Fluorescence was measured as described above. The pH gradient in the gel was determined by direct measurement in the gel layer using the surface pH electrode (LKB, Sweden) through each 1 cm.

## 3. Results

### 3.1. Comparison of intracellular activity and secretion of α-L-fucosidase in fibroblast cultures

Enzyme activity was determined in monolayer cultures of embryonal and postnatal human skin fibroblasts in the normal, after sucrose load and in the cultures of pathological cells with low, medium and high accumulation levels. As seen from Table 1, in the normal state there are considerable differences between embryonal and postnatal fibroblast cultures in both the specific intracellular and secreted α-L-fucosidase activities; however, the secretion levels in the both cell lines were almost the same. With a

Table 1
The activity of the intracellular (I) and secreted (II) α-L-fucosidase from normal (−), sucrose-loaded (+) and pathological fibroblasts

| Cell line | Specific activity (nmol/h per $10^6$ cells) | | Total activity (nmol/h) | | Secreted activity (%) |
|---|---|---|---|---|---|
| | I | II | I | II | II/I + II |
| Embryonal (−) | 32.3 | 12.1 | 266.2 | 99.8 | 27 |
| Embryonal (+) | 31.1 | 3.5 | 282.7 | 32.2 | 10 |
| Postnatal (−) | 4.9 | 1.6 | 13.3 | 4.4 | 25 |
| Postnatal (+) | 1.5 | 2.4 | 7.3 | 11.8 | 61 |
| Pathological cells | | | | | |
| Stage I | 7.2 | 8.3 | 13.0 | 15.0 | 53.6 |
| Stage II | 6.6 | 28.8 | 21.7 | 70.2 | 76.3 |
| Stage III | 9.5 | 0.4 | 28.5 | 1.0 | 3.5 |

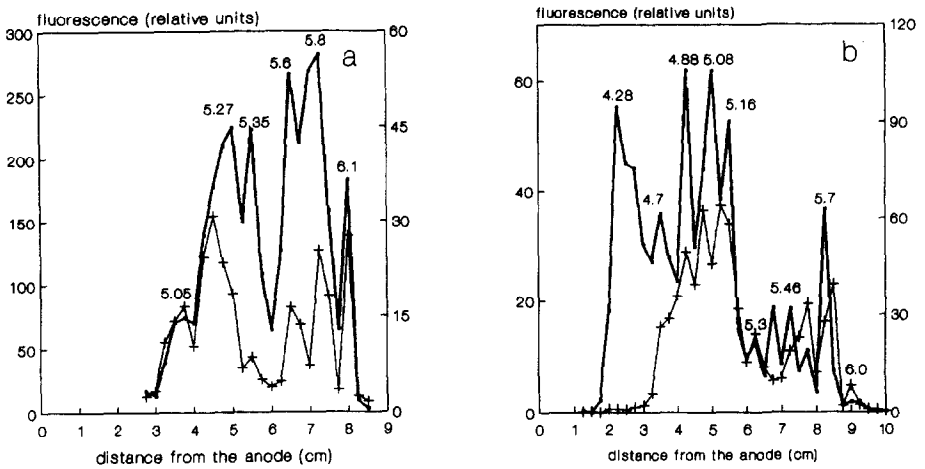


Fig. 1. Isoelectric focusing of intracellular (−, Y1-left) and secreted (-+-, Y2-right) α-L-fucosidase from embryonal human skin fibroblasts in the normal (a) and after sucrose load (b). Numbers near the peaks are p*I* values.

sucrose load, the value of intracellular α-L-fucosidase activity remained virtually unchanged in embryonal fibroblasts and was significantly decreased in postnatal fibroblasts cultures. Intralysosomal sucrose accumulation led to a 3-fold decrease of α-L-fucosidase secretion in embryonal fibroblasts and to its considerable increase in postnatal fibroblasts. In pathological cells, the tendency for the intracellular α-L-fucosidase activity to increase with non-hydrolyzable products' accumulation was clearly seen. The secretion level of α-L-fucosidase from these cells was significantly enhanced at stage II and markedly reduced at stage III, characterized by maximal intracellular accumulation.

### *3.2. Isoelectric focusing of the intracellular and secreted α-L-fucosidases from normal and pathological fibroblasts*

As seen from Fig. 1a, embryonal fibroblasts show similar spectra profiles for the intracellular and secreted enzymes, with the same quantity of isoforms and some variance in the isoform ratios in cells and culture medium. Sucrose load (Fig. 1b) led to significant changes in the isoforms' spectrum of the intracellular α-L-fucosidase: the appearance of new, more acidic enzyme forms with p*I* 4.28, 4.7, 4.88; predominance of acidic enzyme forms over more neutral ones; and virtual disappearance of the most basic form with p*I* 6.1. The isoforms' spectrum of secreted α-L-fucosidase was characterized by similar change in the ratio of acidic to basic forms, but the isoform with pi 4.28 was absent. As seen from Fig. 2a, isoelectric focusing revealed a close similarity between the isoforms' spectra of the intracellular and secreted α-L-fucosidase in normal postnatal fibroblasts. However, postnatal cells were characterized by the presence of two acidic enzyme forms with p*I* 4.4 and 4.7, which were absent from embryonal cells. After sucrose load, no marked changes were observed in the intracellular isoforms' spectrum as compared to normal (Fig. 2b). However, the secreted α-L-fucosidase was char-

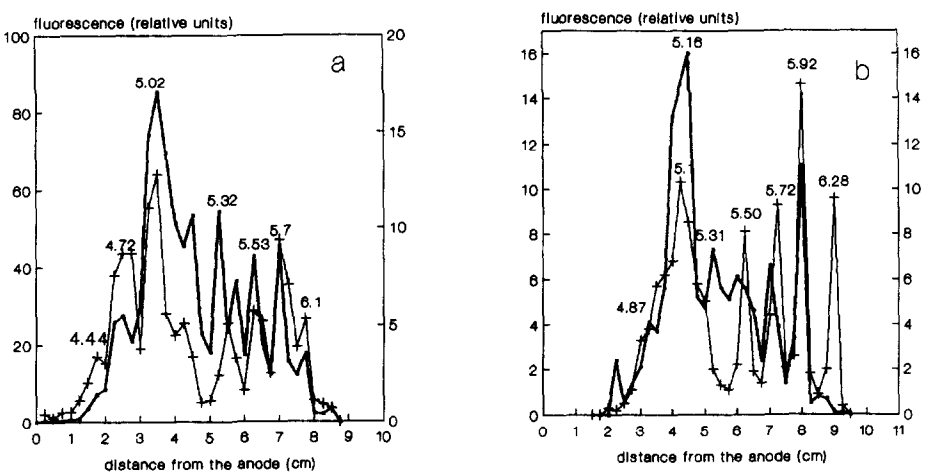


Fig. 2. Isoelectric focusing of intracellular (-, Y1-left) and secreted (-+-, Y2-right) α-L-fucosidase from postnatal human skin fibroblasts in the normal (a) and after sucrose load (b). Numbers near the peaks are p*I* values.

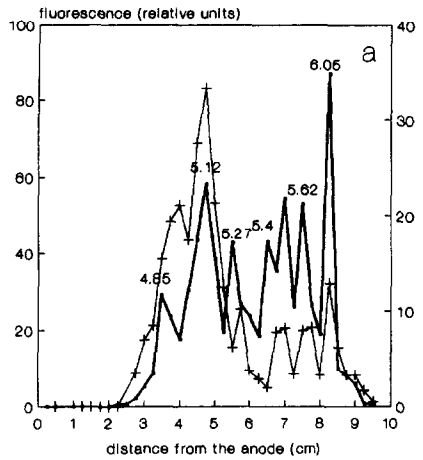


Fig. 3. Isoelectric focusing of intracellular (-, Y1-left) and secreted (-+-, Y2-right) α-L-fucosidase from Fabry patient skin fibroblasts at stage I. Numbers near the peaks are p*I* values.

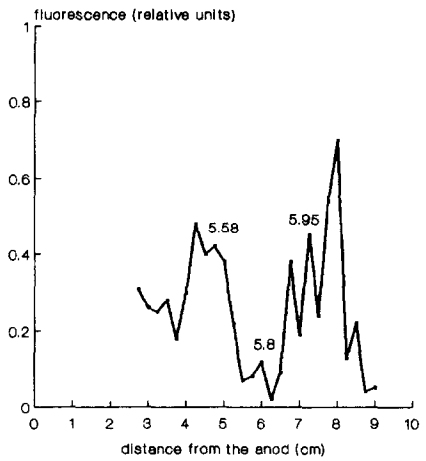


Fig. 5. Isoelectric focusing of α-L-fucosidase from a combined culture medium obtained at stage I and II. Numbers near the peaks are p*I* values.

acterized by a predominant expression of more basic forms and the appearance of an additional basic form with p*I* 6.28, which was not detected in the control.

As seen from Figs. 3 and 4 the isoforms' spectra profiles of the intracellular α-L-fucosidase from pathological cells remained virtually unchanged at stages I and II. However, the isoforms' spectra profiles of secreted α-L-fucosidase significantly varied from one stage to another. The differences were mostly characteristic of the acidic and basic isoforms' ratios. The early monolayer cells having minimal accumulation levels were characterized by a predominance of more acidic isoforms of secreted α-L-fucosidase over more neutral ones. At stage II, on the contrary, an expression of more basic isoforms of α-L-fucosidase was predominant. Isoelectric focusing of a combined medium from cell cultures of early and late monolayers (Fig. 5) revealed isoforms' spectrum characterized by the presence of both acidic and basic isoforms and corresponding on the whole to the intracellular α-L-fucosidase isoforms' spectrum.

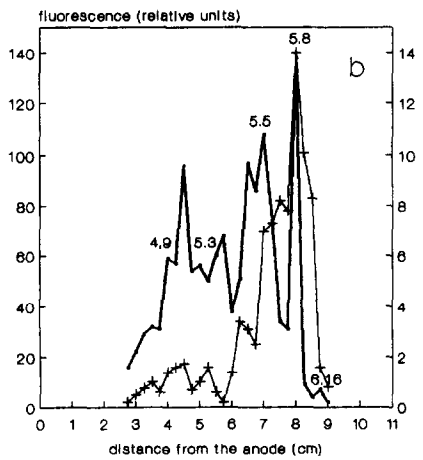


Fig. 4. Isoelectric focusing of intracellular (-, Y1-left) and secreted (-+-, Y2-right) α-L-fucosidase from Fabry patient skin fibroblasts at stage II. Numbers near the peaks are p*I* values.

## 4. Discussion

It was shown earlier that sucrose load leads not only to the quantitative but also to some qualitative changes in secreted enzymes [3]. Our data are also indicative of significant differences between the two fibroblast lines after sucrose load. Thus, the essential shift of α-L-fucosidase profile to the acidic region, as observed in embryonal cells, is probably due to the appearance of more sialylated α-L-fucosidase isoform. Support for this suggestion comes from the following facts: (1) neuraminidase treatment of sucrose-loaded cell extract caused the shift of the enzyme forms to a more neutral region and the disappearance of the acidic form with p*I* 4.28 (data not shown); (2) the pH-dependence of the intracellular α-L-fucosidase activity was also characterized by a slight shift to a more acidic region after sucrose load (data not shown). It is well known that pH-dependence of α-L-fucosidase is correlated with glycosylation, in particular with sialylation of the enzyme [6,14]. Furthermore, we have shown that α-L-fucosidase from two other fibroblast lines (embryonal line No. 845 and postnatal line No. 17) had different isoforms' spectra and pH-dependences; with predominance of more acidic forms an additional activity in more acidic pH values was observed. As for postnatal fibroblasts, we did not find any perceptable changes in the multiple forms' profiles of the intracellular α-L-fucosidase after sucrose load, but the enzyme from the culture medium was characterized by increase in activity of more neutral isoforms, as compared to controls, and by the corresponding changes in the pH-dependence profiles (Fig. 2b). It should be noted that the isoform patterns of α-L-fucosidase undergo certain changes during fetal development owing to an additional sialylation [15]; based on this fact we suggest that the more

pronounced effect of sucrose load on the enzyme from the embryonal cells is due to the insufficient sialylation on the α-L-fucosidase in the normal.

With regard to α-L-fucosidase from fibroblasts of a patient with Fabry's disease, the isoforms' spectrum of the intracellular α-L-fucosidase was similar to that from normal postnatal cells after sucrose load and was virtually unchanged throughout the accumulation of the natural compounds in the lysosomes. Unlike the intracellular α-L-fucosidase, the multiple forms' spectrum of the secreted enzyme was changed with the increase of fibroblasts' cultivation time and was characterized by a predominance of acidic forms at stage I and of more neutral ones at stage II – as the case of sucrose-loaded embryonal and postnatal fibroblasts, respectively. It should be noted that the shift of isoforms of some serum lysosomal hydrolases to a more basic region was also observed in the carbohydrate deficient glycoproteins syndrome [16], which is similar to the lysosomal storage disease.

Thus, the data obtained demonstrate that the sucrose load, which models the accumulation of nonhydrolyzable exogenous products, as well as the storage of natural compounds in the cells, both significantly affect the intracellular activity, secretion levels and isoforms' spectra of secreted α-L-fucosidase from human skin fibroblasts. The different responses of embryonal and postnatal fibroblasts to sucrose load and the accumulation of natural compounds in cells are probably due to the interrelated causes, in particular to the nature of a fibroblasts strain and of accumulated product, level of accumulation and other factors. Apparently, changes in the multiple forms' spectra of α-L-fucosidase show that intralysosomal accumulation of nonhydrolyzable compounds influences the α-L-fucosidase posttranslational processing, which appears to have some peculiarities in various cell lines. Whether these peculiarities are typical and regular for definite cell lines remains to be clarified. For this purpose it is necessary to investigate a greater number of normal and pathological cells with hypertrophied lysosomal compartment.

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