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Isoenzyme Profiles of α-Mannosidase and β-Galactosidase in Leukemic Cells*)

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ABSTRACT

The isoenzyme profiles of α-mannosidase and β-galactosidase in leukemic cells were examined with DEAE-Sephadex chromatography. A clear difference of these constituent isoenzymes was found between normal lymphocytes and granulocytes. In leukemic cells, the components of α-mannosidase eluting early (fraction I to III) from the chromatography were specific for myelogenous leukemia. No case of acute non-T/non-B lymphocytic leukemia (ALL) or T-cell leukemia demonstrated these components. Leukemic cells except for T-cell leukemia also demonstrated significant increase of an α-mannosidase component which eluted with 0.2 M of KCl (fraction VI).

There was no type specific alteration of β-galactosidase isoenzymes among examined leukemias. ALL and T-cell leukemia had isoenzymes of β-galactosidase similar to those of normal lymphocytes. However, an increase of an β-galactosidase component eluted with 0.3 M of KCl (fraction VII) was found in acute myelocytic leukemia and chronic myelo (mono) cytic leukemia.

These enzyme analysis may be useful for investigating intrinsic abnormalities in leukemic cells.

INTRODUCTION

Studies of lysosomal enzymes are a useful way of classifying leukemic cells, however, they have generally been limited to cytochemical analysis. Recently, biochemical analysis of constituent enzymes has been applied to malignant cells[5,7,9,13]. In previous communications we reported that activities of lysosomal hydrolases in leukemic cells showed characteristic quantitative alterations in different types of leukemia[15]. The level of activities of β-hexosaminidase, α-mannosidase and β-galactosidase indicated a clear difference between lymphocytic and myelo (mono) cytic leukemias[15]. We also demonstrated the abnormal expressions of β-hexosaminidase isoenzymes in acute non-T/non-B lymphocytic leukemia (ALL), which was consistent with the findings reported by Ellis et al.[8], and subtle differences in the profiles of acute myelocytic leukemia (AML)[16].

In this study, we undertook to analyse the isoenzyme profiles of α-mannosidase and β-galactosidase using DEAE-Sephadex chromatography and to show the qualitative alterations in leukemic cells.

MATERIALS AND METHODS

The diagnosis of leukemia was made according to established criteria[10]. Thirty-five patients with leukemia included in this study were reported previously[15]. Informed consent was obtained from the patients and/or guardians. Details of materials and methods used in this study were reported previously[15,16]. Leukemic cells were separated by gradient centrifugation using sodium metrizoate-Ficoll solution[2]. The purity of leukemic cells used in this chromatographic analysis was than 85%. Control lymphocytes were obtained from healthy laboratory

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personnel (24-35 years of age; n=8).

Cells were homogenized in 0.1% (w/v) triton X-100. The supernatant after centrifugation at 450×g for 10 min. at 4°C was used as an enzyme sourse (whole homogenate). The activities of α-mannosidase and β-galactosidase were determined by a method using 4-methylumbelliferyl-α-D-mannopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside[14]. The concentration of the substrate in reaction mixture (0.2 ml) was 0.1 mM in 0.1 M acetate buffer, pH 4.5 (α-mannosidase) and pH 4.0 (β-galactosidase). The reaction was terminated by the addition (3.3 ml) of 50mM glycine-NaOH buffer, pH 10.4, with 5mM EDTA. The fluorescence was measured in a fluorescence spectrophotometer at an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

Ion-exchange chromatography was performed according to the method reported previously with the following modification[16]. The apparatus and columms were identical to those used for β-hexosaminidase isoenzyme resolution[16]. The cell lysate (1 ml) in 10 mM sodium phosphate buffer (pH 6.0) was applied on a 8 mm×18 mm columm of DEAE-Sephadex A-50 (OH-type; Pharmacia) equilibrated with the same buffer. The fractions were eluted by the following discontinuous KCl gradients in 10mM sodium phosphate buffer (pH 6.0); fractions I & II (without KCl gradient), fraction III (0.01 M of KCl), fraction IV (0.05 M), fraction V (0.1 M), fraction VI (0.2 M), fraction VII (0.3 M), fraction VIII (0.4 M), fraction IX (0.5 M), fraction X (0.6 M) and fraction XI (1.0 M). The separated three tubes consisted of each fraction (0.7 ml of the eluate×3). KCl (Cl^-) has an activating effect on the β-galactosidase activity[11,17] and in this study the recovery of the activity after chromatography was 126±19.6 (SD)% (n=41). On the other hand, KCl showed no effect on α-mannosidase activity, however, the recovery after the chromatography was 76.9±8.8 (SD)% (n=54). The low recovery of α-mannosidase activity was probably the result of membrane associated enzymes[1] which could not be eluted from this chromatography. In this study we used a crude homogenate as an enzyme sourse. As reported previously, under the same conditions the recovery of the representative lysosomal enzyme (β-hexosaminidase) was almost 100%[16].

Statistical comparisons were carried out using Student's t-test.

RESULTS

α-mannosidase isoenzymes

A clear difference in the isoenzyme profile of α-mannosidase of granulocytes and lymphocytes was observed; normal granulocytes had early components (fraction I to III) eluted from the chromatography but normal lymphocytes did not (Table 1). Some other isoenzymes also showed significant difference between lymphocytes and granulocytes (Table 1). Leukemic ALL cells resemble lymphocytes and had no activity in the early eluted components. However, in ALL cells the activity of fraction V decreased significantly ($p<0.01$) and the activity of fraction VI increased ($p<0.001$) compared with those of the normal lymphocytes. The ratio of the activities in the two fractions (VI/V) was 2.78±1.9 (mean±SD), which was significantly higher than that of normal lymphocytes (0.89±0.2; $p<0.01$). The isoenzymes of acute myelocytic leukemia (AML) cells had early eluted components similar to normal granulocytes. However, the relative activities in fractions IV to VI showed significant difference ($p<0.001$) from those of normal granulocytes. The activity of fraction VI in AML was significantly higher than that of the granulocytes and the ratio of activities of fractions V and VI (1.63±0.80) was significantly higher than that of the granulocytes (0.53±0.16; $p<0.01$). The isoenzyme profiles of α-mannosidase differed between ALL and AML.

The profile in T-cell leukemia was similar to those of normal lymphocytes (Table 2). On the other hand, the chronic myelo (mono) genous leukemic cells had early eluted components of α-mannosidase, although they were significantly lower levels than those of normal granulocytes (Table 2). The increase of the activity in fraction VI was also obtserved in this leukemia, as in ALL and AML.

It was a characteristic finding that the early eluted components (fraction I-III) of α-mannosidase appeared in myelogenous cells and that the leukemic cells of both ALL and AML possessed relatively increased activities in fraction VI more than those of their respective control cells.

β-galactosidase isoenzymes

Table 1. α-mannosidase isoenzymes in normal lymphocytes, granulocytes and leukemic cells of acute non-T/non-B lymphocytic leukemia and acute myelocytic leukemia

fraction	isoenzyme activity (%)			
	lymphocytes (n=8)	granulocytes (n=8)	ALL (n=14)	AML (n=11)
I	0	7.18± 1.22	0	6.10± 2.80
II	0	4.19± 0.95	0	3.66± 1.19
III	0	6.13± 1.47	0	4.92± 1.30
IV	8.99± 1.96[2]	18.60± 3.35	7.19± 3.88	11.36± 3.57[5]
V	45.59± 6.25	33.99± 4.08	27.41±13.91[3]	25.60± 6.80[5]
VI	39.28± 3.15[2]	20.56± 5.05	53.93±11.15[4]	37.51± 8.76[5]
VII	5.78± 4.01	2.10± 1.34	10.76± 9.23	7.42± 5.61
VIII	0	0.23± 0.38	0	0.74± 0.83
IX	0	0	0	0
X	0	0	0	0
XI	0	0	0	0
VI/V	0.89± 0.20[2]	0.53± 0.16	2.78± 1.90[3]	1.63± 0.80[6]

abbreviations: ALL, acute non-T/non-B lymphocytic leukemia
AML, acute myelocytic leukemia
Data is shown as mean±SD.
[1]Isoenzyme activity is expressed as percent of the total activity.
[2]$p<0.001$ vs. granulocytes [3]$p<0.01$ vs. lymphocytes
[4]$p<0.001$ vs. lymphocytes [5]$p<0.001$ vs. granulocytes
[6]$p<0.01$ vs. granulocytes

Table 2. α-mannosidase isoenzymes in T-cell leukemia and chronic myelo (mono) cytic leukemia

fraction	isoenzyme activity (%)[1]	
	T-cell leukemia (n=4)	CM (Mo) L (n=6)
I	0	3.67± 1.47[4]
II	0	1.77± 0.7[4 5]
III	0	3.09± 1.19[3]
IV	10.03± 2.70	12.58± 1.45[3 6]
V	43.00±10.93	40.67± 4.47
VI	36.13± 4.58[2]	35.12± 5.64[4]
VII	4.82± 4.32	3.20± 1.20
VIII	0	0
IX	0	0
X	0	0
XI	0	0
VI/V	0.89± 0.72[2]	0.88± 0.21[3]

abbreviation: CM (Mo) L, chronic myelo (mono) cytic leukemia
[1]Isoenzyme activity is expressed as percent of the total activity (Mean±SD).
[2]$p<0.01$ vs. ALL [3]$p<0.01$ vs. granulocytes
[4]$p<0.001$ vs. granulocytes [5]$p<0.01$ vs. AML
[6]$p<0.001$ vs. AML

The profiles of β-galactosidase in lymphocytes differed from that in granulocytes (Table 3). The activities in fraction V and VI were significantly higher in granulocytes than in lymphocytes; the activities in fractions VII to IX were significantly higher in lymphocytes than in granulocytes. The ratio of the activities of fractions VI to VII showed a clear difference between lymphocytes (0.55±0.2) and granulocytes (3.06±1.01; $p<0.001$) (Table 3).

The isoenzyme profiles of ALL cells were similar to those of normal lymphocytes. AML cells showed different profiles from those of granulocytes. In AML the activity in fraction VI was decreased, compared with that of granulocytes ($p<0.001$), however, the activities in fractions VII and VIII increased ($p<0.001$ and $p<0.01$, respectively). The ratio of the activities between fractions VI and VII in AML was 0.85±0.59, which was significantly lower than 3.06±1.01 of the granulocytes ($p<0.001$).

The profile of β-galactosidase in T-cell leukemia was similar to that of normal lymphocytes. (Table 4). In chronic myelo(mono)genous leukemia, the activity of fraction VI was decresed and the activity of franction VII was

Table 3. β-galactosidase isoenzymes in normal lymphocytes, Granulocytes and leukemic cells of acute non-T/non-B lymphocytic leukemia and acute myelocytic leukemia

fraction	isoenzyme activity (%)[1]			
	lymphocytes (n=8)	granulocytes (n=8)	ALL (n=14)	AML (n=11)
I	0	0	0	0
II	0	0	0	0
III	0	0	0	0
IV	0	0	0	0
V	0.24± 0.26	3.81± 2.18[2]	0.24± 0.42	1.12± 1.69
VI	27.31± 8.22	68.49± 5.70[4]	25.31±11.80	35.95±17.03[6]
VII	51.44± 8.47[4]	24.08± 5.99	60.12± 9.84	48.96±11.81[6]
VIII	13.35± 3.75[4]	3.16± 2.28	11.76± 7.88	11.06± 7.60[5]
IX	3.44± 1.59[5]	0.38± 0.50	2.30± 2.42	2.96± 3.31
X	0	0	0	0
XI	0	0	0	0
VI/VII	0.55± 0.20	3.06± 1.01[3]	0.45± 0.25	0.85± 0.59[6]

Abbreviations: ALL, acute non-T/non-B lymphocytic leukemia
AML, acute myelocytic leukemia
[1]Isoenzyme activity is expressed as percent of the total activity (Mean±SD).
[2]$p<0.01$ vs. lymphocytes [3]$p<0.001$ vs. lymphocytes
[4]$p<0.001$ vs. granulocytes [5]$p<0.01$ vs. granulocytes
[6]$p<0.001$ vs. granulocytes

Table 4. β-garactosidase isoenzymes in T-cell leukemia and chronic myelo (mono) cytic leukemia

fraction	isoenzyme activity (%)[1]	
	T-cell leukemia (n=4)	CM (Mo) L (n=6)
I	0	0
II	0	0
III	0	0
IV	0	0
V	0.40±0.49	0.90±0.73
VI	25.40±7.88	55.20±4.81[2,4]
VII	53.45±4.02	36.58±2.81[3]
VIII	13.58±3.93	8.45±5.59
IX	5.40±2.48	0.97±1.11
X	0	0
XI	0	0
IV/VII	0.47±0.13	1.54±0.30[2]

abbreviation: CM (Mo) L, chronic myelo (mono) cytic leukemia [1]Isoenzyme activity is expressed as percent of the total activity (mean±SD).
[2]$p<0.01$ vs. granulocytes
[3]$p<0.001$ vs. granulocytes
[4]$p<0.01$ vs. AML

increased significantly, however, the change was not as prominent as was seen in AML (Table 4)

DISCUSSION

Previous studies of lysosomal enzymes in leukemic cells have demonstrated abnormal activities and abnormal expressions of the isoenzyme profiles[3,6,7]. The abnormal expression of β-hexosaminidase (the intermediate forms) is well known as a characteristic of common ALL. We reported not only a heterogeneity of β-hexosaminidase isoenzymes in ALL but also a characteristic change in myelocytic leukemia[16]. In this study we extended the isoenzyme analysis to α-mannosidase and β-galactosidase, because these activities differed clearly between lymphogenous and myelogenous leukemia[15]. The assay was carried out at an acidic pH so as to detect lysosomal forms of α-mannosidase (pH 4.5) and β-galactosidase (pH 4.0). The DEAE-Sephadex chromatography using in this study made clear a difference in the isoenzyme profiles between the normal lymphocytes and granulocytes (Tables 1 and 3).

In respect to α-mannosidase, three types isoenzymes (two acidic forms and a neutral form) have been reported in other assay systems.

This study elucidated, at least, three forms of α-mannosidase isoenzymes, early eluted components, and the components eluted in fractions V and VI. In preliminary study, these components were lysosomal forms because of acidic optimal pH and heat stability (data was not shown), which were compatible with a report by Butterworth[4]. The early eluted components were seen only in granulocytes and myelogenous leukemia. This finding was thought to be a myelocytic character. In addition, there was a significant increase of α-mannosidase isoenzyme activity in fraction VI, which was observed in all leukemias examined except T-cell leukemia. The increase seemed to relate to their malignant transformation, although its significance is not uncertain.

To our knowledge, there has been no report of β-galactosidase isoenzymes in leukemia. The isoenzyme profiles differed significantly between lymphocytes and granulocytes (Table 3). ALL cells had no significant difference in the profiles from normal lymphocytes, however, an increase of the activity in fraction VII was remarkable in AML when compared to normal granulocytes. The increase was also significant in chronic myelo(mono)cytic leukemia. However, no type specific alteration between myelocytic and lymphcoytic leukemias was observed. The total activity of β-galactosidase in myelogenous leukemia was increased as reported previously[15]. These qualitative and quantitative studies suggest that in myelogenous leukemia the increased activity results in part from the abnormal elevation of the isoenzyme component in fraction VII.

Of interest is the finding that T-cell leukemia has similar isoenzyme profiles of both α-mannosidase and β-galactosidase to those of normal lymphocytes. As reported reviously, T-cell leukemia also had β-hexosaminidase isoenzymes similar to normal lymphocytes[16]. It seems that the isoenzyme profiles might gave certain relation to the intrinsic differentiation of leukemic cells in spite of their immunological markers.

The findings presented in this communication clearly suggest that the analysis of isoenzyme profiles is useful way examining intrinsic abnormalities in leukemic cells. These enzymes was provide useful enzyme markers for detecting the nature of leukemic cells and complement other immunological and cytochemical markers. It is, however, not known whether the alteration of these isoenzyme expression in leukemic cells reflect abnormalities due to an intrinsic variability in a target cell population of the leukemogenic progenitor cells or to a disturbance in the control mechanisms associated with the malignant transformation. Further attention should be given to these enzymes in a large series of patients with various kinds of leukemia to examine the abnormal expressions of the isoenzyme profiles and to establish the significance of the abnormalities.

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