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ELECTROPHORETIC SEPARATION OF HUMAN EMBRYONIC GLOBIN DEMONSTRATES " α-THALASSEMIA" IN HUMAN LEUKEMIA CELL LINE K562.

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SUMMARY

Human embryonic, fetal, and adult globin chains ($\zeta,\varepsilon,{}^{A}\gamma,{}^{G}\gamma,\beta,\alpha$) can be separated by electrophoresis on Triton Acid urea gels. K562, a human leukemia cell line, was induced with hemin, labelled with [^{3}H]-leucine, and globin synthesis analyzed. All globins except β were produced. $\varepsilon > \zeta$; ${}^{G}\gamma:{}^{A}\gamma$=70:30; non-α:α=>2:1. Thus, hemin-induced K562 synthesized embryonic and fetal globin chains, and had globin synthetic imbalance, with "α-thalassemia."

INTRODUCTION

Human leukemia cell line K562 was developed from the culture of cells obtained from the pleural fluid of a patient with chronic myelogenous leukemia in blast crisis (1). K562 was found recently to contain red cell membrane proteins (2), and to produce hemoglobin following culture with hemin (3). Rutherford et al used hemoglobin electrophoresis to demonstrate the presence of human embryonic and fetal hemoglobins in induced K562 (3). Separation of globin chains by chromatography on carboxymethyl cellulose showed a synthetic ratio of α/γ of 0.08; the embryonic globin chains were not recovered with this method (4,5), and thus overall globin chain synthetic ratios could not be determined.

We have previously reported the use of an electrophoretic technique to separate human globin chains ζ, ${}^{A}\gamma$, ${}^{G}\gamma$, β, and α (6,7,8). We report here that this method, polyacrylamide electrophoresis in urea, acid, and Triton X-100, also separates the ε chain of the embryonic hemoglobin, Gower-1 ($\zeta_2\varepsilon_2$) (5).

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We used this gel system to confirm the production of embryonic and fetal globin chains by K562 following induction with hemin. There was an excess of synthesis of non-α chains, thus suggesting a "thalassemic" defect in K562.

METHODS

Hb Portland ($\zeta_2\gamma_2$) was obtained from the blood of a fetus with homozygous α thalassemia, and was provided by Dr. Bernard Forget (7). Hb Gower-1 ($\alpha_2\varepsilon_2$) was the kind gift of Dr. Rosemary Gale (5). Cord and adult blood samples were obtained according to protocols approved by the Human Investigation Committee at the Children's Hospital Medical Center and the Boston Hospital for Women. Red blood cells were washed with 0.9% NaCl, and lysed with distilled water. Hemolysates or column-purified hemoglobins were electrophoresed on Triton acid urea polyacrylamide gels as described in detail elsewhere (6). This gel electrophoresis method was modified from the technique described by Rovera et al (9). In brief, the gels contained 12% acrylamide, 0.08% bisacrylamide, 6 M urea, 5% acetic acid, and 2% Triton X-100. Slab gels were 15 cm long. Electrophoresis buffer was 5% acetic acid. Pre-electrophoresis was conducted for 1 hour at 200 volts, and repeated for 45 minutes at 150 volts following the addition of 1 M cysteamine to the gel slots. Samples were prepared by the addition of 5 µl of cell lysates plus 5 µg of unlabelled carrier cord blood hemolysate to 20 µl of sample buffer containing 6 M urea, 5% acetic acid, and 0.8 M mercaptoethanol. Electrophoresis was for 20 hrs at 10 mamps. [^{3}H]-leucine labelled globins were identified by fluorography (10) using preflashed film (11) The fluorograms were scanned at 615 nM in a Gilford 2400 spectrophotometer equipped with a linear transporter. The area under each globin peak was determined by drawing a line connecting the lowest points before and after each peak. A perpendicular line was dropped between the ζ and ε chains. The peaks were quantitated by weighing.

Cell line K562 was provided by Dr. Stuart Orkin, who obtained it from Andersson et al (2). The cells were grown in Dulbecco's modification of Eagle's Medium, containing 10% heat-inactivated fetal calf serum (Flow Laboratories), in 10% CO_2 in a water-jacketed 37°C incubator. The cells were passed once a week, at 1/10-1/20 dilution. For incubation, 10^5 cells were placed in 10 ml of medium, containing the appropriate inducing agent (12). The tested inducers included 280 mM dimethylsulfoxide (Fisher Chemical Co.), 1 mM butyric acid (Aldrich Chemical Co.), and 50 µM hemin (Eastman Kodak Co.). Only hemin led to induction of hemoglobin, as determined by staining of cytocentrifuge preparations of cells with benzidine-Wright-Giemsa, or globin synthesis, as determined by the fluorograms. At four or five days, the cells were resuspended in 2 ml of medium without leucine, to which 25-100 µCi of [^{3}H]-leucine were added (New England Nuclear Corp., > 100 Ci/mM). After incubation for four hours, the cells were thrice washed in Hank's balanced salts (Grand Island Biological Co.) containing 1% bovine serum albumin, and lysed in 1 mM phosphate, pH 7.4.

RESULTS AND DISCUSSION

Electrophoresis of human hemoglobins on Triton gels leads to separation of all the major normal globin chains, as shown in figure 1 A. From anode to cathode, the bands are ζ, ε, $^A\gamma$, $^G\gamma$, β, and α. The identity of the bands was determined by electrophoresis of hemoglobins of known globin composition (6,7). This electrophoretic technique provides the first one-step method for

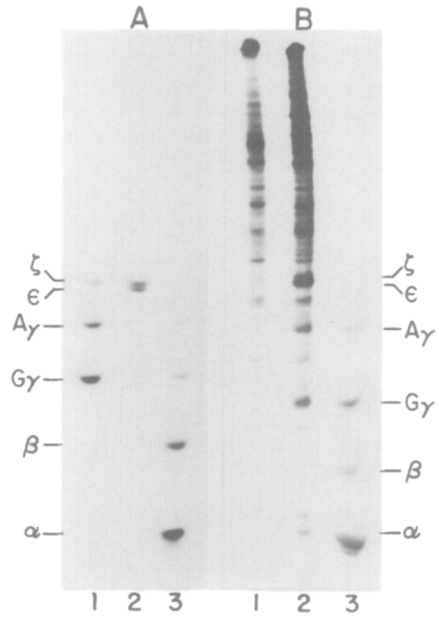

FIGURE 1: A) Separation of globin chains by electrophoresis on 15 cm slab gel containing urea, polyacrylamide and Triton X-100 (6). 5 µg of Hb was applied in each well. Proteins were stained with Coomassie blue.
1) Hbs Portland and Barts, containing ζ, $^{A}\gamma$ and $^{G}\gamma$ globins. From a fetus with homozygous α thalassemia.
2) Hb Gower-1, containing ζ and ε globins.
3) Hbs F and A, containing $^{A}\gamma$, $^{G}\gamma$, β and α globins.

B) Separation of [^{3}H]-leucine-labelled proteins on 15 cm slab gel. Radiactive proteins were located by fluorography (10) using preflashed film (11), and quantitated by densitometry (6).
1) Uninduced K562.
2) K562 induced for 4 days with 50 µM hemin.
3) Hb F and A.

separation of all the globins and for determination of globin synthetic ratios when embryonic hemoglobins are present.

Culture of K562 cells with hemin for four days produced a brown-red cell pellet suggestive of hemoglobin production. The Triton polyacrylamide gel electrophoretic patterns of [^{3}H]-leucine-labelled proteins synthesized by these cells after culture for four days without and with 50 µM hemin are shown in figure 1 B. The densitometry scans are shown in figure 2. There was increased radioactivity in the regions of the embryonic and fetal globin chains, confirming the finding of Rutherford et al (3). Globin synthesis represented

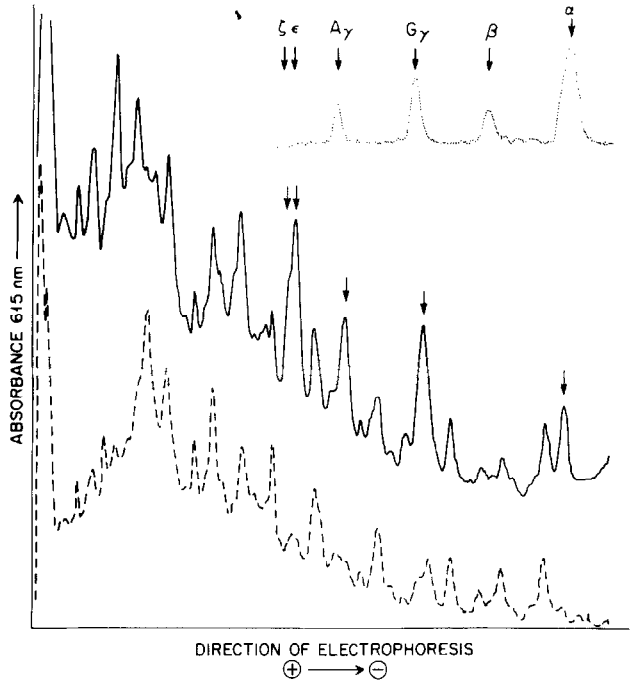

FIGURE 2: Densitometry scans of fluorograms shown in figure 1B.
--- Uninduced K562
___ K562 induced for 4 days with 50 μM hemin
··· Hb F and A

approximately 7.5% of total protein synthesis. Both ζ and ε chains were produced, with ε in excess of ζ. The embryonic globins comprised 30% of total globin synthesis. $^{A}\gamma$ and $^{G}\gamma$ (γ chains with alanine or glycine in position 136) were both synthesized; $^{G}\gamma$ represented 70% of the γ globin chains, identical to the value found in normal fetuses and newborns (8). There was a small amount of α globin chain synthesis, and no apparent increase in radioactivity in the β region. The non-α/α ratio, (ε+γ)/(ζ+α) was ∿ 2 and 4 in 2 experiments, while α/γ was 0.16 and 0.30, similar to the 0.08 ratio found by Rutherford et al (3). After growth for five days with 50 μM hemin, non-α/γ was 2.0, and α/γ was 0.38. $^{G}\gamma$ remained 70% of total γ.

Hemin was the only inducing agent which we tested which led to globin synthesis by K562. Butyric acid and dimethylsulfoxide did not induce globin production. With hemin induction, ε synthesis exceeded ζ, and ε+γ was much greater than ζ+α. Thus hemin-induced K562 appears to have a form of

"α-thalassemia", with a non-α/α ratio similar to that found in HB H (β_4) disease. Rutherford et al (3) showed the presence of Hb Bart's (γ_4) by starch gel electrophoresis. This hemoglobin is seen in newborn infants with α - thalassemia trait. Although some patients with erythroleukemia have been found to have an acquired form of Hb H disease (13), Hb H has only been described in one patient with chronic myelogenous leukemia (14).

There are several possible explanations for the finding of embryonic chains and chain imbalance in cell line K562. The original cell which led to the establishment of the cell line may have been programmed for the production of these globin chains; culture with hemin may have been responsible for selective transcription of the embryonic globin gene. Other inducing agents might lead to different transcription products, as has been reported in murine erythroleukemia cell lines (12). The K562 cell line is a potential in vitro model for studies of human globin chain regulation, of switching from embryonic to fetal and also to adult globin, and of imbalanced globin synthesis. Separation of the globin chains by electrophoresis now provides a method for complete analysis of these studies at the protein level.

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