Two Human Liver cDNAs Encode UDP-Glucuronosyltransferases with 2 Log Differences in Activity toward Parallel Substrates Including Hyodeoxycholic Acid and Certain Estrogen Derivatives†

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Received August 23, 1991; Revised Manuscript Received December 31, 1991

ABSTRACT: Two human liver UDP-glucuronosyltransferase cDNA clones, HLUG25 [Jackson, M. R., et al. (1987) *Biochem. J. 242*, 581–588] and UDPGT$_h$-2 [Ritter, J. K., et al. (1990) *J. Biol. Chem. 266*, 7900–7906] have previously been shown to encode isozymes active in the glucuronidation of hyodeoxycholic acid (HDCA) and certain estrogen derivatives (estriols and 3,4-catechol estrogens), respectively. Here we report that the UDPGT$_h$-2-encoded isoform (udpgt$_h$-2) and the HLUG25-encoded isoform (udpgt$_h$-1) have parallel aglycon specificities. Following expression in COS-1 cells, each isoform metabolized three types of dihydroxy- or trihydroxy-substituted ring structures, including the 3,4-catechol estrogen (4-hydroxyestrone), estriol and 17-epiestriol, and HDCA, but the udpgt$_h$-2 isozyme is 100-fold more efficient than udpgt$_h$-1. udpgt$_h$-1 and udpgt$_h$-2 are 86% identical overall (76 differences out of 528 amino acids), including 55 differences in the first 300 amino acids of the amino terminus, a domain which confers isoform substrate specificity. The data indicate that a high level of conservation in the amino terminus is not required for the preservation of substrate selectivity. Analysis of glucuronidation activity encoded by UDPGT$_h$-1/UDPGT$_h$-2 chimeric cDNAs constructed at their common restriction sites, *Sac*I (codon 297), *Nco*I (codon 385), and *Hha*I (codon 469), showed that nine amino acids between residues 385 and 469 are important for catalytic efficiency, suggesting that this region represents a domain which is critical for catalysis but distinct from that responsible for aglycon selection. In parallel with the existence of liver and kidney microsomal HDCA glucuronosyl transferase activity, mRNA coding for udpgt$_h$-2 is expressed in liver and kidney, whereas that for udpgt$_h$-1 is expressed only in the liver. These data indicate that udpgt$_h$-2 is a primary isoform responsible for the detoxification of the bile salt intermediate as well as the active estrogen intermediates.

The maintenance of normal bile acid content and biliary flow are critical for the proper digestion of dietary fat and for the prevention of gall bladder disease (Greenberger & Isselbacher, 1991). Although the precise regulation of bile acid metabolism is not understood, hyodeoxycholic acid (HDCA) is distinguished from the others by its high rate of glucuronidation (Parquet et al., 1988). Conditions of cholestasis (Fröhling & Stiehl, 1976) and malabsorption (Almé et al., 1978) cause high levels of HDCA and its glucuronide to appear in the urine. The appearance of elevated amounts of this bile acid and its conjugate in urine follows the accumulation of cytotoxic mono- and dihydroxylated bile acids (primarily chenodeoxycholic, cholic, and deoxycholic acids). The increased levels of 12α- and 7α,12α-hydroxylated derivatives of lithocholic acid cause the liver to respond by shifting the hydroxylation to the 6α position to produce the nontoxic HDCA (Bremmelgaard & Sjövall, 1980), which is glucuronidated efficiently by the liver and kidney and less effectively by the intestine (Parquet et al., 1985). These studies suggest that the 6α-hydroxylation/glucuronidation pathway in man represents a detoxifying adaptation in response to abnormal bile acid metabolism (Radomińska-Pyrek et al, 1980. Direct glucuronidation of the toxic lithocholic acid occurs in patients with cholestatic syndromes and leads to an even more toxic and poorly soluble lithocholic 3-*O*-glucuronide (Oelberg et al., 1984). The toxic chenodeoxycholic acid (7α-hydroxy-substituted lithocholic acid) is also poorly glucuronidated in man. [In contrast, the rat efficiently glucuronidates 3α- and 3α,12α-hydroxylated derivatives (lithocholic and deoxycholic acids) and 6β- and 7β-hydroxylated bile acid intermediates (murideoxycholic and β-muricholic acids) (Zimniak et al., 1988).]

Recently, Fournel-Gigleux et al. (1989) reported that a human liver UDP-glucuronosyltransferase cDNA (HLUG25) encodes a 6α-hydroxylated bile acid (HDCA) transferase isoform, a finding confirmed in our laboratory upon expression of an identical coding cDNA, UDPGT$_h$-1.[1] Even though the liver and kidney are known sites of HDCA glucuronidation, little or no UDPGT$_h$-1 mRNA was detectable in kidney by Northern analysis, suggesting that an additional isoform(s) is(are) involved. In order to uncover the transferase isozyme responsible for detoxifying this critical endogenous aglycon, we examined the suitability of a series of bile acid derivatives as substrates for cloned human transferase isoforms isolated in this laboratory. One of these, udpgt$_h$-2, previously shown to glucuronidate estriols and 3,4-catechol estrogens (Ritter et al., 1990), was found to be equally efficient in glucuronidating

† This work was presented at the annual meeting of the American Society of Biochemistry and Molecular Biology in New Orleans, LA, June 5–9, 1990, and appeared as an abstract in *FASEB J. 4*, A1774 (1990).

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[1] Although it was reported that six nucleotide differences exist between HLUG25 and UDPGT$_h$-1 [Ritter, J. K., Sheen, Y. Y., Karkowsky, A., & Owens, I. S. (1988) *J. Cell Biol. 107*, 851a], the sequence of HLUG25 has since been found to be identical to the published sequence of UDPGT$_h$-1 (Genbank Accession Number Y00317).

HDCA. Similarly, udpgt$_h$-1 exhibited parallel glucuronidating activity toward the same three substrates but with 1% the efficiency of udpgt$_h$-2. Because of its more efficient turnover of the bile acid derivative and its tissue distribution, udpgt$_h$-2 necessarily plays an important role in the maintenance of physiological concentrations of the essential bile salts and, thus, the control of cholestatic diseases.

Materials and Methods

Materials. UDP-glucuronic acid, cholic acid, hyocholic acid, and all steroid derivatives were from Sigma Chemical Co. (St. Louis, MO); HDCA was from Aldrich Chemical Co. (Milwaukee, WI); [^{35}S]methionine was from ICN Biomedicals, Inc. (Costa Mesa, CA); [^{14}C]UDP-glucuronic acid was from Amersham (Arlington Heights, IL). Restriction enzymes and other reagents used in molecular biology techniques were from New England Biolabs (Beverly, MA), Pharmacia (Piscataway, NJ), and Bethesda Research Laboratories (Bethesda, MD). The pSVL vector was from Pharmacia, and COS-1 cells were from the American Type Culture Collection (Rockville, MD). The Bluescript plasmids and XL-1 Blue cells were from Stratagene (La Jolla, CA). Reagents used for cell culture studies were from Gibco (Grand Island, NY) or Mediatech (Washington, DC).

Isolation of UDPGT$_h$-1 and UDPGT$_h$-2 from a Human Liver cDNA Library. The two human liver transferase cDNAs, UDPGT$_h$-1 and UDPGT$_h$-2, were isolated (Ritter et al., 1990) from a λgt11 cDNA library by hybridization to the mouse transferase clone ^{32}P-UDPGT$_m$-1 (Kimura & Owens, 1987) which was labeled by the oligo-primed technique using a kit (Pharmacia), [α-^{32}P]deoxycytidine triphosphate, and the supplier's protocol. Each clone was sequenced by the direct plasmid dideoxy protocol as described previously using the Bluescript vector (Ritter et al., 1990). The protein encoded by the clone, HLUG25, which is identical to UDPGT$_h$-1, was previously characterized biochemically by Fournel-Gigleux et al. (1987), and that encoded by UDPGT$_h$-2 was also previously characterized by Ritter et al. (1990). This study uncovered important parallels in aglycon specificity and differences in the catalytic activities of these two proteins.

Expression of Transferase Proteins Encoded by pUDPGT$_h$-1 and pUDPGT$_h$-2. UDPGT$_h$-1 and UDPGT$_h$-2 were subcloned into the pSVL plasmid to produce the expression units pUDPGT$_h$-1 and pUDPGT$_h$-2, with the sense strand downstream of the promoter element in the vector. Each recombinant plasmid was transfected into COS-1 cells as described (Ritter et al., 1990). In order to ensure that the comparisons of activities (Figure 3) were based on equivalent amounts of specific protein, we established the relative level of synthesis of each radiolabeled protein. Cells were transfected with pUDPGT$_h$-1 or pUDPGT$_h$-2 and incubated for 68 h in regular medium, 1 h in methionine-free medium, and, finally, 4 h in [^{35}S]methionine (100 μCi/mL) as previously described (Ritter et al., 1990). The [^{35}S]methionine-labeled transferases were solubilized, immunocomplexed with either control IgG or antimouse transferase IgG, and detergent-washed as already described (Mackenzie et al., 1984). The immunocomplexes were detached and analyzed on a 7.5% polyacrylamide gel and processed for autoradiography as indicated (Ritter et al., 1990).

Glucuronidation Assay Using [^{14}C]UDP-glucuronic acid. Preliminary data indicated that udpgt$_h$-1 and udpgt$_h$-2 glucuronidate similar substrates. In order to determine the relative amounts of glucuronide generated by either udpgt$_h$-1 or udpgt$_h$-2, control and transfected COS-1 cell homogenates were assayed in a 0.075-mL reaction containing 10 or 100 μM aglycon, 50 μM [^{14}C]UDP-glucuronic acid (33 μCi/μM), 50 mM Tris-HCl, pH 7.7, 4 mM $MgCl_2$, 1 mM ascorbic acid, 1 mM phenylmethanesulfonyl fluoride, and 1.0 μg/mL leupeptin. Reactions were incubated for 16 h at 25 °C and analyzed by thin-layer chromatography (TLC). The same amount of specific protein) based on the ^{35}S-labeled immunoprotein study above) was added to each reaction. The products were quantified by scanning the plates on an Ambis radioanalytical system Mark II (Ambis, San Diego, CA). The minimal amount of glucuronide product detected under the assay conditions used is approximately 1.7 pmol (with signal/background ratio of 2) above a background of 50 cpm. Plates were exposed to X-ray film to obtain autoradiograms (Figure 3, panel B). Panel C of Figure 3 shows the results of panel A expressed as picomoles of glucuronide formed per 45 μg of protein in 16 h.

Construction of Chimeric cDNAs between UDPGT$_h$-1 and UDPGT$_h$-2. Chimeras between UDPGT$_h$-1 and UDPGT$_h$-2 were constructed in Bluescript by using the common restriction enzyme sites *Sac*I, *Nco*I, or *Hha*I to create exchanges at codons 297, 385, and 469, respectively. The regions surrounding the endonuclease sites involved in joining the two fragments were sequenced by the dideoxy protocol as described above. The chimeras were then subcloned into pSVL and expressed in COS-1 cells as already described for the parental cDNAs (Ritter et al., 1990). The hybrid transferase proteins were assayed for the glucuronidation of 4-hydroxyestrone and HDCA and compared to the parental forms (Figure 4).

Northern Blot Analysis of mRNA. Messenger RNA was isolated (Chirgwin et al., 1979) from normal human tissue. Messenger RNA samples (2 μg each) were electrophoresed and blotted as described (Ritter et al., 1990). Full-length UDPGT$_h$-2 and the *Eco*RI/*Acc*I 413-bp fragment of UDPGT$_h$-1 were labeled by randomly-primed synthesis in the presence of [α^{32}P]dCTP. After the filter was hybridized to [^{32}P]UDPGT$_h$-2, stripped, and checked for background, it was rehybridized to the [^{32}P]UDPGT$_h$-1 probe (413-bp fragment). The complete coding sequence of UDPGT$_h$-2 cross-hybridizes with the 2.3-kb message encoding UDPGT$_h$-1, but the 413-bp *Eco*RI/*Acc*I fragment (base pairs 127–539) of UDPGT$_h$-1 does not cross-hybridize to the 2.0-kb message encoding UDPGT$_h$-2. The amounts of RNA applied to the gel were normalized (McKinnon et al., 1987) by hybridization to ^{32}P-labeled human cyclophilin cDNA.

Results and Discussion

Deduced Amino Acid Differences between udpgt$_h$-1 and udpgt$_h$-2. The two human liver UDP-glucuronosyltransferase clones, UDPGT$_h$-1 and UDPGT$_h$-2, were originally identified as encoding isozymes active in the glucuronidation of HDCA (Fournel-Gigleux et al., 1989) and estriol and 3,4-catechol estrogens (Ritter et al., 1990), respectively. In Figure 1, it is shown that the two encoded proteins have 76 differences overall; there are 55 in the 300 amino-terminal residues and 21 in the 228 carboxyl-terminal residues. Thus, udpgt$_h$-1 and udpgt$_h$-2 have 82% identity in the first 300 amino acids. Also, udpgt$_h$-2 has a cysteine at position 515 which is not present in udpgt$_h$-1. The observation that the NH_2 termini contain the greatest number of differences between these two clones is consistent with the location of the most variable region between other transferase isoforms (Iyanagi et al., 1986). In a study with chimeric cDNA constructs it was shown (Mackenzie, 1990) that the selection of the acceptor substrate is controlled by the amino terminus (~300 residues). In the case of other transferase isoforms, two human bilirubin and one phenol-metabolizing isoforms contain unique amino ter-

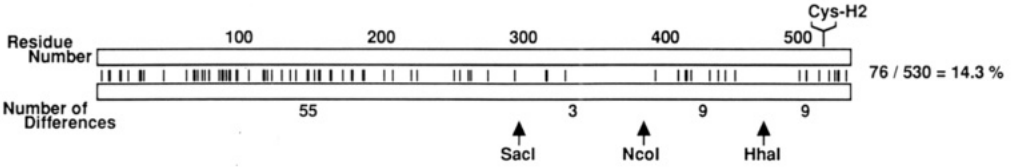


FIGURE 1: Amino acid differences between the udpgt$_h$-1 and udpgt$_h$-2 isozymes. The vertical lines between the two clear strips represent amino acid differences distributed according to the designated residue number between 1 and 528. The number of differences between the restriction enzyme sites is shown below the diagram. Specific restriction enzyme sites which were used in the creation of chimeras are indicated. Cys-H2 refers to a cysteine residue in udpgt$_h$-2 which is absent in udpgt$_h$-1.

mini of 289 residues with 41–48% identity, whereas the 244-amino acid carboxyl termini of the isoforms are identical (Ritter et al., 1991). The conservation in the carboxyl domain suggests that this region of the molecule is involved in a function common to all isozymes, such as the transfer of glucuronic acid from UDP-glucuronic acid.

Relative Levels of Synthesis of udpgt$_h$-1 and udpgt$_h$-2 in Transfected COS-1 Cells. In order to ensure that equal amounts of specific protein were being compared, the levels of synthesis were assessed 72 h after transfection of the individual expression units, pUDPGT$_h$-1 and pUDPGT$_h$-2, into COS-1 cells. The results with ^{35}S-labeled protein show that equal amounts of udpgt$_h$-1 and udpgt$_h$-2 were immunocomplexed (Figure 2, lanes 3 and 2) when similar amounts of cell homogenate were used. With preimmune IgG there was no recovery of immunoprecipitable protein (Figure 2, lanes 1 and 4). The udpgt$_h$-2 isozyme (Ritter et al., 1990) has a higher molecular mass (Figure 2), due most likely to the utilization of three potential asparagine-linked glycosylation sites in the deduced amino acid sequence as compared to one in the udpgt$_h$-1 sequence (Jackson et al., 1987).

Comparison of Glucuronidating Activity of the udgtp$_h$-1 and udpgt$_h$-2 Isozymes. While we were carrying out a more detailed analysis of the substrate selectivity of udpgt$_h$-1 and udpgt$_h$-2 and their chimeras, it became apparent that the two parental forms were metabolizing the same series of acceptor substrates. Both udpgt$_h$-1 and udpgt$_h$-2 (Ritter et al., 1990) had already been extensively screened for glucuronidating activity. udpgt$_h$-1 was examined for acceptor substrate activity upon expression in yeast, as well as in COS-1 cells (data not shown). Our results confirmed the findings in a published study (Fournel-Gigleux et al., 1989) which showed that the HLUG25-encoded protein glucuronidates HDCA at an apparently low rate. While extending these studies, we uncovered the fact that udpgt$_h$-2 also glucuronidates 17-epiestriol and HDCA at a rate comparable to that for 4-hydroxyestrone (Figure 3). Further comparisons of activity showed that the udpgt$_h$-1 isozyme metabolizes each of the four best substrates seen for the udpgt$_h$-2 isoform (Figure 3). A substrate is included from each of the three categories seen for the more active isozyme: an estriol (containing three hydroxy groups), HDCA (a 3α,6α-dihydroxy bile salt derivative), and 4-hydroxyestrone (a 3,4-catechol estrogen; see Figure 4]. The greater activity associated with udpgt$_h$-2 is also determined by the fact that related chemical structures are glucuronidated but at a lower rate. Conversion of these related chemicals to glucuronides by udpgt$_h$-1 was barely detectable with only 5β-androstan-3α,11β,17β-triol and 16,17-epiestriol. On the basis of this study, it is concluded that excellent substrates for the udpgt$_h$-2 protein, i.e., those with greater than 200 pmol produced in 16 h, can be detected as substrates for udpgt$_h$-1 (Figure 3). The most effective substrates of udpgt$_h$-2 are grouped (Figure 4, see pluses) into three categories according to chemical structures: the 3,4-catechol estrogens (line 1), HDCA (line 2), and the estriols (line 3, effectiveness descending from 17-epiestriol, estriol, 16-epiestriol, to 2-hydroxyestriol). A detailed profile of the compounds which are less effective substrates for udpgt$_h$-2 and those below the level of detection for udpgt$_h$-1 is shown in Figure 3. Regio- and stereospecificity of the hydroxy substituents are critical to these substrate selections, on the basis of the ineffectiveness of 2,3-catechol estrogens and 3α,6β- and 3α,7α-dihydroxy-lithocholic acid derivatives and the preference for 16α,17α-estriol over 16α,17β- and 16β,17β-estriols (see Figure 4). The close proximity of dihydroxy substituent groups appears to be a critical requirement. The position of the glucuronide is not known in any of these cases; it is known, however, that 6-*O*-β-D-glucuronide of HDCA is the predominant product excreted by humans (Radomińska-Pyrek, 1987).

FIGURE 2: Relative amounts of udpgt$_h$-1 and udpgt$_h$-2 synthesized in cells transfected with either the pUDPGT$_h$-1 or the pUDPGT$_h$-2 expression unit. Extract from cells transfected with either pUDPGT$_h$-1 or pUDPGT$_h$-2 and cultured in the presence of ^{35}S-labeled methionine were immunocomplexed with preimmune serum (PIS) or immune serum (IS) and prepared for autoradiography as described under Materials and Methods.

It is apparent from this study that a high level of identity in the substrate selection domain, the amino terminus, is not required to maintain acceptor function. This point is further supported by the observation that 100% identity in the carboxyl termini of two human bilirubin transferase isoforms with only 48% identity in the amino termini has maintained substrate specificity.

Recently, a report (Coffman et al., 1990) described a cloned human liver estriol UDP-glucuronosyltransferase which contains 75% identity to udpgt$_h$-2. The basis for multiple

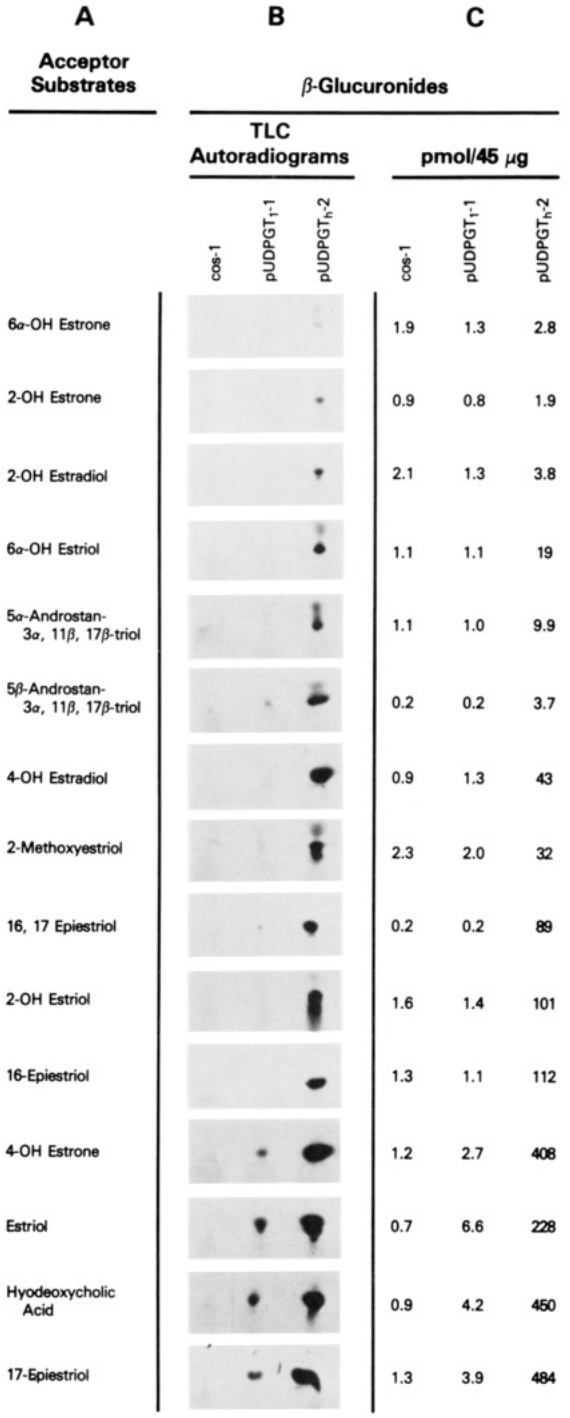


FIGURE 3: Comparison of the relative udpgt$_h$-1- and udpgt$_h$-2-catalyzed activity in transfected COS-1 cells. Transferase activity with either pUDPGT$_h$-1- or pUDPGT$_h$-2-transfected or control cells was assayed for glucuronidation of the acceptor substrates listed. The assays were carried out with both 10 and 100 μM (shown) acceptor substrate concentration using conditions described under Materials and Methods. The level of glucuronidation (panel C) was quantitated as indicated under Materials and Methods.

estriol-glucuronidating enzymes may relate to important differences in kinetic parameters, overlapping substrate specificities, and responsiveness of either gene transcriptional unit to regulatory signals.

Relative Activity of the Chimeric Transferase Molecules. The diagram in Figure 5 shows that the chimeras with exchanges at codon 469 (see bottom two lines) did not change glucuronidation compared to the intact isoforms except that udpgt$_h$-2 activity for HDCA appears to increase. There are nine amino acid differences between these carboxyl termini

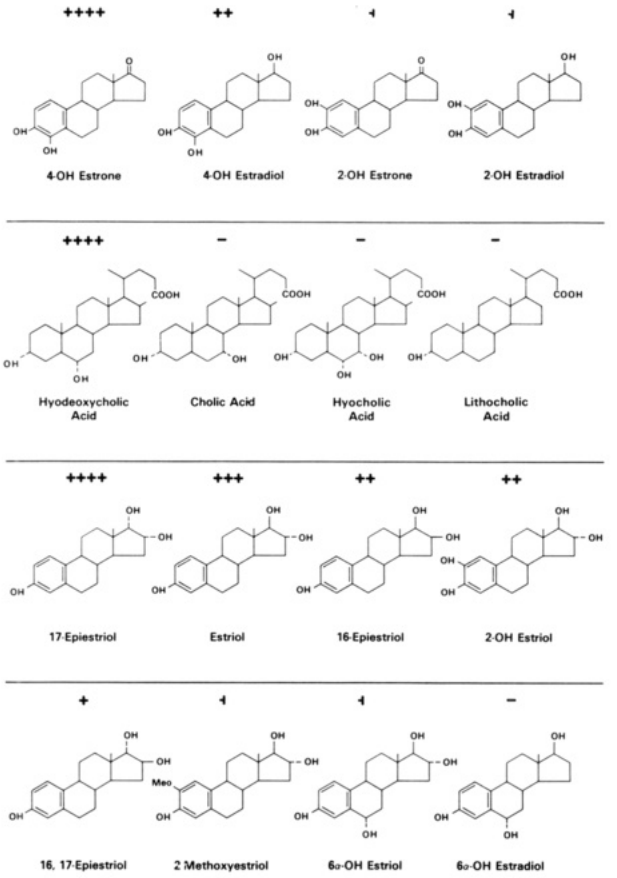
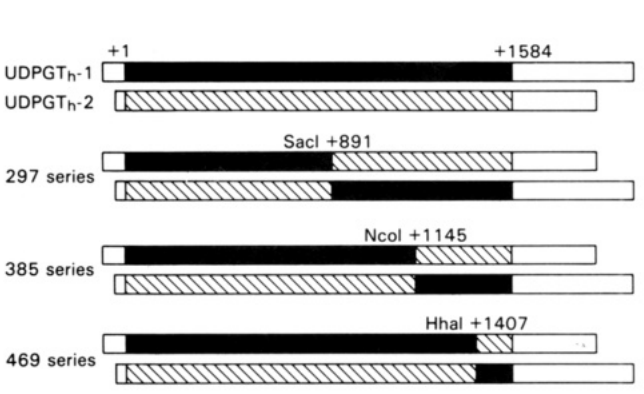


FIGURE 4: Structure of the acceptor substrates for udpgt$_h$-1 and udpgt$_h$-2. The relative substrate specificity of udpgt$_h$-1 and udpgt$_h$-2 and catalytic efficiencies indicate that three categories of chemical structures, 3,4-catechol estrogens (line 1), HDCA (line 2), and estriols (lines 3 and 4), are preferred and are equally effective. For the results with udpgt$_h$-2 the relative effectiveness of structures in each category is shown by the number of plus signs (+) above the compound.

(see Figure 1). Exchanges made at codons 297 and 385 (Figure 5, second and third set of lines, respectively) completely abolished activity of udpgt$_h$-2 without an appreciable effect on that of udpgt$_h$-1. Between chimeras udpgt$_h$-2/385 and udpgt$_h$-2/469, there are also nine amino acid differences compared to this same region of udpgt$_h$-1. This region between residues 385 and 469 excludes the unique amino terminus (~300 residues) and the conserved region between residues 481 and 499 seen in all characterized transferases [detailed in Ritter et al. (1991)]. The fact that udpgt$_h$-2/469 did not show a decrease in activity suggests that this conserved region is not a factor in the low turnover observed with udpgt$_h$-1. One can deduce that the nine amino acid differences between 385 and 469 represent a domain in the carboxyl terminus which has undergone a critical substitution(s) in udpgt$_h$-1. A consideration of the amino acids in this region (Figure 1) shows the following differences between udpgt$_h$-1 and udpgt$_h$-2: valine/isoleucine at codon 393, lysine/arginine at 409, serine/arginine at 414, leucine/valine at 415, histidine/asparagine at 418, threonine/arginine at 431, leucine/serine at 437, alanine/valine at 442, and histidine/glutamine at 449. Because five of the changes are neutral, the loss in activity is most likely due to one or more of the four nonneutral substitutions. The failure of udpgt$_h$-1/297 and udpgt$_h$-1/385 (with the carboxyl segment from udpgt$_h$-2) to show improvements in the rate of conversion to product suggests that there are defects in the udpgt$_h$-1 molecule up to at least residue 469.

Although all the critical domains in the UDP-glucuronosyltransferase have not been established, sequence com-



	β-GLUCURONIDES (pmol/50 μg protein)	
	4-OH-Estrone	HDC
UDPGTh-1	4.6	3.6
UDPGTh-2	107.5	64.5
297 series	0.9	0.9
	17.0	2.1
385 series	1.6	1.2
	4.7	1.6
469 series	2.4	2.8
	104.1	147.7

FIGURE 5: Intact and chimeric constructs of $UDPGT_h$-1 and $UDPGT_h$-2 and the relative activity associated with each construct expressed in COS-1 cells. Chimeric constructs with exchanges at the common *Sac*I, *Nco*I, and *Hha*I sites in the cDNA generated exchanges at amino acids 297, 385, and 469, respectively. The intact and chimeras were subcloned into pSVL and transfected into COS-1 cells as indicated under Materials and Methods. The level of glucuronidation (right) by each encoded transferase (left) was assessed after 8 h of incubation as described under Materials and Methods.

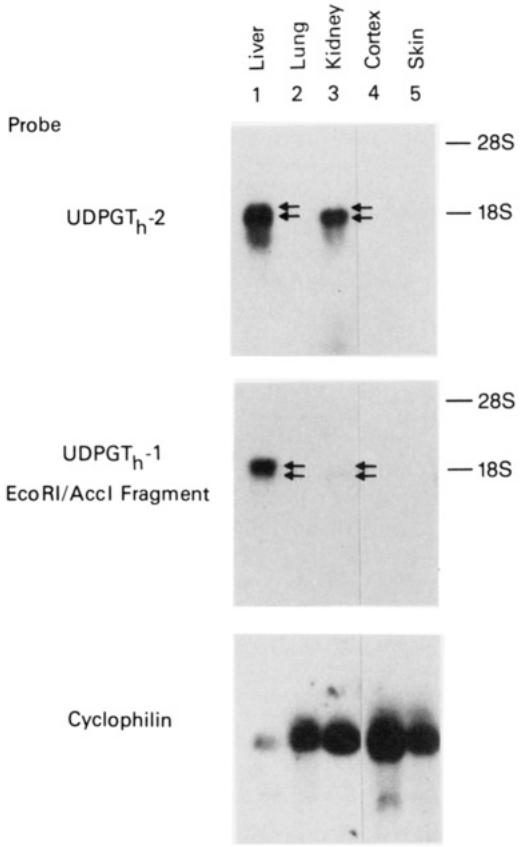


FIGURE 6: Tissue distribution in humans of mRNA encoding $UDPGT_h$-1 and $UDPGT_h$-2. Messenger RNA was isolated from human tissues as described under Materials and Methods. In addition to hybridizing to its corresponding message (2.0 kb, lower arrow), the complete coding sequence of $UDPGT_h$-2 also cross-hybridizes to the message (2.3 kb, lower arrow) encoding $UDPGT_h$-1. The $UDPGT_h$-1 (413–bp) probe does not cross-hybridize significantly to any other species of RNA. The RNA samples were electrophoresed, blotted onto ZetaBind membrane, and hybridized to the ^{35}P-labeled specific probes (shown on the left of each panel) as described under Materials and Methods.

parisons (O'Reilly & Miller, 1989) of three different mammalian transferase isoforms to the protein encoded by the baculovirus *egt* gene helped to uncover an ecdysteroid UDP-glucosyltransferase which catalyzes an analogous transfer of glucose from UDP-glucose to ecdysteroid to block effectively ecdysteroid-dependent molting of infected insects. The two evolutionarily distant but analogous enzymes have 21% overall amino acid sequence identity and contain many discontinuous regions of identity and similarity. Although the viral-encoded enzyme lacks a membrane-spanning region, the overall comparisons point to many conserved regions (including a signal peptide), which strongly suggests that each is functionally significant. On this basis one might argue that the membrane-bound UDP-glucuronosyltransferase has many important domains not yet understood.

Tissue Distribution of Human mRNA Coding for $UDPGT_h$-1 and $UDPGT_h$-2. In Figure 6 the results show that the mRNA coding for $UDPGT_h$-2 previously shown (Ritter et al., 1990) to be 2.0 kb (lower arrow) is present in liver and kidney, whereas mRNA coding for $UDPGT_h$-1 previously shown to be at least 2.3 kb (Jackdon et al., 1987) is seen only in liver (Figure 6, upper arrow). The results of the Northern blot analysis (Figure 6) show that $UDPGT_h$-2 hybridizes to both the 2.0- and 2.3-kb messages in liver. The longer 2.3-kb message is not present in kidney. This distribution of $UDPGT_h$-2 mRNA parallels the biochemical data showing efficient liver and kidney microsomal HDCA glucuronidation (Parquet et al., 1988).

The apparent equal preference of $udpgt_h$-2 for HDCA, 17-epiestriol, and 3,4-catechol estrogen points to the structure and configuration of an enzyme active site which confers overlapping specificity due to chemical similarities. This conclusion is supported by the demonstration that the same specificity has been maintained in the low-activity $udpgt_h$-1 isoform. Amino acids necessary for this selectivity have been conserved in spite of the 82% difference in the amino terminus. Amino acids affecting the rate of conversion to product have undoubtedly undergone substitutions in $udpgt_h$-1. The relative effectiveness of the $udpgt_h$-2 enzyme in conjugating three different metabolites is not understood. On the basis of preliminary data, the physiological role of $udpgt_h$-2 in detoxification mechanisms possibly relates to discrimination made at the kinetic level and, thus, effective metabolism of the three categories of physiological compounds. Importantly, this study points to the presence of isoforms with severely reduced catalytic efficiency, which appear to have been superseded by more active isozymes.

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Interaction of Lysophospholipid/Taurodeoxycholate Submicellar Aggregates with Phospholipid Bilayers†

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Received October 22, 1991; Revised Manuscript Received January 14, 1992

ABSTRACT: The equilibrium partitioning and the rate of transfer of monoacylphosphatidylethanolamines (lysoPEs) between phospholipid bilayers and lysoPE/taurodeoxycholate submicellar aggregates (SMAs) were examined with a series of environment-sensitive fluorescent-labeled *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1-monoacylphosphatidylethanolamine (*N*-NBD-lysoPE) probes of differing acyl chain length. Our previous work has demonstrated the formation of SMAs between bile salts and lysophospholipids [Shoemaker & Nichols (1990) *Biochemistry 29*, 5837-5842]. The experiments in the current work demonstrate that SMAs can coexist with phospholipid vesicles and can function as shuttle carriers for the transfer of lysophospholipids between membranes. The formation of submicellar aggregates of *N*-NBD-lysoPE and taurodeoxycholate (TDC) in equilibrium with 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) vesicles was determined from the increase in fluorescence generated upon addition of TDC to POPC vesicles containing 3 mol % *N*-NBD-lysoPE and 3 mol % *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine (*N*-Rh-PE) as a nonextractable fluorescence energy-transfer quencher. The fraction of lysolipid extracted increased as a function of decreasing acyl chain length of the *N*-NBD-lysoPE molecule. The half-time for equilibration was independent of acyl chain length and averaged 44 ms at 10 °C. The delivery of *N*-NBD-lysoPE from preformed *N*-NBD-lysoPE/TDC SMAs into POPC vesicles containing the energy-transfer quencher *N*-Rh-PE was measured by the rate of fluorescence decline. The initial rate of insertion increased with decreasing acyl chain length of the *N*-NBD-lysoPE molecule and as a function of vesicle concentration. The measured rates of *N*-NBD-lysoPE/TDC SMA interaction with vesicles indicate that the dynamic interaction of SMAs with membranes can facilitate intermembrane lysolipid transfer in the presence of nonlytic concentrations of TDC.

The currently accepted role of bile salts in the absorption of phospholipids, cholesterol, fatty acids, and other lipids in the intestine is to solubilize these lipids in mixed micelles that facilitate their transfer through the unstirred water lining the surface of the intestine (Carey et al., 1983; Erlinger, 1987; Wilson, 1981, 1991). Due to the hydrophobic nature of lipids, the unstirred water layer adjacent to the enterocytes of the upper small intestine is the major permeability barrier for their absorption (Wilson et al., 1971; Sallee & Dietschy, 1973; Westergaard & Dietschy, 1974). The amphiphilic structure of bile salts allows them to aggregate with various lipid species in the form of mixed micelles ultimately resulting in the solubilization of large amounts of intestinal lipid (Cabral & Small, 1989). These bile salt/lipid mixed micelles augment

† This study was supported by U.S. Public Health Service Grants GM32342 and DK40641 to J.W.N.
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0006-2960/92/0431-3414$03.00/0