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MYELOPEROXIDASES IN CULTURED HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60[1]

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SUMMARY: Human promyelocytic leukemia cells HL-60 contain a small and a large myeloperoxidase with molecular weights of 79,000 and 153,000, respectively. In the cells, 80% of the total myeloperoxidase is small myeloperoxidase and 20% is large myeloperoxidase. Futhermore, 40% of the total myeloperoxidase was recovered in the soluble fraction, and the rest in the granule fraction. However, peripheral blood granulocytes are known to contain only the large myeloperoxidase, which is mainly recovered in the granule fraction. During differentiation of HL-60 cells into granulocytes by dimethylsulfoxide, both the small and large myeloperoxidase decrease, but small myeloperoxidase decreases preferentially.

INTRODUCTION

Myeloperoxidase, a main hemoprotein constituting 2-5% of the dry weight of granulocytes (1, 2), functions in killing bacteria taken up by granulocytes (3). The molecular weights of the enzyme purified from various sources, including humans, ranged from 130,000 to 160,000 (2, 4-7). Recently we found a novel small myeloperoxidase with a molecular weight 79,000 in solid tumors of human promyelocytic leukemia cells HL-60 grown in athymic nude mice (8). About 8% of the total myeloperoxidase in the tumors is this small myeloperoxidase, and the rest is a large myeloperoxidase with a molecualr weight 153,000. It seemed interesting to investigate whether this small myeloperoxidase was specific to promyelocytic leukemia HL-60 cells, or to precursors of granulocytes. Collins et al. showed that HL-60 cells can be induced to

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Abbreviations used; TPA, 12-o-tetradecanoyl-phorbor-13-acetate; CETAB, cetyltrimethylammonium bromide.

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differentiate into granulocytes by various reagents, such as dimethylsulfoxide and retinoic acid (9-11). Thus we studied the myeloperoxidase in the cells in relation to differentiation of HL-60 cells into granulocytes.

We report here that the small myeloperoxidase is the main myeloperoxidase in HL-60 cells in culture with only a little of the large myeloperoxidase, but that it decreases more rapidly than the large enzyme during differentiation of HL-60 cells.

MATERIALS AND METHODS

Chemicals. TPA was obtained from Midland Corp. Horse heart cytochrome c was from Sigma Chemical Co. Human I_gG was from Miles Laboratories, Inc. Superoxide dismutase was a gift from Dr. O. Hayaishi, Kyoto University, Kyoto.

Cells and cell culture. The HL-60 cell line (12) was a generous gift from Dr. R. C. Gallo, National Institute of Health, MD. The cells were grown in suspension in a 174 cm^2 Nunc culture flask containing 75 ml of RPMI-1640 medium (Flow Laboratories) supplemented with 15% heat-inactivated fetal calf serum and Kanamycin (50 μg/ml). For induction of differentiation of the cells into granulocytes, HL-60 cells were seeded at 4.5×10^5 cells/ml and grown in the absence or presence of 1.25% dimethylsulfoxide for 75 h. Differentiated characters were examined as described below.

Assay of phagocytosis. The phagocytic cells were determined as described previously (13), except that the cells were incubated with human I_gG-coated polystyrene latex particles (1 μm in diameter) for 1.5 h. The I_gG-coated particles were prepared by the method of Segal and Coade (14).

Assay of superoxide. The assay mixture consisted of 0.5 ml phosphate-buffered saline (pH 7.4) containing 25 nmol of cytochrome c, $0.2 - 2 \times 10^5$ cells, and 0.8 nmol of TPA and was incubated in the presence or absence of 4000 units of superoxide dismutase for 20 min at 37°. The formation of reduced cytochrome c which could be inhibited by superoxide dismutase was determined by measuring the absorbance at 550 nm (10).

Assay of myeloperoxidase. Myeloperoxidase activity was determined using guaiacol (8). One unit of myeloperoxidase was defined as the amount of enzyme causing increase of 1 unit in the absorbance at 470 nm in 1 min at room temperature under the assay conditions.

Extraction and subcellular fractionation of myeloperoxidase. HL-60 cells (1×10^8) incubated without or with dimethylsulfoxide were resuspended in 1 ml of 6.7 mM sodium phosphate buffer (pH 6.3), 3 mM NaCl, 1 mM $MgCl_2$, 0.5 mM phenylmethylsulfonylfluoride and 0.3% Triton X-100, stood for 20 min in ice water, and then broken by 15 strokes of a Dounce homogenizer with a tightly fitting pestle. The homogenate was divided into two portions. One portion was mixed with CETAB at a final concentration of 1%, stirred for 1.5 h and then centrifuged at 20,000 x g for 20 min. All the myeloperoxidase activity was found in the supernatant fluid, named total extract. The other portion was centrifuged at 20,000 x g for 20 min. The resulting supernatant and pellet fracitons, named the soluble and granule fractions (15), respectively, were each extracted with 1% CETAB in the same way as the total extract.

TABLE I

Induction of Differentiation of Human Promyelocytic Leukemia HL-60 cells by Dimethylsulfoxide[a]

Treatment	Cell number	O_2^- produced		Phagocytic cells
		+TPA	-TPA	
	(per ml)	(nmol/20 min/10^6 cells)		(% of total cells)
Control	2.5×10^6	0.0	0.0	6.8
Dimethyl-sulfoxide	2.0×10^6	16.7	1.4	78.8

[a]The cells were grown in the presence or absence of 1.25% dimethylsulfoxide for 75 h.

RESULTS

HL-60 cells were grown in the presence or absence of dimethylsulfoxide for 3 days. Table I shows that in the dimethylsulfoxide-treated culture, 79% of the cells were phagocytic and that the cells had the ability to produce O_2^- in the presence of TPA, indicating that HL-60 cells were induced to differentiate into granulocytes under these conditions. However, in the control culture, only 7% of the cells were phagocytic and the cells did not produce significant O_2^-.

The myeloperoxidase activity in dimethylsulfoxide treated cells decreased to 23% of that in control cells (Table II). To examine the localization of myeloperoxidase in the cells, we fractionated HL-60 cells and peripheral blood granulocytes into soluble and granule fractions. Table II shows that myeloperoxidase in the human granulocytes from the peripheral blood was present exclusively in the granules. In contrast, about 40% of the myeloperoxidase in the cells from the control culture was found in the soluble fraction and the rest was in the granules. During differentiation of HL-60

TABLE II

Subcellular Distribution of Myeloperoxidase in HL-60 Cells and Peripheral Blood Leukocytes[a]

Fraction	HL-60 cells	HL-60 cells induced to differentiate	Peripheral blood leukocytes
		(units/10^8 cells)	
Homogenate	56.1	13.8	5.1
Total extract	162	36.5	197
Soluble	65.2	6.9	1.8
Granules	88.1	25.2	285

[a]HL-60 cells with or without induction of differentiation were the same as described in Table 1. Human leukocytes from the peripheral blood of a healthy adult man were prepared by exposing the leukocyte-rich fraction to buffer containing 155 mM NH_4Cl-0.1 mM EDTA-10 mM $KHCO_3$ (pH 7.0) to lyze red cells (2). HL-60 cells and peripheral blood granulocytes were suspended at 10^8 cells/ml and disrupted. Extraction and subcellular fractionation of myeloperoxidase were as described in Materials and Methods.

cells, the amount of myeloperoxidase in the soluble fraction decreased more rapidly than that in the granules.

The amounts of myeloperoxidase in the whole cell extract, and the soluble and granule fractions of HL-60 cells and human granulocytes from peripheral blood were analyzed by sucrose density gradient centrifugation (Fig. 1). The extract of peripheral blood granulocytes contained only myeloperoxidase of 7.9s (Fig. 1a), as reported by others (2, 4-7), but that of control HL-60 cells contained two activities, a large molecular enzyme of 7.9s, and a small molecular one of 5.2s (Fig. 1b). The small molecular myeloperoxidase amounted to 77% of the total enzyme. On subcellular fractionation of the cells, the myeloperoxidase activity recovered in the soluble fraction was entirely in the form of the small myeloperoxidase and that in the granule fraction was present 28% as the large enzyme and 72% as the small

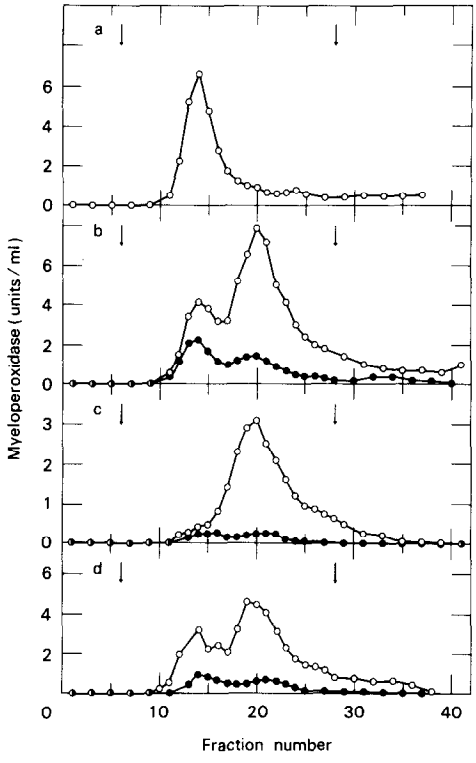

Fig. 1. Sucrose density gradient centrifugation of myeloperoxidase from HL-60 cells and their induced, differentiated cells. The total extract, and the soluble and granule fractions of the cells described in Table II were loaded on 5 ml of a linear gradient of 5% to 20% (w/v) sucrose in 0.2 M potassium phosphate buffer (pH 7.8) and centrifuged at 40,000 rpm in an SW 50.1 rotor in a Beckman ultracentrifuge at 3° for 18 h. The centrifugal force was from right to left. a, total extract of peripheral blood leukocytes from the adult man; b, total extract of HL-60 cells; c, soluble fraction of HL-60 cells; d, granule fraction of HL-60 cells. o, myeloperoxidase of HL-60 cells in the control culture; •, that of HL-60 cells treated with dimethylsulfoxide. Arrows from left to right indicate the positions of bovine liver catalase (11.3s) and horse heart cytochrome c (1.9s).

enzyme (Fig. 1c and d). In the dimethylsulfoxide treated cells, the large myeloperoxidase decreased to 50% of that in control cells, and the small myeloperoxidase decreased to 14% of that in control cells (Fig. 1b). This indicates that the small myeloperoxidase was lost more rapidly than large one. The large and small myeloperoxidases in the induced, differentiated cells were estimated to contribute 50% each to the total activities in the whole-cell extract, and the soluble and granule fractions (Fig. 1).

The small myeloperoxidase from the soluble fraction of HL-60 cells had absorption bands at 427 and 570 nm before reduction, and at 473 and 638 nm

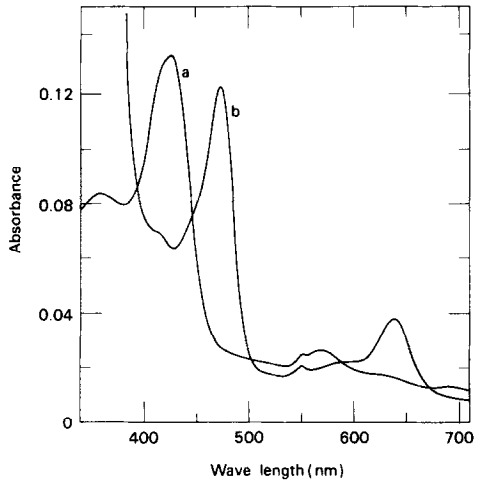

Fig. 2. Absorption spectra of the small myeloperodidase. The soluble fraction of HL-60 cells from the control culture was loaded on a DEAE-Sepharose CL-6B column (0.8 x 0.8 cm) which had been equilibrated with 0.025 M potassium phosphate (pH 7.8) containing 0.02% CETAB. The breakthrough fraction was collected and treated with 60% ethanol. The precipitate was dissolved in, and dialyzed against, 0.02 M potassium phosphate (pH 7.8) containing 0.02% CETAB. The absorption spectra of the dialyzed sample were recorded in a Shimazu double-beam spectrophotometer, model UV-300. a, untreated sample; b, sample reduced with $Na_2S_2O_4$.

after reduction (Fig. 2). The preparation was contaminated with a little of a compound absorbing visible light. The pyridine ferrohemochromogen, derived from the reduced enzyme by treatment with 30% pyridine and 0.15 M NaOH, had an α-band at 588 nm and γ-band at 435 nm. These spectra were identical to those of the leukocyte myeloperoxidase described by others (7, 16).

DISCUSSION

This work showed that the small molecular myeloperoxidase with a molecular weight 79,000 was the main myeloperoxidase in cultured HL-60 cells and that it disappeared during differentiation of the cells. Previously we found that a small amount of the small myeloperoxidase was purified with the large enzyme with a molecular weight 153,000 from solid tumors of HL-60 cells grown subcutaneously on athymic nude mice (8). Small myeloperoxidases, like the large enzyme, can be dissociated into two protein components with molecular weights 59,000 and 10,000 by treatment with 2-mercaptoethanol and sodium

dodecylsulfate (8, 17). The small myeloperoxidase might be the active portion of the large enzyme (8). But shown in this work and by others (2, 4-7), only the large myeloperoxidase was detected in an extract of human peripheral blood granulocytes under our experimental conditions. Moreover, a purified preparation of the large myeloperoxidase of HL-60 cells can not be dissociated into the small enzyme under the same conditions (8). Furthermore, the small myeloperoxidase differes immunologically from the large myeloperoxidase (8). These findings do not necessarily exclude the possibility that the small myeloperoxidase is formed during synthesis of the large myeloperoxidase in HL-60 cells. Further work is needed to show whether the small myeloperoxidase differs in amino acid sequence from the large myeloperoxidase.

HL-60 cells differed from peripheral granulocytes not only in having the small myeloperoxidase, but also in the subcellular localization of their myeloperoxidase: myeloperoxidase of peripheral blood granulocytes was present exclusively in the granules, as reported by others (1, 15), whereas that in HL-60 cells was located in the soluble fraction as well as the granule fraction. When HL-60 cells were induced to differentiate, the small myeloperoxidase in the soluble fraction disappeared almost completely, while the large myeloperoxidase in the granules decreased only 50%. Thus after differentiation, the large myeloperoxidase in the granules became predominant. Loss of the small myeloperoxidase probably explains why only the large enzyme is present in peripheral blood granulocytes. Myeloperoxidase must be located in the correct granules of the cells to exert its physiological function (18). Thus it is unknown whether the small myeloperoxidase, recovered in the soluble fraction, functions in killing bacteia in the cells.

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