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Backbone assignment of the apo-form of the human C-terminal domain of UDP-Glucuronosyltransferase 1A (UGT1A)

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Abstract

A major component of phase II drug metabolism is the covalent addition of glucuronic acid to metabolites and xenobiotics. This activity is carried out by UDP-glucuronosyltransferases (UGT) which bind the UDP-glucuronic acid donor and catalyze the covalent addition of glucuronic acid sugar moieties onto a wide variety of substrates. UGTs play important roles in drug detoxification and were recently shown to act in an inducible form of multi-drug resistance in cancer patients. Despite their biological importance, structural understanding of these enzymes is limited. The C-terminal domain is identical for all UGT1A family members and required for binding to UDP-glucuronic acid as well as involved in contacts with substrates. Here, we report the backbone assignments for the C-terminal domain of UGT1A. These assignments are a critical tool for the development of a deeper biochemical understanding of substrate specificity and enzymatic activity.

Keywords

drug resistance; Gli1-inducible drug glucuronidation; UGT; backbone resonance assignment

Biological Context

UDP-glucuronosyltransferases (UGTs ; EC 2.4.1.17) play critical roles in drug detoxification and metabolite clearance in vertebrates (Rowland et al. 2013; Tukey and Strassburg 2000). The UGT family is divided into the UGT1A1 and UGT2 subfamilies. There are nine members of the UGT1A family (UGT1A1–UGT1A9) all of which share a common C-terminal domain through an exon-sharing mechanism. Interestingly, UGT1A enzymes were recently found to play roles in the onset of drug resistance in some cancers. Specifically, elevation of the glioma-associated Gli1 transcription factor led to the elevation of the UGT1A family of enzymes in cancer cells and further, elevated Gli1 and UGT1A protein levels were observed in patients correlating with drug resistance and concomitant clinical relapse (Assouline et al. 2015; Zahreddine et al. 2014). Indeed, elevation of Gli1 and UGT1A led to glucuronidation of ribavirin and cytarabine as observed by mass spectrometry

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and the resistance of cancer cells to these drugs (Zahreddine et al. 2014). Targeting of Gli1 with the FDA approved compound Vismodegib or through genetic means led to reduced Gli1 levels, reduced UGT1A protein levels, reduction in levels of glucuronidated substrates and restored drug sensitivity (Zahreddine et al. 2014). These studies strongly suggest that this Gli1-mediated drug glucuronidation could occur in a wide variety of clinical contexts and be harbingers of patient relapse (Zahreddine and Borden 2015). Thus, it is important to establish means to directly target the UGT1A enzymes. A key first step in such an endeavour is to obtain structural information. Unfortunately, there is no structural information to date for the UGT1A family and only one crystal structure of the C-terminal domain of UGT2B7 (Miley et al. 2007), PDB code 2O6L. UGTs are part of the glycosyltransferase 1 (GT1) super-family (Campbell et al. 1997; Coutinho et al. 2003), and much of the structural information for UGTs is based on plant glycosyltransferases (Miley et al. 2007; Offen et al. 2006; Shao et al. 2005). Thus, it is imperative to develop tools that permit studies on the human UGT1As with a view to identifying compounds that could selectively inhibit UGT1A activity. As a first step, we expressed, purified and assigned the C-terminal domain of UGT1A.

Methods and experiments

Protein expression and purification

The C-terminal domain of human UGT1A4 (UGT1A4-Ct, residues 284–450: note UGT's 1A1 to 1A9 have identical residues in this region) was inserted into the pET-26b vector between the EcoRI and XhoI restriction sites. The pET-26b vector codes the pelB signal sequence for periplasmic localization, which is then cleaved leaving an extra 8 residues at the N-terminus (MDIGNSDP). In addition, a TEV protease cleavage site was introduced upstream to the C-terminal His-tag leaving the sequence (ENLYFQ) attached to the C-terminus of the protein. The plasmid was overexpressed in *Escherichia coli* BL21(DE3)-RIPL competent cells in minimum media (see below) at 37°C and induced overnight with 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) at 20°C. The reducing agent, DTT was used in all buffers to prevent any possible formation of disulfides and dimers from the cysteine residue. Cells were harvested by centrifugation and stored at –80°C until use. The frozen cells were resuspended in PBS buffer supplemented with 360 mM NaCl, 0.5 mM DTT, 0.3% Sarkosyl, 1mg/ml lysozyme, cocktail of protease inhibitor (GE), 15mM imidazole, and lysed by sonication. After adding DNase and RNase, the lysate was mixed overnight end-over-end at 4°C. The lysate was cleared by centrifugation (30 min, 20,000 rpm, 4°C) and purified over Ni-NTA beads (Qiagen) onto a gravity flow column. After the resin was extensively washed in PBS, 360 mM NaCl, 0.5 mM DTT, 30 mM imidazole, the protein was unfolded on the beads with 6M Guanidine HCl in 50 mM Hepes pH 7.5, 1 mM DTT, 30 mM imidazole and eluted with the same buffer containing 500 mM imidazole. The protein was quickly refolded by slowly diluting it into cold buffer containing 50 mM Hepes pH 7.5, 200 mM NaCl, 1 mM DTT, 1 M 3-(1-Pyridinio)-1-propanesulfonate (NDSB201). The final UGT1A4-Ct concentration was kept lower than 0.1mg/ml. After mixing for 1h at 4°C, the refolded protein was concentrated to 20 ml and dialyzed against 50 mM sodium phosphate (pH 7.2), 300 mM NaCl, 1 mM DTT. The tag was further removed by the TEV protease. High level of purity (>95%) was achieved by gel filtration on Superdex 75pg

column (GE-Biosciences) in 50 mM sodium phosphate (pH 7.2), 300 mM NaCl, 1 mM DTT.

Uniformly ^{15}N , ^{13}C samples were obtained by growing the cells in M9 minimal medium containing 2g/L of $^{15}\text{N}\text{-NH}_4\text{Cl}$ and 2g of ^{13}C -labeled glucose as the sole source of nitrogen and carbon respectively (Sigma-Aldrich). Deuterium adaption for a uniformly ^{15}N , ^{13}C , ^2H UGT1A4-Ct sample was achieved by gradually increasing the deuterium content. Briefly, freshly transformed colonies were used to start a 25ml culture in LB medium/ H_2O , followed by successive pre-cultures in M9 media containing 0%, 50% and 100% D_2O .

NMR spectroscopy and data processing

NMR samples of UGT1A4-Ct (~ 200 μM) were prepared in 50 mM phosphate buffer (pH 7.2), 300 mM NaCl, 1 mM DTT, 0.02% NaN_3 in 93% H_2O /7 % D_2O and acquired at 20°C on a Bruker AVANCE III HD 600 spectrometer with a QCIP Z-axis gradient cryoprobe. Backbone assignments were obtained from the standard Bruker sequences for HNCO, HN(CA)CO, HNCA, HNCACB, CBCA(CO)NH and TROSY versions of HNCA, HNCACB, HNCOCACB for deuterated samples. The HNCACB and HN(CO)CACB were optimized for $\text{C}\beta$ transfers. All triple resonance experiments were acquired using non-uniform sampling schemes (typically between 15% and 20% sampling) with a sine weighted multidimensional Poisson Gap schedule (Hyberts et al. 2013). All spectra were processed with NMRPipe (Delaglio et al. 1995) or SMILE (Ying et al. 2017) for NUS spectra and analyzed with NMRViewJ (Johnson and Blevins 1994).

Extent of assignments and data deposition

The human UGT1A4-Ct construct we used consisted of 182 residues. The protein exhibited aggregation over a small amount of time for protein concentrations above 200 μM and in conditions of low ionic strength. We therefore used a high salt concentration of 300 mM NaCl which necessitated the use of a shaped sample tube to achieve maximum sensitivity. We assigned the majority of backbone residues (92% HN, 88% N, 92% $\text{C}\alpha$, 90% $\text{C}\beta$, 85% CO, Fig. 1). The chemical shift assignments have been deposited in the Biological Magnetic Resonance Bank (BMRB) under the accession number 27446. Secondary structure prediction using the TALOS-N webserver (Shen and Bax 2015) indicated that the secondary structure elements in the UGT1A-Ct family are very similar to the C-terminus of the UGT2B7 which has ~ 60% identity (Fig. 2). We note that the final 2 helices ($\alpha 5$, $\alpha 6$) in UGT2B7 X-ray structure are separated by an aspartate and proline (a helix breaker) at residue 435-436 (2B7 numbering) which enables the helix $\alpha 6$ to make stabilizing contacts with the UGT2B7 core. This proline is replaced by a lysine (435, UGT1A4 numbering) in UGT1A4-Ct, and TALOS-N still predicts a break between these helices at this region thereby maintaining these interactions. We were unable to assign a sequential region comprising residues S307-K317 presumably due to broadening which arose from flexibility. Indeed, this region in the UGT2B7-Ct crystal structure is a loop with high B-factors and further, residues 314-317 (UGT1A4 numbering) are missing in one of the two models. In all, our UGT1A-Ct assignments will provide a powerful tool for studying this important family of proteins.

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Fig. 1. ^1H - ^{15}N HSQC spectrum of 200 μM UGT1A4-Ct in 50 mM sodium phosphate, 300 mM NaCl, 1 mM DTT (pH 7.2) at 293 K. Assignment of the backbone amide resonances are shown for each residue: residues with an asterisk (*) denote folding in the ^{15}N dimension. We note that residue M366 (10.96 ppm, ^1H and 118.13 ppm, ^{15}N) is out of this spectral window and not shown.

Fig. 2.

B. Secondary structure prediction of UGT1A4-Ct from TALOS-N (Shen and Bax 2013) using backbone ^{15}N , $^{13}\text{C}'$, $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$ chemical shifts. The probability of α -helical (blue) or β -sheet (red) structure is represented by the height of the bars as predicted by the TALOS-N software. For comparison the secondary structure elements observed for the crystal structure of UGT2B7-Ct is represented in panel A.