CYSTIC FIBROSIS: DEPRESSED α-FUCOSIDASE ACTIVITY IN CULTURED LYMPHOBLASTS

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1. Introduction

Cystic fibrosis (CF) is the most common genetically determined disease among Caucasians [1]. Obstruction of the epithelial-lined respiratory, gastrointestinal and genital–urinary tracts due to alterations in mucus secretions implicates an alteration in glycoprotein metabolism in the etiology of the disease. Dische and coworkers [2,3] first reported elevations of the fucose content of some mucoid fractions of duodenal secretions. Although these findings have not been consistently reproduced [4,5] the involvement of changes in the metabolism of complex carbohydrates in cystic fibrosis remains probable, with reports of changes involving fucose [6–9].

We have attempted to determine whether there may be either an elevated biosynthetic incorporation of this monosaccharide into complex carbohydrates or a reduced cleavage of the sugar from these molecules using cultured cells from CF patients [10]. We report here some evidence for the latter possibility from experiments employing cultured lymphoblasts. These are permanent cell lines established by transformation of peripheral blood lymphocytes with Epstein-Barr virus according to [11]. We found that the activity of α-L-fucosidase, an enzyme which cleaves fucose from glycolipids and glycoproteins is reduced 2–3-fold in cultured lymphoblasts derived from patients with cystic fibrosis relative to normals. This change may be involved in the alterations in the fucose content of some of the abnormal glycoproteins which contribute to the respiratory and glandular obstructions in the disease. Levels of activity of other acid glycosidases are not substantially altered.

2. Materials and methods

Lymphoblast cultures were diluted to 2×10^5 cells/ml (total vol. 1 l) with modified MEM (Eagle) supplemented with 15% fetal calf serum according to [12] and grown in spinner flasks at 37°C. At 24 h intervals 50 ml aliquots were removed, pelleted at $400 \times g$ for 10 min and washed once with 50 ml phosphate-buffered saline (PBS) and once with 15 ml of the same. The washed cells were resuspended in 0.5 ml PBS, frozen, thawed and homogenized with 15 strokes in a Potter homogenizer. The homogenates were assayed for protein as in [13] and for α-L-fucosidase according to [14] with incubation for 60 min at pH 5.5 in 1 mM 4-methylumbelliferyl-α-L-fucoside (Koch-Light). The pH dependencies of the enzyme in both normal and CF cell homogenates were determined and optimal activity was between pH 5.0 and 6.0. The other enzymes were assayed in 40 mM citrate–sodium phosphate (pH 4.1) using amounts of cell protein within the linear range of dependence of activity on protein and at saturating substrate concentration as follows: 1 mM 4-methylumbelliferyl-α-D-glucopyranoside for α-glucosidase; 10 mM 4-methylumbelliferyl α-D-mannopyranoside for α mannosidase; 3 mM 4-methylumbelliferyl-2-acetamide-2-deoxy-β-glucopyranoside for hexosaminidase; and 1 mM 4-methylumbelliferyl-β-D-glucuronide for β-glucuronidase.

3. Results

Initial experiments in which α-L-fucosidase activities were assayed in homogenates of cells at a single

stage of cell growth consistently showed lower levels in CF cell lines than normals. However, the magnitudes of these differences were variable. Therefore, specific enzyme activities were determined at various points along the entire growth curve. Fig.1a illustrates the growth curves and specific fucosidase activities for one age- and sex-matched pair of CF and normal cells. The specific activity in the dilute cells at the beginning of the growth curve was ~ 25% less in the CF than the normal. While the activity of the normals increased till the end of log phase and then began to decline during stationary phase, the activity in the CF cells gradually decreased over the entire growth curve. As a result, the difference increased to ~ 3-fold at the transition from log to stationary phase. To establish that the difference was really a manifestation of the CF genotype rather than unique to this particular pair, 5 normal cell lines and 5 CF lines were utilized for the same type of experiment (fig.1b). The two groups of cells clearly fall into distinctly different categories having characteristics similar to those of the normal and CF profiles in fig.1a. The activities in the 5 normal lines rise with growth and are higher than all 5 CF lines. The other two most notable features include the diminished growth dependence of the enzyme activity in the CF cell lines and the smaller degree of variability among the different CF lines than among the normals. The latter observation tends to strengthen the correlation between the properties of this enzyme and the CF genotype.

We do not yet know why the deficiency of this enzyme in CF lymphoblasts is quantitative rather than absolute. However, although metachromatic inclusions have been claimed in CF cells [15], there is no indication of extensive intracellular accumulation of glycolipids or glycoproteins such as occurs in some lysosomal storage diseases including fucosidosis [16,17]. Hence, it seems likely that fucose-containing compounds in general are degraded at a reduced level or that only the cleavage of some of them is reduced in CF. Experiments with a variety of natural substrates rather than the synthetic one employed in these experiments will be required to test the latter possibility. It is notable that elevated sweat chloride, the diagnostic feature of CF [18] is also sometimes seen in fucosidosis where α-L-fucosidase is more totally lacking [19].

We were also able to detect reduced enzyme activity in partially purified lysosomal fractions of CF lymphoblasts. Other lysosomal glycosidases were also assayed in homogenates of the same cells used in the fucosidase

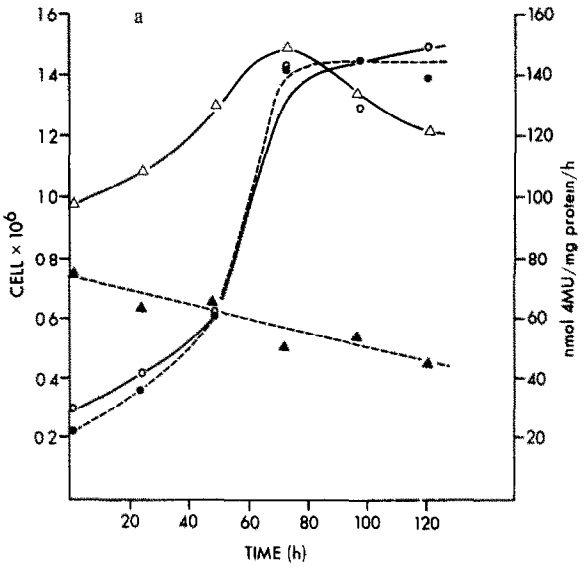

Fig.1a. Effect of cell density on α-L-fucosidase specific activity in normal (△) and CF (▲) lymphoblasts. The corresponding growth curves of the normal (○) and CF (●) cells are shown as well. The normal cell line was no. 93 derived from a female aged 4. The CF line was no. 115 derived from a patient with cystic fibrosis of the same age and sex.

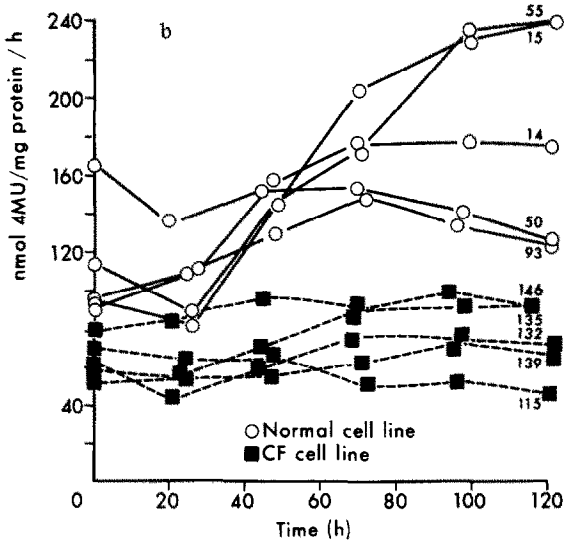

Fig.1b. Results of the same types of experiments described in fig.1a for other cell lines: (○) normal; (■) CF lines. The cell lines which could be matched as to sex of the donor and roughly according to age were as follows. Normals: no. 14, male aged 22; no. 15, male aged 3; no. 50, male aged 9; no. 55, male aged 11; no. 93, female aged 4. Patients with cystic fibrosis: no. 115, female aged 4; no. 132, male aged 18; no. 135, male aged 9; no. 139, male aged 9; no. 146, male aged 14.

Table 1
Mean specific activities of several glycosidases assayed on the same cell lines as in fig.1b

Enzyme	Growth time	Specific activity ± SD (nmol . mg protein^{-1} . h^{-1})					
		0 h	22 h	44 h	71 h	96 h	120 h
α-Fucosidase	Normal	115 ± 30	109 ± 19	144 ± 40	171 ± 61	177 ± 65	177 ± 64
	CF	56 ± 18	53 ± 21	60 ± 24	67 ± 25	70 ± 26	71 ± 29
β-Hexosaminidase	Normal	302 ± 66	250 ± 59	323 ± 79	399 ± 65	414 ± 90	415 ± 91
	CF	265 ± 90	277 ± 14	312 ± 13	370 ± 89	356 ± 69	378 ± 70
α-Glucosidase	Normal	4.6 ± 2.5	3.9 ± 2.5	4.2 ± 3.0	5.7 ± 5.6	6.4 ± 6.1	7.0 ± 6.4
	CF	8.1 ± 6.5	7.0 ± 1.2	7.5 ± 1.2	8.0 ± 0.9	8.6 ± 0.6	9.5 ± 0.6
β-Glucuronidase[a]	Normal	11.6 ± 5.1	19.5 ± 11.7	27.3 ± 13.2	50.7 ± 5.2	67.3 ± 6.6	77.3 ± 13.6
	CF	12.0 ± 6.0	29.2 ± 3.1	38.2 ± 3.2	39.5 ± 4.0	56.6 ± 14.2	69.7 ± 8.4
α-Mannosidase[a]	Normal	1.5 ± 1.0	12.5 ± 12.0	12.2 ± 5.8	19.3 ± 5.2	23.7 ± 5.4	36.3 ± 8.9
	CF	2.3 ± 0.4	11.3 ± 4.2	17.7 ± 6.2	16.5 ± 0.5	35.0 ± 7.0	53.0 ± 11.7

[a] For these two enzymes the mean values were obtained from only 3 of the 5 pairs of cell lines

experiments (table 1). In contrast to the clear-cut reduction in α-fucosidase activity in the CF cells, no significant differences were observed between normal and CF cells in the other 4 enzymes. It is notable that there was a trend to higher levels of α-glucosidase in CF cells even though the standard deviations overlapped those of the normal cells; the level of this enzyme is elevated in CF sera [20]. All of these enzymes except α-mannosidase (and α-L-fucosidase) had been measured earlier in CF lymphoblasts and were also not found to differ from normals [21]. Thus the depressed level of α-L-fucosidase appears to be a highly specific change in CF.

4. Discussion

At present it seems likely that this enzymic change in transformed lymphoid cell lines will be more valuable as an experimental tool for deeper delving into the biochemical mechanism of the disease rather than as a marker for diagnosis or screening. This is because the enzyme may be expressed differently in different tissues [22] and its activity may not be reduced in cultured fibroblasts from CF patients [23]. This latter point remains to be firmly established, however, since fibroblast fucosidase has been reported to be: elevated [23]; unchanged or moderately diminished [24]; or after stimulation of cells, reduced [25]. Nevertheless, even if the enzyme is not found to be depressed in fibroblasts, there are other well-documented examples of variable alterations of lysosomal hydrolases in different tissues in storage diseases such as I-cell disease in which a multiple deficiency of lysosomal acid hydrolases is seen in fibroblasts but not in lymphoid cells [26].

Although there may be an enhanced leakage of some lysosomal enzymes in CF [25], in our experiments α-L-fucosidase was not released from CF lymphoblasts to any greater extent than from normals [27]. The results of other studies which we have performed to assess the nature of the depression of α-L-fucosidase activity in CF lymphoblasts [27] may be summarized as follows. Mixing experiments failed to detect any inhibitor of normal enzyme activity in CF cells. Kinetic experiments indicated that K_m 62 μM was not different between normal and CF cells. The lower activities in CF cells were not due to a larger pool of cryptic enzyme since they persisted after total extraction of the enzyme with detergent or by sonication. Furthermore, when purified from CF lymphoblasts, the enzyme has qualitatively similar isoelectric forms and subunit M_r to the normal. Hence, while the magnitude of the enzyme activity is reduced in CF lymphoblasts, several properties are qualitatively unchanged.

Acknowledgements

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