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Short communication

Glucosidation of hyodeoxycholic acid by UDP-glucuronosyltransferase 2B7

Peter Mackenzie[a,*], Joanna M. Little[b], Anna Radominska-Pandya[b]

[a]Department of Clinical Pharmacology, Flinders University and Flinders Medical Center, Bedford Park, SA 5042, Australia
[b]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

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Abstract

Previous studies have shown that several endogenous compounds, such as bilirubin and certain bile acids, are glucosidated in human liver. In this work, we have identified human UDP-glucuronosyltransferase 2B7 (UGT2B7) as the isoform that catalyzes the glucosidation of hyodeoxycholic acid (HDCA). The glucosidation by UGT2B7 was specific for HDCA and was not observed with the other bile acids examined, lithocholic acid, chenodeoxycholic acid, and ursodeoxycholic acid. The kinetics of HDCA glucuronidation and glucosidation by UGT2B7 were characterized. The K_m values for glucuronidation and glucosidation of HDCA were 11.6 and 17.9 μM, respectively, with V_{max} values of 4.15 nmol/min/mg protein for glucuronidation and 3.28 nmol/min/mg for glucosidation. At a fixed concentration of HDCA, the apparent K_m for UDP-glucuronic acid was 89 μM with a V_{max} of 3.53 nmol/min/mg. The corresponding parameters for UDP-glucose were 442 μM and 1.98 nmol/min/mg, respectively. UGT2B7 catalyzed the addition of the glucose and glucuronic acid moieties to an hydroxyl group on HDCA and also possessed some capacity to use UDP-xylose as sugar donor. The two polymorphic variants of UGT2B7, UGT2B7*1 and UGT2B7*2 could both glucosidate HDCA. This is the first report that identifies UGT2B7 as the enzyme responsible for the glucosidation of the bile acid, HDCA.

Keywords: UDP-glucuronosyltransferase 2B7; Bile acid glucosidation; Glucuronidation

1. Introduction

The UGTs are a family of membrane-bound enzymes that glucuronidate both endogenous and xenobiotic compounds such as bilirubin, steroids, drugs, and environmental pollutants. The major function of glucuronidation is to change hydrophobic compounds into soluble derivatives, thus facilitating their detoxification and excretion [1]. However, UGTs can also synthesize glucuronides that are biologically active or more toxic than their parent compound [2].

UGTs show very high specificity toward the cofactor UDP-GlcUA [1]. However, glucosides of several endogenous compounds, such as bilirubin and HDCA, have been identified previously. The most detailed information is on the glucosidation of bilirubin. After initial identification of bilirubin glucosidation in rat and human liver microsomes [3,4], Senafi *et al.* [5] demonstrated that, in addition to UDP-GlcUA, recombinant human UGT1A1 could utilize UDP-Glc and UDP-Xyl to biosynthesize the corresponding conjugates. Mammalian UGTs that glucosidate phenols and steroids have also been described [6–8]. In addition, bile acid glucosides could be isolated from human urine [9] and are formed *in vitro* [10]. Our detailed studies carried out with human liver microsomes demonstrated that HDCA can be conjugated with glucose and xylose and, more importantly, that HDCA was the only bile acid that underwent this conjugation [11]. This work also demonstrated using NMR and FAB-MS spectrometry that the glucose derivative was almost exclusively attached to the hydroxyl function of HDCA and that human UGT2B4, which has high specificity toward HDCA, was not involved in the glucosidation reaction.

The purpose of the present study was to identify and characterize the human UGT isoforms responsible for the

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* Corresponding author. Tel.: + 61-8-8204-5394; fax: +61-8-8204-5114.

E-mail address: peter.mackenzie@flinders.edu.au (P. Mackenzie).

Abbreviations: UGT, UDP-glucuronosyltransferase; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; UDP-GlcUA, uridine 5′-diphosphate glucuronic acid; UDP-Glc, uridine 5′-diphosphate glucose; UDP-Xyl, uridine 5′-diphosphate xylose; TLC, thin layer chromatography.

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biosynthesis of HDCA glucosides. The data presented in this work show that UGT2B7 is involved in HDCA glucosidation.

2. Materials and methods

2.1. Materials

[^{3}H]HDCA was synthesized in our laboratory as previously described [12,13]. [^{14}C]UDP-GlcUA and [^{14}C] and [^{3}H]UDP-Glc were from Perkin-Elmer Life Science. The bile acids and other substrates, unlabeled UDP-sugars, Brij 58, HEPES and alamethicin were purchased from Sigma.

2.2. Recombinant UGT2B7

Human UGT2B7 (Tyr268) [14] was expressed in HK293 cells [15] and was provided by Dr. T. Tephly, Department of Pharmacology, University of Iowa. A membrane fraction enriched in UGT2B7 was prepared as previously described [16] and aliquots were stored at −80° until used. In some experiments, a second source of UGT2B7 (Tyr268)-expressing HK293 cells was used. These UGT2B7 stably expressing cells were prepared using the pEFIRES-P expression vector and puromycin selection as described [17]. The data obtained with both sources of recombinant UGT2B7 were equivalent.

2.3. Enzymatic assays

HDCA activity was assayed with [^{3}H]HDCA (100 μM final concentration) and UDP-GlcUA or UDP-Glc (4 mM final concentration) as the sugar donor as described in detail previously [11–13]. Briefly, HDCA was solubilized in micelles with the detergent Brij 58 (final Brij concentration, 0.05%) and incubated with UDP-sugar and human recombinant UGT2B7 (50 μg protein) in 100 mM HEPES buffer, containing 5 mM $MgCl_2$ and 5 mM saccharolactone, pH 7.4 for 10 min. The other bile acids assayed (lithocholic acid (LCA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA)) were treated in the same manner. The remaining compounds, androsterone, estriol, 17α-estradiol, borneol, 4-methylumbelliferone, ketoprofen, and mycophenolic acid (100 μM) were incubated with 1 mM [^{14}C]UDP-GlcUA or UDP-Glc for 1 hr at 37° as described previously [18].

The glucuronidated or glucosidated products and the unreacted substrate were separated by development in chloroform–methanol–glacial acetic acid–water (65:25:2:4, v/v). Radioactive compounds were localized on TLC plates by autoradiography at −80°. Zones corresponding to the glucuronide bands were scraped into scintillation vials and radioactivity was measured by liquid scintillation counting (Beckman Model LS5000TD; Beckman Instruments). In some experiments, glucuronides were visualized and quantified by use of a PhosphoImager (Molecular Dynamics, Inc.).

Identification of the position of glucuronidation (hydroxyl or carboxyl group) was assessed by alkaline hydrolysis as previously described [12].

Kinetic analysis was carried out at a constant UDP-sugar concentration (4 mM) with HDCA concentrations from 5 to 200 μM or at constant HDCA concentration (100 μM) and UDP-sugar concentrations from 50 μM to 3 mM with an incubation time of 10 min. Kinetic parameters were determined using EnzymeKinetics software (Trinity Software).

3. Results and discussion

Bile acids are synthesized in the liver from cholesterol and have a major role in the solubilization of fats and fat absorption. Many are also ligands for the nuclear receptors, PXR and FXR [19,20]. Following their secretion into the intestinal lumen, bile acids can be further metabolized by bacteria to secondary bile acids. We have demonstrated previously that LCA, which can be hydroxylated at position 6 by CYP3A4, yielding HDCA, can then be efficiently glucuronidated on the 6-OH group [13,21,22]. The coupled hydroxylation–glucuronidation system is considered an efficient detoxification pathway for toxic bile acids, since defects in bile acid metabolism can be associated with serious diseases, including cholestasis [23].

Certain bile acids can be effectively glucuronidated by two human UGT2B isoforms, UGT2B4 and UGT2B7 [11,21,24,25]. In addition to conjugation with glucuronic acid, glucosides of HDCA have also been shown to be synthesized by human liver microsomes [11]. However, the specific human isoform(s) involved in the latter conjugation processes has not been identified.

In this study, we show that human UGT2B7 has the capacity to glucosidate HDCA. Membrane fractions from HK293 cells transfected with UGT2B7 catalyzed the conversion of HDCA to HDCA glucuronide and glucoside (Fig. 1). The rates of HDCA glucuronide synthesis were approximately double those of glucoside formation (Table 1). Of the other bile acids utilized, only LCA

Table 1
Glucuronidation and glucosidation of bile acids by UGT2B7

Substrate	Activity (nmol/mg/min)		
	UDP-GlcUA	UDP-Glc	UDP-Xyl
HDCA	2.01 ± 0.65	0.96 ± 0.11	0.13
LCA	0.014	nd[a]	–[b]
CDCA	nd	nd	–
UDCA	nd	nd	–

Assays were carried as described in the text with 100 μM substrate and 4 mM UDP-sugar. Results are the mean (±SD) of at least two determinations.

[a] nd, not detected (the activity was less than 0.15 pmol/mg/min).

[b] Not determined.

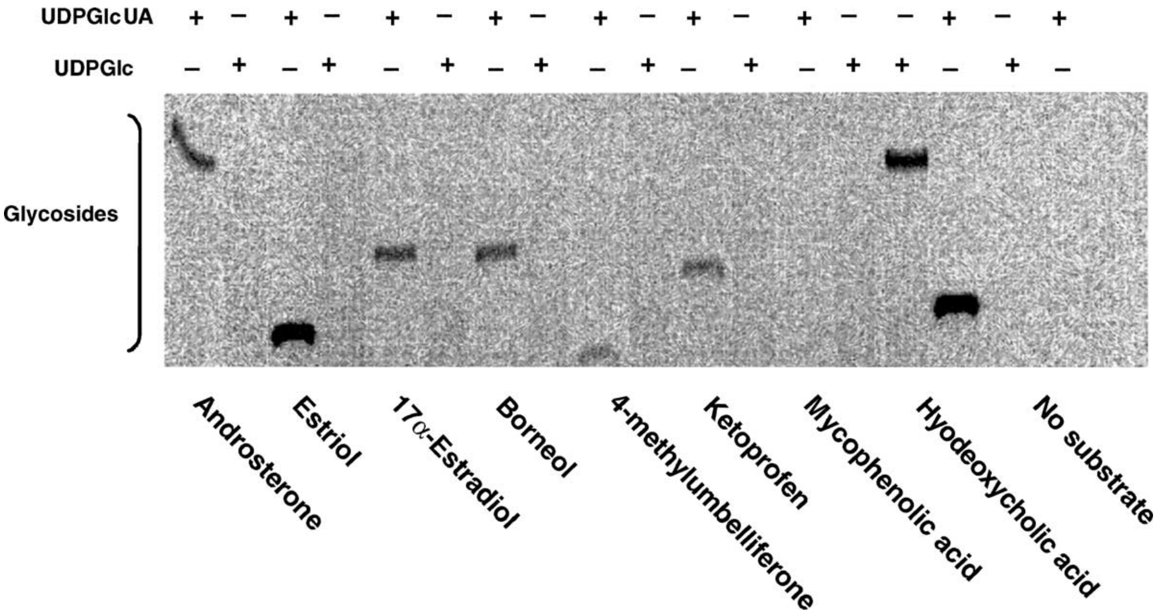

Fig. 1. Conjugation of HDCA and other compounds by UGT2B7. The capacity of UGT2B7 to conjugate various compounds with glucose or glucuronic acid (as indicated at the top of the figure) was assessed as described in Section 2. An autoradiograph of the area of the TLC plate containing the conjugates is shown.

was glucuronidated by UGT2B7. In contrast, UGT2B7-mediated glucosidation of LCA and the other bile acids was not observed. UGT2B7 was also incapable of glucosidating other well characterized substrates of UGT2B7 [26], including, androsterone, estriol, 17α-estradiol, borneol, 4-methylumbelliferone and ketoprofen (Fig. 1). Phenols and some steroids were recognized previously as targets for microsomal conjugation with glucose, but our results would indicate that isoforms other than UGT2B7 are responsible for this. The glycosylation of mycophenolic acid by recombinant UGT2B7 was also not seen (Fig. 1), even though glucuronides and glucosides of this compound have been detected in microsomal preparations from human liver and kidney [27]. Thus, our present studies demonstrate a very high specificity of UGT2B7 toward HDCA glucosidation.

There are two polymorphic forms of UGT2B7, UGT2B7*1 with a histidine at position 268 and UGT2B7*2 with a tyrosine in this position [26]. Both polymorphic forms have the capacity to glucosidate HDCA as shown by assays of microsomal samples of livers homozygous for either UGT2B7*1 or UGT2B7*2 [28]. The activities of three UGT2B7*1 homozygote samples were 2.49, 1.62 and 2.30 nmol HDCA glucuronide formed/min/mg protein and 1.04, 0.72 and 0.87 nmol HDCA glucoside-formed/min/mg protein, respectively. The corresponding values for three UGT2B7*2 homozygote samples were 2.54, 2.55, 3.55 nmol HDCA glucuronide formed/min/mg protein and 1.04, 1.07, 1.39 nmol HDCA glucoside-formed/min/mg protein for glucuronide and glucoside formation, respectively.

The kinetics of HDCA glucuronidation and glucosidation by UGT2B7 microsomes were characterized. Formation of both HDCA conjugates follows Michaelis–Menten kinetics over a bile acid concentration range of 5–150 μM. Representative kinetics are summarized in Table 2. The apparent K_m for the formation of HDCA glucuronide by UGT2B7 was slightly lower than that for HDCA glucoside and the V_{max} for HDCA glucuronidation was approximately 30% higher than that for glucosidation. The apparent K_m and V_{max} for the formation HDCA glucuronide and glucoside over a range of UDP-GlcUA and UDP-Glc concentrations of 50–3000 μM also followed Michaelis–Menten kinetics. The apparent K_m value with UDP-Glc was approximately 5-fold higher than that for UDP-GlcUA, indicating significantly higher affinity for glucuronide

Table 2
Apparent kinetic constants (K_m and V_{max}) for glucuronidation and glucosidation of HDCA by UGT2B7[a]

	$K_{m(HDCA)}$ (μM)	$V_{max(HDCA)}$ (nmol/mg/min)	$K_{m(UDP\text{-}sugar)}$ (μM)	$V_{max(UDP\text{-}sugar)}$ (nmol/mg/min)	$V_{max}/K_{m(HDCA)}$ (mL/mg/min)
UDP-GlcUA	11.6	4.15	89	3.53	358
UDP-Glc	17.9	3.28	442	1.98	183

Note: Kinetic parameters for UDP-GlcUA and UDP-Glc determined with human liver microsomes (from a 32-year-old female who died of anoxia) have been previously reported [11]: these were K_m, 127 and 280 μM and V_{max}, 3.8 and 1.8 nmol/mg/min for UDP-GlcUA and UDP-Glc, respectively.

[a] Apparent K_m and V_{max} values were determined at 5–200 μM HDCA and 4 μM UDP-GlcUA or UDP-Glc as well as at 50–3000 μM UDP-GlcUA or UDP-Glc and 100 μM HDCA. Results are the means of two duplicate determinations.

formation. The corresponding $V_{\max}$ for UDP-GlcUA was approximately 80% higher than that for UDP-Glc. Kinetic analysis of HDCA glucuronidation and glucosidation at constant concentrations of HDCA by human liver microsomes has been documented by us previously and the kinetic data reported (see Note in Table 2) are qualitatively similar to the results presented here. However, the kinetics with a variable concentration of HDCA and a constant concentration of nucleotide sugar have been evaluated for the first time in these studies.

Our previous studies have shown that HDCA is glucosidated at the 6-OH position [11]. Alkaline hydrolysis (data not shown) indicated that both glucuronidation and glucosidation of HDCA results in the formation of a hydroxyl-linked conjugate, presumably at the 6-OH position.

UGT2B7 also has some capacity to use UDP-Xyl to form HDCA xylosides (Table 1). Thus, UGT2B7 may also be responsible for the formation of HDCA xylosides in human liver microsomal preparations as has been observed previously [11].

Comparison of the two human bile acid-specific isoforms, UGT2B4 [11] and UGT2B7 (the present studies) clearly indicates that only the latter has the ability to form HDCA glucosides. As other UGT forms, including UGT1A3, UGT1A8 and UGT2B28 have some capacity to glucuronidate HDCA, it is possible they may also glucosidate this bile acid. One can speculate on the physiological significance of HDCA glucoside formation in humans. One possibility is that the formation of glucosides represents a back-up detoxification mechanism in pathologic situations where an accumulation of certain toxic bile acids, e.g., LCA, is observed. Extensive glucuronidation may result in total depletion of the available pool of UDP-GlcUA. When the need for bile acid detoxification persists, conjugation with glucose from readily available UDP-glucose stores would take over. This scenario may also apply to the formation of bilirubin glucosides. The excretion of bilirubin, a toxic heme breakdown product, in the form of the glucuronide or glucoside is of physiological importance [3,4]. Both bilirubin and bile acids are present in the body in gram quantities and their proper excretion is critical, especially under pathological conditions. The higher K_m values for HDCA glucosidation could indicate that the switch from glucuronide to glucoside represents an additional detoxification mechanism activated under pharmacological conditions by the accumulation of potentially toxic endogenous compounds. The involvement of UGT2B7 in HDCA glucosidation is a novel discovery.

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