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Ligand orientation governs conjugation capacity of UDP-glucuronosyltransferase 1A1

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UDP-glucuronosyltransferase 1A1 (UGT1A1) is an endoplasmic reticulum membrane protein that catalyses glucuronidation. Mutant UGT1A1 possesses a different conjugation capacity, and the molecular mechanisms regulating these conjugation reactions are as yet unclear. To elucidate these molecular mechanisms, we simulated and analysed the glucuronidation of wild-type UGT1A1 and six UGT1A1 mutants, with bilirubin as the substrate. We found that only the orientation of the substrates correlated with the conjugation capacity in *in vitro* experiments. Inasmuch as glucuronidation is an intermolecular rearrangement reaction, we find that the conjugation reaction proceeds only when the hydroxyl group of the substrate is oriented towards the coenzyme, which allows the proton transfer to occur.

Keywords: conjugation capacity/docking simulation/glucuronidation/molecular modeling/substrate orientation/UGT1A1.

Abbreviations: RMS, root mean square; UDPGA, UDP-glucuronic acid; UGT1A1, UDP-glucuronosyltransferase 1A1.

UDP-glucuronosyltransferases (UGTs) constitute a membrane-bound enzyme family whose members catalyse glucuronidation, which is an important process for the clearance of drugs, endogenous compounds, dietary chemicals and environmental pollutants from the body. UGTs also facilitate excretion of the products of phase I metabolism. UGTs are classified into three subfamilies—UGT1A, UGT2A and UGT2B—on the basis of sequence homology and gene structure. UGT1A1, one of the nine isoforms of the UGT1A subfamily, was generated by alternative splicing of exon 1 to the four common exons (exons 2–5) on chromosome 2q37 (*1*). UGT1A1 is the only enzyme involved in bilirubin glucuronidation (*2*), and numerous mutations of the UGT1A1 gene have been discovered in patients with Gilbert's syndrome and Crigler–Najjar syndrome, which are characterized by jaundice (*1*). Although *in vitro* analyses of the conjugation capacity of UGT1A1 mutants have been reported (*3–5*), the molecular mechanisms of this conjugation reaction are still unclear.

The three-dimensional (3D) structure of human UGT1A1 was determined via homology modelling, by using a grape flavonoid glucosyltransferase (*Vitis vinifera* 3-*O*-glucosyltransferase, VvGT1), UGT71G1 from *Medicago truncatula*, and UGT2B7 from *Homo sapiens* as the template (*6–8*). The X-ray crystallographic structures used as templates in these studies provided data including the binding of the coenzyme to the glycosyltransferases, specifically, the binding sites of UGT1A1 and coenzymes and the reactive sites of conjugation with substrates (*9*). Moreover, recent advances in high-performance computer technology have enabled practical applications of molecular simulation programs (*10–15*) for protein analysis. In this study, we analysed the molecular mechanisms of the conjugation reaction of UGT1A1 by using *in silico* simulations. The aim of this study was to elucidate, by means of both molecular simulation and *in vitro* experimental results, the molecular mechanisms of the conjugation reaction of UGT1A1 and the key factors involved in the reaction to define the conjugation capacity of UGT1A1.

We performed three molecular simulations for each glucuronidation step: docking of UGT1A1 with UDPGA, induced fit for the docking model of UGT1A1 with UDPGA and docking to elucidate bilirubin orientation (Fig. 1). For docking models with a coenzyme, we similarly analysed the orientation of bilirubin for each model structure and then calculated the average results of the number of simulations that had the hydroxyl group of bilirubin oriented towards UDPGA, which we named the hydroxyl orientation. We calculated the mean ± SD of docking energy for each UGT1A1 mutant with the hydroxyl orientation of bilirubin and the other orientations.

We compared our simulation results with the *in vitro* conjugation capacity of each mutant UGT1A1 as determined on the basis of three previous reports. Specifically, values for G71R, F83L and I294T, as a percentage of the wild-type UGT1A1 value, were

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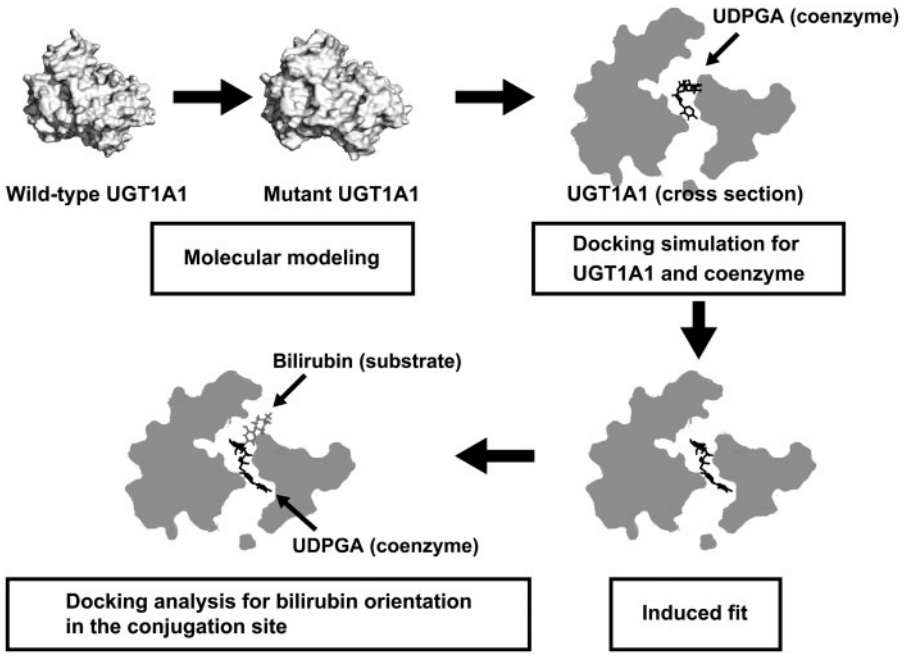

Fig. 1 Schematic drawing of the glucuronidation-based molecular simulation for conjugation of bilirubin and UDPGA. Boxes provide details of the molecular simulation process.

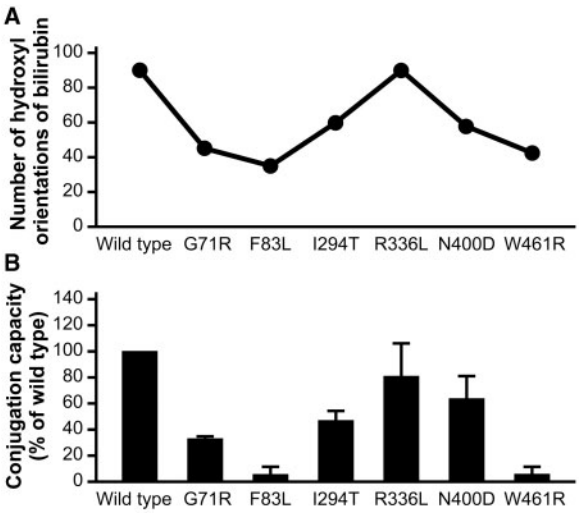

Fig. 2 Correlation between the orientation and the conjugation capacity. Orientation of the bilirubin to UDPGA (*i.e.* number of orientations in which the hydroxyl group of bilirubin points toward UDPGA) (A) and the *in vitro* conjugation capacity (B), as related to wild-type UGT1A1 and UGT1A1 mutants.

based on data from Yamamoto *et al.* (*5*), Udomuksorn *et al.* (*4*) and Ciotti *et al.* (*3*), respectively. We also calculated the values of bilirubin conjugation capacity, as a percentage of the wild-type value, for R336L, N400D and W461R, which cause Crigler–Najjar syndrome type I or II (the classification of type I or II depends on the blood concentration of bilirubin).

As a result of comparison with the values for *in vitro* conjugation capacity, we found a good correlation only for the number of hydroxyl orientations of bilirubin (Fig. 2). The other results of our simulations—that is, the number of native orientations of UDPGA, the docking energy of UDPGA with UGT1A1 and the docking energy of bilirubin into UGT1A1—had no correlation with *in vitro* conjugation capacity. Table I shows a summary of our analysis of the molecular simulations. For both docking energy results, no significant differences between the orientations were observed. Our results therefore suggested that the conjugation capacity of UGT1A1 was controlled by the hydroxyl orientation of the substrate.

As shown in Fig. 3, we propose that the hydroxyl orientations of bilirubin governs the conjugation capacity for glucuronidation, with the mechanism based on the hypothetical mechanism of glycoconjugation achieved by the glycosyltransferase (*16*, *17*). During glucuronidation with bilirubin in the hydroxyl orientation, the hydroxyl group is deprotonated by the acidic amino acid of UGT1A1, and the resultant oxygen atom of bilirubin and the glycosyl group of UDPGA undergo a nucleophilic reaction, which produces bilirubin glucuronide. The oxygen at the cleavage site of glucuronic acid of UDPGA is then protonated by NH_2 (the amino group) of a basic amino acid (Fig. 3B). During glucuronidation with bilirubin in another orientation, its hydroxyl group cannot be deprotonated because it does not point towards the acidic amino acid of UGT1A1, and no side chain for deprotonation exists (Fig. 3C). We therefore concluded that this specific molecular mechanism governs UGT1A1 conjugation capacity.

In this article, we present a new model for a molecular mechanism of bilirubin glucuronidation by UGT1A1. Substrate orientation is a key factor in the glucuronidation reaction of UGT1A1 with bilirubin, as well as with substrates other than bilirubin (*18*). Indeed, our experimental results of the simulation for

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Table I. Results of molecular simulations of bilirubin conjugation with wild-type UGT1A1 and UGT1A1 mutants.

Measure	Orientation	Wild type	G71R	F83L	I294T	R336L	N400D	W461R
No. of native orientations of UDPGA (50 simulations)		12	12	11	10	18	15	10
Docking energy of UGT1A1 and UDPGA (kcal/mol)	Reactive	−3.81 ± 1.48	−4.15 ± 1.15	−2.28 ± 0.58	−2.97 ± 1.92	−3.42 ± 2.08	−2.36 ± 1.18	−1.98 ± 3.06
	Other	−3.82 ± 1.39	−3.48 ± 1.18	−2.93 ± 1.11	−2.59 ± 2.81	−2.25 ± 2.32	−1.70 ± 2.38	−2.37 ± 1.60
No. of hydroxyl orientations of bilirubin (300 simulations)		91	46	34	59	88	58	42
Docking energy of UGT1A1 and bilirubin (kcal/mol)	Hydroxyl	−7.15 ± 0.95	−7.16 ± 3.17	−6.79 ± 1.32	−8.01 ± 1.09	−7.19 ± 1.05	−6.83 ± 1.23	−7.63 ± 1.11
	Other	−7.53 ± 0.90	−7.17 ± 3.24	−8.49 ± 1.36	−7.87 ± 1.15	−7.12 ± 1.15	−7.09 ± 1.17	−7.62 ± 1.03

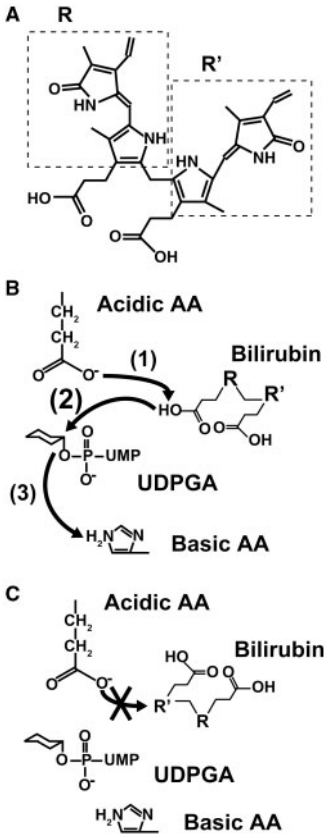

Fig. 3 The mechanism of bilirubin glucuronidation by means of the conjugation reaction of UGT1A1. The bilirubin molecule has two dipyrrole moieties (referred to as R and R′) with two hydroxyl groups (A). With bilirubin in the hydroxyl orientation, glucuronidation of bilirubin with glucuronic acid occurs in three steps: (1) deprotonation of the hydroxyl group, (2) nucleophilic reaction with UDPGA and (3) protonation of UDP (B). With bilirubin in another orientation, this glucuronidation mechanism cannot operate (C).

SN-38 indicated a correlation between substrate orientation and *in vitro* conjugation capacity: comparison among Wild, G71R, P229Q and L233R showed that 100%, 49%, 9%, 11% for *in vitro* conjugation capacity (*19*) and 66, 30, 17, 19 for hydroxyl orientations in 100 simulations, respectively. In addition, 17β-estradiol undergoes glucuronidation at only the one-tailed hydroxyl group (the 3-OH on the A ring) (*20*). These results suggest that the conjugation capacity of UGT1A1 is mainly controlled by substrate orientation. In addition, our simulation findings did not depend on the particular simulation program, because we confirmed the correlation between substrate orientation and *in vitro* conjugation capacity by also using the MOE program (data not shown).

We therefore propose a molecular mechanism of conjugation capacity that is governed by substrate orientation, as analogous to reaction mechanisms of glycoconjugates in the glycosyltransferase reaction (*17*). This proposed mechanism of glucuronidation (Fig. 3) suggests that the hydroxyl group of the substrate is required for the transfer reaction of glucuronic acid to occur.

Mutant UGT1A1 is known to cause not only hyperbilirubinaemia but also adverse effects of chemotherapy (*21*). Indeed, patients in the United States and Japan are encouraged to undergo a genetic test for UGT1A1 before beginning chemotherapy (*22*). Being able to predict *in silico* conjugation capacity of UGT1A1 is important because more than 70 mutants have already been reported, with other mutations expected to be found in the future. We believe that these molecular simulation results, primarily related to substrate orientation, may lead to prediction of the conjugation capacity of other UGT1A1 mutants.

Supplementary Data

Supplementary Data are available at JB online.

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Conflict of interest
None declared.

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