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ANTIBODIES TO A SYNTHETIC PEPTIDE THAT REACT SPECIFICALLY WITH A COMMON SURFACE REGION ON TWO HYDROCARBON-INDUCIBLE ISOENZYMES OF CYTOCHROME P-450 IN THE RAT

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Abstract—An antibody that reacts with two hydrocarbon-inducible isoenzymes of rat cytochrome P-450 (*c* and *d*) in the rat was produced by immunising with a synthetic peptide, Leu-Ile-Ser-Lys-Phe-Gln-Lys-Leu-Met, which has the same primary structure as that of a region of both of these isoenzymes. There was no crossreactivity with hydrocarbon-inducible isoenzymes in liver microsomes from rabbit, mouse or in man. Nor was there any crossreactivity detected with liver microsomes from uninduced rats, or rats induced with phenobarbitone or isonicotinic acid hydrazide. This is consistent with the primary structure of these isoenzymes in the regions aligned with amino acids 174–182 (the immunising peptide) in rat isoenzyme *c* and demonstrates the ability to produce antibodies of defined specificity against isoenzymes of cytochrome P-450 by using synthetic peptide. As the antibody preparation is able to bind to isoenzymes *c* and *d* in their native conformations, either as partially purified enzymes, or in microsomes, it is suggested that this region is present on the surface of these cytochromes P-450.

Cytochromes P-450 comprise a superfamily of proteins. They are important in the metabolism of many endogenous and exogenous substrates and are inducible by a number of substances, including polycyclic aromatic hydrocarbons, phenobarbitone, alcohol and steroids [1]. The genes responsible for the synthesis of at least 65 isoenzymes have been sequenced from which the primary structures of the proteins can be deduced [2, 3].

A major problem in the study of cytochrome P-450 is the difficulty in purifying these membrane-bound enzymes to homogeneity. Although major inducible forms in a number of species have been purified, the yields are low [4]. Purification from uninduced animals and humans is even more difficult. One way to avoid the difficulties of purification is to raise antibodies using purified isoenzymes from induced animals and to identify crossreacting isoenzymes in the same and other species. In this way a monoclonal antibody that recognises a human hydrocarbon-inducible isoenzyme has been produced using a rat isoenzyme as an immunogen [5]. The main draw-back with this approach is that the cross-reactivity is not always observed and cannot be predicted.

One solution to this problem is to use as an antigen a synthetic peptide representing a short linear sequence of the protein of interest. The resulting antibodies would be expected to cross-react with the target protein in its native conformation providing that the peptide sequence chosen occurs on the surface of the protein and does not form an inaccessible secondary structure [6].

In this paper we describe the successful use of this approach. A hydrophilic area of cytochrome P-450 was chosen that is unique to only two of the known isoenzymes in the rat. The antibody raised against the peptide cross-reacted only with these two isoenzymes.

MATERIALS AND METHODS

Materials. *N*-α-Fluorenylmethoxycarbonyl (Fmoc) amino acids were purchased from MilliGen (Boston, MA) and *t*-butylthio cysteine from Novabiochem AG (Switzerland). All gel filtration and ion-exchange resins were from Pharmacia (Uppsala, Sweden). Hydroxylapatite and all SDS-polyacrylamide gel electrophoresis reagents were from Bio-Rad Laboratories (Watford, U.K.) except for pre-stained molecular weight standards which were from Sigma (Poole, Dorset, U.K.). Haemocyanin was from Calbiochem (San Diego, CA). Enzyme-linked immunosorbent assay plates were from Dynatech Laboratories (Billinghurst, U.K.) and conjugates of horseradish peroxidase with immunoglobulins were from ICN Biomedicals Ltd (High Wycombe, U.K.). Acetonitrile (grade S) was from Rathburn Chemicals (Walkerburn, Scotland). All other chemicals were purchased from Sigma or BDH Ltd. (Poole, U.K.) and were of AnalaR grade or the best equivalent.

Selection of synthesis of peptides. The cytochrome P-450 sequences were examined for (1) homology between the rat isoenzymes *c* and *d* and (2) potential surface areas, determined as an index of possible

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surface occurrence using the scale of Janin [7] and by calculating the mean index for every five consecutive residues throughout the complete amino acid sequence of the cytochromes P-450. The area with the highest score for (2) was at 357–362 in rat *c* (351–356 in rat *d*), but this was rejected because it contained three arginine residues and as such was likely to cause problems in synthesis [8–10]. Therefore, the area at 176–180 in rat *c* (173–177 in rat *d*) which scored second highest was chosen. The peptide synthesised was extended by two amino acids at both ends (Table 1).

All peptides (Table 1) were synthesised manually by a solid-phase method using a polyamide support and Fmoc amino acids [11]. Amino acids were coupled either as preformed symmetrical anhydrides or as pentafluorophenyl esters [12]. For the purpose of conjugation to proteins cysteine was added to the N-terminus as *t*-butylthio cysteine. After completion of synthesis, side chain protecting groups were removed from amino acids by treatment with 13 M trifluoroacetic acid in the presence of cation scavengers [13–15]. The resulting peptides were purified by gel filtration on a Sephadex G-25 column (2.2 × 45 cm) in 8 M-acetic acid to give a product of >85% purity, as determined by high pressure liquid chromatography, using a μBondapak column (Millipore U.K. Ltd, Harrow, U.K.), a gradient of 9–54% (v/v) acetonitrile in the presence of 0.1% (v/v) trifluoroacetic acid over 20 min at a flow rate of 1 ml/min, and constant monitoring of the eluent at 210 nm. All of the peptides had the correct amino acid analysis, and the correct molecular weights as confirmed by mass spectrometry (M-Scan Ltd., Ascot, U.K.). Deprotection of thiol-containing peptides was accomplished by the method described by Atherton and co-workers [15]. The thiol content of the lyophilised peptides was between 30 and 80% [16].

Coupling of peptides to protein carriers. Peptides were coupled to a carrier protein lysozyme (from chicken egg white) through the N-terminal cysteine of the peptide, using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) as the coupling reagent [17]. The MBS-derivatised lysozyme was added to peptides at a ratio of 1 mole of lysozyme for 3 moles of thiol peptide, the pH was adjusted to 7.3 and the mixture stirred for 3 hr and then dialysed against phosphate buffered saline (PBS: 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.5). The degree of conjugation was assessed by SDS/polyacrylamide gel electrophoresis using 15% (w/v) gels and was, on average, 2.5 moles of peptide per mole of lysozyme. Keyhole limpet haemocyanin (KLH) conjugates and a "null" conjugate, in which cysteine was substituted for peptide, were made in the same way as described above.

Immunisation. Male New Zealand White rabbits (see below for details) were immunised with repeated injections of 200 μg of peptide conjugates in Freund's adjuvant in a total volume of 1 ml. The rabbits were immunised subcutaneously with complete adjuvant and subsequently with incomplete adjuvant subcutaneously 14 days later and intraperitoneally after 21 days. Blood samples were taken after 28 days.

Purification of antibodies. The immunoglobulin fraction in rabbit serum was prepared by ammonium sulphate precipitation at 35% saturation on ice. The precipitate was washed twice in the same volume of ice-cold 35% saturated ammonium sulphate, 50 mM potassium phosphate, pH 7.0 as that of the original serum, and centrifuged (950 *g* for 15 min at 4°). The precipitate was resuspended in, and dialysed against PBS.

The production of monoclonal antibodies 3/4/2 and 12/2/3/2 has been described elsewhere [5, 18]. The monoclonal antibodies were grown up in mouse ascitic fluid and then purified as described above, except that 50% saturated ammonium sulphate was used and dialysis was against 20 mM triethanolamine–HCl, pH 7.7. Further purification was by fast protein liquid chromatography using a MonoQ chromatography column (Pharmacia, Milton Keynes, U.K.). A gradient of 0–0.35 M NaCl in 20 mM triethanolamine–HCl, pH 7.7 was used at a flow rate of 1 ml/min over a period of 20 min. The

Table 1. The primary structure of cytochromes P-450 in the region of positions 174–182 in rat cytochrome P-450 isoenzyme *c* and the equivalent regions aligned for maximum amino acid homology in the gene families P450IA1 and P450IA2 in rat, human, rabbit and mouse

Species	Protein	Sequence	Position	Source
Gene Family P450IA1				
Rat	*c*	-Leu-Ile-Ser-Lys-Phe-Gln-Lys-Leu-Met-	174–182*	[27]
Human	not named	-Leu-Ile-Ser-Thr-Leu-Gln-Glu-Leu-Met-	170–178†	[28]
Rabbit	LM6	-Leu-Ile-Gly-Arg-Phe-Gln-Glu-Leu-Met-	119–127§	[29]
Mouse	P1	-Leu-Val-Ser-Lys-Leu-Gln-Lys-Val-Met-	174–182	[30]
Gene Family P450IA2				
Rat	*d*	-Leu-Ile-Ser-Lys-Phe-Gln-Lys-Leu-Met-	171–179*	[31]
Human	not named	-Leu-Ile-Ser-Arg-Leu-Gln-Glu-Leu-Met-	172–180‡	[32]
Rabbit	LM4	-Leu-Ile-Ser-Arg-Phe-Gln-Glu-Leu-Met-	171–179	[33]
Mouse	P3	-Leu-Val-Ser-Lys-Leu-Gln-Lys-Ala-Met-	171–179	[30]

* Primary structure is the same in rat isoenzymes *c* and *d*. The synthetic peptide with this structure is referred to as rat *c* 174–182.

† The synthetic peptide with this structure is referred to as human *c* 170–178.

‡ The synthetic peptide with this structure is referred to as human *d* 172–180.

§ Approximately 10% of the N-terminal region has not been sequenced.

retention times of the antibodies were 9.0 min and 8.2 min for 3/4/2 and 12/2/3/2, respectively, and these were greater than 95% pure as determined by SDS–polyacrylamide gel electrophoresis.

Immunological analyses. Enzyme linked immunosorbent assay (ELISA) plates were coated with either protein or peptide conjugate diluted to a concentration of 5 μg/ml or unconjugated peptide at 2 μg/ml, in 50 mM sodium carbonate, pH 9.5. Where indicated, 8 M urea was added to this buffer. The assay was performed as described previously [19], with horseradish peroxidase conjugated goat anti-rabbit IgG or goat anti-mouse, as appropriate. After each stage of the assay the plates were washed extensively with PBS containing 0.05% (v/v) Tween 20 and 0.05% (w/v) bovine serum albumin (BSA). Development was with 100 μl of a solution of 2.8 mM 1,3-phenylenediamine dihydrochloride, 4.4 mM H_2O_2 in 0.05 M citric acid/0.1 M Na_2HPO_4 buffer, pH 5.0. After 10 min the absorbance of the coloured product was determined at 492 nm using an automatic colorometric plate reader (Titretek Multiskan, Flow Laboratories, U.K.).

Immunoblot analysis was carried out essentially as described by Towbin and co-workers [20]. The samples, including pre-stained standards, were subjected to SDS–polyacrylamide gel electrophoresis using a 8.5% (w/v) gel [21] and then transferred to nitrocellulose. After blocking with PBS containing 3% (w/v) BSA, the nitrocellulose was incubated with either 1 mg anti-peptide antibody or 15 μg monoclonal antibody in 15 ml of PBS containing 0.1% (w/v) BSA. After washing the nitrocellulose sheet, immunopositive bands were visualised following incubation with either a goat anti-rabbit IgG-horseradish peroxidase conjugate or a goat anti-mouse IgG-horseradish peroxidase conjugate (as appropriate) and then in 2.8 mM 4-chloro-1-naphthol, 4.4 mM-H_2O_2 in 25 mM Tris–HCl, pH 7.4. The intensity of stained bands was quantified by reflectance densitometry using a GS 300 Transmittance/Reflectance Scanning Densitometer (Hoefer Scientific Instruments, San Francisco, CA) coupled to a Waters 840 chromatography data station (Millipore, U.K. Ltd, Harrow, U.K.).

Treatment of animals with inducing compounds. Male Wistar rats (200–250 g from Olac), male New Zealand White rabbits (3 kg from Froxfield), and female Balb/c mice (6–10 weeks from Tuck) were housed and maintained as previously described [22]. Groups of animals were treated with inducing compounds as follows: 3-methylcholanthrene (3-MC), 80 mg/kg by intraperitoneal injection 48 hr before death; phenobarbitone sodium (PB), 80 mg/kg by intraperitoneal injection daily for 4 days, the last dose being administered 24 hr before death; isonicotinic acid hydrazide (isoniazid), 0.1% (w/v) in the drinking water (pH adjusted to 7.0) for 10 days and 50 mg/kg by intraperitoneal injection 3 hr before death. Animals were killed by stunning and exsanguination, the livers were removed and microsomal fractions were isolated as previously described [5].

Preparation and analysis of human microsomes. Human liver samples were obtained from wedge biopsies taken at laparotomy and were surplus to histological requirements. Local research ethical committee permission was obtained for the use of these samples. After collection, they were frozen immediately in liquid nitrogen and subsequently stored at −80°. Microsomal fractions were prepared, after thawing the samples on ice, as described above. A complete medical history including their smoking habits was obtained from each of the patients. The microsomes had been previously analysed by immunoblotting using the monoclonal antibody 3/4/2 [23]. In several donors an increase in an immunoreactive protein was found and this was related to a history of smoking cigarettes. The induced protein is most likely the human cytochrome P-450 isoenzyme gene product of P450IA2 [23]. Microsomes containing this induced protein and those in which it was absent were used in this study.

Purification of rat cytochromes P-450 c *and* d. Cytochromes P-450 were isolated from hepatic microsomal fraction of rats treated with 3-MC as described above. The methods used were based on those described by Guengerich and co-workers [4, 24]. The ion-exchange step was altered in that a Q-fast flow column (2.2 × 13 cm) was used in the presence of 0.2% (w/v) lubrol PX and 7.5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate). A gradient of 0–1 M NaCl was used at a flow rate of 5 ml/min over a period of 200 min. Rat isoenzyme *d* was not retained on the column and rat isoenzyme *c* was eluted with 0.25 M NaCl. The purification was monitored by immunoblotting using 12/2/3/2 (as described above). Final specific contents were 11 and 10 nmol P-450/mg of protein for isoenzymes *c* and *d*, respectively. Both isoenzymes were 85–90% pure, as determined by SDS-polyacrylamide gel electrophoresis; the isoenzyme *c* preparation contained no isoenzyme *d* and the isoenzyme *d* preparation was free from isoenzyme *c*.

RESULTS

The results presented here were all obtained using a peptide–lysozyme conjugate as an antigen. The affinity of the anti-peptide antibody for the peptide antigen was determined by examining binding to a conjugate of the synthetic peptide with KLH, i.e. a protein other than the one used as a carrier for immunisation. The antibody bound to KLH-rat *c* 174–182, but not to KLH alone (Fig. 1). Although, there was some binding to KLH conjugates with the analogue peptides equivalent to the two human isoenzyme sequences (Table 1), this was very similar to that seen with a "null" conjugate of cysteine coupled to KLH (Fig. 1). Synthetic peptides were adsorbed directly to the ELISA plate and recognition of rat *c* 174–182 by the antibody was retained (Fig. 2). Under these conditions, the affinity constant for binding of the anti-peptide to the homologous peptide was 2.7×10^{-2} g/l, which is close to that for binding to the KLH-rat *c* 174–182 conjugate, 2.5×10^{-2} g/l (Fig. 1). Binding was specific to the homologous peptide, there being no significant cross-reactivity with an unrelated peptide or with two analogue peptide sequences (Fig. 2) from two human isoenzymes (Table 1). The difference between this experiment and the cross-reactivity obtained when

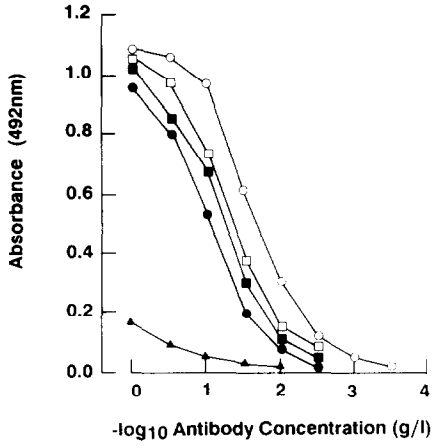

Fig. 1. The relative affinity of binding of the anti-peptide antibody to various peptide conjugates. Microtitre wells were coated with either KLH-rat *c* 174–182 (○), KLH-human *c* 170–178 (□), KLH-human *d* 172–180 (■), KLH-cysteine (●), or unconjugated KLH (▲). To each group a series of dilutions of the anti-peptide antibody was added and the relative binding was determined as described in the text.

the analogue peptides were coupled to a protein carrier (Fig. 1) was the presence of the coupling group benzoyl succinimidyl cysteine. The antibody preparation also contained antibodies to lysozyme (data not shown), which had a binding affinity constant of 6.3×10^{-3} g/l.

The specificity of binding of the anti-peptide antibody with liver microsomes from variously induced rats was determined by immunoblotting, and the profile of binding compared with that of two monoclonal antibodies of known binding characteristics (Fig. 3). The identity of the immunoreactive isoenzymes was determined by comparison with the binding of the monoclonal antibodies 12/2/3/2 and 3/4/2, with pre-stained molecular weight standards transferred to the nitrocellulose paper, and by comparison with polyacrylamide gels run in parallel and stained for protein. There was specific binding of the anti-peptide antibody to rat forms *c* and *d*, but no detectable crossreactivity with any of the other inducible or constitutive isoenzymes in the rat. Further, there was no detectable reactivity with any of the constitutive or hydrocarbon-inducible forms of cytochrome P-450 in the other species examined (Fig. 3).

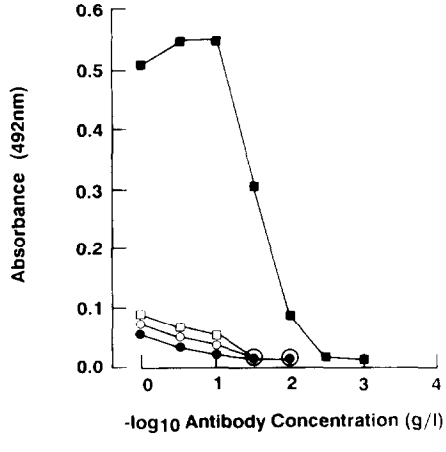

Fig. 2. The relative affinity of binding of the anti-peptide antibody to various unconjugated peptides. Microtitre wells were coated with either rat *c* 174–182 (■), human *c* (170–178 (○), human *d* 172–180 (●), or Lys-Lys-Ala-Asn-Gly-Gln-Ser-Met-Thr-Phe-Asn (Lys-Lys-rat c 120–128) (□). To each group a series of dilutions of the anti-peptide antibody was added and the relative binding was determined as described in the text.

The relative binding of the anti-peptide antibody to rat isoenzymes *c* and *d* was quantified by reflectance densitometry and was compared with the binding of 12/2/3/2 (Fig. 3). With liver microsomes from 3-MC-induced rat, 12/2/3/2 bound to isoenzymes *c* and *d* with a ratio of 1:1, whereas the anti-peptide antibody bound to the same isoenzymes at a ratio of 1:5. Assuming equal quantities of rat isoenzymes *c* and *d* in the 3-MC-induced rat liver microsomes, based on the relative intensities of the cytochrome P-450 bands on protein-stained gels, the anti-peptide antibody appears to bind preferentially to rat isoenzyme *d*. However, in ELISA under non-denaturing conditions, equal binding to purified isoenzymes rat *c* and rat *d* was found with both the anti-peptide antibody and 12/2/3/2 (Fig. 4). The anti-peptide antibody showed the same binding characteristics to the urea-denatured isoenzymes (Fig. 4), but 12/2/3/2 showed reduced binding under these conditions (Fig. 4). 3/4/2 bound equally well to both native and denatured rat isoenzyme *c* (Fig. 4a); this monoclonal antibody does not bind to isoenzyme rat *d* [18].

The relative affinity of the anti-peptide antibody to cytochrome P-450 in liver microsomes from 3-MC-induced rat was determined by ELISA (Fig. 5). The affinity of binding was the same for native and 8 M-urea denatured microsomes, consistent with a linear epitope. The binding of the anti-peptide antibody was specific to liver microsomes from 3-MC-induced rat, there was no significant binding to liver microsomes from uninduced, PB-induced or isonicotinic acid hydrazide-induced rats (Fig. 5, or data not shown).

DISCUSSION

A polyclonal antibody was raised to a synthetic peptide corresponding to a linear sequence of amino acids common to rat cytochrome P-450 isoenzymes *c* and *d* (residues 174–182 in rat isoenzyme *c*), unique to these two isoenzymes amongst those isoenzymes of cytochrome P-450 where the sequences are known. This anti-peptide antibody binds to rat isoenzymes *c* and *d* in liver microsomes from 3-MC-induced rat, but there is no significant binding with microsomes from uninduced, PB-induced or isonicotinic acid hydrazide-induced rats. Thus, the anti-peptide shows no detectable crossreactivity with rat isoenzymes *b*, *e* or *j*, nor with any of the major male constitutive isoenzymes. Nor is there any reactivity with other constitutive or hydrocarbon-inducible forms in the other species examined. This excludes

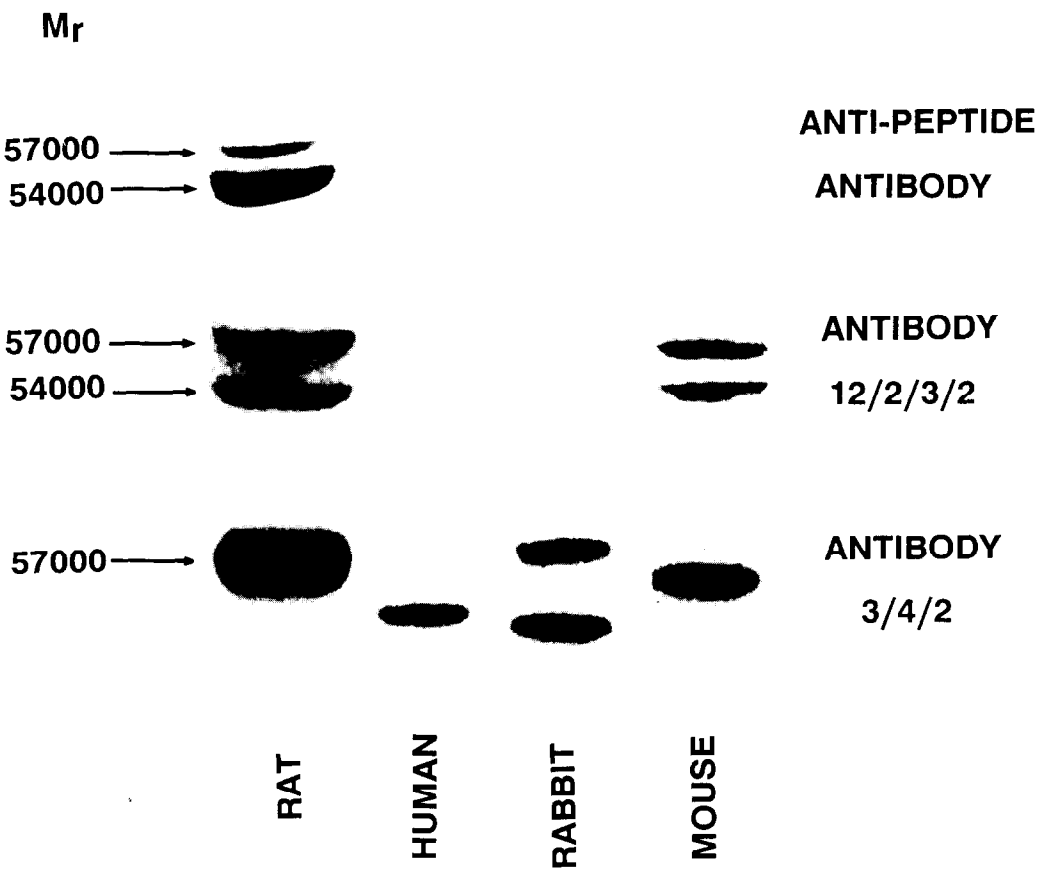

Fig. 3. Immuno-blots of cytochromes P-450. Regions of M_r 50,000–60,000 of three immuno-blots following SDS-polyacrylamide gel electrophoresis of hepatic microsomes from rat, human, rabbit and mouse. All animals had been induced with 3-MC, except the human, which was obtained from a cigarette smoker. The immuno-blots were developed with either the anti-peptide antibody, monoclonal antibody 12/2/3/2 or monoclonal antibody 3/4/2.

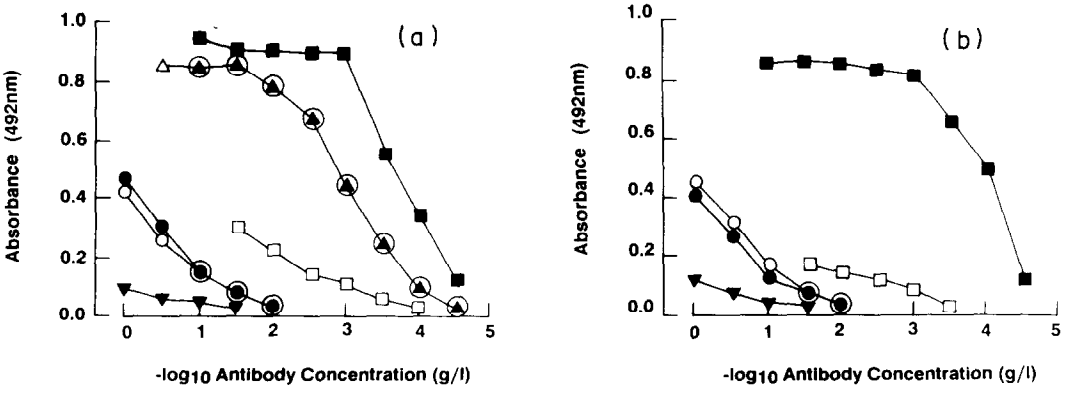

Fig. 4. The relative affinity of binding of the anti-peptide antibody, 3/4/2 and 12/2/3/2 to purified rat cytochromes P-450 isoenzyme *c* (a) and isoenzyme *d* (b). Purified cytochromes were coated onto microtitre wells, in either a native state (solid symbols) or denatured in 8 M urea (open symbols). To both isoenzymes a series of dilutions of anti-peptide antibody (○, ●), 12/2/3/2 (□, ■), 3/4/2 (△, ▲) or an antibody preparation from an unimmunised rabbit (▼) was added and the relative binding determined as described in the text.

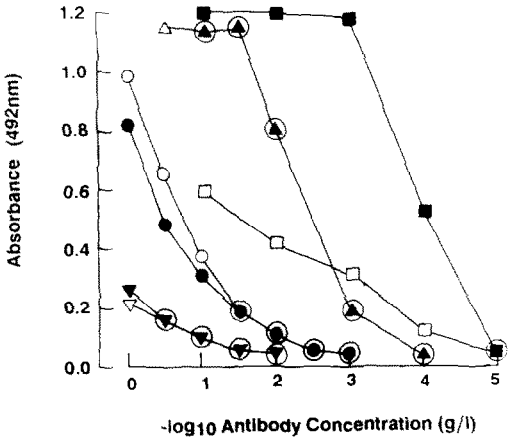

Fig. 5. The relative affinity of binding of the anti-peptide antibody, 3/4/2 and 12/2/3/2 to various hepatic microsome preparations. Microsomes obtained from rats treated with various inducing compounds were coated onto microtitre wells. To each group a series of dilutions of anti-peptide antibody, 12/2/3/2 or 3/4/2 was added and the relative binding determined as described in the text. When 3-MC induced microsomes were used, significant binding was obtained using 12/2/3/2 (■), 3/4/2 (▲) and the anti-peptide antibody (●). When the microsomes were denatured with 8 M urea 3/4/2 (△) and anti-peptide antibody (○) binding was unaffected, but 12/2/3/2 binding was significantly reduced (□). Similarly, there was no significant binding of the anti-peptide antibody to control microsomes whether native (▼) or denatured (▽). Microsomes from rats treated with phenobarbitone or isonicotinic acid hydrazide gave similar binding curves to that shown for control rats.

binding of the antibody to isoenzymes LM4 and LM6 in the rabbit and isoenzymes P_1 and P_3 in the mouse. Binding to the human hydrocarbon-inducible isoenzyme (orthologous to isoenzyme *d* in the rat) is also excluded because of the lack of binding to either liver microsomes from cigarette smokers or the free synthetic peptides corresponding to the same sequences in the human isoenzymes. Thus, binding of the anti-peptide antibody is, as far as could be determined, specific to the rat isoenzymes *c* and *d*.

The pattern of cross-reactivity obtained with the anti-peptide antibody was that expected from the primary structures of the isoenzymes of cytochrome P-450. Table 1 shows region 174–182 in rat isoenzyme *c* and the aligned sequences from the other hydrocarbon-inducible isoenzymes present in the liver microsomes examined in this study. Rat isoenzyme *d* is identical to isoenzyme *c* at this region. For the other isoenzymes there are two or three differences in their amino acid compositions at this region from that of rat isoenzyme *c*. Overall, there is close homology amongst the sequences, with most substitutions being conservative. However, at three positions there are notable differences. These occur at the positions equivalent to 177, 178 and 180 in rat isoenzyme *c*. At position 177 lysine in rat isoenzyme *c* is replaced by threonine in the gene product of the human P450IA1 gene and by arginine in the product of the human P450IA2 gene and in both rabbit isoenzymes. At position 178, phenylalanine is replaced by leucine in both of the human and both of the mouse isoenzymes. A charge change occurs at position 180 where lysine is replaced by glutamic acid in both of the rabbit and both of the human isoenzymes. These changes would explain the lack of cross-reactivity with the rabbit and human isoenzymes. The lack of cross-reactivity with the mouse isoenzymes is probably the result of the cumulative effect of three relatively conservative changes.

There was a difference in the relative binding of the anti-peptide antibody to rat isoenzymes *c* and *d* in immunoblotting. It has been shown that the conformational changes in protein structure caused by SDS can result in an increase in their helical content [25, 26]. It is possible that such an effect influences the binding of the anti-peptide antibody.

In ELISA, the anti-peptide antibody binds equally well to native and denatured rat isoenzymes *c* and *d* or liver microsomes from 3-MC-induced rat. It is likely, therefore, that the antibody is directed to a surface region of the proteins, as predicted, and that the regions 174–182 in rat isoenzyme *c* and 171–179 in rat isoenzyme *d* are wholly or largely at the surface of the membrane-bound native isoenzymes.

At least three populations of antibodies were produced against the antigen conjugate, i.e. antibodies to (i) the protein carrier, lysozyme, (ii) the coupling group, benzoyl succinimidyl cysteine, and (iii) the synthetic peptide. To produce specific high affinity antibodies to cytochromes P-450 monoclonal antibodies against synthetic peptides could be raised and selected by screening for the target cytochrome P-450 isoenzyme.

The present study has shown the feasibility of producing antibodies of defined specificity against isoenzymes of cytochrome P-450, based only on the predicted primary sequence of the proteins. The reactivity of such antibodies will be with a known epitope. Thus should prove invaluable in analysing the structure–function relationships of the isoenzymes of cytochrome P-450, in addition to their use in those applications where antibodies of defined specificity are required, including immunoquantification, immunoidentification or immunolocalisation.

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