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Identification of the human liver cytochrome *P*-450 responsible for coumarin 7-hydroxylase activity

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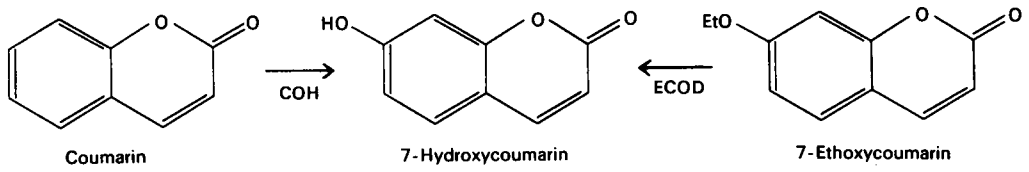
1. We have constructed a full-length human liver cytochrome P450IIA cDNA from a partial-length clone by oligonucleotide-directed mutagenesis, and subcloned it into the monkey kidney (COS-7) cell expression vector, pSVL. 2. The cDNA encodes a 49 kDa protein with coumarin 7-hydroxylase (COH) activity which cross-reacts with antisera to the mouse cytochrome *P*-450 isoenzyme responsible for COH activity and comigrates with a human liver microsomal protein. 3. Western blot analysis of a panel of human livers indicates that the level of the 49 kDa protein, detected using antisera to either the mouse COH *P*-450 or rat P450IIA1 protein, correlates very highly with COH activity. 4. Antisera to the rat P450IIA1 protein can inhibit COH activity in human liver microsomes. Taken together, these data indicate that a member of the P450IIA subfamily is responsible for most, if not all, of the COH activity in human liver.

INTRODUCTION

Cytochrome *P*-450-dependent mono-oxygenases (*P*-450s) are a supergene family of enzymes that catalyse the oxidation of lipophilic chemicals through the insertion of one atom of molecular oxygen into the substrate. They play a central role in the metabolism of a wide variety of endogenous molecules as well as foreign compounds (Wolf, 1986; Gonzalez, 1989). Coumarin occurs naturally in several plants, and has been used as an odour-enhancer in food and cosmetic preparations; derivatives such as bishydroxycoumarin (dicumarol) and warfarin are used therapeutically as anticoagulant agents. Metabolism of coumarin in man and mouse occurs by cytochrome *P*-450-mediated 7-hydroxylation, free or conjugated 7-hydroxy derivatives being the predominant excretion products (Scheme 1; Shilling *et al.*, 1969; Wood & Conney, 1974). In the rat, by way of contrast, the amount of excreted 7-hydroxycoumarin is less than 1% of the dose administered (Kaighen & Williams, 1961). Coumarin 7-hydroxylase (COH) activity is higher in human liver than in mouse or other species, and is virtually absent from the rat (Pelkonen *et al.*, 1985; Kaipainen *et al.*, 1985; Raunio *et al.*, 1988*a*).

The cytochrome *P*-450 responsible for COH has been purified from mouse liver (Kaipainen *et al.*, 1984; Juvonen *et al.*, 1985, 1988), and antisera towards it cross-react strongly with microsomal protein from human liver and effectively inhibit COH activity in both mouse and human (Raunio *et al.*, 1988*a*). However, these antisera do not cross-react with rat liver microsomal protein, nor do they inhibit the low level of COH activity present (Raunio *et al.*, 1988*b*). In these respects human COH appears more similar to that of mouse than rat. In the mouse, COH activity is strain-dependent and inherited as a single autosomal trait (Wood & Conney, 1974; Wood, 1979; Wood & Taylor, 1979). The *Coh* locus, encoding COH, is located on mouse chromosome 7 close to *Gpi-1* (glucose phosphate isomerase; Wood & Taylor, 1979). *P*-450 genes from the major phenobarbital-inducible P450IIB subfamily (*Cyp2b*) were found to map very close to *Coh*, there being no cross-overs in recombinant inbred mouse lines between *Cyp2b* and *Coh*, suggesting that COH was a member of this subfamily (Simmons & Kasper, 1983). However, anti-COH antisera do not cross-react with rat P450IIB protein, nor do anti-P450IIB antisera react with COH, indicating that COH is unrelated to the P450IIB subfamily (Raunio *et al.*, 1988*a*,*b*). [We have used the *P*-450 nomenclature system of Nebert *et al.* (1989) throughout this paper.]

In man the P450IIA gene subfamily (*CYP2A*) maps to chromosome 19q12 → 13.2, close to *GPI* and in a region which shows synteny homology with the part of mouse chromosome 7



Scheme 1. Metabolism of coumarin

Abbreviations used: COH, coumarin 7-hydroxylase, or cytochrome *P*-450 with this activity; ECOD, 7-ethoxycoumarin *O*-de-ethylase; DMEM, Dulbecco's modified Eagle's medium; SV40, simian virus 40.

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containing *Coh* (Davis *et al.*, 1986; Brook *et al.*, 1987; Nadeau & Reiner, 1989). We, and others, have subsequently mapped the human P450IIB gene subfamily (*CYP2B*) to this same region of chromosome 19 (Miles *et al.*, 1988; Santisteban *et al.*, 1988). Further work from this laboratory showed that in man *CYP2A* and *CYP2B* are present within 350 kb (Miles *et al.*, 1989), and that in mouse the P450IIA gene subfamily (*Cyp2a*) is very closely linked to *Cyp2b* and *Coh* (Miles *et al.*, 1990). From all of these data we suggested that in man and mouse COH was more likely to be a member of the P450IIA subfamily than the P450IIB subfamily (Miles *et al.*, 1989). Recently, Negishi and his co-workers have shown that in the mouse there are two members of the P450IIA subfamily (Type I and Type II testosterone 15α-hydroxylases) which differ in only 11 amino acid residues out of 494; the Type II enzyme is mouse COH (Lindberg *et al.*, 1989; Negishi *et al.*, 1989). In this paper we show that COH is also encoded by a member of the P450IIA gene subfamily in man.

EXPERIMENTAL

Cell lines, plasmids, bacteriophages and *Escherichia coli* strains

COS-7 (transformed monkey kidney) cells were kindly donated by Professor B. Burchell, Department of Biochemical Medicine, Ninewells Hospital, Dundee, Scotland, and were maintained under standard cell culture conditions in Dulbecco's modified Eagle's medium (DMEM). Plasmid pMP81 containing a partial-length human P450IIA cDNA has been described previously (Miles *et al.*, 1989), and the simian-virus-40 (SV40)-based expression vector, pSVL, was purchased from Pharmacia–LKB. Bacteriophage M13mp18 was from Boehringer and was propagated in *E. coli* strain JM101 for sequencing (Messing, 1979) and in strain BW313 (*dut ung*) for the production of uracil-containing templates (Kunkel, 1985).

Oligonucleotide-directed mutagenesis

The 'two-primer-extension-plus-ligation' method for mutagenesis was performed with the 54 mer oligonucleotide P1 (see Fig. 1 for sequence) and universal primer in accordance with Zoller & Smith (1984), except that a uracil-containing template was used (Kunkel, 1985). Mutant strand selection was by transfection of *E. coli* JM101 (*ung*$^+$) cells. Mutant phages were identified by the dot-hybridization procedure with the ^{32}P-labelled mutagenic primer as a probe (Zoller & Smith, 1983), and their sequences were confirmed using a series of synthetic primers and the dideoxy chain termination method with [α-^{35}S]thio-dATP (Sanger *et al.*, 1980; Biggin *et al.*, 1983; Miles *et al.*, 1989).

Transfection of COS-7 cells

Plasmids containing (1) full-length (pMP113 and pMP114), (2) partial-length, missing the first four codons (pMP103) and (3) no P450IIA cDNA (pSVL) were purified by CsCl gradient-density centrifugation. DNA (40 μg) was used to transfect COS-7 cells in 25 cm^2 flasks by the calcium phosphate procedure (Parker & Stark, 1979) essentially as described by Gorman (1986), except that no glycerol was used. A period of 24 h was allowed for the cells to recover following transfection before new DMEM was added. Cells were harvested by trypsin treatment at 24, 48 or 72 h after refeeding and resuspended in 0.5 ml of 10 mM-sodium phosphate (pH 8.0)/2 mM-$MgCl_2$/2 mM-dithiothreitol/1 mM-EDTA. Samples for Western blot analysis were hand-homogenized and separated into crude pellet and supernatant fractions by centrifugation; samples for COH assays were prepared by sonication using an MSE Soniprep (two 5 s bursts at an amplitude of 12 μm with samples kept on ice).

Other molecular biology methods

DNA manipulations, bacterial transformations, RNA preparation and Northern blots were by standard methods (Maniatis *et al.*, 1982).

Preparation of human liver microsomes

Human liver samples were obtained post-mortem from kidney transplant donors and the panel used in these experiments have been described previously (Hall *et al.*, 1989). Microsomal fractions were prepared as described by Adams *et al.* (1985). Protein determinations were made according to the procedure of Lowry *et al.* (1951).

Western blot analysis

Proteins were separated by SDS/PAGE (Laemmli, 1970), transferred to nitrocellulose and probed with antibodies as described by Towbin *et al.* (1979) and modified by Lewis *et al.* (1988). Antibodies to mouse COH and rat P450IIA1 (Levin a) have been described previously (Wolf *et al.*, 1986; Raunio *et al.*, 1988*a*). Cross-reacting protein was quantified for comparative purposes by scanning the autoradiographs using a Joyce Loebl Chromoscan 3 laser densitometer. Standard curves establishing the linearity of the method were made by comparing the band intensities obtained on serial dilution of a microsomal sample. Molecular masses were estimated using 'rainbow' markers from Amersham International as standards. For the human liver microsomal preparations 7.5 μg of protein was analysed, whereas for COS cell transfectants 100 μg of crude supernatant or membrane fractions was used.

COH assays

The method of Creaven *et al.* (1965), which distinguishes 7-hydroxycoumarin from 3-hydroxycoumarin, was modified as follows. COH activity was measured in reaction mixtures (0.5 ml) containing 25 mM-potassium phosphate, pH 7.2, 2.5 mM-$MgCl_2$, 50 μM-coumarin and 750 μM-NADPH. For human liver microsomal preparations 50 μg of protein was used, whereas for the transfected cells 350 μl of the disrupted cell suspensions containing between 1.1 and 3.4 mg of protein was used. The reactions were carried out for 15 min at 37 °C for the microsomal preparations, or for various time periods for the cell preparations. Reactions were stopped by the addition of 0.5 ml of 6% (w/v) trichloroacetic acid, and precipitated protein was removed by centrifugation. The supernatant (0.5 ml) was then added to 3 ml of 0.8 M-Tris/0.8 M-glycine, pH 9.0. The amount of 7-hydroxycoumarin formed from coumarin was measured fluorimetrically at $\lambda_{em.} = 460$ nm and $\lambda_{ex.} = 376$ nm using a Perkin–Elmer LS-3 fluorescence spectrometer, and compared with 7-hydroxycoumarin standards made up in the same reaction/stop mixture but with no protein added.

Materials

Amersham International supplied [α-^{32}P]dATP, [α-^{35}S]thio-dATP, [γ-^{32}P]ATP and ^{125}I-Protein A. Coumarin and 7-hydroxycoumarin were from Aldrich Chemical Co. Other reagents were from the usual suppliers.

RESULTS

Construction of a full-length human P450IIA cDNA

We have cloned and sequenced previously a member of the human P450IIA subfamily (pMP81) which, by comparison with mouse P450IIA cDNAs, appeared to lack the first four codons (Miles *et al.*, 1989). This supposition was confirmed when

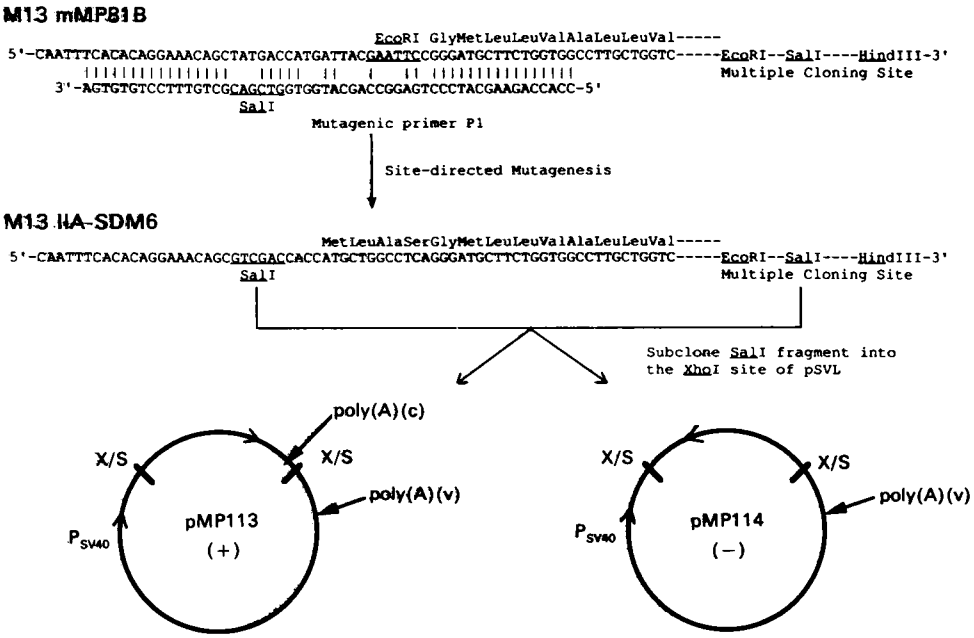

Fig. 1. Construction of a full-length human P450IIA cDNA

The 54-mer oligonucleotide P1 was used to introduce a *Sal*I site, 5 bp of 5′ untranslated region and the first four codons of the human P450IIA cDNA into the M13 clone mMP81B by site-directed mutagenesis generating IIA-SDM6. The 1.8 kb *Sal*I fragment from IIA-SDM6 was subcloned into pSVL, the SV40-based COS cell expression vector in both orientations to give pMP113 (forward) and pMP114 (reverse). The *Xho*I/*Sal*I (X/S) hybrid junction, polyadenylation signals in the cDNA (c) and vector (v) and the SV40 promoter (P_{SV40}) are shown.

Yamano *et al.* (1989) described a full-length human P450IIA cDNA which contains four additional codons and differs from the pMP81 sequence by only three nucleotides in the coding region and two in the 3′ non-coding region. We filled in the ends of the partial-length *Eco*RI fragment of pMP81 and subcloned it into the *Sma*I site of the SV40-based vector pSVL in both possible orientations to give pMP103 (reverse orientation) and pMP104 (forward orientation). Plasmid pMP103 has been used as a negative control in transfection experiments (see below), but rather than try and express the partial-length cDNA, it was decided that a full-length clone was required for functional studies.

Instead of cloning a full-length version of pMP81 cDNA from a library, we have introduced the missing four codons, derived from Yamano *et al.* (1989), using oligonucleotide-directed mutagenesis (Fig. 1). The 1.76 kb *Eco*RI fragment from pMP81 was subcloned into M13mp18 to give mMP81B, such that the coding region was on the same strand as the *lacZ′* gene of the vector. The 54-mer oligonucleotide, P1, was designed to introduce the missing codons plus 5 bp of the 5′ non-coding sequence considered important for translational initiation plus a *Sal*I site to help in subcloning (Fig. 1). A uracil-containing single-stranded DNA template of mMP81B, prepared by the method of Kunkel (1985), was used in the mutagenesis method of Zoller & Smith (1984) with mutagenic oligonucleotide P1 and universal primer. From the 17 plaques recovered, 12 were positive when probed with mutagenic oligonucleotide. Four of the positive clones were plaque-purified, and one (IIA-SDM6) was chosen for further analysis. Sequencing the whole insert of IIA-SDM6 showed that the desired mutations were the only ones to have taken place.

Replicative-form DNA of IIA-SDM6 was made and the 1.8 kb *Sal*I fragment containing the full-length cDNA was subcloned into the unique *Xho*I site of pSVL (Fig. 1). Plasmids containing inserts with the forward and reverse orientations for expression were named pMP113 and pMP114 respectively, and were used together with pMP103 in subsequent experiments.

Expression of P450IIA in COS-7 cells

Western blot analysis of COS-7 cells using anti-COH (mouse P450IIA) or anti-(rat P450IIA1) antiserum or Northern blot analysis with pMP81 cDNA as a probe indicates that these cells contain little or no endogenous P450IIA-related protein or mRNA, and therefore are suitable recipient cells for expressing the human P450IIA cDNA (Fig. 2, lanes 0). Several other groups have found COS cells suitable for the expression of *P*-450s, as they contain active *P*-450 reductase (see Gonzalez, 1989, for a review). RNA was isolated from mock-, pMP113- and pMP114-transfected cells and subjected to Northern blot analysis with pMP81 as a probe. Fig. 2(*a*) shows that pMP113- and pMP114-transfected cells contain messages which hybridize to the P450IIA cDNA, whereas the mock-transfected cells do not. The size of the transcript in the pMP113-transfected cells (2.4 kb) is consistent with the use of the cDNA-encoded polyadenylation signal, whereas pMP114, transcribing the opposite strand, uses the polyadenylation signal present in the vector to give a message of 3.6 kb (Figs. 1 and 2*a*).

Crude supernatant and pellet fractions from mock-, pMP103- and pMP113-transfected cells were analysed by Western blotting using anti-COH antiserum. [Initially we had difficulty cloning the *Sal*I fragment from IIA-SDM6 in the reverse orientation into pSVL to give pMP114, so pMP103 was used as the negative control. Later, Western blots with pMP114-transfected cells indicated that no anti-P450IIA cross-reacting protein was produced (results not shown).] Only those cells harbouring plasmid pMP113 expressed a cross-reacting protein. This protein was largely in the pellet (membrane) fraction and comigrates with the major protein identified with the antibody in human liver microsomes (49 kDa; Fig. 2*b*). From both Northern and

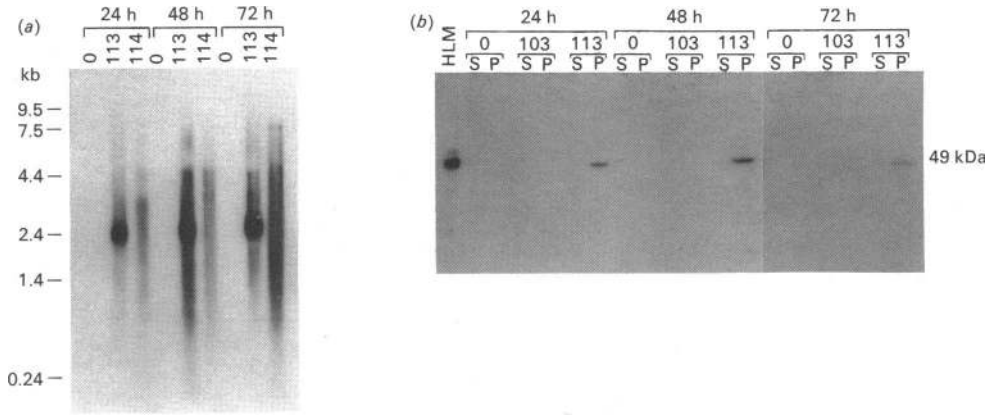

Fig. 2. Expression of human P450IIA in COS cells

(*a*) Northern blot analysis of RNA (6 μg) from mock-, pMP113- and pMP114-transfected cells (designated 0, 113 and 114 respectively) taken at 24, 48 and 72 h after refeeding and hybridized with the human P450IIA cDNA, pMP81, as a probe. No message is detected in mock-transfected cells; the 2.4 kb message in the pMP113-transfected cells is consistent with the use of the cDNA polyadenylation signal. The message from pMP114 (reverse orientation) is more degraded than that from pMP113, probably due to instability of the normally non-transcribed strand, but the mRNA at 3.6 kb is consistent with the use of the vector polyadenylation signal. (*b*) Western blot analysis of protein (100 μg) from crude supernatant (S) and pellet (P) fractions of mock-, pMP103- and pMP113-transfected cells (designated 0, 102 and 113 respectively) using anti-COH antisera. HLM is 7.5 μg of human liver microsomal protein (LIV). The 49 kDa protein, expressed only by the pMP113-transfected cells, is associated predominantly with the pellet fraction and comigrates with the protein present in human liver microsomes.

Western blot analysis, it is seen that the amount of P450IIA message and protein increases up to 48 h but then declines (Fig. 2); 48 h samples were used for further analysis.

COH activity of transfected COS-7 cells

The COH activity of sonicates from mock-, pMP113- and pMP114-transfected cells was measured in the 48 h samples. The mean activity present in triplicate transfections, determined following a 30 min incubation, was [in pmol·min^{-1}·(mg of protein)$^{-1}$]: 0.06 for the mock-transfected cells, 0.23 for the pMP114-transfected cells and 6.8 for the pMP113-transfected cells. This level of activity in the expressing (pMP113-transfected) cells is comparable with that obtained by Negishi *et al.* (1989) with the mouse Type II P450IIA (COH) cDNA when expressed in COS cells [14 pmol·min^{-1}·(mg of protein)$^{-1}$]. Why the pMP114-transfected cells should show a higher fluorescence than the mock-transfected cells is unclear. The formation of 7-hydroxycoumarin by the pMP113-transfected cells was time-dependent, but it is not linear over a 45 min time period, suggesting that the protein loses activity on incubation in the assay conditions (results not shown). It was also found that

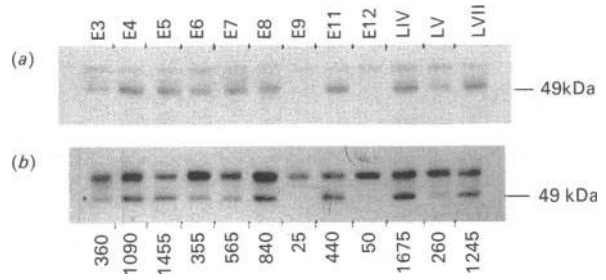

Fig. 3. Correlation of COH activity with the 49 kDa P450IIA protein in human liver microsomes

Microsomal preparations (7.5 μg of protein) from the panel of human livers were analysed by Western blotting using (*a*) anti-COH (mouse P450IIA) antiserum and (*b*) anti-(rat P450IIA1) antiserum. The COH activity of each sample was determined and is given below (b) in pmol·min^{-1}·(mg of protein)$^{-1}$. The amount of 49 kDa protein, which comigrates with that expressed from pMP113 in COS cells and is detected by both antisera, correlates very highly with COH activity.

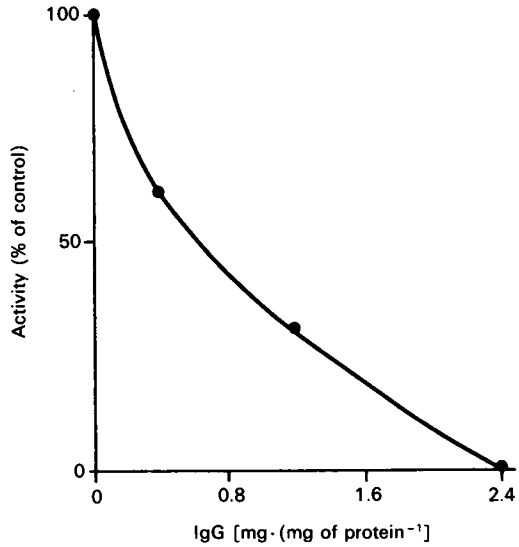

Fig. 4. Inhibition of human liver microsomal COH activity by anti-UT$_1$ antiserum

COH assays were carried out as described in the Experimental section, with the exception that the rat anti-P450IIA antibody or pre-immune serum were incubated with the microsomal fraction for 15 min at 4 °C prior to the addition of the other assay components. The amounts of serum were always adjusted to the same value by the addition of pre-immune serum. Data are expressed as % of control rates obtained in the presence of pre-immune serum; however, pre-immune serum had no significant effect on the reaction rate. The experiment shown was carried out using sample E8 (Fig. 3). The experiment was also carried out on LIV and two other liver samples from another panel. At 2.4 mg of IgG·(mg of microsomal protein)$^{-1}$, 100 % inhibition was observed in each case.

freezing and thawing the sonicated cell samples led to approx. 10-fold decrease in specific COH activity (results not shown).

These cDNA transfection experiments show that a member of the human P450IIA subfamily will catalyse the 7-hydroxylation of coumarin. In order to determine whether the P450IIA subfamily is the major contributor to this activity, we have undertaken an analysis of a panel of human livers.

Correlation of COH activity in human liver with anti-P450IIA cross-reactivity

Antisera raised to the purified rat *P*-450 P450IIA1 and the mouse COH *P*-450 (P450IIA subfamily) were used to probe Western blots of a panel of twelve human liver microsome preparations (Fig. 3). The anti-P450IIA1 serum recognizes two proteins in human liver microsomes of 49 kDa and 55 kDa (Fig. 3*a*). Three proteins are recognized in human liver microsomes by anti-COH (49, 51 and 55 kDa; Fig. 3*b*), the 49 kDa protein migrating with that encoded by pMP113 (Fig. 2*b*). It is clear from Fig. 3 that samples E9 and E12 have the lowest amount of cross-reacting 49 kDa protein detected by both antisera, and that sample LIV has the highest. Indeed, the very high correlation between the amount of 49 kDa protein cross-reacting with both antisera is given by the Spearman rank correlation coefficient, $r_s = 0.921$ ($P < 0.001$), thus suggesting that the two antisera are recognizing the same 49 kDa protein. In support of this, the anti-P450IIA1 serum also recognizes the same protein detected by anti-COH in the pMP113-transfected cells, albeit less strongly (results not shown). Fig. 3(*c*) shows the COH activity associated with each of the samples; the lowest levels of activity being found in E9 and E12 and the highest in LIV. The correlation between COH activity and the 49 kDa protein detected by anti-COH is $r_s = 0.923$ ($P < 0.001$), and that between COH activity and the 49 kDa protein detected by anti-P450IIA1 is $r_s = 0.837$ ($P < 0.001$). There is no statistically significant correlation between the levels of the 49 kDa protein in the human liver panel and the metabolism of methoxyresorufin, ethoxyresorufin, benzyloxyresorufin, 2-aminofluorene to mutagenic products, testosterone at the 6β position or mephenytoin (results no shown).

COH activity can be inhibited completely in human liver microsomes using the anti-P450IIA1 serum (Fig. 4). Previous studies with the anti-COH antiserum have shown that it can also almost completely inhibit COH activity in human liver microsomes, and can also inhibit 7-ethoxycoumarin *O*-deethylase (ECOD) by about 40% (Scheme 1; Raunio *et al.*,

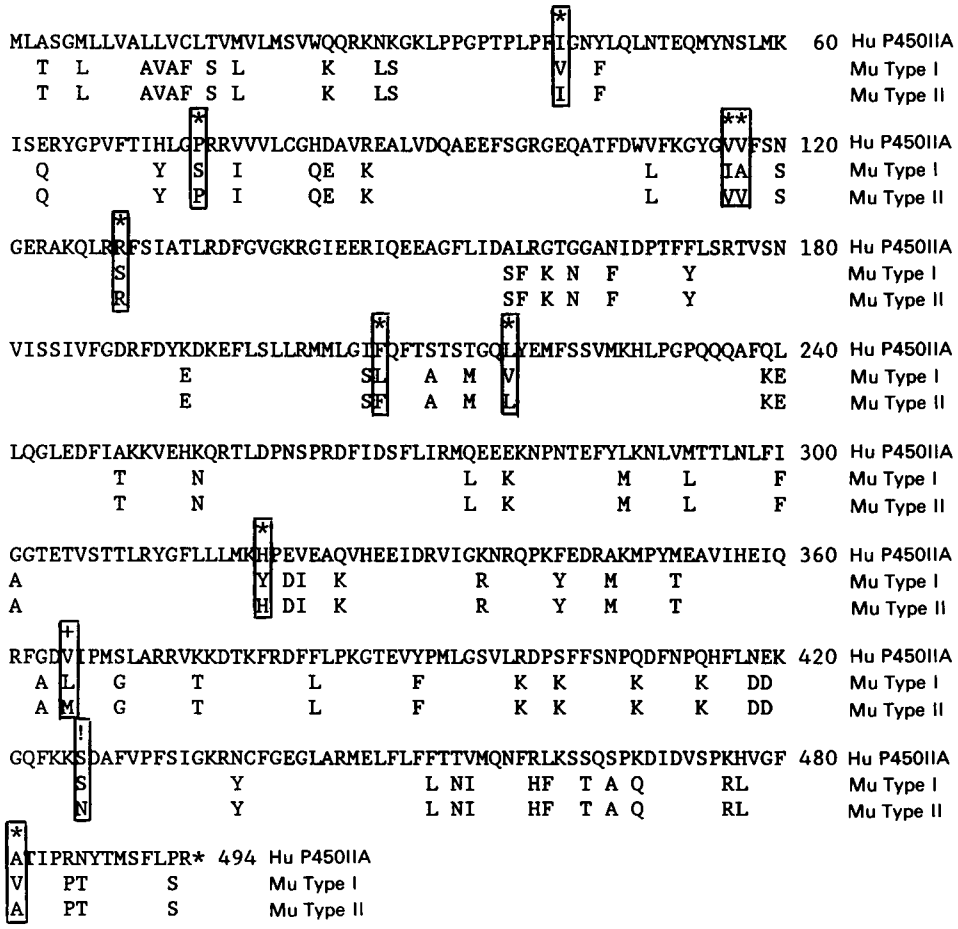

Fig. 5. Comparison of the human P450IIA amino acid sequence with mouse Type I and Type II P450IIA sequences

The human P450IIA amino acid sequence is shown in full, but only those residues that differ are given in the mouse sequences. The 11 amino acid residues that differ between the Type I (testosterone 15α-hydroxylase) and Type II (COH) sequences are highlighted by boxes. ! indicates that the human sequence has the same residue as Type I; * indicates it has the same residue as Type II; and + that it has a residue different to both at these positions. Two of the three residues that are important for COH activity in the mouse Type II protein (Val-117 and Phe-209) are conserved in the human P450IIA sequence, including the critical Phe-209 residue.

1988*a*). These findings, coupled with the high correlation of COH activity with levels of the 49 kDa protein reacting with anti-P450IIA antisera and with the ability of a P450IIA cDNA to express COH activity, strongly suggest that, in man, a member of the P450IIA subfamily is responsible for most, if not all, of the COH activity.

DISCUSSION

The cDNA transfection experiments coupled with the immunological data strongly suggest that a member of the P450IIA subfamily is responsible for most, if not all, of the COH activity in human liver microsomes. Based on Southern blot analysis and cDNA sequencing, there are probably two or three genes within the P450IIA subfamily (Phillips *et al.*, 1985; Miles *et al.*, 1989), so although the pMP81 cDNA encodes COH activity, our results do not preclude the possibility that there is another member of the P450IIA subfamily with this activity. In the mouse there are two P450IIA proteins which differ in only 11 residues out of 494 (Fig. 5). The Type I sequence has testosterone 15α-hydroxylase activity but no COH activity, whereas the Type II sequence has COH activity but only 1% of the testosterone 15α-hydroxylase activity of the Type I sequence (Negishi *et al.*, 1989; Lindberg *et al.*, 1989; Lindberg & Negishi, 1989). Of the 11 amino acid differences between the Type I and Type II proteins, the pMP81-encoded human P450IIA sequence has nine of the residues associated with Type II (COH), one which is associated with Type I, and one which differs from both Type I and Type II (Fig. 5). More interestingly, two out of the three residues important for COH activity in the mouse Type II sequence, as deduced from site-directed mutagenesis experiments (Val-117 and Phe-209; Lindberg & Negishi, 1989), are conserved in the human P450IIA sequence, including the critical Phe-209 residue. There is 83% overall amino acid sequence identity between the mouse and human P450IIA proteins, one of the highest degrees of similarity between human and rodent drug-metabolizing *P*-450s noted (Miles *et al.*, 1989). This present work shows that there is also conservation of substrate specificity, and in this context it will be interesting to explore the degree of functional similarity of the mouse and human proteins by making the corresponding site-directed mutants in the human proteins which convert COH to testosterone 15α-hydroxylase in the mouse. Similarly, it will be interesting to determine whether there is any P450IIA-related testosterone 15α-hydroxylase activity in human liver.

We have noted previously that there is considerable inter-individual variation of P450IIA-related transcripts in human liver, and because they tend to vary with P450IIB-related transcripts, we suggested that the P450IIA and P450IIB genes may be regulated by common factors in man (Miles *et al.*, 1989). There is considerable variation in the level of the 49 kDa P450IIA protein and COH activity in the human liver samples (up to 80-fold and 70-fold respectively; Fig. 3), suggesting that expression of the protein may be regulated by environmental factors. However, genetic factors may also influence the level of expression of the P450IIA protein. A restriction-fragment-length polymorphism is associated with the *CYP2A* locus in man (Wainwright *et al.*, 1985), but whether this is linked to the levels of expression of COH is not known. It is interesting to note that the metabolism of the coumarin derivative, dicumarol, appears to be under genetic control (Vessell & Page, 1968). With the expression of the human P450IIA isoenzyme responsible for COH activity in COS cells, it will now be possible to explore its activity towards various coumarin derivatives and other xenobiotics and to begin to investigate its potential role in chemical carcinogenesis.

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