UDP GT cDNA EXPRESSION AND UDP GT1 IN HUMAN LIVER


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ABSTRACT — Following expression of UDPGT1 and UDPGT2 in Cos-1 cells, each isoform metabolized three types of dihydroxy- or trihydroxy-substituted ring structures, including the 3,4-catechol estrogen (4-hydroxyestrone), estradiol and 17-epiestriol, and hydroxycholic acid (HDCA), but the UDPGT2 isozyme was 100-fold more efficient than UDPGT1. UDPGT1 and UDPGT2 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 isomform substrate specificity. The data indicate a high level of conservation in the amino terminus is not required for the preservation of substrate specificity. Analysis of glucuronidation activity encoded by UDPGT1/UDPGT2 chimeric cDNAs constructed at their common restriction sites, Sac I (codon 279), 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 actalysis but distinct from that responsible for aglycon selection. Screening of leukocyte DNA cosmid library with human UDPGT-Br1 (1-470bps) or UDPGT-Br2 (1-450bps) resulted in three overlapping clones, which were isolated and mapped by endonucleases.

Construction of subclones and DNA sequencing, Southern blot analysis revealed that a cluster of 4 exons (132, 88, 220, 1032 bps in one clone) encodes the entire region of 3' identity shared between human UDPGT-phenol, human UDPGT-Br1 and human UDPGT-Br2. A similar strategy but using probes which correspond to the unique regions of human UDPGT-Br1 and human UDPGT-Br2 showed that the exon 1 of UGT1A and UGT1D encodes the unique region of human UDPGT-Br1 and human UDPGT-Br2 and is located 5.6 and 49 Kb, respectively, upstream of the 4 common exons.

KEY WORDS: UDP-glucuronosyltransferase cDNA, UDPGT1, UDPGT2, HULG25 Chimeric cDNA

INTRODUCTION

UDP-glucuronosyltransferase (UDPGT) was a family of integral membrane enzymes that catalyzed the glucuronidation of many potentially toxic xenobiotics and endogenous compounds. An undetermined number of isoforms catalyzed the transfer of glucuronic acid from UDP-glucuronic acid to any of a large number of lipophilic acceptor substrates to generate water-soluble glucuronides which had enhanced rate of excretion from cells (Dutton, G. J., 1980). Due to the difficulties associated with the purification of this labile and membrane-bound class of proteins (Burchell, B. 1981; Lilienblum, W. el al., 1985; Chowdhury, R. J. et al., 1986), very little in the literature existed regarding purification of human transferases. Complementary DNAs isolated from rat (Izumi, E. N., 1980; Iwanaga, T. et al., 1986), mouse (Kumura, T., and Owens I.S., 1987), and human (Jackson, M. R. et al., 1987; Harding, D., et al., 1988) had been reported which encoded transferases that glucuronidate primar estrogens, or xenogenous compounds such as ω-nitrophenol (Jackson, M.R. et al., 1987), 4-methylumbelliferone (Kimura, T., and Owens I.S., 1987). Fournal-Giguex et al. (1989) reported that a human liver UDP-glucuronosyltransferase cDNA (HULG25) encoded a ωα-hydroxylated bile acid (hydroxycholic acid, HDCA) transferase isocform, a finding confirmed in our laboratory upon expression of an identical coding cDNA, UDPGT1. Even though the liver and kidney were the known sites of HDCA glucuronidation, little or no UDPGT1

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mRNA was detectable in kidney by Northern analysis, suggesting that an additional isoform(s) was involved. In order to understand the transferase isozyme responsible for detoxifying the critical endogenous glycone, we examined the suitability of a series of bile acid derivatives as substrates for cloned human transferase isoforms isolated in the laboratory.

MATERIALS AND METHODS

Materials

UDP-glucuronic acid, cholic acid, hyocholic acid, and all steroid derivatives were from Sigma Chemical Co. (St. Louis, MO USA); HDCA was from Aldrich Chemical Co. (Milwaukee, WI USA); [35S] methionine was from ICN Biomedicai, Inc. (Costa Mesa, CA USA); [14C]UDP-glucuronic acid was from Amersham (Arlington Heights, IL USA). Restriction enzymes and other reagents used in molecular biology techniques were from New England Biolabs (Beverly, MAS USA), Pharmacia (Piscataway, NJ USA), and Bethesda Research Laboratories (Bethesda, MD USA). The pSVL vector was from Pharmacia, and COS-1 cells were from the American Type Culture Collection (Rockville, MD USA). The Bluescript plasmids and XL-1 Blue cells were from Stratagene (La Jolla, CA USA). Reagents used for cell culture studies were from Cibco (Grand Island, NY USA) or Mediatech (Washington, DC USA). Isolation of UDPGTh-1 and UDPGTh-2 from a human liver cDNA Library. The two human liver transferase cDNAs, UDPGTh-1 and UDPGTh-2, were isolated from a λgt II cDNA library by hybridization to the mouse transferase clone 32P-UDPGTm-1 (Kimura & Owens, 1987) which was abeled by the oligo-primed technique using a kit (Pharmacia), [ a 32P]deoxycytidine triphosphate, and the supplier's protocol. Each clone was sequenced by the direct plasmid deoxy protocol.

Expression of Transferase proteins Encoded by pUDPGTh-1 and pUDPGTh-2

UDPGTh-1 and UDPGTh-2 were subcloned into the pSVL plasmid to produce the expression units pUDPGTh-1 and pUDPGTh-2, with the sense strand downstream of the promoter element in the vector. Each recombinant plasmid was transfected into COS 1 cells. In order to ensure that the comparisons of activities were based on equivalent amounts of specific protein, we established the relative level of synthe-
RESULTS AND DISCUSSION

Deduced Amino Acid differences between UDPGTh1 and UDPGTh2

The two human liver UDP-glucuronosyltransferase clones, UDPGTh1 and UDPGTh2., were originally identified as encoding isozymes active in the glucuronidation of HDCA (Fournel-Gigleux et al., 1989) and estriol and 3,4-catechol estrogens. 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. The observation that the amino termini contained the greatest number of differences between these two clones was 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 (Jackson, M., 1987) that the selection of the acceptor substrate was controlled by the amino terminus (~300 residues). In the case of other transferase isoforms, two human bilirubin and one phenol-metabolizing isoforms contained unique amino termini with 41-48% identity, whereas the 244-amino acid carboxyl termini of the isoforms were identical. The conservation in the carboxy domain suggested that this region of molecule was involved in a function common to all isozymes, such as the transfer of glucuronic acid from UDP-glucuronic acid.

Relative levels of synthesis of UDPGTH1 and UDPGTH2 in transfected COS1 cells

In order to ensure that equal amount of specific protein were being compared, the levels of synthesis were assessed 72 hours after transfection of the individual expression units, pUDPGTh1 and pUDPGTh2 and into COS1 cells. The results with [35S]-labeled protein showed that equal amounts of UDPGTh1 and UDPGTh2 were immunocomplexed when similar amounts of cell homogenate were used.

Comparison of Glucuronidating Activity of the UDPGTH1 and UDPGTH2 isozymes

While we were carrying out a more detailed analysis of the substrate selectivity of UDPGTh1 and UDPGTh2 and their chimeras, it became apparent that the two parental forms were the same series of acceptor substrates. Both UDPGTh1 and UDPGTh2 had been screened for glucuronidating activity. UDPGTh1 was examined for acceptor substrate activity upon expression in yeast, as well as in COS1 cells. 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. Comparisons of activity showed that the UDPGTh1 isozyme metabolized each of the four best substrates seen for the UDPGTh2 isoform. A substrate was included from each of the three categories seen for the more active isozyme: an estriol (containing three hydroxy groups), HDCA (a 3a 6a -dihydroxy bile salt derivatives), and 4-hydroxyestrone (a 3,4-catechol estrogen). The greater activity associated with was also determined by the fact that related chemical structures were glucuronidated but with a low rate.

Conversion of these related chemicals to glucuronides by UDPGTh1 was barely detectable with only 5β -androstan-3β, 11β, 17β -tril and 16β, 17-epiestriol. On the basis of this study, it was concluded that excellent substrates for the UDPGTh2 protein, i.e., those with greater than 200 pmol produced in 16 hours, could be detected as substrates for UDPGTh1. Regio- and stereospecificity of the hydroxy substituents were critical to these substrate selections, on the basis of the ineffectiveness of 2,3-catechol estrogens 3a, 6β, 16α, 17β and 3α, 7α, 16β, 17β -dihydroxyphenolic acid derivatives and preference for 16α, 17α -estriol. The close proximity of dihydroxy substituent groups appeared to be a critical requirement. The position of the glucuronide was not required to maintain acceptor function. This point was as 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. The basis for multiple estriol-glucuronidating enzymes many related 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 chimeras with exchange at codon 469 did not change glucuronidation compared to the intact isoforms except that UDPGTh2 activity for HDCA appeared to increase.

There were nine amino acid differences between these carboxyl termini. Exchanges made at codons 297 and 385 completely abolished activity of without an appreciable effect on that of UDPGTh1. Between chimeras UDPGTh2/385 and UDPGTh2/469, there were also nine amino acid differences compared to
this same region of UDPGTh1. This region between residues 385 and 469 excluded the unique amino acid terminus (≈ 300 residues) and conserved region between residues 481 and 499 seen in all characterized transferases. The fact that UDPGTh2/469 did not show a decrease in activity suggested that this conserved region was not a factor in the low turnover with UDPGTh1. On this basis one might argue that the membrane-bound UDP-glucuronosyltransferase had many important domains not yet understood. The apparent equal preference of UDPGTh2 for HDCA, 17-epiesterol, and 3,4 catechol estrogen pointed the structure and configuration of an enzyme active site which conferred overlapping specificity due to chemical similarities. This conclusion was supported by the demonstration that the same specificity had been maintained in the low activity UDPGTh1 isoform. Amino acid necessary for this selectivity had been conserved in spite of the 82% difference in the amino acid terminus.

Amino acids affected the rate of conversion to product had undoutrly undergone substitution in UDPGTh1. The relative effectiveness of the UDPGTh2 enzyme in conjugating three different metabolites was not understood.

UDPGT1

To determine the organization of the gene in humans, the unique ends of the UDPGTbr1 and UDPGTbr2 cDNAs were used to screen a genomic cosmid library. Three overlapping clones were isolated and mapped by endonucleases. Construction of subclones, Southern blot analysis with the common region of the cDNAs, and sequencing revealed that a cluster of 4 exons encodes the entire region of identity shared between UDPGTP1, UDPGTbr1, and UDPGTbr2. A similar strategy but using probes which correspond to the unique regions of UDPGTbr1 and UDPGTbr2 showed that the exon 1 of UGT1A, UGT1D, and UGT1F encodes the entire unique regions of UDPGTbr1, UDPGTbr2, and UDPGTP1 and is located 5.6, 49, and 73 Kb, respectively, upstream of the 4 common exons. Furthermore, three additional unique exons were identified 19, 38, and 54 Kb upstream of the 4 common exons.

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REFERENCES