Propylthiouracil, a selective inhibitor of NADH-cytochrome b_5 reductase

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Propylthiouracil inhibited the activity of NADH-cytochrome b_5 reductase of rat liver microsomes using potassium ferricyanide as electron acceptor. On the other hand, NADPH-cytochrome P-450 reductase activity was not affected by the compound. NADH-supported reduction of cytochrome b_5 was also inhibited by propylthiouracil in the reconstituted system consisting of cytochrome b_5 and partially purified NADH-cytochrome b_5 reductase.

Propylthiouracil *Enzyme inhibition* *NADH-cytochrome* b_5 *reductase* *Cytochrome* b_5 *Microsome*

1. INTRODUCTION

NADH-cytochrome b_5 reductase (fp_1, EC 1.6.2.2), a flavoprotein, transfers the electron from NADH to cytochrome b_5 [1] which concerns fatty acid desaturation [2] and cytochrome P-450-mediated mixed-function oxidations [3]. This enzyme was purified from liver microsomes [4–6] and its enzymatic properties were also well characterized. However, no findings relating to a selective inhibitor of the enzyme have been reported.

In [7], we found that repeated administration of propylthiouracil (PTU) at a dose of 5 mmol/kg induced cytochrome b_5 and fp_1 but not cytochrome P-450 and NADPH-cytochrome P-450 reductase (fp_2) in rat liver microsomes. Alternatively, PTU treatment may facilitate electron flow from NADH to cytochrome b_5. These findings prompted us to study the effect of PTU on the activity of fp_1 in vitro. This paper describes that PTU is a selective inhibitor of fp_1 of rat liver microsomes.

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2. EXPERIMENTAL

Liver microsomes of Sprague-Dawley rats were prepared as described [7]; cytochrome b_5 was purified according to Kamataki et al. [8]. The specific content of purified cytochrome b_5 was 39 nmol/mg protein. The fp_1 was partially purified from rat liver microsomes according to Mihara and Sato [5]. The second DEAE-Sephadex fraction (spec. act. 43 μmol/mg protein per min) was used in figs 2 and 3. Judging from SDS-polyacrylamide gel electrophoresis [9], the partially purified fp_1 was free from cytochrome b_5 and fp_2. The activity of fp_1 was determined according to Takesue and Omura [10]. The reaction medium was preincubated for 10 min at 25°C, since it was found in a preliminary experiment that PTU inhibition required preincubation. Unless otherwise indicated, the reaction was started by adding NADH, the reduction of ferricyanide being monitored at 420 nm using a Hitachi model 100-21 spectrophotometer. The blank which contained neither enzyme nor NADH was also measured. In the reconstituted system, 5 μM cytochrome b_5 and partially purified fp_1 were used instead of ferricyanide

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and microsomes, respectively. The reduction of cytochrome b_5 was measured as described by Omura and Sato [11]. The activity of fp_2 was determined using cytochrome c as electron acceptor [12].

Protein was assayed by the method of Lowry et al. [13].

3. RESULTS AND DISCUSSION

Fig.1 shows that PTU inhibited the activity of fp_1 of rat liver microsomes in a concentration-dependent manner. A significant decrease in enzyme activity was seen at 0.25 mM PTU. At 5 mM, PTU caused 70% inhibition of enzyme activity. Inhibition of the enzyme activity by PTU was also observed when dichlorophenolindophenol was used as electron acceptor instead of ferricyanide (not shown). On the other hand, the activity of fp_2 was not affected by PTU at up to 5 mM (fig.1). Although atebrin is well known as an inhibitor of flavoprotein as flavin analogue [14], this compound inhibits not only fp_1 but also fp_2, which was also observed in our preliminary experiment. In addition, sulfhydryl blockers caused a decrease in fp_1 activity [15]. These compounds also inactivate fp_2 [16]. Thus, PTU is the first selective inhibitor of fp_1 but not of fp_2 in rat liver microsomes.

As the endogenous electron acceptor of fp_1 is cytochrome b_5, the effect of PTU on the reduction of cytochrome b_5 induced by partially purified fp_1 and NADH was examined. Fig.2 shows that PTU inhibited fp_1 activity using cytochrome b_5 as electron acceptor (50% at 5 mM). Thus, PTU is considered to lower fp_1 activity under physiological conditions. Since cytochrome b_5 is also reduced non-enzymatically by dithionite, the effect of PTU on the chemical reduction of cytochrome b_5 by this compound was studied. Even at 5 mM, PTU had no effect on the reduction of cytochrome b_5 induced by dithionite (not shown). This indicates that PTU does not modify cytochrome b_5 itself.

As shown in fig.3, PTU decreased the enzyme activity after preincubation with ferricyanide. On the other hand, preincubation in the presence of NADH abolished inhibition of the enzyme by PTU. Thus, the inhibition of fp_1 by PTU was antagonized by NADH. Therefore, PTU seems to interact with the NADH-binding site of the enzyme and/or may act on the oxidized form of FAD but not the reduced one.

On the basis of the present results, it has been demonstrated for the first time that PTU is a selec-

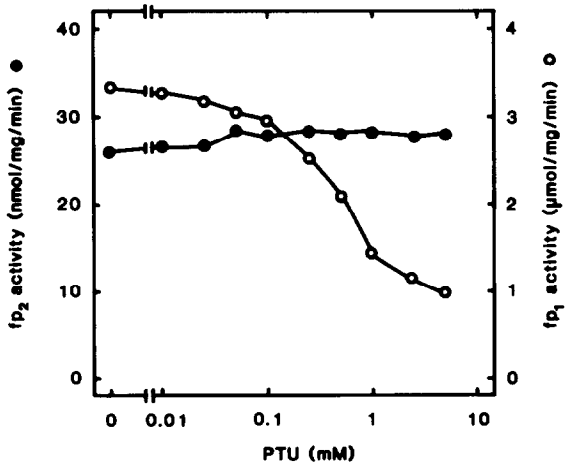

Fig.1. Effect of PTU on the activities of fp_1 (○) and fp_2 (●) of rat liver microsomes. After preincubation with PTU for 10 min, the reaction was started by the addition of NADH. The values are expressed as an average of four experiments.

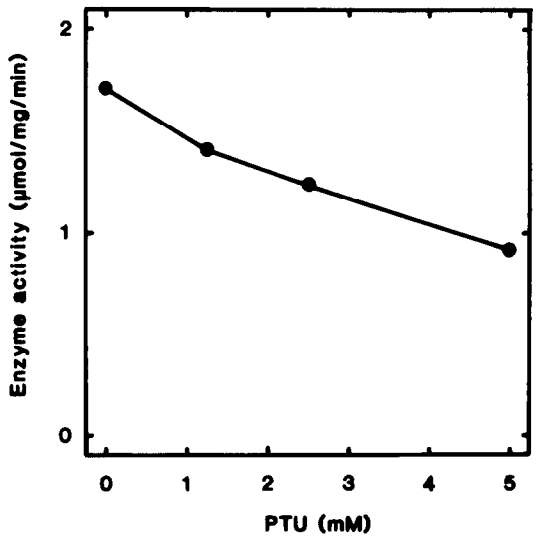

Fig.2. Effect of PTU on the reduction of cytochrome b_5 induced by NADH and partially purified fp_1. The enzyme was preincubated with PTU for 10 min as detailed in fig.1.

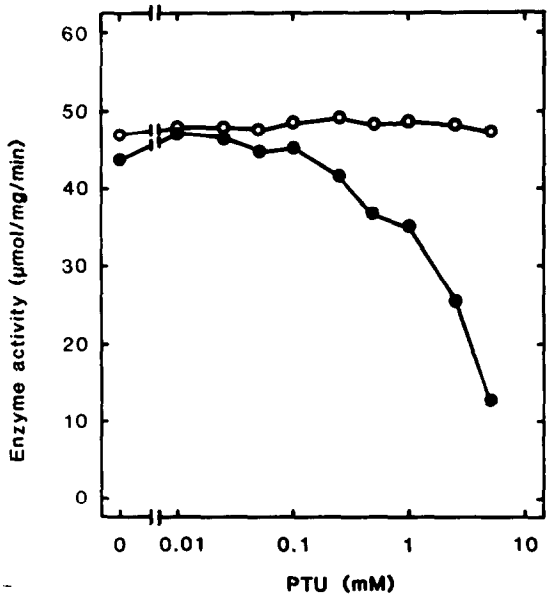

Fig.3. Effect of preincubation with NADH or ferricyanide on inactivation of partially purified fp_1 induced by PTU. The reaction of fp_1 was started by the addition of NADH or ferricyanide after preincubation with ferricyanide (●) or NADH (○), respectively.

tive inhibitor of fp_1 but not of fp_2. Therefore, addition of PTU to the microsomal electron transfer system causes the selective obstruction of the electron flow from NADH to cytochrome b_5. As the cytochrome b_5 pathway is involved in cytochrome P-450-mediated monooxygenations [3], PTU will be a useful tool for studying the electron transfer system in microsomes. In addition, it should be noted that the inhibitory effect of PTU was antagonized by NADH. Experiments are in progress to clarify the mechanism of inhibition of the enzyme by PTU and the antagonistic effect of NADH.

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