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ACCELERATED COMMUNICATION

An Active and Water-Soluble Truncation Mutant of the Human UDP-Glucuronosyltransferase 1A9

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ABSTRACT

The UDP-glucuronosyltransferases (UGTs) are integral membrane proteins, and previous attempts to generate a water-soluble UGT by removing the single *trans*-membrane helix yielded inactive and membrane-bound proteins. We have now replaced the 45 C-terminal amino acids of the human UGT1A9, including its *trans*-membrane helix, with a fusion peptide ending with six His residues. Detergent-free extraction of insect cells expressing this mutant, UGT1A9Sol, released scopoletin glucuronidation activity into the supernatant, and subsequent ultracentrifugation did not sediment that activity. UGT1A9Sol was purified by immobilized metal affinity chromatography (IMAC) in the absence of detergents throughout the entire process. The IMAC purification increased somewhat the apparent K_m of UGT1A9 toward scopoletin and rendered the enzyme sensitive to freezing. The activity of UGT1A9Sol in the cell extract was partly inhibited by Triton X-100, irrespective of the presence or absence of phospholipids. UGT1A9Sol exhibited a relatively high rate of scopoletin glucuronidation, whereas its activity toward 1-naphthol, entacapone, umbelliferone, and 4-nitrophenol was much lower. The kinetics and substrate specificity of UGT1A9Sol resembled the detergent-suspended full-length UGT1A9 rather than the membrane-bound UGT1A9. The apparent K_m value of UGT1A9Sol for scopoletin was similar to that of the full-length UGT1A9 in the presence of detergent, but much higher than the respective value in the membrane-bound enzyme. The results suggest that either the detergent binding to the *trans*-membrane helix within the full-length UGT1A9, or the removal of this helix by gene manipulation, affect the interaction of the enzyme with its aglycone substrate in a similar manner.

UDP-glucuronosyltransferases (UGTs) are a family of membrane-bound proteins of the endoplasmic reticulum. UGTs catalyze glucuronic acid transfer from UDP-glucuronic acid (UDP-GA) to acceptor molecules, aglycones, mostly small lipophilic organic molecules that are either xeno- or endobiotics (for review, see Meech and Mackenzie, 1997a; Radominska-Pandya et al., 1999; Tukey and Strassburg, 2000). Glucuronidation renders the aglycones more water-soluble and stimulates considerably their excretion from the body through the urine or bile. The human genome contains some 16 functional UGT genes that are divided into two main subfamilies, UGT1A and UGT2B (Tukey and Strassburg, 2000). Genetic variation and polymorphism have been identified in several UGTs (e.g., mutations that lead to low expression level or low activity of the UGT1A1 enzyme result in poor glucuronidation of bilirubin and in the development of Crigler-Najjar or Gilbert's syndromes) (Clarke et al., 1997, Yamamoto et al., 1998; Kadakol et al., 2000; Tukey and Strassburg, 2000).

At the protein level, the UGTs can be divided into two domains of rather similar size, a more variable N-terminal half that is encoded by exon 1 of the respective genes, and a highly conserved C-terminal half that is encoded by exons 2 to 5 (UGT1A subfamily) or exons 2 to 6 (UGT2B subfamily) (Tukey and Strassburg, 2000). In the UGT1A subfamily, the C-terminal part is identical, because the exons 2 to 5 are shared among all of them (Tukey and Strassburg, 2000). The aglycone specificity of individual UGTs seems, therefore, to be determined by the N-terminal domain, whereas the binding site of the sugar donor, UDP-glucuronic acid, is located primarily within the C-terminal domain. However, this divi-

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ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; GA, glucuronic acid; IMAC, immobilized metal affinity chromatography; PAGE, polyacrylamide gel electrophoresis.

sion of labor may be superficial because the UGTs are oligomeric, and monomer-monomer interactions may affect the binding of substrates (Ikushiro et al., 1997; Meech and Mackenzie, 1997b; Ghosh et al., 2001; Ishii et al., 2001; Kurkela et al., 2003).

The UGTs are bound to the endoplasmic reticulum membrane so that most of their mass is located on the luminal side of the membrane. A short *trans*-membrane segment is present near the C terminus of these 50- to 60-kDa proteins, and the last 20 to 26 amino acids are exposed on the cytoplasmic side of the membrane (Meech and Mackenzie, 1997a). The single C-terminal *trans*-membrane segment, however, is not the only way that the UGTs bind to the membrane. Recombinant UGTs that were truncated just before the *trans*-membrane helix were still bound to the membrane but were catalytically inactive (Meech and Mackenzie, 1998; Ouzzine et al., 1999). Nevertheless, we have recently demonstrated that UGT1A9 is an exception among the human UGTs in its resistance to detergent-induced inactivation (Kurkela et al., 2003). The mechanism of detergent inhibition of the UGTs is presently unclear, but one could suggest that the single *trans*-membrane helix of the protein is involved. If this indeed is the case, then the deletion of the C-terminal segment of UGT1A9, including its *trans*-membrane helix, might not fully inactivate the enzyme, because it was not inactivated by the addition of detergent (Kurkela et al., 2003). We have thus truncated UGT1A9 immediately upstream from the putative beginning of the *trans*-membrane helix and replaced the last 45 C-terminal amino acids of the enzyme with a fusion peptide encoding the cleavage site of enterokinase and a His tag. The resulting mutant, UGT1A9Sol, was expressed in baculovirus-infected insect cells and analyzed as described below.

Materials and Methods

Materials. Scopoletin, saccharolactone, UDP-GA, and phospholipids (phosphatidylcholine type X-E), α-naphthyl-β-D-glucuronide, 4-nitrophenyl-β-D-glucuronide, and umbelliferyl-β-D-glucuronide were purchased from Sigma. Radiolabeled [^{14}C]UDP-GA was from PerkinElmer Life and Analytical Sciences. Entacapone was kindly provided by Orion Pharma (Espoo, Finland), whereas entacapone and scopoletin glucuronides were synthesized in our laboratory (Luukkanen et al., 1999).

Gene Manipulation and Mutant Expression in Insect Cells. The single *trans*-membrane helix of the human UGT1A9 is predicted to start at Val 488. The truncation was thus done by inserting a SalI restriction site into this location by polymerase chain reaction using the reverse (antisense) oligonucleotide 5′-CGAAGTCGACGTCCAAGGAATGGTACTGGT-3′ (the SalI restriction site is underlined) and a forward oligonucleotide from about the middle of the coding region of the gene. The amplified DNA was digested with SalI and BstE2 and used to replace the BstE2-SalI fragment of the full-length UGT1A9 that was previously cloned in modified pFastBac as a fusion construct, including segment coding for the enterokinase cleavage site and six His residues (Kurkela et al., 2003). As a result of this manipulation, the resulting UGT1A9Sol has the same C-terminal tag as the full-length recombinant UGT1A9, but the new mutant is 45 amino acids shorter (Table 1). Generation of recombinant baculovirus and expression optimization were performed as described previously (Kurkela et al., 2003).

Cells Lysis and Affinity Purification. The washed frozen cells were thawed and suspended in ice-cold H_2O to about half the culture volume before collection. The cells were homogenized and further diluted by cold H_2O to about three times the original culture volume. The suspension was mixed on ice for 30 min followed by centrifugation at 40,000*g* for 1 h. The supernatant was collected, and in the preliminary experiments, it was used directly for activity assays. For kinetic assays, however, the supernatant was concentrated using a Centriplus YM-50 device (Millipore Corporation, Bedford, MA). For enzyme purification by immobilized metal affinity chromatography (IMAC), the pellet from the first centrifugation of disrupted cells was collected and subjected to a second round of suspension, homogenization, dilution, and centrifugation as described above. The first and second extracts were finally pooled, supplemented with Tris-HCl, pH 7.5, to a final concentration of 20 mM, NaCl to 500 mM (from a 5 M stock solution), and imidazole to 50 mM. The mixture was then filtered using a Steritop device (0.22 μm; Millipore), and loaded onto a 5-ml nickel-charged HiTrap chelating high-performance liquid chromatography column (Amersham Biosciences), and developed by an imidazole gradient from 50 to 400 mM. Fractions containing scopoletin glucuronidation activity were pooled and concentrated using a Centriplus YM50 filter device (Millipore). The concentrated UGT1A9Sol was diluted in a buffer containing 25 mM Tris-HCl and 0.5 mM EDTA, pH 7.5, concentrated again, and placed at +4°C.

Activity Assays. The assay mixtures contained 50 mM phosphate buffer, pH 7.4, 10 mM $MgCl_2$, 5 mM saccharolactone, 5 mM UDP-GA, 500 μM aglycone substrate, unless stated otherwise, and 1 mg/ml of sonicated phospholipids or 0.2% Triton X-100 when indicated. The amount of enzyme in the assay was adjusted in each case to achieve on one hand a good signal-to-noise ratio and on the other hand to avoid the consumption of more than 10% of the aglycone during the assay. The activity assays were performed at 37°C for 45 min. The reactions were terminated and the proteins precipitated by the addition of ice-cold perchloric acid to a final concentration of 365 mM. After chilling in an ice bath for 10 min, a 5-min centrifugation at 15,700*g* was conducted. Aliquots of the supernatants were subjected to high-performance liquid chromatography analyses. The results were fitted to Michaelis-Menten equation using SigmaPlot (enzyme kinetics module 1.1).

HPLC Conditions. The liquid chromatographic system included the Agilent 1100 series degasser, binary pump, autosampler, thermostated column compartment, multiple wavelength detector, and fluorescence detector. The 1-naphthol, entacapone, 4-nitrophenol, scopoletin and umbelliferone, and their respective glucuronides, were separated and quantified as described previously (Kaivosaari et al., 2001; Kurkela et al., 2003), except that we have presently employed Hypersil BDS-C18 150 × 4.6-mm column (Hewlett Packard, Palo Alto, CA) instead of the symmetry C18 150 × 3.9 mm column.

Miscellaneous. Protein concentrations were measured by a bicinchoninic acid system (Pierce), using bovine serum albumin as a

TABLE 1

C-terminal sequences of native human UGT1A9, recombinant full-length enzyme with a fusion peptide (1A9XHC), and the new mutant, UGT1A9Sol

The hydrophobic segment that forms the single trans-membrane helix of the enzyme is indicated in bold (A and B), and the sequences of the C-terminal extensions of 1A9XHC (Kurkela et al., 2003) and UGT1A9Sol are italic.

Protein	C-Terminal Sequence
UGT1A9	W_{480}YQYHSLD**VIGFLLAVVLTVAFITF**KCCAYGYRKCLGKKGRVKKAHKSKTH$_{530}$
1A9XHC	W_{480}YQYHSLD**VIGFLLAVVLTVAFITF**KCCAYGYRKCLGKKGRVKKAHKSKTH*SEVDKDLYDDDDKDHHHHHH*$_{550}$
UGT1A9Sol	W_{480}YQYHSLD*VDKDLYDDDDKDHHHHHH*$_{505}$

standard. SDS-PAGE analyses were carried out essentially according to Laemmli (1970) in the Mini PROTEAN 3 apparatus (Bio-Rad, Hercules, CA), using 12% gels.

Results

The 45 C-terminal amino acids of UGT1A9 include the single *trans*-membrane helix of the enzyme and the cytoplasmic "tail" of the enzyme (Table 1). To generate a water-soluble UGT mutant, we have deleted this segment and replaced it with a tag for affinity purification. This was achieved by changing Ile490 of UGT1A9 to Asp, thereby generating an in-frame SalI restriction site within the Val489 and the new Asp490. The latter SalI site, in combination with the BamH1 site just upstream from the first ATG of UGT1A9, was employed for subcloning the gene into the pFastBac derivative pFBXHC. This was followed by the preparation of recombinant baculovirus, as described previously (Kurkela et al., 2003). As a result of this cloning strategy, the C-terminal extension of the new mutant, UGT1A9Sol, is almost identical to that of the recombinant full-length UGT1A9 (1A9XHC in Table 1) that was previously prepared and purified by IMAC (Kurkela et al., 2003). It may be noted that the same fusion peptide was added to all the recombinant UGTs that have recently been produced in our laboratory (Kurkela et al., 2003, Kuuranne et al., 2003), and perhaps the stretch of Asp and Lys residues within the fusion peptide (Table 1) contributed somehow to the water-solubility of UGT1A9Sol.

The water solubility of the new mutant was initially tested by subjecting the washed cells to osmotic shock and homogenization followed by centrifugation, as described previously for the purpose of membrane preparation (Kurkela et al., 2003). Similar treatment was performed to the cells expressing the recombinant full-length UGT1A9 (1A9XHC; Table 1), and the supernatants from the two samples were assayed for the activity of scopoletin glucuronidation. The supernatant from the cells expressing UGT1A9Sol exhibited scopoletin glucuronidation activity, whereas the equivalent supernatant sample of 1A9XHC-expressing cells was completely inactive. To rule out that the observed activity arose from very small membrane particles rather than the water-soluble enzyme, the UGT1A9Sol supernatant was subjected to ultra centrifugation at 120,000*g* for 1 h. This produced a tiny pellet, but the activity in the supernatant remained at the same level as before ultracentrifugation. These results demonstrated that UGT1A9Sol is indeed a water-soluble enzyme.

UGT1A9Sol carries a C-terminal His-tag (Table 1), and it was thus purified by IMAC in a manner similar to the previous purification of recombinant UGT1A9 (1A9XHC; Table 1) with one major difference: in the case of UGT1A9Sol, all the buffers and solutions were detergent-free. An SDS-PAGE analysis of the purified UGT1A9Sol, alongside with the full-length UGT1A9 (1A9XHC), is shown in Fig. 1. The electrophoretic mobilities of the two purified versions of UGT1A9 are in good agreement with the calculated (assuming identical glycosylation) mass difference between them, 4981 Da.

The purified UGT1A9Sol glucuronidated scopoletin, further strengthening the notion that this is an active and water-soluble enzyme. The extensive washing and the elution using buffers with a high salt content did not abolish the activity. The activity of the purified UGT1A9Sol remained stable for about 2 weeks at +4°C. However, freezing to −20°C, in either the presence or the absence of 10% glycerol, resulted in a significant loss of activity. The latter is in contrast with the full-length 1A9, where the addition of 10% glycerol to the purified enzyme largely prevented the loss of activity upon freezing (Kurkela et al., 2003).

The availability of UGT1A9Sol prompted us to examine the effect of detergent on the activity of enzyme that lacks the *trans*-membrane helix. Concentrated cell extract was used in these experiments, rather than the purified UGT1A9Sol, to eliminate the potential adverse effects of high concentrations of NaCl and imidazole (see below). Hence, kinetic analyses of the scopoletin glucuronidation activity of UGT1A9Sol were carried out in the presence and absence of Triton X-100 or sonicated phospholipids. The results showed that the addition of the detergent roughly doubled the apparent K_m value, and reduced the V_{max} by about 20% (Fig. 2). The addition of phospholipids had a minor effect in the opposite direction; i.e., V_{max} was slightly increased and K_m decreased.

The kinetics of scopoletin glucuronidation was also determined for the purified UGT1A9Sol in the presence of sonicated phospholipid (Fig. 3). The apparent K_m of the purified UGT1A9Sol for scopoletin was higher than in the concentrated extract, indicating that the IMAC purification of UGT1A9Sol affected its apparent K_m for scopoletin similarly to the addition of detergent (Fig. 2). From these results (Figs. 2 and 3), it was concluded that as far as the affinity for a substrate is measured, the concentrated cell extract is a better subject than the purified UGT1A9Sol.

The activity of UGT1A9Sol toward several aglycones is shown in Table 2, alongside the respective activities of the membrane-bound UGT1A9 (1A9XHC; i.e., the His-tagged enzyme) in the absence and presence of Triton X-100. The

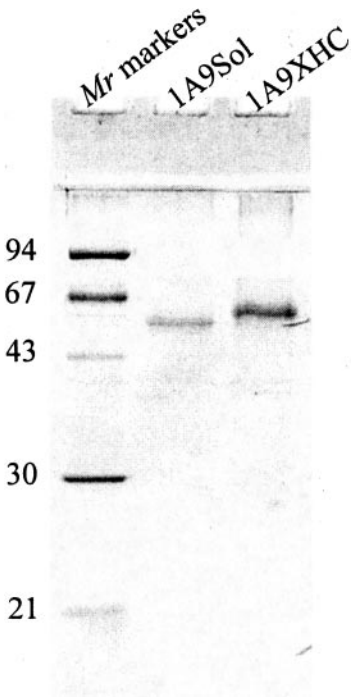

Fig. 1. SDS-PAGE analysis of purified UGT1A9Sol. The IMAC-purified mutant (0.4 μg) was examined alongside the purified full-length 1A9XHC (0.9 μg) on a 12% gel.

activity of the water-soluble UGT1A9Sol toward entacapone, a very good and specific substrate (among the liver UGTs) for the membrane-bound UGT1A9 (Lautala et al., 2000; Kurkela et al., 2003), is very low in comparison to its scopoletin glucuronidation rate (Table 2). Nevertheless, the entacapone glucuronidation activity of UGT1A9Sol in the concentrated cell extract was sufficiently high to carry out kinetic analyses. The apparent K_m of UGT1A9Sol for entacapone (447 μM; Fig. 4A) was about nine times higher than in the membrane-bound, full-length UGT1A9 (Fig. 4B), a finding that may explain at least part of the drop in the activity.

Discussion

Obtaining a water-soluble and active UGT has long been a desirable objective. It was earlier reported that deleting the 12 N-terminal amino acids rendered the mouse UDPGT$_m$-1 (currently called UGT2B5) cytoplasmic and water soluble (Toghrol et al., 1990). However, these results were compromised in part by technical difficulties and may have to be repeated using tools that were not available at that time. Several laboratories have tried to prepare C-terminal truncation mutants, but the disappointing results were inactive

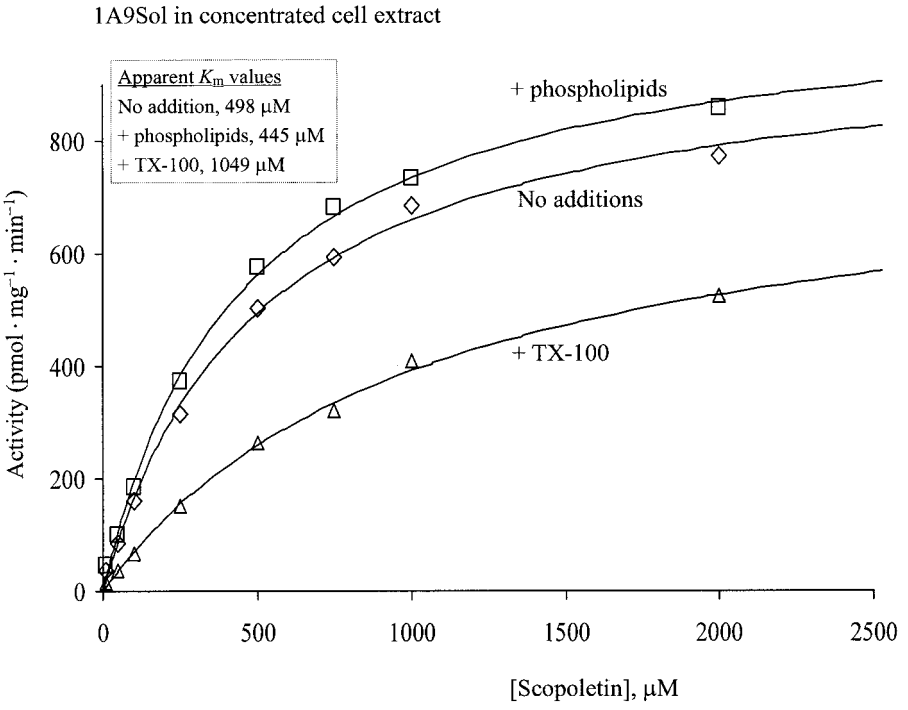

Fig. 2. Kinetic analyses of scopoletin glucuronidation by the UGT1A9Sol in a concentrated cell extract. The final concentrations of the sonicated phospholipids and TX-100, when added, were 1 mg/ml and 0.2%, respectively. The protein concentration was 600 μg/ml.

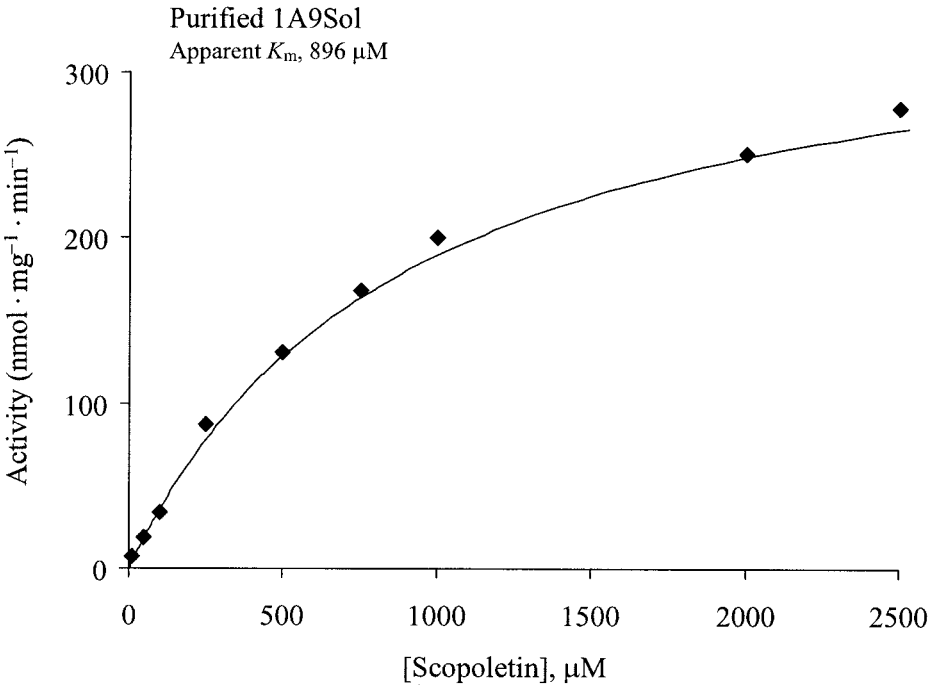

Fig. 3. Kinetics of scopoletin glucuronidation by the purified UGT1A9Sol (0.9 μg/ml) in the presence of 1 mg/ml sonicated phospholipids.

mutants that were still attached to the membrane (Meech and Mackenzie, 1998; Ouzzine et al., 1999). Nevertheless, the importance of producing a water-soluble and active mutant for future structural studies prompted us to try to prepare such a mutant from UGT1A9. It was hoped that the currently unknown property of UGT1A9 that leads to its exceptional (partial) resistance to detergents (Kurkela et al., 2003) would also contribute to the stability of a C-terminal truncation mutant of this isoform. The results of this work proved that attempting such a mutagenesis was rewarding.

It should be pointed out that not all the expressed UGT1A9Sol was extracted into the supernatant by the simple and mild lysis treatment. We could not liberate more than about 40% of the scopoletin glucuronidation activity into the supernatant in the first round of lysis, and about 30% of the remaining activity in the first pellet could be extracted by the second round of dilution, homogenization, and centrifugation. To test whether the UGT1A9Sol in the pellet was loosely attached to the membrane and behaved as a peripheral membrane protein, we tested the effect of salt on the extraction efficiency. Addition of NaCl (up to 300 mM) during the first or second rounds of extraction had no significant effect on the release of UGT1A9Sol into the supernatant. Hence, UGT1A9Sol is neither an integral nor a peripheral membrane protein, and the remaining activity in the pellet is probably trapped inside microsomes and unbroken cells.

We have previously shown that the purified full-length UGT1A9 glucuronidated scopoletin at a rather high rate, but its apparent K_m for this aglycone was much higher than in the case of membrane-bound enzyme. The entacapone glucuronidation activity of purified 1A9XHC was measurable, but it was much lower than that of the native enzyme (Kurkela et al., 2003). The sharp decrease in entacapone activity appeared immediately upon the addition of 0.2% Triton X-100 to the reaction mixture containing the membrane-bound UGT1A9. Interestingly, the magnitude of the difference in the apparent K_m for entacapone resembled the change of the apparent K_m for scopoletin between the membrane-bound UGT1A9 and the UGT1A9Sol (Figs. 2 and 4; Kurkela et al., 2003).

The UGT1A9Sol behaved in many respects like the full-length UGT1A9 in the presence of 0.2% Triton X-100 (Table 2), which suggests that the binding of detergent to the *trans*-membrane helix may dislocate it from its native position or conformation and affect the enzymatic activity in the same manner as the complete removal of the *trans*-membrane helix and the cytoplasmic "tail". On the other hand, the detergent binding to the *trans*-membrane helix could affect the conformation of the protein segment that immediately precedes this helix. The latter segment was shown to be involved in the enzyme's activity, because a mutation within this region of UGT1A1, Y486D, may cause Crigler-Najjar syndrome and reduce the enzymatic activity of both UGT1A1 and UGT1A6 in vitro (Yamamoto et al., 1998; Gagne et al., 2002; Ito et al., 2002; Jinno et al., 2003).

The presence of Triton X-100 somewhat affected the activity of UGT1A9Sol and increased its apparent K_m toward scopoletin, even though it lacks the *trans*-membrane helix (Fig. 2). This relatively minor effect of the detergent, Triton X-100, on this water-soluble UGT may have arisen from

TABLE 2

Glucuronidation activities of UGT1A9Sol.

The protein concentrations in the assays were either 0.18 mg/ml (UGT1A9 ± Triton X-100) or 0.8 mg/ml (UGT1A9Sol), and the detergent concentration, when present, was 0.2% (w/v). The concentration of the different aglycones was 0.5 mM, and that of the UDP-GA was 5 mM. Note that the amounts of protein and UGT in the two 1A9XHC samples were identical. The UGT1A9Sol was assayed using a concentrated cell extract; hence, the protein composition of this fraction is completely different from the membrane sample. Estimations of the relative amount of UGT per protein by immunodetection suggested that the concentration of enzyme per protein in the cell extract was about 100 times lower than in the membranes.

Aglycone	Specific Activity		
	UGT1A9Sol	Membrane-Bound UGT1A9 (1A9XHC)	1A9XHC plus Triton X-100
		pmol/mg/min	
Scopoletin	131.3 ± 1.0	2882.0 ± 131.9	7780.0 ± 518.3
1-Naphthol	23.3 ± 0.9	208.5 ± 12.8	243.2 ± 9.5
Entacapone	7.6 ± 0.1	9846.0 ± 423.5	508.6 ± 94.4
Umbellipherone	5.8 ± 0.3	3323.0 ± 323.3	376.9 ± 45.8
4-Nitrophenol	2.1 ± 0.1	1518.0 ± 98.7	271.3 ± 24.4

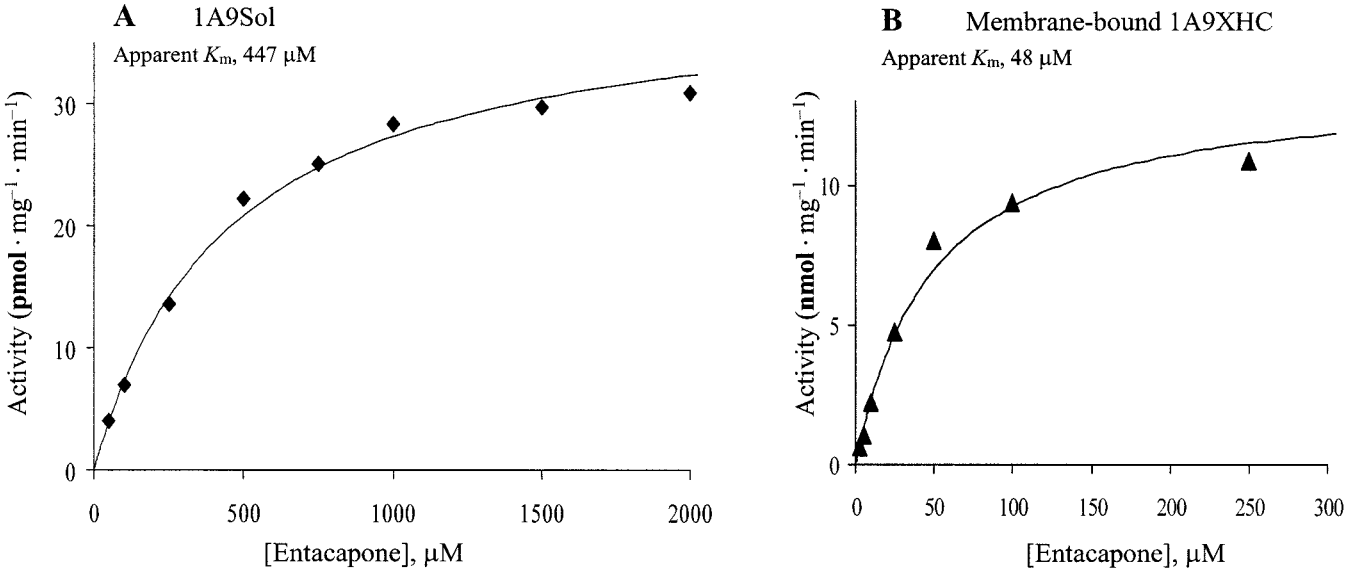

Fig. 4. Kinetics of entacapone glucuronidation by the UGT1A9Sol (A), and the membrane-bound 1A9XHC (B). A concentrated cell extract, 800 μg/ml, was used for the UGT1A9Sol assays (A). The full-length enzyme (B) was analyzed in washed membranes, at a protein concentration of 90 μg/ml. Note the differences between the activity scales in A and B (*y*-axis is in pmol in A and nmol in B), and in the range of entacapone concentrations (*x*-axis).

binding either to hydrophobic surface(s) of the protein, at the substrate binding site, or perhaps even from direct binding of aglycone molecules to a detergent micelle. Further studies are needed to resolve this question.

One intriguing question that emerges from this study is why the UGT1A9 is water-soluble and enzymatically active in the absence of the *trans*-membrane helix and the cytoplasmic "tail", whereas truncations of the equivalent segment from either UGT2B1 (Meech and Mackenzie, 1998) or UGT1A6 (Ouzzine et al., 1999) rendered them inactive and still attached to the membrane. Does UGT1A9 lack the additional membrane-binding (but not *trans*-membrane) regions the other UGTs possess? Or does the relatively long fusion peptide that we have added to the C-terminal of UGT1A9Sol, a segment rich in both basic and acidic residues (Table 1), somehow neutralize the affinity to the membrane of the *trans*-membrane-lacking UGT? Presently, there is no conclusive explanation(s) for these observations, and further studies that will attempt to resolve the differences between these UGTs are likely to yield interesting information about the structure and function of the UGTs. However, a clue may be found in our previous results about the sensitivity of the UGTs to the inhibition by detergents (Kurkela et al., 2003). In that study, UGT1A9 was an exception in its partial resistance to such inhibition, and it may be suggested that the same property that made it more resistant to Triton X-100 is also involved in the current finding that UGT1A9Sol is water soluble and active. In this respect, it may be interesting to note that the primary structures of the human UGTs 1A7, 1A8, and 1A10 are much more similar to UGT1A9 than either UGT1A6 or UGT2B1 (Tukey and Strassburg, 2000). The UGTs 1A7, 1A8, and 1A10 were not included in the study, where we have examined the sensitivity of most of the human hepatic UGTs (1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, and 2B15) to detergent inhibition (Kurkela et al., 2003). We have recently (Kuuranne et al., 2003) cloned and expressed the human UGTs 1A7, 1A8, and 1A10 as fusion proteins that carry the same C-terminal fusion peptide as our recombinant UGT1A9 (Table 1). UGTs 1A7, 1A8, and 1A10 catalyze entacapone at high rates; in contrast to UGT1A9, their scopoletin glucuronidation activity was sensitive to Triton X-100 (results not shown). This may suggest that only one or few amino acids within the N-terminal half of UGT1A9 are responsible for its relative resistance to detergent inhibition and perhaps also for its water-solubility in the absence of the single *trans*-membrane helix and cytoplasmic "tail".

Finally, in this work, we have shown for the first time that an active and water-soluble truncation mutant of a UGT can be produced. This is a major breakthrough toward solving the high-resolution structure of any UGT, even though further experiments and mutagenesis are needed to equal the substrate specificity and glucuronidation kinetics of the water-soluble UGT to those of its membrane-bound counterpart.

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