CONCISE REPORT

Synthesis of Adult-Type Hemoglobin in Human Erythremia Cell Line

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KMOE-2/05 cells, derived from a patient with acute erythremia, became benzidine-positive after the addition of cytosine arabinoside (CA). Radioimmunoassays using anti-human hemoglobin antibodies revealed an elevated amount of hemoglobin in the CA-exposed cells over that in the control cells (without CA). Isoelectric focusing of the CA-exposed cell lysate formed benzidine-positive foci in the positions of human adult Hb (HbA) and human fetal Hb (HbF). To determine the types of globin synthesized in the CA-exposed cells, globin chains internally labeled with \(^{3}H\)-leucine were purified by carboxy-methyl (CM)-Sephadex column chromatography, immunoadsorption by Sepharose-coupled anti-human Hb antibodies and Sephadex G-100. The labeled globin chains were finally separated by CM-cellulose chromatography in urea. Two distinct peaks of radioactivity were shown in exactly the same fractions as carrier human globin \(\alpha\) and \(\beta\)-chains. These observations indicate that these KMOE-2/05 cells synthesize HbA.

CONTINUOUS human erythroid cell lines provide good model systems elucidating specific events that occur during erythroid cell maturation. K562 and HEL cells respond to hemin or butyrate, resulting in induction of hemoglobin synthesis.\(^1\)\(^2\) However, hemoglobin synthesized by induction was reported to be only fetal or embryonic. A clonal cell line, KMOE-2/05, separated from the original KMOE cell line,\(^3\) developed benzidine-positive cells after the addition of cytosine arabinoside (CA), mitomycin C, and daunomycin.\(^4\) We report here the results of analysis of synthesized Hb after the cells were exposed to CA.

MATERIALS AND METHODS

Radioimmunoassay of Hb in the Cell Lysate

Antihuman Hb antibodies were purified from antiserum of guinea pigs that were hyperimmunized with human Hb using immunoadsorbent prepared by coupling human Hb to cyanogen bromide-activated Sepharose 6B. The antibodies of IgG2 class were obtained from the purified antibodies by chromatography on DEAE-cellulose according to the method of Yagi.\(^5\) The IgG2 antibodies and radioiodinated human Hb thus obtained were used for radioimmunoassay (RIA).

Isoelectric Focusing of the Cell Lysates

KMOE-2/05 cells were grown for 10 days in alpha-medium (Flow Labs, McLean, Va), supplemented with 10% fetal bovine serum (FBS, Flow) with or without \(10^{-3}\) mol/L CA (Sigma Chemical Corp., St. Louis, Mo). K562 cells were grown for 4 days in the same medium with 50 \(\mu\)mol/L hemin (Type I, Bovine, Sigma). Five \(\times\) \(10^{8}\) KMOE-2/05 cells, or \(1.3 \times 10^{7}\) K562 cells, were washed and lysed by three cycles of freezing and thawing in 0.5 mL of distilled water. Insoluble proteins were removed by centrifugation at 500 g for 15 minutes and 140,000 g for 45 minutes. Thirty-five microliters of lysates from unexposed or CA-exposed KMOE-2/05 cells and hemin-exposed K562 cells, 15 \(\mu\)L of HbA (500 \(\mu\)g/mL) and HbF (500 \(\mu\)g/mL) were subjected to isoelectric focusing on a slab of acrylamide gel with a pH range of 3.5-9.5 in an LKB multiphor unit\(^6\) (LKB Instruments, Gaithersburg, Md), and stained with benzidine. HbA and HbF were separated by carboxy-methyl (CM)-Sephadex from adult and cord blood erythrocytes, respectively.

Chromatography of Cell Lysates on CM-Sephadex

Lysate from CA-exposed \(5 \times 10^{7}\) of KMOE-2/05 cells was dialyzed against 0.05 mol/L phosphate buffer (PB) pH 6.0, and was applied on a 0.9 \(\times\) 1.7 cm column of CM-Sephadex (C-50, Pharmacia Fine Chemicals, Piscataway, Nj), equilibrated against the same buffer.\(^7\) After unbound material was washed away with 6 mL of buffer, HbF and HbA fractions were eluted with 60 mmol/L NaCl and 300 mmol/L NaCl in 0.05 mol/L PB, pH 6.0, respectively. One-milliliter fractions were collected at 4°C. An aliquot of each fraction was subjected to RIA.

Chromatography of Globin Chains on CM-Cellulose in Urea

KMOE-2/05 cells (\(1.5 \times 10^{6}\) cells) were incubated for 24 hours with 1 \(\mu\)Ci of \(^{3}H\)-leucine (New England Nuclear, Boston, Mass) and \(10^{-3}\) mol/L CA in 100 \(\mu\)L of leucine-free Eagle’s minimal essential medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% dialyzed fetal bovine serum on day 8 from exposure to \(10^{-3}\) mol/L CA. Lysate from the \(^3\)H-labeled KMOE-2/05 cells were separated by CM-Sephadex. Fractions containing HbA were pooled and immunologically adsorbed to Sepharose coupled with antihuman Hb IgG2 antibodies. K562 cells were incubated for 48 hours with 100 \(\mu\)Ci of \(^{14}C\)-leucine (New England Nuclear) and 50 \(\mu\)mol/L hemin in 100 \(\mu\)L of leucine-free Eagle’s MEM with \(10^{-3}\) dialyzed FBS. \(^{14}C\)-labeled hemoglobin was adsorbed to Sepharose-haptoglobin (from human serum, Green Cross Corporation, Tokyo, Japan) using the method derived by Tsapis et al.\(^8\) Labeled globin chains were eluted from the Sepharose-anti-Hb-\(^3\)H–Hb or Sepharose-haptoglobin-\(^{14}C\)-Hb complexes with 5% formic acid after removal of the heme with acetone/HCl (2%). Eluted globin chains were further purified by gel-filtration chromatography at room temperature, on a 1.5 \(\times\) 50 cm column that was packed with Sephadex G-100 (Pharmacia) and equilibrated in 20%
formic acid. The labeled globin chains were then separated on CM-cellulose (CM-52, Whatman, Clifton, NJ) in 8 mol/L urea and 0.05 mol/L 2-mercaptoethanol with a linear gradient of Na₂HPO₄ from 5 to 30 mmol/L at pH 6.7. Twenty milligrams of unlabeled globin was added initially as a carrier. Fractions (3 mL) were collected at a flow rate of 1 mL/min. An aliquot (1 mL of each fraction) was used for the determination of radioactivity after mixing it with 10 mL of Aquasol II (New England Nuclear).

RESULTS

KMOE-2/05 cells were cultured for 10 days with 10⁻⁴ mol/L CA. Approximately 2,500 cells per 1 x 10⁵ cells were benzidine-positive. The amount of Hb in the cells cultured for 10 days in the presence of 10⁻⁴ mol/L CA was quantitated by RIA. Approximately 1 μg of Hb per 1 x 10⁶ cells was detected (data not shown). Cells from control cultures without the addition of CA contained less than 10 ng of Hb per 10⁶ cells.

Lysates from CA-exposed KMOE-2/05 cells (3.5 x 10⁶ cells) and hemin-exposed K562 cells (1 x 10⁶ cells) were subjected to Hb analysis by isoelectric focusing on slabs of polyacrylamide gels (Fig 1). A major benzidine-positive focus was formed at the region of human HbA, and a minor benzidine-positive focus at that of HbF. Both foci were also stained by Coomassie Blue. Cell-lysate from control KMOE-2/05 cells cultured without the addition of CA did not form any benzidine-positive focus. The benzidine-positive focus of hemin-exposed K562 cell lysate was not observed in the region of HbA (data not shown).

Further analysis was performed to reveal HbA synthesis in CA-exposed KMOE-2/05 cells. Hb in KMOE-2/05 cells, exposed to CA for 8 days, were internally labeled by [³H]leucine. The radiolabeled lysate of the cells was subjected to CM-Sephadex column chromatography. The main radioactive peak appeared in fractions of carrier HbA (Fig 2). The fractions containing HbA (tube Nos. 3, 4, and 5 after elution with 300 mmol/L NaCl in Fig 2A) were pooled and applied to Sepharose 6B coupled with antihuman Hb antibodies. The labeled globin chains were eluted from the Sepharose after removal of the heme. Eluates collected exactly as above (A) were monitored in a Hitachi (Tokyo, Japan) 100-40 spectrophotometer at 540 μM for detection of Hb.

![Fig 1. Isolelectric focusing of the lysate from 3.5 x 10⁶ KMOE-2/05 cells exposed to 10⁻⁴ mol/L CA. Authentic HbA and HbF were separated by CM-Sephadex from adult and cord blood erythrocytes, respectively. Lanes 1 and 5, HbA; lane 2, HbA plus HbF; lane 3, lysate from CA-exposed KMOE-2/05 cells; lane 4, HbF.](image-url)

![Fig 2. Chromatography of lysate from CA-exposed KMOE-2/05 cells on CM-Sephadex. (A) Fractionation of the lysate from CA-exposed 5 x 10⁶ cells. (B) Eleven milligrams of cord blood Hb and 13 mg of adult blood Hb were applied to a reference column. Eluates collected exactly as above (A) were monitored in a Hitachi (Tokyo, Japan) 100-40 spectrophotometer at 540 μM for detection of Hb.](image-url)
CA is a well-known inhibitor of DNA synthesis. However, inhibitors of DNA synthesis, such as 5-fluorouracil, were not effective for the induction of benzidine-positive cells, so mechanisms other than the inhibition of DNA synthesis are supposed to be responsible for the induction of Hb-synthesizing cells.

Chromatographic patterns of [3H]leucine-labeled globins from CA-exposed KMOE-2/05 cells suggested a balanced synthesis of \( \beta/\alpha \)-globin chains (\( \beta/\alpha = 1.14 \)) (Fig 3A), which roughly agrees with the value of in vivo synthesis of HbA in human adult erythrocytes.

We compared the Hb synthesis in hemin-exposed K562 and CA-exposed KMOE-2/05 cells. The K562 cells start to synthesize Hb after 4 days of exposure to hemin. In contrast, KMOE-2/05 cells need to be exposed to CA for 10 days for Hb synthesis to begin. The most striking difference between the two cell lines was the number of benzidine-positive cells after exposure to the Hb inducer. More than 50% of K562 cells were benzidine-positive, but the percentage of benzidine-positive-KMOE-2/05 cells was only 2.5% after exposure to inducers. The hemoglobin content in 10^6 KMOE-2/05 cells was 1 g after 10 days of culture with CA as determined by RIA. Rutherford et al reported that the amount of Hb in 10^6 K562 cells after exposure to hemin for six days was 3.5 \( \mu \)g, which was determined by visible-absorption spectrum analysis. The difference in the sensitivity to triggers for Hb synthesis was also observed between two cell lines. Hemin, or butyrate, which was effective for induction of embryonic and/or fetal type Hb on K562 and HEL cells, did not show any Hb-synthesizing effect on KMOE-2/05 cells. Butyrate had a positive effect on original KMOE cells, but not on the clone used in the experiment.

The human erythremia cell line KMOE-2/05 will be a unique source of material for investigating the molecular mechanisms of biosynthesis of human HbA.

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REFERENCES


4. Manuscript in preparation


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