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Hexosaminidase I Indicates Maturation Disarrangement in Acute Leukemias *

J. R. Novotny [1], S. Brendler [2], H. J. Kytzia [2], K. Sandhoff [2], and G. Gaedicke [1]

A. Introduction

For a number of lysosomal hydrolases, abnormally expressed forms have been found to occur in childhood and adult leukemia. Most of these abnormalities are confined to the physicochemical properties of the enzymes, e.g., abnormal electrophoretic mobility, change of isoelectric point, and alteration of isoenzyme pattern. All this has been described for the hexosaminidase system in human leukemia cells. The most-investigated phenomenon is the occurrence of hexosaminidase I [1–3, 5, 6, 19]. Other abnormalities, such as the anodic shift of the Hex A isoenzyme [2, 6] or a relative decrease of the Hex B form [1], are found in various leukemia subtypes.

In our study, we found that the Hex I isoenzyme is present in excess in cells from typical cALL, pre B-ALL, AUL, and AML. This has also been described in T-ALL, pre T-ALL, T-CLL, and multiple myeloma cells. However, this isoenzyme has so far not been found to be raised in CML and B-CLL. An anodic shift of Hex A and a decrease in Hex B activity appeared independently in a few cases from among all investigated leukemia forms.

In normal leukocytes, hexosaminidases occur in two isoenzymatic variants: Hex A and Hex B. Hex A is a heteropolymer enzyme composed of one alpha subunit and one beta subunit, whereas Hex B is a homopolymer enzyme consisting of two beta subunits [16, 17].

Since there are only few data available about the nature of the hexosaminidase I or the "shifted" hexosaminidase A in human leukemia cells, we have investigated their subunit composition and enzymatic properties.

B. Methods

All methods used are described elsewhere [4, 7, 8, 10–15].

C. Results

I. Biochemical and physicochemical properties of hexosaminidase I demonstrate that Hex I belongs to the hexosaminidase system and behaves like Hex B, whereas the "shifted" hexosaminidase A resembles Hex A. cALL cells and REH-6 cells contain an excess of hexosaminidase I isoenzyme, as compared to normal lymphocytes.

II. Immunochemical analysis of preparations from such cells shows that Hex I is a homopolymer of beta subunits. Since the molecular weight is about 100 000 daltons, it must be made up of two beta subunits. The "shifted" Hex A is a heteropolymer of one alpha and one beta subunit.

III. The processing of the enzyme subunits in REH-6 cells shows striking differ-

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[1] Abteilung Pädiatrie II, Universitäts-Kinderklinik, Prittwitzstraße 43, 7900 Ulm/Donau, FRG
[2] Institut für Organische Chemie und Biochemie der Universität, Gerhard-Domagk-Straße 1, 5300 Bonn, FRG

Table 1. Comparison of hexosaminidase I and "shifted A" hexosaminidase from leukemia cells with the hexosaminidase isoenzymes A and B

Property	Hexosaminidase A	Hexosaminidase B	Hexosaminidase I	"Shifted" hexosaminidase A
Isoelectric point	5.0	7.3	about 6.5	about 4.6
Stability at 50 °C	−	+	+	−
Precipitation with antibodies to hexosaminidase B	+	+	+	+
Precipitation with specific antibodies to hexosaminidase A	+	−	−	+
Molecular weight	About 100000	About 100000	About 100000	Not determined
Proposed composition	Alpha/beta	Beta/beta	Beta/beta	Alpha/beta
Hydrolysis of:				
MUF-/pNP-Glc-NAc	+	+	+	+
MUF-/pNP-Gal-NAc	+	+	+	+
MUF-/pNP-Glc-NAc-6-S	+	−	−	+
Ganglioside GM_2*	+	−	−	+

* In presence of the physiological activator

Table 2. Molecular weights of precursor proteins, intermediate forms, and "mature" polypeptides of beta hexosaminidase from human cells (modified from 9, 18)

Enzyme	Cell system	Enzyme precursor	Intermediate form	"Mature" enzyme subunit
Beta hexosaminidase Alpha subunit	Fibroblasts Macrophages Monocytes Lymphocytes Granulocytes Smooth-muscle cells Endothelial cells	67		54
Beta hexosaminidase Beta subunit	Fibroblasts Macrophages Monocytes Lymphocytes Granulocytes Smooth-muscle cells Endothelial cells	63	52	29
	Leukemia cells (REH-6 cell line)	66	51.5	Not formed

In leukemia cells (REH-6 cell line), hexosaminidase alpha precursors and mature alpha subunits are formed only in traces.

ences in quantitative and qualitative synthesis, in comparison to normal fibroblasts. Thus, an abnormal precursor of the hexosaminidase beta chain (about 1000 daltons larger than the regular beta precursor) and an abnormal beta subunit (slightly smaller than the regular one) are formed, while no normal alpha or beta precursors or alpha subunits occur in REH-6 cells. Mature beta chains do not appear in these cells.

D. Discussion

Although a raised Hex I level has been found in most cases of cALL, pre B-ALL, AML, and roughly half of the cases of AUL, it has also been demonstrated in cases of other leukemia subtypes (e.g., T-ALL, T-CLL, and multiple myeloma). The abovementioned abnormalities, such as the shifted Hex A or the decreased Hex B, seem not to be strictly confined to certain leukemia subtypes. It is therefore suggested that the abnormalities described might be more general, as they affect a number of lysosomal enzymes in various leukemia subtypes.

Hexosaminidase I is an isoenzyme which shows a different physicochemical behavior, as compared to isoenzymes A and B. Our results from heat inactivation of isoenzyme I activity, as well as the lack of any specific activity against sulfated synthetic substrates or ganglioside GM_2, clearly demonstrate that hexosaminidase I must be largely composed of beta subunits. This is further strongly supported by our immunodiffusion assays using antisera against pure hexosaminidase B and specific antibodies against hexosaminidase A. Thus, we conclude from our data that hexosaminidase I from cALL cells is a homopolymer polypeptide like hexosaminidase B and is composed of hexosaminidase beta units only. In contrast, shifted hexosaminidase A is a heteropolymer enzyme like the normal Hex A and consists of one alpha and one beta unit.

Data from determination of apparent molecular weight by gel filtration do not reveal major differences. On the basis of these findings, we favor the hypothesis that the difference may be due to an abnormal post-translational processing of the beta subunits of isoenzyme I, as compared to that of the regular isoenzyme B. This view is supported by labeling experiments in which we were able to show an abnormal beta precursor and beta subunit of Hex I, as well as a lack of mature beta chains in cALL cells. These differences could be due to an altered composition of the carbohydrate chains of the molecules. Corresponding mechanisms may lead to the anodic shift of hexosaminidase A. In summary, we conclude that the findings described may indicate maturation disarrangement in acute leukemias.

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