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Patterns of α-L-fucosidase in acute myeloid leukemia cells. Comparison with promyelocytic HL-60 cell line *

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ABSTRACT

Changes were observed in α-L-fucosidase forms in cells from acute myelocytic leukemias (AML). Total α-L-fucosidase activity was not significantly different for normal granulocytes and leukemic cells, but enzymic profiles obtained by chromatofocusing are quite different. In granulocyte profile, two main peaks are present (B and more acidic A) which were eluted at pH 5.2 with a shoulder at pH 4.6. In AMLs the B form is present but weakly expressed, whereas the more acidic forms are the major ones. This pattern may be related either to the malignancy character or to the stage at which the differentiation is stopped. Experiments on an HL-60 cell line (promyelocytic cells corresponding to the AML 3 type) showed that differentiation induced by dimethyl sulfoxide leads to the appearance of the B form present in normal mature cells. Thus the repartition of the enzyme forms seems to be related to the stage of differentiation of the myelocytic cells.

INTRODUCTION

α-L-Fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51), an acid hydrolase that has been ubiquitously found in human tissues and extracellular fluids, is a cellular enzyme being located in lysosomes[1–4]. It plays an important role in glycoprotein and glycolipid catabolism, and its generalized deficiency leads to an inborn error of metabolism, fucosidosis.

Several accounts of alterations to the isoenzymes of different glycosidases in human leukemic cells were reported[5,6]. Particularly abnormal patterns of α-L-fucosidase expression have been recognized in different types of leukemias. In cultured lymphoid cells, α-L-fucosidase is synthesized as a precursor form that is

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processed to mature forms either intra- or extra-cellularly. All three enzymes forms are glycoproteins with a common polypeptide chain but with different carbohydrate components.

We describe herein the changes observed in some leukemic cell populations representative of acute myelocytic leukemias (AML). HL-60 cells provided a useful tool to determine whether the observed modifications are related to the malignancy or to the state of differentiation of the cells, as HL-60 is a promyelocytic cell line that is able to proliferate in vitro without changes in its state of differentiation[7]. Differentiation along the pathway towards granulocytes can be induced by treating the cells in culture with dimethyl sulfoxide[8] (Me_2SO), and the effects of Me_2SO on α-L-fucosidase patterns was examined.

EXPERIMENTAL

Materials and methods.—4-Methylumbelliferyl α-L-fucopyranoside was purchased from Sigma Chemical Co (St Louis, MO). Polybuffer exchanger PBE-94, polybuffer 74, and Ficoll–Hypaque were from Pharmacia-LKB (Bois d'Arcy, France). RPMI 1640 medium was obtained from Seromed Biochrom KG (Berlin, FRG) and fetal calf serum (FCS) from Biological Industries (Israel).

Leukemic cells were obtained from peripheral blood samples of patients with acute myelocytic leukemias (AML 1–4 according to the FAB classification: AML 1, undifferentiated acute myeloblastic leukemia; AML 2, well differentiated acute myeloblastic leukemia; AML 3, acute promyelocytic leukemia; and AML 4, acute myelomonoblastic leukemia). Leukemic cells were used without purification since the percentage of blasts in the peripheral blood was $> 90\%$ of total leucocytes in each case.

Cell culture.—The HL-60 cell line was cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal calf serum in an humidified atmosphere containing 5% CO_2. Treatment of cells with Me_2SO was performed as follows. Cells were subcultured in fresh medium (2×10^5 cells/mL) and, after 24 h, when the cell concentration was 3×10^5 cells/mL, Me_2SO was added to give a final concentration of 1.25% (v/v). The cells were harvested after 3, 4, and 5 days of exposure to Me_2SO.

Preparation of cell lysate and enzyme extraction.—The cells (2×10^7), after three washings with 0.9% NaCl (2 mL), were disrupted by successive freezing and thawing, after which 0.02% (v/v) Triton X-100 was added. After 30 min at 4°, the mixture was centrifuged 20 min at 2000*g*. All procedures were carried out at 4°. The supernatant was used as cell lysate.

Enzyme assay.—The enzymic activity was determined with mM 4-methylumbelliferyl α-L-fucopyranoside in 0.1 M sodium citrate–sodium phosphate buffer, pH 5.4. After incubation at 37° and alcalinization with a 0.2 M glycine–NaOH (pH 10) buffer, the liberated 4-methylumbelliferone was measured at an excitation wavelength of 356 nm and an emission wavelength of 432 nm. The results are expressed in units corresponding to nmol of substrate hydrolyzed h^{-1} mL^{-1} or $(10^6 \text{ cells})^{-1}$.

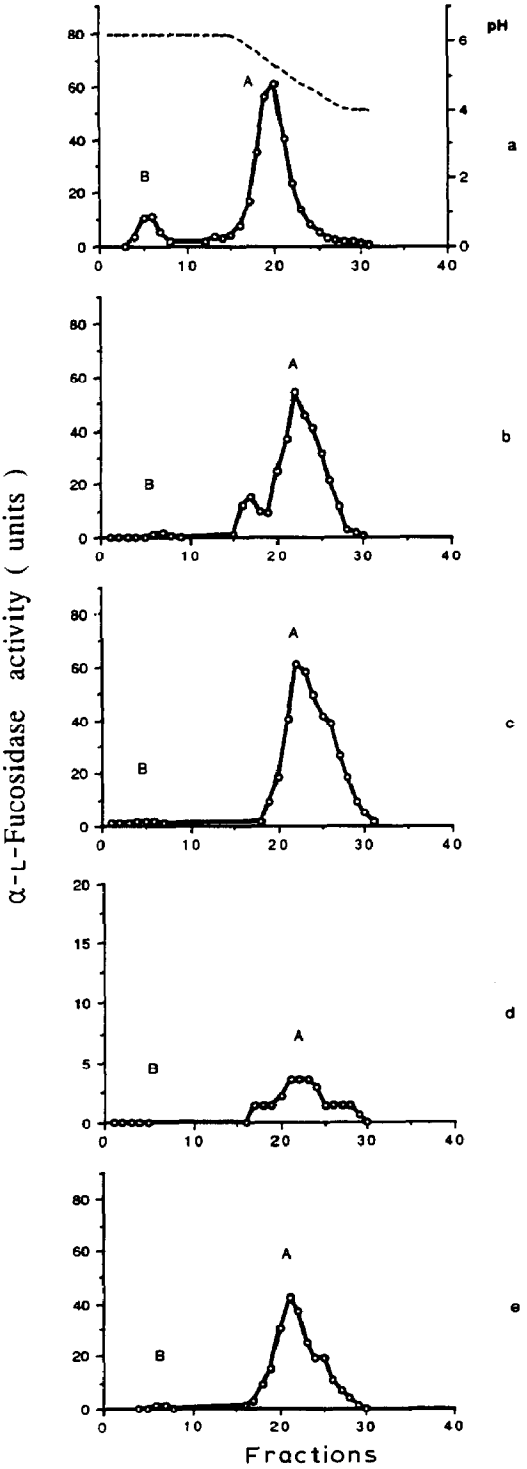

Fig. 1. Chromatofocusing profiles on PBE-94 of α-L-fucosidase of: (a) normal granulocytes, (b) AML 1, (c) AML 2, (d) AML 3, and (e) AML 4; (— — —) pH gradient generated by a PB 74 buffer, pH 4.0 and (○———○) α-L-fucosidase activity [nmol h^{-1} (10^6 cells)$^{-1}$].

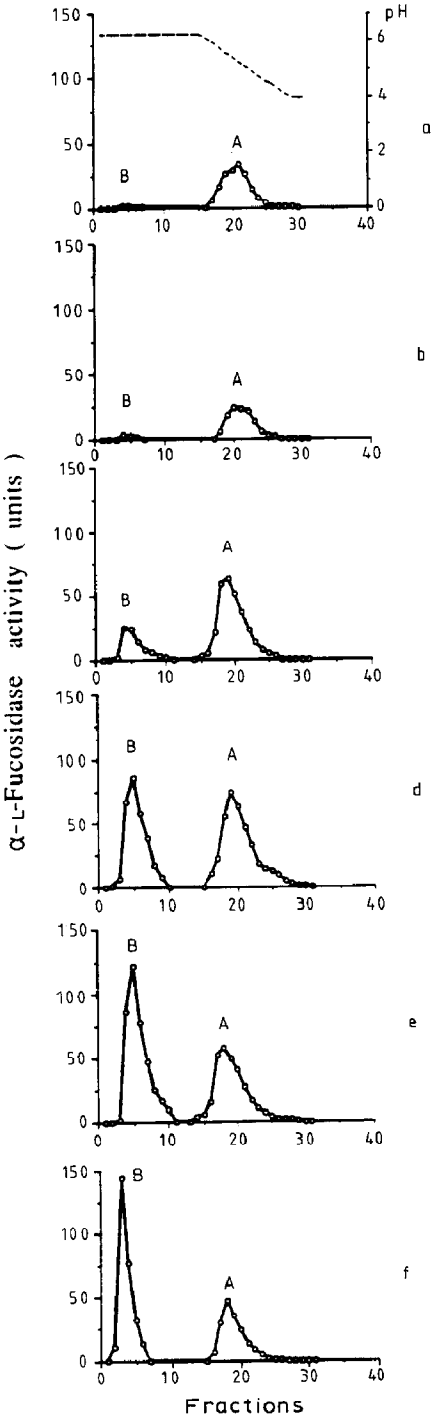

Fig. 2. Chromatofocusing profiles on PBE-94 of α-L-fucosidase of HL-60 cells: (a) without Me_2SO, (b), (c), (d), (e), and (f) in the presence of Me_2SO, after 2, 3, 4, and 5 days, respectively; (— — —) pH gradient (as in Fig. 1), and (○———○) α-L-fucosidase activity [nmol h^{-1} (10^6 cells)$^{-1}$].

Chromatofocusing.—Chromatofocusing was performed at room temperature on an anion exchanger PBE-94 column (1 × 5 cm) equilibrated with a 25 mM histidine–HCl buffer, pH 6.2. A pH gradient (pH 6.2–4.0) was formed automatically as the elution buffer (PB-74 buffer, pH 4.0, 1.5-fold the column volume) titrated the charged groups on the ion exchanger. After the sample (1 mL of an appropriate dilution corresponding to at least 10^7 cells) had been adsorbed, the elution was performed with the PB-74 buffer at a 20 mL h^{-1} flow rate. Fractions (2.5 mL) were collected at 4°C.

RESULTS

Total α-L-fucosidase activity, expressed as units $(10^6 \text{ cells})^{-1}$, was not significantly different for normal granulocytes and leukemic cells (5 ± 1.5).

Enzymic profiles from different types of leukemic cells and normal cells appear different. In granulocytes, two main peaks were separated, one (B) not retarded by the column, and one (A) eluted at a more acidic pH. The complex shape of the A peak suggested the presence of more than one component, one major peak eluted at pH 5.2 with a shoulder at pH 4.6. Profiles from AML cells varied from case to case, both in their elution pH and in their height relative to the other components (Fig. 1). In AML cells, the B form was present but weakly expressed, whereas the more acidic forms were the major ones (pH 5.2, 4.6). Moreover, the shoulder, observed at pH 5.8 in granulocytes and in AML 1 cells, disappeared in the other myeloid leukemic cells. Differences in profiles of AML cells, when compared to normal granulocytes corresponding to the last step of differentiation of the myeloid cell line, could be related either to the stage of differentiation or to the malignancy.

α-L-Fucosidase enzymic profiles from HL-60 cells grown in the absence of, and in the presence of 1.25% Me_2SO (Fig. 2), show that the main effect of Me_2SO treatment was a progressive increase of the B form during cell growth. In the absence of Me_2SO, the B form represented $< 25\%$ of the total activity, whereas after five days of growth, it was greatly increased to $> 50\%$. On the other hand, the shape of the A peak was unchanged.

DISCUSSION

Altered enzymic profiles were found for numerous glycosidases in leukemic cells, mainly for *N*-acetyl-β-D-hexosaminidase[9–11], α-L-fucosidase profiles could distinguish lymphoid from myeloid cells[11]. In order to determine whether quantitative and qualitative changes observed in α-L-fucosidase activity were related to the step of differentiation of leukemic cells, we studied myeloid cells corresponding to AML 1, AML 2, AML 3, and AML 4. As compared with normal granulocytes, all AML cells possessed similar total activities, a finding confirming those of Besley et al.[12] and Orlacchio et al.[13]. To characterise further possible qualitative modifica-

tions in these leukemic cells, enzymic forms were separated by chromatofocusing. The less acidic B form was not expressed in leukemic cells corresponding to the more differentiated stages.

The abnormalities observed in lysosomal hydrolase profiles seem to be a general phenomenon since lysosomal enzymes have certain features in common. The precise reason for the alteration of lysosomal enzyme activities or patterns of expression in leukemia is not well known. However, lysosomal enzymes are glycoproteins and a number of changes involving the oligosaccharide structure of glycoproteins and glycolipids have been associated with forms of malignancy and cellular differentiation.

To study the possible role of differentiation, we used the promyelocytic HL-60 cell line whose differentiation into granulocytes can be induced by Me_2SO. In untreated cultured cells, profiles similar to those obtained with AML cells were observed, whereas during growth in the presence of Me_2SO the B form present in mature cells progressively increased; 0.4% metamyelocytes were present in untreated cells, whereas mature cells represented, respectively, 23, 42, and 50% after 3, 4, and 5 days of exposure to Me_2SO. After 72 h of culture in the presence of Me_2SO the B form appeared and greatly increased after 4 and 5 days. These results are consistent with the observation that the first distinct morphological changes appear two days after addition of the inducer, and that full maturation into metamyelocytes and mature granulocytes occurs only after 3–4 days[14,15]. Moreover, similar changes in enzymic profiles have been reported for *N*-acetyl-β-D-hexosaminidase. Thus the B form seems to be related to the maturation steps.

The experiments reported herein do not clarify the mechanism of the effect described. Nevertheless, it is clear that induction of differentiation of HL-60 cells with Me_2SO restores the formation the α-L-fucosidase B form present in normal differentiated cells.

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