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STEROID UDP GLUCURONOSYLTRANSFERASES

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Summary—The glucuronidation of steroids is a major process necessary for their elimination in the bile and urine. In general, steroid glucuronides are biologically less reactive than their parent steroids. However, in some cases often associated with disease and steroid therapy, more reactive or toxic glucuronides may be formed. The concentrations of specific steroid glucuronides in the blood may also indicate hormonal imbalances and may function as diagnostic markers of genetic defects in steroid synthesis and metabolism. In this review, the forms of UDP glucuronosyltransferase involved in steroid glucuronidation are described in terms of their specificities, functional domains and regulation. The available evidence suggests that steroid glucuronidation is mainly carried out by members of the UGT2B subfamily which are encoded by genes containing 6 exons. Members of this subfamily exhibit a regioselectivity in their glucuronidation of steroids that is mediated by domains in the amino-terminal half on the protein encoded by exons 1 and 2. Although much of this review will describe studies in the rat, preliminary evidence indicates that a similar situation may exist in humans.

THE ROLE OF GLUCURONIDATION IN STEROID METABOLISM

Glucuronidation is a major process converting steroids to more polar derivatives and is often a prerequisite for their removal from the cell. The formation of a glucuronide is illustrated with estradiol-17β as substrate (Fig. 1). Binding of UDP glucuronic acid to the enzyme UDP glucuronosyltransferase destabilizes the α-bond between UDP and the anomeric carbon atom of glucuronic acid. This bond is converted to a β-linkage between the glucuronosyl moiety and the nucleophile to form a β-glucuronide [1]. The covalent addition of a bulky sugar acid renders the steroid more hydrophilic and aids in its excretion in the bile or urine. In adults, many steroids from all major classes including androgens, estrogens, progestins, corticoids, bile acids and vitamins, such as vitamin D3 are excreted as glucuronides. Glucuronic acid is linked to 3α- and 21-hydroxyl groups of C21 steroids and the 3-(C18) or 3α-(C19) and 17β-hydroxyl groups of C18 and C19 steroids [2]. Steroids with 3β-hydroxyl groups such as dehydroepiandrosterone are generally excreted as sulfates. In neonates, the late maturation of the glucuronidation pathway [3] results in the excretion of a considerable proportion of unconjugated steroids.

The glucuronides of steroids are in most cases less biologically reactive than their parent steroid. However, under certain conditions often associated with disease and therapeutic steroid intervention, the formation of specific glucuronides may lead to increased toxicity. A classic example is the steroid D-ring glucuronides such as estradiol-17β, estriol-17β, estriol-16α and testosterone-17β (β-D-glucuronides) [4]. The intravenous administration of these glucuronides in rats reduces bile flow by up to 50% in a dose-dependent and reversible manner; an effect that is postulated to explain the induction of intrahepatic cholestasis by estrogenic and anabolic steroids. Estradiol-17β(β-D-glucuronide) has also been demonstrated to increase hepatic tight junctional permeability [5]. Paradoxically, estradiol-17β containing the glucuronosyl group on the 3-hydroxyl, increases bile flow compared to the parent steroid [4]. The effects of bile acid glucuronides on bile flow are more varied. It has been reported that lithocholic acid-3α(β-D-glucuronide) can decrease bile flow more than lithocholate [6], whereas, in contrast, the 3α-glucuronide of ursodeoxycholate is more effective than its parent bile acid at increasing bile flow [7]. Tetrahydrocorticoid glucuronides may also be more biologically reactive than their parent steroids as reflected in their more potent inhibition of angiogenesis [8].

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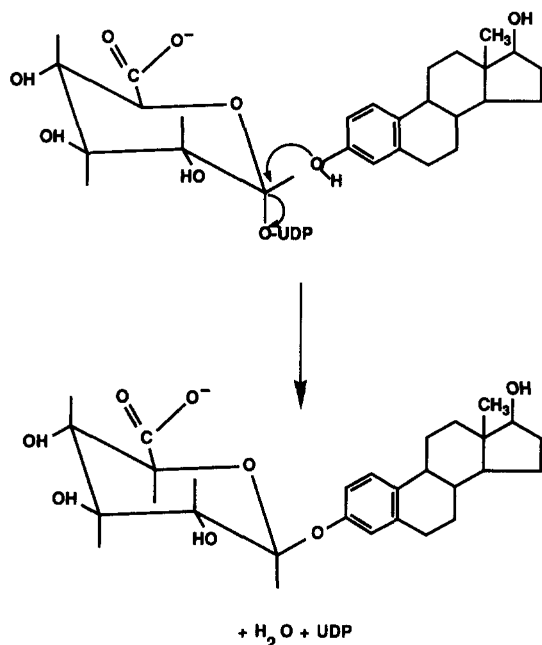

Fig. 1. UDP glucuronosyltransferase catalyzed glucuronidation of estradiol-17β.

Steroid glucuronides may, in some cases, be important markers of hormone status. For example, the levels of androsterone and 5α-androstane-3α,17β-diol glucuronides are elevated in the serum of hirsute women [9, 10] and that of the latter glucuronide in 21-hydroxylase deficiency [11, 12]. The bile alcohol glucuronide, 5β-cholestane-3α,7α,12α,25-tetrol-3-*O*-β-D-glucuronide is an important marker for the diagnosis of cerebrotendinous xanthomatosis patients [13].

As steroid glucuronides have different biological properties and their levels vary with age and hormonal status, it is important that the forms of UDP glucuronosyltransferase involved in their biosynthesis are defined and their tissue distribution and regulation characterized.

UDP GLUCURONOSYLTRANSFERASES AND THEIR STEROID SPECIFICITIES

The UDP glucuronosyltransferases are integral proteins in the endoplasmic reticulum of the liver and several extrahepatic tissues including the kidneys, gastrointestinal tract and lung [3, 14–18]. More than 14 forms (Fig. 2) have been identified in the rat by cDNA cloning and purification studies [19–21]. An analysis of their deduced amino acid sequences suggests that they can be divided into two families. The sequences of members of one family are more than 50% similar to each other but less than 50% similar to those in the second family. The members of family 1 are all derived by alternate splicing from a single gene [22] located on human chromosome 2 [23]. Members of this family glucuronidate small planar phenols [24, 25] and bilirubin [26, 27]. Steroid substrates for family 1 enzymes have not been identified to date. Family 2 members are encoded by separate genes and comprise two subfamilies. The 2A subfamily consists of a form that is specific for the olfactory epithelium and has activity towards at least five odourants [28]. Steroid substrates have not been tested. The 2B subfamily contains forms that are more than 65% similar in sequence. Three of these forms, UGT2B1, 3 and 6, glucuronidate testosterone [29–31], whereas the fourth, UGT2B2, glucuronidates androsterone and bile acids [32–34].

Using cDNA expression systems we have assessed the capacity of five forms of UDP glucuronosyltransferase, UGT2B1, 3, 5 and 6 and UGT1*06 to glucuronidate a range of natural and synthetic steroids (unpublished data). The results of these studies are summarized in Table 1. In general it appears that UGT2B1, 3 and 6 are active towards the 17β-hydroxyl group of C18 and C19 steroids. The addition of an extra 7α-, 16α- or 11β-hydroxyl to the steroid nucleus produces better substrates for UGT2B1 but poorer substrates for UGT2B3 and 6. In contrast, UGT2B2 is more active towards the 3α-hydroxyl group of C19 steroids and bile acids and is less active towards those steroids containing additional 7α- and 11β-hydroxyl groups. The UGT1*06 form was inactive towards all the steroids tested. Although glucuronidated by microsomal UDP glucuronosyltransferases, esterone and its hydroxylated metabolites were not glucuronidated to any great extent by the five rat forms tested in this study. This is in contrast to human family 2B forms that are active in the glucuronidation of estriol and its 2-, 4- and 6-hydroxylated derivatives [35].

CATALYTIC DOMAINS OF UDP GLUCURONOSYLTRANSFERASES

A comparison of UDP glucuronosyltransferase sequences reveals that they all have

*The names of UDP glucuronosyltransferase forms described in this paper follow the recommended nomenclature recently published [19].

Table 1. Steroid substrates of rat liver UDP glucuronosyltransferases

Steroid	Microsome UGTs	UGT2B1	UGT2B3	UGT2B2
17β-Hydroxy-4-androsten-3-one (testosterone)	+[a]	+	+	
17β-Hydroxy-5α-androstan-3-one	+	+	+	
1-Dehydro-17α-methyltestosterone	+		+	
3α-Hydroxy-5α-Androstan-17-one	+			+
3α,11β-Dihydroxy-5α-androstan-17-one	+			
3α,16α-Dihydroxy-5α-androstan-17-one	+			+
3α-Hydroxy-5β-androstan-17-one	+			+
3α,11β-Dihydroxy-5β-androstan-17-one	+			
3α-16α-Dihydroxy-5β-androstan-17-one	+			+
5α-Androstan-3α,17β-diol	+	+		+
5-Androstene-3β,17β-diol	+	+		
Estradiol-17β	+	+	+	
Diethylstilbestrol	+	+		
3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one	+			+
3α,17α,21-Trihydroxy-5β-pregnane-11,20-dione	+			+
3α,11β,21-Trihydroxy-5β-pregnan-20-one	+			+
5β-Pregnane-3α,11β,17α,20β,21-pentol	+			+
3α,17α,20β,21-Tetrahydroxy-5β-pregnan-11-one	+			+

[a] A "+" indicates that the enzyme is active towards the listed steroid (Stranks and Mackenzie, unpublished data).

a signal peptide that is cleaved when inserted into the endoplasmic reticulum and a carboxyl-terminal hydrophobic segment of 17 residues that is sufficient to traverse a membrane. The remainder of the polypeptide chain of about 30 residues rich in basic amino acids is probably exposed on the cytoplasmic face of the membrane [36]. This topological model is supported by *in vitro* transcription/translation experiments demonstrating that the mature enzyme formed

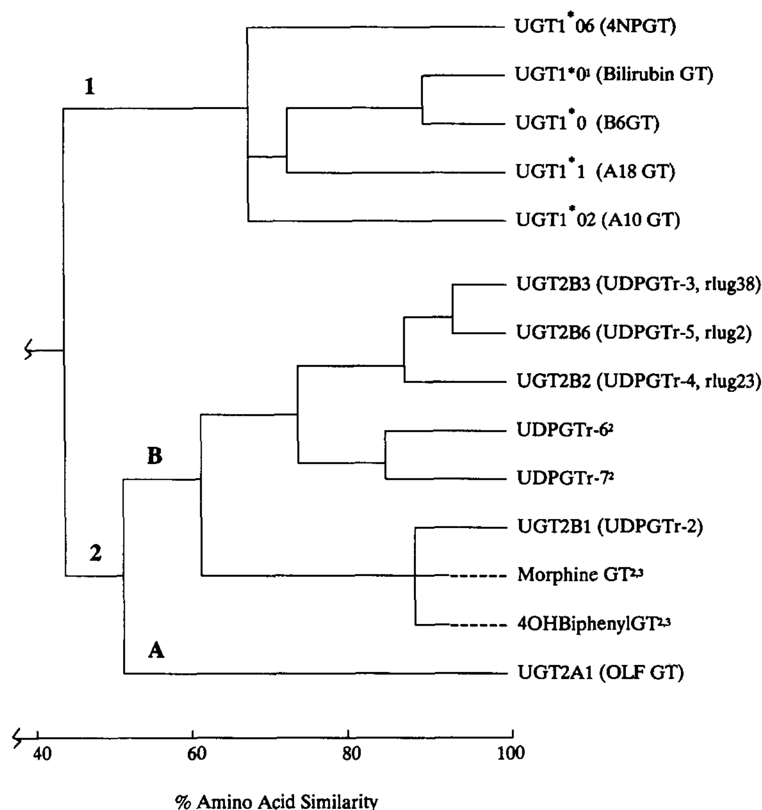

Fig. 2. The rat UDP glucuronosyltransferase family. The deduced amino acid sequences of UGT1*06 [24], UGT1*0 [bilirubin GT, 27], UGT1*0 [B6GT, 44], UGT1*1 [44], UGT1*02 [44], UGT2A1 [28], UGT2B3 [30, 55], UGT2B6 [31], UGT2B2 [32, 56] and UGT2B1 [29] were compared to generate the figure. [1] Bilirubin GT and B6GT are distinct gene products. The final numeral of their systematic names will be assigned when the structure of the gene encoding all UGT1 forms is fully characterized. [2] Systematic names have not been assigned as only partial sequences are available [unpublished data]. [3] Morphine GT [54] and 4-hydroxybiphenyl GT [21] are purified proteins that may be closely related to UGT2B1 (this is indicated by a broken line).

in the presence of dog pancreatic microsomes, is not removed from the membrane by alkaline pH treatment, a process that destroys the vesicular structure of the membrane and releases luminal proteins, and is not accessible to externally added antibody [30, 37]. The resistance of microsomal bilirubin UDP glucuronosyltransferase to protein digestion in the absence of detergent is also consistent with this model [38]. It would thus appear that more than 95% of the polypeptide chain, including the catalytic site is located on the luminal aspect of the endoplasmic reticulum.

A luminal location is also consistent with the presence of N-linked oligosaccharide side chains on some forms of UDP glucuronosyltransferase such as UGT2B1 and 2 and UGT1*06 [29, 39]. N-Linked glycosylation is known to modulate protein structure, function and stability. However, this posttranslational modification is not essential for UDPGT catalytic activity and does not appear to play a role in substrate selection or protein stabilization [40]. Subtle effects of glycosylation on the kinetic parameters of the enzyme can not be discounted.

The domains of UDP glucuronosyltransferase that are responsible for steroid selection are found in the amino-terminal half of the polypeptide chain. Evidence for this was obtained from the synthesis and analysis of chimeric proteins formed from two forms that had different steroid specificities [41]. Furthermore, it appears that the domains responsible for steroid selection are encoded by the first one or two exons of the UGT2B genes. The genes encoding two members of this family, UGT2B1 and 2 have been cloned and sequenced [42, 43]. Both genes consist of six exons and have identical intron/exon boundaries (Fig. 3). The sizes of the genes and their corresponding introns vary. The members of the UGT1 family in contrast, are derived by the alternate splicing of a single gene consisting of a set of at least five first exons followed by exons 2 to 5 [22]. Although this gene has not been fully characterized, it appears that the five UGT1 enzymes characterized to date share an identical carboxy-terminal 246 amino acid domain encoded by exons 2 to 5 that is probably involved in the binding of the common co-substrate UDP glucuronic acid [15, 44]. Their highly variable amino-terminal domains of 285–289 amino acids are about 40% similar in sequence and are encoded by the set of five, first exons. Although experimental evidence is lacking, this region may be also responsible for binding the planar phenols or bilirubin characteristic of each form. The first two exons of the UGT2B genes encoding the steroid binding domains correspond to the variable first exons of the UGT1 gene.

The carboxy-terminal stretch of 17 hydrophobic amino acids and terminating region rich in basic residues is encoded in the last exon of all UDP glucuronosyltransferase genes characterized to date. As mentioned above, this region is thought to anchor the protein to the membrane with the basic tail exposed on the cytoplasmic face of the endoplasmic reticulum (ER). The lysine residues in the third and fourth or fifth position from the carboxy-terminus are necessary for maintaining the enzyme in the ER and preventing its egress from this intracellular compartment [45]. We have preliminary evidence to suggest that this region is also

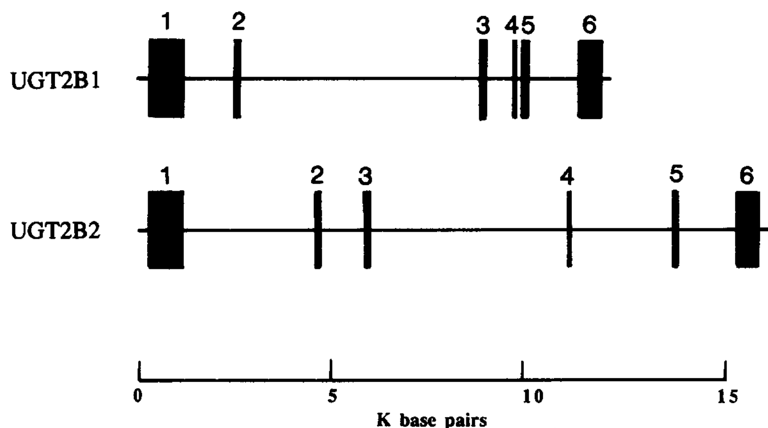

Fig. 3. UGT2B1 and UGT2B2 gene organization and functional domains. Exons are numbered. Exons 1 and 2 contribute to the steroid binding domain. Exons 4 and 6 contribute to the UDP glucuronic acid binding domain. The putative transmembrane domain and cytoplasmic region containing the endoplasmic retention signal are encoded in exon 6.

important for catalytic activity as proteins that are missing the carboxyl-terminal 31 residues are inactive (unpublished data).

REGULATION OF STEROID UDP GLUCURONOSYLTRANSFERASES

The mRNAs encoding UGT2B subfamily forms are more than 20-fold higher in the liver compared to the small intestine, lung, kidneys, brain and testes [30, 36]. Other tissues such as the skin have yet to be examined. This apparent liver specific expression appears to be mediated by the presence of several DNA motifs, including a HP1 site, that are found in many liver specific genes [46 and references therein] and in the 5′-flanking regions of the UGT2B1 and 2 genes [42, 43]. Consistent with this hypothesis is the finding that a 323 bp promoter fragment of the UGT2B1 gene was sufficient to drive the transcription of a reporter gene in rat liver cell lines but not in cell lines derived from other tissues [42].

Although the UGT2B enzymes are constitutively expressed in the liver, their levels may be altered by environmental chemicals. For example, UGT2B1 mRNA levels are elevated 5-fold in the livers of rats treated with phenobarbital [47], whereas forms related to UGT2B2, 3 and 6 are induced to a lesser extent by dexamethasone, rifampicin, imidazole, methylpyrazole and pyrazole [36]. In preneoplastic nodules induced by chemical carcinogens, the UGT2B mRNAs appear to be unchanged or decreased whereas UGT1*06 mRNA levels are elevated 10- to 15-fold [48, 50].

Initial evidence from enzyme activity studies demonstrated that the glucuronidation of testosterone and estradiol-17β was first evident in rats at birth and then increased to adult levels [51, 52], whereas that towards androsterone appeared after weaning [53]. The increased glucuronidation of these steroids after birth is reflected in increased levels of UGT2B1 mRNA [2, our unpublished data] and UGT2B2 mRNA levels [43]. A similar postnatal development in the capacity to glucuronidate steroids has also been documented in human livers [3]. Although the liver is a major organ involved in steroid metabolism, the steroid specificities of UDP glucuronosyltransferases in extrahepatic tissues will need to be assessed when the enzymes in these tissues have been identified and isolated.

CONCLUSIONS

The glucuronidation of steroids plays a major role in the termination of their biological activities and in their removal from the cell. Steroids are mainly glucuronidated by UDP glucuronosyltransferases belonging to the UGT2B subfamily. These forms appear in the liver after birth and their levels are modulated by phenobarbital and other drugs. Steroids containing a 17β-hydroxyl group are mainly glucuronidated by UGT2B1 and those containing a 3α-hydroxyl group are glucuronidated by UGT2B2. Steroid specificity is determined by the protein domains encoded by exons 1 and 2 of the UGT2B genes and is not altered by the presence of N-linked oligosaccharides. The carboxyl-terminal hydrophobic and basic regions of the UDP glucuronosyltransferase protein are necessary for catalytic activity in addition to their role in membrane anchoring and retention of the enzyme in the endoplasmic reticulum.

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