Electrofocusing Patterns of Fucosyltransferases in Plasma of Patients with Neoplastic Disease

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ABSTRACT

Electrofocusing patterns of plasma fucosyltransferases provide information concerning marrow status of patients with myeloproliferative disorders. Three enzymes were detected in normal plasmas using an acceptor terminat
ning in N-acetylglucosamine-galactose. The enzyme which focused at pH 4.7 was elevated during rapid proliferation of myeloid cells, e.g., acute myelogenous leukemias and certain infectious diseases. Activity at pH 5.1 was decreased in acute myelogenous leukemia patients, and from other observations, appears related to the level of erythropoietic activity. Acceptor studies show this enzyme to be specified by the H gene. A third enzyme focused at pH 5.5 and appeared to be correlated with a later step in granulocytes maturation.

Two other plasma fucosyltransferases (pH 5.6 and 8.3) were detected with a high-molecular-weight acceptor terminating in N-acetylglucosamine. This activity was markedly elevated during regeneration of a normal marrow population during drug-induced remission of acute myelogenous leukemia. Additional isoenzymes were detected, using this acceptor, in plasma of patients with certain solid tumors and multiple myeloma. However, the new isoelectric points observed (pH 6.0, 6.9, and 7.8) suggest these enzymes are probably not derived from hematopoietic tissues.

INTRODUCTION

The fucosyltransferases are a group of enzymes which catalyze the transfer of the sugar fucose from a GDP-fucose donor onto specific sugar acceptors (15). Both low-molecular-weight acceptors, i.e., mono- and disaccharides (5, 13, 14, 16) and high-molecular-weight acceptors derived from glycoproteins (1-4, 6, 12, 19) can be utilized by different transferases. At least 3 plasma fucosyltransferases have been described. One such enzyme, specified by the H gene, transfers fucose onto N-acetylglucosamine (12). The first 2 enzymes are inhibited by the SH reagent NEM, while the third is insensitive to NEM (6). Elevated levels of fucosyltransferase activity have been reported in plasma of patients with solid tumors (1, 2) and in tumor tissue of animals bearing experimental neoplasms (3, 4).

MATERIALS AND METHODS

GDP-[14C]-L-fucose (170 to 200 Ci/mol) was purchased from New England Nuclear, Boston, Mass., and from Amersham/Searle, Arlington Heights, Ill. Fetuin was provided by Calbiochem, Los Angeles, Calif.; terminal sialic acid and (where specified) subterminal Gal residues were removed as described by Spiro (17). Phenyl-β-galactoside was obtained from Sigma Chemical Co., St. Louis, Mo. Ampholytes and Ultrodex (a purified form of ultrafine Sephadex G-75) were obtained from LKB Instruments, Inc., Silver Spring, Md., as was the Multiphor apparatus used for horizontal bed electrofocusing. The ion-exchange resin AG 1-X8 (a purified form of Dowex 1) was purchased from Bio-Rad Laboratories, Richmond, Calif.

Plasmas were chilled to 0°C after collection. Erythrocytes were removed by centrifugation for 5 min at 1,000 × g, and platelets were removed by a subsequent centrifugation at 10,000 × g for 10 min. The resulting preparations were stored at −70°C until needed.

Methodology used for assessing plasma fucosyltransferase levels using high-molecular-weight acceptors and an ion-exchange procedure has been described (6). The product was isolated by ion-exchange chromatography after 1-hr incubations at 37°C. This procedure was used both for an initial estimation of plasma fucosyltransferase activity and for assay of electrofocusing fractions. Enzyme activity in plasmas and in electrofocused fractions was also assayed using phenyl-Gal as acceptor (11). Electrofocusing was carried out on a 11 × 23-cm flat bed of Ultrodex containing a 5% ampholyte concentration. The NEM-sensitive enzyme was analyzed using 2.5% of pH 3.5 to 5 plus

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3 The abbreviations used are: Gal, galactose; GlcNAc, N-acetylglucosamine; NEM, N-ethylmaleimide; AML, acute myelogenous leukemia.

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D. Kessel et al.

2.5% of pH 5 to 7 ampholyte. The NEM-insensitive enzyme was analyzed using 2.5% pH 5 to 7 plus 2.5% pH 7 to 9 ampholytes. Before focusing, 3-ml plasma samples were dialyzed at 4°C for 6 hr against the appropriate 5% ampholyte mixture. The resulting fine precipitate was removed by centrifugation, and the plasma was applied to the center of the gel bed as described in LKB Applications Note 198. Electrofocusing was carried out over a 16 hr interval using a constant power of 7 watts (ISCO Model 492 power supply) with the gel bed chilled to 4°C. The bed was then divided into 30 fractions, their pH was measured, and the individual gel sections were eluted with 3-ml portions of distilled water. The eluates were concentrated to 300 μl at 4°C using a B-15 minicon concentrator (Amicon Corp., Lexington, Mass.). Assays for fucosyltransferase activity utilizing the 2 high-molecular-weight acceptors and the low-molecular-weight phenyl-Gal acceptor were carried out as described in Ref. 6 using 100 μl of concentrated fraction for each assay.

RESULTS

Enzyme Levels in Plasma Samples. Before electrofocusing, fucosyltransferase levels in plasmas were determined; results are shown in Table 1. The NEM-sensitive enzyme activity was determined using a desialated fetuin acceptor and can represent incorporation of fucose onto either the terminal Gal or the subterminal GlcNAc residue (16). Transfer of fucose onto the Gal residue of phenyl-β-galactoside was also measured. The NEM-insensitive enzyme activity was measured with a GlcNAc-terminal acceptor (asialoagalactofetuin) and involves transfer of fucose onto an internal GlcNAc residue (19). In Table 1 are shown the enzyme levels found in plasmas shown in Charts 1 to 3, and the range of values from other plasmas which were electrofocused during the course of this study.

Electrofocusing Patterns

Gal-Terminal Acceptor. The patterns shown in Chart 1 were obtained using a desialated fetuin acceptor. Typical focusing patterns are shown using plasmas from a normal donor (Blood Group A), a patient with tetracycline-induced agranulocytosis, <60 granulocytes/cu mm (Blood Group A), and a patient with Crohn’s disease and intraabdominal abscesses, blood group unknown.

The major peaks of enzyme activity focused at pH 4.7, 5.1, and 5.6. The first peak was generally elevated in plasmas of patients with infectious diseases and was markedly decreased in the single example of agranulocytosis. A second peak of activity at pH 5.1 was substantially elevated in the latter patient. A third isoenzyme with pI = 5.5 was also detected. The relative level of this enzyme, compared with the normal controls, was elevated in infectious disease and chronic myelogenous leukemia and decreased in agranulocytosis.

Patterns shown in Chart 2 were obtained from 2 plasmas of patients with untreated AML. The bottom pattern was obtained from a patient with 20% marrow myeloblasts (Blood Group A1) who later achieved a drug-induced remission. The top pattern was obtained from plasma of a patient with 60% marrow myeloblasts (Blood Group O) who failed to respond to drug therapy. These results are typical of those seen in several other

| Table 1
---|---|---|
| **Fucosyltransferase levels in plasma samples**

<table>
<thead>
<tr>
<th>Donor status</th>
<th>Value for sample focused</th>
<th>Value for sample focused</th>
<th>Value for sample focused</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-GlcNAc-Gal</td>
<td>382</td>
<td>304–555</td>
<td>307</td>
</tr>
<tr>
<td>R-GlcNAc</td>
<td>95</td>
<td>375</td>
<td>225</td>
</tr>
<tr>
<td>Phenyl-Gal</td>
<td>870</td>
<td>657–1350</td>
<td>820</td>
</tr>
<tr>
<td>AML (3)</td>
<td>1650</td>
<td>1421–2793</td>
<td>130</td>
</tr>
<tr>
<td>AML remission (3)</td>
<td>340</td>
<td>263–372</td>
<td>1774</td>
</tr>
</tbody>
</table>

*a* The abbreviations used are: R-GlcNAc-Gal, desialated fetuin; R-GlcNAc, asialoagalactofetuin; Phenyl-Gal, phenyl-β-galactoside; AML³, untreated patients with 50 to 80% marrow myeloblasts; AML⁴, untreated patients with 20 to 45% marrow myeloblasts.

*b* Numbers in parentheses, number of donors.
studies with the level of the enzyme activity with \( p_1 = 4.7 \) proportional, and the activity at \( p_1 = 5.5 \) inversely proportional to percentage of marrow blasts. The enzyme activity at \( p_1 = 5.1 \) was markedly decreased, compared with controls, in both plasmas. All enzyme activity shown in Charts 1 and 2 was abolished by addition of 10 \( \text{mm} \) NEM to incubation mixtures.

**GlcNAc-Terminal Acceptors.** Enzyme activity was measured with asialoagalactofetuin as acceptor in the presence of 10 \( \text{mm} \) NEM. The patterns obtained with 5 plasmas from normal donors closely conformed to that shown for a typical such plasma (Blood Group A1) in Chart 3 (solid line) with the major enzyme activity focusing at \( pH 8.3 \) and a minor peak at \( pH 5.6 \). In plasmas from 3 patients with AML, in drug-induced remission, the enzyme \( p_1 \) did not significantly differ from controls. A typical result is shown in Chart 3 (dotted line); plasma was obtained from a patient with Blood Group B.

Plasmas from several patients with solid tumors, or multiple myeloma, also contained high levels of fucosyltransferase activity which required a GlcNAc-terminal acceptor. Electrofocusing studies of 3 samples obtained from myeloma patients revealed a different pattern from that found with normal donors or leukemia patients. The predominating isoenzyme activities showed \( p_1 \) values of 5.9, 6.6, and 7.5 (data not shown).

**Phenyl-Gal Acceptor.** In all plasma samples examined, we found a major peak with \( p_1 = 5.1 \) (Charts 1 and 2). The data of Table 1 indicate an elevation of this enzyme activity in plasma of the patient with agranulocytosis. Marrow examination indicated a predominantly erythroid population. In contrast, this enzyme activity was markedly reduced in AML patients who exhibited varying degrees of suppression of erythropoiesis.

**DISCUSSION**

The data summarized in Table 1 show an elevation of total NEM-sensitive fucosyltransferase, measured with a desialated fetuin acceptor, in plasma of patients with AML and infectious diseases. In contrast, the leukemic plasmas showed a subnormal enzyme level when the low-molecular-weight acceptor phenyl-Gal was used. The latter finding indicates the importance of choice of acceptor in the delineation of glycosyltransferase activities. Data shown in Chart 2 indicate that the portion of this enzyme activity which demonstrates an isoelectric point of \( pH 4.7 \) is correlated with percentage of marrow myeloblasts and may, therefore, derive from such a marrow cell population. Further evidence of the correlation of myeloproliferation versus level of \( p_1 = 4.7 \) enzyme was provided by examination of plasma samples from a patient with agranulocytosis; enzyme activity was markedly diminished.

We interpret the results shown in Table 1 and Charts 1 and 2 to confirm the finding initially reported by Kuhns et al. (11, 18) of decreased activity of the \( H \) gene-specified 2'-fucosyltransferase in plasma of patients with AML. An elevated level of this enzyme was found in plasma from a patient with agranulocytosis. Microscopic examination of a marrow sample from this patient revealed a high proportion of erythroid precursors. Since impaired formation of RBC precursors is often associated with AML, we interpret the results shown here to suggest that the plasma level of the fucosyltransferase focusing at \( pH 5.1 \) may be derived from erythroid precursors in marrow.

The other major plasma fucosyltransferase which can utilize an acceptor with the desialated fetuin configuration is the 3'-fucosyltransferase which transfers fucose onto the 3'-position of a subterminal GlcNAc (16). We cannot conclude whether the plasma fucosyltransferase detected with desialated fetuin, which focuses at \( pH 5.5 \), represents the 3'-fucosyltransferase. Using a low-molecular-weight acceptor, Watkins* has reported that plasma 3'-fucosyltransferase was not elevated in AML.

The fucosyltransferase with an isoelectric point of 5.5 (Charts 1 and 2) appears to be correlated with the number of circulating mature granulocytes, e.g., the level is decreased in agranulocytosis and AML and elevated in infectious disease. The enzyme activity was also markedly elevated in plasma of chronic myelogenous leukemia patients (data not shown). This activity may, therefore, derive from a postmyeloblast marrow precursor, e.g., the promyelocyte.

We reported elevated levels of another fucosyltransferase activity which required a GlcNAc-terminal acceptor.

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* W. Watkins, personal communication.

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**Electrofocusing Patterns**

**Chart 2.** Electrofocusing patterns of NEM-sensitive fucosyltransferase activity in plasmas from patients with AML. Top, untreated AML, 60% marrow myeloblasts, Blood Group O; bottom, untreated AML, 20% marrow myeloblasts, Blood Group A1. Data represent incorporation of radioactive fucose by fractions which focused at specified \( \text{pH} \) values into a desialated fetuin acceptor (---) or phenylgalactoside (----). Left ordinate, enzyme activity in fractions of \( \text{pH} <5 \); right ordinate, fractions of \( \text{pH} >5 \).

**Chart 3.** Electrofocusing patterns of NEM-resistant fucosyltransferase measured with an asialo-agalactofetuin acceptor, using a normal plasma (-----) and a plasma from a patient in drug-induced remission of AML (- - - - - - - - -). Data represent incorporation of radioactive fucose into acceptor during 4-hr incubations by enzyme fractions which focused at the specified \( \text{pH} \) values.

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* W. Watkins, personal communication.
activity in plasma of patients in drug-induced remission of AML during intervals of marrow hyperplasia following successful marrow-suppressive drug therapy (8). This activity requires a GlcNAc-terminal, fetuin-derived acceptor, is NEM insensitive (6), and presumably represents the α-6 core fucosyltransferase described by Wilson et al. (19). Electrofocusing patterns indicate an elevation of the 2 major isoenzymes (pI = 5.6 and 8.3) of this enzyme activity during such remissions (Chart 3).

In the course of this study, we have also studied plasma samples from several patients with solid tumors. In such patients, the marrow status can vary widely, depending on prior use of drugs which affect the hematopoietic system, concurrent infection, and marrow infiltration by tumor. The results of this ongoing study will be reported separately. Preliminary data indicate elevation of the NEM-sensitive fucosyltransferase focusing at pH 4.7 whenever patients had a history of myelosuppressive drug therapy. This result may derive from a temporary narrow myeloid hyperplasia following such therapy. In many studies, we found an elevated level of NEM-insensitive enzyme activity with p'I's of 5.8 and 7.5 (GlcNAc-terminal acceptor). These do not correspond to any enzyme shown in Chart 3 and may be a tumor product.

We cannot yet detect an effect of blood group upon electrofocusing patterns. Watkins (18) has described a higher average level of the H gene-specified fucosyltransferase in plasma of A1, A2, and B donors compared with other groups.

The data described above indicate that the altered levels of fucosyltransferases in plasma of patients with hematological cancers (6-9, 11, 18) derive from alterations in production or maturation of populations of normal marrow components. Analysis of such plasma enzyme levels continues to provide a useful assessment of marrow status in the leukemia patient undergoing chemotherapy.

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REFERENCES

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