Hemin Control of Heme Biosynthesis and Catabolism in a Human Leukemia Cell Line

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Hemin treatment of the Philadelphia chromosome positive leukemia cell line, K562, accentuates a number of erythroid phenotypic characteristics. The nature of this heme effect was investigated by examining heme production and heme biosynthetic and catabolic enzyme activity in untreated and 0.05 mM hemin-treated cells. Activities of δ-aminolevulinic acid synthetase (ALAS), the rate limiting heme synthetic enzyme, and δ-aminolevulinic dehydratase (ALAD) were detectable in both uninduced and induced K562 cells. However, cells treated with hemin showed a significant increase in ALAS and ALAD activity by 2.5 days of treatment compared to untreated cells, and both enzyme activities further increased over the remainder of the incubation period. Incorporation of 3H-ALA into heme was also monitored. 3H-ALA was incorporated into heme in both untreated and treated cultures, but this incorporation was significantly greater in hemin-treated cells. Incorporation of 3H-ALA into heme indicates that cellular heme biosynthesis accounts for a portion of the heme utilized for hemoglobin production and that hemin stimulates heme synthesis by increasing enzyme activities distal to ALAS in the heme biosynthetic pathway. Heme oxygenase, the rate-limiting enzyme in mammalian heme degradation, was also studied and found to be present in large amounts in treated and untreated K562 cells. However, heme oxygenase activity was significantly decreased following hemin treatment. The demonstration of heme oxygenase activity in K562 cells documents the presence of this heme catabolic enzyme in human erythropoietic stem cells. The fall in heme oxygenase activity that accompanies hemin treatment might reflect an effort by erythroid cells to conserve heme during erythroid differentiation. These studies indicate the central role that heme synthesis and catabolism play during human erythroid differentiation.

K562 CELLS ARE DERIVED from a Philadelphia chromosome positive cell line initiated from the pleural effusion of a patient with chronic granulocytic leukemia in terminal blast crisis. When cultured in the presence of hemin or sodium butyrate, these cells undergo considerable erythroid maturation in vitro. This involves not only the synthesis of hemoglobin but also membrane and enzymic changes typical of normal human embryonic-fetal erythroid differentiation. Thus, K562 cells are potentially a useful system for studying the dynamics of human erythropoiesis and for analysis of expression of specific genes during erythroid differentiation.

An increase in heme synthesis is a pivotal event occurring during erythroid differentiation. Sassa has examined changes in heme biosynthetic enzyme activities and heme concentration during dimethyl sulfoxide (DMSO) induced differentiation of murine Friend leukemia virus transformed cells. His data suggest that a sequential induction by DMSO of these enzyme activities occurs during the process of erythroid differentiation. Granick and Sassa have also examined the effect of hemin treatment on Friend virus transformed cells, finding that exogenous hemin is not only directly incorporated into the heme of the newly synthesized hemoglobin but that it also stimulates the synthesis of cellular heme. This article describes the effects of hemin treatment on heme synthesis of the human leukemia cell line K562 and provides further information concerning changes in heme synthetic enzyme activity during the process of human erythroid differentiation.

Heme oxygenase (HO) is the rate-limiting enzyme in heme catabolism. This enzyme is found in the microsomal fraction of a number of organs, and its activity is inducible in a variety of tissues including kidney, liver, and bone marrow. Ibrahim and coworkers have recently reported that HO activity is not limited to phagocytic cells. They have demonstrated HO activity in murine erythroid colonies. We have also detected HO activity in K562 cells and have shown that HO activity actually decreases as these leukemia cells accumulate hemoglobin. These data suggest that HO activity might play an important role during erythroid differentiation and that it is responsive to different regulatory factors than HO activity of phagocytic cells.

MATERIALS AND METHODS

Cell Line

K562 cells were a kind gift of Drs. T.R. Rutherford and J.B. Clegg; these cells have been shown to produce embryonic and fetal hemoglobin in response to hemin. Cells were maintained at 37°C in a humidified atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum. “Induction” was accomplished by adding...
a 100-fold concentrated stock of hemin to a final concentration of 0.05 mM. Hemin was prepared as described by Rutherford et al.\textsuperscript{12} and sterilized by filtration prior to addition to the cultures. Cell growth was continued for 5.5 days after the addition of hemin. Exposure to hemin under these conditions did not reduce cell viability as determined by trypan blue exclusion. Aliquots of cells were removed after 1.5, 2.5, 3.5, 4.5, and 5.5 days of hemin exposure and assayed for d-aminolevulinic acid synthetase (ALAS), d-aminolevulinic acid dehydratase (ALAD), HO activity, and for \(^3\)H-ALA incorporation into heme.

**Enzyme Assays**

The activity of ALAS was determined according to the radiochemical assay method of Ebert et al.\textsuperscript{13} using 10^7 cells/assay. ALAD activity was determined spectrophotometrically by the micromethod of Sassa et al.\textsuperscript{14} The pH optimum for the activity of K562 ALAD was narrowly defined; the enzyme exhibited maximal activity at pH 6.0–6.2. HO activity was assayed according to the method of Ibrahim and Levere.\textsuperscript{15}

**Incorporation of \(^3\)H-ALA Into Heme**

K562 cells were grown in 850 sq cm plastic roller bottles at a concentration of 0.3–1.5 \times 10^6 cells/ml in the presence of 4 \mu Ci/ml of \(^3\)H-ALA. Aliquots of 10^7 cells were harvested by centrifugation at daily intervals, washed with saline containing 1% trichloroacetic acid and subsequently washed twice with saline. After washing the cells with acetone 4 times, cellular radioactive heme was extracted by the methods of Bonkowsky et al.\textsuperscript{16} The heme was crystallized and washed with distilled water at 4°C. The extracted heme was dissolved in 5.9 M ammonium hydroxide and bleached with 30% aqueous H\textsubscript{2}O\textsubscript{2}. \(^3\)H-heme was counted by a Beckman scintillation counter (LS-250).

**RESULTS**

ALAS and ALAD activity in untreated and hemin-treated K562 cells during a 5.5-day period of incubation is shown in Fig. 1. ALAS activity was detectable in untreated cultures on day 1.5 of incubation, and this activity increased 30% over the incubation period. ALAD activity was also studied in these cultures. Significant levels of ALAD activity were assayed after 1.5 days of incubation of untreated cells, and the enzyme activity increased by 55% after 5.5 days of incubation (Fig. 1). The presence of detectable amounts of ALAS and ALAD activity in untreated K562 cells suggests that differentiation of these cells toward the erythroid pathway may take place spontaneously but occurs at a low rate.

In contrast to untreated cells, hemin-treated cells showed a significant increase in ALAS and ALAD activity by 2.5 days (Fig. 1). Activity of both these enzymes increased progressively over the 5.5-day incubation period. These data indicate that hemin treatment of K562 cells results not only in an increase of hemoglobin formation, but also leads to an increment of ALAS and ALAD activity.

In order to determine if endogenous heme synthesis is stimulated by hemin treatment of leukemia cells, \(^3\)H-ALA incorporation into heme was investigated. We found that \(^3\)H-ALA was utilized by both untreated and hemin-treated K562 cells to produce heme (Fig. 2). The rate of \(^3\)H-ALA incorporation was significantly higher in hemin-treated cells after 2.5 days and continued to further increase during the induction period. These results indicate that endogenous cellular heme biosynthesis accounts for at least a fraction of heme incorporated into the hemoglobin produced by these cells. Hemin treatment does not solely supply exogenously added heme for incorporation into hemoglobin, but also stimulates heme synthetic enzyme activity and cellular heme biosynthesis. The increased incorporation of \(^3\)H-ALA into heme indicates that hemin stimulates the synthesis of
HEME METABOLISM IN K562 CELLS

Fig. 2. Changes in the incorporation of $^3$H-ALA into heme. Cells (K562) were grown in the absence (*) or in the presence of 0.05 mM (A). Data are the mean of duplicate assays.

heme by increasing enzyme activity from ALAD to ferrochelatase.

HO, the rate-limiting enzyme in heme degradation, was also studied in untreated and hemin-treated K562 cells (Fig. 3). HO activity was present in both sets of incubations. In the untreated cells, HO activity fell by 2.5 days and then proceeded to oscillate over the remainder of the incubation period. Hemin-treated K562 cells showed significantly less amounts of HO activity after 1.5 days of incubation, and this activity further diminished by 3.5 days, remaining at this low level for the rest of the incubation period.

DISCUSSION

Erythroid differentiation is an extremely complex phenomenon involving multiple regulatory events. The development of in vitro culture techniques utilizing semisolid media have provided a useful means for studying the clonal proliferation and differentiation of erythropoietic stem cells. These assay systems provide progeny of human stem cells for analysis, but unfortunately, the hematopoietic stem cells themselves are not identifiable or present in sufficient numbers to allow detailed biochemical analysis during the early stages of erythroid differentiation. Recently, the human leukemia cell line, K562, has been shown to express a number of erythroid properties when exposed to appropriate in vitro culture conditions. These cells appear to possess a proliferative capacity that resembles that of the early erythroid progenitor cell, the burst-forming unit-erythroid, BFU-E. This cell line has allowed us to analyze biochemical changes during erythroid differentiation.

Rutherford et al. have rigorously established that K562 cells after exposure to hemin produce embryonic hemoglobins (Hb Portland and Hb Gower) as well as small quantities of fetal hemoglobin (HbF). Benz and coworkers have analyzed K562 mRNA by cell-free translation and molecular hybridization with authentic globin cDNA and have demonstrated that steady-state levels of embryonic globin mRNA are increased in these cells after exposure to hemin. In addition, cDNA synthesized from K562 cell mRNA was cloned in bacterial plasmids by recombinant DNA technology. A number of recombinants were obtained that hybridized to globin cDNA, and restriction endonuclease analysis of some of these individually cloned cDNAs yielded results consistent with those expected for $\alpha$, $\zeta$, or $\gamma$ globin cDNAs. All of these studies confirm the erythroid nature of the K562 cell line.

It was the purpose of this study to clarify the role of hemin in the induction of erythroid differentiation in K562 cells. The results of this study demonstrate that hemin treatment of these cells results not only in an increase of hemoglobin concentration but also an increment in heme biosynthetic enzyme activity. Our data also clearly demonstrate that hemin stimulates cellular heme synthesis. Direct evidence has been obtained for an augmentation of ALAS and ALAD
activity following hemin exposure. Hemin also appears to stimulate other enzymes in the heme biosynthetic pathway, including ferrochelatase as judged by the increased incorporation of $^3$H-ALA into heme.

The heme degradative potential of the K562 cell line was also measured by assaying for HO activity. HO is the rate-limiting enzyme in human heme catabolism. Both untreated and hemin-treated K562 cells possessed HO activity at a higher order of magnitude than that previously reported for bone marrow cells. Surprisingly, hemin treatment resulted in a significant drop in HO activity. This decrement is contrary to that reported in mice with normoblastosis, where an enhanced release of heme from hemoglobin was associated with a marked induction of hepatic microsomal HO activity. The fall in HO activity by differentiating K562 cells is in agreement with data recently reported by Ibrahim and coworkers in studies of HO activity in erythropoietin-stimulated murine erythroid colonies. Our data suggest that the decrement in erythroid HO activity is an important feature of the erythroid differentiation process, perhaps permitting the conservation of heme for eventual incorporation into hemoglobin.

Hemin is known to suppress hepatic heme synthetic activity. By contrast, hemin induces the same pathways in murine erythroleukemia cells and also augments the erythroid colony-forming capacity of normal mouse bone marrow cells. Similarly, hemin stimulates heme biosynthetic activity in K562 cells. The mechanism by which hemin stimulates ALAS activity in erythroid cells is unclear. It appears that heme biosynthesis in K562 leukemia cells and perhaps in normal human erythroid stem cells is subject to a different control system than that reported for hepatocytes.

REFERENCES

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