THE EFFECT OF HEMIN ON THE EXPRESSION OF β GLOBIN GENES IN FRIEND CELLS

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1. Introduction

The hemoglobin beta chain (Hbb) locus of the mouse is on chromosome 7 [1]. In several strains of mice, this locus controls the structure of at least two types of β globin chains (β major and β minor) that differ only in a few amino acids [2–5]. It is possible that the genes coding for these chains are the result of reduplication of an ancestral gene [5], but detailed linkage studies are not presently available.

It is not yet clear whether the expression of the two β globin genes is independently regulated at the transcriptional level or whether translational mechanisms favor the expression of one β gene over the other. That regulatory mechanisms do exist, however, is suggested by the difference in the ratio of the two β gene products in differently treated cells. For example, in the erythrocytes of adult DBA/2 mice the ratio of β major to β minor chains is 4 [6]; in Friend cells (clone 745) induced to differentiate either by dimethylsulfoxide (Me_2SO), hexamethylene bisacetamide, or butyric acid, the ratio ranges from 3–1, respectively [7,8].

In this paper we report that it is possible to completely dissociate the expression of the β minor gene from that of the β major gene. Using a new method of globin chain separation, we have investigated the effect of several inducers of erythroid differentiation on Friend erythroleukemia cells derived from DBA/2 mice (clone 745), and have observed that hemin, a good inducer of hemoglobin synthesis [9], causes the synthesis of only β minor and not β major globin chains. In contrast, almost equal amounts of both β minor and β major globin chains are expressed in Friend cells treated with either of two other inducers of differentiation, Me_2SO or hypoxanthine. Both chains are expressed but the β minor predominates in *N*-methylacetamide (NMA)-treated Friend cells and in a spontaneously differentiating variant clone of Friend cells (clone D). Thus, two β globin genes, probably the result of duplication, have independent mechanisms regulating globin expression. The system described here could provide an excellent tool for investigating in detail the mechanisms regulating the expression of the Hbb locus.

Abbreviations: NMA, *N*-methylacetamide; Me_2SO, dimethylsulfoxide; B+, benzidine-positive; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; Hbb, hemoglobin beta chain

2. Materials and methods

Friend erythroleukemia cells (clone 745) of DBA/2 mouse origin [10] were grown as previously described [11]. Less than 0.5% benzidine-positive (B+) cells were present in the population at the time of the experiments reported here. Cells were plated at a concentration of 1×10^5 cells/ml in the presence of one of the following inducers of hemoglobin synthesis: Me_2SO (230 mM), hemin (0.1 mM), hypoxanthine (6 mM) and NMA (13.7 mM). After 4 days the cells were refed with medium containing the inducers and labeled for 48 h with 1 μCi/ml of a ^{14}C-labeled amino acid mixture (Amersham). The percentage of hemoglobin-containing cells present in the induced cultures at the time of harvest was determined in cell suspension using benzidine reaction [12]. Me_2SO-treated cultures had 83% B+ cells, hypoxanthine-treated cultures 78%, and NMA-treated cultures 62%. The percentage of B+ cells in hemin-treated cells was not assayed because the presence of free heme interferes in the assay.

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Clone D cells, which spontaneously differentiate without inducers, were routinely grown in the presence of 1.6×10^{-7} M TPA to avoid reversion [13]. These cells were released into the normal medium at the time of the experiments and labeled as described above 4 days later. From 60–75% B+ cells were present at the time of harvest at day 6 as determined by the suspension benzidine method [12]. Hemoglobin was solubilized from 5×10^7 cells using 1 ml lysis buffer (0.3% Triton X-100, 50 mM Tris, pH 7.0, 25 mM KCl, 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol) as described by Kabat et al. [7]. Globin chains were analyzed after denaturation of the lysates in 4 M urea, 5% acetic acid and 5% β mercaptoethanol. The proteins present in the lysate (specific activity 330–540 cpm/μg) were separated electrophoretically using the Triton-acidic urea gel system described by Zweidler [14]. The concentrations of Triton X-100 used are described in the text. Globin chains were identified using as markers either total red cell lysates or isoelectric-focusing purified hemoglobin of adult DBA/2 mice and of 13-day-old DBA/2 mouse embryos [15]. After fluorography, the samples were exposed for 24–48 h at −70°C using preflashed Kodak RR-Royal X-Omat film [16,17]. The amount of globin chains present in each band was determined by scanning either autoradiograms or gels stained with Amido Black at 600 nm with a Corning model 750 scanning densitometer equipped with integrating capabilities. No differences in the ratios were detected when stained gels and autoradiograms were compared.

3. Results

Globin chains were separated by electrophoresis on polyacrylamide gels containing acidic urea and Triton X-100. In order to determine the concentration of Triton X-100 most suitable for separating the various globin chains, slabs containing a horizontal gradient of Triton (0–2%) were utilized. Figure 1a shows separation of globins of DBA/2 adult mouse erythrocytes. All the globin chains migrated more slowly in the presence of higher Triton concentrations. The α globin moved fastest toward the cathode, and β globins slowest. The α and β globins were distinguished by comparing this pattern to that obtained from red cell lysates of 13-day-old DBA/2 mouse embryos lacking β globins [15]. Characteristically, we resolved β globins progressively better in this gel system by increasing the Triton X-100 concentration. The β major was the slowest migrating globin chain. The β minor migrated between α and β major. The distance of β minor from α was constant throughout the gradient of nonionic detergent whereas its distance from β major increased with increasing Triton concentration. Figure 1b shows the pattern obtained on the same type of Triton gels from a lysate of untreated Friend cells. No appreciable amounts of globin chains could be detected. Figure 1c shows the type of globin chains found in Friend cells induced to differentiate by Me_2SO. Although a number of polypeptides were present in the gels in this case, the globin chains were among the fastest to migrate toward the cathode. Again, as in the case of DBA/2 adult mice, the β chains could be resolved into a β major and a β minor chain. In contrast, cells treated with 1×10^{-4} M hemin contained α globin and only one β globin chain (fig.1d). This β globin has been identified as β minor since it migrated throughout the Triton gradient at a constant distance from the α chain and comigrated with β minor when a mixture of lysates of DBA/2 adult mouse and hemin-induced Friend cells were electrophoresed.

Figure 2 shows a comparison of the electrophoresis profiles of globin chains from Friend cells treated with Me_2SO, hypoxanthine, NMA and hemin and from clone D spontaneously differentiating cells. In this experiment the samples were electrophoresed in parallel on gel slabs containing 2% Triton.

Both β major and β minor chains were present in all but the hemin-treated cells. Some other polypeptides migrating near β were also present in NMA-treated cells and in cells of clone D. However, these polypeptides could be readily identified as non-β globin chains by examining the corresponding slab gels containing a horizontal gradient of Triton X-100.

Figure 3 shows the relative amounts of β major and β minor globin chains in Friend cells induced to synthesize hemoglobin by different drugs and in DBA/2 adult mouse cells. Gel slabs were scanned using a Corning model 750 scanning densitometer, and the surface area under each peak was integrated by computer. The ratio of β major to β minor globin chains (average of 3 experiments) was 3.8 in DBA/2 adult mice, 1.2 in Me_2SO-treated, 0.91 in hypoxan-

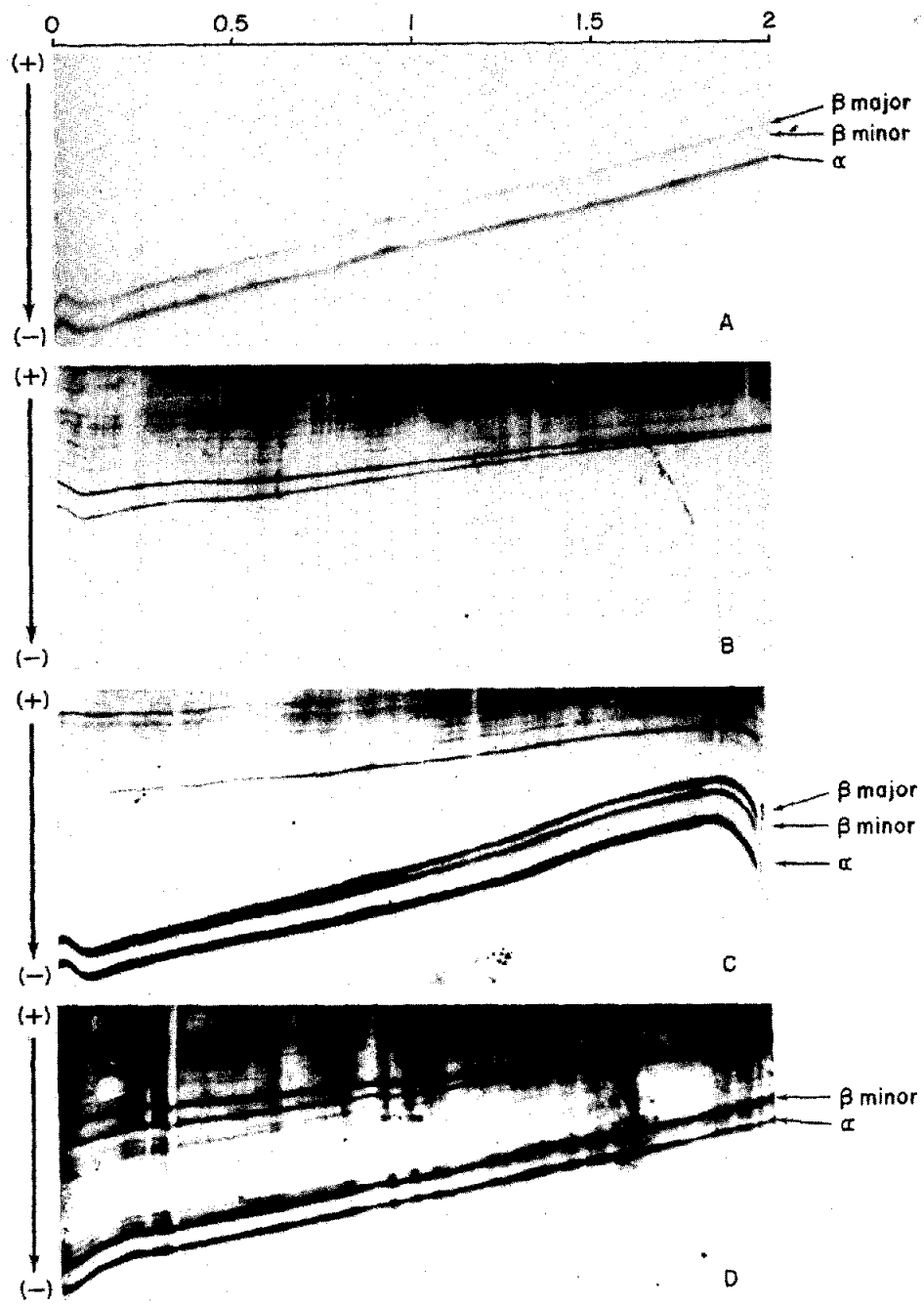

Fig.1. Electrophoretic separation of globin chains on polyacrylamide gel slabs containing a horizontal gradient of Triton X-100. Abscissa: Triton concentration. Protein samples (200–400 μg) prepared as described in Materials and methods were elctrophoresed on each slab for 9 h at 140 V. (a) Adult DBA/2 erythrocytes; (b) Untreated Friend erythroleukemia cells; (c) Friend erythroleukemia cells treated for 6 days with 230 mM Me_2SO; (d) Friend erytholeukemia cells treated for 6 days with 1×10^{-4} M hemin.

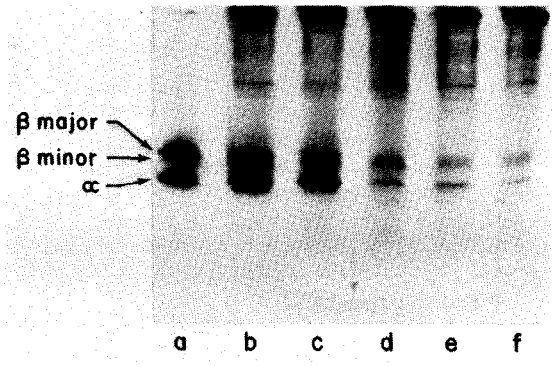

Fig.2. Electrophoretic separation of globin chains on polyacrylamide gel slabs containing 2% Triton X-100. Lysates from: (a) DBA/2 mouse erythrocytes; (b–e) Friend cells (clone 745) treated for 6 days with Me_2SO (230 mM), hypoxanthine (6 mM), hemin (1×10^{-4} M) and NMA (13.7 mM), respectively, and (e) untreated, spontaneously differentiating cells of clone D. Gels were stained with Amido Black and destained by diffusion.

thine-treated, 0.69 in NMA-treated Friend cells and 0.30 in clone D cells.

4. Discussion

In this paper we report that nonallelic variants of β globin polypeptides in DBA/2 mice can be readily resolved by Triton urea gel electrophoresis even without prior purification of the hemoglobins and that the induction of synthesis of the two β globin chains can be dissociated in Friend cells of DBA/2 origin induced to differentiate. Nonionic detergents have been reported to affect the migration rate of proteins in an electrophoretic field, by interacting with the hydrophobic groups of the proteins and forming mixed micelles that have a slower electrophoretic migration [13,18]. This principle has been utilized here in order to completely resolve the β major from the β minor chain of mouse DBA/2 hemoglobin.

In Friend cells (clone 745) hemin induces the synthesis of only β minor and not β major globin. Kabat et al. [7] and Nudel et al. [8] reported that the β major to β minor ratio is variable in Friend cells treated with Me_2SO, butyric acid or hexa-

Fig.3. Relative amounts of β globin chains: (a) In adult DBA/2 mouse erythrocytes; (b–e) In Friend cells treated for 6 days with Me_2SO, hypoxanthine, hemin, NMA, respectively; (f) Untreated cells of spontaneously differentiating clone D; (g) Untreated Friend cells, clone 745.

methylene bisacetamide. Nudel et al. [8], using a wheat germ translation system, have shown that no translational defects are responsible for this variability, which suggests that alteration in the expression of β globin chains in induced Friend cells reflects an alteration in the transcription of β globin genes. That this is also the case in hemin-treated cells remains to be established, especially because of the known effect of hemin on translation [19,20]. Our results confirm that the expression of the β globin genes in Friend cells varies using different inducers [7,8], and identify hemin (and therefore its reduced form, heme) as specifically involved in the regulation of the expression of the β minor globin chain. Using hemin as an inducer, the expression of a single globin gene can be analyzed in more detail. Further, if induction is due to transcription of the β minor gene, a β minor mRNA could be isolated free of β major mRNA contamination.

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