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IDENTIFICATION OF THE EPITOPE OF AN ANTI-PEPTIDE ANTIBODY WHICH BINDS TO CYP1A2 IN MANY SPECIES INCLUDING MAN

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Abstract—An anti-peptide antibody was raised against the sequence Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-Asn-Tyr-Lys which occurs at positions 283–294 in the rat cytochrome P450 enzyme CYP1A2. Compared with its binding to the peptide used for immunization, the antibody bound with only slightly reduced affinity to the truncated peptides Thr-Gly-Ala-Leu-Phe-Lys-His-Ser and Leu-Phe-Lys-His-Ser. However, binding to the peptide Ser-Glu-Asn-Tyr-Lys-Asp-Asn, which overlaps with the C-terminal region of the immunizing peptide, was very low. Thus, a major epitope for the anti-peptide antibody is Leu-Phe-Lys-His-Ser, which corresponds to a region of CYP1A2 that is conserved in many species. The antibody was tested by immunoblotting for its ability to bind to hepatic microsomal fractions from a number of species. Where possible animals were treated with compounds which induce CYP1A2 and the results compared with those with untreated animals. It was found that the antibody bound to rat, mouse, rabbit, hamster, guinea pig, pig, marmoset monkey and human CYP1A2. No evidence was found for binding to dog CYP1A2. The region corresponding to the major epitope at residues 286–290 of rat CYP1A2 was identical in mouse, hamster, rabbit and human CYP1A2. The sequence of marmoset and guinea pig CYP1A2 are not known but are predicted to be very similar to the sequence in the rat. The lack of binding of the antibody to dog CYP1A2 may be explained by two differences in this region compared with rat CYP1A2. Maximum inhibition of CYP1A2 activity by this antibody, as measured by high-affinity phenacetin *O*-deethylase activity, was 20%. This is in contrast to a previously described anti-peptide antibody directed to an adjacent region which caused 65% inhibition of this activity. Thus, the edge of an inhibitory region on the surface of cytochrome P450 has been identified. The ability of the antibody to bind to CYP1A2 from a number of animals should make this antibody of use for studying the levels of CYP1A2 apoprotein in many species.

The diversity of xenobiotic compounds that can be oxidized by eukaryotic organisms is a consequence of the multiplicity of the cytochrome P450 (P450†) enzymes present, each with characteristic specificity. Metabolism by the P450 system is essential for the detoxication and elimination of many drugs and other xenobiotic compounds, as well as for the biosynthesis of a number of endogenous substances [1, 2].

The specific content of an individual P450 enzyme can be estimated by measuring its activity. The reliability of this approach depends upon the use of highly specific substrates, however, the oxidation of very few compounds is catalysed solely by one P450 enzyme. Hence, although differences in some monooxygenase activities may provide an indication of changes in the level of expression of a single form of P450, interpretation is often likely to be complicated by the contribution of other P450 enzymes to the activity in question. This will be particularly so when forms of P450 normally present at very low levels are increased by inducing agents to a significant proportion of the total P450 content [3]. In addition, factors other than the level of expression can affect the activity of a P450 enzyme, for example the presence of inhibitor compounds or perturbation of the structure of the lipid composition of the endoplasmic reticulum. Further, the ability of orthologous P450 enzymes to metabolize a given substrate can vary greatly amongst species, so that an activity that is diagnostic of the levels of a P450 enzyme in one species may not provide an accurate measure of the levels of that P450 in another species [4].

The expression of specific P450 apoproteins can be studied using antibodies. In contrast to most enzyme activities, antibodies can be produced that are specific to a single P450 enzyme [5]. Although there can be problems of specificity with polyclonal and monoclonal antibodies [6], anti-peptide antibodies targeted to a region unique to a single P450 sequence are enzyme specific. We have used this approach successfully to produce antibodies against rat P450 enzymes CYP1A1 and CYP1A2 [7]. Depending on the amino acid sequence targeted, it is possible to design an anti-peptide antibody that will bind to the orthologous P450 from other species [8].

Here, we have raised an anti-peptide antibody against rat CYP1A2. We present evidence that the major epitope within the peptide coincides with a

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† Abbreviations: P450, cytochrome P450; KLH, keyhole limpet haemocyanin; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; POD, phenacetin *O*-deethylase.

region of the enzyme that is highly conserved in CYP1A2 from many species. Hence, the antibody will be of use in studies of the expression of CYP1A2 in a number of species, including humans.

MATERIALS AND METHODS

Materials. All SDS–polyacrylamide gel electrophoresis reagents were from National Diagnostics (Aylesbury, U.K.). Polystyrene 96-well microtitre plates were from Dynatech Laboratories (Billinghurst, U.K.), and conjugates of immunoglobulins with horseradish peroxidase were from ICN-Flow (High Wycombe, U.K.). Cyanogen bromide-activated Sepharose was from Pharmacia (Milton Keynes, U.K.). Pepsyn resin, 4-hydroxymethylphenoxyacetic acid, *N*-α-9-fluorenylmethoxycarbonyl amino acid pentafluorophenyl and 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine esters were purchased from Millipore Ltd (London, U.K.) or Calbiochem-Novabiochem (Nottingham, U.K.). Acetonitrile (grade S) was from Rathburn Chemicals (Walkerburn, U.K.). Keyhole limpet haemocyanin (KLH) was also from Calbiochem-Novabiochem. 2,3,5,6-D_4-Paracetamol was obtained from MSD Isotopes (Montreal, Canada). All other chemicals were purchased from the Sigma Chemical Co. (Poole, U.K.) or Merck-BDH (Lutterworth, U.K.) and were of analytical grade or the best equivalent.

Peptide synthesis, conjugation to carrier protein and production of antibodies. Synthesis of peptides, their conjugation to a carrier protein, KLH, and the immunization of rabbits were as described previously [9]. The peptides synthesized (Table 1) were Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-Asn-Tyr-Lys (peptide 1, the immunizing peptide), Thr-Gly-Ala-Leu-Phe-Lys-His-Ser (peptide 2), Leu-Phe-Lys-His-Ser (peptide 3) and Ser-Glu-Asn-Tyr-Lys-Asp-Asn (peptide 4). Cysteine was added to the *N*-terminus of peptides for the purpose of conjugation to KLH. A null conjugate (KLH–Cys) was produced by substituting cysteine for the thiolated peptide. All peptides were >90% pure as determined by reversed-phase high pressure liquid chromatography under the conditions described previously [9]. They had the correct composition by amino acid analysis and the expected molecular mass determined by fast atom bombardment mass spectrometry.

Antibodies against two other peptides were also used. The sequence Gly-Arg-Asp-Arg-Gln-Pro-Arg-Leu (peptide 5) occurs in both rat CYP1A1 and CYP1A2 and an antibody raised against this peptide reacts with both P450 enzymes [19]. In the dog, CYP1A1 and CYP1A2 both contain a very similar sequence (Gly-Arg-Ala-Arg-Gln-Pro-Arg-Leu), thus it is likely that this antibody will bind to both dog P450 enzymes. The sequence Gln-Asp-Arg-Arg-Leu-Asp-Glu-Asn (peptide 6) occurs in rat CYP1A1, but not rat CYP1A2 and an antibody raised against this peptide coupled to KLH through its *N*-terminus binds specifically to CYP1A1 in several species [8]. The equivalent region in CYP1A2 is quite different in all known sequences, including dog. The major epitope for this antibody is the *C*-terminal region Arg-Leu-Asp-Glu-Asn. The equivalent region of dog CYP1A1 (Gln-Asp-Lys-Arg-Leu-Asp-Glu-Asn) is very similar to that in the rat and contains the major epitope. Thus, this antibody would very likely bind to dog CYP1A1, but not to CYP1A2.

Immunological methods. Immunoblotting was performed using antiserum diluted 1:500 in 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) containing 0.1% (w/v) bovine serum albumin as described previously [9]. Enzyme-linked immunosorbent assay (ELISA) was performed as described before [9] using antiserum depleted of antibodies against carrier protein by affinity adsorption using KLH–Cys coupled to Sepharose as described previously [20]. ELISA microtitre plates were coated with 2 μg/mL of conjugates of KLH coupled to peptide or cysteine dissolved in PBS.

Treatment of animals, preparation of hepatic microsomal fractions. Male Wistar rats (200–250 g) were obtained from Harlan Olac Ltd (Bicester, U.K.) and male New Zealand White rabbits (3 kg) were from Froxfield Farms Ltd (Petersfield, U.K.). Male Balb/c mice (6–10 weeks) and male Dunkin–Hartley guinea pigs (250–300 g) were from A Tuck and Son Ltd (Battlebridge, U.K.). Male Golden Syrian hamsters (90–110 g) were supplied by Belgrave Trading Ltd (Lambourn, U.K.). Groups of animals were left untreated or were administered 80 mg/kg of 3-methylcholanthrene (MC) dissolved in corn oil. Groups of rats were also treated with sodium phenobarbitone, pregnenolone 16α-carbonitrile, clofibrate or isoniazid as described by Rich *et al.* [21]. Animals were killed humanely in accordance with approved Home Office procedures appropriate to the species, the livers were rapidly removed and microsomal fractions prepared as described previously [22]. Liver tissue from male greyhounds (24–28 kg) was kindly provided by the Department of Surgery (R.P.M.S., U.K.). Typically, these animals had received sodium thiopentone, halothane, gallamine, heparin and protamine sulphate within 6 hr of being killed. Liver tissue from minipigs was provided by Dr K. Moore (Department of Clinical Pharmacology, R.P.M.S., U.K.). Microsomal fractions were prepared from these liver samples as described above. Hepatic microsomal fractions from male marmosets that were untreated or treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were generously provided by Dr Thomas Schulz-Schalge (Institut für Toxikologie und Embryopharmakologie, FU Berlin, Germany).

Preparation of microsomal fractions from human liver. Human liver samples were obtained from the human tissue bank at the Royal Postgraduate Medical School [22]. Local Ethics Committee approval and Coroner's permission were obtained for their use in these studies. The preparation of the microsomal fraction was as described previously [22].

Measurement of phenacetin O-deethylase (POD) activity. High-affinity POD activity of hepatic microsomal fraction from MC-treated rats was determined using a phenacetin concentration of 4 μM as described by Murray and Boobis [23] incorporating the modifications of Edwards *et al.* [19]. Under these conditions POD reflects CYP1A2 activity [3].

Table 1. Aligned sequences of CYP1A2 from six species, rat CYP1A1, and four synthetic peptides compared with the predicted secondary structure of CYP1A2 in this region

Species	Sequence	Residues	Reference
Rat 1A2	-Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-------Asn-Tyr-Lys-	283–294	[10]
Mouse 1A2	- -Ser- - - - - - - - - ------- - - -	283–294	[11]
Hamster 1A2	- - - - - - - - - - ------- -Ser- -	283–294	[12]
Rabbit 1A2	- - - - - - - - - - -Lys- -Ser- -	283–295	[13]
Human 1A2	- - - - - - - - - -Lys-Lys-Gly-Pro-Arg-	284–296	[14]
Dog 1A2	- - - - -Leu- - -Asn- -Lys-Ser-Ser-Arg-	270–282*	[15]
Rat 1A1	- -Asp-Ser- - Ile -Glu- -Cys-Gln-Asp-Arg-Arg-Leu-	286–298	[16]
Peptide 1	Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-------Asn-Tyr-Lys	283–294	This paper
Peptide 2	Thr-Gly-Ala-Leu-Phe-Lys-His-Ser	283–290	This paper
Peptide 3	Leu-Phe-Lys-His-Ser	286–290	This paper
Peptide 4	Ser-Glu-------Asn-Tyr-Lys-Asp-Asn	290–296	[17]
Predicted structure	----------G-helix----------**********loop**********		[18]

The amino acid sequences, except for that of rabbit CYP1A2, are predicted from their cDNA sequences and are numbered from the *N*-terminal methionine or the first known amino acid residue. The synthetic peptides are numbered with respect to rat CYP1A2.

* The cDNA used to predict this sequence lacked an initiation codon, hence it is likely that the *N*-terminus is missing from this sequence making it 13 or 14 residues shorter than the other CYP1A2 sequences.

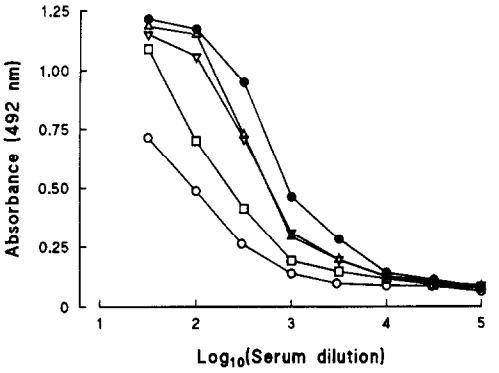

Fig. 1. Relative binding of antiserum to synthetic peptides. Microtitre plate wells were coated with conjugates of KLH coupled to peptide 1 (●), peptide 2 (△), peptide 3 (▽), peptide 4 (□), or cysteine (○). Antiserum, which had previously been substantially depleted of antibodies against KLH–Cys by adsorption using Sepharose-KLH–Cys as described in Materials and Methods, was added to the microtitre plate wells in a series of dilutions. Antibody binding was determined as described in the text. Each point is the mean of two measurements and the data shown are from a typical experiment representative of three experiments with similar results.

RESULTS

The binding of the anti-peptide antibody produced in this study to the immunizing peptide and truncated analogues was investigated in ELISA. The antibody bound to the peptide used to prepare the immunogen, and this was clearly strongest amongst the peptides tested (Fig. 1). In comparison, there was a decrease in binding to the peptide truncated at the *C*-terminus (peptide 2). However, binding to this peptide was similar to peptide 3, which was further shortened from the *N*-terminus (Fig. 1). Binding to the peptide corresponding to the five *C*-terminal residues of the immunizing peptide extended by Asp-Asn (peptide 4) was very weak, and was only slightly greater than to the null conjugate (Fig. 1).

The anti-peptide antibody was tested by immunoblotting for its ability to bind to proteins in hepatic microsomal fractions from a number of mammalian species. The antibody bound to a single protein band in hepatic microsomal fractions from untreated rat, mouse, rabbit, hamster, guinea pig, pig, marmoset monkey and human (Fig. 2). No binding was found with dog hepatic microsomal fraction (Fig. 2). Following treatment with MC, the intensity of the band in rat, mouse, rabbit, hamster and guinea pig was markedly increased (Fig. 2). A second, closely running band of higher molecular mass than CYP1A2 was also apparent in the guinea pig after MC treatment (Fig. 2). Several additional bands of lower molecular mass were seen in the MC-treated rabbit (Fig. 2). In this case, there may have been proteolytic fragmentation of rabbit CYP1A2, due to the high levels of the enzyme in the MC-treated animal. Immunotitration showed that the intensity of these bands was much lower than that of the intact enzyme (data not shown). Treatment of marmosets with TCDD resulted in an increase in the intensity of the band recognized by the antibody (Fig. 2). Neither pig nor dog was treated with MC or TCDD in these studies, so that the effects of polycyclic aromatic hydrocarbon inducers on antibody reactivity could not be determined in these species.

In immunoblotting the antibody raised against peptide 5 bound to a single band in three dog hepatic microsomal samples (Fig. 3a). The antibody also bound to one band in untreated rat liver, corresponding to CYP1A2 [19], and to two bands, corresponding to CYP1A1 and CYP1A2 [19], in liver from rats treated with MC. In contrast, an identical blot developed with an antibody raised against peptide 6, did not bind to any of the dog samples, nor to untreated rat liver (Fig. 3b). Only in the liver from MC-treated rat was there binding and this was to a single band corresponding to CYP1A1 [8].

The effects of the antibody to peptide 1 on the activity of CYP1A2 was investigated. In hepatic microsomal fraction from MC-treated rat, high affinity POD is catalysed exclusively by CYP1A2 [24]. Hence, POD activity of this microsomal fraction was used as a measure of CYP1A2 activity. Microsomal fraction was incubated with saturating amount of the antiserum. Control samples were incubated with the same amount (250 μL) of pre-immune serum. Control activity was 1081 ± 57 pmol/mg microsomal protein/min (mean ± SD, N = 3). In the presence of the antibody, POD activity was significantly ($P < 0.01$, Student's *t*-test) reduced, to 860 ± 17 pmol/mg microsomal protein/min (mean ± SD, N = 3), i.e. 20% inhibition. Increasing the amount of antibody present in the incubation produced no further reduction in enzyme activity.

DISCUSSION

An antibody was successfully targeted to CYP1A2 by immunizing a rabbit with a conjugate of the peptide Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-Asn-Tyr-Lys, corresponding to residues 283–294 of the rat enzyme. This sequence is unique to rat CYP1A2 amongst all of the proteins in the SWISS-PROT database [25] as of February 1993. Although, as expected, the anti-peptide antibody binds most strongly to the immunizing peptide (peptide 1), binding to peptide 2, which lacks the *C*-terminal 4 amino acids, is only slightly less. This demonstrates that the *C*-terminus is not critical for binding to the major epitope for this antibody. Further truncation of the peptide by removing the *N*-terminal 3 residues, yielding peptide 3—the central 5 residues of peptide 1 (Table 1), does not further affect binding, which is almost identical to peptide 2. This indicates that the major epitope for the antibody is at or around residues 286–290 of rat CYP1A2. In contrast to binding to these peptides, binding to peptide 4, corresponding to the 4 *C*-terminal residues of the immunizing peptide extended by Asp-Asn, is very poor. This confirms the above findings, that the *C*-terminal region of the peptide does not appear to contain a major epitope for the antibody, although there does appear to be a minor epitope in this region.

In immunoblotting, the antibody recognized a single protein band in hepatic microsomal fractions from rat, mouse, rabbit, hamster, guinea pig, pig,

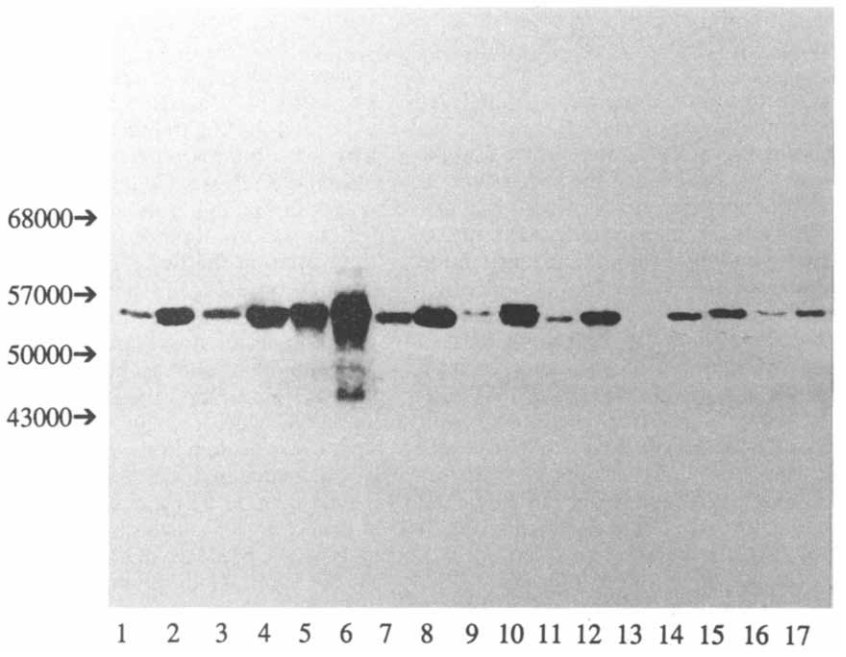

Fig. 2. Immunoblot showing the binding of an antibody raised against the peptide Thr-Gly-Ala-Leu-Phe-Lys-His-Ser-Glu-Asn-Tyr-Lys (peptide 1). Microsomal fractions were prepared from the livers of untreated animals and animals after treatment with MC or TCDD. To each lane 10 μg of protein was applied as follows: untreated rat (lane 1), MC-treated rat (lane 2), untreated mouse (lane 3), MC-treated mouse (lane 4), untreated rabbit (lane 5), MC-treated rabbit (lane 6), untreated hamster (lane 7), MC-treated hamster (lane 8), untreated guinea pig (lane 9), MC-treated guinea pig (lane 10), untreated marmoset (lane 11), TCDD-treated marmoset (lane 12), untreated dog (lane 13), untreated pig (lane 14), human sample 03007 (lane 15), human sample 03008 (lane 16), human sample 03009 (lane 17). The arrows indicate the positions of electrophoretic molecular mass standards. The immunoblot was developed with the anti-peptide antibody as described in the text. The results shown are representative of samples taken from at least three animals in each case, except for the pig where only two samples were obtained.

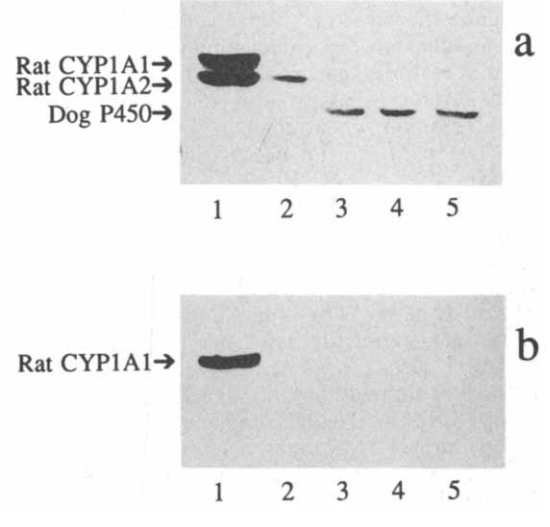

Fig. 3. Immunoblots showing the binding of antibodies directed to (a) CYP1A2 and CYP1A1, and (b) CYP1A1 specifically, in the rat and dog. To each lane was applied 25 μg of hepatic microsomal fraction prepared from MC-treated rat (lane 1), untreated rat (lane 2), and three untreated dogs (lanes 3–5). The immunoblot was developed with (a) an anti-CYP1A2/CYP1A1 antibody, or (b) an anti-CYP1A1 antibody, as described in the text. Only the central section of the blots, which contain the immunoreactive proteins, are shown.

marmoset and human. The bands correspond to CYP1A2 as determined from their relative electrophoretic migration, where known, and by comparison of their reactivity with other anti-CYP1A2 antibodies [9, 17, 19]. In the human samples, the intensity of the band varied amongst individuals (Fig. 2), in agreement with previous studies demonstrating marked interindividual variation (1–27 pmol/mg microsomal protein) in the level of expression of this enzyme [26]. In rat and rabbit the intensity of the immunoreaction was increased following treatment of the animals with MC in accordance with previous studies which showed an increase from 9 to 124 pmol/mg microsomal protein in the rat [3] and from 102 to 524 pmol/mg microsomal protein in the rabbit [27]. There was also an increase in immunoreactivity corresponding to CYP1A2 in mouse and hamster after treatment with MC in agreement with previous studies [28, 29]. Similarly, TCDD treatment of the marmoset increased the intensity of the immunoreactive band. This is consistent with the known effects of polycyclic aromatic hydrocarbons in this species [30]. Although only one band was apparent in the untreated guinea pig, following treatment of the animals with MC, two immunoreactive bands were visible. It is likely that the lower band, which was also apparent in the untreated animals, corresponds to CYP1A2. Whether the upper band is a variant of CYP1A2 or another P450

remains to be determined. Of the species studied, only in the untreated dog was there no binding of the antibody.

The relative electrophoretic migration of CYP1A1 has been determined previously in rat, rabbit, hamster and humans, using a number of specific antibodies [8, 19, 29]. In none of the species tested was there any binding of the anti-peptide 1 antibody to CYP1A1. This was not unexpected, as the region of CYP1A1 corresponding to the immunizing peptide is quite dissimilar in the rat (Table 1) and other species. In particular, the region corresponding to the major epitope at residues 286–290 of rat CYP1A2 varies in 3 out of 5 amino acid residues compared with rat CYP1A1.

In microsomal fractions from rats treated with inducers of other forms of P450, i.e. phenobarbitone, pregnenolone 16α-carbonitrile, clofibrate and isoniazid, the antibody also bound to only a single band corresponding to CYP1A2, although the intensity of the band was slightly reduced compared with untreated rats (results not shown).

The ability of the antibody to bind to CYP1A2 in a number of species is readily explicable by consideration of the amino acid sequences of the enzymes at the region corresponding to the immunizing peptide (Table 1). Mouse CYP1A2 differs by only one residue from the rat sequence, Ser instead of Gly, towards the *N*-terminus. The hamster enzyme is also very similar, with only a single amino acid difference from the immunizing peptide, Ser for Tyr towards the *C*-terminus. Neither of these single amino acid substitutions appears to have any significant effect on antibody binding.

In rabbit CYP1A2, there are two differences, both towards the *C*-terminus of the region, Ser for Tyr as found in the hamster, and also the insertion of Lys between Glu and Asn. Of particular interest, human CYP1A2 varies even more in the *C*-terminal region of this sequence, with all 5 of the residues at the *C*-terminus differing from those in the immunizing peptide. Despite these differences between rat CYP1A2 and the rabbit and human orthologues, the antibody clearly binds strongly to CYP1A2 in both rabbit and human. Thus, differences at the *C*- and *N*-termini of the sequence do not appear to have any significant effect on antibody binding. This is consistent with the location of the major epitope at residues 286–290 of rat CYP1A2 as indicated above. Indeed, this sequence is conserved in rat, mouse, hamster, rabbit and human CYP1A2.

Although the sequences of guinea pig and marmoset CYP1A2 are not known at present, the ability of the antibody to bind to these proteins indicates that the major epitope corresponding to residues 286–290 of rat CYP1A2 is also conserved in guinea pig and marmoset CYP1A2.

In contrast, the antibody does not appear to recognize dog CYP1A2. The region of canine CYP1A2 corresponding to the immunizing peptide differs by six residues. Four of these differences are at the *C*-terminus and are very similar to the differences seen in other species in which binding was retained. Hence, it is very unlikely that these changes have very much effect on immunoreactivity. However, the other two changes, Leu for Pro and Asn for Ser, occur in the region corresponding to residues 286–290 of rat CYP1A2. This is the only species in which there are any differences in these core residues. Based on the likely identity of the major epitope for the antibody, it is probable that these substitutions prevent binding of the antibody to canine CYP1A2. Confirmation of this conclusion by lack of binding in the dog would not be possible if there was an absence or low level of expression of the P450 in the liver. This seems unlikely as the antibody raised against peptide 5 which recognizes CYP1A2 and CYP1A1 bound to a single band in immunoblots of dog hepatic microsomal fractions indicating that one or both of these enzymes is present. However, the antibody raised against peptide 6, which recognizes only CYP1A1, did not bind to any protein in the dog microsomal fractions. This indicates that the band seen with the antibody against peptide 5 is indeed CYP1A2. In addition, Uchida *et al.* [15] have shown, by northern blotting analysis, the presence of mRNA coding for CYP1A2 but not CYP1A1 in the liver of untreated dogs.

We and others have previously found that anti-peptide antibodies tend to be directed towards the end of the peptide opposite that from the site of coupling to carrier protein [8, 31–33]. The peptide used here was coupled to KLH through its *N*-terminus, and so strong binding to the *C*-terminal region was anticipated. This was clearly not so. The reasons for the difference between the immunogenicity of this peptide and those used previously may be its size and secondary structure. The 12-residue peptide used here is larger than those used in our previous studies, in which peptides of <10 residues were used. It is possible that the 12-residue peptide adopts a relatively stable secondary structure in solution and that antibodies preferentially recognize this structure. Whatever the explanation, it is clear that larger peptide immunogens have more complex epitopes than smaller peptides and that the terminal directedness of antibodies, which appears to be common when small peptides are used as immunogens, need not necessarily apply to larger peptides.

In our model of the structure of eukaryotic P450 [18, 34], the region corresponding to the immunizing peptide comprises the *C*-terminal end of the G-helix and the loop region leading to the I-helix (there appears to be no H-helix in CYP1A). The ends of helices are frequently associated with stable turn structures [35]. The intensity of binding of the antibody in immunoblotting suggests that the major epitope is linear. Hence, it appears that the major epitope in CYP1A2 enzymes recognized by this antibody corresponds to a turn region adjacent to the G-helix (Table 1).

The antibody inhibits POD activity of hepatic microsomal fraction of MC-treated rats, a source of high levels of CYP1A2, by 20%. We have previously shown that an anti-peptide antibody raised against residues 290–296 of CYP1A2 (peptide 4) inhibited this activity by 65% [17]. Although the peptide used in the present study overlaps the pro-inhibitory region at residues 290–296, it appears that the major epitope for the antibody against residues 283–294 of rat CYP1A2 is located at residues 286–290, which is

adjacent to, but barely overlaps, the binding site of the inhibitory antibody. This localizes the pro-inhibitory region of this part of the enzyme to residues C-terminal to residue 290, i.e. more towards the I-helix than the G-helix.

In conclusion, we have produced an anti-peptide antibody which binds specifically to CYP1A2 in a number of species including human. This general reactivity with CYP1A2 can be explained by binding of the antibody to a sequence in the protein common to the CYP1A2 enzymes. The only exception is the dog, in which the putative binding site is altered. This antibody should be of general value in studies of the expression of CYP1A2 in many species.

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