Expression and Functional Domains of Rabbit Liver UDP-glucuronosyltransferase 2B16 and 2B13*

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Southern blot analysis has demonstrated that the 5′ portion of the rabbit liver dexamethasone-inducible UDP-glucuronosyltransferase (UGT) 2B13 RNA is related in sequence to a family of UGT genes (Tukey, R. H., Pendurthi, U. R., Nguyen, N. T., Green, M. D., and Tephy, T. R. (1993) J. Biol. Chem. 268, 15260–15266). To identify these additional gene transcripts, rabbit liver cDNA libraries were screened with a 5′ conserved 330-base pair UGT2B13 cDNA fragment, resulting in the isolation and characterization of several rabbit liver UGT cDNAs. One such clone, called pGT11, encodes a putative glycoprotein that is 78% similar to rabbit UGT2B13. The new UGT has been designated UGT2B16. The UGT2B16 gene is expressed as a single 4200-base RNA transcript that is regulated only in adult rabbits. The predicted NH2-terminal 25 amino acids of UGT2B16 are identical to that of rabbit liver UGT2B13, with the remainder of the protein being 77% similar to UGT2B13. Expressed UGT2B16 protein in COS-1 cells was active toward 4-hydroxybiphenyl, similar to that of UGT2B13. However, UGT2B16 efficiently conjugated 4-hydroxyestrone and 4-tert-butylphenol, substrates that are not efficiently catalyzed by UGT2B13. To further characterize the functional domains of UGT2B16 and UGT2B13, a series of chimeric cDNAs were constructed that contained portions of both UGT2B16 and UGT2B13. Chimeric 2B16αα–2B13ββ31, which contained the amino-terminal UGT2B16 amino acids 1–300 followed by amino acids 301–531 of UGT2B13, as well as chimeric 2B16αα300–2B13ββ531 and 2B16αα434–2B13ββ531 proteins, catalyzed the glucuronidation of 4-hydroxyestrone, indicating that the carboxyl terminus of UGT2B13 could substitute for those same regions on UGT2B16. However, the replacement of the carboxyl end of UGT2B16 with 2B16αα–531 or 2B16αα434–531 dramatically impaired the catalytic function of the chimeric proteins. These results indicate that the carboxyl end of UGT2B13 plays an important role in the functional and possible conformational state of the protein.

Glucuronidation is an important biochemical process of detoxification that leads to the removal of endogenous agents such as steroid hormones, bilirubin, and bile salts, in addition to thousands of xenobiotics and dietary by-products (1). The transfer of glucuronic acid from the cosubstrate UDP-glucuronic acid to the aglycones renders the products water-soluble, a process that eliminates potential biological activities and facilitates excretion of the glucuronides from the cell. The UDP-glucuronosyltransferases (UGTs, EC 2.4.1.17) are glycoproteins (2), which are channeled by a hydrophobic leader sequence (3) to the luminal surface of the endoplasmic reticulum (4). The diversity in the selection of structurally diverse compounds for glucuronidation results from a relatively large “superfamily” of UGTs, many of which have been identified through recombinant DNA techniques (5). Based upon overall structural similarities, the UGTs have been classified into either the UGT1 or UGT2 gene families (6, 7). All of the UGT1 proteins are transcribed from a single locus (8), with each protein containing a variable amino-terminal half and an identical carboxyl region. In contrast, the UGT2 proteins are believed to be transcribed from independent structural genes (9).

The UGT2 proteins share a high degree of similarity in their carboxyl regions with significant amino acid diversity occurring in the amino-terminal region of the proteins. Through the construction and expression of chimeric cDNA clones containing amino-terminal and carboxyl-terminal regions of different UGTs, it has been suggested (10) that the amino-terminal half is important in dictating which of the many different substrates will be selected for conjugation. Because of the high degree of similarity in the carboxyl portion of the UGTs, it has been proposed that this region dictates the conformational properties that underlie the binding of the cosubstrate UDP-glucuronic acid. Under this type of model, it is presumed that a substrate or aglycone binding pocket and a separate UDPGA domain interact to coordinate transfer of glucuronic acid to the facilitating substrate. Such a model would indicate that the UDPGA binding domain and possibly the secondary structure responsible for forming this region is closely related in all of the UGTs.

Previous experiments in our laboratory utilizing cDNAs encoding UGT2B13 and UGT2B14 have demonstrated the existence of a large UGT2 family in rabbits (11). UGT2B13, which has an identical amino-terminal sequence to the purified rabbit liver estrone UGT, catalyzes the glucuronidation of small phenolic agents like 2-naphthol, as well as the bulkier phenols such as 4- and 2-hydroxybiphenyl. Interestingly, UGT2B13 has limited ability to conjugate steroids. The estrone UGT cDNA has not yet been identified. UGT2B13 is expressed constitutively as an adult RNA. However, in neonatal rabbits, UGT2B13 is expressed in a fashion concordant with the expression of rabbit CYP3A6, being induced in newborns with agents such as dexamethasone and macrolid antibiotics (11). Northern blot analysis also indicated the possibility that sequence-
labeled UGT2B13 transcripts were regulated in a similar fashion to that of UGT2B16, suggesting that UGT2B13-like genes may be under similar modes of regulation. Combined, these results indicated that UGT2B13 could be used to selectively identify related rabbit UGT cDNAs. Since there appeared to be a high degree of nucleic acid sequence similarity to other regulated UGTs, experiments were undertaken to clone and characterize additional UGT2B13-like cDNAs, with the intent of examining how these genes are regulated as well as investigating the catalytic properties of the related proteins.

**EXPERIMENTAL PROCEDURES**

**Materials—**Restriction endonucleases, T4 polynucleotide kinase, T4 DNA ligase, avian myeloblastosis virus reverse transcriptase, and the RΝase inhibitor RNAse were purchased from New England Biolabs or Life Sciences (St. Petersburg, FL). Oligo(dT)-cellulose was purchased from Boehringer Mannheim. Nitroplus 2000 nitrocellulose hybridization paper was purchased from Micron Separations Inc (Westwood, MA). The Erase-a-Base DNA sequence kit was obtained from Promega (Madison, WI), while DNA sequencing kits were purchased from U. S. Biochemical Corp. DNA nick translation kits, [α-32P]dCTP (3000 Ci/ mmol), [γ-35S]ATP (3000 Ci/mmol), and [α-35S]dATP (400 Ci/mmol) were provided at 50% of these activities and then stored at −20 °C. After annealing, 10 μl of 3 M sodium acetate, pH 7.0, and 275 μl of ethanol were added. The precipitate was resuspended in 25 μl of 50 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM MgCl2, 1 mM dNTPs, 1 mM dithiothreitol, 1 unit/μl plasmidial RNAse inhibitor, 50 μg/ml actinomycin D, and 1 unit/μl avian myeloblastosis virus reverse transcriptase, and the reaction incubated at 42 °C for 2 h. The mRNA was then digested with RNase A, and the extension products extracted with phenol/chloroform in 6% polyacrylamide-DNA sequencing gels. To determine the exact length of the extended products, a parallel DNA sequencing reaction was included in the gel.

**Construction of Plasmids for Expression in COS-1 Cells—**the cDNA that encodes the COOH-terminal end of the UGTs. The entire coding sequence was then removed by digesting the plasmid with XhoI and BlpI, and then cloning this fragment into the XhoI and XbaI sites in the eukaryotic expression vector pSVL. The vector is identified as p2B16.SVL. From previous work, the expression of UGT2B13 was carried out using p2B13.SVL (11).

Using p2B13.SVL and p2B16.SVL, a number of UGT2B13 and UGT2B16 chimeric cDNAs were constructed. Chimeric cDNAs were constructed by using the restriction enzyme sites SacI, BamHI, or AccI to create exchanges at codons 300, 358, or 434, respectively. For the SacI site exchange, both plasmids were digested with SacI and the small and large fragments were recovered from 1% low melting agarose gels. The large SacI fragments contained linearized pSVL vector in addition to coding region that spanned amino acids 300–434, while the small SacI fragments encoded a COOH-terminal end of the UGTs. The large SacI fragments from one UGT plasmid was ligated with the smaller SacI fragments from the other UGTs, generating plasmids p2B16S13 and p2B13S16. Plasmid p2B13S16 encodes a complete transcript covering amino acids 1–300 of UGT2B13 and 301–531 of UGT2B16, and the expressed protein is identified as 2B13_300B16_531, while plasmid p2B18S13 encodes the chimera protein 2B16_300B13_531.
A BamHI restriction enzyme site at codon 358 is present in both p2B16.SVL and p2B13.SVL along with a single BamHI site that is located 3’ of the cDNA. Plasmids p2B13.SVL and p2B16.SVL were each digested with BamHI and the fragments purified, followed by ligation of the 3’ BamHI fragment from p2B15 with the 5’ portion of p2B16. This construct, named p2B16B213A14, encoded a chimeric protein called B216...B213, that encoded UGT2B16 from amino acids 1 to 358 and UGT2B13 from amino acids 359 to 531. After digesting p2B13S16Ba13 with BamHI and purifying the 5’ cDNA/fragment vector, the 3’ BamHI fragment from p2B15 was mixed with this fragment in a ligation reaction to generate plasmid p2B13S16Ba13, which encodes the chimeric protein 2B133002B163582B13531.

The pSVL vector contains two AseI sites, and there is a single AseI site in both p2B13.SVL and p2B16.SVL at codon 434. Both plasmids were digested with AseI under conditions that generated partial restriction enzyme digestion, and the different fragments removed from low melting agarose. A p2B13 fragment that contains a portion of the vector and the 5’ portion of the cDNA was ligated together with the 5’ portion of p2B16. A similar ligation was constructed with the 5’ portion of p2B16 and the 3’ portion of p2B13. These plasmids are designated as p2B16A13 and p2B13A16, and encode chimeric proteins that are identified as B216...B213 and B213...B216, respectively. The proper orientation, chimeric nature, and the absence of mutations at the ligation site were confirmed by restriction map analysis and partial DNA sequence analysis. All of the pSVL clones were transfected and expressed in COS-1 cells as described previously (13).

35S-Labeling and Immunoprecipitation—After 48 h of transfection, COS-1 cells from a single 35-mm tissue culture dish were washed three times with Hank’s balanced salt solution and incubated for 4 h in methionine-free modified Eagle’s medium supplemented with 100 μCi/ml Tran35S-label. The cells were washed twice in phosphate-buffered saline and the cells lysed on ice in 600 μl of RIPA solution containing 1% Triton X-100, 1% sodium deoxycholate, 1% SDS, 150 mM NaCl, 1 mM phenylmethanesulfonyl fluoride, and 50 μM Tris-HCl, pH 7.5. Cellular DNA was broken by shearing through a 25-gauge needle and was removed with other cellular debris by centrifugation at 6,000 × g for 10 min. The cleared supernatant was incubated for 4 h at 4 °C with 22 μg of Tris-HCl, pH 7.5, containing 10 μg Mcl-1, by gel retardation assay with the UGT2B16 oligonucleotide 32P-labeled, as described in "Materials and Methods." To confirm the DNA sequence at both the 5’ and 3’ termini of the cDNA, designated as plasmid pGT11, was selected and further characterized by DNA sequence analysis. Clone pGT11 was 2832 base pairs in length (Fig. 1). There exists an open reading frame that encodes a protein of 523 amino acids, followed by a 3’-untranslated region of 1260 bases. There is no predicted 5’-untranslated region or identifiable 3’ RNA termination signals, indicating that the original RNA transcript is larger than the cloned cDNA. When the predicted amino acid sequence encoded by pGT11 was examined for similarity with the other rabbit UGTs, it displays 78% similarity to UGT2B13 (Fig. 2) and 74% similarity to UGT2B14 (not shown). Based upon the high degree of similarity to the rabbit UGT2B sequences and the guidelines for classifying new UGTs (7), the predicted protein that is encoded by pGT11 has been designated as UGT2B16.

Expression of UGT2B16 Transcripts in Rabbit Liver—To examine the relative levels of RNA expression of UGT2B16 RNA as well as transcript size, primer extension analysis was conducted with RNA isolated from both neonatal and adult rabbits. Since there exists such a high degree of similarity in the 5’ portion of pGT11 with the UGT2B13 RNA, the first divergent region between the two RNAs was selected to identify antisense oligonucleotides, as indicated under "Experimental Procedures." Primer extension with the UGT2B16 oligonucleotide generated transcripts of 224 and 227 bases, while the UGT2B13 oligonucleotide generated a single transcript of 256 bases (Fig. 3).

RESULTS

Identification of the Rabbit Liver UGT2B16 cDNA—Southern blot analysis demonstrated that the 5’ portion of the
dexamethasone-inducible 4-hydroxybiphenyl UGT2B13 RNA is related in sequence to additional UGT genes (11). In attempts to identify additional UGT2B13-like RNA transcripts, a 5’ portion of the UGT2B13 cDNA covering bases 111–442 was employed as a probe to screen a rabbit liver cDNA library constructed in λZAP. Our initial screen resulted in the purification of over 20 λZAP cDNA clones. Each cDNA was initially characterized by PCR analysis to assure that the size of the inserts were at least 1600 bases, the minimum length needed to encode the 530-amino acid UGTs. A second screen was then conducted to determine the RNA sequence at both the 5’ and 3’ locations of the inserts to assure that each recombinant would encode a full-length protein. From these initial screens, one cDNA, designated as plasmid pGT11, was selected and further characterized by DNA sequence analysis. Clone pGT11 was 2832 base pairs in length (Fig. 1). There exists an open reading frame that encodes a protein of 523 amino acids, followed by a 3’-untranslated region of 1260 bases. There is no predicted 5’-untranslated region or identifiable 3’ RNA termination signals, indicating that the original RNA transcript is larger than the cloned cDNA. When the predicted amino acid sequence encoded by pGT11 was examined for similarity with the other rabbit UGTs, it displays 78% similarity to UGT2B13 (Fig. 2) and 74% similarity to UGT2B14 (not shown). Based upon the high degree of similarity to the rabbit UGT2B sequences and the guidelines for classifying new UGTs (7), the predicted protein that is encoded by pGT11 has been designated as UGT2B16.

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In examining the expression of UGT2B16, Northern blot analysis was conducted using two cDNA fragments as probes, one that spanned the coding region and the other that encompassed just the noncoding 3’ region of the cDNA. As demonstrated in Fig. 4A, the noncoding region of UGT2B16 identified a major 4.2-kb RNA transcript that begins to be expressed in 15 day old neonatal rabbits but exists primarily as an adult RNA transcript. When the 5’ coding region was used, the 4.2-kb fragment and several other transcripts were also identified. This would be expected since the coding region shares significant homology to other rabbit UGT RNA, such as UGT2B13. To examine this possibility, the same Northern blot was stripped and reprobed with the 5’ portion of the UGT2B13 cDNA. Two distinct transcripts, primarily expressed in adult RNA, are present as 4 and 1.9 kb, and migrate in the same location as two of the transcripts identified with the 5’ portion

2 Based upon a recommended nomenclature at the recent "VIII International Workshop on Glucuronidation and UDP-glucuronosyltransferases" (Iowa City, IA, May 19–22, 1996), the designation of the protein encoded by pGT11 is UGT2B16. The rabbit liver UGT1.6 is now designated as UGT1A6.
of the UGT2B16 cDNA. This result indicates that the 4- and 1.9-kb transcripts identified with the 5’ portion of UGT2B16 insert encode the UGT2B13 RNA. Another transcript, migrating at 2.2 kb and which is also identified with the 5’ portion of the UGT2B16 cDNA, most likely represents another UGT2B gene product.

Although UGT2B13 is expressed primarily in adults, we had previously demonstrated that this gene was regulated in a fashion similar to that of CYP3A6, being induced in neonatal rabbits with dexamethasone and macrolid antibiotics (11). To determine if UGT2B16 is regulated by these same inducers, RNA isolated from neonatal rabbits treated with dexamethasone was used to quantitate the levels of UGT2B16 RNA. As shown in Fig. 4B, dexamethasone had no effect on the ability to induce the 4.2-kb transcript that encodes UGT2B16 RNA. However, when the 5’ coding region of the UGT2B16 cDNA was used as a probe, it did detect induction of the UGT2B13 RNA transcripts in addition to the unidentified 2.2-kb RNA. Induction of UGT2B13 and CYP3A6 were verified in duplicate Northern blots using the respective cDNAs as probes. It should be noted that while CYP3A6 is usually expressed in adult rabbits, it exhibits variable levels of expression (15). The sample used in Fig. 4 was from an animal that exhibited a low level of expression. Although UGT2B16 and UGT2B13 RNAs are related in sequence, these results indicate that the cis-acting regulatory elements associated with the induction of the UGT2B13 gene are not conserved in the UGT2B16 gene.

Expression of UGT2B16 cDNA in COS-1 Cells—The predicted amino acid sequence of UGT2B16 is 523 amino acids with approximately 8 amino acids missing in the leader sequence when compared with UGT2B13 (Fig. 2). Since the leader sequence and the first 25 amino acids are identical between UGT2B16 and UGT2B13, a 186-basepair fragment of the UGT2B13 cDNA that encoded all of the leader sequence was used to exchange this same region of the UGT2B16 cDNA, generating a cDNA that encoded the additional 7 amino acids plus the initiation methionine (see “Experimental Procedures” for details). This chimeric UGT2B16 cDNA was then transferred to the plasmid pSVL and transfected into COS-1 cells to examine protein expression and catalytic activity.
The predicted amino-terminal end of the processed UGT2B16 is identical to UGT2B13 and the amino-terminal sequence of the purified rabbit liver estrone UGT. After confirming by pulse-chase analysis with [35S]methionine/cysteine-labeled protein that UGT2B16 and UGT2B13 are synthesized in COS-1 cells (Fig. 6B), UGT activity was examined in whole cell extracts using substrates catalyzed by the rabbit liver UGTs, as shown in Fig. 5. Expressed UGT2B16 catalyzes the glucuronidation of bulky phenols such as 2- and 4-hydroxybiphenyl, as well as smaller phenolic compounds like 2-naphthol. The bulky phenols are also conjugated by UGT2B13. However, UGT2B16 was able to glucuronidate 4-tert-butylphenol, a bulky phenol that did not serve as an efficient substrate for expressed UGT2B13. Interestingly, there were two substrates that showed strict selectivity to either UGT2B13 or UGT2B16. Octylgallate, a large nonplanar phenol was conjugated only by UGT2B13, while 4-hydroxyestrone served as a substrate for expressed UGT2B16. Neither UGT2B13 nor UGT2B16, which contain an amino-terminal region identical to that of the rabbit liver estrone UGT, were able to conjugate estrone.

The Expression of Chimeric UGT2B16 and UGT2B13—The largest degree of divergence between the two proteins resides at amino acids 64–226, with overall amino acid similarity from amino acids 1–64 and 229–530 being greater than 85%. It has been proposed that the substantial variation in amino acid sequence observed in the amino-terminal half of the transferases contributes to the diversity in substrate specificity (10). Since UGT2B16 and UGT2B13 share the ability to glucuronidate some common substrates, yet also show selective substrate specificity, the variable regions between these two proteins may underlie the ability of UGT2B16 and UGT2B13 to display differences in catalytic activity. To examine this possibility, regions of UGT2B16 and UGT2B13 cDNAs were exchanged and expressed in COS-1 cells to generate chimeric proteins that encoded the different regions of the two proteins.

UGT2B16, UGT2B13, and the chimeric proteins that are expressed in COS-1 cells are schematically shown in Fig. 6A. With 2B16, switches were made at amino acids Ser-300, Trp-358, and Val-434, while with 2B13 switches were made at amino acids Ser-300 and Val-434. In addition, amino acids Ser-300 through Trp-358 of 2B13 were replaced with 2B16. Proteinsynthesis of each chimeric protein, evaluated in COS-1 cellswas evaluated with 4-hydroxyestrone, octylgallate, and 4-hydroxybiphenyl, as shown in Fig. 7(A–C). 4-Hydroxyestrone is a selective substrate for UGT2B16, and octylgallate serves as a selective substrate for UGT2B13. The expression of UGT2B16 and those UGT2B16 chimeric proteins that share UGT2B13 carboxyl portions were catalytically active in the presence of 4-hydroxyestrone. There are 36 amino acid differences between UGT2B16 and UGT2B13 in the carboxyl region between Ser-300 and Asp-531, but switching this region on UGT2B16 (2B163002B13531) actually makes a more efficient protein in the glucuronidation of 4-hydroxyestrone when compared to that of the parent UGT2B16. Chimeric 2B163582B13531 and 2B164342B13531 were as functional as the parent UGT2B16. Since all of the chimerics that contained 2B16 in the amino-terminal were functional toward the glucuronidation of 4-hydroxyestrone, an activity that is not supported by UGT2B13, these results indicate that the amino-
The terminal region of UGT2B16 supports the structural requirements necessary for the specific glucuronidation of 4-hydroxyestrone.

Since both UGT2B16 and UGT2B13 catalyze the glucuronidation of some common substrates such as 4-hydroxybiphenyl (Fig. 5), it was anticipated that the replacement of the carboxyl region of UGT2B13 with that of UGT2B16 would have little impact on catalytic activity toward 4-hydroxybiphenyl. The cDNAs that encoded chimeric proteins $2B_{13300-2B_{16358}}$ and $2B_{13434-2B_{16531}}$ efficiently led to the production of immunoprecipitable UGTs, as displayed in Fig. 6B. However, when these expressed proteins were examined for catalytic activity, the UGT2B13 chimerics were not capable of conjugating 4-hydroxybiphenyl (Fig. 7B). In addition, these two chimerics were inactive with other substrates that were readily conjugated by UGT2B13, such as octyl gallate (Fig. 7C). Even the replacement of amino acids 300–358 from UGT2B13 in this same region of UGT2B16 (chimeric $2B_{13300-2B_{16358}}$) dramatically interrupted the glucuronidation activity toward octyl gallate. While there are 15 amino acid differences between UGT2B13 and UGT2B16 from Val-434 to Asp-531, substituting UGT2B13 with the UGT2B16 sequences completely inactivates the protein. It would appear that rigid structural requirements for the sequences between Val-434 and Asp-531 are needed to maintain UGT2B13 in an active conformation.

**DISCUSSION**

The UGT2B family in rabbits is composed of the UGT2B13, UGT2B14, and the 4-hydroxyestrone UGT2B16. Enzyme purification and NH$_2$-terminal analysis of estrone UGT demonstrates that the NH$_2$-terminal sequence is identical to that of both UGT2B13 and UGT2B16, but the mRNA encoded by the estrone UGT has not yet been identified. Northern blot analysis indicates that UGT2B16 is encoded by a large RNA of approximately 4.2 kb, and is not inducible in neonatal rabbits in a fashion similar to that of P4503A6.

The expression of UGT2B16 has catalytic activity that overlaps that of UGT2B13. Both enzymes glucuronidate small phenolic agents such as 2-naphthol and 4-methylumbelliferone, as well as bulky phenolic compounds like 2-hydroxy- and 4-hydroxybiphenyl. The larger phenolic agents are preferentially...
metabolized by UGT2B13. However, in contrast to UGT2B13, UGT2B16 had significant catalytic activity toward the polyhydroxylated estrogen 4-hydroxyestrone, but was catalytically inactive toward estriol and 17β-estradiol. In humans, 4-hydroxyestrone has been reported to be conjugated by UGT2B7, UGT2B8, UGT2B9, and UGT2B11, while UGT2B8 and UGT2B11 also conjugate 4-hydroxybiphenyl. UGT2B16 is unable to conjugate hyodeoxycholic acid, which also cannot be conjugated by UGT2B8 and UGT2B11. Based upon a comparison of the catalytic activities with UGT2B16 and the human UGT2 enzymes, UGT2B16 is most similar to UGT2B8 and UGT2B11. However, the 4-hydroxyestrone UGT activity observed with UGT2B16 was equivalent to the 4-tert-butylphenol UGT activity from expressed UGT2B16, which has been reported to be conjugated only by human UGTHP4. Therefore,
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UGT2B16 appears to have a much broader substrate specificity range than any of the UGT2 proteins identified in humans.

The observation that UGT2B16 and UGT2B13 share substrate specificity toward 4-hydroxybiphenyl, while UGT2B16 is selective toward 4-hydroxyestrone and UGT2B13 is specific for octylgallate, provided us the opportunity to examine the ability of chimeric proteins containing portions of both UGTs to glucuronidate these substrates. The results suggest that the amino-terminal portion of the proteins, as reported previously (10), are important for aglycone selection. This was demonstrated by linking the amino-terminal region of UGT2B16 with the carboxyl region of UGT2B13 and demonstrating that these chimerics were active in the conjugation of 4-hydroxyestrone. In one instance, a more efficient enzyme was derived using the amino-terminal 300 amino acids of UGT2B16 with the carboxyl-terminal 231 amino acids of UGT2B13.

However, the construction of UGT2B13-UGT2B16 chimeric proteins indicates that the carboxyl region of the UGTs may play a critical role in catalytic activity. For example, when the same NH2-terminal region of UGT2B13 was spliced to the carboxyl region of UGT2B16, there was a complete loss of catalytic activity. Even the replacement of amino acids 300–358 of UGT2B13 with UGT2B16 drastically reduced catalytic activity. Although these proteins are efficiently manufactured in the cells (Fig. 6B), the replacement of virtually any portion of the carboxyl region of UGT2B13 with UGT2B16 impaired enzyme function. This is clearly shown with 2B134342B16531, where amino acid residues 434–531 of UGT2B13 have been replaced with the same region from UGT2B16. It has been suggested (16) that amino acids 352–408 may be important in the recognition of UDP-glucuronic acid. If one proposes that the amino-terminal region, for example amino acids 1–300, are critical for substrate specificity while the region from 352–408 is important for UDP-glucuronic acid binding, amino acids 434–531 would appear to play an important role in some aspect of conformational stability. This is supported in experiments using chimeric 2B134342B16531, which was shown to be inactive when compared to the progenitor UGT2B13 in the glucuronidation of octylgallate.

Analysis of the carboxyl region of UGT2B13 and UGT2B16 by the method of Kyte and Doolittle (17) to identify membrane-spanning regions demonstrates that amino acids 495–519 are hydrophobic. This hydrophobic domain is flanked by two charged areas, which are characteristic of the halt-transfer signals of transmembrane proteins (18) as well as all UGTs (19), and plays a role in securing the protein in the membrane. Aside from the importance of amino acids 494–531 in positioning the protein in the membrane, this region may have a minor impact on substrate recognition and catalysis. With this assumption and based upon the negative catalytic activity of expressed 2B134342B16531, it would appear that amino acids 434–495 might be critical to UGT2B13 for activity. Interestingly, when UGT2B13 and UGT2B16 are aligned, there are conserved amino acid differences at positions 463, 468, and 488, and two nonconserved differences at Asn-443 and Arg-461. It may be that the nonconserved changes between UGT2B13 and 2B134342B16531 at Asn-443 and Arg-461 underlie the dramatic reduction in catalytic activity associated with 2B134342B16531.

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