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Protein-Protein Interactions between Rat Hepatic Cytochromes P450 (P450s) and UDP-Glucuronosyltransferases (UGTs):
Evidence for the Functionally Active UGT in P450-UGT Complex

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Summary: The interaction between cytochrome P450s (CYP, P450) and UDP-glucuronosyltransferases (UGTs) was studied by co-immunoprecipitation. P450 isoform-selective antibody was used as a probe to co-precipitate UGTs with the P450s from solubilized rat liver microsomes. Antibodies toward CYP3A2, CYP2B2, CYP2C11/13 and CYP1A2 co-precipitated UGTs with corresponding P450s. However, calnexin, a type-I membrane protein, in the endoplasmic reticulum was not co-precipitated by anti-P450 antibodies. UGT activity toward 4-methylumbelliferone was detected in all co-precipitates, suggesting that UGT in the complex with P450s is functionally active. Repeated washing of co-immunoprecipitates revealed differences among P450 isoforms with regard to the affinity for UGT. Larger amounts of UGT1A1 and UGT1A6, compared with UGT2B1, were washed out from UGTs-CYP2C11/13 co-precipitates, whereas UGT-CYP3A2 and UGT-CYP2Bs complexes were resistant to thorough washing. Thus, CYP2C11/13 could associate with UGTs, but the affinity is assumed to be weaker than that of CYP2B/3As. These results suggest that there is isoform specificity in the interaction between P450s and UGTs.

Key words: protein-protein interaction; cytochrome P450; UDP-glucuronosyltransferase; UGT; immunoprecipitation; glucuronidation; endoplasmic reticulum; functional interaction

Introduction

Drug metabolizing enzymes which are present abundantly in the liver play important roles in the biotransformation both of endogenous and exogenous compounds to polar products in order to facilitate their elimination. These reactions are categorized into Phase I and Phase II reactions.1,2) Cytochrome P450 (P450), CYP and the UDP-glucuronosyltransferase (UGT), the
catalytic domains of which are localized in the cytosolic and luminal sides of the endoplasmic reticulum (ER) membrane, respectively, are two major enzyme groups responsible for Phase I and II reactions.

Accumulating data have suggested that the function of drug metabolizing enzymes is modified by their protein-protein interactions. In relation to this, we have investigated the interaction between several UGTs and CYP1A1 by CYP1A1-immobilized affinity chromatography. Recently, we have demonstrated a protein-protein interaction between UGT2B7 and CYP3A4 and a change in the function of UGT2B7 due to this interaction. Furthermore, CYP2C9 and CYP1A2 also affect UGT2B7 function in vitro by a mechanism differing from CYP3A4. Other workers have also reported protein-protein interactions between UGTs and CYP3A4 by co-immunoprecipitation. However, the isomeric specificity in the interaction of P450 and UGT is largely unknown. It also remains to be clarified further whether UGT in a UGT-P450 complex is catalytically active. Our previous study only observed a change in UGT function when P450 was expressed simultaneously with UGT or when exogenous P450 was added. Therefore, there is no definitive evidence at present regarding the function of UGT/P450 in the form of a complex.

In this study, protein-protein interactions between rat hepatic P450s and UGTs were studied by co-immunoprecipitation. Antibodies selective for several isoforms of P450 were used as probes to assess the specificity of P450 in the interaction with the UGT. In addition, we examined whether UGTs in the immunoprecipitates are catalytically active.

Materials and Methods

Materials: Cholic acid, 4-methylumbelliferone (4-MU) and L-α-phosphatidylcholine (egg yolk, type XI-E) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phenobarbital sodium salt, 3-methylcholanthrene, and Freund’s adjuvants (complete and incomplete) were obtained from Wako Pure Chemical Industries, Co., Ltd. (Osaka, Japan). UDP-glucuronic acid (trisodium salt) was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Protein assay reagents were provided by Bio-Rad (Hercules, CA, USA). Protein A-Sepharose CL4B was obtained from GE Healthcare Biosciences (Tokyo, Japan). Emulgen 911 was a generous gift from Kao-Atlas Co. (Tokyo, Japan). All other reagents were of analytical grade and used without further purification.

Animals and treatments: All experimental procedures conducted in this study were approved by the Ethics Committee for Animal Experiments of the Graduate School of Pharmaceutical Sciences, Kyushu University. Male Wistar rats (150–200 g) were purchased from Charles River Japan (Yokohama, Japan). Animals were housed in cages for a week before treatment. Sodium phenobarbital (PB) (80 mg/kg/day in saline) was administered i.p. for 4 days while control rats received vehicle. 3-Methylcholanthrene (3-MC) (20 mg/kg/in corn oil) was administered i.p. for 4 days while control rats received vehicle. Each group was composed of 4 animals. Then, 20 hr after the last injection, their livers were removed. Livers from each group were combined for preparation of microsomes. Livers were homogenized in 1.15% KCl and microsomes were prepared by procedures described elsewhere. Microsomes were suspended in 25 mM Tris-HCl (pH 7.4) buffer containing 20% glycerol and 0.1 mM dithiothreitol. The protein content was determined by Bradford’s procedures.

Preparation of anti-P450 antibodies: Purified rat CYP1A1 and CYP2B2 were diluted to a protein concentration of 10 μg/mL with saline and mixed with an equal volume of complete Freund’s adjuvant (Wako, Tokyo, Japan) to produce a homogenous emulsion. Emulsified antigen (1 μg/100 μL) was intraperitoneally injected into male BALB/cAnNCrj mice (17–22 g) obtained from CLEA Japan (Tokyo, Japan). Two weeks later, mice were boosted by an intraperitoneal injection of the same amount of the antigen emulsified with Freund’s incomplete adjuvant (Wako, Osaka, Japan). Mice were bled 1 week after the booster injection, and serum was collected. Every antiserum was verified by Western blotting for its specificity using P450s (CYP3A2, rat CYP1A1, rat CYP1A2, CYP2B2 and CYP2C11) purified in our laboratory. The antibodies raised in this study were suitable for Western blotting but not for co-immunoprecipitation.

Western blotting: Proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 7.5–12.5% gels) were electroblotted to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). Following antibodies were used to immunoprecipitate target P450s indicated: CYP3As, rabbit anti-rat CYP3A21,22 capable of reacting with CYP3A2 and cross-react with other CYP3As; CYP2C11/13, rabbit anti-rat CYP2C1123 capable of reacting with CYP2C11 and CYP2C13; CYP2B1/2, rabbit anti-rat CYP2B24,23 capable of reacting with CYP2B and CYP2B2; CYP1A1/2, rabbit anti-rat CYP1A124,25 capable of reacting with CYP1A1 and CYP1A2. The following primary antibodies for Western blotting were used to detect the target proteins indicated: CYP3As, goat anti-rat CYP3A2 (Daichii Kagaku Yukuhin, Co., Tokyo, Japan); CYP2C11/13, goat anti-rat CYP2C11 (Daichii Kagaku Yukuhin, Co., Tokyo, Japan); CYP2B1/2, mouse anti-rat CYP2B2 (described above); CYP1A1/2, mouse anti-CYP1A1 (described above); UGT isoforms, goat anti-mouse low pI form UGT antibody26 or rabbit antibodies towards...
individual rat UGT isoforms (UGT1A1, UGT1A6, UGT2B1) and calnexin, rabbit anti-calnexin antibody (StressGen Biotechnologies, Victoria, B. C., Canada). The immunogen for this antibody is a calnexin synthetic peptide conserved among canine, mouse, human and rat. Antibody used for the Western blotting of P450 and UGT (around 50 kDa) in the immunoprecipitate should be prepared by immunizing an animal which is different from the species supplying the antibody used in the immunoprecipitation. This is because the very intense band due to the heavy chain (around 50 kDa) of IgG used in the immunoprecipitation masks the IgG coupled to the target protein. Because of this limitation, Western blotting of UGT in the immunoprecipitates obtained by treating with rabbit antibody against P450 was carried out with goat anti-UGT antibody. Similarly, goat or mouse antibody was used for detection of P450s. Immunochemical staining was performed with alkaline phosphatase-labeled secondary antibody.

**Immunoprecipitation:** Solubilization of rat liver microsomes with sodium cholate was performed according to the method described elsewhere. UGT and/or P450 in solubilized rat liver microsomes were immunoprecipitated according to the method described previously. Briefly, solubilized microsomes (30 μg protein except in Fig. 1) were mixed with a rabbit anti-P450 antibody (65 μg protein) attached to Protein A-coupled Sepharose CL-4B, and gently shaken for 30 min at 4°C. After low speed centrifugation, the supernatant was removed and the precipitates were washed for 5 min with 10 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol, 0.05% Emulgen 911 and 200 mM NaCl. Unless otherwise mentioned, washing of the immunoprecipitates was repeated 3 times under the above conditions. The immunoprecipitates obtained were then subjected to SDS-PAGE followed by Western blotting. Rabbit anti-P450 antibodies used for immunoprecipitation did not react with UGT2B7 and human UGT1A7 (Data not shown). The anti-low pl form UGT antibody did not react with the rat P450s tested in this study (Data not shown).

**Measurement of UGT activity in immunoprecipitates:** UGT activity was measured with 4-methylumbelliferone (4-MU) as a substrate basically according to the method of Hanioka et al. Immuneoprecipitates with Protein A-Sepharose CL-4B resin were suspended in 100 μL 1.15% KCl and mixed with 200 μL incubation cocktail consisting of 1 mM 4-MU, 50 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl₂, 150 μg L-α-phosphatidylcholine and 2 mM UDP-glucuronic acid (final concentrations in 300 μL). The enzymatic reaction was carried out at 37°C for 2 h. The suspension was gently mixed by vortexing every 15 min during the incubation period. The sample was chilled on ice and centrifuged at 4°C and 3,000 rpm for 5 min. A portion (250 μL) of the supernatant was mixed with 83 μL 1 M trichloroacetic acid and kept on ice for 30 min. Following centrifugation at 4°C and 15,000 rpm for 15 min, the resulting supernatant was subjected to high performance liquid chromatography (HPLC). The HPLC system used consisted of a HITACHI L6200 intelligent pump, an L-7200 auto-sampler and an

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Fig. 1. Co-immunoprecipitation of CYP3A2 and UGTs with anti-CYP3A2 antibody. Sodium cholate-solubilized rat liver microsomes (solubilized RLM: 100 μg protein) were subjected to immunoprecipitation with rabbit anti-rat CYP3A2 antibody. The immunoprecipitates obtained were analyzed by Western blot analysis with primary antibodies, goat anti-rat CYP3A2 (A), goat anti-mouse UGT (B) or rabbit anti-calnexin (C), respectively. Normal rabbit serum was used as a negative control (Cont.). Sol. RLM (10 μg protein) was also subjected to Western blotting.
Co-immunoprecipitation of P450 and UGTs with anti-P450 antibodies. Sodium cholate-solubilized liver microsomes (RLM from saline (S)-, phenobarbital (PB)- and 3-methylcholanthrene (MC)-treated Wistar rats: 30 µg protein respectively) were subjected to immunoprecipitation with resin-coupled rabbit anti-CYP2B2 (A), rabbit anti-CYP2C11/13 (CYP2Cs) (B) and rabbit anti-rat CYP1A2 (C), respectively. The immunoprecipitates obtained were analyzed by Western blotting with primary antibodies, mouse anti-CYP2B2 (A, upper panel), goat anti-CYP2C11 (B, upper panel) and mouse anti-rat CYP1A1 (C, upper panel). Each middle and lower panel shows Western blots with primary antibodies, goat anti-mouse UGT and rabbit anti-calnexin, respectively. Normal rabbit serum attached to resin and resin alone were used as negative controls (Cont. and Resin). In A, solubilized RLM (PB) proteins were also subjected to Western blotting without antibody treatment (extreme left lanes: upper panel, 3 µg; middle panel, 1 µg; and lower panel, 10 µg). Similarly, solubilized RLM (S) (2, 1 and 10 µg protein) and solubilized RLM (MC) (10, 1 and 10 µg protein) were electrophoresed and blotted in Experiments B and C (extreme left lanes).
**Co-Immunoprecipitation of Rat P450s and UGTs**

![Graph](image)

**Fig. 3.** The activity of 4-methylumbelliferone (4-MU) glucuronide formation by UGT immunoprecipitated (IP) from solubilized microsomes by anti-P450 antibodies. Sodium cholate-solubilized liver microsomes [solubilized RLM from saline (S)-, phenobarbital (PB)- or 3-methylcholanganthrene (MC)-treated groups: 30 µg protein] were subjected to immunoprecipitation with rabbit anti-P450 antibodies. Solubilized RLM (S) were subjected to immunoprecipitation with rabbit anti-CYP3A2 (A) or rabbit anti-CYP2C11/13 (CYP2Cs) (B) antibody. Solubilized RLM (PB) and RLM (MC) were subjected to immunoprecipitation with rabbit anti-CYP2B2 (C) and rabbit anti-CYP1A2 (D) antibodies, respectively. Glucuronidation activity toward 4-MU in UGTs-P450 co-immunoprecipitates was measured as described in Materials and Methods. Normal rabbit serum was used as a negative control. Each value represents the mean ± S.D. of three immunoprecipitates each of which was obtained separately. Significantly different from the normal serum group in solubilized RLM (S) (*, p < 0.05; ***, p < 0.001); from the normal serum group in solubilized RLM (PB) (†††, p < 0.001); from the normal serum group in solubilized RLM (MC) (###, p < 0.001).

Effect of extensive washing on the P450-UGT complex formation: In order to assess the difference in the affinity of the UGT-P450 complex, we then examined the washing conditions which are needed to dissociate the complex. If the affinity toward UGTs is different between P450 isoforms, it is expected that extensive washing of UGT-P450 co-immunoprecipitate will result in a different recovery of UGTs in the immunoprecipitates. Figure 4 shows how extensive washing affects the recovery of UGTs and P450s in immunoprecipitates. The band intensity of UGT was compared after washing immunoprecipitates three, five or ten times. Following five washes, almost complete recoveries of P450 and UGT were observed in immunoprecipitates obtained using rabbit anti-CYP3A2, anti-CYP2B2 and anti-rat CYP1A2 antibodies (Panels A, C and D). However, only a trace or no UGT was detected in UGT-CYP2C co-immunoprecipitates after five washes while CYP2C remained even after the same treatment. It is, therefore, suggested that the affinity toward UGT varies among the P450 isoforms. Probably, CYP2Cs exhibit a rather lower affinity toward constitutively expressed UGT isoforms than CYP3As. Because of the easy dissociation of the CYP2C-UGT complex, we then recovered and concentrated the washing solution to estimate the UGT isoform bound to CYP2Cs. The concentrated washing solution was subjected to Western blotting with isoform-selective antibodies against rat UGT1A1, rat UGT1A6 and UGT2B1 (Fig. 5). The immunoblotting detected the above three UGT isoforms in the washing solution, although the amounts differed between the isoforms. The amounts of UGT1A1 and 1A6 found in the washings were higher than that of UGT2B1 when compared to solubilized microsomes. Further, Western blotting with isoform-non specific anti-UGT antibody
Fig. 4. Effects of repeated washing on the removal of UGTs and P450s in immunoprecipitates from solubilized rat hepatic microsomes. Sodium cholate-solubilized liver microsomes [solubilized RLM from saline (S)-, phenobarbital (PB)- or 3-methylcholanthrene (MC)-treated groups; 30 mg protein] were subjected to immunoprecipitation with rabbit anti-P450 antibodies. Solubilized RLM (S) were treated with rabbit anti-CYP3A2 (A) or rabbit anti-CYP2C11/13 (B) antibody. Solubilized RLM (PB) and RLM (MC) were treated with rabbit anti-CYP2B2 (C) and rabbit anti-CYP1A2 (D) antibodies, respectively. Then, the immunoprecipitates obtained were washed three, five or ten times as indicated in the figure. Western blot analysis was carried out with goat anti-mouse UGT and goat or mouse anti-rat P450 antibodies. Band intensity was quantified by using NIH Image software (version 1.62) and indicated relative to the value (= 1) observed after washing three times. Although each figure shows an immunoblot image from one experiment, the reproducibility of the data shown was confirmed by separate experiments.

Fig. 5. Western blot analysis of UGT isoforms in the concentrated washing solution of immunoprecipitates obtained by rabbit anti-CYP2C11/13 antibody treatment. RLM (S) proteins were subjected to Western blotting (A, 17 μg; B, 17 μg; C, 5 μg; and D, 2 μg). Concentrated sol. represents the concentrated washing solution of immunoprecipitates: the precipitates were washed twice and washed further eight times (total ten times), and washing solutions obtained by third to tenth washing were pooled and concentrated by Amicon Microcon (Millipore, Bedford, MA, USA). This sample was subjected to Western blotting at an amount equal to 5 μg protein. The proteins transferred onto PVDF membranes were treated with primary antibodies, rabbit anti-UGT1A1, rabbit anti-UGT1A6, rabbit anti-UGT2B1, or goat anti-mouse UGT, respectively.
(Fig. 5D) showed that the UGT isoform pattern in the washing solution is distinctive when compared to solubilized microsomes.

Discussion

Protein-protein interactions between the P450 and UGT in rat liver microsomes were studied by co-immunoprecipitation using P450 isoform-specific antibodies. Rabbit antibodies against rat CYP1A2, 2B2, 2C11/13 and 3A2 were examined as probes to see whether UGTs were co-immunoprecipitated with the respective P450 or not. The data obtained here indicated that UGTs are co-immunoprecipitated with all P450 isoforms tested. It has been reported that UGT1A1, UGT1A6 and UGT2B7 are co-immunoprecipitated with CYP3A4 from solubilized human liver microsomes. Similarly, we observed the concomitant precipitation of UGTs with CYP3As from solubilized microsomes of untreated rat liver by rabbit anti-CYP3A2 antibody. Therefore, the interaction between CYP3As and UGTs is suggested to occur universally from species to species. Previously, we have shown that there are protein-protein interactions between rat CYP1A1 and several UGTs by means of CYP1A1-immobilized affinity chromatography. The present study also suggests that this is also true for the association of rat CYP1A2 and UGTs. Although the previous study was carried out using solubilized hepatic microsomes from PB-treated rats, the present study used a preparation from 3-MC-treated rats. Thus, hepatic UGTs both in 3-MC- and PB-treated rats seem to interact with CYP1As. We also examined whether any other P450 isoforms are involved in the interactions with the UGT. We found, for the first time, in this study that CYP1A2, CYP2B2 and CYP2C2 are also involved in the P450-UGT interaction. Since the abilities of rabbit anti-P450 antibodies to precipitate P450-UGT complex were different, there are limitations about the assumption of P450 isoform selectivity in the interaction with UGT. Keeping such limitations in mind, Western blots suggested that the sort of UGT isoform and the amount in the co-precipitates depend on the rabbit anti-P450 antibody used. This seems to support the isoform selectivity in the protein-protein interaction between P450 and UGT.

It has been suggested that human P450s modulate the activity of UGT2B7-catalyzed morphine glucuronidation and the modification potentials differ between CYP3A4, 2C9 and 1A2. The present study indicated that the UGTs in the P450-UGT complex are catalytically active, supporting the previous work mentioned above. It is, therefore, suggested that UGTs exhibit their function even when they form a complex with P450. In our previous studies, we observed both the activation and suppression of UGT2B7 function depending on P450 isoforms examined and on the solubilization statuses of enzyme sources (recombinant UGT2B7 in COS cells). Since the experimental conditions are greatly different from present study in which 4-MU glucuronidation by rat liver microsomes was used as the index, it should be careful for the comparison of both studies. Keeping this in mind, it is possible that UGT activity in the immunoprecipitates given by treatment with anti-P450 antibody is a combination of the activity of multiple UGTs each of which is being activated or suppressed by P450s. This possibility may partially explain the reason why there is poor relationship between 4-MU UGT activity detected in the immunoprecipitates and the amounts of UGTs yielded by immunoprecipitation: that is, it may be varied from a P450-UGT couple to the other pairs whether the activation/suppression occurs. Other possibilities concerning the inconsistency between the activity and UGT amounts in the precipitates are discussed later. On comparing UGT-CYP3A and UGT-CYP2C complexes, almost double the UGT activity was found in the former complex than in the latter. This difference was consistent with the different UGT levels in the co-immunoprecipitates (Fig. 1, 2). However, there is the possibility that liberation of some extent of UGT from UGT-CYP2C complex during incubation cannot be excluded because CYP2C shows rather lower affinity to UGTs. While rabbit anti-CYP2B2 antibody precipitated UGTs from solubilized liver microsomes of PB-treated rats, rabbit anti-CYP1A2 antibody pulled down UGTs from those of 3-MC-treated rats. Although the Western blot showed different electrophoretic patterns of UGT in the immunoprecipitations (Fig. 2), the UGT activities in the UGT-CYP2B and UGT-CYP1A complexes were comparable. The antibody used to detect UGTs by Western blot was raised in goat against the low pI form UGT which was purified from mouse liver. The substrate specificity of the enzyme is similar to rat UGT2B1. Although the antibody used in this study non-specifically recognizes multiple UGT isoforms, it shows higher reactivities toward UGT2B2 isoforms than human UGT1A isoforms (data not shown). As can be seen in Fig. 2, the precipitates obtained by treatment with anti-CYP2B antibody contained greater amounts of UGTs compared with the precipitates by rabbit anti-CYP1A IgG. Thus, it is likely that the UGT isoforms mainly involved in UGT-CYP2B co-immunoprecipitates are different from those in UGT-CYP1A co-immunoprecipitates. This observation suggests that the CYP2B2 has a higher affinity for UGT2B than CYP1A subfamilies. UGT1A6, UGT2B1 and UGT2B12 are capable of glucuronidating 4-MU. Furthermore, UGT1A6 is low in constitutive and highly inducible by 3-MC. UGT2B1 and UGT2B12 are expressed constitutively and are PB-inducible. Although the UGT isoforms involved in the complex with P450 could not
be identified, UGT2B1 and UGT2B12 are possible candidates for the UGT-CYP2B complex since these isoforms are PB-inducible and abundant. Although UGT1A6 is 3-MC-inducible, a previous study does not support the functional interaction between UGT1A6 and CYP1A1. If this is the case, UGT1A7, another 3-MC-inducible form, may be involved in the interaction with CYP1A1. Our previous study has suggested that CYP1A1 behaves as a kind of effector for the glucuronidation of 3-hydroxybenzo[a]pyrene which is mainly catalyzed by UGT1A7. This observation seems to support the above possibility. Although the UGT1A6-CYP1A2 interaction may also be possible, it remains to be confirmed. Thus, UGT2B1 and UGT2B12 are candidate partners of CYP2B2s while UGT1A6 and UGT1A7 are possibly associated with the UGT-CYP1A complex. However, it cannot be excluded that both CYP2B2s and CYP1As interact with constitutive forms of UGT.

Similarly as above, although CYP2B2-associated UGTs showed similar intensity to CYP3A2-associated UGTs in immunoblotting, the formers had the activity twice as high as the latters (Fig. 1–3). The composition of UGT isoforms expressed in liver is probably different between untreated and PB-treated rats. Higher activity in the CYP2B2-bound UGTs seems to be due, at least partially, to the PB-induction of UGT2B1 and UGT2B12 which catalyze 4-MU glucuronidation. Further, CYP1A2-associated UGTs had the intermediate activity of those of CYP2B2 and CYP3A2-associated UGTs, nevertheless the blot intensity was far lesser than the latter two (Fig. 1–3). Both UGT1A6 and UGT1A7 that are capable of catalyzing 4-MU glucuronidation are highly inducible by 3-MC. However, as described above, antibody used in Western blot was specific to UGT2Bs rather than UGT1As. The composition of UGT isoforms expressed in liver would also differ from untreated rats to 3-MC-treated rats. It is, therefore, likely that UGT1A6 and UGT1A7 induced by 3-MC contribute to the high activity in CYP1A2-associated forms of UGT, although the immunoblotting under the present conditions could not identify these isoforms. Experiments involving the washing conditions needed to dissociate the P450-UGT complex showed that there are noticeable differences in affinities between P450 isoforms and UGTs. Some isoforms of UGT are induced in hepatic microsomes from 3-MC- or PB-treated rats. However, the resistance of UGT-P450 complex to repeated washing would depend on not only the expression level of UGT but also that of partner P450. It is, therefore, reasonable to consider that the amount of P450 immnoprecipitated is one of the determinants for the amount of UGTs co-immunoprecipitated. Figure 4D shows that CYP1A1/2 interact with several UGT isoforms. The UGT ‘‘L’’ exhibited lower affinity to CYP1A1/2 than the UGT ‘‘U’’ (Fig. 4D). On the other hand, CYP3A2 also interacts with several UGT isoforms in untreated rat liver (Fig. 4A). However, in this case, co-precipitated UGTs ‘‘U’’ and ‘‘L’’ exhibited comparable affinity toward CYP3As (Fig. 4A). Similarly, co-precipitated UGTs ‘‘U’’ and ‘‘L’’ showed no marked difference in the affinity toward CYP2B1/2 (Fig. 4C). Affinity of UGTs toward P450s seems to be markedly different. These results strongly suggest that the resistant character of UGT-P450 complex against extensive wash is not mainly due to the levels of UGTs in microsomes. It is suggested that CYP2Cs exhibit a lower affinity toward UGTs than any other P450 isoforms tested in this study. Although the UGTs were easily dissociated from the CYP2C-UGT complex, it is likely that a relatively high amount of UGT1A1/6 was bound to CYP2C while a relatively small amount of UGT2B1 was bound to CYP2C, on the basis of their contents in microsomes. It is not clear why the migration of UGT1A6 in the washing solution is slightly different from that of solubilized microsomes. It is assumed that it is due to the conformational change during the washing and/or concentrating process.

In this study, we examined whether there was an interaction between rat P450s and UGTs. As described above, it is suggested that the interaction between UGTs and P450s takes place in the form of many combinations. Many kinds of protein-protein interactions could occur in the endoplasmic reticulum but such interactions were not observed between P450 and calnexin under the conditions used. Since the interaction between P450s and UGTs can be detected by co-immunoprecipitation, it is a relatively tight interaction. Functional alteration of both enzymes by the interaction is also great concern. Although we did not carry out experiments on functional alterations caused by the UGTs-P450 interaction, there could be functional significance on the basis of our previous work in human UGT and P450 isoforms. As the bottom line of this study, we have found for the first time that UGTs-P450s co-immunoprecipitates are catalytically active in glucuronidation. Further studies are needed to elucidate the functional protein-protein interaction between UGTs and P450s.

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