Biochemical Pharmacology, Vol. 31, No. 9, pp.1815-1817, 1982.
Printed in Great Britain.

0006-2952/82/091815-03$03.00/0

IMMUNOPURIFICATION OF CYTOCHROME P-448 FROM MICROSOMAL FRACTIONS OF RABBIT LIVER WITH RETENTION OF METABOLIC ACTIVITY

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(Received 10 February 1982; accepted 24 February 1982)

With the realisation that the haemoprotein cytochrome P-450 exists in multiple forms (reviewed in 1), considerable attention has been directed towards determining their substrate specificity. Progress has been hindered by the difficulty of purifying individual forms of the enzyme to homogeneity, particularly where the form represents a small proportion of the total cytochrome P-450 present. One possible solution is to capitalise on the high specificity that antibodies normally exhibit towards their corresponding antigens. However, immunopurification of cytochrome P-450 has been hampered by two problems.

i) to raise a specific antibody by conventional techniques a homogeneous antigen is required (2)

ii) attempts to purify cytochrome P-450 using such antibodies (which are polyclonal), with recovery of the antigen, have always led to inactivation of the haemoprogein (e.g. 3)

The use of monoclonal antibodies against cytochrome P-450 could overcome both of these problems. We have therefore raised monoclonal antibodies to a form of rabbit liver cytochrome P-450 to investigate the feasibility of such an approach.

Male New Zealand white rabbits were induced with 3-methylcholanthrene (3-MC) (4) and cytochrome P-448 (mol wt on SDS-polyacrylamide gel electrophoresis 55,000 daltons; also designated LM4) purified from the hepatic microsomal fraction by polyethylene glycol precipitation followed by hydroxylapatite-cellulose chromatography (5). The specific content of the final preparation was 16.6 nmol mg^{-1}.

BALB/c mice were immunised with this protein and 4 monoclonal antibodies to cytochrome P-448 were obtained as previously described (6). When incubated with a reconstituted benzo(a)pyrene hydroxylase system, one of these antibodies, MBS 105, had no effect on activity i.e. this was a so-called "null" antibody. In previous studies both polyclonal (2) and monoclonal (7) antibodies inhibited monooxygenase activity.

When MBS 105 was incubated overnight at 4°C with microsomal fractions from 3-MC treated rabbit liver a single protein of mol. wt. 55,000 daltons, which co-chromatographed with cytochrome P-448, was adsorbed (6). This null antibody was then covalently bound to CH-activated sepharose and the antibody-sepharose complex incubated overnight at 4°C with cholate solubilised microsomal fractions from liver of 3-MC treated rabbits

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and rats and control rabbits. A protein of molecular weight 55,000 daltons was absorbed from solubilised microsomal fractions of control and 3-MC treated rabbit liver but no protein was adsorbed from rat liver microsomes. It was not possible to elute adsorbed proteins from the gel with conditions milder than glacial acetic acid, and this denatured the protein.

Although it was not possible to dissociate cytochrome P-448 from the immobilised antibody in an active form a reconstituted system (6) comprising the immobilised cytochrome P-448, NADPH-cytochrome P-450 reductase and dilauryl-α-lecithin was capable of oxidising benzo(a)pyrene (Table 1).

Table 1. Reconstitution of immobilised cytochrome P-448 in benzo(a)pyrene hydroxylase system

Incubation system	Source of cytochrome P-448	Benzo(a)pyrene hydroxylase activity (pmol/incubation)
Complete*	purified preparation	211
No NADPH	" "	12
No reductase	" "	10
No cytochrome	-	8
Complete	3-MC treated rabbit liver	222
Complete	Control rabbit liver	46
Complete	3-MC treated rat liver	28
No NADPH	" " " "	22

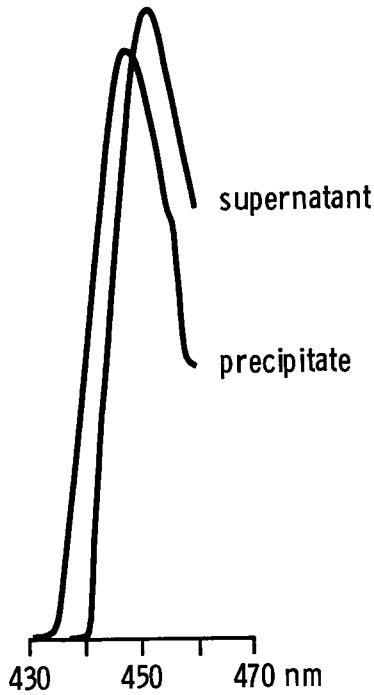
*Sepharose-"null" antibody-cytochrome P-448 complex, NADPH-cytochrome P-450 reductase, lipid and NADPH.
Values are mean of two determinations, which in each case were within 5% of each other.

The results in Table 1 indicate that the antibody-sepharose complex was capable of selectively adsorbing cytochrome P-448 from solubilised microsomal fractions of rabbit liver. Activity required the addition of reductase and no activity was observed after the antibody-sepharose complex was incubated with microsomal fractions of rat liver.

Cholate solubilised microsomes from the livers of 3-MC treated or control rabbits were incubated with the null antibody at 4°C for 16 h. The antibody, and any bound antigen, were then immunoprecipitated by the addition of a polyclonal anti-mouse immunoglobulin antibody. The resultant precipitates were collected by centrifugation and resuspended in phosphate buffered saline. The reduced versus CO-reduced difference spectrum showed a peak absorption at 447 nm (Fig. 1). This was true whether microsomes were from 3-MC treated or control rabbits. In the latter case the reduced versus CO-reduced difference spectrum in the supernatant remaining after addition of the second antibody had a peak absorption at 450 nm.

It was thus possible to use a null antibody to cytochrome P-448 to immunopurify the cytochrome. The antigen could not be dissociated from the antibody in an active form. However, the cytochrome P-448 bound to the null antibody immobilised on sepharose gel exhibited substantial benzo(a)pyrene hydroxylase activity in a reconstituted system.

Thus the approach suggested for the purification of an active form of cytochrome P-450 using a monoclonal antibody has succeeded. The techniques described should prove very useful in the quantitation and characterisation of different forms of cytochrome P-450.



Reduced versus CO-reduced difference spectra of immunoprecipitate and supernatant obtained by incubation of solubilised control rabbit liver microsomal fraction with monoclonal antibody MBS 105 followed by addition of anti-mouse immunoglobulin.

Acknowledgements

This work was supported by a grant from the Medical Research Council and by a Joint Research Scheme Award from Imperial Chemical Industries Limited.

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