Development of Cytochrome b and an Active Oxidase System in Association with Maturation of a Human Promyelocytic (HL-60) Cell Line

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ABSTRACT The human HL-60 myeloid leukaemia cell line developed, during maturational changes induced by dimethyl sulphoxide, an enhanced capacity for phorbol myristate acetate-stimulated oxidative activity and acquired a cytochrome b. Titration of the absorbance at 559 nm at potentials of −190 to −370 mV indicated that this cytochrome had a very low potential, differentiating it from mitochondrial and endoplasmic reticulum cytochromes and identifying it as the cytochrome b$_{245}$ that has been recently found in other phagocytic cells. Subcellular fractionation studies of mature HL-60 cells showed that cytochrome b had a dual distribution within the cell. The lighter peak of activity was associated with the plasma membrane markers, adenylate cyclase and receptors for the N-formyl-L-methionyl-L-leucyl-L-phenylalanine (f-Met-Leu-Phe) peptide. The denser components localized with the mitochondria but were distinct from mitochondrial cytochromes because whereas the activity of cytochrome c oxidase fell during HL-60 cell maturation, that of this cytochrome b was markedly increased. Concentrations of myeloperoxidase were unrelated to activity of the oxidase system and decreased as the cells matured. The increase in the concentrations of cytochrome b with cellular maturation paralleled the increase in the stimulated nonmitochondrial respiratory activity of these cells. The turnover of the hexose monophosphate shunt of immature cells was increased by the oxidising agents, methylene blue and tert-butylhydroperoxide, indicating that these immature cells have the capacity to generate the putative substrate of the oxidase system. The development of stimulated nonmitochondrial respiratory activity by maturing HL-60 cells is associated with, and is probably dependent upon, the aquisition by these cells of the cytochrome b$_{245}$ oxidase system.

The respiratory burst of stimulated phagocytes is mediated by a nonmitochondrial oxidase system that generates hydrogen peroxide, superoxide, and other species of reduced oxygen (17). This oxidase system is an electron transport chain that contains a very unusual terminal low potential cytochrome b (31) that has been identified in neutrophils, monocytes, macrophages, and eosinophils, but is not obviously apparent in nonphagocytic cells (34). The natural product of the oxidase system is thought to be hydrogen peroxide (14), which then acts as a substrate for myeloperoxidase-mediated halogenation (16).

The HL-60 cell-line is a human cell line, thought to be of primitive myeloid lineage. When incubated in the presence of dimethyl sulfoxide (DMSO), these cells mature from immature promyelocytes to cells that resemble relatively mature granulocytes (6). This maturation includes the development of a functional oxidase system as evidenced by enhanced superoxide production and hexose monophosphate shunt activity when the cells are stimulated by phorbol myristate acetate (PMA) (12-O-tetradecanoylphorbol-13-aceitate) (24).

This study was conducted to investigate whether HL-60 cells contained a low potential cytochrome b and, if they were found to do so, to relate the concentration of this cytochrome to the development of oxidase activity and to the concentration of myeloperoxidase in the cells as they mature.
Materials and Methods

Cell Cultivation and Preparation

HL-60 cells were a kind gift of Dr. R. C. Gallo (National Cancer Institute) and were maintained in culture as described (24). 1.25% (vol/vol) DMSO (Sigma Chemical Co., St. Louis, MO) was added to the incubation medium to induce maturation (6). Cells were harvested after 0, 4, 7, 11, and 14 d by centrifugation at 150 g for 5 min at 15°C, washed twice and resuspended in a sterile 0.1 M Krebs-Ringer phosphate solution, pH 7.4, containing 5 mM glucose, 1.2 mM MgSO4, and 0.9 mM CaCl2 (KRPG) (19). Cell viability was assayed by trypan blue exclusion and by the release of lactate dehydrogenase (LDH) (28). Differential counts were made in Leishman's stained cytospinflower preparations (Shandon Scientific Instruments Ltd., Caversham, Bucksmshire, England).

Spectroscopic Measurement of Cytochrome b and Myeloperoxidase

Reduced minus oxidized difference spectra were recorded in a spectrophotometer (SP 6-200 UV/VIS, Pye Unicam, Cambridge, England) on intact cells at a concentration of 1 × 10⁶/ml as previously described (8). The concentration of cytochrome b was calculated from the height of the 559-nm peak, taking the extinction coefficient of cytochrome b as E(cm-1) = 21.6 mmol cm⁻¹ (9). Dithionite difference spectra were re-run after the cell membranes in both sample and reference cuvettes had been disrupted by addition of Triton X-100 (0.1%) (Sigma Chemical Co.). The concentration of myeloperoxidase was estimated from the height of its 475-nm peak using the formula E = 89 mmol cm⁻¹ (3). The cytochrome b was identified as a low potential cytochrome by titration between potentials of -190 and -370 mV as previously described (8, 34).

Oxidase and Metabolic Activity

Activity was stimulated with PMA (1 μg, in a solution containing 1 μg DMSO/ml), polystyrene latex beads (0.8 μm diameter, 50 particles/cell) opsonized with human gamma globulin (IgG) (Lister Institute, Elstree, England) as described previously (32). (All agents were obtained from Sigma Chemical Co.). Superoxide production was measured by the superoxide dismutase inhibitable reduction of ferricytochrome c (1) and oxygen consumption with an oxygen electrode (32). The oxidation of 1°-C and 6°-labeled glucose to carbon dioxide was measured by the method of Skeel et al. (35), modified to make multiple simultaneous analyses: 1 ml reaction mixtures contained 4 × 10⁶ cells in KRP, 0.25 μCi [1-14C]glucose (0.064 mM) (sp act 3.9 mCi/mmol; Amersham International, England) and 1 μg PMA. Reactions were performed in 2 ml volume multwell plastic Linbro tissue culture plates (Flow Laboratories, Irvine, Ayrshire, Scotland) with the wells sealed by 2.1-cm diameter filter papers (Grade I; Whatman Ltd., Maidstone, England); soaked in 100 μl potassium hydroxide (2 M). The plates were incubated for 60 min in a humidified atmosphere at 37°C with continuous swirling on a horizontal mixer (Ludell Ltd., Burgess Hill, Sussex, England). Reactions were stopped by addition of 0.5 ml of hydrochloric acid (1.0 M) and the plates were incubated for 30 min at 20°C. The filters were placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear Chemicals, Dreishich, W. Germany), and emissions were counted in a 460 CD Automatic Tricarb liquid scintillation counter (Packard Instruments Ltd., Caversham, Buckinghamshire, England). Oxidative activity was expressed as the stimulated rate minus the resting rate per milligram of protein per hour.

3-O-methyl Glucose Uptake during Metabolic Activation

Cells (4 × 10⁷) were incubated in 1 ml of KRP containing glucose (0.064 mM) and 1 μCi of 3-O-methyl-[1-14C]glucose (sp act 3.0 Ci/mmol; Amersham International) in multwell Linbro plates (Flow Laboratories, Scotland) for 60 min at 37°C in a humidified atmosphere with continuous shaking. The samples were harvested by filtration through 0.45-μm pore diameter cellulose-nitrite filters (Sartorius GmbH, D-3490 Gottingen, W. Germany) using a multisample filtering manifold (Millipore, Bedford, MA). The filters were washed three times with 2.0 ml KRP, placed in scintillation vials and dissolved in 5 ml Filter Count (New England Nuclear Chemicals GmbH, 6072 Dreishich, W. Germany), and emissions were counted in a liquid scintillation counter (Packard Instruments Ltd., Buckinghamshire, England).

Subcellular Fractionation Studies

Cells (5 × 10⁷ uninduced and 2 × 10⁷ 12-d induced) were pelleted by centrifugation and washed twice in cold sucrose (11.2% wt/wt). The cells were suspended in cold sucrose (5 ml of 11.2%) and disrupted in a Dounce homogenizer with 50 strokes of a tight-fitting (B) pestle (Kontes glass, Vineland, NJ). The homogenates were centrifuged at 600 g at 4°C for 10 min to remove the nuclei and unbroken cells, and the postnuclear supernatants (6 ml) were layered onto 27-mM linear gradients of sucrose from 11 to 55% (wt/wt) (density 1.045–1.266 g/ml) with a 3-mll cushion of 60%. The gradients were centrifuged at 25,000 rpm in an AN 627 swinging bucket rotor in an OTD 50B Sorvall Centrifuge (Dupont U.K. Ltd., Stevenage, Hertfordshire, England) (integrated angular velocity 4 × 10⁶ radians/s², g, = 125,000, g, = 54,000, g, = 98,900). 25 × 1.1 ml fractions were collected from each gradient by upward displacement with 60% sucrose. All sucrose solutions contained EDTA (1 mM, pH 7.4) and preservative-free heparin (5 IU/ml; Weddel Pharmaceuticals, London, England).

Analyses Conducted on Fractionated Cells

Cytochrome b was measured by reduced minus oxidized difference spectroscopy on aliquots (400 μl) of the fractions in 11.0% sucrose, and compared with that of the following components measured as follows: protein by the method of Lowry et al. (20) using bovine serum albumin as standard; 5° nucleotidase by the hydrolysis of [H-AMP] (11); adenylate cyclase by the generation of cyclic AMP (cAMP) from ATP, using the method of Wisher and Evans (37), minimally modified by the addition of guanosine triphosphate (10 mM) and sodium fluoride (10 mM) to the incubation medium; cAMP by a competitive binding radioassay (Cyclic AMP Assay Kit, Amerham International, Buckinghamshire, England); receptors for N-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Ph) by a f-Met-Leu-[14C]-Ph-binding assay (26); cytochrome c oxidase by the spectrophotometric method of Cooperstein and Lazarow (7); glucose-6-phosphate dehydrogenase by the production of radio-labeled phosphate from D-glucose-[14C]-6-phosphate potassium salt (Amerham International) (13, 22); lysozyme spectrophotometrically by the lysis of Micrococcus lysodeikticus (22) using egg white lysozyme (Sigma Chemical Co.) as a standard; myeloperoxidase by the peroxidation of o-dianisidine as described by Breet and Baggoloni (5), using horse radish peroxidase (HRPO) (Type II, Sigma Chemical Co.) as a standard.

Rate Sedimentation Analysis of Fractions Containing Mitochondria

Organelles of HL-60 cells 12 d after the induction of maturation with DMSO were separated on sucrose density gradients by analytical subcellular fractionation as described above. Aliquots of the two fractions in which most of the mitochondria were located (densities of 1.148 and 1.161) were pooled and diluted with water containing EDTA (1 mM) and heparin (5 IU/ml) to a density of 1.079. The combined fractions (1.8 ml) were layered onto 82 ml of 20% sucrose in a sucrose solution containing EDTA (1 mM, pH 7.4) and preservative-free heparin (5 IU/ml; Weddel Pharmaceuticals, London, England). Mitochondrial fractions were separated on sucrose density gradients by analytical subcellular fractionation conducted on fractionated cells.

RESULTS

Oxidase Activity of Stimulated Cells

Morphological maturation (Table I) and an increase in stimulated oxidative activity (Fig. 1) were observed during DMSO-induced maturation of HL-60 cells, as has been previously described (23, 24). Little change in PMA-stimulated oxygen consumption, superoxide production, or heoxo-monophosphate shunt activity (HMPs) activity was observed during the first 4 d after maturation induction. Subsequently, there was a sharp rise in all parameters to maximal activity at day 10 (Fig. 1). A decrease in activity at day 14 to 80%–90% of...
TABLE 1
Concentration Changes after DMSO Induction

| Maturation time | Morphology* | Protein† | Cytochrome c oxidase activity§ | Cytochrome b¶ | Spectral activity¶ | Peroxidase activity¶ |
|-----------------|-------------|----------|-------------------------------|--------------|-------------------|----------------------|
| d               | % metamyelocytes | mg/10⁶ cells | mL/mg protein | pmol/mg protein | pmol/mg protein | U/mg protein |
| 0               | 0           | 1.66 ± 0.11 | 13.9 ± 4.3       | 6 ± 2        | 226 ± 25          | 0.299 ± 0.015       |
| 3               | 7.6 ± 0.5   | 1.26 ± 0.08 | 4.1 ± 1.7        | 8            | 176               | 0.224 ± 0.018       |
| 5               | --          | --         | --               | 22 ± 24      | 112, 160          | --                  |
| 7 or 8          | 31.3 ± 1.9  | 0.94 ± 0.09 | 2.7 ± 1.1        | 41 ± 35      | 186, 66           | 0.202 ± 0.015       |
| 10              | 42.0 ± 1.5  | 0.73 ± 0.16 | 1.3 ± 0.7        | 38, 49       | 246, 143          | 0.208 ± 0.011       |
| 14              | 51.0 ± 3.3  | 0.78 ± 0.18 | 1.6 ± 0.0         | 105 ± 46     | 232 ± 50          | 0.203 ± 0.018       |

Changes in the concentration of cytochrome b-245 cytochrome oxidase, myeloperoxidase, and in the morphological appearance of HL-60 cells at various times after the induction of maturation with DMSO.

* Mean ± SE, n = 11.
† Mean ± SE, n = 4.
§ Mean ± SE, n = 5.
¶ Mean ± SE, n = 3. Individual results where less than three experiments were performed.
|| Mean ± SE, n = 8.

maximal values was possibly due to a small increase in the proportion of promyelocytes (88%, 53%, 27%, 15%, and 20%, at days 0, 4, 7, 10, and 14, respectively), which had no stimulated oxidase activity. PMA-stimulated oxidation of 6-[^14]C]glucose increased with the same kinetics as the other parameters of oxidase activity.

Cytochrome b

Reduced minus oxidized spectroscopy revealed the presence of a cytochrome b with α- and γ-peaks of absorption at 559 and 428 nm in mature HL-60 cells (Fig. 2). It was identified as a low potential cytochrome by an increase of 64% in the absorbance at 559 nm, as the potential was reduced from -190 to -370 mV. There was no increase in absorbance at 559 nm in uninduced HL-60 cells below -100 mV, at which mitochondrial cytochromes are fully reduced, indicating the absence from these cells of detectable low potential cytochrome b. Their absorption spectrum showed peaks between 443 and 452 nm and at 548-559 nm, which are consistent with mitochondrial cytochromes (Fig. 2).

In the uninduced cells the distribution of cytochrome components corresponded with that of the mitochondria, with a peak absorbance at a density of ~1.148-1.161 g/ml (Figs. 3 and 4). In comparison the induced cells showed two peaks of cytochrome distribution. In two experiments the dense peaks were also associated with the mitochondria at densities of 1.148 and 1.161, whereas the light peaks had densities of 1.127 and 1.138 g/ml. The lighter density peak of cytochrome b was one fraction heavier than the 1.125 g/ml density peak of the membrane markers, adenylate cyclase, and f-Met-Leu-Phe receptor binding. 5' nucleotidase was unsatisfactory as a membrane marker because insufficient activity was detectable. Glucose-6-phosphatase, a marker enzyme for “rough” endoplasmic reticulum (21), was widely distributed over fractions containing plasma membrane, mitochondria and azurophil granules. The dense peak of cytochrome b was further resolved by rate
Activity stimulated by both agents increased with cellular maturation, with similar kinetics to other parameters of oxidase activity (Fig. 1), with the exception that at day 7 the increase in activity was slightly higher than that of either superoxide production or oxygen consumption. Control experiments using DMSO (1 µg/ml) as a stimulant showed that it did not increase HMPS activity at any stage of maturation (Table II).

To identify whether the increase in HMPS activity during maturation was due to an increase in the enzymes of the HMPS or an enhanced capacity to respond to a stimulus to the oxidase system, further studies were performed. The cells were exposed

![Figure 3 Reduced minus oxidized difference spectra of fractions collected after the fractionation of disrupted HL-60 cells by centrifugation on continuous sucrose gradients. Postnuclear supernatants were prepared from 3 x 10^6 uninduced cells (90% promyelocytes) and 6 x 10^6 induced cells (16% promyelocytes, 28% myelocytes, 56% metamyelocytes) each of which contained ~20 mg of protein. Absorbance scale marker equals 0.0025. The numbers indicate the density of sucrose in the respective fractions.

![Figure 4 The density distribution profile of components of homogenized induced (12 d after induction of maturation with DMSO, differential count as in legend to Fig. 3) HL-60 cells fractionated on continuous sucrose gradients. Data are expressed as a percentage of the total activity in the gradients. The percentage of recovered activity in the gradients were: cytochrome b, 85; adenylate cyclase, 97; f-Met-Leu-Phe receptors, 86; cytochrome c oxidase, 85; glucose-6-phosphatase, 97; lysozyme, 70; and myeloperoxidase, 98.

![Figure 5 Rate sedimentation analysis of cytochrome components localized with the mitochondria after analytic subcellular fractionation of HL-60 cells, 12 d after induction with DMSO. The two fractions with densities of 1.148 and 1.161 shown in Fig. 3 were combined and centrifuged gently through a 20% sucrose gradient (see Materials and Methods). Reduced-minus-oxidized difference spectra of the rapidly sedimenting (a) and slowly sedimenting (b) components are shown. The mitochondrial components (a) sedimented more rapidly than the other components which contained more nonmitochondrial cytochrome b (b).

Hexose Monophosphate Shunt Activity (Oxidation of 1-L[14C]Glucose)

HMPS activity in the maturing HL-60 cell model was stimulated by PMA and IgG-opsonized latex particles (Table II).
TABLE II

Changes in the Metabolic Responses

| Parameter                         | Stimulus    | No. of studies | Mature cells | Immature cells | Induced cells |
|-----------------------------------|-------------|----------------|--------------|----------------|---------------|
|                                   |             |                | Unstimulated | Stimulated     | Unstimulated | Stimulated   |
| Oxygen consumption (nmol/min/10^7 cells) | PMA         | 3              | 9.7          | 11.5           | 4.8           | 19.9         |
| Superoxide production (nmol/min/10^7 cells) | PMA         | 2              | 0.1          | 0.4            | 0             | 18.5         |
| 1-[^14]C]glucose oxid" (pmol/10^6 cells/h) | PMA         | 8              | 230 ± 10     | 484 ± 17       | 215 ± 19      | 4,360 ± 610  |
| 1-[^14]C]glucose oxid" (pmol/10^6 cells/h) | DMSO        | 2              | 213          | 256            | 165           | 220          |
| 1-[^14]C]glucose oxid" (pmol/10^6 cells/h) | t-Butyl-hydroperoxide | 3  | 188 ± 26     | 1,816 ± 160    | 185 ± 38      | 3,555 ± 437  |
| 1-[^14]C]glucose oxid" (pmol/10^6 cells/h) | Methylene blue | 2         | 239          | 1,085          | 150           | 2,546        |
| 1-[^14]C]glucose oxid" (pmol/10^6 cells/h) | IgG-latex    | 2              | 343          | 389            | 166           | 1,336        |

Changes in the metabolic responses of unstimulated and stimulated HL-60 cells, before and 10 d after the induction of maturation with DMSO. Results are expressed as mean of two separate experiments or mean ± SE when more than two experiments were performed. Oxid" oxidation.

To two oxidizing agents, tert-butylhydroperoxide (BHP) (1 mM) which acts as a substrate for glutathione peroxidase, and oxidizes glutathione which in turn is reduced by glutathione reductase, producing NADPH (15); and methylene blue (MB) (2 mM), which oxidized NADPH by way of a diaphorase (18). In both cases, the NADP is reduced by reducing equivalents originating in glucose and passing through the HMP shunt. Stimulation of HMPS activity in immature cells with these oxidizing agents was fivefold greater than that stimulated by PMA or latex (Table II), indicating that HMPS activity was not the limiting factor with regard to the degree of activation in these cells. However, an increase in HMPS activity was observed with these agents during maturation although this increment was less marked than PMA-stimulated activity.

To investigate whether the increase in I-[^14]C]glucose oxidation during maturation could be explained by an increased uptake of radio-labeled glucose into the cell, the 3-O-methyl analogue of D-glucose was incubated with the cells during PMA-stimulated oxidative activity. This has almost identical transport characteristics to D-glucose (2) but is not metabolised and thus accumulates within the cell (27). In these experiments there was an inhibition of glucose uptake during stimulation with PMA and the degree of inhibition increased as the cells matured (Fig. 6). Therefore the increase in HMPS activity during maturation was not due to an increase in uptake of labeled glucose, but, in fact, because the uptake of extracellular glucose is actually decreased with maturation, the observed increase in I-[^14]C]glucose oxidation is a gross underestimate of the actual enhancement of activity.

Release of LDH from HL-60 cells was measured before and after stimulation with PMA to exclude the possibility that accumulated intracellular 3-O-methyl glucose was leaking out during PMA-induced membrane perturbations. At all stages of maturation except day 10, PMA caused a small increment in LDH release (Fig. 6), but there was no significant increase in release as the cells matured. The concentration of lactate dehydrogenase was 3.2, 2.8, 2.2, 2.7, and 2.0 mU/mg protein in uninduced HL-60 cells at 3, 7, 10, and 14 d after induction of maturation, respectively. (1 U is equivalent to 1 μmol NADH2 oxidized per min.)

Oxidation of 6-[^14]C]glucose to 6-[^14]CO2, via the Embden-Meyerhof-Parnas pathway followed by oxidation of pyruvate, increased with the same kinetics during maturation as the other parameters of oxidative activity (Fig. 1), yet less radio-labeled glucose was oxidised via this route to carbon dioxide than through the HMPS (Table II), as previously reported (24, 30).

DISCUSSION

We found, in confirmation of the work of Newburger et al. (24) and Mendelsohn et al. (23), that HL-60 cells in the course of maturation from cells that resemble promyelocytes to more mature metamyelocytes, develop an increase in stimulated
oxidase activity. The rate of increase in this activity corresponds very closely with the acquisition by the cells of a low potential cytochrome b. This observation provides further support for the functional role of cytochrome b as an integral part of the oxidase system of phagocytic cells.

We investigated whether the increase of oxidase activity during cell maturation was due to the development of other factors besides cytochrome b, such as the generation of substrate by the HMPS, or increased glucose metabolism. We therefore used methylene blue and tert-butylhydroperoxide to provide oxidizing conditions, since these agents stimulate the HMPS directly, bypassing the membrane oxidase. These experiments indicated that immature HL-60 cells which do not exhibit stimulated oxidase activity can increase their HMPS activity tenfold to 36% of the maximal level seen in mature cells. Thus, the oxidase activity in these cells does not appear to be limited by their ability to generate sufficient substrate. The increase in measured HMPS activity was not simply an apparent increase resulting from the preferential use of extracellular radioactive glucose rather than intracellular carbohydrate stores in mature cells, because experiments using the 3-O-methyl glucose analogue showed an inhibition of glucose uptake upon PMA stimulation and that this became more apparent as the cells matured. Others have reported a similar inhibitory effect of PMA on the transport of nutrients into mature neutrophils (10).

Other factors that might prevent the metabolic response of immature cells to stimuli include the absence of plasma membrane receptors for these different stimuli. The development of phorbol receptors has not yet been measured in this cell line; however, the development of receptors for IgG has been measured in maturing HL-60 cells by the rosetting of IgG coated erythrocytes (29). They were found to be present on 30% of uninduced cells, 30% of cells at day 3 and 67% of cells at day 10. Thus, these receptors for IgG are present at the initiation of induction of maturation at a time when IgG coated particles do not stimulate a respiratory response. This could indicate that the receptor density is too low at this stage to activate a response, but it seems more likely that the respiratory burst is limited at this stage by the failure of the cell to complete synthesis of the cytochrome b and possibly other related redox components.

The cytochrome b that developed in HL-60 cells has similar spectral characteristics to cytochrome b-245 found in other phagocytic cells (33, 34). At least 60% of the total cytochrome b in mature cells has a potential below -190 mV, indicating that the mature cells contain ~50 pmol of low potential cytochrome b for each milligram of protein (Table I), a figure not substantially below that of 80 pmol/mg found with mature neutrophils (34).

The distribution of cytochrome b in day HL-60 cells (50% metamyelocytes) subjected to analytical subcellular fractionation on continuous sucrose density gradients was different from that found in normal human neutrophils (33). In normal human neutrophils the cytochrome b has a bimodal localization associated with the specific granules (33). In day 12 induced HL-60 cells the cytochrome was distributed with the plasma membranes and mitochondria, and of that cytochrome b that distributed with the mitochondria at least half was not mitochondrial. Thus most of the cytochrome b in the induced HL-60 cells was in the region of the membranes. It was difficult to accurately identify these as plasma membranes because the plasma membrane markers have not been characterized in the HL-60 cells. The usual markers used in human neutrophils gave a roughly similar distribution to the cytochrome b, although they were also detected in heavier fractions containing mitochondria, endoplasmic reticulum, and azurophil granules. The absence of both a dense cytochrome b component and the classical specific granule contents (36) in HL-60 cells (24, 25) is further evidence for the localization of the cytochrome in these granules in normal neutrophils. An alternative explanation would be the fusion of the specific granules with the plasma membrane in induced HL-60 cells, but this is unlikely as specific granule markers were undetected in both the cells and extracellular medium (25), making seclusion of the specific granules unlikely.

Myeloperoxidase was present in similar concentrations in immature and mature cells and this contrasts with the marked increase in cytochrome b and stimulated oxidase activity. It is interesting that the synthesis of this enzyme should be so different from that of the other proteins that are responsible for the generation of its substrate.

The maturation of HL-60 cells is associated with a marked increase in their stimulated respiratory capacity. This does not appear to be due to an increase in the enzyme pathways generating the putative substrate of the oxidase system. It could be due to the synthesis of the electron transport chain including cytochrome b-245.

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