Purification and Characterization of Small Molecular Weight Myeloperoxidase from Human Promyelocytic Leukemia HL-60 Cells†

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ABSTRACT: Human myeloperoxidase was purified to homogeneity from human promyelocytic leukemia HL-60 cells. A small molecular weight myeloperoxidase was found in these cells and was separated from three other forms of myeloperoxidase of large molecular weight by carboxymethyl-Sepharose CL-6B column chromatography and Sephacryl S-200 gel filtration. The $s_{20,w}$ values of the molecular weights of the small and large myeloperoxidases were found to be 5.2 and 8.07 S, respectively, by sucrose density gradient centrifugation. From these $s_{20,w}$ values, the molecular weights of the small and large myeloperoxidases were estimated to be 79 000 and 153 000, respectively. On electrophoresis in sodium dodecyl sulfate–polyacrylamide gel, the small and large myeloperoxidases each gave two bands of protein corresponding to molecular weights of 59 300 and 10 150. The small myeloperoxidase could not be distinguished from the large enzymes by the Ouchterlony double immunodiffusion test, but it could be distinguished from them by the microcomplement fixation text. One of the three large molecular weight myeloperoxidases was eluted at a lower concentration of methyl α-D-mannoside than the other two on concanavalin A–Sepharose chromatography. This suggested that the heterogeneity of the myeloperoxidases with large molecular weight may be partly due to differences in their sugar moieties.

Myeloperoxidase [donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7] is present in granulocytes and is involved in the bacteriocidal function of these cells (Klebanoff, 1975; Rosen & Klebanoff, 1977; Harrison & Schultz, 1976; Sbarra et al., 1976).

Myeloperoxidases from bone marrow cells of guinea pigs have been studied in relation to granulocytes differentiation (Himmelhoch et al., 1969; Desser et al., 1972). Bone marrow cells are a mixture of several kinds of hematopoietic cells at various stages of differentiation. It is difficult to follow the differentiation of the progenitor cells to granulocytes in vitro, but the human leukemia line HL-60 cells are promyelocytic cells and can be induced to differentiate into granulocytes or macrophages by dimethyl sulfoxide, 12-*O*-tetradecanoylphorbor-13-acetate, or tunicamycin (Collins et al., 1978; Huberman & Callaham, 1979; Rovera et al., 1979; Nakayasu et al., 1980). Induction of differentiation of HL-60 cells is associated with appearance of the characteristics of normal terminally differentiated cells, such as phagocytosis, superoxide-producing activity, and bacteriocidal function (Collins et al., 1979; Newburger et al., 1979).

Myeloperoxidase activity has been demonstrated in HL-60 cells by histochemical staining before and after differentiation (Collins et al., 1978), and its activity has been found to change markedly during in vitro cell differentiation (Rovera et al., 1979; Nakayasu et al., 1980). Myeloperoxidase of HL-60 cells has not yet been purified and characterized, but it has been purified from various other sources such as human leukocytes (Schultz & Shmukler, 1964; Olsson et al., 1972; Bakkenist et al., 1978), pig leukocytes (Odajima & Yamazaki, 1972), guinea pig bone marrow cells (Desser et al., 1972), canine pus (Agner, 1958; Harrison et al., 1977), and rat chloroma tissue (Schultz et al., 1957). The molecular weight of these myeloperoxidases is between 130 000 and 160 000, regardless of source.

In this work we purified and characterized this enzyme from HL-60 cells and found that it consisted of a small size myeloperoxidase, M_r 79 000, in addition to large size myeloperoxidases, M_r 153 000. The small myeloperoxidase differed in immunological properties from the large myeloperoxidases, and the large myeloperoxidases appeared heterogeneous on CM-Sepharose and Con A–Sepharose[1] column chromatography.

Materials and Methods

Chemicals. CM-Sepharose CL-6B, Sephacryl S-200, and Con A–Sepharose were purchased from Pharmacia Fine Chemicals. Phenylmethanesulfonyl fluoride, methyl α-D-mannoside grade III, bovine liver catalase, bovine serum albumin, yeast alcohol dehydrogenase, and horse heart cytochorme *c* were obtained from Sigma Chemical Co. Hemolysin and guinea pig complement were from Flow Laboratories. Agarose was from Seakem, Marine Colloids, Inc. Sheep erythrocyts were from Nippon Bio Test Laboratories. Freund's adjuvant was from Difco Laboratories.

Growth of HL-60 Cells. The human promyelocytic leukemia cell line HL-60 was established by Collins et al. (1977). HL-60 cells were transplanted subcutaneously into the back of male athymic nude mice (nu/nu) with a BALB/C genetic background and solid tumors were obtained from the animals 1.5 months later. The tumors were kept frozen at −80 °C until use.

Purification of Myeloperoxidase from HL-60 Cells. The whole purification procedure was carried out at 0 to 4 °C.

Preparation of Homogenate and Extract. Solid tumors (18.4 g) were thawed, chopped up with scissors, and homogenized in 60 mL of buffer A, consisting of 6.7 mM sodium phosphate (pH 6.6), 3 mM NaCl, 1 mM $MgCl_2$, 0.5 mM phenylmethanesulfonyl fluoride, and 0.3% Triton X-100, first by ten strokes of a pestle in a glass–Teflon homogenizer and then in a Polytron operated for two 15-s periods. The homogenate was filtered on four layers of gauze and the filtrate was centrifugated at 20000*g* for 20 min. The pellet was washed with 60 mL of bufer A without Triton X-100 with stirring overnight and then centrifuged at 20000*g* for 20 min. The washed pellet was extracted with 80 mL of 1% CETAB in 0.1 M potassium phosphate (pH 7.8), first by homogeni-

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[1] Abbreviations used: Con A–Sepharose, concanavalin A covalently bound Sepharose 4B; CETAB, cetyltrimethylammonium bromide.

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zation in a Polytron for two 15-s periods and then by stirring for 2 h. The suspension was then centrifuged at 20000*g* for 20 min and the resulting pellet was again extracted with 25 mL of the same CETAB solution, first by homogenization in a Polytron for four 15-s periods and then by stirring for 1 h. The suspension was centrifuged at 20000*g* for 20 min. The two supernatants were combined, named "extract", and used for the next step of purification.

CM-Sepharose CL-6B Column Chromatography. The extract (95 mL) was loaded on a CM-Sepharose CL-6B column (2.4 × 8.5 cm) which had been equilibrated with 0.1 M potassium phosphate (pH 7.8) containing 0.02% CETAB. The column was washed with 150 mL of the same buffer and then the enzyme was eluted with 500 mL of a linear gradient of 0.1 M to 0.5 M potassium phosphate (pH 7.8) containing 0.02% CETAB. Fractions of 10 mL were collected. In this way myeloperoxidase activity was separated into three fractions, I, II, and III. Each fraction was brought to 70% saturation of ammonium sulfate, stirred for 1 h, and centrifuged at 27000*g* for 20 min. The pellet was dissolved in 3 mL of 0.2 M potassium phosphate (pH 7.8) containing 0.02% CETAB and dialyzed against 300 mL of the same buffer overnight with one change of the buffer.

Sephacryl S-200 Column Chromatography. The concentrated CM-Sepharose fractions I, II, and III were placed on columns (2.6 × 70 cm) which had been equilibrated with 0.2 M potassium phosphate (pH 7.8) containing 0.02% CETAB, and the enzyme was eluted with the same buffer at a flow rate of 30 mL/h. Fractions of 4.5 mL were collected. In this way myeloperoxidase I was further separated into two enzymes, I_A and I_B. The fractions containing the enzyme activity were brought to 70% saturation of ammonium sulfate, and the precipitates were dissolved in, and dialyzed against, 0.02 M potassium phosphte (pH 7.8).

Assay of Myeloperoxidase. Myeloperoxidase activity was determined by the method of Chance & Maehly (1955). The reaction mixture (3 mL) contained 1 mL of 50 mM sodium phosphate (pH 7.3), 2 mL of 20 mM guaiacol, 20 μL of 40 mM H_2O_2, and the enzyme. The reaction was started by adding H_2O_2, and increase in absorbance at 470 nm was followed in a Hitachi spectrophotometer, Model 200-20. 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 20 °C under these conditions.

Sucrose Density Gradient Centrifugation. The $s_{20,w}$ value of myeloperoxidase was determined by sucrose density gradient centrifugation as described by Martin & Ames (1961). Myeloperoxidases I_A, I_B, II, and III were each placed on 5 mL of a linear gradient of 5% to 20% (w/v) sucrose in 0.2 M potassium phosphate (pH 7.8). Bovine liver catalase (87 μg), yeast alcohol dehydrogenase (200 μg), bovine serum albumin (500 μg), and horse heart cytochrome *c* (300 μg) were included as s value markers in each tube. The samples were centrifuged at 40 000 rpm for 18 h at 3 °C in an SW 50.1 rotor of a Beckman ultracentrifuge. Then fractions of 154 μL were collected from the bottom of the tube.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out by the method of Weber & Osborn (1969). Myeloperoxidase was dissolved in 1% sodium dodecyl sulfate solution containing 2% mercaptoethanol and 0.01 M sodium phosphate (pH 7.2). The samples with 10% glycerol and 0.1% bromphenol blue were placed on 13% polyacrylamide slab gel of 1-mm thickness and subjected to electrophoresis. After electrophoresis, proteins were stained with 0.2% Coomassie brilliant blue in 20% methanol–7% acetic acid for 3 h and destained with the same solution without dye.

Con A–Sepharose Column Chromatography. Myeloperoxidase (26 units) was applied to a Con A–Sepharose column (0.4 × 2.0 cm) which had been equilibrated with 0.2 M potassium phospahte (pH 7.8) containing 0.02% CETAB. The column was washed with 1.5 mL of the same buffer and then material was eluted with 10 mL of a linear gradient of 0–0.1 M methyl α-D-mannoside in the same buffer. Fractions of 0.5 mL were collected at a flow rate of 10 mL/h.

Preparation of Antiserum against Myeloperoxidase III. Myeloperoxidase III (300 μg) was mixed with complete Freund's adjuvant and injected into the foot pads of a rabbit. After 3 weeks, the rabbit was given a booster injection of the same antigen in incomplete Freund's adjuvant, and blood was taken 1 week later. The serum was used without fractionation for experiments.

Ouchterlony Double Immunodiffusion Test. Double immunodiffusion in gel was performed by modification of the method of Ouchterlony (Himmelhoch et al., 1969). Gel contained 1% agarose in 1 M NaCl with 0.01 M sodium phosphate (pH 7.4).

Microcomplement Fixation Test. The microcomplement fixation test was performed essentially as described by Wasserman & Levine (1961). The reaction mixture (0.6 mL) consisted of 0.1 mL of guinea pig complement (1:258 dilution), 0.1 mL of antiserum against myeloperoxidase III (1:30,000 dilution), and various amounts of myeloperoxidase samples in isotonic Veronal buffer (pH 7.5). The mixtures were incubated for 18 h at 4 °C and then incubated further with 0.1 mL of sensitized sheep erythrocytes (5×10^7 cells/mL) for 1 h at 37 °C. After centrifugation at 1000*g* for 7 min, the percentage hemolysis was determined by measuring the absorbance at 414 nm. The isotonic Veronal buffer (pH 7.5) consisted of 5 mM Veronal buffer, 0.145 M NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.1% gelatin, and it was used to dilute all reagents for complement fixation. Sheep erythrocytes (1×10^8 cells/mL) were sensitized by incubation with an equal volume of hemolysin (1:4000 dilution) at 37 °C for 15 min.

Results

Purification of Myeloperoxidase from Promyelocytic Leukemia HL-60 Cells. Human myeloperoxidase was extracted with 1% CETAB from the particulate fraction precipited at 20000*g* from an HL-60 cell homogenate. The extract was loaded on a CM-Sepharose CL-6B column and material was eluted with a gradient of 0.1–0.5 M potassium phosphate (pH 7.8) containing 0.02% CETAB. In this way myeloperoxidase activity was separated into three fractions, I, II, and III, which were recovered in tubes 30–33, 35–38, and 39–44, respectively (Figure 1). Fractions I, II, and III were then each loaded on a Sephacryl S-200 column and eluted with 0.2 M potassium phosphate (pH 7.8) containing 0.02% CETAB. Figure 2 shows the gel filtration profiles of myeloperoxidases I, II, and III on Sephacryl S-200. Myeloperoxidase I was separated into two distinct activities, named I_A and I_B, on the column. Myeloperoxidase I_A was eluted first in a position corresponding to a molecular weight of 150 000, and myeloperoxidase I_B was eluted later, indicating that it had a smaller molecular weight. On gel filtration, all the activity of myeloperoxidases II and III was eluted in exactly the same position as myeloperoxidase I_A, and none in fractions corresponding to myeloperoxidase I_B.

Table I summarizes the purification of human myeloperoxidases from promyelocytic leukemia HL-60 cells. Myeloperoxidases I_A, I_B, II, and III had ratios of absorbance at 430

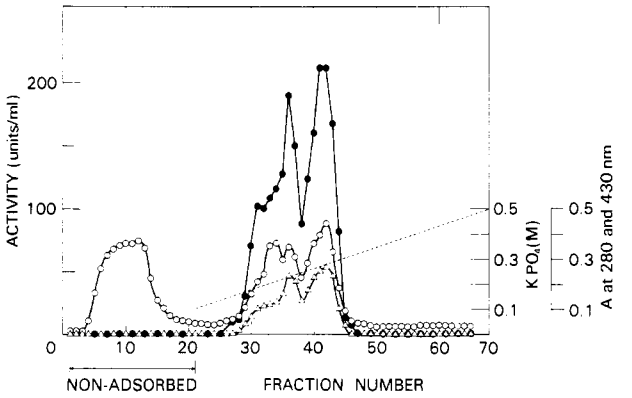

FIGURE 1: CM-Sepharose CL-6B column chromatography of myeloperoxidase. A CETAB extract of the particulate fraction of human promyelocytic leukemia HL-60 cells was loaded on a CM-Sepharose CL-6B column (2.4 × 8.5 cm) equilibrated with 0.1 M potassium phosphate (pH 7.8) containing 0.02% CETAB and washed with the same solvent. Myeloperoxidase was eluted with 500 mL of a linear gradient of 0.1–0.5 M potassium phosphate (pH 7.8) in 0.02% CETAB. Fractions of 10.4 mL were collected. (●) Myeloperoxidase activity; (○) absorbance at 280 nm; (△) absorbance at 430 nm.

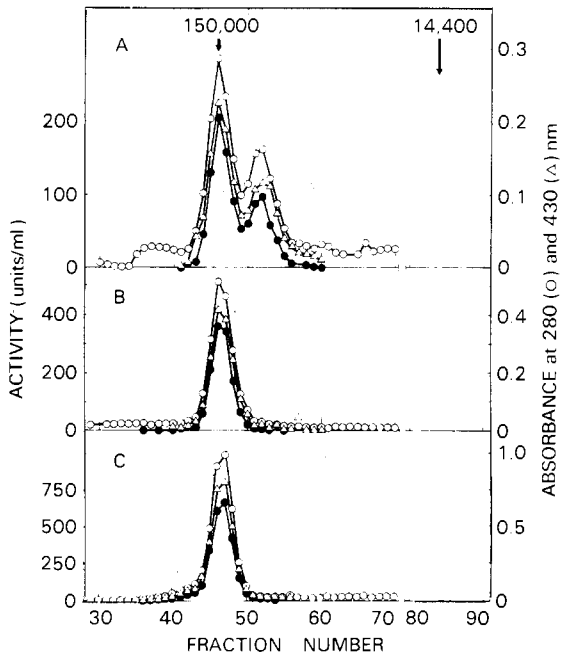

FIGURE 2: Sephacryl S-200 gel filtration of myeloperoxidase. Concentrated solution from CM-Sepharose CL-6B (3 mL) was loaded on a Sephacryl S-200 column (26 × 70 cm) which was equilibrated with 0.2 M potassium phosphate (pH 7.8) containing 0.02% CETAB. Myeloperoxidase was eluted with the same solution and fractions of 4.5 mL were collected. (●) Activity of myeloperoxidase; (○) absorbance at 280 nm; (△) absorbance at 430 nm. (A) CM-Sepharose CL-6B myeloperoxidase fraction I; (B) CM-Sepharose CL-6B myeloperoxidase fraction II; (C) CM-Sepharose CL-6B myeloperoxidase fraction III. 150 000 and 14 400 are the molecular weights of alcohol dehydrogenase and lysozyme, respectively, added as molecular weight markers.

nm to that at 280 nm of 0.81. The specific activity of the small myeloperoxidase I_B was similar to those of the large molecular weight myeloperoxidases, I_A, II, and III. Myeloperoxidase I_B activity amounted to 8% of the total myeloperoxidase activity at the final stage of purification. The yield of total activity of myeloperoxidase was 62% from the extract. The myeloperoxidase activity of the extract was as much as 2.5 times that of the homogenate, possibly due to inaccurate measurement of the granule bound myeloperoxidase activity in the homogenate.

Table I: Purification of Myeloperoxidase from Human Promyelocytic Leukemia HL-60 Cells[a]

fraction	total protein (mg)	total act. (units)	spec act. (units/mg)	yield (%)
homogenate	2538	11655	4.6	
extract	192	29295	152.6	100
CM-Sepharose CL-6B				
I	13.9	3936	283.2	13.4
II	11.4	5359	470.1	18.3
III	19.4	10822	557.8	36.9
Sephacryl S-200				
I_A	3.2	2600	812.5	8.9
I_B	2.1	1425	678.6	4.9
II	7.3	5047	691.4	17.2
III	12.4	9140	737.1	31.2

[a] Solid tumors (18.4 g) of HL-60 cells were used as a source of myeloperoxidase.

Table II: Absorption Spectra of Human Myeloperoxidase[a]

myeloperoxidase	absorption bands (nm)
I_A, native	280, 429, 568
reduced	473, 637
I_B, native	279, 427, 567
reduced	472, 637
II, native	279, 427, 568
reduced	473, 637
III, native	280, 428, 568
reduced	473, 637

[a] Absorption spectra of human myeloperoxidase in 0.1 M potassium phosphate (pH 7.0) were taken in a Shimazu double-beam recording spectrophotometer UV-300. Myeloperoxidase was reduced by addition of sodium dithionite.

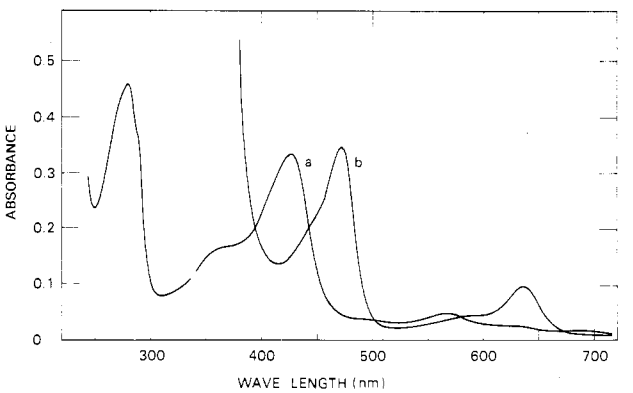

FIGURE 3: Absorption spectra of myeloperoxidase I_B. The absorption spectra were recorded as described in Table II. (a) Native myeloperoxidase; (b) myeloperoxidase reduced with $Na_2S_2O_4$.

Absorption Spectra of Small and Large Molecular Weight Myeloperoxidases. The absorption spectra of native and reduced myeloperoxidase I_B, shown in Figure 3, were nearly identical with those of the large myeloperoxidases I_A, II and III. The absorption bands of native and reduced myeloperoxidases I_A, I_B, II, and III are shown in Table II. Although the small molecular weight myeloperoxidase has not been described before, its absorption spectra indicated that it is a myeloperoxidase, not an eosinophil peroxidase.

Molecular Weights of Myeloperoxidases I_A, I_B, II, and III. Figure 4 shows the result of sucrose density gradient centrifugation of myeloperoxidases I_A, I_B, II, and III. On the basis of the $s_{20,w}$ values of the standards, catalase, alcohol dehydrogenase, bovine serum albumin, and cytochrome *c*, the $s_{20,w}$ values of myeloperoxidases I_A, I_B, II, and III were 8.2, 5.2, 7.9, and 8.1, respectively. The molecular weight of

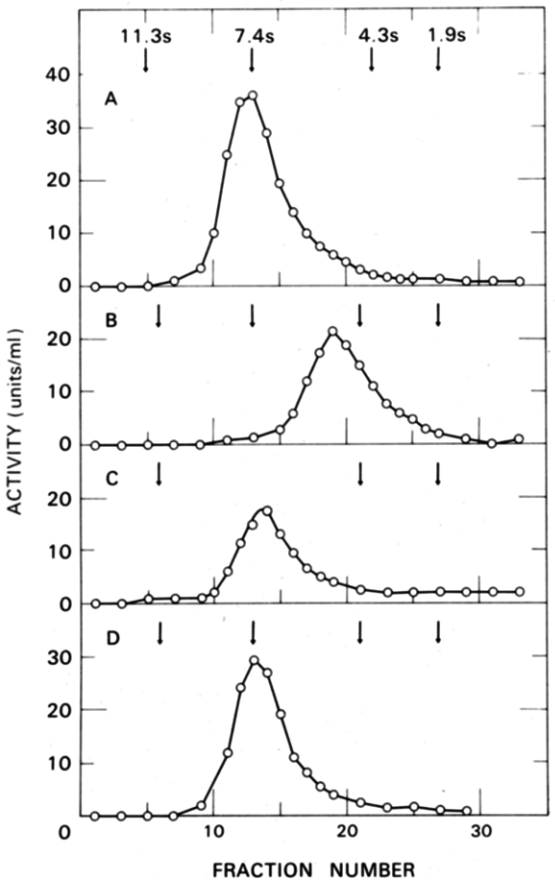

FIGURE 4: Sucrose density gradient centrifugation of myeloperoxidase. Myeloperoxidase, I_A, I_B, II, and III (20 μL each) were loaded on a 5-mL linear gradient of 5–20% sucrose in 0.2 M potassium phosphate (pH 7.5) and centrifuged at 40 000 rpm for 18 h at 3 °C in the SW 50.1 rotor of a Beckman ultracentrifuge. Fractions of 154 μL were collected from the bottom of the tubes. Arrows from left to right indicate the positions of bovine liver catalase (11.3 S), yeast alcohol dehydrogenase (7.4 S), bovine serum albumin (4.3 S), and horse heart cytochrome *c* (1.9 S), which were included in each tube as standards. (A) Myeloperoxidase I_A; (B) myeloperoxidase I_B; (C) myeloperoxidase II; (D) myeloperoxidase III.

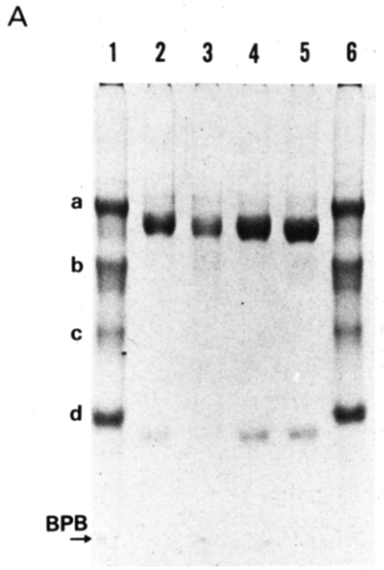

FIGURE 5: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of myeloperoxidase. Myeloperoxidases I_A, I_B, II, and III were loaded on a 13% polyacrylamide gel plate and subjected to electrophoresis for 4.5 h. (A) Slots 1 and 6 were standard molecular weight proteins: (a) bovine serum albumin (78 000); (b) ovalbumin (43 000); (c) chymotrysinogen (23 250); (d) horse heart cytochrome *c* (11 700); BPB, bromophenol blue; slot 2, myeloperoxidase I_A; slot 3, myeloperoxidase I_B; slot 4, myeloperoxidase II; slot 5, myeloperoxidase III.

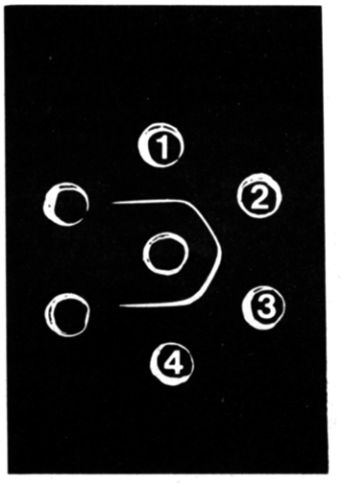

FIGURE 6: Double-diffusion immunoprecipitation of myeloperoxidase. Myeloperoxidase and rabbit antiserum against myeloperoxidase III were incubated in a 1% agarose containing 1 M NaCl and 0.01 M sodium phosphate (pH 7.4) at 17 h at 20 °C. The center well contained the antiserum, and wells 1, 2, 3, and 4 contained myeloperoxidase I_A, myeloperoxidase I_B, myeloperoxidase II, and myeloperoxidase III, respectively.

myeloperoxidase I_B was calculated to be 79 000 from the $s_{20,w}$ value of 5.2 S and the molecular weights of myeloperoxidases I_A, II, and III were calculated to be 153 000 from their average $s_{20,w}$ of 8.07 S. Thus, the molecular weights of the large myeloperoxidases I_A, II, and III are twice that of myeloperoxidase I_B. The large myeloperoxidases I_A, II, and III did not dissociate into the small myeloperoxidase, and the small myeloperoxidase I_B did not aggregate into a large molecule in 0.2 M potassium phosphate (pH 7.8) on sucrose density gradient centrifugation (Figure 4).

Figure 5 shows the results of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of myeloperoxidases I_A, I_B, II, and III. Two bands of proteins at positions corresponding to molecular weights of 59 350 and 10 150 were obtained with all four myeloperoxidases. The sum of the molecular weights of the two bands is 69 500.

Con A–Sepharose Column Chromatography. The large molecular weight myeloperoxidases appeared heterogeneous when charged on CM-Sepharose CL-6B column chromatography, as described above. This heterogeneity of these myeloperoxidases was confirmed by Con A–Sepharose column chromatography as follows. Myeloperoxidases I_A, II, and III were loaded on a Con A–Sepharose column which had been equilibrated with 0.2 M potassium phosphate (pH 7.8) containing 0.02% CETAB, and then the enzyme activity was eluted with a linear gradient of 0–0.1 M methyl α-D-mannoside in the same buffer (data not shown). Myeloperoxidase I_A was eluted at 24 mM methyl α-D-mannoside, and myeloperoxidases II and III were eluted at 47 and 44 mM methyl α-D-mannoside, respectively. The small myeloperoxidase I_B was eluted at 20 mM methyl α-D-mannoside under the same conditions.

Immunological Studies on Small and Large Myeloperoxidases. Figure 6 shows the immunological reactions on agarose gel of myeloperoxidases I_A, I_B, II, and III with rabbit antiserum against myeloperoxidase III. All four myeloperoxidases reacted with antiserum against myeloperoxidase III. All four myeloperoxidases formed single precipitin lines against the antiserum, and these lines fused with each other. Thus, no difference among these four myeloperoxidases could be seen

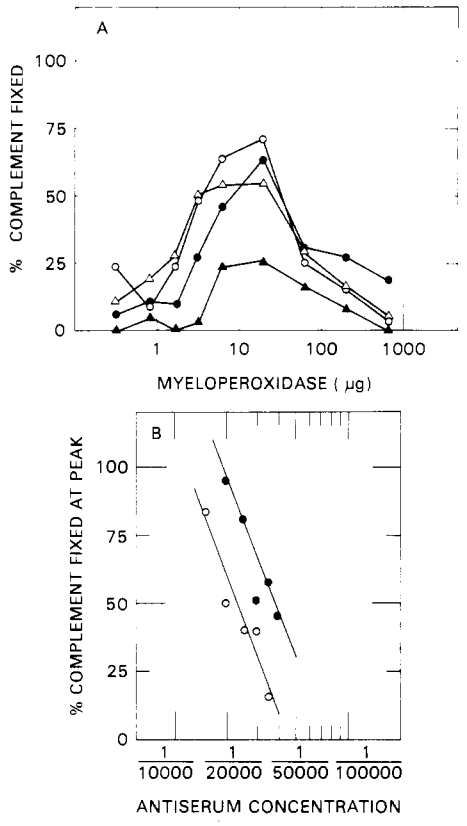

FIGURE 7: Microcomplement fixation by myeloperoxidases with antiserum against myeloperoxidase III. (A) Antiserum against myeloperoxidase III (0.1 mL, 1:30,000 dilution) was allowed to react with various amounts of myeloperoxidase in the presence of guinea pig complement (1:258 dilution) for 18 h at 4 °C: (△) Myeloperoxidase I_A; (▲) myeloperoxidase I_B; (○) myeloperoxidase II; (●) myeloperoxidase III. (B) Myeloperoxidases I_B and III were allowed to react with various amounts of antiserum against myeloperoxidase III in the presence of guinea pig complement for 18 h at 4 °C. Each point represents the peak heights of the complement fixation curve for a particular antiserum concentration. The peak height was determined as described in (A): (●) myeloperoxidase III; (○) myeloperoxidase I_B.

in the Ouchterlony double diffusion test.

Figure 7A shows the amounts of complement fixed by the antigen–antibody formed in the reactions of various amounts of myeloperoxidases I_A, I_B, II, and III with antiserum against myeloperoxidase III. The amounts of complement fixed by the antiserum against myeloperoxidase III with the large myeloperoxidases I_A, II, and III were not significantly different, but the amount fixed by reaction of various amounts of small myeloperoxidase I_B was much less (Figure 7A).

Moreover, the largest amounts of complement fixed by reaction of the antiserum with the small myeloperoxidase I_B and with the large myeloperoxidase III were distinctly different (Figure 7B). These results indicate that myeloperoxidase I_B is different in immunological properties from myeloperoxidases I_A, II, and III.

Discussion

In previous studies human myeloperoxidase has usually been purified from leukocytes of pooled peripheral blood from several donors. Heterogeneity has been observed in the purified myeloperoxidase obtained in this way (Felberg & Schultz, 1972; Bakkenist et al., 1978), possibly due to the heterogeneity of its source. To avoid this problem, in this work we purified myeloperoxidase from human leukemia HL-60 cells because they are homogeneous cells, more than 85% being promyelocytic cells, and can be grown both in culture and in athymic nude mice (Collins et al., 1978). HL-60 cells were found to be a good source of myeloperoxidase.

We found two kinds of myeloperoxidase with molecular weights of 153 000 and 79 000 in HL-60 cells and purified these enzymes to homogeneity from the particulate fraction. The yield of these enzymes was 62% of the total activity of myeloperoxidase in the particulate fraction. The small myeloperoxidase amounted to 8% of the total activity. In the homogenate, 64% of the myeloperoxidase activity was present in the particulate fraction. From these findings, myeloperoxidases were calculated to constitute 2.5% of the total protein in HL-60 cells. All the myeloperoxidases so far purified from various sources including human cells have molecular weights of 130 000 to 160 000 (Ehrenberg & Agner, 1958; Harrison et al., 1977; Odajima & Yamazaki, 1972; Desser et al., 1972; Zglicyzyński et al., 1968; Bakkenist et al., 1978).

The absorption spectra of the small myeloperoxidase in the native and reduced forms were identical with those of a large myeloperoxidase from HL-60 cells and with those reported by others (Agner, 1958; Schultz & Shmukler, 1964; Newton et al., 1965; Odajima, 1980; Bakkenist et al., 1978). Moreover, the absorption spectra of this small myeloperoxidase differed from those of eosinophilic peroxidase (Archer et al., 1965; Desser et al., 1972). These findings indicate that the small peroxidase was actually a myeloperoxidase. The molecular weight of the small myeloperoxidase was calculated to be 79 000 and 70 000, by sucrose density gradient centrifugation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis, respectively. These values are about half those of the large myeloperoxidases. The small myeloperoxidase could not be dissociated from the large myeloperoxidase by exposure to the concentrations of up to 1% Triton X-100 and 1% CETAB in 0.2 M potassium phosphate (pH 7.8) used in the purification procedure. Moreover, the crude extract of HL-60 cells also showed both small and large myeloperoxidase activities on sucrose density gradient centrifugation (data not shown). Therefore, the small myeloperoxidase was not formed during the purification procedure, but was actually present in HL-60 cells.

Large and small myeloperoxidases when treated with 2-mercaptoethanol and sodium dodecyl sulfate gave rise to two protein components of M_r 10 150 and 59 300, respectively. Harrison et al., (1977) reported that the subunit structure of myeloperoxidase consists of two light chains of M_r 10 500 and two heavy chains of M_r 57 500. In addition, Andrews & Krinsky (1979) reported that treatment of myeloperoxidase with dithiothreitol produced active subunits of M_r 70 000. Thus, further studies are needed to determine whether the small myeloperoxidase is related to an active subunit of the large myeloperoxidase.

The microcomplement fixation test has been shown to be sensitive to small differences in the amino acid sequences of proteins, such as closely related primate albumins, bird lysozymes, and bird lactic dehydrogenases, and it can detect changes of even a single amino acid in human hemoglobins (Wilson et al., 1964; Sarich & Wilson, 1966; Arnheim & Wilson, 1967). In this work the small myeloperoxidase could be distinguished from the large myeloperoxidases by the microcomplement fixation test, but not by the Ouchterlony double immunodiffusion test, which is consistent with the possibility that its amino acid sequence differs from that of the large myeloperoxidases. This hypothesis is supported by the finding of a charge heterogeneity of both the light and heavy chains of myeloperoxidase (Harrison et al. 1977) which may also be indicative of differences in amino acid sequences.

Several forms of human myeloperoxidase have been separated by polyacrylamide gel electrophoresis (Felberg & Schultz, 1972; Bakkenist et al., 1978), but it is not known whether this heterogeneity is due to differences in amino acid sequences or to modifications of the enzyme with carbohydrates and others compounds. Three forms of large myeloperoxidase, I_A, II, and III, were isolated from HL-60 cells. These forms could not be distinguished by Ouchterlony double immunodiffusion or the microcomplement fixation test, but they were eluted at different positions from a Con A–Sepharose column with a gradient of methyl α-D-mannoside. This result suggests that their heterogeneity is partly ascribable to differences in their carbohydrate moieties. This possibility is compatible with the fact that human and canine myeloperoxidases contain carbohydrate (Bakkenist et al., 1978; Harrison et al., 1977).

Further studies are needed to compare the enzymatic properties of the small and large myeloperoxidases.

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