MEMBRANE-BOUND REDOX PROTEINS OF THE MURINE FRIEND VIRUS-INDUCED ERYTHROLEUKEMIA CELL

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ABSTRACT

We have obtained and studied a 105,000-*g* pellet from T-3-C1-2 cells, a cloned line of Friend virus-induced erythroleukemia cells. By difference spectrophotometry, the pellet was shown to contain cytochrome b_5 and cytochrome P-450, hemeproteins that have been shown to participate in electron-transport reactions of endoplasmic reticulum and other membranous fractions of various tissues. The pellet also possesses NADH-cytochrome *c* reductase activity which is inhibited by anti-cytochrome b_5 γ-globulin, indicating the presence of cytochrome b_5 reductase. This is the first demonstration of membrane-bound forms of these redox proteins in erythroid cells. Dimethyl sulfoxide-treated T-3-C1-2 cells were also shown to possess membrane-bound cytochrome b_5 and NADH-cytochrome *c* reductase activity. We failed to detect soluble cytochrome b_5 in the 105,000-*g* supernatant fraction from homogenates of untreated or dimethyl sulfoxide-treated T-3-C1-2 cells. In contrast, erythrocytes obtained from mouse blood were shown to possess soluble cytochrome b_5 but no membrane-bound form of this protein. These findings are supportive of our hypothesis that soluble cytochrome b_5 of erythrocytes is derived from endoplasmic reticulum or some other membrane structure of immature erythroid cells during cell maturation.

KEY WORDS erythroleukemia · cytochrome b_5 · NADH-cytochrome *c* reductase · cytochrome P-450 · erythrocytes

The murine Friend virus-induced erythroleukemia cell line has been used as a model to study erythroid differentiation (10). The T-3-C1-2 cloned line of these erythroleukemia cells has a very low level of spontaneous differentiation (18, 34). Upon treatment with dimethyl sulfoxide (DMSO), however, this cell line (like other erythroleukemia lines) undergoes changes similar to those associated with erythroid differentiation. The changes include the appearance of erythroid membrane antigen (17), accumulation of mRNA for globin synthesis (34), and synthesis of globin chains and hemoglobin (18).

In the present study, we have used the Friend virus-induced erythroleukemia T-3-C1-2 line as a model for early erythroid cells to study cytochrome b_5, a protein found in reticulocytes and mature erythrocytes. In non-nucleated erythroid cells, cytochrome b_5 (16, 31) and cytochrome b_5 reductase (16, 20, 30, 39) exist in the cytoplasm as soluble molecules. Erythrocyte cytochrome b_5 is similar to cytochrome b_5 solubilized from microsomes of the liver. The amino acid composition of bovine erythrocyte cytochrome b_5 I is in very good agreement with a segment (residues 1–97) of bovine liver microsomal cytochrome b_5 (6). Tryptic digestions

of bovine erythrocyte cytochrome b_5 and bovine liver microsomal cytochrome b_5 yield core heme-peptides which are indistinguishable on the basis of electrophoretic migration and amino acid composition (6, 15).

It is our contention that, in the immature erythroid cell, cytochrome b_5 is associated with membranes, and that sometime during the maturation process this hydrophobic membrane-bound protein is converted to a water-soluble molecule. In this paper we will present evidence that cytochrome b_5 in an immature erythroid cell system exists as a membrane-bound protein. Some of these results have been presented previously in abstract form (36).

MATERIALS AND METHODS

Growth and Preparation of Friend Erythroleukemia Cells

Friend virus-induced erythroleukemia cells, clonal line T-3-C1-2, were obtained from Dr. S. Orkin of the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md. The cells had been maintained in culture in the laboratory of Dr. R. Ruddon, Department of Pharmacology, The University of Michigan. This clonal line has been described as a "genetically homogeneous population of erythroleukemic cells" (27). These cells were assayed periodically by Dr. Allen Lau, using the uridine phosphorylase assay (22), and were found to be free of mycoplasma contamination (21).

The cells were grown in suspension with RPMI-1640 powdered tissue culture medium (9.4 g/liter) to which $NaHCO_3$ (1.8 g/liter), penicillin (0.07 g/liter), streptomycin (0.1 g/liter), and 1 N HCl (3.4 ml/liter) had been added. The medium was also supplemented with 10% fetal calf serum (KC Biological, Inc., Lenexa, Kans.). The growth medium was sterilized by filtration through a 0.22-μm Millipore filter (Millipore Corp., Bedford, Mass.). All cultures were initiated at a concentration of 3×10^4 cells/ml of medium in a total vol of 200 ml. The cultures were maintained in one l Corning plastic flasks (Corning Glass Works, Science Products Div., Corning, N.Y.). The flasks were briefly gassed with 5% CO_2–95% air and incubated at 37°C. When cells were treated with DMSO, the cultures were initiated in the same way except for the addition of DMSO to a final concentration of 1.2%. All cells were incubated for 96 h. Cells were harvested by centrifugation at 600 *g* for 10 min. Pellets were washed with 0.9% NaCl and frozen at −70°C.

Cell counts were performed in hemocytometers with 0.9% NaCl as diluent. Cell viability was determined by Erythrosin B dye exclusion. Hemoglobin was stained as follows:[1] Immediately before use, 5.0 ml of H_2O and 0.5 ml of 30% H_2O_2 were combined with 1.0 ml stock benzidine solution (3% benzidine in 90% acetic acid). Two drops of stain were added to 0.2 ml of cell suspension. After 15 min, the number of benzidine-positive cells were counted in a hemocytometer.

[1] This procedure suggested by Dr. S. Orkin.

Electron Microscopy

The cells were collected by centrifugation at 600 *g* for 10 min to form a pellet of ~1 mm thickness. To the pellet, 3% glutaraldehyde containing 0.1 M phosphate buffer, pH 7.2, was added. The pellet was allowed to fix in the solution for 30 min. The pellet was then washed three times with 0.1 M cacodylate buffer, pH 7.3, containing 0.2 M sucrose. The pellet remained in this solution in the cold until it was postfixed in 2% osmium tetroxide-0.1 M cacodylate buffer, pH 7.2, and then it was dehydrated in a graded ethanol series. The dehydrated pellet was washed in propylene oxide, soaked in propylene oxide and Epon, and then embedded in Epon. Sections were prepared, post-stained with uranyl acetate (9) and lead citrate (33), and examined with an AEI Corinth 275 electron microscope.

Preparation of a 105,000-g Pellet

A 105,000-*g* pellet was prepared from untreated and DMSO-treated cells by a modification of the method of Omura and Sato (26). The cells were homogenized with 3 vol of cold 0.25 M sucrose containing 0.001 M EDTA, pH 7.6, in a Potter-Elvehjem homogenizer equipped with a Teflon pestle (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.). The homogenate was centrifuged at 12,100 *g* for 25 min in a Sorvall RC-2B centrifuge at 0°C (DuPont Instruments-Sorvall, DuPont Co., Newton, Conn.). The precipitate was discarded and the supernatant fraction was centrifuged at 105,000 *g* for 1 h in a Beckman L5-65 preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The resulting pellet was then resuspended in 1.15% KCl with a Dounce homogenizer (Kontes Co., Vineland, N.J.) and recentrifuged at 105,000 *g* for 1 h. The washed, 105,000-*g* pellet was resuspended in 0.1 M potassium phosphate, pH 7.0. Protein determinations were made by the method of Lowry et al. using bovine serum albumin as standard (23).

Difference Spectra of the 105,000-g Pellet

Difference spectra of the 105,000-*g* pellet preparations were measured in an Aminco Chance DW-2 spectrophotometer (American Instrument Co., Travenol Laboratories Inc., Silver Spring, Md.) using cuvettes of 1-cm optical path. The pellets from either untreated or DMSO-treated cells were placed in both the sample and reference compartments. After recording the baseline, NADH was added to the sample cuvette and the difference spectrum was recorded. The amount of cytochrome b_5 present was calculated from the reduced minus oxidized difference spectrum using $\Delta\epsilon_{mM}$(424–409 nm) = 185 according to

Omura and Sato (26). To obtain the reduced CO-complex minus reduced difference spectrum, CO was bubbled through the sample cuvette and then solid sodium dithionite was added to both sample and reference cuvettes. The content of cytochrome P-450 was determined from the reduced CO-complex minus reduced difference spectrum using $\Delta\epsilon_{mM}$ (450–490 nm) = 91 (26).

NADH Cytochrome c Reductase Assay

The NADH cytochrome *c* reductase activity of the 105,000-*g* pellet was assayed by measuring the rate of reduction of cytochrome *c* (horse heart type III; Sigma Chemical Co., St. Louis, Mo.) using NADH (24). A value of 21.0 for the change in the millimolar extinction coefficient at 550 nm for ferrocytochrome *c* was used in the calculation of specific activity (25). The reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM KCN (to inhibit contaminating mitochondria), 0.1 mM cytochrome *c*, microsomes (10 μl of 1 mg/ml solution), and water to a total vol of 1 ml. The reaction was started by the addition of NADH and the absorbance changes at 550 nm were recorded at 30°C. In experiments where anti-cytochrome b_5 γ-globulin was added, all components (minus NADH) were pre-incubated for 20 min at 30°C with the γ-globulin and the reaction was then started with the addition of NADH. The rabbit anti-cytochrome b_5 γ-globulin (a generous gift from Dr. T. Omura and Dr. G. Mannering) had been prepared against trypsin-solubilized rat liver microsomal cytochrome b_5 which showed a single band on acrylamide gel electrophoresis.

Isolation of Soluble Cytochrome b_5 from Mouse Erythrocytes

Soluble cytochrome b_5 was isolated from mouse erythrocytes by a modification of the procedure used to isolate soluble cytochrome b_5 from the supernatant fraction of bovine erythrocytes (6). The isolation procedure was carried out at 4°C using deionized-distilled water throughout. Mouse whole blood (Type I, fresh, in citrate) was obtained from Pel Freez Biologicals Inc., Rogers, Ariz. The blood was centrifuged at 3,000 *g* for 10 min. The supernatant fraction and leukocytes (along with some erythrocytes) were removed by aspiration. The packed cells were washed in 0.9% NaCl and centrifuged at 3,000 *g* for 10 min. The resulting packed erythrocytes (4.5 ml) were then lysed with 3 vol of water. The hemolysate was stored at −70°C. The hemolysate was thawed, the pH was adjusted to 6.0, and the stromal fraction was removed by centrifugation at 12,100 *g* for 30 min. The supernatant fraction was diluted with 2 vol of water and applied to a 0.5 × 5-cm DEAE-cellulose column which had been previously equilibrated with 0.003 M potassium phosphate buffer, pH 7.2. After a wash with the same buffer, the column was eluted sequentially with 0.01 M KH_2PO_4-0.01 M KCl, 0.05 M KH_2PO_4, and finally 0.2 M KH_2PO_4. Absolute and difference spectra of the isolated cytochrome b_5 were recorded with a Cary 14 spectrophotometer (Cary Instruments, Fairfield, N.J.).

Attempted Isolation of Soluble Cytochrome b_5 from the 105,000-g Supernatant Fraction of T-3-C1-2 Cell Homogenate

The 105,000-*g* supernatant fraction of the erythroleukemia cell homogenate was diluted with 3 vol of water and then subjected to the same column chromatographic procedure that was used for the isolation of soluble cytochrome b_5 from mouse erythrocytes. Absolute and difference spectra of the eluted fractions were recorded with a Cary 14 spectrophotometer.

RESULTS

Morphology of Untreated and DMSO-Treated Murine Friend Erythroleukemia Cells, Clonal Line T-3-C1-2

Fig. 1 shows electron micrographs of both untreated and DMSO-treated T-3-C1-2 cells harvested after 96 h of growth. The untreated T-3-C1-2 cell (Fig. 1 *A*) shows a subcellular pattern similar to that of very early erythroid cells (proerythroblast to basophilic erythroblast), as has been previously reported (35). The cell has a high nuclear-to-cytoplasmic ratio, numerous mitochondria, and a very electron-dense cytoplasm due to the presence of large numbers of ribosomes. Rough endoplasmic reticulum is present in small amounts.

The DMSO-treated cell (Fig. 1 *B*) has a subcellular pattern similar to that of the late erythroblast cells (polychromatophilic to orthochromatophilic erythroblast). The cell still possesses ribosomes, mitochondria, and small amounts of rough endoplasmic reticulum, but it has a lower nuclear-to-cytoplasmic ratio. One prominent difference between the untreated and the DMSO-treated cell is that the latter cell exhibits the presence of complex vacuolar structures containing numerous virus particles. Also, an increase in the numbers of budding viruses is seen. These changes are characteristic of erythroleukemia cells induced to differentiate by DMSO (11, 12, 35). Fig. 1 *C* shows a portion of a DMSO-treated cell which shows several budding viruses.

Detection of Membrane-bound Cytochrome b_5 and Cytochrome P-450

We obtained a 105,000-*g* pellet from both untreated and DMSO-treated erythroleukemia cells.

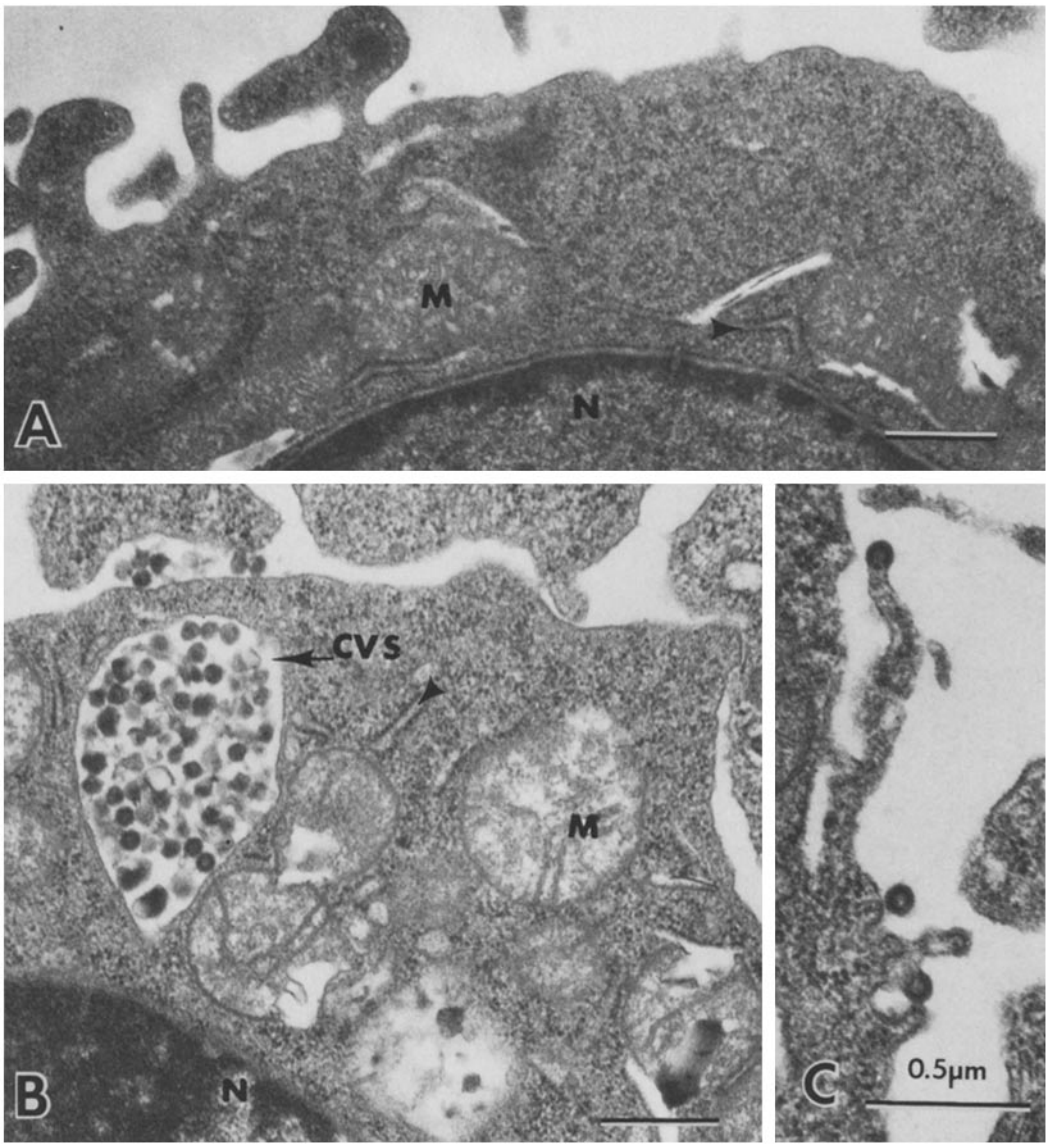

FIGURE 1 Electron micrographs of T-3-C1-2 erythroleukemia cells. (*A*) An untreated cell after 96 h of growth. The cell has a large nucleus (*N*), numerous mitochondria (*M*), and traces of rough endoplasmic reticulum (arrow head). Bar, 0.5 μm. × 30,000. (*B*) A T-3-C1-2 cell after 96 h of DMSO treatment. The cell retains a nucleus (*N*), mitochondria (*M*), and low levels of rough endoplasmic reticulum (arrow head). Complex vacuolar structures (*CVS*) containing numerous virus particles are seen frequently. An increase in the number of budding viruses is also seen. Bar, 0.5 μm. × 30,000. (*C*) A portion of a T-3-C1-2 cell after 96 h of DMSO treatment which shows several budding viruses. Bar, 0.5 μm. × 45,000.

The yield of protein in the 105,000-*g* pellet was 8.5 mg/10^9 cells for untreated cells and 5.0 mg/10^9 cells for DMSO-treated cells. The visible spectral properties of the 105,000-*g* pellet from the erythroleukemia cells are summarized in Table I. The NADH reduced minus oxidized difference spectrum (Fig. 2) shows absorbance maxima at 426, 526, and 559 nm. The spectrum corresponds to that of mouse liver microsomal cytochrome b_5 observed in this study and to the spectrum of microsomal cytochrome b_5 of other tissues. A similar NADH reduced minus oxidized spectrum was

TABLE I

Comparison of Visible Spectral Properties of the 105,000-g Pellet from Erythroleukemia Cell with Liver Microsomal Cytochrome b_5, Liver Microsomal Cytochrome P-450, and Erythrocyte Soluble Cytochrome b_5

	Absorbance maxima	
	Reduced minus oxidized	Reduced CO-complex minus reduced*
	nm	
Pellet (105,000 *g*) from untreated T-3-Cl-2 cells	426, 526, 559‡	451
Pellet (105,000 *g*) from DMSO-treated T-3-Cl-2 cells	427, 526, 559‡	419, 535, 571
Mouse liver microsomal cytochrome b_5	426, 528, 559‡	none
Mouse liver microsomal cytochrome P-450	—	451
Mouse erythrocyte soluble cytochrome b_5	424, 525, 558*	—

* Reduced with sodium dithionite.

‡ Reduced with NADH.

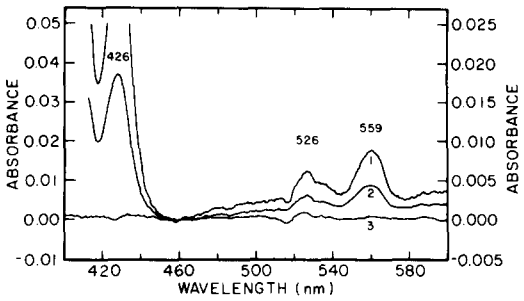

FIGURE 2 The NADH reduced minus oxidized difference spectrum of the 105,000-*g* pellet obtained from T-3-C1-2 erythroleukemia cells. The sample and reference cuvettes contained pellet (7 mg protein/ml) suspended in 0.1 M phosphate buffer, pH 7.0. NADH was added to the sample cuvette and the spectral difference measured. Curve *1* was recorded with a full scale absorbance of 0.05 (scale on the right). Curve *2* was recorded with a full scale absorbance of 0.1 (scale on the left). Curve *3* represents the recorded oxidized vs. oxidized baseline.

observed with the 105,000-*g* pellet from the DMSO-treated cells.

The reduced CO-complex minus reduced difference spectrum of the 105,000-*g* pellet from untreated T-3-C1-2 cells (Fig. 3) shows an absorbance maximum at 451 nm which is typical of the spectrum of cytochrome P-450, the CO-inhibited hydroxylase of microsomes. The reduced CO-complex minus reduced difference spectrum of the pellet from the DMSO-treated cells corresponded to that of carbonmonoxyhemoglobin minus deoxyhemoglobin. This indicates that the pellet had not been washed completely free of the hemoglobin synthesized by T-3-C1-2 cells as a response to the DMSO treatment. No peak or shoulder at 450 nm could be seen. However, we cannot determine from this spectrum whether cytochrome P-450 was absent in the 105,000-*g* pellet of DMSO-treated cells or whether the spectrum of cytochrome P-450 was masked by the contaminating hemoglobin.

The quantitation of cytochrome b_5 and cytochrome P-450 in these cells is summarized in Table II. The amounts of cytochrome b_5 in the untreated and DMSO-treated cells are similar (0.014 and 0.016 nmol/mg protein, respectively). These amounts (expressed either on the basis of number of cells or milligrams of pelleted protein) are small compared to the amount of microsomal cytochrome b_5 in mouse liver. The amount of membrane-bound cytochrome P-450 in the erythroleukemia cell is likewise small relative to the amount of microsomal cytochrome P-450 in the liver cell. The ratio of cytochrome b_5 to cytochrome P-450 in erythroleukemia cells is comparable to that of mouse liver microsomes.

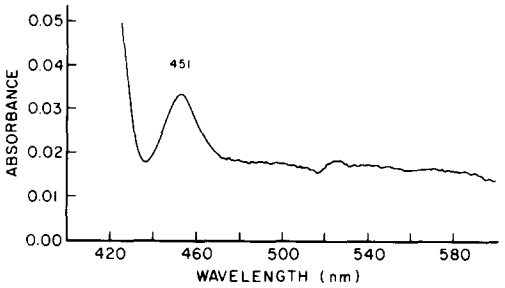

FIGURE 3 The reduced CO-complex minus reduced difference spectrum of the 105,000-*g* pellet obtained from untreated T-3-C1-2 erythroleukemia cells. The sample and reference cuvettes contained pellet (7 mg protein/ml) suspended in 0.1 M phosphate buffer, pH 7.0. Carbon monoxide was bubbled through the sample cuvette and then sodium dithionite was added to both sample and reference cuvettes.

TABLE II

Amounts of Membrane-Bound Redox Proteins in Mouse Erythroleukemia Cells

Protein (units)	Source			
	Pellet from untreated T-3-Cl-2 cells	Pellet from DMSO-treated T-3-Cl-2 cells	Mouse liver microsomes*	Mouse erythrocytes
Total protein (mg/10^9 cells)	8.5	5.0	93.5	none
Cytochrome b_5				
(nmol/10^9 cells)	0.12	0.08	50.5	0.04
(nmol/mg protein)	0.014	0.016	0.54	—
Cytochrome P-450				
(nmol/10^9 cells)	0.22	—	150	none
(nmol/mg protein)	0.025	—	1.60	none
NADH-cytochrome *c* reductase activity				
(μmol/min/10^9 cells)	0.70	0.75	97	—
(μmol/min/mg protein)	0.082	0.15	1.04	—
(μmol/min/10^9 cells in the presence of anti-cytochrome b_5 γ-globulin)	0.16	0.15	—	—
(μmol/min/mg protein in the presence of anti-cytochrome b_5 γ-globulin)	0.019	0.029	—	—

* From the unpublished data of Dr. Kostas Vatsis, Department of Biochemistry, The University of Michigan. The values per 10^9 cells were calculated using the value of 15.9 mg microsomal protein/g of liver and the reported value of 1.7×10^8 hepatocytes/g of liver (40).

Evidence for Membrane-bound Cytochrome b_5 Reductase

The 105,000-*g* pellets from both untreated and DMSO-treated cells were shown to possess NADH-cytochrome *c* reductase activity (Table II). Strittmatter and Velick (38) have shown that in liver microsomes this activity is dependent upon both cytochrome b_5 reductase and cytochrome b_5, and thus can be used as evidence that both proteins are present. Further evidence for the presence of cytochrome b_5 in the pellets from untreated and DMSO-treated erythroleukemia cells was provided by demonstration of inhibition of the NADH-cytochrome *c* reductase activities with anti-cytochrome b_5 γ-globulin (Table II). Approx. 80% inhibition of the reductase activity of these pellets was observed when 40 mg of anti-cytochrome b_5 immunoglobulin were used/mg of protein. At this concentration the same immunoglobulin preparation showed 80% inhibition of the reductase activity using solubilized liver microsomal proteins.[2]

[2] Personal communication from Dr. S. Kuwahara and Dr. R. Schwen of the laboratory of Dr. G. Mannering.

Cytosolic Cytochrome b_5: Its Presence in Mouse Erythrocytes and Its Absence in Mouse Erythroleukemia Cells

Soluble cytochrome b_5 was detected and isolated for the first time from mouse erythrocytes. Fig. 4 shows the DEAE-cellulose elution profile for a preparation of soluble cytochrome b_5 derived from 4.5 ml of packed erythrocytes from mouse blood. The oxidized, reduced, and reduced minus oxidized spectra are similar to the spectra of soluble cytochrome b_5 from human, bovine, and rabbit erythrocytes (6, 31). The reduced minus oxidized difference spectrum (Fig. 5) shows absorbance maxima at 424, 525, and 558 nm. The amount of soluble cytochrome b_5 calculated from this spectrum was 0.87 nmol/ml of packed erythrocytes or 0.04 nmol/10^9 erythrocytes.

When this procedure was carried out on the 105,000-*g* supernatant fraction from homogenates of untreated and DMSO-treated erythroleukemia cells, no soluble cytochrome b_5 was detected. This finding indicates an absence or very low levels of cytochrome b_5 in the cytosol of these cells even after DMSO treatment. Oxyhemoglobin was spectrally identified in the initial fractions from

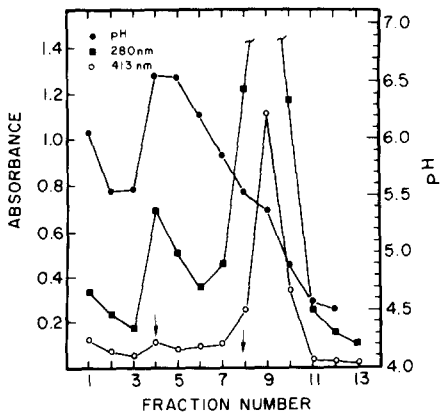

FIGURE 4 Partial purification of mouse erythrocyte cytochrome b_5 by chromatography on DEAE-cellulose. The diluted, stroma-free mouse erythrocyte hemolysate was applied to a 0.5 × 5-cm DEAE-cellulose column, and the column was then eluted sequentially with 10 mM KH_2PO_4-10 mM KCl, 50 mM KH_2PO_4, and 0.2 M KH_2PO_4 as described in the text. The arrow on the left denotes the beginning of the 50 mM KH_2PO_4 wash and the arrow on the right the beginning of the 0.2 KH_2PO_4 wash.

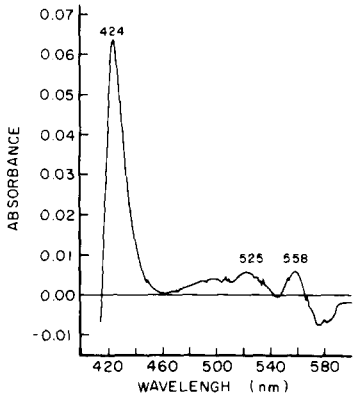

FIGURE 5 Reduced minus oxidized difference spectrum of mouse erythrocyte cytochrome b_5. Fractions 8, 9, and 10 from the DEAE-cellulose column (Fig. 4) were pooled and added to the sample and reference cuvettes. The material in the sample cuvette was reduced by the addition of sodium dithionite.

DEAE-cellulose chromatography of the 105,000-*g* supernatant fraction of homogenates from DMSO-treated T-3-C1-2 cells. This finding suggests that, in the erythroleukemia cell, hemoglobin is present in its ferrous form.

DISCUSSION

We have isolated a 105,000-*g* pellet from Friend virus-induced erythroleukemia cells and have demonstrated that the pellet contains cytochrome b_5, cytochrome P-450, and NADH-cytochrome b_5 reductase activity. This is the first report of the presence of membrane-bound forms of these proteins in erythroid cells. The levels of these proteins in erythroleukemia cells are low. Nonetheless, there is no doubt that they arose from these cells, because there were no suggestions that contaminating cells were present in this culture.

Cytochrome b_5, cytochrome P-450, and cytochrome b_5 reductase have been shown to be major constituents of microsomes from kidney, lung, and other tissues (13). Our detection of these proteins in the 105,000-*g* pellet might suggest that we have isolated microsomes from erythroleukemia cells. The small amounts of these membrane-bound redox proteins in erythroleukemia cells, relative to hepatocytes, would be in keeping with the relative amounts of endoplasmic reticulum which can be observed by electron microscopy within these cells.

However, our data do not allow us to establish whether these redox proteins are derived from endoplasmic reticulum rather than from contaminating mitochondrial, nuclear, or other membrane fractions. Both erythroleukemia cells and normal erythroid cells, at various stages of maturation, have been shown by electron microscopy to possess large nuclei and numerous mitochondria, in addition to small amounts of endoplasmic reticulum (1, 28, 35). Peroxisomal and outer mitochondrial membranes (but not inner mitochondrial membranes) of rat liver have been shown to possess cytochrome b_5 (7, 32, 37), and mitochondria from adrenal glands contain substantial amounts of cytochrome P-450 (4). Moreover, cytochrome P-450, NADPH-cytochrome *c* reductase activity, cytochrome b_5, NADH-cytochrome *c* reductase activity, or combinations of these redox proteins have been detected in nuclear membrane preparations from the livers of a variety of species (2, 8, 19, 41).

In this paper, we also report the isolation of soluble cytochrome b_5 from the cytoplasmic fraction of mouse erythrocytes. Soluble cytochrome b_5 had been isolated previously from beef (6), human (14, 16, 31), rabbit (31), and pork (5) erythrocytes. In contrast to the detection of soluble cytochrome b_5 in the mature erythrocytes of the mouse, no soluble cytochrome b_5 could be detected in the cytoplasmic fraction of mouse erythroleukemia cells.

The Friend virus-induced erythroleukemia cell serves as a model for early immature cells of the erythroid series. Morphologically, this cell has many of the characteristics of immature erythroblasts. Approx. 1% of the erythroleukemia cells spontaneously differentiate to the level of polychromatophilic and orthochromatophilic erythroblasts and synthesize hemoglobin (11, 12). Upon treatment with DMSO, Friend virus-transformed cells exhibit changes that are analogous to those seen in the differentiation of normal erythrocyte precursors. The polychromatophilic- and orthochromatophilic-like cells that result from DMSO stimulation (35) develop erythrocyte membrane antigen (17), accumulate mRNA for globin synthesis (34), and synthesize globin chains indistinguishable from those of the adult mouse (3, 29).

It is our contention that the soluble cytochrome b_5 and cytochrome b_5 reductase present in the cytoplasm of mature erythrocytes are derived from membraneous structures (endoplasmic reticulum, mitochondria, or nuclei) of immature erythroid cells by proteolysis during the maturation process. The endoplasmic reticulum and nuclei, along with most of the other subcellular organelles (excluding mitochondria), have disappeared from the cell by the late orthochromatophilic erythroblast or reticulocyte stage. Because the erythroleukemia cell is a model for a normal immature erythroid cell, our demonstration of membrane-bound cytochrome b_5 but no soluble cytochrome b_5 in the erythroleukemia cell suggests that only particulate cytochrome b_5 is present in normal, early immature, erythroid cells. The finding that the amount of membrane-bound cytochrome b_5 present in mouse erythroleukemia cells is greater than the amount of soluble cytochrome b_5 present in the cytoplasm of mouse erythrocytes is compatible with membrane structures being the origin of the soluble cytochrome b_5.

As has been reported by other workers, we found that DMSO caused the erythroleukemia cells to differentiate, as evidenced by positive benzidine staining of the cells, the red color of the cell pellet, the spectral demonstration of hemoglobin, and the orthochromatophilic-like morphological features of the cells. Electron microscopy of these DMSO-treated cells revealed small amounts of endoplasmic reticulum to be still present. Cytochrome b_5 and cytochrome b_5 reductase were detected in the 105,000-g pellets of the DMSO-treated cells, and no cytochrome b_5 was detected in the 105,000-g supernatant fraction. Apparently, DMSO-induced differentiation of erythroleukemia cells to the polychromatophilic or orthochromatophilic erythroblast stage does not result in the solubilization of cytochrome b_5. The exact stage at which solubilization of membrane-bound cytochrome b_5 occurs during normal erythroid maturation remains to be elucidated.

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