MOUSE FETAL HEMOGLOBIN SYNTHESIS IN MURINE ERYTHROLEUKEMIC CELLS

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1. Introduction

In humans, hemoglobin F (Hb F) is the predominant hemoglobin from week 6-32 of the gestational period. After week 32 of gestation, the percentage of β-globin synthesis increases while γ-globin production decreases [1]. The switch from Hb F to hemoglobin A (Hb A) occurs during normal hematopoietic development. Individuals with severe β-globin hemoglobinopathies, such as victims of sickle cell anemia or β-thalassemia, have a lower severity of disease, if there is an enhancement of Hb F synthesis. The regulatory mechanism that controls the Hb F to Hb A switch is not known.

Murine erythroleukemic cells (MELC) have been used as an in vitro model to start erythroid differentiation [2,3]. The hemoglobins synthesized in the clones derived from the DBA/2 mouse are indistinguishable from the host animal [4,5]. The DBA/2 adult has two hemoglobins which have been designated β-major and β-minor hemoglobins [6]. After induction of MELC with dimethylsulfoxide (DMSO) [4,7–9], butyric acid (BA) [8–10], or hemin [9,11–13], the different types of hemoglobins are synthesized in different quantities. Therefore, the clones derived from the DBA/2 mouse have been postulated as a useful model system to study gene switching [9].

The T3C12 clone was derived from the DDD mouse [14]. This clone has been shown not to have a β-minor hemoglobin upon induction [15]. In this report, one hemoglobin in addition to the adult hemoglobin was found by the induction of BA in T3C12 cells. The data suggest that this additional hemoglobin may be the fetal hemoglobin of DDD mouse and the synthesis of the fetal hemoglobin may be dependent on the inducer concentration during the transcriptional and post-transcriptional processing.

2. Materials and methods

Two murine erythroleukemic cell lines, clone 745 (GM86) and clone T3C12 (GM979) were obtained from the Institute for Medical Research, Camden, NJ. The cultures were grown in suspension in α-medium, containing 10% fetal calf serum and antibiotics, at 37°C and 5% CO_2 atmosphere. For erythroid induction, the cells were cultured with DMSO or BA for 5 days. Hemoglobin was obtained by washing the cells with Dulbecco's phosphate-buffered saline (PBS) twice and lysing them with water.

C57BL/6 and DBA/2 mice were obtained from the Mammalian Genetics Branch, National Cancer Institute, USA. Male adult DDD mice were a gift from Drs Kiyoshi Suzuki and Yasuo Kawanishi, the Institute of Medical Science, University of Tokyo. Adult mouse blood was collected in a heparinized syringe by heart puncture. Fetal mouse blood was collected by incision of the jugular vein of fetuses. Hemoglobin was obtained by washing the erythrocytes 3 times with PBS and lysing them with water.

All hemolysates were centrifuged at 20 000 × g for 30 min to remove cell debris. Hemoglobin samples were then saturated with CO and treated with KCN to 1%. Isoelectric focusing was run at 400 V for 4 h in 4% polyacrylamide gel containing ampholytes 6–8 [16]. The gel was scanned later at 420 nm on a spectrophotometer.

3. Results and discussion

After erythroid induction by either 1.5% DMSO or 1 mM BA, clone 745 cells synthesized two hemoglobins which were indistinguishable by isoelectric point (pI) from the two adult hemoglobins of DBA/2 mouse (fig.1). The two hemoglobins produced in clone 745 cells have also been shown indistinguishable from these produced in the adult DBA/2 mouse by using the techniques of chromatography and electrophoresis [4,5,8].

Clone T3C12 line was derived from DDD mouse [15]. In contrast to DBA/2 mouse, there is only one hemoglobin in the adult DDD mouse (fig.2C). Upon induction by 1.5% DMSO, T3C12 cells synthesized one hemoglobin with a pI similar to the adult hemoglobin of DDD mouse (fig.2A). Induced by 1 mM BA, T3C12 line produced two hemoglobins; one of which had a similar pI as the adult hemoglobin and the other had a higher PI (fig.2B).

A fetal hemoglobin has been reported in mouse [17,18]. Work in our laboratory has also shown that C57BL/6 and DBA/2 fetal mouse contains a fetal hemoglobin ([19], unpublished observation). Comparing the pI of these fetal hemoglobins with that of the hemoglobin synthesized in T3C12 cells, it appears that the T3C12 hemoglobin with a higher pI may be the fetal hemoglobin of DDD mouse (fig.3). The C57BL/6 mouse contains one adult hemoglobin and one fetal hemoglobin during gestation. Since the adult hemoglobin of DDD mouse has the similar pI as that of C57BL/6 mouse it is conceivable that DDD mouse may also have a fetal hemoglobin which has the similar pI as the fetal hemoglobin of C57BL/6 mouse. However, the possibility cannot be excluded that before a fetal hemoglobin is found in the fetal DDD

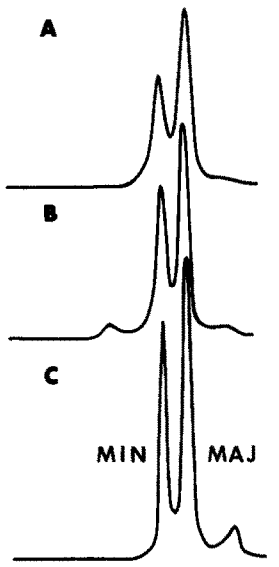

Fig.1.

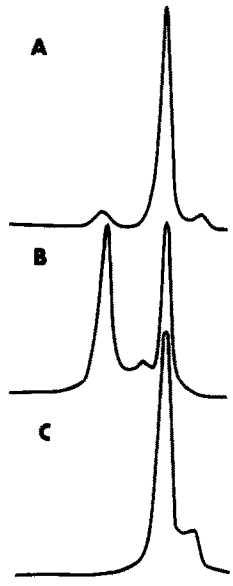

Fig.2.

Fig.1. Spectrophotometric scans at 420 nm of isoelectric focusing of hemoglobin in polyacrylamide gels between pH 6 and 8. (A) Hemoglobins in 745 cells induced by DMSO; (B) hemoglobins in 745 cells induced by BA; (C) major (MAJ) and minor (MIN) adult hemoglobin of adult DBA/2 mouse.

Fig.2. Spectrophotometric scans at 420 nm of isoelectric focusing of hemoglobin in polyacrylamide gels between pH 6 and 8. (A) Hemoglobin in T3C12 cells induced by DMSO; (B) hemoglobins in T3C12 cells induced by BA; (C) adult hemoglobin of DDD mouse.

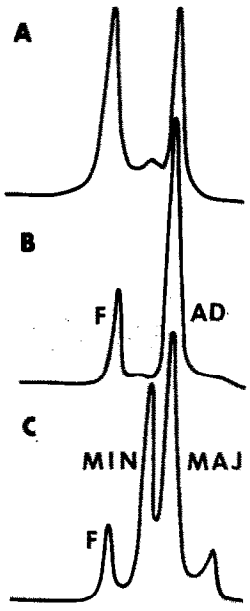

Fig.3.

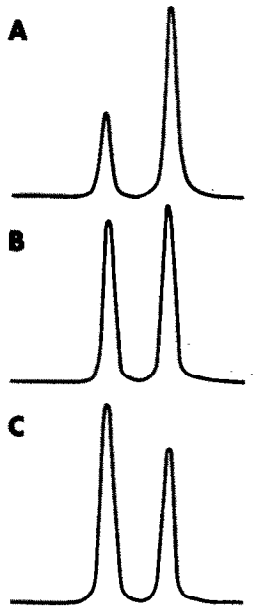

Fig.4.

Fig.3. Spectrophotometric scans at 420 nm of isoelectric focusing of hemoglobin in polyacrylamide gels between pH 6 and 8. (A) Hemoglobins in T3C12 cells induced by BA; (B) fetal (F) and adult (AD) hemoglobin of day 17 fetal C57BL/6 mouse; (C) fetal (F), major (MAJ) and minor (MIN) hemoglobin of day 18 fetal DBA/2 mouse.

Fig.4. Spectrophotometric scans at 420 nm of isoelectric focusing of hemoglobin in polyacrylamide gels between pH 6 and 8. Hemoglobins in T3C12 cells induced by (A) 0.5 mM, (B) 1 mM, (C) 2 mM BA for 5 days.

mouse, this higher pI hemoglobin may be the minor hemoglobin of the adult DDD mouse described [15]. Nevertheless, those investigators did not find the minor hemoglobin in the clones T3C12 and 5000 upon the induction by BA.

The synthesis of the fetal and adult hemoglobin was varied by various concentrations of the inducer. As seen in fig.4, at 0.5 mM BA for 5 days, 34% of the total hemoglobin which the cells synthesized was the fetal hemoglobin; at 1 mM BA, 47% of the total was the fetal hemoglobin; and at 2 mM BA, 64% of the total was the fetal hemoglobin. On the other hand, when the cells were cultured with 2 mM BA for 2 days, then in fresh medium without inducer for 3 days, only 50% of the total hemoglobin was the fetal hemoglobin. These data lead us to suggest that the synthesis of the fetal or adult hemoglobin may be regulated by the inducer concentration during the transcriptional and post-transcriptional processing.

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