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MECHANISM OF INACTIVATION OF MYELOPEROXIDASE BY PROPYLTHIOURACIL

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Abstract—The mechanism of inactivation of myeloperoxidase purified from rat bone marrow by propylthiouracil (PTU) was studied. PTU inhibited not only the peroxidase activity but also the chlorinating activity of myeloperoxidase in a concentration dependent manner. When myeloperoxidase was treated with PTU and hydrogen peroxide (5 μM), inactivation of the enzyme was still observed after the excess reagents were removed by a column of Sephadex G-25. The treatment of the enzyme with PTU in the absence of hydrogen peroxide caused a slight inhibition of the enzyme activity. In addition, [^{14}C]PTU became bound to myeloperoxidase in the presence of hydrogen peroxide. Difference spectrum of myeloperoxidase incubated with the small (0.1 mM) and large (2 mM) amounts of hydrogen peroxide revealed the formation of compounds II and III, respectively. Difference spectrum of myeloperoxidase treated with PTU in the presence of a low concentration of hydrogen peroxide (5 μM) was similar to that of compound II. Therefore, these results indicate that PTU inactivates myeloperoxidase through binding to the enzyme and the conversion to a compound II-like form in the presence of hydrogen peroxide.

Myeloperoxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) is present in mammalian neutrophil [1–3]. This enzyme is also observed in the bone marrow and is a marker in the neutrophilic granulocytes [4]. There are numerous reports concerning myeloperoxidase in the mature neutrophils. This peroxidase plays a role in the bacteriocidal function through the formation of hypochlorous acid which is catalysed by chlorinating activity of myeloperoxidase [5–7].

Recently, we have found that the treatment of rats with PTU, an antithyroid drug, inhibited the peroxidase activity of bone marrow with a concomitant loss of leukocyte number [8]. The inactivation of myeloperoxidase was accompanied by the change in the heme structure analysed by EPR spectra [9]. Thus, PTU may be useful for the study of the reaction mechanism of myeloperoxidase. With this in mind we have investigated the inactivating mechanism of myeloperoxidase by PTU. This paper describes that PTU inhibited not only the peroxidase but also chlorinating activities of myeloperoxidase in the presence of hydrogen peroxide through the binding to the peroxidase and the conversion of the native enzyme to a compound II-like form.

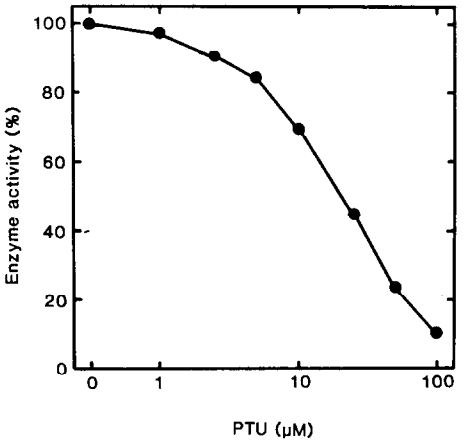

Fig. 1. Effect of various concentrations of PTU on myeloperoxidase purified from rat bone marrow. The enzyme activity was determined as the oxidation of guaiacol. The specific activity of myeloperoxidase was 200 units/mg protein.

MATERIALS AND METHODS

Materials. PTU was purchased from the Sigma Chemical Co. (St Louis, MO). Hydrogen peroxide, guaiacol and KI were from Nakarai Tesque, Inc. (Kyoto, Japan). 6-Propyl-2-[2-^{14}C]thiouracil (53 mCi/mmol) was from Amersham International (Tokyo, Japan). NAP-10 (Sephadex G-25 DNA grade) was obtained from Pharmacia (Uppsala, Sweden). Water was purified by the use of a NANOpure II-4P organic free system (Barnstead, Boston, MA). All other reagents were analytical grade available. The concentration of hydrogen peroxide was determined by iodometric titration.

Preparation of purified myeloperoxidase. Myeloperoxidase was purified from rat bone marrow as described previously [10]. The ratio between the absorption at the Soret peak (430 nm) and that at 280 nm (the *RZ* value) of the purified enzyme was more than 0.8.

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† Abbreviation: PTU, propylthiouracil.

Assay of the peroxidase and chlorinating activities. The peroxidase activity was determined using guaiacol or KI as the electron donor [10] and the chlorinating activity was assayed using monochlorodimedone as the substrate [11].

Treatment of the peroxidase with PTU. The standard incubation mixture for the treatment of myeloperoxidase with PTU consisted of 100 mM potassium phosphate buffer (pH 7.0), 5 μM hydrogen peroxide, 40 nM myeloperoxidase and various concentrations of PTU in a total volume of 0.5 mL. Unless otherwise indicated, the reaction was started by adding hydrogen peroxide and carried out at 25° for 10 min. After the incubation, the mixture was immediately applied to a column of Sephadex G-25 (NAP-10) equilibrated with 25 mM potassium phosphate buffer (pH 7.0) to remove the excess reagents. The column was washed with 0.5 mL of the same buffer and then myeloperoxidase was eluted with 1.5 mL of the buffer. The recovery of myeloperoxidase was more than 85%.

Spectral analysis. Difference spectra were recorded on a Shimadzu UV-2100 recording spectrophotometer, in the range between 340 and 700 nm as described by Hoogland *et al.* [12].

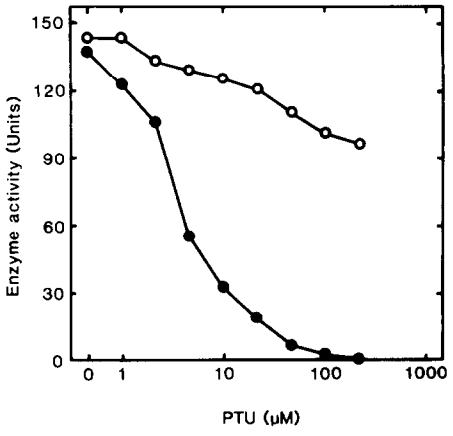

Fig. 2. Effect of the treatment of PTU on the myeloperoxidase purified from rat bone marrow. The treatment of myeloperoxidase with PTU was carried out in the presence (●) and absence (○) of 5 μM hydrogen peroxide. The excess reagents were removed as described in Materials and Methods. The enzyme activity was determined as the oxidation of guaiacol.

RESULTS

When PTU was added to the reaction mixture for the assay of the peroxidase activity, this compound inhibited the activity of myeloperoxidase purified from rat bone marrow in a concentration dependent manner (Fig. 1). The concentration of PTU required to produce 50% inhibition of the peroxidase activity was calculated as about 20 μM. At the concentration of 100 μM, the enzyme activity was almost completely inactivated by PTU. The inhibition of the enzyme activity using KI as the electron donor instead of guaiacol by PTU was also observed (data not shown).

To understand whether PTU inactivated the peroxidase irreversibly or not, effect of the treatment of myeloperoxidase with PTU on the peroxidase activity was examined. Figure 2 shows that PTU inhibited the enzyme activity a little when the compound was removed after the incubation with myeloperoxidase in the absence of hydrogen peroxide. However, in the presence of 5 μM hydrogen peroxide, which had no effect on the enzyme activity, PTU treatment dramatically inactivated the enzyme in a concentration dependent manner (Fig. 2). A maximum inhibition of the enzyme activity was observed by the treatment with 100 μM PTU. Figure 3 shows the inhibition of the peroxidase activity by PTU treatment was dependent on the incubation time. More than 50% inhibition by PTU treatment was observed at 1 min after the incubation. The treatment of myeloperoxidase with 100 μM PTU for more than 10 min caused almost complete inactivation of the enzyme. The treatment of myeloperoxidase with PTU also inhibited the peroxidase activity toward KI (data not shown). The chlorinating activity was also decreased by PTU treatment in an incubation time-dependent manner (Fig. 3).

The binding of [^{14}C]PTU to myeloperoxidase in the presence or absence of hydrogen peroxide was

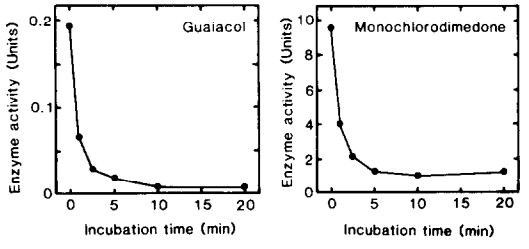

Fig. 3. Time course of inactivation of myeloperoxidase by the treatment of PTU. The enzyme activities were determined as the oxidation of guaiacol and the chlorination of monochlorodimedone. The treatment of myeloperoxidase with PTU (20 μM) was carried out in the presence of 5 μM hydrogen peroxide. At the time indicated, the mixture was immediately loaded onto a NAP-10 column to remove excess reagents.

studied to obtain the further information about inactivation of the peroxidase by the compound. Figure 4 shows the chromatogram of myeloperoxidase treated with [^{14}C]PTU on Sephadex G-25. In the presence of hydrogen peroxide, the radioactivity of [^{14}C]PTU was detected in the fraction eluted with myeloperoxidase. On the other hand, no binding of radioactivity to the peroxidase was observed in the absence of hydrogen peroxide. Thus, PTU binds myeloperoxidase in the presence of hydrogen peroxide.

Finally, the effect of PTU on the status of heme was examined. Figure 5 shows that compound II was formed by a small amount of hydrogen peroxide (0.1 mM). On the other hand, a higher concentration of hydrogen peroxide (2 mM) caused the formation of compound III. These difference spectra agreed with the previous report [12]. Figure 5 also shows that difference spectrum of myeloperoxidase in the presence of PTU (100 μM) plus a low concentration

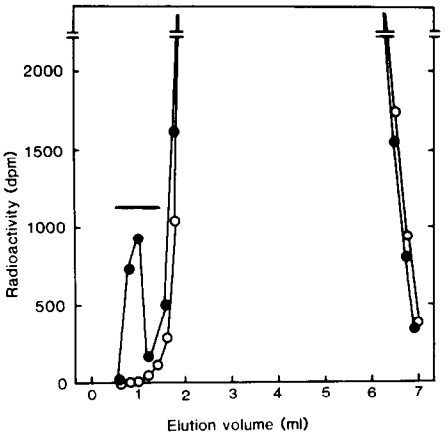

Fig. 4. Chromatogram of Sephadex G-25 of myeloperoxidase treated with [^{14}C]PTU. The treatment of myeloperoxidase with [^{14}C]PTU (10 μM) in the presence (●) and absence (○) of 5 μM hydrogen peroxide was carried out for 10 min, following application onto a NAP-25 column. Marker bar indicates the fraction eluted with myeloperoxidase.

of hydrogen peroxide (5 μM), which had no effect on the spectrum of the peroxidase, were similar to those of compound II, the inactive form of myeloperoxidase.

DISCUSSION

The present results indicate that PTU inhibited the peroxidase and chlorinating activities of myeloperoxidase purified from rat bone marrow. It is important that a slight amount of hydrogen peroxide was essential for the inactivation of myeloperoxidase by PTU. In addition, binding of [^{14}C]PTU to myeloperoxidase was also observed in the presence of hydrogen peroxide. This low concentration (5 μM) of hydrogen peroxide had no effect on both the peroxidase activity and difference spectrum of myeloperoxidase. The higher concentrations of hydrogen peroxide more than 10 μM caused inactivation of myeloperoxidase owing to the conversion of the native to compounds II or III. Indeed compounds II and III were formed by the incubation of myeloperoxidase with 0.1 and 2 mM hydrogen peroxide, respectively. Moles of [^{14}C]PTU bound per mole of myeloperoxidase was calculated as approximately 1.0 from the experiment of [^{14}C]PTU binding, suggesting that 1 mole of PTU binds 1 mole of myeloperoxidase. Thus, inactivation of myeloperoxidase by PTU was closely related to the binding of [^{14}C]PTU to the peroxidase in the presence of hydrogen peroxide.

In the absence of hydrogen peroxide, the treatment of myeloperoxidase with the higher concentrations of PTU resulted in the slight but significant inhibition of the enzyme activity. In addition, a significant binding of [^{14}C]PTU to myeloperoxidase was observed using a higher concentration of [^{14}C]PTU (data not shown). It is possible that PTU itself has a relative low binding affinity to myeloperoxidase. Hydrogen peroxide is likely to potentiate the inactivation of the peroxidase by PTU. Therefore, the higher concentrations of PTU in the absence of hydrogen peroxide may be required for the inactivation of the enzyme.

Doerge [13, 14] reported that thioamide compounds inhibited lactoperoxidase through forming their S-oxygenated intermediates that covalently bind to the enzyme. Therefore, myeloperoxidase might be inactivated by PTU metabolite(s), produced by the reaction of the compound with the peroxidase and hydrogen peroxide, since peroxidase catalyses oxidation of some organic compounds in the presence of hydrogen peroxide. However, this possibility can be excluded by the following: (i) PTU is not able to be metabolized in this experimental condition which the treatment of myeloperoxidase with PTU was carried out at pH 7.0 and in the absence of chloride, since this compound can be metabolized by myeloperoxidase–hydrogen peroxide–chloride system at acidic pH [11]; (ii) methionine, an inhibitor of metabolism of PTU catalysed by myeloperoxidase system [11], had no effect on the inactivation of myeloperoxidase by PTU (data not shown); and (iii) the inhibitory effects of the S-oxygenated metabolites of PTU such as PTU-SO_2 and PTU-SO_3 [11] were lower than that of PTU itself (data not shown).

The binding of PTU to myeloperoxidase in the presence of hydrogen peroxide made us examine the heme status of the peroxidase. There are several forms of heme status of myeloperoxidase: native enzyme, compounds I, II and III. Compound I, produced by the reaction of native myeloperoxidase with hydrogen peroxide, is highly unstable and catalytically active complex [15, 16]. Although the detection of compound I has not succeeded yet, compound I is converted to compound II, an inactive form, by an excess hydrogen peroxide [15]. Compound III, an inactive form, was formed from compound II by the higher level of hydrogen peroxide [17, 18] or from the reaction of native enzyme with superoxide anion [18] or of reduced enzyme with molecular oxygen [19]. Recently it has been reported that compounds II and III were distinguished by difference spectra [12]. The analysis of the difference spectra can reveal the inactive form of myeloperoxidase treated with PTU in the presence of hydrogen peroxide. The difference spectrum of myeloperoxidase treated with PTU and a low concentration of hydrogen peroxide was similar to those of compound II. In addition, compound III is active for chlorination of monochlorodimedone [20]. Therefore, the inactivation of PTU is likely to be due to the irreversible binding of PTU to myeloperoxidase in the presence of hydrogen peroxide and the conversion to the compound II-like form of the enzyme.

Inactivation by thioureylene antithyroid drugs including PTU has been well documented in thyroid peroxidase [21–25]. The requirement of hydrogen peroxide for the inhibition of iodination catalysed by thyroid peroxidase by thioureylene antithyroid compounds was also mentioned. Inactivation of thyroid peroxidase by these compounds involves a reaction between the compound and the oxidized heme group produced by interaction between thyroid per-

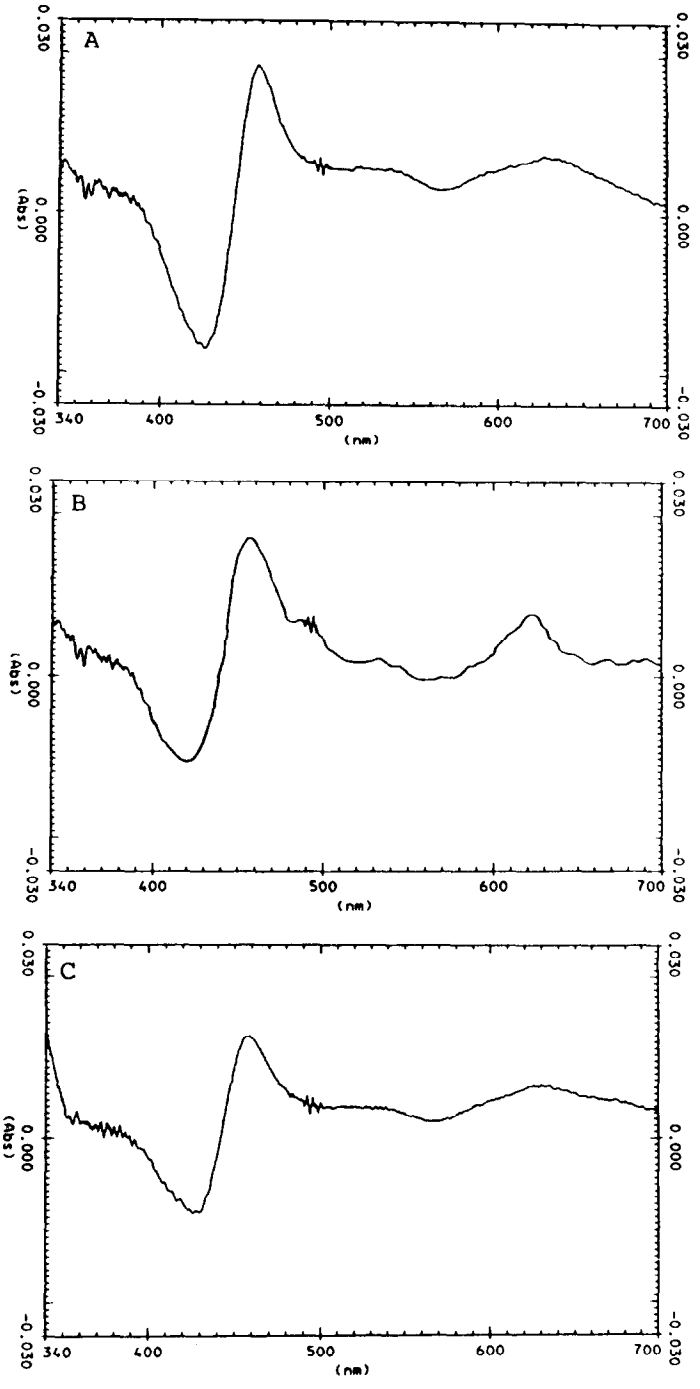

Fig. 5. Difference spectra of compound II, compound III and myeloperoxidase treated with PTU and hydrogen peroxide. Compounds II and III were formed by the incubation of myeloperoxidase with 0.1 and 2 mM hydrogen peroxide, respectively. Myeloperoxidase was also treated with 100 μM PTU in the presence of 5 μM hydrogen peroxide (A) Compound II minus native enzyme; (B) compound III minus native enzyme; (C) myeloperoxidase incubated with PTU and hydrogen peroxide minus native enzyme.

oxidase and hydrogen peroxide. However, little is known about the inactive form produced by oxidized thyroid peroxidase and PTU. In view of this point, in this study it has been demonstrated that inactive form is identified a compound II-like enzyme. Therefore, we propose that the mechanism of inactivation of myeloperoxidase by PTU is the binding of the compound to the oxidized peroxidase, which is produced by the reaction of native enzyme with a slight amount of hydrogen peroxide, following the irreversible conversion to compound II-like enzyme, an inactive form of myeloperoxidase. However, it is

uncertain about the oxidized peroxidase produced by a low concentration of hydrogen peroxide. Although Ohtaki *et al.* [24] reported that this oxidized form was compound II, difference spectrum of compound II was not observed after the incubation of myeloperoxidase and 5 μM hydrogen peroxide in this experiment. PTU is likely to bind an unknown oxidized myeloperoxidase but not compound II. Further studies are needed on this oxidized form of myeloperoxidase. In conclusion, PTU is a potent inhibitor of myeloperoxidase and this compound will be useful for the study of the reaction mechanism of the enzyme and of the role of the peroxidase in the bone marrow.

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