Demonstration of Phosphoglucomutase 1 in a Subclone of the K-562 Cell Line

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ABSTRACT

Phosphoglucomutase 1, an enzyme mapping on the short arms of chromosome 1, is constantly missing in the leukemic cell line K-562 in spite of the presence of three No. 1 chromosomes. In the present work, a subclone of the cell line, K-562 (S)P, is described, where the enzyme can be demonstrated, thus excluding a small deletion as the cause for the lack of expression of phosphoglucomutase 1.

The relationship between the presence of the enzyme and the karyotype changes in this subclone is analyzed. Addition of several inducers to the standard K-562 line failed to elicit expression of the enzyme.

INTRODUCTION

The pluripotent stem cell line K-562 is a useful model in the study of the expression of differentiated proteins such as hemoglobin, glycophorin, l-i antigen, and acetylcholinesterase (1, 6, 8, 15). Several chemicals induce an increased synthesis of these proteins, while other enzyme activities are unaffected. Also, gene dosage can be investigated in the K-562 cell line. This cell line is near triploid, and the location of structural genes for several enzymes on different chromosomes is known (11).

During investigation on gene dosage, the activity of PGM3 in this line was found to be significantly lower than in another leukemic cell line (1). This finding could be explained by the observation that one of the electrophoretic forms of the enzyme, i.e., PGM 1, is constantly missing (13), and only the electrophoretic isoenzymes PGM 2 and PGM 3 are represented in these cells. Since PGM 1 isoenzyme constitutes about 90% of the whole enzyme level, the lack of this electrophoretic form explained the decreased total activity. The absence of PGM 1, however, is quite surprising, because chromosome 1, where this isoenzyme maps, is constantly trisomic in K-562 cells, and no gross deletion is detectable on standard cytogenetic examination. On the other hand, PGM activity may be under regulatory control, since the appearance and relative proportion of different isoforms show tissue specificity (10); PGM 1 may therefore be a differentiative marker, potentially responsive to inducers. Prerequisite to the evaluation of the inducibility or regulatory mechanisms acting on this enzyme is to exclude a deletion as the cause for the absence of the enzyme.

In the present paper, we describe the constitutive expression of PGM 1 in a clone of the K-562 cell line. The karyotype analysis of this clone is presented. Attempts to induce enzyme expression with potential inducers of differentiation on the standard K-562 line are also described.

MATERIALS AND METHODS

Cell Lines. Line HL 60 was kindly provided by Dr. A. Donelli (University of Modena, Modena, Italy), line K-562 was given by Dr. M. Gianni (University of Milan, Milan, Italy); subclone K-562 (S) was provided by Dr. L. Pegoraro (University of Torino, Torino, Italy). During the periodic tests of uniformity of this clone, a subclone, K-562 (S)P, was identified and characterized for PGM 1 and for karyotype (see Results).

Cells were grown in suspension in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum in humidified atmosphere with 5% CO2. For induction studies, cells (1 x 10^6/ml) were cultured for different times in the presence of various inducers as summarized in Table 1. Growth rate was measured daily, and cell viability was monitored by the trypan blue exclusion test under all culture conditions.

Electrophoresis Procedure. Cells were collected, washed with 0.15 M NaCl, and then diluted (1:4, v/v) in lysis buffer (2.7 mM EDTA:0.7 mM 2-mercaptoethanol, pH 7.0), lysed by 3 cycles of freezing-thawing, and centrifuged at 40,000 x g for 30 min at 4°C; the supernatant was used for analysis. Samples were then submitted to electrophoresis for 45 min at 250 V at 25°C using a Titan III, 60 x 76 mm strips (Helena Laboratories, Beaumont, TX), equilibrated for 10 min in the running buffer, in a homemade electrophoresis chamber equipped with an LBK (LBK Instruments, Bromma, Sweden) power supply. The running buffer consisted of 10 mM Tris-10 mM maleic anhydride, pH 7.0. The enzymatic staining was performed according to the method of Van Someren et al. (16). Briefly, the staining mixture consisted of 100 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 4.5 mM EDTA (pH 7.0), 0.25 mM KCl, 0.6 mM NADP, 8 mM glucose 1-phosphate, 30 mM glucose 1,6-diphosphate, 27 units glucose-6-phosphate dehydrogenase, 0.85 mM 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and 0.25 mM Phenazinomethasulfate. The staining mixture was applied to the strip for 30 sec; then the strip was incubated at 37°C in a humidified chamber; and after 10 min, the enzyme bands were visible.

Chromosome Analysis. Chromosome analysis was performed by standard techniques, with the following changes: the time of colchicine exposure was 20 min; after hypotonic shock for 2 min with 75 mM KCl prewarmed at 37°C, methanol/acetic acid (3:1, v/v) was added once. Air-dried slides were stained with Giemsa; then, after a selection of mitoses, they were destained with ethanol:acetic acid (1:1, v/v) for 10 min and with absolute ethanol overnight. Mitoses were photographed in Q-banding and, when indicated, stained for C-banding after treatment with Mcr-Ivaine’s buffer (pH 7.0) overnight and distilled water for 24 hr.

RESULTS

Clone K-562 (S), used in our laboratory to study the coordinated expression of hemoglobin and acetylcholinesterase (7), differs from the original line because of an increased level of hemoglobin and adherence. To secure the uniformity of this clone, the karyotype and several enzyme markers were periodically controlled. Several factors may in fact alter the composition of the cell line. Subsampling to perpetuate the line not only
Table 1

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Concentration</th>
<th>Days of treatment</th>
<th>Acetylcholinesterase</th>
<th>Hemo-</th>
<th>PGM</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemin</td>
<td>50 μM</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>1.25 mM</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>5-Azacytidine</td>
<td>1 μM</td>
<td>1 or 2</td>
<td>NT*</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>1.3% (v/v)</td>
<td>4</td>
<td>NT</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1 mM</td>
<td>4</td>
<td>NT</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>12-O-Tetradecanoylphorbol-13-acetate</td>
<td>20 μM</td>
<td>4</td>
<td>NT</td>
<td>NT</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Platelet-derived growth factor 0.5–1.0 unit/ml
Platelet-derived growth factor 10–20 μg/ml

*NT, not tested.

Porcine purified platelet-derived growth factor (Speywood, Ltd, Nottingham, England) with an activity of 235 units/ml and a specific activity of 0.49 unit/mg of protein.

Extract of human platelets containing partially purified platelet-derived growth factor obtained as described by Pledger et al. (12).

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Fig. 1. PGM pattern in K-562 (S)P subclone. A, RBC; B, HL-60 cells; C, K-562 cells; D, K-562 (S)P cells. Hb, hemoglobin.

PGM3
PGM2
PGM1
Hb

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We attempted to induce PGM 1 expression in the standard K-562 line with several chemicals. They were chosen as known inducers of other proteins in the same line (hemin, sodium butyrate, 5-azacytidine) or in other cell lines (dimethyl sulfoxide, retinoic acid, 12-O-tetradecanoylphorbol-13-acetate). Platelet-derived growth factor was used as a possible inducer of tyrosine phosphorylation (5), since induction of tyrosine phosphorylation was described as required for phosphoglycerate mutase function (4). Table 1 lists the compounds that were used. While hemoglobin and acetylcholinesterase were induced by hemin and butyrate, no expression of PGM 1 was observed with all the compounds used.

DISCUSSION

Lack of PGM 1 expression in the K-562 line may have different explanations. One of them predicts a small deletion, cytologically
one chromosome should induce the expression of the structural gene for the enzyme. The isolation of a clone derived from K-562 and showing the PGM 1 band strongly suggests that this is not the case. The reasons for the reappearance of PGM 1 in our clone, however, are not understood. Contamination with another cell type, as occasionally reported in some laboratories in spite of careful handling of the cell lines, was excluded on several criteria. De novo PGM 1 expression could either be related to chromosomal rearrangements which were detected during the karyotype evolution of the cell line or could either be related to chromosomal rearrangements which derived from K-562 and showing the PGM 1 band strongly the structural gene for the enzyme. The isolation of a clone analysis revealed that one of the 3 No. 1 chromosomes was in fact involved in a gross rearrangement, having a deletion in the short arms, where PGM 1 gene maps at 1p32 or 1p22–p311 or 1p33–p34 (11). There is, however, no reason why a deletion on one chromosome should induce the expression of the structural genes in the remaining 2 chromosomes, otherwise silent.

Deletion of one of the structural PGM 1 genes could be irrelevant, and the breakage of chromosome 1 at this particular site be significant only to release repression. Alternatively, rearrangements of other chromosomes could be critical in regulating PGM 1 expression, quite independently on the deletion on chromosome 1. Chromosome changes per se may also be irrelevant to PGM 1 expression. The clone was fortuitously isolated presumably because of increased adherence and hemoglobin level, and PGM 1 expression may be correlated with these properties. The concomitant increased expression of PGM 3 which is peculiar to differentiated tissues (10) further indicates a regulatory involvement in the expression of this isozyme. Finally, although several inducers failed to restore PGM 1 expression in the original cell line, other chemicals or growth factors remain to be tested to verify a possible PGM 1 induction.

A successive test for Mycoplasma contamination, performed as a standard control after the paper was submitted for publication, revealed the presence of Mycoplasma in both presently available K-562 and K-562 S(P) cells. However, electrophoretic analysis for PGM 1 confirmed the presence of PGM 1 only in clone K-562 S(P).

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REFERENCES

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