CONCISE REPORT

Hemin-Induced Conversion of Pyruvate Kinase Isozymes in K562 Cells

By S. Takegawa, T. Shinohara, and S. Miwa

The effects of hemin on the conversion of pyruvate kinase (PK) isozymes from the M_2-type to the L-type in K562 cells were investigated. Immunofluorescence, ion exchange chromatography, and electrophoretic studies showed that the untreated K562 cells contained only the M_2-type PK, while eight to 20 days after induction with hemin, concomitant with hemoglobin F synthesis, L-type PK levels increased while M_2-type PK levels decreased. Electrophoretic study revealed three hybrid isozymes of the L-type and M_2-type PK. We conclude that the conversion of PK isozymes from the M_2-type to the L-type in erythroid precursor cells occurs in the early stage of maturation.

WE HAVE RECENTLY REPORTED the presence of M_2-type pyruvate kinase (PK) in the proerythroblast stage and conversion of the M_2-type PK to L-type PK with erythroid maturation[1] and demonstrated a compensatory production of M_2-type PK in the orthochromatic erythroblasts of PK-deficient patients.[2] Lozzio and Lozzio[3] found that the K562 cell line obtained from the pleural effusion of a patient with Philadelphia chromosome-positive chronic myelogenous leukemia (CML) in blastic crisis can be differentiated into erythroid precursors after the addition of hemin.[4] Hence, this cell line seems to be suitable for examining changes in PK isozymes in erythroid precursor cells. In this report, we describe conversion of PK isozymes from the M_2-type to the L-type in K562 cells after exposure to an inducing agent, hemin.

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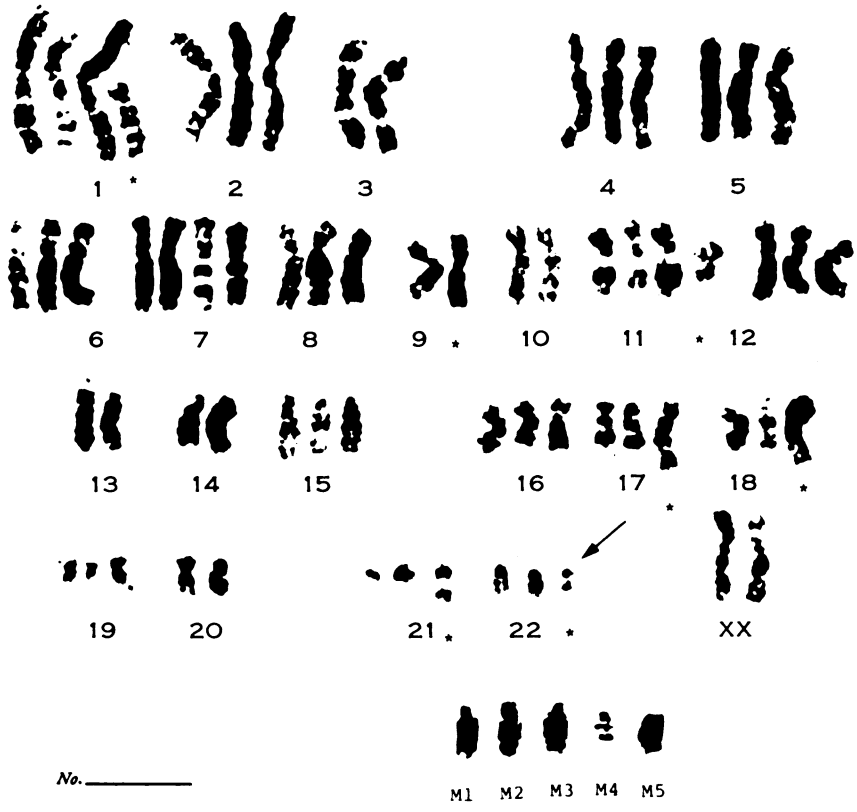

Fig 1. Karyotype of a pseudotriploid cell with 46 chromosomes stained with the Giemsa-banding technique. The Philadelphia chromosome (Ph^1) is identified by the arrow. *, abnormal chromosomes; M_{1-5}, markers.

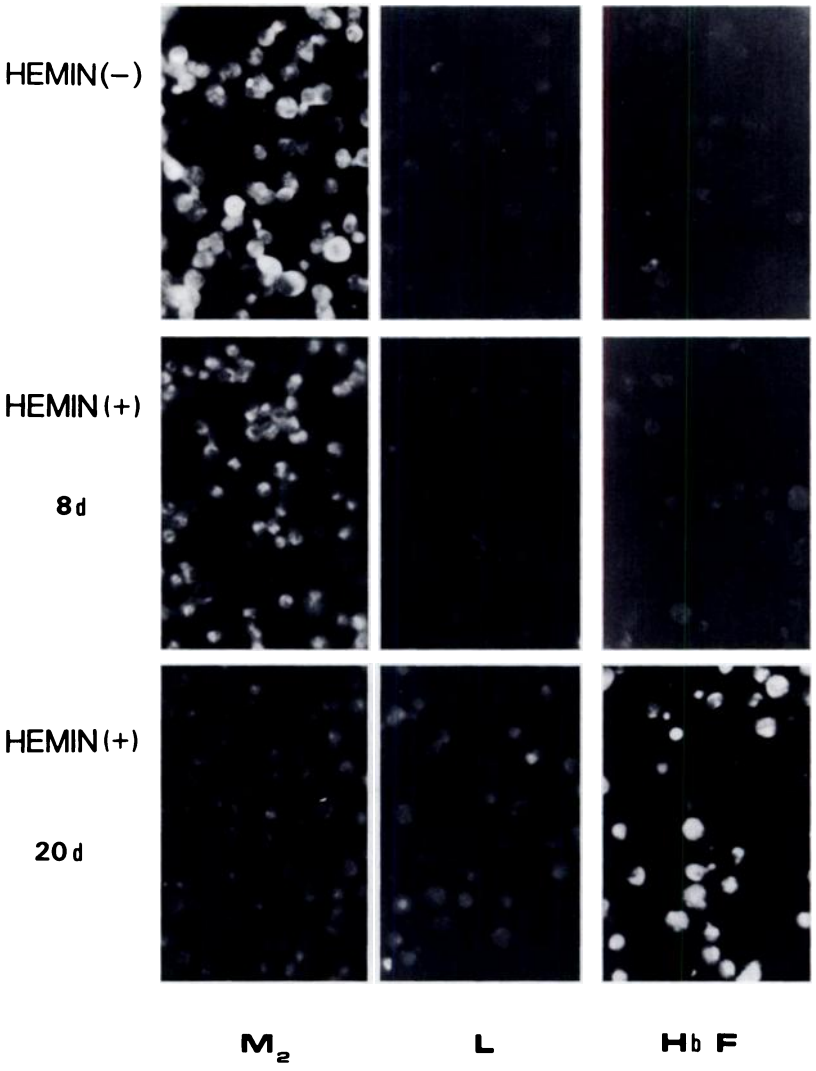

Fig 2. Immunofluorescent stain of K-562 cells. K-562 cells reacted with FITC-labeled anti-M_2-type PK (M_2), anti-L-type PK (L), and anti-hemoglobin F (Hb F). HEMIN(−), untreated K-562 cells; HEMIN(+) 8d, cells on day 8 of induction; HEMIN(+) 20d, cells on 20th day of induction.

MATERIALS AND METHODS

The K562 cells were a kind gift of Dr Yoshikura at the Department of Bacteriology, The University of Tokyo. Chromosome analysis was performed with the trypsin-Giemsa banding technique. The cultures contained 10^5 cells per milliliter in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum with or without 50 μmol/L hemin (Sigma Chemical Co, St Louis).[4] After 20 days the cells were harvested by centrifugation at 4 °C. Lysis was performed by addition of four volumes of 10 mmol/L sodium phosphate buffer, pH 6.0, containing 2 mmol/L $MgSO_4$, 0.2 mmol/L fructose-1,6-diphosphate (FDP) and 0.1% 2-mercaptoethanol (buffer A) and by sonication for two minutes. The cellular debris was removed by centrifugation at 4 °C for 30 minutes at 20,000 g.

Pyruvate kinase activities were determined at 37 °C according to the International Committee for Standardization in Haematology[5] (ICSH) recommended methods[5] using a Gilford Model 250 spectrophotometer. The column was loaded with 0.08 to 0.1 U of the enzyme preparation. For linear gradient elution, a CM-Sephadex C-50 (Pharmacia Fine Chemicals, Piscataway, NJ) column (1 × 18 cm) was equilibrated with buffer A. A 70-mL linear gradient of sodium phosphate buffer from 10 to 100 mmol/L was used for elution; 60 fractions were collected. All the operations were carried out at 4 °C. Pyruvate kinase electrophoresis was performed using polyacrylamide thin layer gel.[7]

Detection of L-type and M_2-type PK by the immunofluorescent method was described previously.[1] Hemoglobin F was determined by the immunofluorescent technique described by Wood et al.[6]

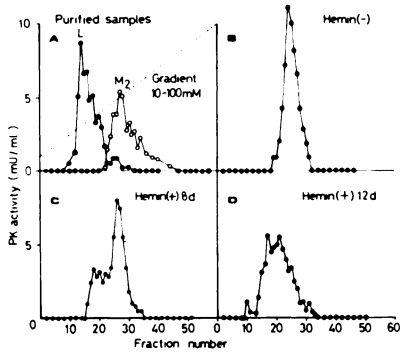

Fig 3. Change of PK in K-562 cell. CM-Sephadex column chromatography. Abbreviations used are the same as those in Fig 2.

RESULTS

Cytogenetic Studies

The K562 cells were aneuploid with a prominent, near-triploid mode (66 to 72). The karyotypic analysis revealed that all the cells contained at least seven abnormal chromosomes and three to five markers (Fig 1). The Philadelphia chromosome was identified, but the 9q+ chromosome was not observed. The banding pattern showed that the 17q+ chromosome was probably a translocation between one chromosome 15 and one chromosome 17.

Immunofluorescent Studies

Figure 2 shows the results of the immunofluorescent studies. The fluorescence of M_2-type PK was decreased after eight days of hemin induction, while that of L-type PK and hemoglobin F was increased with induction duration.

Ion Exchange Chromatography

The chromatographic patterns are shown in Fig 3. Purified L-type PK was eluted at an earlier position than the M_2-type PK (Fig 3A). In the sample from uninduced K562 cells, the elution profiles showed a single peak at the same position as M_2-type PK. On the other hand, in hemin-induced cells, a broad fraction was seen with a peak in a position between L- and M_2-type PK (Fig 3D).

Pyruvate Kinase Electrophoresis

Electrophoretic studies revealed three bands in addition to the L-type PK band, the mobilities of which were between L-type and M_2-type PK (Fig 4).

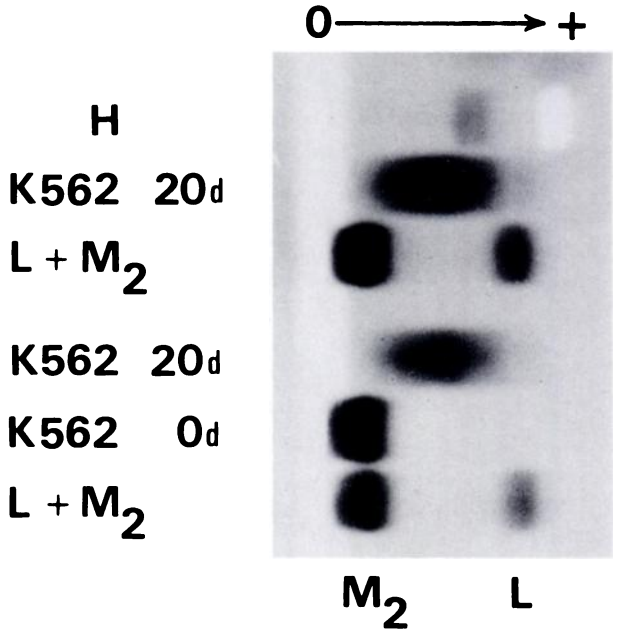

Fig 4. Pyruvate kinase electrophoresis. H, hemolysate; M_2, M_2-type PK; L, L-type PK.

DISCUSSION

We have reported the conversion of PK isozymes from the M_2-type to the L-type during early maturation of normal and PK-deficient erythroblasts.[1,2] K562 cells established by Lozzio and Lozzio[3] are known to differentiate into erythroid cells after the addition of hemin.[4] Many studies concerning embryonic hemoglobin synthesis and membrane proteins have been reported, but little is known about the glycolytic enzymes of this cell line.

Our immunofluorescent studies and CM-Sephadex chromatographic study confirm that conversion of PK isozymes occurs by induction with hemin, ie, M_2-type PK decreased and L-type PK increased. Furthermore, electrophoretic studies showed hybrid enzymes between M_2 and L. Cardenas et al[8] detected five isozymes of bovine PK by using the hybridization technique. We applied a similar technique to 20-day samples, but the zymogram showed only four bands (data not shown). There appears to be two reasons for this result. First, the ratio of M_2-type PK to L-type PK was not equal. Second, the condition of dissociation of PK subunits was not appropriate, and homotetramer isozyme, ie, $(M_2)_4$ could not form.

Recently, Jansen et al[9] showed the presence of L-type PK in K562 cells, but they failed to demonstrate the conversion of PK isozymes,[9] possibly because of conditions such as the batches of serum used and concentration of the inducers, which are of great importance in cell cultures.[10] Furthermore, the K562 cells used by Jansen et al[9] exhibited different karyotypic characteristics from our K562 cells.

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