Abstract

UDP-glucuronosyltransferases (UGTs) are membrane bound glycosyltransferases located in the endoplasmic reticulum (ER). They catalyse the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to endogenous or exogenous compounds. As a consequence the aqueous solubility of the substrate (aglycone) is increased and it is more easily excreted from the body. UGTs consist of two domains located on the lumenal side of the ER membrane, a single-pass transmembrane helix and a short cytosolic tail. 19 different UGTs are known in man and, based on sequence and gene organisation, they are divided into three subfamilies: UGT1A, UGT2A, and UGT2B. The functional unit of UGTs probably consists of two or more monomers.

Bilirubin, a degradation product of haemoglobin, is glucuronidated by UGT1A1. The UGT1A1 Y486D mutation causes hyperbilirubinemia and, due to exon sharing in the UGT1A subfamily, individuals that are homozygous for the 1A1-Y486D mutation carry the corresponding mutation in all their nine different UGT1As. Recombinant UGT1A6 carrying this mutation, 6YD, had drastically decreased glucuronidation activity and increased K_m for both the aglycone and UDPGA. Co-infection of the 6YD mutant with UGT1A4 partially restored the glucuronidation activity and decreased the K_ms of substrates to close to wild-type levels. Strong evidence for the formation of a tight hetero-oligomer was provided by the co-purification experiment of 6YD and UGT1A4.

In the absence of a three-dimensional structure of the full-length UGT, I used other approaches to obtain structural information. Binding of the UDPGA was studied in UGT1A6, UGT1A9, UGT1A10 and UGT2B7 by site-directed mutagenesis, modelling, single-point activity and kinetic measurements with several aglycone substrates. The results indicated that residues H371, E379, D395 and Q396 (UGT1A6 numbering) are involved in UDPGA binding. The effects of the mutations on the glucuronidation rate were substrate- and isoform-dependent.

UGTs belong to the GT1 family of glycosyltransferases. Many enzymes in this family use a serine protease-type catalytic mechanism, where histidine and aspartate form a “catalytic dyad”. Such a pair in human UGT1A9 could be H37 and either D143 or D148. Histidine 37 is not totally conserved among human UGTs, being replaced by proline in UGT1A4 and leucine in UGT2B10. Both latter isoforms are more specialised in *N*-glucuronidation. The roles of H37, D143 and D148 were investigated in UGT1A9 by site-directed mutagenesis, single-point activity and kinetic measurements. H37 was shown to be more important in *O*- than in *N*-glucuronidation activity. D148 also played a different role in these two types of glucuronidation reactions. The results implied that H37-D143 is the catalytic dyad in UGT1A9, but could not exclude the possible role of D148 in this dyad.