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Full Length Article

Cloning and Site Directed Mutagenesis of UGT76E1 Leads to Changed Substrate Activity in *Arabidopsis thaliana*

Humara Naz Majeed[1, 2*], **Muhammad Anjum Zia**[1], **Min Yang**[2], **Munir Ahmed Sheikh**[1] **and Ijaz Ahmad Bhatti**[1]
[1]*Department of Biochemistry, University of Agriculture Faisalabad, Pakistan*
[2]*Department Pharmaceutical & Biological Chemistry, School of Pharmacy, University College London, UK*
[*]For correspondence: humaranazmajeed@yahoo.com; ucnvhnm@ucl.ac.uk

Abstract

Glycosyltransferases are ubiquitous enzymes play vital role in numerous aspects of living organisms and are involved in secondary metabolism. Also known as uridine diphosphate (UDP) glycosyltransferases (UGTs) are engaged in detoxification in living organisms. UGT76E1 is a member of 76E family of UGT comprising of 453 amino acids. The gene for glycosyltransferase, UGT76E1 from *Arabidopsis thaliana* was expressed and purified through affinity chromatography.Site directed mutagenesis was performed to change hydrophilic threonine (T) into hydrophobic alanine (A) at 134 position. Afterwards the activity of mutant enzyme was analyzed through mass spectrometry. Novobiocin and kaempherol were used as acceptor moleculesfor activity tests. After mutagenesis the mutant UGT76E1T134A lost its activity with its donor sugar UDP glucose, as no peak was observed for the required products glc-novobiocin and glc-kaempherol at 773 and 447 in mass spectrum respectively. The mutant did not attainany new activity with UDP rhamnose also. Complete loss of activity of UGT76E1T134A with UDP glucose as donor sugar suggested for the presence of significant peptides at the site of mutation.The current results showed that glycosyltransferases can be modified to use different substrates by site directed mutagenesis. This UGT enzyme modification could open new horizon in the development of new drugs for cancer treatment.

Keywords: Glycosyltransferases; UDP glycosyltransferases UGTs; UGT76E1; UGT76E1T134A

Introduction

Glycosyltransferases are ubiquitous enzymes involved in a variety of essential processes including energy storage, metabolism of carbohydrates, cellular trafficking, protein glycosylation, biosynthesis of cell wall and the synthesis of medicinally relevant natural products. Among these, glycosylation is a key process for cell homeostasis in all living organisms that defines the location of small molecules, their bioactivity and cellular metabolic activities (Lim and Dianna, 2004; Offen *et al.,* 2006). Glycosyltransferases (GTs) participate in glycosylation reaction and change the hydrophilicity of living molecules. This change helps to detoxify the natural products along with their stabilization and also regulates the cellular signaling (Sakakibara, 2009; Bowles and Lim, 2010; Barvkar *et al.*, 2012).

Glycosyltransferases are classified in families 1-69 on the basis of substrate specificity. In plants, the most common substrate for transferring reaction is UDP-glucose (Lim *et al.,* 2004).Typical donors of plant GTs are UDP-glucose (UDP-Glc), UDP-rhamnose (UDP-Rha), UDP-galactose (UDP-Gal), UDP-xylose (UDP-Xyl) and UDP-glucuronic acid (UDP-GlcUA). Glycosylation may occur at single or multiple groups (-OH, -COOH, -NH_2, -SH and C-C) of acceptor molecules (Bowles *et al.,* 2006). The acceptor molecules for GTs in plants are their own metabolites and xenobiotics, mevalonate derivatives, polyketides, amino acid derivatives and certain other synthetic chemicals (Bowles and Lim, 2010). Transfer of sugar molecule to any acceptor molecule changes its biochemical properties as well as its bioactivity (Vogt and Jones, 2000; Lim and Bowles, 2004). So GTs has importance in biotechnology because of their regio-selective way in which they transfer sugar molecule to acceptor molecule (Lim *et al.,* 2004).

For the last few years, enzymatic engineering emerged as a potent tool in making of new UGTs with enhanced activity. Thus, the biocatalysis system of a microbe has been used to develop plant UGTs with desired properties (Lim *et al.,* 2004; Lim, 2005). In addition, domain exchange has also been described to improve UGTs catalytic activity. Subsequently, a domain exchange between UGT78D2 and UGT78D1 improved the catalytic activity of enzymes and exhibited dual sugar selectivity (Kim *et al.,* 2013). Moreover, most of the natural products have important pharmaceutical properties (Rates, 2001; Cordel, 2002). These natural products can be specifically glycosylated to

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serve as potential pharmaceutical agents (Kren, 2001; Mijatovic *et al*., 2007). Hence, plant UGTs help to improve the pharmacological properties of certain drugs and used in pharmaceutical industry (Weymouth-Wilson, 1997; Ahmed *et al*., 2006). Similarly, the processes of glycosylation and deglycosylation catalyzed by UGTs have implication in the synthesis of activated sugars (Zhang *et al*., 2006; Modolo *et al*., 2007).

In vitro studies of glycosyltransferases revealed that single gene product can glycosylate a number of substrates; as well as more than one enzyme can glycosylate the same substrate. This clearly shows that *in vivo* function of enzymes can only be determined by substrate availability (Bowles *et al.*, 2006). A comprehensive study of UGTs, both *in vitro* and *in vivo*, can facilitate in making of engineered UGTs. As UDP glucose is a donor sugar for almost all the UGTs.Thus, these new UGTs and also by changing the substrate specificity, UGTs can be broadly used in the production of medicines for cancer therapy and health promoting edible fruits and vegetables (Butelli *et al*., 2008; Thorson *et al.*, 2004).

The application in the biosynthesis of natural products and drug discovery and, also due to the vital role of glycosylation in biological processes necessitates the research work on glycosyltransferases.*Arabidopsis thaliana* proved as a good model for plant UGTs and have already been used to analyze the activity of higher plant UGTs (Li *et al.*, 2001). The genomic classification showed that approximately one to two percent of *Arabidopsis thaliana* genome encodes GTs (more than 500 completely sequenced organisms of carbohydrate active enzymes (CAZy) database) (Lim *et al.*, 2005; Lairson *et al.*, 2008). It was hypothesized that genetic engineering through site directed mutagenesis can be a potent tool to change the enzyme activity towards its substrate. Thus, the current research work was designed to perform cloning and site directed mutagenesis in UGT76E1 from *Arabidopsis thaliana*. This study was conducted to synthesize a modified UGT76E1 T134A which may use different substrates with enhanced catalytic activity. The findings of the current study could serve as a milestonetowards new horizons in cancer therapy.

Materials and Methods

UDP glycosyltransferases has been known to use UDP rhamnose as one of donor molecule *in vivo*. In order to find-out the key peptide that binds to UDP rhamnose as substrate, this *in vitro* study was designed using site directed mutagenesis technique. The experimental work was performed in Pharmaceutical and Biological Chemistry Department, School of Pharmacy, University College London.

Chemicals and Reagents

The following chemicals and reagents were used: Ampicillin (FLUKA), Isopropyl β-D-1 thiogalactopyranoside (IPTG; SIGMA), LB broth (SIGMA), XL-1 Blue competent cells (AGILENT TECHNOLOGIES UK LTD), Agar (FLUKA), 1Kb DNA ladder (NEW ENGLAND BIOLABS), PCR nucleotide mix, *Taq* buffer and *Taq* polymerase X (PROMEGA), Primers (EUROFINS MWG OPERON), GStrap FF (GE HEALTHCARE), Phenol blue (SIGMA-ALDRICH), 10X Tank buffer (BIO-RAD), N-N-N-N-tetramethylethylenediamine (TEMED) (SIGMA), Ammonium per sulphate (APS) (SIGMA), Sugar donor UDP-Glucose (Sigma X), novobiocin, kaempferol and quercetin (SIGMA), UDP Rhamnose (a kind gift from Prof. Rob Field in University of East Anglia), UGTs plasmids (University of York) and Miniprep kit from QIAGEN. The mass spectrometry was performed in School of Pharmacy, UCL and DNA sequencing was performed at Wolfson institute for Biomedical Research and Cancer, UCL.

Transformation for UDP Glycosyltransferase

One µL of recombinant plasmids of UDP glycosyltransferases was added into 20 µL XL1-Blue competent cells and gently mixed up and down for homogenous mixing. Then the mixture was incubated on ice for 30-40 min and heat shocked for 40 seconds at 42°C and cooled on ice for 2 min. Next, the cells were mixed with 200 µL of LB media and incubated in a shaker of 220 rpm at 37°C. After 1h, 100 µL mixture was spread on agar plate containing 50 µg/mL Ampicillin. The agar plates were then placed in incubator at 37°C for overnight. After 24-hours, a single colony was selected from the agar plates and put into 1L of LB media containing 50 µg/mL Ampicillin. The flask containing the mixture, then incubated in a shaker of 220 rpm at 37°C for overnight. After addition of equal volume of 80% glycerol, dispensed into 1 mL aliquots and stored at -80°C (Froger and Hall, 2007).

Protein Expression for UGTs

Colonies stored in glycerol were inoculated in 25 mL of LB media with 50 µg/mL of Ampicillin as the seed culture and grown overnight in a shaker at 37°C and 160 rpm. Then, the seed culture was used to be inoculated in 1 L of LB broth with 50 µg/mL of Ampicillin and grown in a shaker, 160 rpm at 37°C until the O.D value reached the range of 0.4-0.6 at 600 nm wavelength, i.e. the cells had entered into log phase. After that, the protein production was induced by adding 0.1 mM Isopropyl-β-D-1 thiogalactopyranoside (IPTG) and incubated in a shaker, 160 rpm at 20°C overnight.

Protein Extraction and Purification for UGTs

Cells were harvested by centrifuging at 9000 rpm for 30 minutes. The pellet was collected and then resuspended in appropriate volume of 20 mMTris buffer pH 7.8. The pellets were sonicated to break the cells and to release the proteins

into the solution, and then, centrifuged at 14000 × *g* for 1 h. The supernatant was purified using glutathione S-transferase (GSTrap) Fast Flow Column because the UGT genes were expressed as fusion proteins with glutathione S-transferase (GST).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to test if the purification was good enough. Separating gel (10%) and stacking gel (4%) were prepared and assembled in Bio-Rad Miniproteon kit to perform electrophoresis. For each mixture, 5 µL sample, 5 µL loading buffer and 2 µL DTT (1M) were prepared and heated to 95°C for 10 min to denature proteins thoroughly. Then the samples were loaded in the already prepared gel. Electrophoresis was performed at 40V in first twenty minutes to let samples reach separating gel and then voltage was increased to 150V till the end of electrophoresis. In the end, the gel was stained with Instant Blue for one hour and rinsed with distilled water for another one hour and imaged to find protein molecular weight against standard protein marker.

Mass Spectrometric Enzyme Activity Test

Variety of methods has been used to measure the activity of an enzyme ranging from radiochemicals to mass spectrometry. The mass spectrometry was used to test the activity of glycosyltransferases by monitoring selected ions, resulting in the determination of both substrate depletion and product accumulation simultaneously (Hoffmann and Stroobant, 2007). Ten µL 1 mM UDP-glucose, 10 µL 1mM novobiocin and 10 µL enzyme were added into a sterile eppendorf tube and incubated over night at 37°C. After incubation, the samples were centrifuged for 5 minutes with acetonitrile till no pellet can be seen. This step was for removing all protein remnants. Then, mass spectrometry with full scan type was used to test the presence of the aimed product.

Sequence Alignment of Glycosyltransferases

The sequence alignment used to arrange sequences of DNA, RNA or proteins to identify regions of similarity according to their function and structure. UGT76E1 was aligned through ClustalX software. Although structures of UGTs were not clear yet; another enzyme catalysing glycosylation from donor UDP-glucose (*Vv*GT, UDP-glucose: flavonoid 3-O-glycosyltransferase from red grape; *Vitis vinifera*) which has been already analysed in previous research was used as reference enzyme in sequence alignment (Offen *et al.,* 2006).

Site Directed Mutagenesis

Kit method was used for site directed mutagenesis. Q5 kit (Q5 Hot start High fidelity 2X master mix) was used from New England Biolabs. Forward primer; "CCTCTTCAGCgcgACAAGTGCTA" and Reverse primer; "ACGCTAGGAAGTTGAAACTCTTTAAC" for UGT76E1 T134A were designed as directed by SDM kit manual (Nebbasechanger.neb.com). The vector pGEX-2T (provided by GE healthcare) was used to insert gene of interest (4948 bp). This bacterial vector is mostly used to express GST fusion proteins with a thrombin site.PCR reactions were performed in 25 µL volume. After PCR, transformation was performed for this purpose a tube of NEB 5-alpha Competent *E. col*i cells was thawed on ice. Then 5 µL of the KLD mix from was added to the tube of thawed cells and the tube was carefully flicked 4-5 times to mix. The mixture was placed on ice for 30 min. And heat shocked at 42°C for 30 seconds; followed by 5 min ice incubation. An addition of 950 µL of SOC was made then to the mixture and incubated at 37°C for 60 min with shaking (250 rpm) at room temperature. The cells were mixed thoroughly by flicking the tube and inverting, then spread 50-100 µL onto a selection plate and incubated overnight at 37°C. Agarose gel electrophoresis was performed after every SDM for Original plasmid; PCR product before digestion and PCR product after digestion (Stellwagen, 2009).

Miniprep for Positive Mutants

QIAprep Spin Miniprep kit was used for the DNA extraction. 1-5 mL overnight bacterial culture was centrifuged at >8000 rpm for 3 min at room temperature and collected the pellet. Resuspended pellet in 250 µL of buffer P1 and transferred to microcentrifuge tube. 250 µL of buffer P2 was added in pellet, it was mixed thoroughly by inverting tube 4-6 times until the mixture turned clear. If lyse blue reagent was used then the solution became blue. 350 µL of N3 buffer was added and mixed immediately by inverting 4-6 times. Centrifuged for 10 minutes at 13000 rpm and the supernatant was applied to QIA prep spin column by decanting or pipetting. Centrifuged for 30-60 seconds and discarded the flow through. Washed the QIA prep spin column by adding 750 µL of PE buffer centrifuged for 30-60 seconds and discarded the flow through. Centrifuged for 1 min to remove residual wash buffer and then placed QIA prep column in a clean 1.5 mL micro centrifuge tube. DNA was eluted by adding 50 µL of EB buffer or double distilled water to the center of column. Let it stood for 1 min and centrifuged for 1 min. At last plasmid was extracted.

DNA Sequencing and Analysis

DNA sequencing was performed at Wolfson institute for biomedical research and the UCL cancer institute, UCL. Afterwards Finch TV software was used to analyze the DNA sequence of mutant to find whether the particular nitrogen base was changed or not.

Results

Cloning of Recombinant Plasmids after Site Directed Mutagenesis (SDM)

Site-directed mutagenesis (SDM) is a technique to create cloned DNAs with modified sequences and the purpose behind this modification is to examine the significance of specific residues in protein structure and function. SDM symbolized as the prime method to engineer proteins and for changing enzyme substrate selectivity (Edelheit *et al.*, 2009). The importance of SDM in protein engineering by changing a specific amino acid and creating a modified sequence was used in the UGTs to change a specific amino acid and studied its effects on the enzyme substrate specificity.

UGT76E1 and MS based Activity Test for the Mutant UGT76E1T134A

UGT76E1 is a putative glycosyltransferase and belongs to family 76E. The gene for UGT76E1 is present on chromosome-5. Other names for UGT76E1 are quercetin 3-*O*-glycosyltransferase and quercetin 3-*O*-glycosyltransferase. Best matched protein for UGT76E1 in *Arabidopsis thaliana* is UGT76E2. Donor sugars for UGT76E1 are UDP glucose, UDP GlcNAc and UDP5S glucose, respectively (Yang *et al.*, 2005).

Amino acid sequence of UGT76E1 also known as glucuronosyl transferase comprises of 453 amino acids. It has molecular weight of 50.8 kD. The hydrophilic Threonine (T) was changed to hydrophobic Alanine (A) at 134 position. Codon ACG for Threonine was changed to GCG for Alanine (A) (given below).

AAVKEFQLPSVLFSTTSATAFVCRSVLSRVN 150
AAVKEFQLPSVLFSATSATAFVCRSVLSRVN 150

Forward primer (CCTCTTCAGCGCGACAAGTGCTA) and reverseprimer (ACGCTAGGAAGTTGAAACTCTTTAAC) were designed according to the changed codon for Alanine. After mutation transformation was done and it was successful. Fig. 1 shows the formation of colonies (Panel A-C). Afterwards expression and purification of cloned enzyme was performed and the purified enzyme with changed amino acid sequence (T134A) was run on SDS-PAGE to get the confirmation of molecular weight. The gel showed a band of proteins of approximately 76 kDsize which indicated infusion of the protein with glutathione S transferase (GST). GST has a molecular weight of 26kD and after subtraction calculations; 50 kD was left behind which is the molecular weight of UGT76E1. It is to be noted that Threonine was changedwith Alanine, so there was not so much difference in molecular weight after mutation (Panel D, Fig. 1).

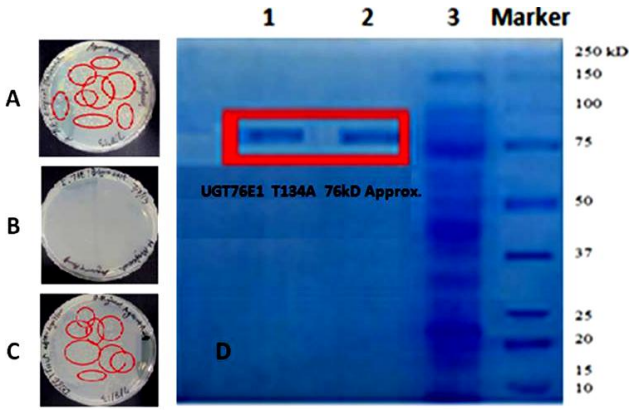

Fig. 1: Clones of wild type UGT76E1, mutant UGT6E1 T134A and SDS-PAGE analysis after expression and purification
A: Original plasmid B: PCR product C: After digestion
D: UGT76E1 approximately molecular weight 50kD and infusion with GST showed band at 76kD

UDP glucose is a nucleotide sugar and act as donor molecule in reactions catalyzed by UGTs. In addition, Kaempherol and novobiocin are the acceptor molecules with molecular weights of 286 and 612 g/mole, respectively. The Mass spectrum in Fig. 2A showed a peak of 447 which confirms the presence of Glc-Kaempherol which means that UGT76E1 transferred the glucose to kaempherol from UDP glucose. However, for mass spectrum in Fig. 2B, no peak was detected for Rham-Kaempherol which indicated that UDP rhamnose is not donor sugar for UGT76E1.On the other hand in Fig. 3C, when UDP glucose reacted with novobiocin (molecular weight 612) in the presence UGT76E1, a peak was obtained at 773 in mass spectrum which indicated that a product was formed and the glucose was transferred from UDP glucose to novobiocin (Fig 5). In fact, the peak of 773 is actually the accumulative molecular weight of novobiocin and glucose after the activity performed in the presence of UGT76E1. However, Fig. 3D showed no peak for rham-novobiocin which confirms the fact that UGT76E1 does not use UDP rhamnose as donor sugar.

The activity tests were also performed with UGT76E1 after mutation i.e. UGT76E1T134A. The mutant UGT76E1T134A lost its activity with its donor sugar UDP glucose and acceptor molecule kaempherol.When the reaction was done with UDP rhamnose as donor sugar and kaempherol as acceptor molecule, it did not get any peak for rham-kaempherol, so there is no new activity with UDP rhamnose (Fig. 4A and B).

After kaempherol as acceptor molecule UGT76E1T134A was used to check for activity with novobiocin as acceptor molecule, UDP glucose and UDP rhamnose were used as donor sugar respectively. In both cases UGT76E1T134A lost its activity with original donor sugar, UDP glucose and it also did not get any new activity with UDP rhamnose as donor sugar (Fig. 4C and D).

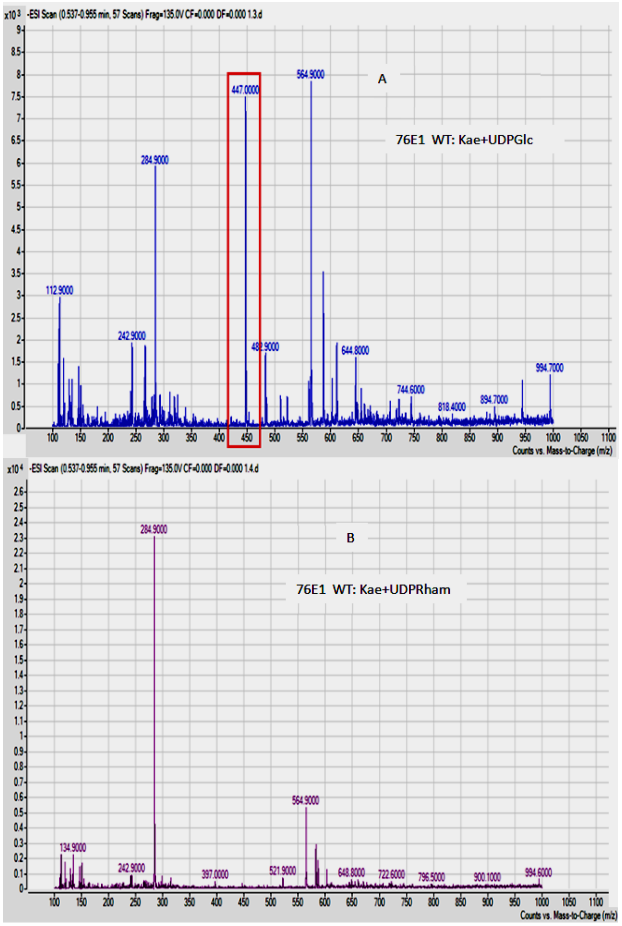

Fig. 2: MS based activity test of UGT76E1 wild type with UDP glucose, UDP rhamnose donor sugars and kaempherol as acceptor molecule

A: Mass spectrum ES-: A peak of 447 confirms the presence of Glc-Kaempherol. B. Mass spectrum ES-: no peak was detected for Rham-Kaempherol

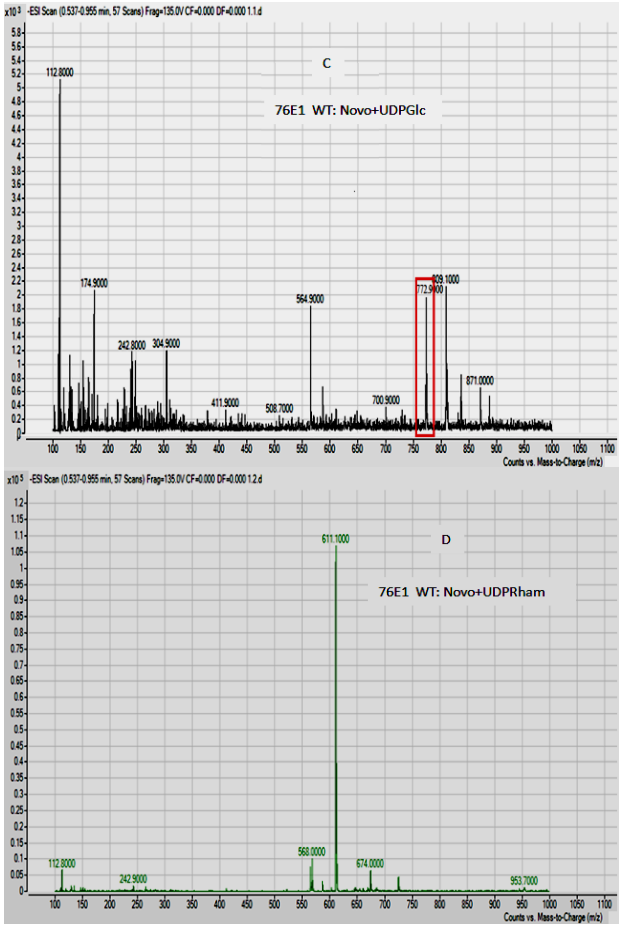

Fig. 3: MS based activity test of UGT76E1 wild type with UDP glucose, UDP rhamnose donor sugars and novobiocin as acceptor molecule

C. Mass spectrum ES-: A peak of 773 confirms the presence of Glc-Novobiocin. D. Mass spectrum ES-: No peak is present for Rham-Novobiocin

Discussion

Glycosyltransferases (GTs) are enzymes that catalyze glycosylation which is a vital process in living cells linked to the transfer of an activated sugar molecule to an acceptor molecule. Nucleotide diphospate (NDP)-sugars are normally the activated donor sugar molecule (Coutinho *et al.*, 2003). Glycosyltransferases (GTs) synthesize flavanol rhamnosides using thymidine diphosphate or uridine diphosphate rhamnose as the sugar donor and a flavonoid as the sugar acceptor (Dong *et al.*, 2003). Flavonoid O-rhamnosides holds enhanced antiviral and antibacterial activities. In addition, kaempferol-3-*O*-rhamnoside is an active anticancer agent which inhibits cell proliferation by activating apoptosis (Diantini *et al.*, 2012; Kim *et al.*, 2012). Therefore, keeping in view the role of flavonoid rhamnosides in therapeutics, more UGTs can be generated through site directed mutagenesis which can use UDP rhamnose as sugar donor.

In this study, wild type UGT76E1 was tested with UDP-glucose and the acceptors novobiocin and kaempherol.UGT76E1 is a member of Family-1 UDP-glucosyltransferases (UGTs) which isknownto catalyze the glycosylation of small molecular weight molecules (Nishimura *et al.*, 2010). The mutant of UGT76E1 (T134A) was synthesized and subjectedfor *in vitro* activity test to confirm the substrate specificity after expression and purification of proteins.It has already been described that UGT enzymes represent a prototype for structure-function relationships and any change in the sequence of amino acids alters enzyme specificity (Ouzzine *et al.*, 2002; Lim *et al.*, 2004; Breton *et al.*, 2012). UGT76E1 was mutated at specific site T134A using VvGT1 as reference enzyme because of sequence identification with GTs of *Arabidopsis thaliana*. VvGT1 is a red grape enzyme UDP-glucose: flavonoid 3-O-glycosyltransferase and its structure have shown key amino acid residues involved in donor specificity (Lim *et al.*, 2004).

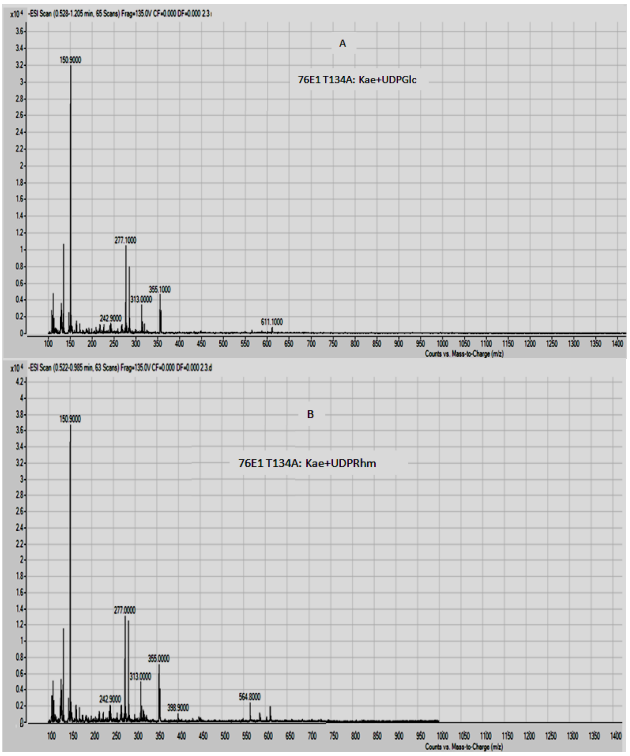

Fig. 4: MS based activity test of mutant UGT76EI T134A with UDP glucose, UDP rhamnose donor sugars and kaempherol as acceptor molecule

A. Mass spectrum ES-: No peak was detected for Glc-Kaempherol. B. Mass spectrum ES-: No peak was detected for Rham-Kaempherol

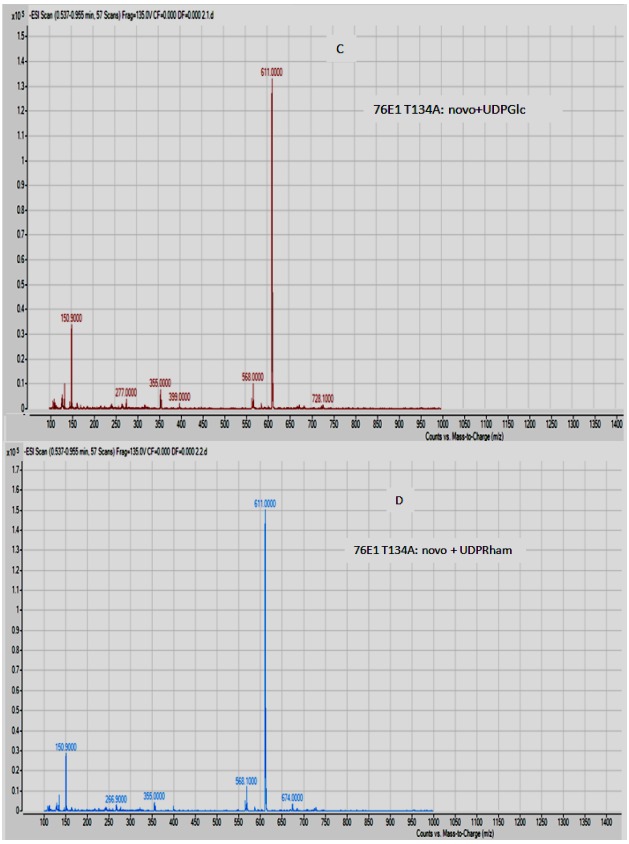

Fig. 5: MS based activity test of mutant UGT76EI T134A with UDP glucose, UDP rhamnose donor sugars and novobiocin as acceptor molecule

C. Mass spectrum ES-: No peak is present for Glc-Novobiocin. D. Mass spectrum ES-: No peak is present for Rham-Novobiocin

In present study, the site directed mutagenesis in UGT76E1 at 134 position where a hydrophilic amino acid therionine was changed to hydrophobic amino acid alanine resulted in complete loss of its activity with UDP glucose with both acceptors novobiocin and kaempherol respectively. It was revealed that UGT76E1T134Adid not use UDP rhamnose as donor sugar which is consistent with the VvGT1 enzyme (Offen *et al.*, 2006). Subsequently, mutation in VvGT1 at 375 position (Q-N and Q-H) completely suppressed the catalytic activity of VvGT1 (Offen *et al.*, 2006). Similarly, a hydrophilic amino acid Arginine was replaced with a hydrophobic amino acid Tryptophan (R140W) in VvGT5. The resultant mutant changed the activity and used UDP glucose and UDP galactose as its donor sugars. The wild type VvGT5 actually uses UDP glucuronic acid as its donor sugar (Ono *et al.*, 2010). The current results are in accordance to the findings of Ibrahim (2011) who used VvGT1 as a reference enzyme for alignment. Corresponding to the position in VvGT1 enzyme, site directed mutagenesis was conducted in UGT73C5 at aspartic acid 397. The catalytic activity was observed when the sugar donor was UDP glucose. During the present study, only glucose was used as a donor molecule but she also attempted to add galactose to novobiocin using wild type UGT73C5 enzyme. It was, then, revealed that UGT73C5 was unable to use UDP-galacltose as a sugar donor and novobiocin as sugar acceptor. In another study, enzymatic engineering of UGT85H2 showed that change of Ile305 to threonine enhanced the catalytic activityof enzyme many folds (Modolo *et al.*, 2009).

In addition, site directed mutagenesis with change of arginine to tryptophan in *Sesamum indicum* UGT88D6 and *Antirrhinum majus* UGT88D4 at position corresponding to Arg350 of *Perillafrutescents* UGT88D7 resulted in the change of sugar donor specificity from UDP-glucuronic acid to UDP-glucose (Noguchi *et al.*, 2009). Ramakrishnan and Qasba (2002) reported changed donor specificity in two GTsβ 1, 4 glycosyltransferase and β1,3-glucuronosyltransferase due to single amino acid replacement. The β 1, 4 glycosyltransferase was found to use UDPGalNAc at a rate equivalent to UDPGaldue to mutation of Tyrosine to Leucine at 289 position. Whereas, β1,3-glucuronosyltransferase with site directed mutation of Histidine to Arginine at 308 position efficiently consumed UDP-Glc, UDP-Man and UDP-GlcNAc and UDP-GlcA donor (Ramakrishnan and Qasba, 2002). In another study, there was a complete reversal of donor specificity in

glycosyltransferase B in human blood when it was mutated at 234 position; P234S (Sandra *et al.,* 2003). Thus, the structural based enzymatic engineering can overall be applied to other UGTs for making effective mutants with different donor sugars and acceptor molecules.

Conclusion

From current experimental data it is concluded that the mutant enzyme UGT76E1T134A completely lost its activity with UDP glucose as donor sugar when novobiocin and kaempherol were used as acceptor molecules individually. This is an evidence reported for the first time for the presence of significant peptides at the site of mutation in UGT76E1.

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