*Biochemical Pharmacology*, Vol. 35, No. 21, pp. 3827–3830, 1986.
Printed in Great Britain.

0006–2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

# STRUCTURAL COMPARISON OF MONOCLONAL ANTIBODY IMMUNOPURIFIED PULMONARY AND HEPATIC CYTOCHROME P-450 FROM 3-METHYLCHOLANTHRENE-TREATED RATS

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(*Received* 5 *December* 1985; *accepted* 20 *March* 1986)

**Abstract**—A pulmonary cytochrome P-450 was purified from lung microsomes of 3-methylcholanthrene (MC)-treated rats by immunoaffinity chromatography using a monoclonal antibody to MC-induced rat liver cytochrome P-450. The isolated pulmonary cytochrome P-450 was MC-inducible and had an apparent molecular weight of 57 kD on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The molecular weight, as well as the $NH_2$-terminal sequence of the first nine amino acids of the pulmonary cytochrome P-450, was identical to that of an epitopically related MC-induced rat liver cytochrome P-450. In addition, partial proteolysis of both cytochromes P-450 yielded indistinguishable peptide patterns on SDS-Page. Treatment of rats with MC, therefore, induces a pulmonary cytochrome P-450 which is structurally identical to the MC-induced hepatic enzyme by several criteria.

The cytochromes P-450 metabolize a wide variety of exogenous and endogenous compounds, including chemical carcinogens, drugs, and steroids [1–3]. The individual cytochrome P-450 isozymes differ in their substrate specificities and reactivities. The types and amounts of isozymes in a particular tissue, therefore, influence the metabolic conversion of substrates to either nontoxic products or toxic metabolites. Progress in the determination of cytochrome P-450 phenotype in tissues has been hindered, however, by the difficulties often encountered in distinguishing closely related multiple forms of cytochrome P-450. A powerful approach to the multiplicity problem involves the use of monoclonal antibodies (MAbs)† as tools for studying these structurally related proteins. We have thus prepared MAbs to a variety of cytochromes P-450 [4–7] and have used these MAbs to phenotype tissue cytochrome P-450 by enzyme inhibition measurements [8–9] and radioimmunoassay [10–12]. MAbs have also been used for immunopurification of cytochrome P-450 from tissues of 3-methylcholanthrene (MC)-treated animals [11, 13], and the $NH_2$-terminal amino acid sequences of several hepatic isozymes have been determined [14].

While a number of hepatic cytochromes P-450 have been purified and characterized [3], relatively few extrahepatic forms have been examined extensively, owing to their low concentrations in extrahepatic tissues. Studies on pulmonary cytochromes P-450 are particularly important, since the lung is a portal of entry for various environmental chemicals metabolized by cytochrome P-450. In this report we characterize a rat lung cytochrome P-450 which was immunopurified with an MAb to MC-induced rat liver cytochrome P-450. The isolated cytochrome P-450 was MC-inducible and had the same molecular weight and $NH_2$-terminal amino acid sequence as the major MC-induced hepatic cytochrome P-450. In addition, proteolytic digestion of cytochrome P-450 from liver and lung generated similar peptide fragments on SDS–PAGE. The pulmonary and hepatic enzymes thus appear quite similar by several criteria. MAb-directed immunopurification thus provides an efficient means for obtaining purified extrahepatic cytochromes P-450 that are suitable for subsequent structural analyses.

## MATERIALS AND METHODS

*Preparation of rat lung microsomes.* Male Sprague–Dawley rats (200–230 g, Charles River) were pretreated with MC (30 mg/kg, i.p. for 3 days). After the animals were killed, the lungs were excised and rinsed with 0.15 M NaCl. Microsomes were prepared by differential centrifugation [15], suspended in phosphate-buffered saline (PBS) containing 25% glycerol, and stored at −70°.

*Preparation of monoclonal antibodies and immunoaffinity resins.* Production of hybridoma cells was essentially as described [6]. Monoclonal antibody 1-7-1 to MC-induced cytochrome P-450 was prepared from ascites fluid by precipitation in 1.6 M ammonium sulfate and chromatography on Affigel Blue (Bio Rad). The coupling of MAb 1-7-1 to

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† Abbreviations: MAb, monoclonal antibody; MC, 3-methylcholanthrene; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS, 10 mM phosphate-buffered 0.9% saline (pH 7.4); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BCA, bicinchoninic acid; and DMAA, *N,N*-dimethyl-*N*-allylamine buffer (pH 9.5).

CNBr-activated Sepharose 4B was carried out as described [13].

*Immunoaffinity purification.* Lung microsomes obtained from a total of 150 rats were suspended in PBS containing 25% glycerol to a final concentration of 5 mg/ml. The microsomes were solubilized by the addition of 10% Emulgen 911 (Kao-Atlas) to a final concentration of 0.5%, with stirring for 30 min at 4°. The mixture was centrifuged at 100,000 *g* to remove insoluble material. The solubilized microsomes were then applied to a 5-ml Sepharose-MAb 1-7-1 column previously equilibrated with PBS, 25% glycerol, 0.5% Emulgen. The resin was then washed with the following solutions, each of which contained 0.1% Emulgen and 25% glycerol: 50 ml PBS; 50 ml PBS, 1 M NaCl; 50 ml PBS. The immunoadsorbed material was then eluted with 30 mM sodium phosphate (pH 3.0). The eluent was adjusted to pH 7.5 by addition of 1 M Tris (pH 8.5) and was dialyzed against 30 mM sodium phosphate (pH 7.2) containing 25% glycerol and 0.1% sodium cholate. The sample was then applied to a small hydroxylapatite column (Bio Rad) equilibrated with this buffer. After washing the column, cytochrome P-450 was eluted with 0.4 M sodium phosphate, 25% glycerol, 0.1% sodium cholate, and was then dialyzed with several changes for 3 days against 30 mM sodium phosphate buffer (pH 7.2) containing 25% glycerol.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli [16]. Proteins and peptides were visualized by staining with Coomassie Brillant Blue R-250 or silver (Bio Rad). Protein content was measured by the Pierce BCA protein assay (Pierce).

*$NH_2$-terminal sequence determination.* Amino acid sequencing was carried out by Applied Biosystems (Foster City, CA) on a 470A Gas Phase Sequencer [17]. Immunopurified protein was dialyzed for 24 hr against 500 ml of 0.05% SDS, 0.4 mM DMAA in pyridine/water (3/2) (Pierce Co.). Afterwards, the sample was dialyzed for 24 hr against 0.4 mM DMAA. The sample was then lyophilized, dissolved in 0.1 ml of 62.5% trifluoroacetic acid (Sigma), and applied to the sequencer. Anilinothiazolinoneamino acids were converted to phenyl thiohydantoin-derivatives and were identified and quantitated as described [18].

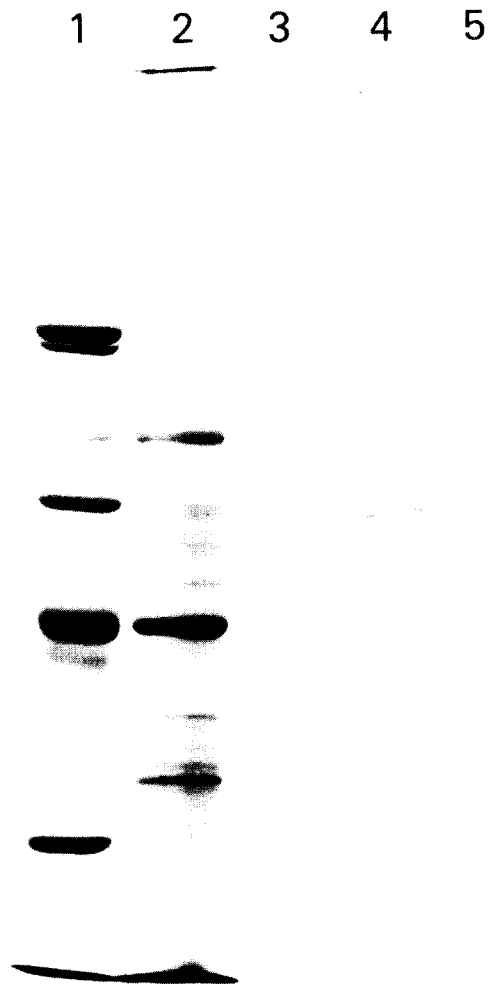


Fig. 1. SDS–PAGE of cytochrome P-450 purified by MAb-immunopurification. Lane 1 contains molecular weight standards. Lane 2 contains lung microsomes from MC-induced rats. Lane 3 contains 57 kD cytochrome P-450 immunopurified from lung microsomes of MC-treated rats. Lane 4 contains 57 kD MC-induced cytochrome P-450 immunopurified from rat liver. The sample loaded in lane 5 was obtained from an immunopurification performed on lung microsomes from control (untreated) rats. Proteins were visualized by staining with Coomassie blue.

## RESULTS AND DISCUSSION

Immunopurification with MAbs specifically isolates cytochromes P-450 on the basis of their epitopic content. We have shown previously that this technique as applied to liver microsomes from MC-treated animals yields cytochromes P-450 which are electrophoretically pure [11] and which exhibit a single major $NH_2$-terminal amino acid sequence [14]. We now report structural studies of a rat pulmonary cytochrome P-450 prepared by this technique.

Immunopurification experiments have shown that two MAbs to MC-induced liver cytochrome P-450, 1-7-1 and 1-31-2, each bind to a 57 kD cytochrome P-450 from both liver and lung microsomes from MC-treated rats [11]. These hepatic and pulmonary cytochromes P-450 are thus immunochemically related by at least two common MAb-specific epitopes. SDS–PAGE of the immunopurified polypeptides from lung and liver are shown in Fig. 1 (lanes 3 and 4). The purified protein from lung was a minor component of total lung microsomal protein (lane 2). When immunopurifications were carried out using lung microsomes from untreated rats, no protein was detected (lane 5), indicating that the isolated protein was MC-inducible.

The purified hepatic and pulmonary polypeptides were compared by peptide mapping on SDS–PAGE. The peptide fragments generated by partial proteolytic degradation using α-chymotrypsin are shown in Fig. 2. Although some intensity differences were observed, the same fragments were present in both

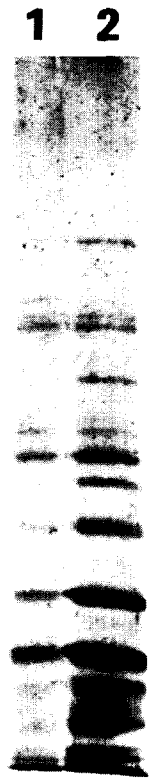


Fig. 2. SDS–PAGE analysis of peptide generated by proteolytic digestion of MAb-immunopurified pulmonary and hepatic cytochromes P-450. Lanes 1 and 2 contain α-chymotryptic digests of proteins from lung and liver respectively. A 15-μg sample of protein was incubated at 22° with 0.06 μg α-chymotrypsin. After 30 min, reaction was stopped by addition of 2% SDS and boiling, followed by application of digest to sample well. Peptides were visualized by silver stain.

digests, indicating that the two polypeptides have a high degree of structural homology.

Amino-terminal sequence analysis by automated Edman degradation was carried out on the isolated pulmonary cytochrome P-450. The first nine degradative cycles yielded positively identifiable major amino acids, for which the yields are shown in Table 1. The amino terminal sequence of the immunopurified polypeptide is:

Pro-Ser-Val-Tyr-Gly-Phe-Pro-Ala-Phe

This sequence is identical to that previously reported for the 57 kD immunopurified rat liver cytochrome P-450 [14] and to the sequence deduced from the rat cDNA sequence of hepatic MC-P-450 [19].

Our previous report [14] demonstrated that MAb-based immunopurification can provide hepatic cytochromes P-450 suitable for primary sequence analysis. The present report shows that the method may also be successfully applied to the cytochromes P-450 that are present at relatively low levels in

Table 1. Automated sequencer analysis of MAb-immunopurified rat pulmonary cytochrome P-450

| Cycle | Amino acid | pmoles |
|---|---|---|
| 1 | Pro | 197 |
| 2 | Ser | 50 |
| 3 | Val | 215 |
| 4 | Tyr | 159 |
| 5 | Gly | 189 |
| 6 | Phe | 179 |
| 7 | Pro | 179 |
| 8 | Ala | 176 |
| 9 | Phe | 152 |

A 2-nmole sample of protein was applied to the sequencer.

extrahepatic tissues such as lung. We have shown that the hepatic and pulmonary isozymes we isolated are identical by several criteria: (1) apparent molecular weight on SDS–PAGE; (2) presence of two common epitopes, recognized by MAbs 1-31-2 and 1-7-1; (3) inducibility by MC; (4) peptide mapping; and (5) amino-terminal sequence.

Extrahepatic cytochromes P-450 have not been studied as extensively as the corresponding hepatic enzymes. Purification and structural studies in particular are hindered by their limited availability, owing to their low levels relative to the hepatic enzymes. The most studied extrahepatic system has been that of rabbit lung, from which several cytochromes P-450 have been purified and characterized [20–23]. The pulmonary P-450$_{\mathrm{I}}$ isozyme, present constitutively and inducible by phenobarbital [23], is indistinguishable from the major phenobarbital-induced hepatic cytochrome P-450, form 2 [20, 21]. A pulmonary TCDD-inducible isozyme which corresponds to hepatic form 6 has also been identified [24]. In addition, an MC-inducible pulmonary cytochrome P-450 has been purified [22], although its relationship to form 6 has not yet been ascertained.

Purification of a pulmonary cytochrome P-450 from MC-treated rats has been reported previously [25], although whether the isolated isozyme is MC-inducible was not reported. Its molecular weight and peptide patterns differed from that of the major hepatic MC-induced isozyme. The pulmonary isozyme we isolated has the same molecular weight and a peptide pattern similar to the hepatic form and therefore differs from the previously reported pulmonary isozyme. The complexity of the rat pulmonary cytochrome P-450 system, including the number of isozymes and their regulation and relation to the hepatic isozymes, remains largely uncharacterized at present. In addition to studies of purified cytochromes P-450, immunological studies of rat lung and liver microsomes have previously revealed immunologic similarities between the cytochromes P-450 present in these tissues [26, 27].

MAb-based immunopurification of cytochromes P-450 from extrahepatic tissues will aid in establishing the relatedness among the various isozymes present in these tissues and in liver. This method is rapid and efficient and is potentially useful for the isolation of specific cytochromes P-450 present in

tissues at low levels, and hence difficult to obtain by conventional purification methods. Subsequent structural studies of the purified extrahepatic cytochromes P-450, such as amino acid sequence analysis and peptide mapping, will be useful tools for elucidating intertissue differences in cytochrome P-450 content.

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