PROTEIN AND GLYCOPROTEIN SYNTHESIS
BY FRIEND ERYTHROLEUKAEMIC CELLS
DURING ERYTHROID DIFFERENTIATION

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SUMMARY

The proteins synthesized by Friend erythroleukemic cells (line 707B) have been studied by high-resolution polyacrylamide gel electrophoresis at various stages after the induction of erythroid differentiation by addition of dimethyl sulphoxide to the medium. After 24 h of differentiation, the rate of synthesis of most of the more abundant proteins is reduced, correlating with the reduction in the proliferation rate of the cells. However, the qualitative composition of the major proteins synthesized by control and differentiating cells remains very similar until at least 96 h after the induction of differentiation. Proteins characteristic of the mature erythrocyte membrane are an exception to the reduction in synthesis in line with the proliferation rate, for they continue to be synthesized at similar rates to those found in control cells. Only two major proteins (an as yet unidentified cytoplasmic protein and globin) are reproducibly induced, while the synthesis of a few proteins is almost completely abolished. In contrast to the similarity of the synthesis of the major proteins in the control and differentiating cells, the synthesis of glycoproteins that bind to Concanavalin A is markedly changed. The synthesis of many Concanavalin A-binding glycoproteins is abolished and they disappear from differentiating cells. The synthesis of a major glycoprotein is, however, induced early in differentiation. This protein, which has an apparent molecular weight of 100,000, becomes the most abundant Concanavalin A-binding glycoprotein in the cell.

INTRODUCTION

Friend erythroleukemic (FL) cells differentiate in vitro along the erythroid differentiation pathway when an inducing agent such as dimethyl sulphoxide (DMSO) or sodium butyrate is included in the growth medium. In response to the inducer, cell proliferation and total protein synthesis are reduced (for a review see Harrison, 1977), but the synthesis of several proteins characteristic of the mature erythrocyte is either initiated or much increased. These proteins include globin (Friend, Scher, Holland & Sato, 1971) and, in some cell lines, spectrin and glycophorin (Eisen, Bach & Emery, 1977a; Rossi et al. 1978).

High-resolution 2-dimensional polyacrylamide gel electrophoresis has allowed the study of the synthesis of a large number of the most abundant FL cell proteins (Peterson & McConkey, 1976b; Reeves & Cserjesi, 1979a). These studies have shown that, after several days in the presence of DMSO, few changes are detectable in the patterns of the resolved proteins; only the relative intensities of some spots on the
L. M. Marshall and R. C. Hunt

autoradiograms have changed (Peterson & McConkey, 1976b). However, when sodium butyrate is used as the inducing agent, a large number of alterations in the gel patterns occur (Reeves & Cserjesi, 1979b). While it is known that the rate of total protein synthesis falls after the induction of erythroid differentiation (Harrison, 1977), it is not known whether this reduction of synthesis applies equally to most of the major proteins of the differentiating cell or whether the reduction is selective; nor is it known whether synthesis is terminated coordinately or at various times after the onset of erythroid differentiation. In addition, little is known about the order of initiation of the synthesis of induced proteins, except that initiation of spectrin synthesis precedes that of globin (Eisen et al. 1977a) in those cells in which spectrin synthesis is inducible.

We (Hunt & Marshall, 1979) and others (Eisen et al. 1977b; McDonald, Letarte & Bernstein, 1978; see also Harrison et al. 1978) have demonstrated that changes occur in the surface proteins of FL cells in response to the addition of DMSO to the growth medium. For example, differentiating cells change in their response to lectins (Eisen et al. 1977b; Hunt & Marshall, 1979), plant proteins that bind to surface carbohydrates and which often cause intercellular agglutination. We have shown that the agglutination of the 707B line of FL cells by Concanavalin A (Con A) is markedly reduced between 24 and 48 h after induction by DMSO. This fall in agglutination is not the result of a change in the number of Con A receptors on the cell surface, nor of a change in their distribution over the cell; rather, there seems to be a change in the types of Con A-binding glycoproteins associated with the cell.

In this report we show that high-resolution polyacrylamide gel electrophoresis resolves approximately 300 of the most abundant proteins of non-induced FL cells when they are pulse-labelled with [35S]methionine. Differentiating cells also make the vast majority of these proteins, although to a greatly reduced extent after 24 h in the presence of DMSO. The synthesis of only one of the resolved proteins is increased, but some proteins, notably actin, glycoporphin and spectrin, all components of the mature erythrocyte plasma membrane, are made almost to the same extent by differentiating as by control cells, thereby becoming a much larger proportion of the total protein synthesis of differentiating cells. The cellular complement of glycoproteins that bind Con A is markedly changed during the course of differentiation. In particular, the synthesis of a major glycoprotein is induced during the first 24 h after induction by DMSO and this becomes the major Con A receptor by the end of the differentiation process.

MATERIALS AND METHODS

Materials

Ampholytes were obtained from LKB, Bromma, Sweden; ultra-pure urea from Schwartz-Mann, Orangeburg, N.Y.; [35S]methionine (1380 Ci/nmol) and Na181I (11–17 mCi/µg I) from the Radiochemical Centre, Amersham, England; Con A (type IV) and Con A-agarose (300 mg Con A/g agarose) from Sigma Chemical Co., St Louis, Missouri.
Friend cell protein synthesis

Cells

The 707B line of FL cells was obtained from Dr T. Rutherford, Radcliffe Infirmary, Oxford, England (the history of this cell line is given by Harrison et al. 1978). Cells were grown in Nunc plastic tissue culture flasks in Ham’s F-10 medium (Flow Laboratories), supplemented with 5% foetal calf serum (Gibco), BME amino acids (Gibco), antibiotics and glutamine.

For induction of erythroid differentiation, DMSO was added to the medium of actively proliferating cells to give a final concentration of 230 mM.

Pulse-labelling of cells

For the total protein synthesis studies, 10^6 cells were harvested by centrifugation at 670 g for 5 min and resuspended in 25 ml methionine-free Ham’s F-10 medium supplemented with 10 μCi [35S]methionine.

For the experiments involving the synthesis of Con A-binding proteins, approx. 10^7 cells were harvested as above and resuspended in 50 ml methionine-free Ham’s F-10 medium supplemented with 50 μCi [35S]methionine. After incubation at 37 °C for 2 h (in the case of the total protein synthesis studies) or 6 h (in the case of studies of the synthesis of Con A-binding glycoproteins) the cells were harvested and washed in Dulbecco’s phosphate-buffered saline (pH 7.4) lacking calcium (PBS).

Extraction of Con A-binding glycoproteins

Approximately 10^7 [35S]methionine-labelled control or differentiating FL cells were washed in a solution containing 20 mM-Tris-HCl (pH 7.6), 0.34 M-sucrose, 1 mM-adenosine 5’-triphosphate (ATP), 1 mM-ethylene diamine tetraacetic acid (disodium salt) (EDTA) and 0.2 mM-phenyl methyl sulphonyl fluoride (PMSF) and resuspended in 5 ml extraction buffer: 50 mM-Tris-HCl (pH 7.6), 25 mM-KCl, 5 mM-CaCl2, 5 mM-MgCl2 and 2% Triton X100. After 30 min at 4 °C, during which the cells were occasionally agitation, the extract was centrifuged at 35,000 rev./min in an SW40 rotor for 30 min. The supernatant was then applied to a column containing 3 ml Con A-agarose, which had been washed and pre-equilibrated with extraction buffer. After washing the column with extraction buffer until the eluted radioactivity fell to background, the Con A-binding glycoproteins were specifically eluted with extraction buffer containing 0.2 M-α-methyl mannoside. The eluted proteins were concentrated by precipitation with ice-cold 10% trichloroacetic acid and the recovery checked by scintillation counting. They were then processed for electrophoresis as described below.

Extraction of actin

[35S]methionine-labelled FL cells were homogenized in a buffer containing 4 mM-Tris-HCl (pH 8), 0.2 mM-CaCl2, 0.2 mM-ATP, 0.5 mM-dithiothreitol and 0.2 mM-PMSF. The homogenate was then centrifuged at 8000 rev./min in an MSE18 centrifuge for 5 min and the supernatant applied to a column containing 5 ml DNase I-Sepharose (synthesized according to the method of Axen, Porath & Ermback, 1967). The column was washed with 0.1 M-NaCl, 1 mM-CaCl2, 50 mM-Tris-HCl (pH 7.4) and then with a solution of 0.5 M-sodium acetate, 1 mM-CaCl2 and 30% glycerol (glycerol buffer). The actin was eluted from the column in glycerol buffer containing 3 M-guanidine hydrochloride and dialysed against water. The protein was lyophilized and prepared for electrophoresis as described below.

Sub-cellular fractionation

To prepare nuclei, [35S]methionine-labelled cells were washed in 10 mM-Tris-HCl (pH 8–0) containing 0.34 M-sucrose and 1 mM-EDTA. They were then resuspended in this solution supplemented with 1 mM-ATP, 1 mM-dithiothreitol and 1 mM-PMSF. Nonidet P-40 was added to a final concentration of 1% and the cells homogenized. The homogenate was then further supplemented with an equal volume of 0.5 M-NaCl, 2% 2-mercaptoethanol in 10 mM-Tris-HCl (pH 8–0). The undissolved material was recovered by centrifugation at 20,000 rev./min
in an SW60 rotor for 30 min. The pellet was observed by phase-contrast microscopy to be composed of nuclei.

In order to obtain a cytoplasmic fraction, cells were homogenized in 4 mM-Tris-HCl (pH 8) containing 0.2 mM-CaCl₂, 0.2 mM-ATP, 0.5 mM-dithiothreitol and 0.2 mM-PMSF and the homogenate centrifuged at 45,000 rev./min in an SW60 rotor for 1 h. The supernatant was concentrated by dialysis at 4 °C against polyethylene glycol and dissolved for polyacrylamide gel electrophoresis as described below.

**Single-dimension polyacrylamide gel electrophoresis**

Samples for analysis were dissolved in 5% sodium dodecyl sulphate (SDS) and 2% 2-mercaptoethanol and heated on a boiling water bath for 2 min. They were then analysed on 8.5% or 15% polyacrylamide slab gels using the buffer system of Laemmli (1971).

**Two-dimensional high-resolution polyacrylamide gel electrophoresis**

Cell pellets were frozen and thawed in sonication buffer and dissolved for isoelectric focusing as described by O'Farrell (1975). Trichloroacetic acid precipitates were dissolved by addition of 0.1% SDS, 0.1% 2-mercaptoethanol and heating at 100 °C for 2 min. After cooling, urea was added to give a final concentration of 9 M, followed by an equal volume of O'Farrell's lysis buffer. The samples were then processed for isoelectric focusing as described (O'Farrell, 1975).

Nuclear pellets were found to give poor results using the above method for solubilization and were dissolved using the method described by Peterson & McConkey (1976a). Isoelectric focusing was carried out on 10-cm tube gels containing a mixture of 3-10 and 5-8 ampholytes (O'Farrell, 1975). For the second dimension, 8.5% polyacrylamide slab gels were employed using the buffer system of Laemmli (1971).

The slab gels were dried and autoradiographed for periods of 7, 14 and 21 days. This ensured that spot intensity of highly radioactive proteins correlated with incorporated radioactivity in shorter exposures while minor proteins were detected after longer exposures.

The pH profile of the isoelectric focusing gels was determined by slicing a focused gel into 2-mm segments and eluting for 48 h at 4 °C in 0.5 ml glass-distilled water. The pH of the eluted material was then determined using a glass electrode.

**Detection of Con A-binding glycoproteins using iodinated lectin**

Control and differentiating FL cells were dissolved in SDS/mercaptoethanol as described above and analysed on single-dimension 8.5% polyacrylamide slab gels. After fixation of the gel in 45% methanol/45% water/10% acetic acid, it was washed extensively in PBS and then immersed for 48 h in a solution of 125I-labelled Con A (prepared as described by Arndt-Jovin & Berg, 1971). Excess Con A was then washed away with PBS, the gel dried and autoradiographed (Burridge, 1976). The autoradiograms were scanned with a Joyce-Loebl Chromoscan microdensitometer.

**Detection of erythrocyte membrane proteins by blotting onto nitrocellulose membranes**

FL cell proteins were separated by gel electrophoresis on 8.5% polyacrylamide/SDS gels. The proteins were transferred by diffusion onto nitrocellulose filters using the method of Bowen, Steinberg, Laemmli & Weintraub (1980). Erythrocyte-specific membrane proteins were then detected using rabbit anti-human erythrocyte membrane immunoglobulin G (IgG) (that cross-reacts with the major mouse erythrocyte membrane proteins), peroxidase-coupled goat anti-rabbit IgG (Miles Laboratories) and 3,3’-diaminobenzidine using the method of Glass, Briggs & Hnilica (1981).

**Immune precipitation of membrane proteins**

Approximately 10⁷ control or induced cells were washed in PBS and then in 10 mM-Tris-HCl (pH 8.0) containing 1 mM-EDTA, 0.34 M-sucrose. The pellet of cells was resuspended in 0.8 ml 10 mM-Tris-HCl (pH 8.0) containing 1 mM-EDTA, 0.34 M-sucrose, 1 mM-ATP,
Friend cell protein synthesis

1 mM-dithiothreitol, 1 mM-PMSF and mixed with 0.2 ml 10% Nonidet P-40 solution. After vigorous agitation, c.8 ml of 10 mM-Tris-HCl (pH 8.0) containing 0.5 M-NaCl/2% 2-mercapto-ethanol was added. The solution was then centrifuged in an SW 50.1 rotor at 35,000 rev./min for 60 min. A fraction (10 µl) of rabbit IgG against erythrocyte membranes was added to 150 µl of this solution and incubated overnight at 4 °C. Goat anti-rabbit IgG (20 µl) was then added and a further overnight incubation at 4 °C carried out. The precipitate was thoroughly washed in 10 mM-Tris-HCl (pH 8.0) containing 1 mM-EDTA, 1% NP-40 and analysed on SDS/polyacrylamide gels.

Haemoglobin detection

Haemoglobin was determined as previously described (Hunt & Marshall, 1979).

RESULTS

Changes in protein synthesis during the differentiation of FL cells

In order to determine the manner in which the synthesis of various proteins changed on induction of differentiation in FL cells, the cells were grown in Ham's F-10 medium containing 230 mM-DMSO. After the addition of DMSO, the mean doubling time of the cells was increased to 21.6 h from a control value of 10.8 h and the haemoglobin concentration within the cells rose dramatically, approximately 96-120 h after the induction of differentiation. The final haemoglobin concentration was approximately 25 µg per 10^7 cells.

The changes that occur in the synthesis of the other major proteins of the cells after addition of DMSO were investigated by pulse-labelling with [35S]methionine. On each day during the growth of DMSO-treated and control cells, 10^6 cells of each type were harvested and resuspended in methionine-free medium supplemented with [35S]-methionine. After incubation for 2 h at 37 °C, the cells were harvested and their labelled proteins analysed by the 2-dimensional system of O'Farrell (1975). In this procedure, the proteins are first separated according to their isoelectric points by isoelectric focusing and then, in the second dimension, according to their molecular weights by SDS/polyacrylamide gel electrophoresis. Fig. 1 shows high-resolution gels of the proteins synthesized by control (Fig. 1A) and differentiating (Fig. 1B) FL cells grown for 96 h in the presence of 230 mM-DMSO. With a few differences (see below) the gels of proteins synthesized after 24, 48, 72 and 120 h in the presence of DMSO were very similar to those of the 96-h time point.

Qualitatively, the spectrum of proteins made by control and differentiating cells is very similar. Several changes are, however, apparent when Figs. 1A and 1B are compared.

1) The radioactivity from [35S]methionine incorporated into the majority of proteins by differentiating cells is much reduced when compared to that incorporated by an equal number of control cells, correlating with a difference in proliferation rates. At 96 h [35S]methionine radioactivity incorporated into total protein by the differentiating cells was 37% of the incorporation by control cells. However, we could not detect the complete abolition of the synthesis of any particular protein in the differentiating cells during the time course studied, possibly due to the minority of uninducible cells in the cultures. Prolonged autoradiography of gels such as that shown in Fig. 1B
Fig. 1. Synthesis of [35S]methionine-labelled proteins 96 h after induction of differentiation. FL cells (10⁶) were grown for 96 h in the absence (A) or presence (B) of 230 mM-DMSO and then labelled with [35S]methionine for 2 h. The labelled proteins were analysed by high-resolution 2-dimensional polyacrylamide gel electrophoresis and the gels were dried and autoradiographed for 21 days. The numbers 1–6 indicate proteins whose synthesis is discussed in the text.
Friend cell protein synthesis

Fig. 2. Identification of spot 1 after 2-dimensional polyacrylamide gel electrophoresis of [³⁵S]methionine-labelled FL cells. Actin was isolated from [³⁵S]methionine-labelled FL cells and was analysed by high-resolution 2-dimensional polyacrylamide gel electrophoresis. The major spot of the actin preparation comigrated with spot 1 of FL cells in both dimensions.

Fig. 3. Synthesis of [³⁵S]methionine-labelled proteins during differentiation of FL cells. FL cells were grown for 24 and 72 h in the absence of DMSO (vertical columns c24 and c72, respectively) and for 24, 48, 72 and 96 h in the presence of 230 mM-DMSO (vertical columns d24, d48, d72 and d96, respectively). The cells were then pulse-labelled with [³⁵S]methionine and analysed by 2-dimensional polyacrylamide gel electrophoresis. The gels were autoradiographed for 21 days. Horizontal row A: region of the gel around protein 4; row B: region of the gel around protein 6; row C: region of the gel around protein 2.
revealed that proteins such as protein 6 were still being synthesized by DMSO-treated cells (see Fig. 3). The reduction in the synthesis of most proteins takes place early in differentiation (within the first 24 h).

(2) Some proteins are synthesized as actively or almost as actively by differentiating as by control cells, and, therefore, constitute a much higher proportion of the proteins synthesized by differentiating cells. Proteins that are prominent in this category are numbered 1, 2, 3 and 5 in Fig. 1. Protein 1 comigrates with actin (Fig. 2), but the identity of the other proteins in this category is not known.

(3) The synthesis of protein 4 is much increased in differentiating cells. The high-resolution polyacrylamide gel system does not reveal the synthesis of globin during differentiation because the isoelectric point of this protein lies outside the resolved range and the 8-5 % polyacrylamide gel system used for the second dimension does not resolve proteins with molecular weights of less than 25000.

Changes in the synthesis of particular proteins during the course of differentiation are shown in Fig. 3. The left and right columns (c24 and c72, respectively) show synthesis 24 and 72 h after the cells have been subcultured and grown in the absence of any inducer of erythroid differentiation. The central columns show the same areas of high-resolution gels on which the pulse-labelled proteins of FL cells grown for 24, 48, 72 and 96 h in the presence of DMSO have been analysed.

Row A shows the kinetics of the induction of protein 4, the most prominently induced protein apart from globin that we have detected during the differentiation of FL cells. This protein is made to a small extent by control cells (columns c24 and c72). Twenty four hours after the addition of DMSO to the cells, the radioactivity incorporated from \[^{35}S\]methionine into this protein during the pulse period increases (column d24), and continues to increase at least up to 96 h after the addition of DMSO, the latest time point examined. This is in marked contrast to the kinetics of globin synthesis, which does not start until 96 h after the addition of DMSO.

Row B shows the synthesis of protein 6, which is reduced during differentiation, after 24 h in the presence of DMSO. In the control cells there is some decline in protein 6 synthesis by 72 h of culture. In contrast to the reduced synthesis of protein 6, the synthesis of the protein to the upper left of protein 6 is little affected by the onset of differentiation.

Row C shows the kinetics of synthesis of protein 2. This, too, is little changed during differentiation while the synthesis of the other major proteins in this region of the gel is reduced by 24 h after the addition of DMSO to the medium.

The subcellular location of protein 4

\[^{35}S\]methionine-labelled proteins from the nuclear and cytoplasmic fractions of FL cells were prepared and analysed after the cells had been grown for 48 h in the presence and absence of 230 mM-DMSO. High-resolution gel electrophoresis showed that protein 4 was considerably enriched in the fraction containing the cytoplasmic proteins (Fig. 4A), but was not present in the nuclear preparations (Fig. 4B).
Friend cell protein synthesis

Fig. 4. Sub-cellular location of protein. FL cells were grown for 48 h in the presence of 230 mM-DMSO, pulse-labelled with [35S]methionine and fractionated to yield a cytoplasmic (A) or nuclear (B) fraction. The labelled proteins were separated by high-resolution 2-dimensional electrophoresis and the gel dried and autoradiographed for 21 days.

Synthesis of erythrocyte-specific membrane proteins

The finding that actin, a protein that is associated with the cytoplasmic surface of the plasma membrane of the mature erythrocyte, is synthesized at the same rate in control and differentiating FL cells despite the overall reduction of protein synthesis in the latter, led us to investigate other erythrocyte-specific membrane proteins. FL cells were pulse-labelled with [35S]methionine after 72 h in the presence or absence of 230 mM-DMSO. The cells were then dissolved and erythrocyte-specific
membrane proteins immunoprecipitated using an antiserum raised against purified erythrocyte membranes. Fig. 5 shows the proteins precipitated from the two cell types after resolution by SDS/polyacrylamide gel electrophoresis. Two of the proteins precipitated comigrated with bands 1 and 2 of spectrin (molecular weights, 240000 and 220000; asterisk in Fig. 5). A third polypeptide was also precipitated in the spectrin region of the gel (molecular weight 235000), an observation that has been made by others (Rossi et al. 1978). A less intense band of radioactivity was precipitated from both control and induced cells that migrated on SDS/polyacrylamide gels with glycophorin (2 asterisks in Fig. 5). In the case of both the spectrin polypeptides and glycophorin, no reproducible difference could be observed in the radioactivity precipitated from the same number of control and induced cells, suggesting that, as is the case with actin, no reduction in synthesis occurred in these membrane proteins after the induction of differentiation.

**Changes in Con A-binding glycoproteins during differentiation**

The high-resolution polyacrylamide gel system described above resolves the 200–300 most abundant proteins made by FL cells; however, few changes are apparent during the course of differentiation. It is possible that many of the changes that occur are not revealed by this technique, as they involve minor proteins. Among the minor
proteins that may be expected to change during differentiation are glycoproteins on the outer surface of the cell. These proteins may be involved as receptors of the signals for differentiation and in the interaction of the cell with its surroundings. The glycosylation of these external proteins is likely to further restrict their resolution on 2-dimensional polyacrylamide gel electrophoresis, as the sugar moieties may cause heterogeneity of charge thereby generating diffuse or multiple spots in the isoelectric focusing dimension.

In order to determine whether there is any difference in the glycoproteins of
differentiating and control FL cells, we have used radio-iodinated Con A to stain SDS/polyacrylamide gels on which FL cell proteins have been separated. Fig. 6 shows microdensitometer traces of autoradiograms of gels stained with $[^{125}I]$Con A in this way. Control FL cells, either actively proliferating (48 h after subculture) or in stationary phase (120 h after subculture) have a complex array of Con A-binding glycoproteins ranging in molecular weight from greater than 150,000 to less than 25,000. In stationary-phase cells a few of the Con A-binding glycoproteins are stained to a lesser degree.

After 48 h of erythroid differentiation, the pattern of Con A-binding glycoproteins becomes markedly simplified. Many of the major bands are reduced in amount when

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Fig. 7. Synthesis of Concanavalin A-binding glycoproteins by control and differentiating FL cells. FL cells were grown for 48 h in the absence of inducing agent (control) and for 24, 48, 96 and 120 h in the presence of 230 mM-DMSO. They were then pulse-labelled for 6 h by growth in medium supplemented with $[^{35}S]$methionine and their Con A-binding proteins isolated by affinity chromatography on Sepharose-Con A. The Con A-binding proteins were analysed by 8.5% polyacrylamide gel electrophoresis. The gels were dried, autoradiographed for 21 days and the autoradiograms scanned with a microdensitometer.
Fig. 8. Synthesis of Con A-binding glycoproteins by FL cells early and late in differentiation. FL cells were grown for 24 h (A) and 96 h (B) in the presence of 230 mM-DMSO. The cells were then pulse-labelled with [35S]methionine and the Con A-binding proteins isolated by affinity chromatography. The proteins were analysed by 2-dimensional polyacrylamide gel electrophoresis and the gels dried and autoradiographed for 21 days.

compared to the corresponding control cells; however, a broad band with a molecular weight of approximately 100 000 is much increased in amount. After 120 h of differentiation, the broad 100 000 band is the major Con A-binding glycoprotein in FL cells.

In order to study the changes in the synthesis of Con A-binding glycoproteins as differentiation proceeds, control and DMSO-induced FL cells were pulse-labelled
with $^{35}$S)methionine as described above. The cells were then washed in a solution containing EDTA to remove adherent or extracellular peripheral proteins and dissolved in buffered Triton X-100. After removal of insoluble material by centrifugation, the Con A-binding glycoproteins were purified by affinity chromatography on a column of Con A-agarose. Following elution of the specifically bound proteins from the column, they were analysed by SDS/polyacrylamide gel electrophoresis. Fig. 7 shows a microdensitometer trace of the Con A-binding glycoproteins synthesized 48 h after subculture by control, actively proliferating cells. Several prominent bands are resolved, some of which have similar mobilities to the bands observed in Fig. 6 (top)

![Microdensitometer trace of Con A-binding glycoproteins](image)

**Fig. 9.** Detection of erythrocyte-specific membrane proteins by blotting onto nitrocellulose filters. The total proteins of control and DMSO-induced FL cells were analysed by electrophoresis on an 8.5% polyacrylamide/SDS gel and the separated proteins transferred onto a nitrocellulose filter. The erythrocyte-specific membrane proteins were detected using a specific anti-erythrocyte membrane antiserum together with peroxidase-conjugated goat anti-rabbit IgG. The bound antisera were then detected using 3,3′-diaminobenzidine. The resulting ‘western blot’ was photographed and the photograph scanned with a microdensitometer. Top scan: erythrocyte-specific membrane proteins of control (uninduced) FL cells 48 h after subculture. Bottom scan: erythrocyte-specific membrane proteins of FL cells that had been grown for 48 h in the presence of 230 mM-DMSO.

using $^{181}$I)Con A to stain the gels. Some proteins that bind $^{181}$I)Con A are not revealed by affinity chromatography of $^{35}$S)methionine pulse-labelled Con A receptors. This is particularly the case in the higher molecular weight region of the gel.

After 24 h in DMSO-containing medium, the synthesis of many Con A-binding glycoproteins is greatly diminished in line with the synthesis of the most abundant proteins revealed by the high-resolution gels in Fig. 1. However, the synthesis of one Con A-binding glycoprotein is markedly increased (Fig. 7). This protein has an apparent molecular weight of 100,000, correlating with the newly appearing glycoprotein revealed by $^{181}$I)Con A binding. At 48 h of differentiation and longer, the synthesis of almost all Con A-binding proteins, including the new 100,000 molecular weight protein, is reduced.
In order to determine whether the bands seen on single dimension SDS/polyacrylamide gel electrophoresis were composed of multiple components, the [³⁵S]methionine pulse-labelled Con A-binding glycoproteins synthesized by cells 24 and 96 h after the addition of DMSO were analysed by high-resolution 2-dimensional polyacrylamide gel electrophoresis. Fig. 8A, B shows that each band on the single dimension gel is likely to be composed of several components. By 96 h after the onset of differentiation, the synthesis of the majority of the Con A-binding glycoproteins is abolished.

The synthesis of a new 100 000 molecular weight Con A-binding glycoprotein after the onset of erythroid differentiation in FL cells may reflect the production of a protein characteristic of the mature erythrocyte membrane. However, using the technique of western-blotting we were unable to detect a DMSO-inducible protein that reacted with our anti-membrane antiserum and had a mobility similar to the new Con-A binding protein (Fig. 9). This suggests that either this new protein is not a component of the mature erythrocyte membrane or a change occurs in the carbohydrate portion of a pre-existing erythrocyte membrane protein such that it binds Con A only after the onset of erythroid differentiation.

**DISCUSSION**

We have shown that several changes occur in the synthesis of the major proteins of Friend erythroleukemic cells during the first 24-48 h after the onset of erythroid differentiation. These changes include: (1) a fall in the synthetic rate of most of the major proteins of the cell within 24 h in line with the reduction of the cell proliferation rate; and (2) a rise in the synthesis of some proteins previously made in only small amounts within 24 h of induction of differentiation. These include a major Con A-binding glycoprotein with a molecular weight of 100 000 and the cytoplasmic protein 4. Interestingly, the synthesis of the new Con A-binding protein, initiated within 24 h of induction of erythroid differentiation, is reduced again by 48 h. Thus, this protein is made only at a very specific time during the differentiation process. In contrast, haemoglobin synthesis is initiated later in the differentiation process and continues until at least the last time point studied (96 h after the addition of DMSO). A third class of proteins including spectrin, glycophorin and actin, all constituents of the membrane of the mature erythrocyte, do not exhibit reduced synthetic rates after induction of differentiation. Instead they are synthesized at a similar rate to that in the control cells even after the proliferation rate of the cells has fallen to approximately 50% of the controls. This means that their synthesis is a considerably larger proportion of that of differentiating cells compared to the control cells and correlates with the fact that these proteins are retained in the mature cell while, during the process of differentiation, most proteins are lost.

Our finding of no change in the synthetic rate of membrane proteins, in contrast to most other proteins of 707B FL cells, is similar to results obtained by Gazitt & Friend (1981) using the 745 line of FL cells, but in contrast to the results of Eisen and colleagues (1977a) who found that spectrin synthesis was greatly increased in F4N FL
cells after treatment with DMSO. Gazitt & Friend (1981), however, did not use antisera to detect erythrocyte-specific membrane proteins and only identified proteins as such by their mobilities on polyacrylamide gel electrophoresis.

We have previously shown (Hunt & Marshall, 1979) that agglutination of the 707B line of FL cells by Con A changes during the course of differentiation. Before addition of DMSO to the medium, all cells are highly agglutinable, but between 24 and 48 h after the onset of differentiation, agglutinability falls markedly. Since the synthesis of the 100,000 molecular weight Con A-binding protein and the loss of many other Con A-binding proteins are early events in FL cell differentiation, it is possible that these represent major changes in the cell membrane that subsequently lead to the reduction of Con A-mediated agglutinability. The identity of the new 100,000 molecular weight protein is unknown and it may not be a protein characteristic of the mature erythrocyte membrane for we could find no DMSO-inducible protein associated with the control or induced FL cells with a molecular weight similar to this Con A receptor and that reacted with anti-erythrocyte plasma membrane antiserum. However, it is possible that the effect of DMSO induction is to alter the sugar associated with a pre-existing membrane protein such that it binds Con A in the differentiating cells only. Thus, glycophorin, found by the blotting technique and by immune precipitation to exist in both control and differentiating cells, exactly comigrates with the 100,000 molecular weight Con A-binding glycoprotein. Possibly, then, the sugars of glycophorin are changed during differentiation and experiments are under way to determine whether this is so.

The 707B line of FL cells is blocked in erythroid differentiation at a point after spectrin and glycophorin induction, whereas the F4N line used by Eisen et al. (1977a) is blocked earlier, before spectrin induction. This would correlate with our results on Con A-induced agglutination of 707B FL cells (Hunt & Marshall, 1979) in which we showed a biphasic response to the lectin. In control cells agglutination was 100% at the lowest Con A concentration tested, but fell between 24 and 48 h after DMSO induction. In contrast, the F4N cell line (Eisen et al. 1977b) showed a more complex response to the lectin after induction by DMSO. At first, agglutination was low, but it subsequently rose to almost 100% before falling once again.

Peterson & McConkey (1976b) have compared the nuclear and cytoplasmic proteins of control and differentiating FL cells and also found few differences after the cells had been grown for 3 days in DMSO-containing medium. Two non-histone chromosomal proteins and one cytoplasmic protein, in addition to globin, showed increased synthesis on induction of differentiation, while the synthesis of 4 non-histone chromosomal and 2 cytoplasmic proteins was reduced. Similar observations have been made by Reeves & Cserjesi (1979a). In view of the considerable changes that take place in the growth rate and morphology of differentiating FL cells, it is at first surprising that so few changes are detected. However, the 2-dimensional gel patterns represent only the approximately 300 most abundant proteins in the cells that are likely to represent many of the functions common not only to proliferating and differentiating FL cells, but to other cell types as well. It has, for example, been found that 20% of the cytoplasmic proteins of FL cells comigrate on 2-dimensional electro-
Friend cell protein synthesis

phoresis with proteins from the cytoplasm of HeLa cells (Peterson & McConkey, 1976a, b).

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