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INACTIVATION OF PEROXIDASES OF RAT BONE MARROW BY REPEATED ADMINISTRATION OF PROPYLTHIOURACIL IS ACCOMPANIED BY A CHANGE IN THE HEME STRUCTURE

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Abstract—Myeloperoxidase and eosinophil peroxidase were isolated from the bone marrow cells of rats treated with or without propylthiouracil (PTU) which caused bone marrow depression. PTU treatment decreased the activity of myeloperoxidase but not of eosinophil peroxidase using guaiacol as the electron donor. However, when KI, *N*-*N*′-dimethyl-*p*-phenylenediamine and pyrogallol were used as the electron donor, the activity of only eosinophil peroxidase was inhibited by PTU treatment. EPR spectra indicated that the structure of myeloperoxidase surrounding the heme iron changed from a rhombic form into an axial one by the repeated administration of PTU. Therefore, the inactivation of peroxidases by PTU treatment was accompanied by an alteration of their structures surrounding the heme.

It is well known that peroxidase (EC 1.11.1.7) is a marker enzyme in neutrophilic granulocytes [1] and its activity is variable according to the differentiation stages in HL-60 cell line [2]. Two peroxidases, myeloperoxidase and eosinophil peroxidase, are present in the bone marrow and isolated from guinea pig [3] and rat [4]. In addition, it was found that myeloperoxidase and eosinophil peroxidase in rat bone marrow exhibited different properties in the catalytic activities, the sensitivity to the heme inhibitors and the structures surrounding the heme iron [4]. However, there has been no evidence concerning the variation of the peroxidase activity of the bone marrow in any physiological or pathological conditions. We previously reported that leukopenia in rats induced by repeated administration of PTU‖, which caused bone marrow depression as the adverse reaction besides its antithyroid action, was accompanied by inhibition of peroxidase activity of the bone marrow [5].

In this experiment, the activities of myeloperoxidase and eosinophil peroxidase separated by CM-Sephadex C-50 column were further determined in rats with bone marrow depression induced by the repeated administration of PTU in order to clarify the physiological significance of the enzymes in the tissue. Moreover, the effect of PTU treatment on the structure surrounding the heme iron of myeloperoxidase was also examined using an EPR spectrum.

MATERIALS AND METHODS

PTU and guaiacol were purchased from Sigma Chemical Co. (St. Louis, MO). Pyrogallol and *N*-*N*′-dimethyl-*p*-phenylenediamine were from Wako Pure Chemical Industries (Osaka, Japan) and Kanto Chemicals Co. (Tokyo, Japan), respectively. CM-Sephadex C-50 was obtained from Pharmacia (Uppsala, Sweden). All other chemicals were analytical grade available.

Bone marrow depression was induced by the intraperitoneal treatment of male Sprague-Dawley rats with PTU (1.5 mmol/kg) once a day for 2 weeks. PTU treatment caused a significant decrease in leukocyte but not erythrocyte count [5]. The preparation of hemoglobin-free bone marrow cells were carried out as described in the previous report [5]. Peroxidases in rat bone marrow were solubilized with 0.5% cetyltrimetylammonium bromide (referred to as cetyltrimethylammonium bromide extract) and the enzymes were separated by the use of a CM-Sephadex C-50 column (0.9 × 20 cm) equilibrated with 25 mM acetate buffer (pH 4.7) containing 0.2 M NaCl. Myeloperoxidase and eosinophil peroxidase were eluted with 0.6 and 1.2 M NaCl, respectively [4]. Judging from sodium dodecyl sulfate polyacrylamide gel electrophoresis of myeloperoxidase and eosinophil peroxidase obtained from CM-Sephadex C-50, both peroxidases mainly consisted of the large and small subunits [4] with only a few faint bands. The intensity of protein bands of each peroxidase from control rats were similar to those from rats treated with PTU. These two partially purified enzyme fractions were used throughout this experiment unless otherwise indicated.

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‖ Abbreviations used: PTU, propylthiouracil.

Peroxidase activity was determined as described previously [4]. Protein was assayed by the method of Lowry *et al.* [6]. The EPR spectrum was measured at 4.3 K with an x-band spectrometer (Type: JES-FE-3X) as described elsewhere [7].

RESULTS AND DISCUSSION

The activity of peroxidase in rat bone marrow was found to be in two forms on the CM-Sephadex C-50 column chromatogram (Fig. 1). From the evidence with respect to the visual spectra and subunit composition [4], the activities eluted with 0.6 and 1.2 M NaCl were identified as myeloperoxidase and eosinophil peroxidase, respectively. As reported previously [4], myeloperoxidase shows the high activity toward guaiacol rather than KI, whereas KI is preferentially oxidized by eosinophil peroxidase. Peroxidases in the bone marrow from rats with leukopenia induced by PTU were also divided into two forms. When guaiacol was used as the electron donor (Fig. 1A), PTU treatment resulted in a marked inhibition of the activity of myeloperoxidase but not of eosinophil peroxidase. In the case of KI used as an electron donor (Fig. 1B), the activity of eosinophil peroxidase was decreased by repeated administration of PTU, whereas the activity of myeloperoxidase was not changed. The elution profiles of protein, determined by absorbance at 280 nm, were affected little by PTU treatment (data not shown).

Figure 2 illustrates the effect of PTU treatment on peroxidase activity assayed with different electron donors and enzyme preparations. As shown in Fig. 2B, when guaiacol was used as the electron donor, the specific activity of myeloperoxidase separated by CM-Sephadex C-50 column chromatography was the highest among the other substrates such as KI, *N,N'*-dimethyl-*p*-phenylenediamine and pyragallol. On the other hand, Fig. 2C shows that the specific activity of eosinophil peroxidase toward guaiacol was lower than the other compounds. Thus, both peroxidases exhibited the characteristic substrate specificity; myeloperoxidase predominantly oxidized guaiacol, whereas eosinophil peroxidase preferentially oxidized KI, *N,N'-dimethyl-p*-phenylenediamine and pyrogallol. This finding was also observed in highly purified enzymes from rat bone marrow [4]. We proposed in a recent report that the differences of the enzymatic properties between these peroxidases may result from their characteristic structures surrounding the heme [4].

Bone marrow depression induced by the repeated administration of PTU resulted in a decrease in the activity of myeloperoxidase toward guaiacol but not the poor substrates such as KI, *N,N'*-dimethyl-*p*-phenylendiamine and pyrogallol (Fig. 2B). PTU treatment caused inhibition of the activity of eosinophil peroxidase toward KI, *N,N'*-dimethyl-*p*-phenylenediamine and pyrogallol but little affected the oxidation of guaiacol, a poor substrate for this enzyme (Fig. 2C). Thus, leukopenia induced by PTU was accompanied by inhibition of myeloperoxidase and eosinophil peroxidase activity toward the appropriate substrates.

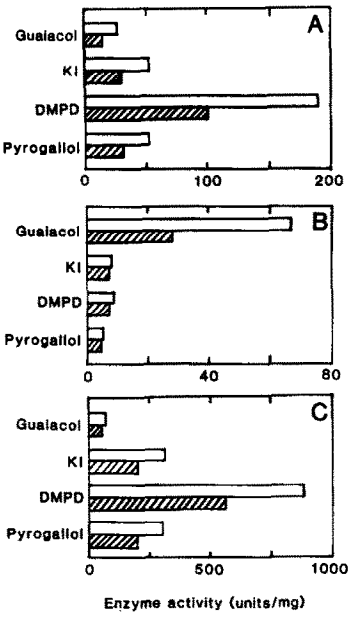

Fig. 2. Effect of the treatment of rats with propylthiouracil on the activity of peroxidase in various enzyme sources. The enzymes from rats treated without (□) or with (▨) propylthiouracil were prepared as described in Materials and Methods: DMPD, *N,N'*-dimethyl-*p*-phenylenediamine; (A) cetyltrimethylammonium bromide extract; (B) myeloperoxidase; (C) eosinophil peroxidase.

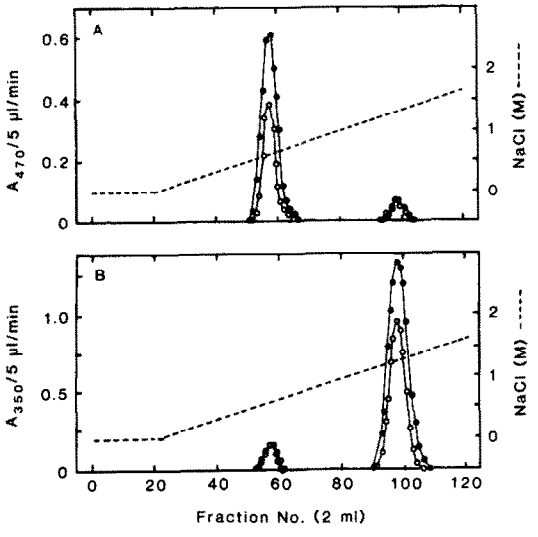

Fig. 1. CM-Sephadex C-50 column chromatograms of the peroxidase activity from the rats treated with or without propylthiouracil. The peroxidases (12 mg protein) solubilized with 0.5% cetyltrimethylammonium bromide were applied to a CM-Sephadex C-50 column (0.9 × 20 cm) equilibrated with 25 mM acetate buffer (pH 4.7), and then the enzymes were eluted with 0.2–2.0 M linear gradient of NaCl in the buffer. Guaiacol and KI were used as an electron donor in A and B, respectively. The values obtained from PTU-treated rats in the small peaks were very similar to the corresponding control values: ●, control; ○, propylthiouracil-treated.

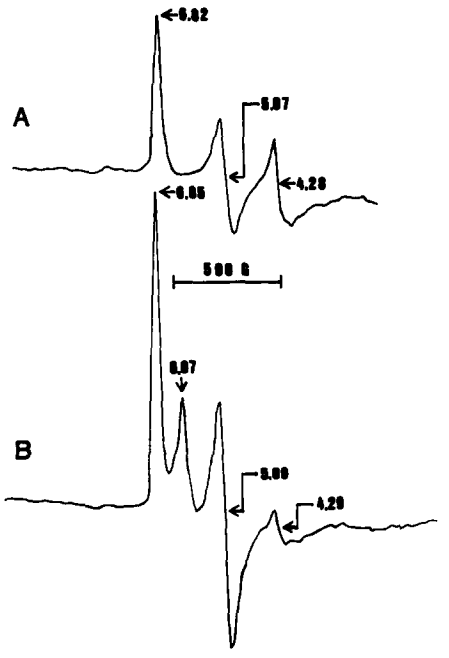

Fig. 3. EPR spectra of myeloperoxidase from rat treated with or without propylthiouracil. EPR spectra of myeloperoxidase (6 mg protein/ml) were recorded in 100 mM potassium phosphate buffer (pH 7.0) containing 0.02% cetyltrimethylammonium bromide and 10% glycerol: (A) control; (B) propylthiouracil-treated.

In the cetyltrimethylammonium bromide extract (Fig. 2A), PTU treatment caused an inhibition of the enzyme activity towards all substrates in similar degrees. The lack of selective inhibition in the cetyltrimethylammonium bromide extract is thought to be due to the mixture of both peroxidases in this preparation.

To obtain the information on the heme structure of myeloperoxidase, EPR spectra of the enzymes from rats treated with or without PTU were measured. As shown in Fig. 3A, the g values of 6.82 and 5.07 were observed in the control rats, indicating that myeloperoxidase has a high spin ferric heme iron with a rhombic symmetry. Because these values were compatible to the data obtained from the highly purified enzyme [4], it is likely that myeloperoxidase used in this experiment is not contaminated with other hemoproteins. Figure 3B shows that an additional signal of $g = 6.07$ was observed on the enzyme from rats treated with PTU. This is the first demonstration that the structure surrounding the heme of myeloperoxidase has been partially changed from a rhombic form to an axial one by the repeated administration of PTU. Thus, leukopenia induced by PTU resulted in the change of the heme structure of bone marrow peroxidase with a concomitant decrease in the enzyme activity. Since the eosinophil peroxidase obtained from the CM-Sephadex C-50 was too little in quantity to analyze by EPR, the effect of PTU on the structure of the peroxidase could not be examined in this experiment.

It is recognized that myeloperoxidase in neutrophil plays a role in antimicrobial function [8]. It is uncertain that the peroxidases in the bone marrow have the physiological roles or not. The present results demonstrated that PTU treatment, which causes bone marrow depression, reduced the electron donor specificity of both peroxidases. Therefore, myeloperoxidase and eosinophil peroxidase may play a significant role in the bone marrow. Although PTU leads to a change in the structure surrounding the heme iron with a concomitant decrease in the activity of the peroxidase, the mechanism of the changes in the structure by the treatment still remains to be elucidated.

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