HUMAN LEUKOCYTIC ACID α-D-MANNOSIDASE IN HEALTH AND IN CHRONIC MYELOID LEUKEMIA

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KEY WORDS: granulocytes; chronic myeloid leukemia; lysosomal enzymes; α-D-mannosidase.

Acid α-D-mannosidase (EC 3.2.1.24) is found mainly in lysosomes, it catalyzes the removal of single mannose residues from the carbohydrate moiety of glycoproteins, and it is one of the principal enzymes involved in the catabolism of glycoproteins, especially those containing oligosaccharides with an oligomannosyl structure, which are components of cell membranes [9, 13]. Since the structure of the cell surface glycoproteins is changed in leukemias, the study of the properties of leukocytic α-mannosidase in this disease is of definite interest. Most information about the properties and composition of acid α-mannosidase isozymes has been obtained by the study of this enzyme isolated from the liver and kidneys [8, 11]. Data on the properties of α-mannosidase of normal leukocytes are sparse and incomplete [6, 12]. There are indications that in some leukemias leukocytic α-mannosidase activity is modified, and that these changes are accompanied in some cases by a change in its isozyme spectrum, but these isozymes have never been characterized [2, 4, 10, 14].

The aim of this investigation was a comparative study of the properties and composition of granulocytic α-mannosidase isozymes from normal blood donors and from myeloid cells of patients with chronic myeloid leukemia (CML).

EXPERIMENTAL METHOD

Leukocytes were isolated from blood of healthy blood donors and patients with CML with a leukocytosis of over 3×10^7 cells/ml. Leukocytes were sedimented with a dextran mixture and fractionated by the method in [1] in a Ficoll–Verografin system. The granulocyte fraction was obtained from the donors' blood after hypotonic hemolysis, whereas a fraction of morphologically immature leukemic cells and morphologically mature granulocytes was obtained from the patients' blood. The cells were counted in a Goryaev's chamber. Extraction was by 0.1% Triton X-100 at 4°C overnight. The extracts were centrifuged at 17,000g for 60 min and the supernatant was used as the source of the enzyme. To determine α-mannosidase activity, 4-methylumbelliferyl-α-D-mannopyranoside (MUMP) was used as the substrate. The incubation mixture (60 µl) contained 1.5 mM MUMP, citrate–phosphate buffer (pH 4.0), and the enzyme preparation. Incubation continued for 30 min at 37°C. Fluorescence was measured on the BIAN-130 fluorometer (wavelength of excitation 365 nm, of emission 436 nm). The unit of activity was taken to be the quantity of enzyme which catalyzed removal of 1 nmole 4-methylumbelliferol in 1 min. The α-mannosidase isozymes were separated on a column with DEAE-cellulose, equilibrated with 0.01M Na-phosphate buffer (pH 6.0). Enzyme not bound with the ion-exchange resin was eluted with the same buffer. Bound mannosidase was eluted by means of a KCl concentration gradient (0–0.5M). Samples containing α-mannosidase activity were pooled, concentrated by filtration on XM-50 membrane (Amicon, The Netherlands), and used for the comparative study of the properties of the enzyme. The relative molecular weight of the mannosidase was determined by electrophoresis in a concentration gradient of polyacrylamide gel (PAG) of 5–23.7% in Tris-Veronal buffer (pH 7.0) for 48 h with a voltage of 150 V, a set of proteins (Pharmacia, Sweden) was used as the standards. Enzyme activity in the gel was detected by incubation with MUMP. Values of K_m for mannosidase were determined graphically by the Lineweaver–Burk method. The results were calculated by a program suggested by V. A. Pekkel' [3]. Neuraminidase treatment was carried out for 3 h at 37°C, by passing the enzyme preparation through a column with immobilized neuraminidase from *Clostridium perfringens* (type VI, Sigma, USA).

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TABLE 1. Acid α-Mannosidase Activity and Ratio between A and B Forms in Donors' Granulocytes and Cells from Patients with CML (n = 8; M ± m)

Type of cells	Activity, $U/10^8$ cells	Ratio between forms
Morphologically immature cells from CML patients	108.6 ± 8.4*	1.31 ± 0.160*
Morphologically mature cells from CML patients	63.0 ± 12.7	0.67 ± 0.065
Donors' granulocytes	49.3 ± 5.7†	0.60 ± 0.056†

Legend. *) Difference between α-mannosidase activity and ratio between forms B/A of morphologically immature and mature cells is statistically significant ($p < 0.01$); †) difference between α-mannosidase activity and ratio between forms B/A of granulocytes and morphologically mature cells from patients is not statistically significant ($p < 0.5$).

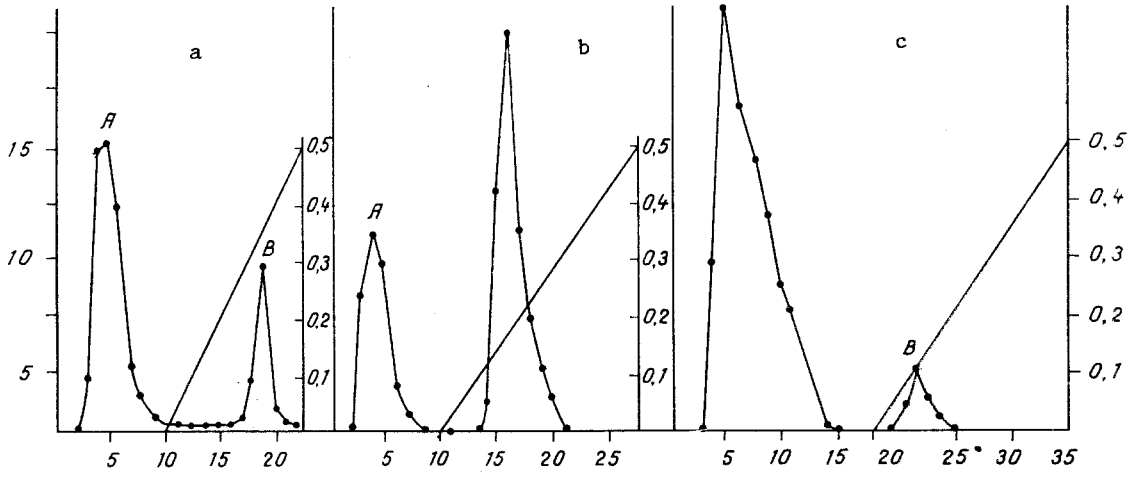

Fig. 1. Fractionation of acid α-mannosidase on DEAE-cellulose. a) α-Mannosidase from donors' granulocytes; b) α-mannosidase of morphologically immature cells; c) α-mannosidase of morphologically immature cells after neuraminidase treatment. A and B) A and B forms of α-mannosidase. Abscissa, no. of fractions; ordinate: on left — α-mannosidase activity (in $U/10^8$ cells), on right — KCl concentration (in M).

EXPERIMENTAL RESULTS

As Table 1 shows, acid α-mannosidase activity of morphologically immature cells from patients with CML was about twice as high as the activity of this enzyme in morphologically mature cells isolated from the blood of the same patient. α-Mannosidase activity in the donors' granulocytes and in the mature cells from CML patients was virtually identical. It can be concluded from these data that increased α-mannosidase activity is characteristic of leukemic cells only, i.e., it is not the result of a general increase in α-mannosidase activity in the leukocytes. Comparative determination of K_m values for α-mannosidase from leukemic cells and normal granulocytes revealed no differences between them. The following values of K_m were obtained: for leukemic cells 0.99 ± 10^{-3} M (SV ± 0.12), and for the patients' granulocytes 0.95 ± 10^{-3} M (SV ± 0.08). The α-mannosidase of both leukemic and normal cells had an optimum of action at pH 4.0, it was stable on heating to 56°C and at pH 6.0 for 30 min, but lost its activity when kept at pH 4.0 for 30 min at 37°C in the absence of substrate. Thus the increased α-mannosidase activity in the leukemic cells was unconnected either with increased affinity of the enzyme for substrate or with increased stability of the enzyme.

Isozymes of acid α-mannosidase were studied by fractionation of the enzyme on DEAE-cellulose and by PAG electrophoresis. Both methods revealed two principal isozymes in both normal and leukemic cells: the A form, with lower electrophoretic mobility and not held up on

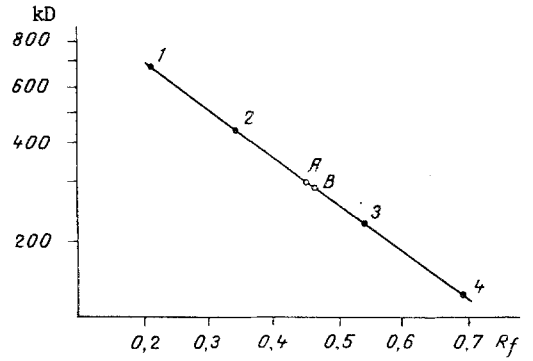

Fig. 2. Determination of relative molecular weight of A and B forms of acid α-mannosidase by PAG electrophoresis. 1) Thyroglobulin (669 kD), 2) ferritin (440 kD), 3) catalase (232 kD), 4) lactate dehydrogenase (140 kD). A and B — A and B forms of α-mannosidase. Abscissa, R_f; ordinate, mol. wt. (in kD).

DEAE-cellulose, and the B form, with higher electrophoretic mobility, and retained in the ion-exchange resin (Fig. 1a, b). According to the parameters mentioned above these enzymes were analogous with the A and B forms found in human liver and fibroblasts [7, 8]. On rechromatography of the separate forms immediately after fractionation, and also after keeping for 10 days in a refrigerator, mutual interconversion of the isozyme was not observed [5]. The ratio between A and B forms in leukemic and normal cells differed significantly (Table 1). Whereas the A form predominated in normal granulocytes and the B/A ratio averaged 0.6, in leukemic cells the B form predominated and the B/A ratio was 1.31. The ratio between α-mannosidase isozymes in granulocytes from healthy blood donors was virtually indistinguishable from that in the patients' mature cells. This was in agreement with data obtained by determination of α-mannosidase activity in these cells. It can be tentatively suggested that the increase in total α-mannosidase activity in leukemic cells is connected with an increase in the content of the B form.

To discover any differences in the degree of sialation of the A and B forms, the enzyme preparation was treated with neuraminidase. Under these circumstances a marked decrease in content of the B form and an increase in the content of the A form were observed (Fig. 1c). This indicated a higher degree of sialation of the B form. Determination of the molecular weights of the A and B forms of α-mannosidase showed that molecular weight of the A form is somewhat higher than that of the B form (A about 300 kD, B about 290 kD; Fig. 2). After treatment with neuraminidase the difference in molecular weights of the A and B forms was still present. The molecular weights of the A and B forms of leukemic and normal cells were virtually identical.

It can be concluded from these results that α-mannosidase of the B form, which is more highly sialated and has a lower molecular weight than the A form, accumulates in the immature cells of leukemia patients, whereas the less sialated A form predominates in mature differentiated granulocytes. Whether this redistribution of α-mannosidase isozymes is a process which accompanies normal differentiation of myeloid cells or whether it is the result of malignant transformation cannot yet be decided.

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EFFECT OF MONONUCLEAR PHAGOCYTE SYSTEM DEFICIENCY AND OF YEAST POLYSACCHARIDE INJECTIONS ON HETEROTOPIC BONE MARROW FORMATION

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Processes of hematopoiesis, controlled by secretion products of monocytes and macrophages [7, 9] and normally taking place in an ectopic focus, are disturbed as a result of blood loss or irradiation of the recipients [4]. The cause of this destabilization of hematopoiesis may also be deficiency of the mononuclear phagocyte system (MPS) which may arise for various reasons (as a result of disease, or created artificially). One model of artificially created MPS deficiency is based on chronic drainage of the peritoneal cavity of mice, with the use of an irrigating solution. Under these circumstances the quantitative and qualitative parameters of function of mononuclear phagocytes are disturbed [6].

This paper describes the results of a study of the effect of MPS deficiency on the formation of a heterotopic focus of hematopoiesis and of the action of yeast heteropolysaccharide, a stimulator of MPS, under these conditions [2].

EXPERIMENTAL METHOD

The extracellular heteropolysaccharide produced by *Cryptococcus luteolus*, strain 228 [1], was used. Experiments were carried out on 120 male (CBA × C57BL)F_1 mice; the peritoneal cavity of 50 mice was drained by the method described in [6] to exhaust MPS. Half of the mice subjected to irrigation in this way were used as donors of bone marrow (donors with MPS deficiency), which was implanted beneath the renal capsule of intact recipients. The other half of these animals were used as recipients of bone marrow from normal donors (the recipients had MPS deficiency). The polysaccharide was injected into these and other recipients intraperitoneally for 30 days after implantation of bone marrow, in a dose of 25 mg/kg every 7 days. Control recipients were given injections of physiological saline.

The heterotopic foci which formed were compared with foci in intact recipients, in which bone marrow from intact donors was implanted. The mice were killed by dislocation of the spine 30 days after implantation of bone marrow. The dimensions of the heterotopic foci formed were estimated from the number of cells and the weight of the bony capsule. The number of hematopoietic cells in the femoral medullary cavity also was determined.

EXPERIMENTAL RESULTS

After drainage for 10 days the number of cells in the peritoneal cavity was appreciably reduced (Table 1). After 30 days the content of peritoneal cells was increased by 1.3 times, although it was still only half the original value.

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