Biosynthesis of hydroxyl-linked glucuroniodes of short-chain bile acids by rat liver 3-hydroxysteroid UDP-glucuronosyltransferase

Anna Radominka,* Mitchell D. Green,† Piotr Zimniak,* Roger Lester,* and Thomas R. Tephly†

Division of Gastroenterology, Department of Internal Medicine,* University of Texas Medical School at Houston, Houston, TX 77225, and Department of Pharmacology,† University of Iowa, Iowa City, IA 52242

Abstract Microsomal preparations from livers of Sprague-Dawley rats catalyze the glucuronidation of 3α-hydroxy-5β-H (3α,5β) short-chain bile acids (C20–C23), predominantly at the hydroxyl group, while the glucuronidation of 3β,5β short-chain bile acids occurs exclusively at the carboxyl group. A similar pattern of conjugation was also observed in Wistar rats having normal levels of 3-hydroxysteroid UDP-glucuronosyltransferase. Significant reductions of formation rates for hydroxyl-linked, but not carboxyl-linked, short-chain bile acid glucuronides were observed in hepatic microsomes from Wistar rats with low 3-hydroxysteroid UDP-glucuronosyltransferase activity. 3-Hydroxysteroid UDP-glucuronosyltransferase, purified to homogeneity from Sprague-Dawley liver microsomes, catalyzed the O-glucuronidation of 3α,5β C20–23 bile acids, as well as of lithocholic and isolithocholic acids (C24). The apparent Michaelis constants (Km) for short-chain bile acids were similar to the value obtained for androsterone. 3α,5β-C21 and 3β,5β-C20 competitively inhibited glucuronidation of androsterone by the purified 3-hydroxysteroid UDP-glucuronosyltransferase. Purified 17β-hydroxysteroid and p-nitrophenol UDP-glucuronosyltransferases did not catalyze the glucuronidation of bile acids. In addition, none of the purified transferases catalyzed the formation of carboxyl-linked bile acid glucuronides. The results show that 3-hydroxysteroid UDP-glucuronosyltransferase, an enzyme specific for 3-hydroxyl groups of androgenic steroids and some conventional bile acids, also catalyzes the glucuronidation of 3α-hydroxy (but not carboxyl) groups of 3α,5β short-chain bile acids. — Radominka, A., M. D. Green, P. Zimniak, R. Lester, and T. R. Tephly. Biosynthesis of hydroxyl-linked glucurononides of short-chain bile acids by rat liver 3-hydroxysteroid UDP-glucuronosyltransferase. J. Lipid Res. 1988. 29: 501–508.

Supplementary key words Sprague-Dawley rats • Wistar rats • Gunn rats • high androsterone Wistar rats • low androsterone Wistar rats • hydroxyl glucuronidation • carboxyl glucuronidation • acyl glucuronide

UDP-glucuronosyltransferases (UDPGTs, EC 2.4.1.17) are a family of isoenzymes that catalyze the conjugation of aglycone acceptor molecules with glucuronic acid supplied by UDP-glucuronic acid. These isoenzymes have been shown to exhibit relatively stringent substrate specificity toward physiologic substrates but possess overlapping specificity toward many xenobiotics. While xenobiotics such as β-nitrophenol, 1-naphthol, or 4-methylumbelliferone are reactive with more than one isoenzyme (1, 2), UDPGTs specific for 17β-hydroxysteroids (1, 3), 3-hydroxysteroids (3), estrone (2, 4, 5), and bilirubin (2, 6, 7) have been purified. The 3-hydroxysteroid UDPGT is specific for the 3α-hydroxyl group of various steroids and normally does not conjugate a 3β-group (1, 3). In addition to neutral steroids, the enzyme is reactive toward bile acids, including mono-and dihydroxylated C24 compounds (8). In the case of acidic steroids, the specificity of the enzyme for 3α versus 3β hydroxyl groups is not absolute: 3β,5β-C24 is glucuronidated, albeit at less than half of the rate found for 3α,5β-C24 (ref. 8 and this report).

It has been demonstrated previously that monohydroxylated short-chain bile acids (C20–C23) are glucuronidated by the rat in vivo (9, 10), and in vitro by rat liver microsomes (11). Short-chain bile acid glucuronides can be conjugated at the hydroxyl group on the steroid ring structure, the carboxyl group of the side chain, or both (10–12). It is not known, however, whether any of the above processes is catalyzed by the 3-hydroxysteroid UDPGT. Three general approaches...
Animals and preparation of microsomes

Blue Spruce Farms, Altamont, NY. The animals were homozygous Gunn rats (200-225 g) were obtained from Charles River Labs., Wilmington, MA. Sprague-Dawley rats (200-250 g) were from Bio-Labs, St. Paul, MN, and Wistar rats. Male Wistar rats (200-250 g) were purchased from Charles River Labs., Wilmington, MA. Sprague-Dawley rats (200-250 g) were from Bio-Labs, St. Paul, MN, and homozygous Gunn rats (200-225 g) were obtained from Blue Spruce Farms, Altamont, NY. The animals were housed over wire mesh in stainless-steel cages and were allowed food (Purina Rodent Chow; Purina, St. Louis, MO) and water ad libitum. The light–dark cycle was 6:00 to 18:00 hr. All rats were fasted overnight prior to the preparation of hepatic microsomal fractions. The rats were killed by cerebral concussion and their livers were perfused in situ with ice-cold 1.15% KCl. Livers were then removed and homogenized in four volumes of 1.15% KCl with a motor-driven Teflon pestle, and microsomes were isolated by differential centrifugation as described (4). The microsomal preparations were stored at −70°C with an overlay of 1.15% KCl.

Enzyme purification

3-Hydroxysteroid UDPGT, 17β-hydroxysteroid UDPGT, and p-nitrophenol UDPGT were purified from Sprague-Dawley rat hepatic microsomes using chromatofocusing and affinity chromatography as described previously (1, 15). All preparations were found to be homogenous by SDS polyacrylamide gel electrophoresis. Furthermore, each enzyme yielded a single and unique sequence of the first 20 N-terminal amino acids (20), indicating the presence of only one protein species in each preparation.

Enzyme assays

Bile acid:UDP-glucuronosyltransferase activity was assayed by two methods (8, 11, 21). For assays with purified enzymes, the substrates (0.1 mM final concentration, prepared in the form of mixed micelles with Brij 58 as previously described (11)) were incubated in a total volume of 60 μl with 0.12–0.18 μg of protein, 5 mM MgCl2, 0.05% Brij 58, 80 μg/ml phosphatidylcholine, 100 mM buffer (see below), and 3.5 mM UDP-[14C]glucuronic acid (200–600 dpm/nmol). Initially, all ten substrates were assayed with the three purified enzymes at two pH values each (HEPES-NaOH, pH 6.5 and 7.5). For subsequent measurements, optimal conditions were used, i.e.: 3α,5β-C20-22, pH 7.5; 3α,5β-C23-24, s and d 3β,5β-C24, pH 6.5. After 10 min at 37°C, the reaction was stopped with cold 0.1 M glycine–trichloroacetate, pH 2.8, and the reaction mixture was passed through a C18 Bond-Elut cartridge. The retained glucuronide was eluted with methanol and its radioactivity was determined by liquid scintillation counting (8, 21).

The identification of the type of glucuronide (hydroxyl- or carboxyl-linked) formed in reactions with the purified enzyme was carried out as follows. The incubation was as described above except that tritium-labeled bile acids (6,000–12,000 dpm/nmol) (19) and unlabeled UDP-glucuronic acid were used. After the reaction was stopped by the addition of an equal amount of ethanol, the entire mixture was applied to TLC plates and the plates were developed to effect a group separation of hydroxyl- and carboxyl-linked glucuronides (11). The plates were evaluated by autoradiography.

Kinetic studies with the purified enzyme were conducted using variable concentrations of the bile acid substrates (12.5–250 μM) in the presence of a fixed concentration of UDP-glucuronic acid (5 mM). Substrate inhibition became noticeable at 125 μM for all bile acids. K_M values were calculated using a numerical version of the direct linear plot method (22–24).

Bile acid glucuronidation in microsomal fractions was measured by the TLC separation technique. Conditions
of incubation and separation were as given previously (11). The UDP-glucuronosyltransferase reaction with microsomes was linear with time for 30 min and with protein concentration up to 1.2 mg/ml.

Androsterone glucuronidation assays were conducted by a modification of the method described previously for estrone glucuronidation (25). The reaction mixture (1 ml) contained ca. 1-2 μg of purified enzyme, 100 μg of phosphatidylcholine, 50 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 5 mM UDP-glucuronic acid, and 56 μM androsterone. Androsterone used in the assay was prepared as follows. One ml of propylene glycol was overlayered with a solution of [³H]androsterone in benzene, and the benzene was evaporated under a stream of nitrogen; 0.25 ml of 22.5 mM unlabeled androsterone in dimethyl sulfoxide was added, followed by 10 ml of 0.5 M Tris-HCl, pH 8.0, containing 0.1 M MgCl₂, and water to 50 ml. The resulting 112 μM stock solution of androsterone was used for the assay to give a final concentration of 56 μM androsterone and the desired concentrations of buffer and MgCl₂. Incubations were carried out for 10 min at 37°C and were terminated by addition of 10 ml of methylene chloride and 1 ml of water. After shaking and phase separation by centrifugation, radioactivity in the aqueous phase was determined. Inhibition of androsterone glucuronidation by short-chain bile acids was measured as described above, except that variable concentrations of androsterone (5.6-56 pM) were used and the bile acid under study was added in dimethyl sulfoxide. At least two concentrations of bile acid were used. The type of inhibition was determined from double-reciprocal plots of initial velocity versus substrate concentration. Kᵢ values for the short-chain bile acids were determined from secondary plots of slope versus inhibitor concentration of data obtained from the primary double-reciprocal plots (26).

Testosterone glucuronidation was measured as described above for androsterone except that the final concentration of the substrate was 50 μM.

Protein was determined using the Bio-Rad dye binding assay originally described by Bradford (27) with bovine serum albumin as standard. Specific activities of enzymes are expressed as nmol of glucuronide formed per mg of protein·min; where applicable, means ± SD are reported.

RESULTS

The glucuronidation rates of C₂₀-C₂₃ bile acids at the 3α-hydroxyl group were similar in hepatic microsomal preparations from Sprague-Dawley and HA Wistar rats (Fig. 1A). The average rate for lithocholic acid (3α,5β-C₂₀) glucuronidation, however, was approximately three times higher in Sprague-Dawley compared to HA Wistar microsomes. For all 3α-hydroxylated substrates, the hepatic microsomal glucuronidation rates at the hydroxyl position were significantly lower in LA Wistar rat liver preparations than in HA Wistar preparations. In contrast, the rates of carboxyl glucuronidation for 3α,5β-C₂₀, C₂₂, and C₂₃ substrates were similar in the three rat substrains (Fig. 1B). As shown previously (11), 3α,5β-C₂₁ and C₂₃ bile acids did not give rise to carboxyl-linked glucuronides. All 3β-hydroxylated short-chain bile acids (C₂₀-C₂₃) produced only carboxyl-linked glucuronides (Fig. 2). The rates of microsomal carboxyl-directed glucuronidation were similar for preparations from Sprague-Dawley and HA Wistar rats. Moreover, no differences in glucuronidation rates were observed when HA and LA Wistar rat microsomal preparations were compared. Among the 3β-hydroxylated substrates, a hydroxyl-linked glucuronide was formed only for the C₂₄ compound (isolithocholate). As in the case of the corresponding 3α-hydroxy acid,
Fig. 2. Rates of enzymatic glucuronidation of 3β,5β bile acids in hepatic microsomal preparations from different rat strains and substrain. See legend to Fig. 1 for details. Upper panel: rates of hydroxyl-directed glucuronidation; lower panel: rates of carboxyl-directed glucuronidation.

lithocholate, Sprague-Dawley-derived microsomes were significantly more active than HA Wistar preparations (Fig. 2A). Unlike lithocholate, however, the activities of HA and LA Wistar microsomes toward 3β,5β-C24 were nearly identical. For the compounds tested, this is the only case in which the two Wistar substrains did not differ in the rate of formation of a hydroxyl-linked glucuronide.

In agreement with previous findings (1), UDPGT isoenzymes could be preparatively separated by chromatofocusing. The 3-hydroxysteroid and the 17β-hydroxysteroid UDPGTs eluted at pH 7.2 and 8.4, respectively, with a peak containing both activities, probably as an aggregate of the two enzymes, between them (Fig. 3A). The elution profiles of activities capable of hydroxyl-directed glucuronidation of three bile acids, 3α,5β-C20, 3α,5β-C22, and lithocholate (Fig. 3B), matched the elution profile of 3-hydroxysteroid UDPGT but not that of 17β-hydroxysteroid UDPGT.

Purified 3-hydroxysteroid UDPGT catalyzed the glucuronidation of all bile acids that gave rise to hydroxyl-linked glucuronides, even with substrates that give rise to both types of glucuronide when incubated with microsomes. Bile acids that form exclusively carboxyl-linked glucuronides in microsomes, i.e., 3β,5β-C20-22, were not substrates for the 3-hydroxysteroid UDPGT. The single 3β-hydroxylated bile acid that was accepted by the enzyme, isolithocholate, reacted at less than half of the rate found for its 3α-hydroxy counterpart, in agreement with literature data (8).

The quantitative pattern of specific activities of the 3-O-glucuronidation reaction (i.e., exclusive of the carboxyl-directed glucuronidation in the case of microsomes) was, in general, similar for microsomes and for the purified 3-hydroxysteroid UDPGT (Fig. 4). The activity of the purified enzyme was dependent on the side chain length of the short-chain bile acids: it was highest for 3α,5β-C20 and decreased gradually to reach a minimum for 3α,5β-C23. The activity for lithocholic acid was slightly higher than that for 3α,5β-C22.

The apparent $K_M$ values for the hydroxyl-directed glucuronidation of short-chain and C24 bile acids by the purified 3-hydroxysteroid UDPGT are given in Table 1.
Fig. 4. Specific activities of microsomal 3-O-glucuronidation (open bars and left scale) and the purified rat liver 3-hydroxysteroid UDP-glucuronosyltransferase (closed bars and right scale) from Sprague-Dawley rats. Bile acid glucuronidation reactions (at 0.2 mM bile acid for microsomal preparations and 0.1 mM for purified enzyme) were carried out as described in Methods. For reactions with microsomes as the enzyme source, products were separated by TLC into carboxyl- and hydroxyl-linked glucuronides (11); only the latter are shown in the figure. The purified enzyme forms only hydroxyl-linked glucuronides. Upper panel: glucuronidation of 3α,5β bile acids. Lower panel: glucuronidation of 3β,5β bile acids. No bars are shown for substrates for which the reaction rate was below the limit of detection (approx. 0.05 nmol/mg min for assays with microsomes, in which labeled bile acids were used, and approx. 0.2 nmol/mg min for assays with the purified enzyme using labeled UDP glucuronic acid).

The apparent $K_M$ values determined using purified enzyme preparations were generally lower than those obtained with microsomes (11). This might be due to a competition for substrate between UDPGT isoenzyme(s) that catalyze carboxyl-specific glucuronidation and the 3-hydroxysteroid UDPGT when microsomes are used, even though alternative explanations are possible, including differential modulation of enzyme activity by phospholipids.

The reaction rates given in Fig. 4 are good approximations of $V_{max}$ since they were obtained at substrate concentrations well above $K_M$. Therefore, the utilization ratio ($V_{max}/K_M$) can be calculated for each substrate; the values are shown in Table 1. The ratios are high for 3α,5β-C$_{20}$ and C$_{21}$ and decrease with increasing side chain length.

As shown above, 3α,5β-C$_{20}$ is glucuronidated at both the 3α-hydroxy and at the carboxyl positions in microsomal preparations; the 3-hydroxyxysteroid UDPGT is responsible for the former, but not the latter process. In contrast, 3β,5β-C$_{20}$ is only glucuronidated at the carboxyl position in microsomes and is not a substrate for the 3-hydroxysteroid UDPGT. Both compounds, however, are competitive inhibitors of androsterone glucuronidation catalyzed by purified 3-hydroxysteroid UDPGT (Fig. 5). The apparent $K_{is}$ value for 3α,5β-C$_{20}$, 43 μM, is similar to its apparent $K_M$ as a substrate (21 μM). The apparent $K_{is}$ for 3β,5β-C$_{20}$ was somewhat higher (85 μM). These data suggest that the two compounds interact at or near the active site of 3-hydroxysteroid UDPGT even though only one acts as a substrate.

None of the bile acids used in this study was glucuronidated by the p-nitrophenol or the 17β-hydroxysteroid UDPGT (data not shown).

### DISCUSSION

3-Hydroxysteroid UDPGT is an enzyme of a relatively restricted substrate specificity. It catalyzes the conjugation of the 3α-hydroxy group of androsterone and etiocholanolone (1), and, at a 10- to 100-fold lower rate, the same group in lithocholic and chenodeoxy-, ursodeoxy-, and deoxycholic acids (8). The 3β-hydroxy group in isolithocholic acid is glucuronidated, albeit at a still lower rate (8). Several carcinogenic arylamines are the only known nonsteroidal substrates of the enzyme (28). In the present work, we demonstrated that the 3-hydroxysteroid UDPGT is also responsible for the glucuronidation of the 3α-hydroxyl group in monohydroxylated steroidal acids with a side chain that is shorter ("short-chain bile acids," (29)) than in the biologically prevalent bile acids. This conclusion is based on three main findings: i) microsomes from LA Wistar rats had a greatly reduced ability to 3-O-glucuronidate short-chain bile acids; ii) purified 3-hydroxysteroid UDPGT was able to conjugate, at the 3-hydroxyl group, all short-chain bile acids examined that

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Apparent $K_M$</th>
<th>Utilization Ratio ($K_M/V_{max}$)</th>
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<tbody>
<tr>
<td>Androsterone</td>
<td>10*</td>
<td>55.0*</td>
</tr>
<tr>
<td>3α,5β-C$_{20}$</td>
<td>21 ± 1.1</td>
<td>12.8</td>
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<tr>
<td>3α,5β-C$_{21}$</td>
<td>9 ± 0.1</td>
<td>9.4</td>
</tr>
<tr>
<td>3α,5β-C$_{22}$</td>
<td>9 ± 0.3</td>
<td>5.6</td>
</tr>
<tr>
<td>3α,5β-C$_{23}$</td>
<td>11 ± 0.1</td>
<td>5.6</td>
</tr>
<tr>
<td>3α,5β-C$_{24}$</td>
<td>28 ± 1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>3β,5β-C$_{24}$</td>
<td>26 ± 1.7</td>
<td>1.9</td>
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Apparent $K_M$ values were determined at 12.5 to 250 μM bile acid and a constant concentration (5 mM) of UDP-glucuronic acid. Means ± SD (n = 4–6, done on two or three different enzyme preparations) are given. For the calculation of the utilization ratios, the activities from Fig. 4 were used as approximations of $V_{max}$ (see text for justification of this approximation).

*Data from (1) and this work.
are 3-O-glucuronidated in microsomes; iii) short-chain bile acids, specifically 3α,5β-C_{20} and 3β,5β-C_{20}, were able to inhibit the glucuronidation of androsterone by the purified 3-hydroxysteroid UDPGT in a competitive manner.

The first finding, the marked reduction of 3-O-glucuronidation of bile acids in LA Wistar as compared to HA Wistar rats, parallels closely a similar reduction in the ability to glucuronidate androsterone. It provided the first strong indication that 3-hydroxysteroid UDPGT is responsible for the formation of hydroxyl-linked glucuronides of monohydroxylated short-chain bile acids. This conclusion was confirmed by direct measurements with the purified enzyme: all bile acids that give rise to hydroxyl-linked glucuronides when incubated with microsomes yielded the same product with the purified 3-hydroxysteroid UDPGT. The apparent Michaelis constants for the 3α,5β short-chain bile acids were in the range of 9-21 μM, the same order of magnitude as the $K_M$ for androsterone (10 μM), the prototypical substrate of the 3-hydroxysteroid UDPGT. The utilization ratios (ratios of $V_{max}$ to $K_M$) for these substrates were between the ratio for androsterone and that for lithocholic acid; the utilization ratio for a dihydroxylated bile acid, chenodeoxycholate, as calculated from data given in reference 8, is still lower. It can thus be concluded that short-chain bile acids are better substrates for the 3-hydroxysteroid UDPGT than "conventional" C_{24} bile acids, but not as good as androsterone.

Competition between androsterone and short-chain bile acids for the purified 3-hydroxysteroid UDPGT serves as the most direct proof of the role of this enzyme in the 3-O-glucuronidation of bile acids; it shows that it is the 3-hydroxysteroid UDPGT, rather than a different, hypothetical enzyme that co-purifies with it, that is responsible for the glucuronidation of bile acids. The fact that 3α,5β-C_{20} acts as a competitive inhibitor of androsterone glucuronidation is not surprising since the compound is a substrate for the enzyme. Competitive inhibition by 3β,5β-C_{20} was less expected: this bile acid gives rise, with microsomes, solely to a carboxyl-linked glucuronide and is not a substrate for the purified 3-hydroxysteroid UDPGT. However, it has been shown previously that some steroids that lack a 3α-hydroxyl group and are not glucuronidated by the 3-hydroxysteroid UDPGT can act as competitive inhibitors of the enzyme (3). The 3β,5β-C_{20} short-chain bile acid appears to be a member of this inhibitory class of compounds.

A comparison of the pattern of glucuronidation of bile acids by rat liver microsomes and by the purified 3-hydroxysteroid UDPGT yields several interesting insights concerning the number of UDP-glucuronosyltransferase isoenzymes present in rat liver. The fact that the specificity spectrum of the 3-hydroxysteroid UDPGT includes

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Fig. 5. Competitive inhibition of androsterone glucuronidation by 3α,5β-C_{20} and 3β,5β-C_{20} bile acids. Inhibition studies were conducted using variable concentrations of androsterone (5.6-56 μM) and 5 mM UDP-glucuronic acid. Solid circles: control androsterone glucuronidation assays. Panel A: 3α,5β-C_{20} was added at 40 μM (open squares) and 80 μM (open circles). Panel B: 3β,5β-C_{20} was added at 85 μM (open squares), 170 μM (open circles), and 340 μM (solid squares).
monohydroxylated \(3\alpha,5\beta\) short-chain bile acids as well as both \(C_{24}\) acids has already been mentioned. The enzyme conjugates only the hydroxyl group in position 3; the formation of the acyl glucuronide on the side chain carboxyl group is not catalyzed by the 3-hydroxyesteroid UDPGT, as evidenced by direct measurements with the purified enzyme, as well as by the fact that microsomes from HA and LA Wistar rats had a comparable activity of carboxyl-linked glucuronide formation. In addition, neither purified \(17\beta\)-hydroxyesteroid UDPGT nor \(p\)-nitrophenol UDPGT catalyzed the acyl glucuronidation of short-chain bile acids. Therefore, the existence of an UDP-glucuronosyltransferase isoenzyme (hereafter not described) specific for the carboxyl group of certain short-chain bile acids must be postulated. This enzyme is not identical with the bilirubin UDPGT, as shown by the fact that bile acid acyl glucuronidation activity is present in Gunn rats, a Wistar-derived mutant strain that lacks bilirubin UDPGT (30-32) (data not shown).

Further conclusions concerning the multiplicity of hepatic UDPGTs can be reached by examining their behavior during purification. For three short-chain bile acids, out of the four that yield a glucuronide with the purified 3-hydroxyesteroid UDPGT, the ratio of the specific activity of the purified enzyme to that of microsomes is the same (in the case of microsomes, only the formation of the 3-O-glucuronide is taken into account for this calculation). For the fourth short-chain bile acid (\(3\alpha,5\beta-C_{24}\)), as well as for both epimers of the \(C_{24}\) acid, this ratio is lower. The higher ratio that applies to the majority of short-chain bile acids, approximately 35, can be treated as the apparent purification factor of the enzyme. Even though the number is unlikely to be the true enrichment factor since it would imply an unreasonably high content of the 3-hydroxyesteroid UDPGT protein in microsomes (ca. 3%), it can be used for purposes of comparison. The fact that with some substrates a significantly lower enrichment is obtained than with others can be explained by assuming that some substrates are glucuronidated (on their 3-hydroxyl group) by more than one UDPGT isoenzyme in microsomes. The additional activities would be separated from the 3-hydroxyesteroid UDPGT during purification. This interpretation is supported by additional data. Isolithocholic acid (\(3\beta,5\beta-C_{24}\)) is the only substrate for which the formation of a hydroxyl-linked glucuronide is only slightly depressed in LA Wistar rats as compared to the HA strain (Fig. 2A). This indicates that, in addition to 3-hydroxyesteroid UDPGT, another enzyme is involved in the glucuronidation of \(3\beta,5\beta-C_{24}\) in microsomes. Since this activity would be absent in the preparation of purified 3-hydroxyesteroid UDPGT, a lowering of the apparent enrichment factor would result. A similar reasoning could be applied to \(3\alpha,5\beta-C_{21}\).

The situation is somewhat different in the case of lithocholic acid (\(3\alpha,5\beta-C_{24}\)). No appreciable amounts of 3-O-glucuronide are formed in LA Wistar microsomes from this compound. On the other hand, it is striking that lithocholic acid is the only substrate for which a several-fold difference in glucuronidation activity was found between Sprague-Dawley and HA Wistar microsomes (Fig. 1A). This could be taken as evidence for an additional UDPGT reacting with lithocholate in Sprague-Dawley but not in Wistar rats, again resulting in a lower apparent enrichment factor for 3-hydroxyesteroid UDPGT isolated from the former strain. Consistent with this reasoning is an observation made previously during the chromatofocusing purification of the 3-hydroxyesteroid UDPGT (8). While the latter enzyme, eluting at pHe 7.8, had a specific activity for androsterone more than twice as high as that for lithocholic acid, another peak, emerging with the void volume of the column, had this ratio reversed (specific activity about five times higher for lithocholic acid than for androsterone, compare Fig. 1 in ref. 8). This indicates that an enzyme specific for the \(3\alpha\) hydroxyl group of lithocholic acid can be physically separated from the 3-hydroxyesteroid UDPGT.

In summary, it can be concluded that a number of bile acid-specific UDPGTs exist in rat liver microsomes. The 3-hydroxyesteroid UDPGT, in addition to the glucuronidation of lithocholic acid, isolithocholic acid, and several dihydroxylated bile acids (8), catalyzes the formation of 3-O-glucuronides of all short-chain \(3\alpha,5\beta\) bile acids (\(C_{20}-C_{22}\)). The latter, except for \(3\alpha,5\beta-C_{21}\), as well as all four \(3\beta,5\beta\) short-chain bile acids give rise to carboxyl-linked glucuronides in a reaction that is catalyzed by an UDPGT isoenzyme distinct from the 3-hydroxyesteroid UDPGT. In Sprague-Dawley rats, a third isoenzyme appears to exist; this UDPGT is specific for lithocholic acid and yields with this substrate the same product as the 3-hydroxyesteroid UDPGT. Finally, more circumstantial evidence suggests that a certain portion of the hydroxyl-linked glucuronides of \(3\alpha,5\beta-C_{21}\) and of \(3\beta,5\beta-C_{24}\) might be formed by one or more UDPGTs different from the 3-hydroxyesteroid UDPGT, even though the bulk of this reaction is carried out by the latter enzyme. Further studies on the separation and purification, structural characterization, and eventually the genetics of these isoenzymes will be needed to clarify the increasingly complex pattern of UDP-glucuronosyltransferases found in rat liver.\[11\]

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