Electrofocusing Patterns of Fucosyltransferase Activity in Plasma of Patients with Chronic Granulocytic Leukemia

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ABSTRACT

Fucosyltransferase activity was measured in plasma samples obtained from patients with chronic granulocytic leukemia in different disease stages. Total enzyme activity was generally elevated in untreated and in stable disease; a marked further elevation was noted 30 to 60 days before development of blast crisis. Electrofocusing studies indicate the presence of three major enzymes. The level of enzyme which focused at pH 5.6 was elevated in untreated disease and rose markedly during blast transformation. A second enzyme focused at pH 5.1 and appeared to be related to marrow erythroid turnover. A third enzyme with pi = 4.7 was elevated only during marrow regeneration which followed myelosuppressive therapy. These data suggest the enzyme with pi = 5.6 to be a specific marker in chronic granulocytic leukemia.

INTRODUCTION

The glycosyltransferases are a group of enzymes which catalyze transfer of individual sugars from nucleotide-sugar donors onto appropriate acceptors (20). Elevated levels of specific enzymes have been reported in plasma of patients with neoplastic disease (1, 8, 9, 11, 14).

We have already reported an association between the level of plasma fucosyltransferase activity versus the percentage of marrow myeloblasts in acute granulocytic leukemia (14). A high-molecular-weight acceptor terminating in the sequence N-acetylglucosamine-galactose was used; all activity was inhibited by the sulfhydryl reagent NEM3 (5). Electrofocusing indicated the presence of 3 enzymes with this acceptor specificity (12). The level of activity with pi = 4.7 was related to marrow myeloid synthesis; an enzyme with pi = 5.1 was associated with erythroid turnover; determinants of activity of a third enzyme were uncertain. The pi of the latter enzyme was initially estimated to be approximately 5.5 but, in the present study, this activity appears to focus at pH 5.6.

In this report, we describe electrofocusing profiles of plasma fucosyltransferases in CGL. The data provide information on determinants of activity of the enzyme with pi = 5.6, along with evidence that measurement of total plasma enzyme activity can aid in predicting the impending development of blastic crisis.

MATERIALS AND METHODS

GDP-L-[14C]Fucose (170 to 200 Ci/mol) was purchased from New England Nuclear, Boston, Mass., and from Amersham/Searle, Arlington Heights, Ill. Phenyl-β-galactoside was purchased from Sigma Chemical Co., St. Louis, Mo. Nonradioactive GDP-fucose was prepared as described previously (7). Fetuin (Calbiochem, Los Angeles, Calif.) was treated as described by Spiro (19) to remove terminal sialic acid. Amphotolins and Ultrodex (a superfine grade of Sephadex G-75) were obtained from LKB Instruments, Inc., Silver Spring, Md. AG 1-X8, a purified form of the anion-exchange resin Dowex 1, was purchased from Bio-Rad Laboratories, Richmond, Calif.

Blood samples were chilled to 0° after collection. Erythrocytes and platelets were removed by sequential centrifugations at 1,500 x g (10 min) and 10,000 x g (20 min), and the cell-free plasma was stored at -70°.

The total level of NEM-sensitive fucosyltransferase activity was measured as described previously using a desialated fetuin acceptor (5). Data are reported in terms of cpm of radioactive fucose incorporated into endogenous plus exogenous acceptors during 60-min incubations at 37°C per 50 μl of plasma.

Electrofocusing was carried on a 11- x 23-cm flat bed containing Ultrodex suspended in 5% pH 4 to 6 amorphine as described in LKB Applications Note 198. Plasmas were dialyzed for 6 hr at 4° against 5% amorphine and the fine precipitate removed by centrifugation. A 3-ml sample of the dialyzed plasma was then applied to the center of the gel bed and a constant power of 7 watts was provided by an ISCO Model 492 power supply. A temperature of 3-5° was maintained.

The bed was divided into 30 equal sections. The pH of the individual sections was measured, and the sections were eluted with 3-ml portions of 10 μM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.0). The eluates were individually concentrated to 300 μl with B-15 Minicon concentrators (Amicon Corp., Lexington, Mass.). All operations were carried out at 4°.

Assay of fucosyltransferase activity in individual fractions was carried out in a 200-μl volume containing 1 μM labeled GDP-fucose (50,000 cpm), 10 mM magnesium acetate, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.0), 0.5 mg of desialated fetuin, and 150 μl of enzyme concentrate. After 20 hr at 37°, incubations were terminated by elution through 0.5- x 2-cm columns of resin (hydroxyl form), and the columns were washed with 1 ml of water. The radioactivity in the eluate plus washings was measured by liquid scintillation counting. In this procedure, GDP-fucose and free fucose are retained by the resin, and the fucosylated product is eluted (5).

The level of the fucosyltransferase specified by the H gene was measured as described previously using phenyl-β-galactoside as acceptor (4). Degradation of radioactive GDP-fucose was monitored by paper chromatography as described in Refs. 17 and 18.
Studies on $K_m$'s of the enzyme which focused at pH 5.6 were carried out (5). For this determination, we used a fraction from an electrofocused preparation of a plasma sample from a patient with CGL in blast crisis.

Hematological data were obtained by microscopic examination of peripheral blood and marrow samples and from bone marrow culture studies.

RESULTS

**Total Fucosyltransferase Activity.** The total level of plasma fucosyltransferase activity in normal controls and CGL patients in different disease stages is shown in Table 1. Data represent enzyme-catalyzed transfer of radioactive fucose from a GDP-fucose donor onto desialated fetuin, in terms of cpm/50 µl of plasma per hr. All such activity was abolished by the addition of 10 mM NEM to incubations. An elevation of total enzyme activity was usually observed in plasmas from patients with CGL whether untreated or in a stable drug-controlled state. A further elevation in enzyme level occurred during blastic transformation.

**Electrofocusing Patterns.** Electrofocusing studies were carried out on plasmas from untreated, preblastic and blast crisis patients and on 4 plasmas from normal donors and from patients with chronic granulocytic leukemia in remission. In normal plasmas, we found 3 major peaks of enzyme activity, with pl values of 4.7, 5.1, and 5.6. Typical results are shown in Chart 1. In patients with untreated CGL, we found an elevation of enzyme activity which focused at pH 5.6 (Chart 2). Upon successful treatment with Myleran, the level of enzyme with pl = 5.6 fell to the level found in normal plasma, while the level of enzyme with pl = 4.7 rose markedly (Chart 3). During blastic transformation (Chart 4), a new pattern was found with a marked elevation in the level of enzyme which focused at pH 5.6. Elevations in the level of enzyme which focused at pH 5.1 were associated with appearance of nucleated RBC in peripheral blood (Chart 4, C and D).

**Substrate Degradation Studies.** The instability of GDP-fucose during incubation in the presence of metal ions and serum has been described (15). In the present study, such degradation was monitored by paper chromatography (17, 18). After incubations for 1 hr for determination of total enzyme activity, the net recovery of GDP-fucose ranged from 50 to 80%. During the 20-hr incubations involved in measurement of enzyme activity in focused samples, the recovery of GDP-fucose ranged from 60 to 80%. Substantial degradation of substrate occurred only during incubation of samples containing enzyme which focused at pH <4.6, presumably reflecting the pl of plasma hydrolases.

$K_m$ studies on the enzyme fraction which focused at pH 5.6 indicated a value of 1.6 µM, using desialated fetuin as acceptor.

Table 1

<table>
<thead>
<tr>
<th>Donor status</th>
<th>No. of patients</th>
<th>Enzyme activity (cpm/50 µl plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>373 ± 63</td>
</tr>
<tr>
<td>CGL untreated</td>
<td>4</td>
<td>758 ± 275</td>
</tr>
<tr>
<td>CGL stable</td>
<td>20</td>
<td>530 ± 185</td>
</tr>
<tr>
<td>CGL preblastic</td>
<td>4</td>
<td>1351 ± 288</td>
</tr>
<tr>
<td>CGL blast crisis</td>
<td>4</td>
<td>1680 ± 480</td>
</tr>
</tbody>
</table>

$^a$ During 60-min incubations.

$^b$ Mean ± S.D.

$^c$ Samples obtained 30 to 60 days prior to development of blastic transformation.

| Chart 1. Electrofocusing patterns of NEM-sensitive fucosyltransferase activity in plasmas from 4 normal donors. |
| Chart 2. Electrofocusing patterns of NEM-sensitive fucosyltransferase activity in plasma of 4 patients with untreated CGL. |
| Chart 3. Electrofocusing patterns of NEM-sensitive fucosyltransferase activity in plasma of 4 patients with CGL undergoing Myleran therapy and in remission. |
| Chart 4. Electrofocusing patterns of NEM-sensitive fucosyltransferase activity in plasma of 4 CGL patients. Plasma A was obtained 2 weeks before clinical evidence of blast crisis, plasmas B, C, D were obtained from patients during blast crisis. Microscopic examination showed the presence of nucleated RBC in the circulating blood of Patients C and D. |
DISCUSSION

Data summarized in Table 1 indicate a general elevation of total NEM-sensitive fucosyltransferase activity in plasma of patients with untreated or stable CGL and a further elevation in activity during blastic transformation. Blast crisis usually occurred within 30 to 60 days after a plasma level of >1000 units of enzyme activity (cpm/50 µl plasma). This assay simultaneously measures 3 enzymes which utilize an acceptor terminating in the N-acetylgalactosamine-galactose sequence (12). Further analysis by electrofocusing was therefore carried out in order to study variations in the different enzymes as a function of disease status.

Electrofocusing delineated 3 enzyme activities with isoelectric points of pH 4.7, 5.1, and 5.6. The level of enzyme with pl = 4.7 was markedly elevated in plasma of Myleran-treated CGL patients (Chart 3). This enzyme was also elevated during early stages of the marrow hyperplasia which followed myelosuppressive drug therapy and in leukemoid reactions associated with infectious disease (12). An elevated level of this plasma enzyme may reflect an increased turnover of early myeloid precursors in marrow, whether malignant or normal.

Using an unambiguous acceptor (4), we found the enzyme which focused at pH 5.1 to be the fucosyltransferase specified by the H gene (12). The variation of enzyme activity in different stages of acute granulocytic leukemia (3, 12), together with other hematological observations (12), suggested a correlation between enzyme activity versus erythroid turnover. In the present study, we observed an elevated level of this activity in plasma of CGL patients in blast crisis when associated with the appearance of circulating nucleated RBC (Chart 4, C and D), a finding in agreement with our previous interpretation. However, Kuhns et al. (16) have provided data which argue against this interpretation.

The level of enzyme with pl = 5.6 was elevated in untreated CGL (Chart 2), returned to a normal value during successful therapy (Chart 3), and was markedly elevated during blastic transformation (Chart 4). These data suggest that this enzyme is a specific marker for the malignant clone in CGL.°

Data provided here and in previous reports (12–14) show a variation in electrofocusing patterns of plasma fucosyltransferase activities as a function of marrow status. These differences may reflect the variation in patterns of glycoprotein synthesis by different normal and malignant cell types described by Gahmberg and Anderson (6).

In another study (10), we reported that the appearance of an elevated level of fucosyltransferase activity in plasma of patients with acute myelogenous leukemia did not reflect the enzyme pattern in isolated myeloblasts. The elevation of specific fucosyltransferases may therefore reflect the operation of a specific secretory process, rather than the nonspecific "shedding" of all enzymes associated with a particular hemato-

Kinetic studies indicate a K_m for the enzyme with pl = 5.6 of 1.6 µM. This enzyme has tentatively been identified as the 3'-fucosyltransferase described previously (2, 17). This value differs from the 62 µM reported for the 3'-fucosyltransferase isolated from milk (6). This difference may relate to use of a different enzyme source or to our use of a high-molecular-weight acceptor for our studies.

The present data provide a means for predicting impending blastic crisis in CGL's, along with information concerning the nature of the fucosyltransferases being measured in this procedure. Current research involves a search for a more specific assay for the enzyme activity which focuses at pH 5.6 to provide an unambiguous marker for CGL.

ACKNOWLEDGMENTS

Excellent technical assistance by Joanne Kaplan and Sue Marier is acknowledged.

REFERENCES


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