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CATALYTIC ACTIVITY OF CYTOCHROMES P-450 PURIFIED BY MONOCLONAL ANTIBODY-DIRECTED IMMUNOPURIFICATION

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The cytochromes P-450 of the microsomal mixed-function oxidases metabolize numerous xenobiotics and endobiotics [1-3], including carcinogens, drugs, steroids, and prostaglandins. Numerous studies have demonstrated cytochrome P-450 multiplicity in the tissues of basal and induced animals [3]. The various isoenzymes differ in their substrate and product specificities and reactivities. Thus, the type and amount of each isoenzyme which is present in a tissue regulate the metabolic conversion of substrates to various metabolites. Since the cytochromes P-450 catalyze numerous types of reactions and act upon a wide variety of substrates, the identification and characterization of individual cytochrome P-450 isoenzymes have implications for studies in many fields, including carcinogenesis, pharmacology, and toxicology.

Enzyme multiplicity and overlapping specificities have thus far limited determination of cytochrome P-450 phenotype in tissues and hindered progress in understanding the role of individual cytochromes P-450 in the metabolism of specific substrates. A new approach to this problem involves the use of monoclonal antibodies (MAbs) as tools for studying these structurally related enzymes. As highly specific probes for antigenic sites, they can be used to immunochemically distinguish and define cytochromes P-450 on the basis of their epitope content. MAbs that inhibit enzymatic activities have proven useful in defining the contribution of specific cytochromes P-450 to the metabolism of individual substrates in human and animal tissues [4,5]. In addition, MAb-based immunopurification procedures for detection and characterization of cytochromes P-450 have been developed in several laboratories [6-10].

The cytochromes P-450 previously prepared by a single step MAb-directed immunopurification procedure have been suitable for electrophoretic [7-9] and primary structure analysis [10]. In these studies, conditions which typically denature enzymes were employed to desorb the purified cytochromes P-450 from the immunoaffinity matrix, and they were therefore not characterized with respect to catalytic activity. We now report that conditions which result in the efficient elution of cytochrome P-450 from the immunoaffinity matrix (0.1 M glycine, pH 3.0) yield purified enzyme which partially retains catalytic activity. We therefore report that, in addition to the previously employed MAb-based methods for the isolation and structural characterization of cytochromes P-450, the MAb-immunopurified cytochromes P-450 may also be characterized enzymatically.

MATERIALS AND METHODS

MAbs were prepared as previously described to purified liver microsomal cytochrome P-450 from rats treated with 3-methylcholanthrene (MC) [11], and phenobarbital (PB) [12]. MAbs were purified from mouse ascites fluid by precipitation with 1.6 M ammonium sulfate

and ion exchange chromatography. They were then covalently coupled to Sepharose to yield the immunoaffinity matrix as described [7]. The MAbs used in this study were 1-7-1, 1-31-2, and 1-36-1 to MC-induced rat liver cytochrome P-450; and 2-66-3, 4-7-1, and 2-8-1 to PB-induced rat liver cytochrome P-450. To immunopurify the cytochromes P-450, 0.6 ml of solubilized rat liver microsomes (4-5 mg/ml) from MC-treated rats was incubated for 5 min with 0.3 ml of Sepharose 1-7-1, 1-31-2, or 1-36-1, and microsomes from PB-treated rats were likewise incubated with Sepharose 2-66-3, 4-7-1, or 2-8-1. The resins were centrifuged and washed with 1 ml of each of the following to remove unbound material: three times with 40 mM potassium phosphate (pH 7.2); twice with 40 mM potassium phosphate, 1 M NaCl; twice with 4 mM potassium phosphate. Immunoabsorbed cytochrome P-450 was eluted with 1.2 ml of 0.1 M glycine (pH 3.0). Samples were immediately adjusted to pH 7.2 by addition of 1 M Tris-HCl (pH 8.5). All the above solutions included 25% glycerol, 0.1% Emulgen 911, and all operations were performed at 4°. Immunopurified proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). They were catalytically characterized by measurements of aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin deethylase (ECD) activities in a reconstituted system [12]. Protein concentrations were determined with the Pierce BCA assay procedure (Pierce), using bovine serum albumin as standard.

RESULTS AND DISCUSSION

SDS-PAGE of the cytochromes P-450 absorbed to the MAb-immunoaffinity matrix showed that MAb 1-31-2 absorbed from MC-microsomes a cytochrome P-450 with M_r 57,000, which was previously shown to be primarily responsible for the AHH activity in these microsomes [8]. The MAb 1-7-1 was shown to adsorb the same M_r 57,000 protein in addition to another cytochrome P-450 of M_r 56,000. MAb 1-36-1 bound only the M_r 57,000 protein (unpublished results). The matrix prepared with MAb 2-66-3 binds cytochrome P-450 with M_r 54,000 [7]. MAbs 4-7-1 and 2-8-1 were found to bind the same cytochrome P-450 as MAb 2-66-3 (unpublished results).

Cytochrome P-450 that is adsorbed to, and immobilized on, a Sepharose-MAb matrix is in the native state, based on CO-reduced difference spectra [7] and catalytic activity [6]. Previously reported conditions used for elution of this enzyme from the immunoadsorbent, however, required strongly denaturing solvents such as glacial acetic acid [6] or SDS [9]. We therefore examined the cytochrome P-450 which elutes under our somewhat milder purification conditions (0.1 M glycine, pH 3.0) to determine whether it retains any catalytic activity.

The AHH and ECD activities of the immunopurified cytochromes P-450 are presented in Table 1. Significant activity was observed for both assay systems. It is evident that the magnitudes of the observed activities were sufficient for characterization of the purified cytochromes P-450 with respect to substrate specificity. The relative efficiencies in catalyzing AHH and ECD varied for the cytochromes P-450 purified using different MAbs. The ratio of AHH to ECD ranged from 0.30 to 2.36. The amount of AHH activity relative to ECD activity was greater for immunopurifications carried out with those MAbs that are specific for MC-induced cytochrome P-450 (1-7-1, 1-31-2, and 1-36-1) as compared to the relative activities obtained with those MAbs that are specific for PB-induced cytochrome P-450 (2-66-3, 4-7-1, and 2-8-1). This result was consistent with the known catalytic properties of these purified enzymes since the MC-induced form is more AHH-active than the PB-induced form [3]. The cytochromes P-450 purified by this method are spectrally inactive and are primarily present as cytochrome P-420 [8]. The P-450 contents of the enzymes purified with MAbs 2-66-3, 4-7-1, and 2-8-1 were 0.08, 0.15, and 0.18 nmol/ml,

Table 1. Aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin (ECD) activities of the cytochromes P-450 purified with Sepharose-MAbs

MAb†	AHH*	ECD*	AHH/ECD
1-7-1 (MC)	11.20	4.73	2.36
1-31-2 (MC)	4.65	3.82	1.22
1-36-1 (MC)	25.00	17.50	1.43
2-66-3 (PB)	2.62	6.39	0.41
4-7-1 (PB)	0.98	3.22	0.30
2-8-1 (PB)	2.00	5.33	0.36

†Treatment of rats (MC or PB) from which liver microsomes were obtained is given in parentheses following each MAb.

*Units: pmoles/ml/min.

respectively. The P-450 contents of purified enzymes obtained with MAbs 1-7-1, 1-31-2, and 1-36-1 were below the limit of spectral detectability. However, all immunopurified cytochromes P-450 retained sufficient catalytic activity for successful measurement and characterization of catalytic activities.

In a previous report a cytochrome P-450 immobilized on a Sepharose-MAb support was shown to retain enzyme activity [6]. Upon elution with a denaturing solvent (acetic acid), however, enzyme activity was lost. Since the activity of the immobilized enzyme was measured in the presence of MAb, this study necessitated an MAb that does not inhibit enzyme activity. In the present report, however, all the MAbs used were inhibitory [11, 12], and the cytochromes P-450 were analyzed after elution from the matrix and, therefore, in the absence of MAb. The elution of cytochrome P-450 with 0.1 M glycine (pH 3.0) prior to enzymatic analysis has the advantages that (a) any MAb, whether inhibitory or noninhibitory, may be used, and (b) the cytochrome P-450 is not immobilized on a solid-state matrix which may influence catalytic activity by limiting accessibility of reductase or other essential cofactors to the enzyme.

These results demonstrate that MAb-based immunopurification provides cytochrome P-450 that is suitable not only for electrophoretic and primary structure analysis, but for characterization of functional enzymatic activity. This procedure is a simple, efficient method that should prove useful for catalytic comparison of a wide range of MAb-specific cytochromes P-450 which may be obtained from different tissues and species under a variety of conditions such as after treatment with inducing agents, and during different developmental or nutritional states.

REFERENCES

1. J.R. Gillette, D.C. Davis and H.A. Sasame, A. Rev. Pharmac. 12, 57 (1972).
2. H.V. Gelboin, Physiol. Rev. 60, 1107 (1980).
3. A.Y.H. Lu and S.B. West, Pharmac. Rev. 31, 277 (1980).
4. T. Fujino, S.S. Park, D. West and H.V. Gelboin, Proc. natn. Acad. Sci. U.S.A. 79, 3682 (1982).

5. T. Fujino, D. West, S.S. Park and H.V. Gelboin, J. biol. Chem. 259, 9044 (1984).
6. K.M. Lewis, A.R. Boobis, M.B. Slade and D.S. Davies, Biochem. Pharmac. 31, 1815 (1982).
7. F.K. Friedman, R.C. Robinson, S.S. Park and H.V. Gelboin, Biochem. biophys. Res. Commun. 116, 859 (1983).
8. K.C. Cheng, H.V. Gelboin, B.J. Song, S.S. Park and F.K. Friedman, J. biol. Chem. 259, 12279 (1984).
9. I. Reubi, K.J. Griffin, J.L. Raucy and E.F. Johnson, J. Biol. Chem. 259, 5887 (1984).
10. K.C. Cheng, H.C. Krutzsch, S.S. Park, P.H. Grantham, H.V. Gelboin and F.K. Friedman, Biochem. biophys. Res. Commun. 123, 1201 (1984).
11. S.S. Park, T. Fujino, D. West, F.P. Guengerich and H.V. Gelboin, Cancer Res. 42, 1798 (1982).
12. S.S. Park, T. Fujino, H. Miller, F.P. Guengerich and H.V. Gelboin, Biochem. Pharmac. 33, 2071 (1984).