Catalytic activity of the membrane-bound methylcholanthrene-inducible cytochrome P-450

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The benzopyrene hydroxylase activity of the methylcholanthrene-inducible form of cytochrome P-450 (P-448) has been studied in native and reconstituted liver microsomal membranes. The data obtained show that the molecular catalytic activity of membrane-bound cytochrome P-448 depends on the molar ratio of the cytochrome to NADPH-cytochrome P-450 reductase and that the optimal ratio for maximal activity of cytochrome P-448 in the microsomal membrane essentially differs from the equimolar one.

Cytochrome P-450 *3-Methylcholanthrene* *3,4-Benzopyrene* *NADPH-cytochrome P-450 reductase* *Cytochrome P-448 antibody*

1. INTRODUCTION

It is generally accepted that the oxidative activity of the cytochrome P-450-dependent microsomal monooxygenase system which catalyzes the oxidation of a wide range of endogenous substrates and xenobiotics of different chemical nature results from the interaction of multiple forms of cytochrome P-450 and NADPH-cytochrome P-450 reductase [1,2].

It is known that liver microsomes contain cytochrome P-450 and NADPH-cytochrome P-450 reductase at a molar ratio of 20:1, which may increase to 30:1 under the inducing influence of xenobiotics [3]. In contrast, kinetic studies of monooxygenase reactions during metabolism of xenobiotics using the reconstituted soluble (non-membranous) system have shown that the maximum oxidative activity of the system is observed at an equimolar ratio of cytochrome P-450 and the reductase [4,5].

Abbreviation: MC, methylcholanthrene; BP, benzopyrene; PB, phenobarbital; 3-OH BP, 3-hydroxybenzopyrene

Here, the molecular BP hydroxylase activity of the MC-inducible form of cytochrome P-450 (P-448) in microsomal membrane has been determined with the use of immunochemical techniques for quantitation of cytochrome P-448 and estimation of cytochrome P-448-dependent BP hydroxylase activity. The molar ratios of cytochrome P-448 and NADPH-cytochrome P-450 reductase giving maximal rates of cytochrome P-448-dependent BP hydroxylation in native and reconstituted microsomal membranes have also been estimated.

2. MATERIALS AND METHODS

Microsomes were prepared from the livers of male Wistar rats by conventional differential centrifugation. The induction of microsomal enzymes was performed by intraperitoneal injections of 3-MC (25 mg/kg body wt) for 3 days and PB (80 mg/kg body wt) for 4 days.

Cytochrome P-448 was purified from MC-treated microsomes as in [6]. The preparations of cytochrome P-448 used in the experiments contained 16–18 nmol per mg protein.

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Antibodies to cytochrome P-448 were obtained from the sera of adult rabbits after immunization by the purified electrophoretically homogeneous cytochrome P-448 as recommended in [7].

Reconstituted microsomal membranes were obtained by self-assembly of liver microsomes solubilized in 4% sodium cholate during gel filtration through Sephadex LH-20 [8]. The isolated cytochrome P-448 was incorporated into the reconstituted membranes by adding certain amounts of the enzyme to solubilized untreated or PB-treated microsomes with subsequent incubation for 15 min at room temperature before chromatography on Sephadex LH-20 [9]. The membranes were also reconstituted from a mixture of the solubilized untreated and MC-treated microsomes.

The total content of cytochrome P-450 was determined according to [10]. The specific content of cytochrome P-448 was determined by rocket immunoelectrophoresis [11].

The activity of NADPH-cytochrome P-450 reductase was measured by the reduction of cytochrome *c* [12]. The reductase content in the microsomes and reconstituted microsomal membranes was determined from the enzyme's molecular mass (78 kDa) and the maximal specific activity of the purified enzyme (60 μmol cytochrome *c* reduced/min per mg protein) [13].

3,4-BP hydroxylase activity was determined from the rates of 3-OH BP accumulation [15]. The metabolism of 3,4-BP in the presence of antibodies to cytochrome P-448 was determined after preliminary incubation of microsomal preparations with the antibodies for 10 min at 37°C [16].

Protein was determined as in [17].

3. RESULTS AND DISCUSSION

To obtain microsomal membranes with different contents of cytochrome P-448 several approaches were used. One consisted of the time-dependent treatment of rats with 3-MC: the duration of the induction was 4, 8, 16 and 72 h. The other comprised variations in the reconstitution of membranes from solubilized microsomes: purified cytochrome P-448 was incorporated into untreated or PB-treated microsomal membranes and the membranes were obtained from mixtures of untreated and MC-treated microsomes solubilized in various proportions. We have previously shown that the main physicochemical properties of the reconstituted membrane-bound monooxygenase system and those of the native one are similar, including the rates of oxidation of xenobiotics. Thus, the activity of BP metabolism in reconstituted membranes was 85–100% of the native microsomes [8].

Preparations of native and reconstituted microsomal membranes were obtained with molar ratios of the MC-inducible form of cytochrome P-450 and NADPH-cytochrome P-450 reductase ranging from 2:1 to 32:1.

To determine the catalytic activity of membrane-bound cytochrome P-448, antibody inhibition studies of BP metabolism were performed. The difference between the rate of inhibition by an excess of antibodies to cytochrome P-448 and the uninhibited rates was attributed to cytochrome P-448. Cytochrome P-448 activity was calculated from the amount of metabolism inhibited by antibodies to cytochrome P-448 divided by the cytochrome P-448 content.

Fig.1 shows that the highest BP hydroxylase activity of cytochrome P-448 was achieved when the molar ratio of cytochrome P-448 to NADPH-

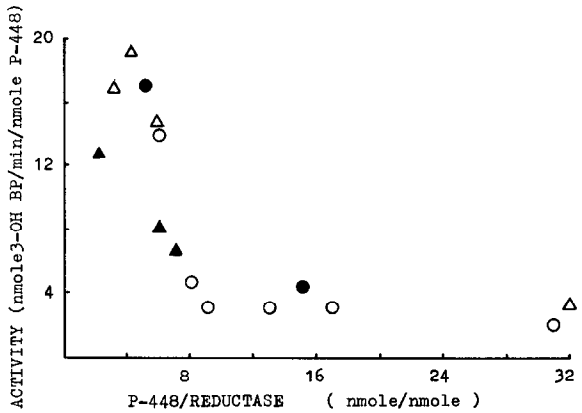

Fig.1. BP hydroxylase activity of MC-inducible cytochrome P-448 in microsomal membranes with different molar ratios of cytochrome and NADPH-cytochrome P-450 reductase. (△) Native microsomes induced with MC, (○) reconstituted membranes from mixtures of untreated and MC-treated microsomes, (▲) reconstituted from PB-treated microsomes with incorporated cytochrome P-448, (●) reconstituted from untreated microsomes with incorporated cytochrome P-448.

cytochrome P-450 reductase in microsomal membranes did not exceed 6:1. There was no enhancement of BP hydroxylase activity in preparations with higher ratios due to increased cytochrome P-448 content.

It has been shown that the dependence between the rates of oxidation of the substrates specific to a certain form of cytochrome P-450 and the molar ratio of this cytochrome form to NADPH-cytochrome P-450 reductase in non-membranous reconstituted systems is described by a parabolic curve, and that the maximum activity of the system is manifested at a 1:1 ratio of the electron carriers [4,5].

Our results demonstrate that in the membrane-bound monooxygenase system there are completely different relationships between the rate of substrate metabolism and the molar ratio of a certain form of cytochrome P-450 and NADPH-cytochrome P-450 reductase. The most effective metabolism of BP by cytochrome P-448 is observed at ratios of the cytochrome and reductase which differ greatly from the equimolar ratio of the carriers.

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