Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8381–8385, November 1988
Biochemistry

Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells

(immunoisolation/alkylphenols/glycoprotein)

DAVID HARDING, SYLVIE FOURNEL-GIGLEUX, MICHAEL R. JACKSON, AND BRIAN BURCHELL

Department of Biochemistry, Medical Sciences Institute, The University, Dundee, DD1 4HN, Scotland

Communicated by Allan H. Conney, June 30, 1988

ABSTRACT **A rat kidney phenol UDP-glucuronosyltransferase cDNA was used to isolate a human liver phenol UDP-glucuronosyltransferase cDNA by screening of a human liver cDNA library in the expression vector λgt11. The 2.4-kilobase cDNA contained an open reading frame of 1593 base pairs coding for a protein of 531 residues. The human liver cDNA was subcloned into the vector pKCRH2. Transfection of this recombinant plasmid into COS-7 cells allowed the expression of a protein of ≈55 kDa. The enzyme synthesized was a glycoprotein, as indicated by a reduction in molecular mass of ≈3 kDa after biosynthesis in the presence of tunicamycin. The expressed enzyme rapidly catalyzed the glucuronidation of 1-naphthol, 4-methylumbelliferone, and 4-nitrophenol. The use of a related series of simple phenols provided an outline description of the substituent restrictions imposed upon the phenolic structures accepted as substrates. The glucuronidation of testosterone, androsterone, and estrone was not catalyzed by this cloned UDP-glucuronosyltransferase.**

Human hepatic microsomal UDP-glucuronosyltransferase [UDPGT; UDPglucuronate β-D-glucuronosyltransferase (acceptor-unspecific); EC 2.4.1.17] has a major role in the conjugation and production of more hydrophilic glucuronides for excretion of drugs, xenobiotics, and endogenous compounds (1). Overwhelming evidence indicates that in rat liver these glucuronidation reactions are catalyzed by a family of isoenzymes. The existence of UDPGT isoenzymes that would specifically glucuronidate bilirubin, bile acids, 5-hydroxytryptamines, and drugs (such as morphine, clofibrate, and chloramphenicol) has been suggested from kinetic analysis of *in vitro* enzyme assays of microsomes prepared from adult (2–6) and developing (7) liver. Inherited defects of bilirubin UDPGT in Crigler–Najjar syndrome (8) have also indicated the existence of different UDPGT isoforms. By using antibodies prepared against purified rat UDPGT antigens, which inhibit several human liver microsomal UDPGT activities, at least five UDPGTs have been detected in human liver microsomes by immunoblot analysis (9, 10). Human liver microsomal UDPGTs have proved difficult to purify, although two distinct UDPGTs with similar properties have been purified to apparent homogeneity (11).

The further analysis of the multiplicity, genetic deficiency, regulation, and development of human UDPGTs requires the use of molecular probes for DNA, RNA, and proteins, and these can be obtained by molecular biological techniques without the need for routine supplies of human liver. The previous expression of a cloned rat UDPGT cDNA (12) has been shown to define distinctive substrate specificities avoiding the possibility of very homologous contaminating UDPGTs, which could occur even after the most rigorous purification procedures. Mechanisms of drug transport, glucuronidation, and excretion can also be studied by using the transferases expressed in cells in culture.

In this paper we report the molecular cloning and sequence of a human liver UDPGT cDNA.* Expression of the cDNA in COS-7 cells and the substrate specificity studies indicate that a phenol UDPGT was encoded by this clone and provide evidence of certain structural restrictions imposed on potential substrates.

MATERIALS AND METHODS

Materials. Anti-UDPGT serum and anti-epoxide hydrolase (EH) serum were prepared as reported (13). ^{32}P- and ^{35}S-labeled nucleotides, [^{35}S]methionine (1000 Ci/mmol; 1 Ci = 37 GBq), [^{3}H]styrene oxide (161 mCi/mmol), [^{14}C]UDP-glucuronic acid (272 mCi/mmol), restriction endonucleases, enzymes, and materials used in sequencing were from Amersham. Tunicamycin and several enzyme substrates were from Sigma. Other UDPGT substrates were obtained from Aldrich or BDH. Silica gel 60 F_{254} plates were from Merck. COS-7 cells (14) were kindly provided by Clare Madin (School of Pathology, Oxford University, Oxford). pKCRH2 (15) was obtained from S. Shibahara (Freidrich Miescher Institüt, Basel). A human liver cDNA library (16) was kindly provided by S. Woo (Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, TX).

METHODS

Isolation of Human Liver UDPGT cDNAs. A full-coding-length rat kidney phenol UDPGT cDNA (D.H. and B.B., unpublished work) was ^{32}P-labeled by the method of Feinberg and Vogelstein (17) and used as a hybridization probe to plaque-screen (18) the human liver cDNA library in phage λgt11 (16), at 58°C in 6× SSPE (19). cDNA inserts from the plaque-purified recombinant phage were subcloned into pUC18 and M13mp18 or -19 (20) for restriction analysis and sequencing by a modified procedure of Sanger's dideoxy method (21, 22). Human microsomal EH cDNA was cloned in this laboratory (23).

The full-coding-length cDNA was excised from M13mp18 with *Eco*RI, reisolated with *Hin*dIII, and subcloned into the *Hin*dIII site of the tissue culture expression vector pKCRH2. Recombinant plasmids were grown in *Escherichia coli* DH1 and plasmids with cDNAs in the correct orientation with respect to transcription from the simian virus 40 early gene were identified by restriction mapping. Plasmid DNA (40 μg) was used to transfect each semiconfluent dish (150 cm^2) of COS cells by calcium phosphate/glycerol shock procedure

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: UDPGT, UDP-glucuronosyltransferase; EH, epoxide hydrolase.

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J04093).

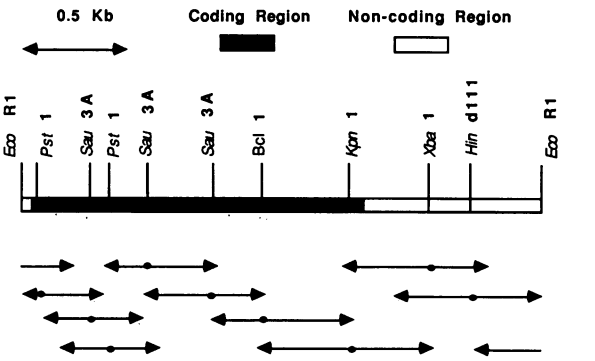

FIG. 1. Partial restriction map and nucleotide sequencing strategy of the phenol UDPGT cDNA insert. The extent and direction of overlapping sequencing are shown by the horizontal arrows. Kb, kilobase(s).

(24) or "lipofection" (25). The cells were harvested 72 hr later, washed with 0.15 M NaCl/5 mM Tris·HCl, pH 7.4, (TBS) and disrupted by gentle hand homogenization in a ground-glass homogenizer. The cell homogenate was assayed for multiple UDPGT activities by TLC as described (26) by incubating 0.1 mg of cell protein, 0.5 mM aglycone, and 18 μM [^{14}C]UDP-glucuronic acid in 100 mM Tris maleate/10 mM $MgCl_2$, pH 7.4, for 30 min at 37°C. Conventional assays with 1-[^{14}C]naphthol as described (27) were also used to assess the success of the transfections. EH was assayed with [^{3}H]styrene oxide as described (28).

Labeling and Immunoprecipitation. COS cells in 20-cm^2 dishes were washed 72 hr after transfection with methionine-free RPMI 1640 medium containing 10% (vol/vol) dialyzed fetal bovine serum and then incubated with 7.5 ml of the same medium in the presence or absence of tunicamycin (2 μg/ml) for 1 hr. This medium was subsequently removed and replaced by 1 ml of the same medium supplemented with 50 μCi of [^{35}S]methionine (1420 Ci/mmol), and cells were incubated for 4 hr. Cells were rinsed twice with ice-cold PBS (138 mM NaCl/2.7 mM KCl/1.5 mM KH_2PO_4/8 mM Na_2HPO_4, adjusted to pH 7.4 with HCl). Cells were harvested and centrifuged at 1500 × *g* for 5 min. The pellets were resuspended and washed with PBS by centrifugation. The pellet was solubilized with 0.5% deoxycholate, 1% Triton X-100, plus 0.1% sodium dodecyl sulfate (SDS) in 10 mM Tris·HCl (pH 7.4) by gentle hand homogenization. The mixture was centrifuged at 16,000 × *g* and the supernatant was incubated with preimmune serum and in sheep anti-rat UDPGT antiserum overnight in ice as described (29). The final products were analyzed by SDS/PAGE on 7.5% polyacrylamide gels (30) followed by fluorography (31).

RESULTS AND DISCUSSION

Isolation and Sequencing of cDNA Clones Encoding a Human Phenol UDPGT. Sixteen recombinant plaques were identified after screening 100,000 recombinants of the λgt11 human liver cDNA library with the ^{32}P-labeled full-coding-length rat kidney phenol cDNA. The sizes of the cDNA inserts were determined on agarose gels and the largest cDNA (≈2.4 kilobases) was further analyzed. The subcloned cDNA insert was restriction-mapped and sequenced according to the strategy indicated in Fig. 1. The nucleotide

```
                TAAAGTAACAACGGTAATAAAGAGTTTTCACTCTCCAAATCACAGGGCTCCTAGGTATTTTCCCTAAACTAACCACTGCTTTCCAGG     -1

ATG GCC TGC CTC CTT CGC TCA TTT CAG AGA ATT TCT GCA GGG GTT TTC TTC TTA GCT CTT TGG GGC ATG GTT GTA GGT GAC AAG CTG CTG     90
Met Ala Cys Leu Leu Arg Ser Phe Gln Arg Ile Ser Ala Gly Val Phe Phe Leu Ala Leu Trp Gly Met Val Val Gly Asp Lys Leu Leu
                            10                            20                            30
GTG GTC CCT CAG GAC GGA AGC CAC TGG CTT AGT ATG AAG GAT ATA GTT GAG GTT CTC AGT GAC CGG GGT CAT GAG ATT GTA GTG GTG GTG    180
Val Val Pro Gln Asp Gly Ser His Trp Leu Ser Met Lys Asp Ile Val Glu Val Leu Ser Asp Arg Gly His Glu Ile Val Val Val Val
                            40                            50                            60
CCT GAA GTT AAT TTG CTT TTG AAA GAA TAC AAA TAC TAC ACA AGA AAA ATC TAT CCA GTG CCG TAT GAC CAA GAA GAG CTG AAG AAC CGT    270
Pro Glu Val Asn Leu Leu Leu Lys Glu Ser Lys Tyr Tyr Thr Arg Lys Ile Tyr Pro Val Pro Tyr Asp Gln Glu Glu Leu Lys Asn Arg
                            70                            80                            90
TAC CAA TCA TTT GGA AAC AAT CAC TTT GCT GAG CGA TCA TTC CTA ACT GCT CCT CAG ACA GAG TAC AGG AAT AAC ATG ATT GTT ATT GGC    360
Tyr Gln Ser Phe Gly Asn Asn His Phe Ala Glu Arg Ser Phe Leu Thr Ala Pro Gln Thr Glu Tyr Arg Asn Asn Met Ile Val Ile Gly
                            100                           110                           120
CTG TAC TTC ATC AAC TGC CAG AGC CTC CTG CAG GAC AGG GAC ACC CTG AAC TTC TTT AAG GAG AGC AAG TTT GAT GCT CTT TTC ACA GAC    450
Leu Tyr Phe Ile Asn Cys Gln Ser Leu Leu Gln Asp Arg Asp Thr Leu Asn Phe Phe Lys Glu Ser Lys Phe Asp Ala Leu Phe Thr Asp
                            130                           140                           150
CCA GCC TTA CCC TGT GGG GTG ATC CTG GCT GAG TAT TTG GGC CTA CCA TCT GTG TAC CTC TTC AGG GGT TTT CCG TGT TCC CTG GAG CAT    540
Pro Ala Leu Pro Cys Gly Val Ile Leu Ala Glu Tyr Leu Gly Leu Pro Ser Val Tyr Leu Phe Arg Gly Phe Pro Cys Ser Leu Glu His
                            160                           170                           180
ACA TTC AGC AGA AGC CCA GAC CCT GTG TCC TAC ATT CCC AGG TGC TAC ACA AAG TTT TCA GAC CAC ATG ACT TTT TCC CAA CGA GTG GCC    630
Thr Phe Ser Arg Ser Pro Asp Pro Val Ser Tyr Ile Pro Arg Cys Tyr Thr Lys Phe Ser Asp His Met Thr Phe Ser Gln Arg Val Ala
                            190                           200                           210
AAC TTC CTT GTT AAT TTG TTG GAG CCC TAT CTA TTT TAT TGT CTG TTT TCA AAG TAT GAG AAA CTC GCA TCA GCT GTC CTC AAG AGA GAT    720
Asn Phe Leu Val Asn Leu Leu Glu Pro Tyr Leu Phe Tyr Cys Leu Phe Ser Lys Tyr Glu Lys Leu Ala Ser Ala Val Leu Lys Arg Asp
                            220                           230                           240
GTG GAT ATA ATC ACC TTA TCA GAG GTC TCT GTT TGG CTG TTA AGA TAT GAC TTT GTG CTT GAA TAT CCT AGG CCG GTC ATG CCC AAC ATG    810
Val Asp Ile Ile Thr Leu Ser Glu Val Ser Val Trp Leu Leu Arg Tyr Asp Phe Val Leu Glu Tyr Pro Arg Pro Val Met Pro Asn Met
                            250                           260                           270
GTC TTC ATT GGA GGT ATC AAC TGT AAG AAG AGG AAA GAC TTG TCT CAG GAA TTT GAA GCC TAC ATT AAT GCT TCT GGA GAA CAT GGA ATT    900
Val Phe Ile Gly Gly Ile Asn Cys Lys Lys Arg Lys Asp Leu Ser Gln Glu Phe Glu Ala Tyr Ile Asn Ala Ser Gly Glu His Gly Ile
                            280                           290                           300
GTG GTT TTC TCT TTG GGA TCA ATG GTC TCA GAA ATT CCA GAG AAG AAA GCT ATG GCA ATT GCT GAT GCT TTG GGC AAA AAC CCT CAG ACA    990
Val Val Phe Ser Leu Gly Ser Met Val Ser Glu Ile Pro Glu Lys Lys Ala Met Ala Ile Ala Asp Ala Leu Gly Lys Asn Pro Gln Thr
                            310                           320                           330
GTC CTG TGG CGG TAC ACT GGA ACC CGA CCA TCG AAT CTT GCG AAC AAC ACG ATA CTT GTT AAG TGG CTA CCC CAA AAC GAT CTG CTT GGT   1080
Val Leu Trp Arg Tyr Thr Gly Thr Arg Pro Ser Asn Leu Ala Asn Asn Thr Ile Leu Val Lys Trp Leu Pro Gln Asn Asp Leu Leu Gly
                            340                           350                           360
CAC CCG ATG ACC CGT GCC TTT ATC ACC CAT GCT GGT TCC CAT GGT GTT TAT GAA AGC ATA TGC AAT GGC GTT CCC ATG GTG ATG ATG CCC   1170
His Pro Met Thr Arg Ala Phe Ile Thr His Ala Gly Ser His Gly Val Tyr Glu Ser Ile Cys Asn Gly Val Pro Met Val Met Met Pro
                            370                           380                           390
TTG TTT GGT GAT CAG ATG GAC AAT GCA AAG CGC ATG GAG ACT AAG GGA GCT GGA GTG ACC CTG AAT GTT CTG GAA ATG ACT TCT GAA GAT   1260
Leu Phe Gly Asp Gln Met Asp Asn Ala Lys Arg Met Glu Thr Lys Gly Ala Gly Val Thr Leu Asn Val Leu Glu Met Thr Ser Glu Asp
                            400                           410                           420
TTA GAA AAT GCT CTA AAA GCA GTC ATC AAT GAC AAA AGT TAC AAG GAG AAC ATC ATG CGC CTC TCC AGC CTT CAC AAG GAC CGC CCG GTG   1350
Leu Glu Asn Ala Leu Lys Ala Val Ile Asn Asp Lys Ser Tyr Lys Glu Asn Ile Met Arg Leu Ser Ser Leu His Lys Asp Arg Pro Val
                            430                           440                           450
GAG CCG CTG GAC CTG GCC GTG TTC TGG GTG GAG TTT GTG ATG AGG CAC AAG GGC GCG CCA CAC CTG CGC CCC GCA GCC CAC GAC CTC ACC   1440
Glu Pro Leu Asp Leu Ala Val Phe Trp Val Glu Phe Val Met Arg His Lys Gly Ala Pro His Leu Arg Pro Ala Ala His Asp Leu Thr
                            460                           470                           480
TGG TAC CAG TAC CAT TCC TTG GAC GTG ATT GGT TTC CTC TTG GCC GTC GTG CTG ACA GTG GCC TTC ATC ACC TTT AAA TGT TGT CCT TAT   1530
Trp Tyr Gln Tyr His Ser Leu Asp Val Ile Gly Phe Leu Leu Ala Val Val Leu Thr Val Ala Phe Ile Thr Phe Lys Cys Cys Pro Tyr
                            490                           500                           510
GGC TAC CCG AAA TGC TTG GGG AAA AAA GGG CGA GTT AAG AAA GCC CAC AAA TCC AAG ACC CAT TGA GAG GTG GGT GGG AAA TAA GGT AAA   1620
Gly Tyr Pro Lys Cys Leu Gly Lys Lys Gly Arg Val Lys Lys Ala His Lys Ser Lys Thr His
                            520                           530
ATTTTGAACCATCCCTAGTCATTTCCAAACTTGAAAACAGAATCAGTGTTAAATTCATTTTATTCTTATTAAGGAAATACTTTGCATAAATTAATCAGCCCCAGAGTGTCTTTAAAAAAT   1740
TCTCTTAAATAAAAATAATAGACTCGCTAGTCAGTAAAGATATTTGAATATGTATCGTGCCCCCTCCGGAGTCTTTGATCAGGATGACATGTGCCATTTTTCAGAGGACGTGCAGACAGG   1860
CTGGCATTCTAGATTACTTTTCTTACTCTGAAACATGGCCTGTTTGGGAGTGCGGGATTCAAAGGTGGTCCCACGGCTGCCCCTACTGCAAATGGCAGTTTTAATCTTATCTTTTGGCTT   1980
CTGCAGATGGTTGCATTGATCCTTAACCAATAATGGTCAGTCCTCATCTCTGTCCTGACTTCATAGGTGCCACCTTGTGGTTTAAAGAAGGGAAGCTTTGTACCTTTAGAGTGTAGGTGA   2100
AATGAATGAATGGCTTGGAGTGCACTGAGAACAGCATATGATTTCTTGCTTTGGGGAAAAAGAATGATGCTATGAAATTGGTGGGTGGTGTATTTGAGAAGATAATCATTGCTTATGTCA   2220
AATGGAGCTGAATTTGATAAAAACCCAAAATACAGCTATGAAGTGCTGGGCAAGTTTACTTTTTTTTCTGATGTTTCCTACAACTAAAAATAAATTAATAAATTTATATAATTCTAAAAA   2340
AAAAAA                                                                                                                     2346
```

FIG. 2. Nucleotide sequence of human phenol UDPGT cDNA and derived amino acid sequence of the transferase. The putative N-glycosylation sites and polyadenylylation sites are underlined. The proposed cleaved signal peptide is indicated by the dashed underline at the N terminus.

sequence is shown in Fig. 2. There was an 86-base-pair (bp) 5′ noncoding region, a 1593-bp coding sequence, and a 754-bp 3′ noncoding region containing two possible polyadenylylation site (AATAAA) 14 and 22 bp upstream of the poly(A) tail.

Predicted Amino Acid Sequence of HLUGP1 and Comparison with Other Human and Rat Liver UDPGT cDNAs. The nucleotide coding sequence was translated from the first methionine (ATG) codon at bp 87 (Fig. 2). This assignment was based on the homology of the initiation codon flanking sequence to the consensus start site (32) and by analogy with the rat phenol UDPGT sequence (33). The amino acid sequence of this human transferase shows 76% sequence identity to the phenol UDPGTs isolated from rat liver (33) and kidney (D.H. and B.B., unpublished work), but only showed 44% identity with human UDPGT H25 (9), which appears to catalyze the glucuronidation of 6α-hydroxy bile acids (S.F.-G., M.R.J., R. Wooster, and B.B., unpublished work). These findings suggest that the two human transferases are members of subfamilies of this gene superfamily. The similarity between the two human sequences was concentrated toward the C terminus, where a potential UDP-glucuronic acid binding site (>65% identity) may be located within residues 300–515, which includes a predicted membrane-anchor sequence (Fig. 3*b*). However, comparison of the hydrophobicity plots of the two human UDPGT sequences (Fig. 3*a*) predicted many common folding patterns despite the apparent lack of overall homology.

Expression of Phenol UDPGT Protein. The cDNA HLUGP1 was subcloned into the expression vector pKCRH2 where transcription was driven by the simian virus 40 promoter. COS-7 cells transfected with this recombinant were labeled with [^{35}S]methionine in the presence or absence of tunicamycin (2 μg/ml). The ^{35}S-labeled proteins were immunopre-

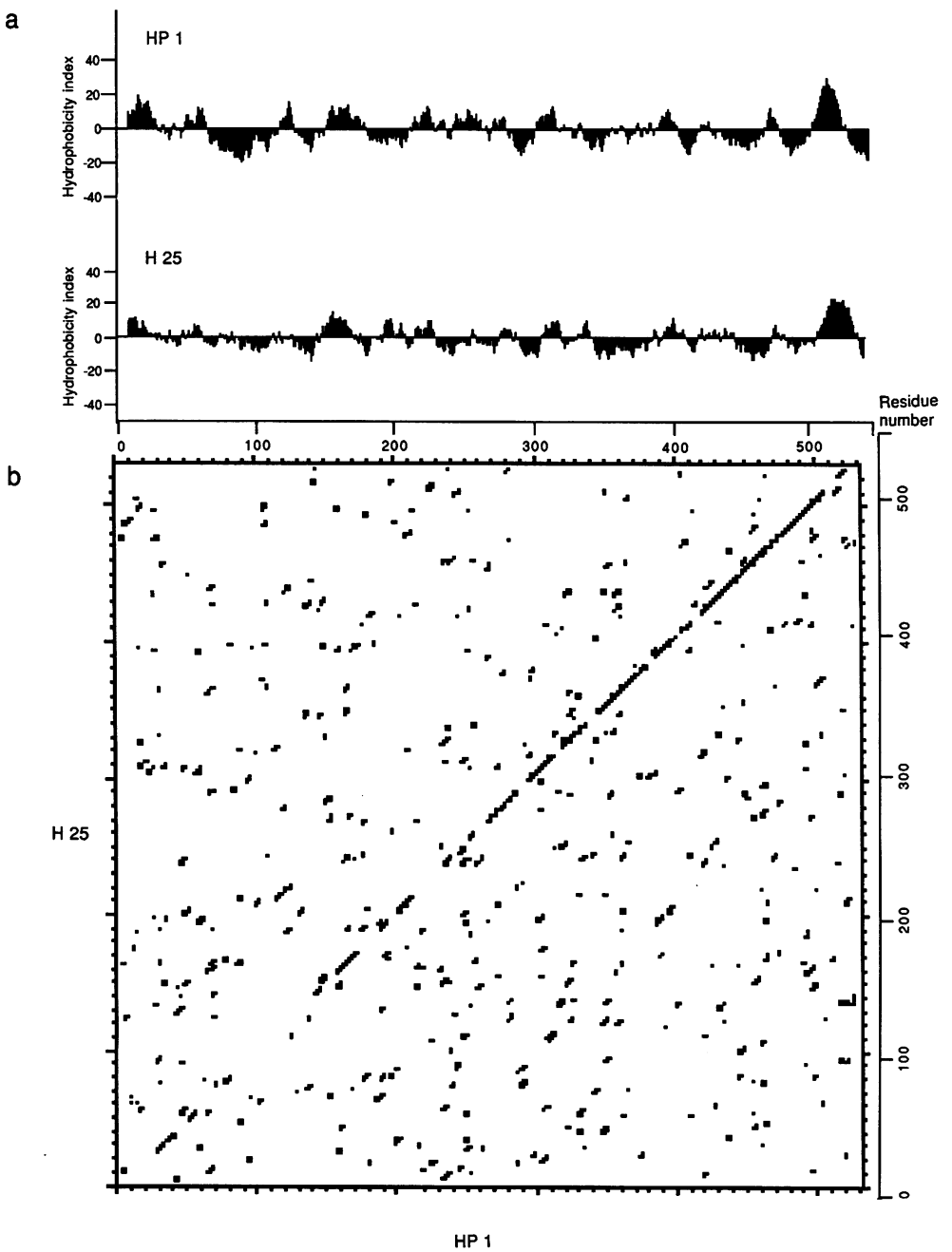

FIG. 3. Hydrophobicity profiles (*a*) and a diagon plot (*b*) comparison of two human UDPGT protein sequences. The amino acid sequences of human phenol and H25 UDPGT were deduced from translation of the open reading frames in the cDNA sequences and were analyzed by computer for hydrophobicity by using the Kyte and Doolittle program (34) with a 15-residue window.

cipitated and analyzed by SDS/PAGE and fluorography. Transfection with recombinant plasmid containing human phenol UDPGT cDNA or human microsomal EH cDNA led to the identification of selectively immunoprecipitated proteins on gels. Fig. 4, lanes 1–3, shows that the expressed human microsomal EH cDNA led to the appearance of a 50-kDa protein product that correlated with the appearance of enzyme activity catalyzing the formation of styrene glycol [0.8 nmol (min/mg of cell protein)$^{-1}$]. The size of the EH synthesized was not affected by the presence of tunicamycin in the cell cultures and, therefore, was not glycosylated. Fig. 4, lanes 3–7, shows the specific expression of a phenol UDPGT (55 kDa). This protein is a similar size to the two phenol UDPGTs purified from human liver (11). The tunicamycin treatment was only partially effective in inhibiting glycosylation of the expressed UDPGT isoenzyme but the mobility of some UDPGTs was increased in the presence of tunicamycin indicating that this protein was glycosylated. Inhibition of glycosylation reduced the size of the protein to 52 kDa. These proteins were not immunoprecipitable from COS cells transfected with the vector alone (Fig. 4).

Expression and Substrate Specificity of Phenol UDPGT. Results from the expression of enzyme activities after transfection of COS-7 cells with vectors containing HLUGP1 cDNA or no inserts are shown in Table 1 and Fig. 5. The data in Fig. 5 illustrate the assay methodology and provide a visual impression of the results. High rates of UDPGT activity toward 1-naphthol and 4-methylumbelliferone were observed. The substrate specificity with a series of closely related phenols was further investigated to provide indications of the chemical structures accepted into the active site of the enzyme. Phenol was a poor substrate, whereas substituents at position 4 such as —NO_2, methyl, or ethyl groups were good substrates. The bulkier 4-alkyl-substituted phenols, 4-propylphenol, and 4-butylphenol were again poor substrates, indicating a size and/or planarity restriction in access of these substrates to the active site of the enzyme as was suggested (35) from studies of the rat fetal liver UDPGT. It would appear that the enzyme in fetal rat liver, the cloned kidney phenol UDPGT (unpublished work), and the cloned human liver phenol UDPGT exhibit a remarkably similar substrate specificity, although the cloned enzymes are only 76% similar in amino acid sequence, suggesting the structure of the active sites of the two enzymes are highly conserved between the species. Adult human liver microsomes catalyzed the glucuronidation of all substituted phenols at a similar rate (Fig. 5), indicating that another UDPGT isoenzyme was contributing toward glucuronidation of propyl- and butylphenol.

Table 1. Substrate specificity of human phenol UDPGT expressed in COS-7 cells

Substrate	UDPGT activity, pmol per min per mg of protein: Vector transfected COS-7 cells	HLUGP1-transfected COS-7 cells	Human liver microsomes
4-Nitrophenol	0.2	17.0 (0.74)	120 (0.68)
Phenol	0.1	1.3 (0.06)	5 (0.03)
4-Methylphenol	0.2	9.6 (0.42)	119 (0.68)
4-Ethylphenol	0.1	9.2 (0.40)	169 (0.95)
4-Propylphenol	0.04	0.8 (0.03)	166 (0.94)
4-Isopropylphenol	0.02	1.2 (0.05)	156 (0.88)
4-*t*-Butylphenol	0.02	1.1 (0.05)	166 (0.94)
1-Naphthol	0.3	23.0 (1.0)	177 (1.00)
2-Naphthol	0.2	15.3 (0.66)	210 (1.19)
5,6,7,8-Tetrahydro-1-naphthol	ND	4.4 (0.19)	139 (0.78)
(1*R*)-1,2,3,4-Tetrahydro-1-naphthol	ND	0.1 (0.00)	6 (0.03)
(1*S*)-1,2,3,4-Tetrahydro-1-naphthol	ND	0.03 (0.00)	1 (0.01)
4-Methylumbelliferone	0.2	12.1 (0.53)	304 (1.72)

The data shown are the mean of measurements of at least two cellular or microsomal preparations. The figures in parentheses show the ratio of glucuronidation of each substrate relative to the rate of glucuronidation of 1-naphthol. ND, not determined.

Table 1 also shows that 1- and 2-naphthol were actively glucuronidated by the expressed transferase but that the 1,2,3,4-tetrahydro-1-naphthols were not substrates. 5,6,7,8-Tetrahydro-1-naphthol was a poor substrate for the cloned enzyme, although rapidly glucuronidated by liver microsomes. This data suggests that the electronic configuration conferred by the unsaturated bonding system was essential

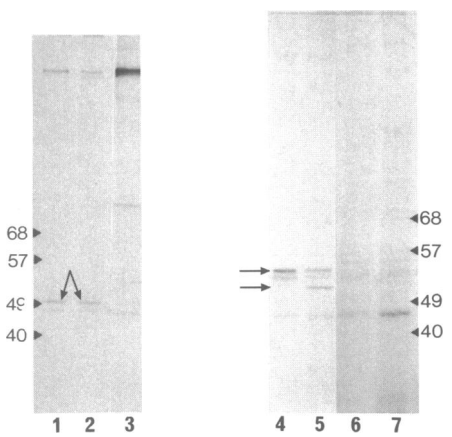

FIG. 4. Expression of human liver phenol UDPGT protein and EH in transfected COS cells. The proteins in COS-7 cells were labeled with [^{35}S]methionine 72 hr after transfection in the presence (lanes 2, 5, and 7) or absence (lanes 1, 3, 4, and 6) of tunicamycin. Immunoprecipitates containing UDPGT protein and human microsomal EH were analyzed by SDS/PAGE and autoradiography. Lanes: 1 and 2, proteins precipitated with anti-human microsomal EH immune serum; 4 and 5, protein isolated with anti-UDPGT antiserum; 3, 6, and 7, proteins isolated with preimmune serum. The mobilities of protein standards, albumin (68 kDa), pyruvate kinase (57 kDa), fumarase (49 kDa), and aldolase (40 kDa), are shown.

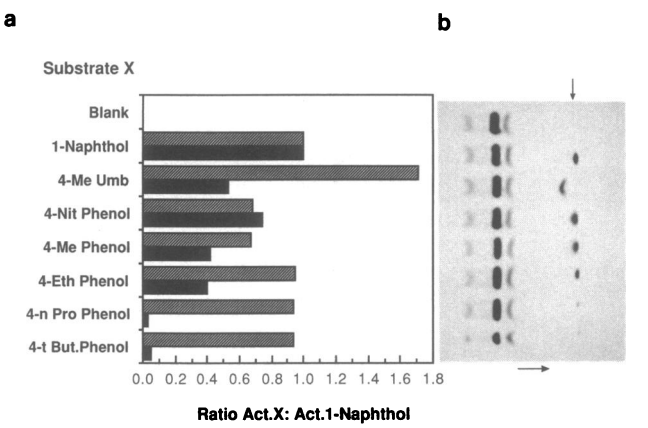

FIG. 5. Glucuronidation of a series of phenols by phenol UDPGT expressed in COS cells. (*a*) Comparison of the rates of UDPGT activity (Act.) toward seven substrates relative to 1-naphthol by using liver microsomes (▨) and cloned transferase in COS cells (■). 4-MeUmb, 4-methylumbelliferone; 4-Nit Phenol, 4-nitrophenol; 4-Me Phenol, 4-methylphenol; 4-Eth Phenol, 4-ethylphenol; 4-n Pro Phenol, 4-propylphenol; 4-t But. Phenol, 4-*t*-butylphenol. (*b*) An autoradiograph of a TLC plate showing the glucuronides formed during assay of the cloned UDPGT with five substrates, as indicated in *a*. The glucuronide spots are indicated by the arrow. Direction of migration was from left to right.

for glucuronidation at the hydroxyl group catalyzed by the cloned phenol UDPGT.

Irshaid and Tephly (11) have purified two human liver UDPGTs that exhibited very similar substrate specificity and that glucuronidate 4-nitrophenol and 4-methylumbelliferone. We were unable to use the discriminating substrate 4-aminobiphenyl (11) to assay the cloned expressed human phenol UDPGT, because use of this carcinogenic compound is prohibited in the U.K. The expressed phenol UDPGT catalyzed the glucuronidation of 4-nitrophenol 1.4 times faster than 4-methylumbelliferone, but not the glucuronidation of estriol, suggesting that this gene product may be the same as the purified human phenol UDPGT(pI 6.2) (11).

Kinetic analysis of phenol glucuronidation also suggests the existence of more than one UDPGT involved in formation of 1-naphthol and 4-methylumbelliferone glucuronides (36). The data presented here (Table 1), where the expressed enzyme was compared with the transferases present in human liver microsomes, would predict that glucuronidation of 4-methylumbelliferone involved possibly two or three microsomal UDPGTs.

The cloned expressed human enzyme did not catalyze the glucuronidation of (−)-morphine, phenolphthalein, or 4-hydroxybiphenyl. Further, the expressed human phenol UDPGT did not catalyze the glucuronidation of endogenous compounds, testosterone, androsterone, and estrone in agreement with the results obtained from purification studies (11). All of these substrates were rapidly glucuronidated by rat liver microsomes (data not shown).

Several other human liver UDPGT cDNAs have been obtained; some have close homology with HLUGP1. Expression of these cDNAs in cell cultures will allow an accurate assessment of the substrate of human UDPGTs in whole cells and their ability to catalyze drug glucuronidation. Preliminary drug screening and improved drug design will be possible by using these systems, although, as only a small proportion of the cells transiently express the transfected cDNA, the establishment of stable cell lines where every cell expresses the cloned UDPGT will greatly improve the effectiveness of this technique.

We thank Linda Gordon for excellent technical assistance, the Medical Research Council, the Wellcome Trust, and Fondation pour la Recherche Medicale et Scientifique for grants supporting this work. B.B. is a Wellcome Trust Senior Lecturer.

1. Dutton, G. J. (1980) *Glucuronidation of Drugs and Other Compounds* (CRC, Boca Raton, FL).
2. Bock, K. W., Brunner, G., Hoensch, H. Huber, E. & Josting, D. (1978) *Eur. J. Clin. Pharmacol.* **14**, 367–373.
3. Mahu, J., Preaux, A., Maurier, P. & Berthelot, P. (1981) *Enzyme* **26**, 93–102.
4. Parquet, M., Pessah, M., Sacquet, E., Solvet, C., Raizman, A. & Infante, R. (1985) *FEBS Lett.* **189**, 183–187.
5. Bock, K. W. & Bock-Hennig, B. S. (1987) *Biochem. Pharmacol.* **36**, 4137–4243.
6. Dragacci, S., Hamar-Hansen, C., Fournel-Gigleux, S., Lafaurie, C., Magdalou, J. & Siest, G. (1987) *Biochem. Pharmacol.* **36**, 3923–3927.
7. Leakey, J. E. A., Hume, R. & Burchell, B. (1987) *Biochem. J.* **243**, 859–861.
8. Arias, I. M., Gartner, L. M., Cohen, M., Ezzer, J. & Levi, A. J. (1969) *Am. J. Med.* **47**, 395–409.
9. Jackson, M. R., McCarthy, L. R., Harding, D., Wilson, S., Coughtrie, M. W. H. & Burchell, B. (1987) *Biochem. J.* **242**, 581–588.
10. Burchell, B., Coughtrie, M. W. H., Jackson, M. R., Shepherd, S. R. P., Harding, D. & Hume, R. (1987) *Mol. Aspects Clin. Med.* **9**, 429–455.
11. Irshaid, Y. M. & Tephly, T. R. (1987) *Mol. Pharmacol.* **31**, 27–34.
12. Mackenzie, P. (1986) *J. Biol. Chem.* **261**, 14112–14117.
13. Coughtrie, M. W. H., Burchell, B., Shepherd, I. M. & Bend, J. R. (1987) *Mol. Pharmacol.* **31**, 585–591.
14. Gluzman, Y. (1981) *Cell* **23**, 175–182.
15. Mishima, M., Kuorsaki, T., Tobimatsu, T., Morimoto, Y., Noda, M., Yamamoto, T., Terao, M., Linstrom, J., Takahashi, T., Kuno, M. & Numa, S. (1984) *Nature (London)* **307**, 604–608.
16. Kwok, S. C. M., Ledley, F. D., DiLella, A. G., Robson, K. J. H. & Woo, S. L. C. (1985) *Biochemistry* **24**, 556–561.
17. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
18. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
19. Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Messing, J., Crea, R. & Seeburgh, P. H. (1981) *Nucleic Acids Res.* **9**, 309–321.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
22. Roe, B. (1987) *BRL Focus* **9**, 10a–10b.
23. Jackson, M. R., Craft, J. A. & Burchell, B. (1987) *Nucleic Acids Res.* **15**, 7188.
24. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31**, 360–369.
25. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7413–7417.
26. Bansal, S. K. & Gessner, T. (1980) *Anal. Biochem.* **109**, 321–329.
27. Otani, G., Abou-El-Makarem, M. M. & Bock, K. W. (1976) *Biochem. Pharmacol.* **25**, 1293–1297.
28. Oesch, F. (1974) *Biochem. J.* **139**, 77–88.
29. Jackson, M. R., Kennedy, S. M. E., Lown, G. & Burchell, G. (1986) *Biochem. Pharmacol.* **35**, 1191–1198.
30. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
31. Skinner, M. K. & Griswold, M. L. (1983) *Biochem. J.* **209**, 281–284.
32. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125–8148.
33. Iyanagi, T., Haniu, M., Sogawa, K., Fujii-Kuriyama, Y. Watanabe, S., Shively, J. E. & Anan, K. F. (1986) *J. Biol. Chem.* **261**, 15607–15614.
34. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
35. Wishart, G. J. & Campbell, M. T. (1979) *Biochem. J.* **178**, 443–447.
36. Miners, J. O., Lillywhite, K. J., Matthews, A. P., Jones, M. E. & Birkett, D. J. (1988) *Biochem. Pharmacol.* **37**, 665–671.