Glycoconj J (2008) 25:49–58
DOI 10.1007/s10719-007-9060-1

Glyco-array technology for efficient monitoring of plant cell wall glycosyltransferase activities

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Received: 27 April 2007 / Revised: 11 June 2007 / Accepted: 19 June 2007 / Published online: 1 August 2007
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Abstract The plant cell wall is a complex network of polysaccharides. The diversity in the linkage types connecting all monosaccharides within these polysaccharides would need a large set of glycosyltransferases to catalyze their formation. Development of a methodology that would allow monitoring of glycosyltransferase activities in an easy and high-throughput manner would help assign biochemical functions, and understand their roles in building this complex network. A microarray-based method was optimized for testing glycosyltransferases involved in plant wall biosynthesis using an α(1,2)fucosyltransferase involved in xyloglucan biosynthesis. The method is simple, sensitive, and easy to implement in any lab. Tamarind xyloglucan polymer and trimer, and a series of cello-oligosaccharides were immobilized on a thin-coated photo-activable glass slide. The slide with the attached sugars was then used to estimate the incorporation of [^{14}C]Fuc onto xyloglucan polymer and trimer. [^{14}C]-radiolabel incorporation is revealed with a standard phosphoimager scanner, after exposure of the glycochip to a phosphor screen and detection. The method proved to be sensitive enough to detect as low as 45 cpm/spot. Oriented anchoring of small oligosaccharides (trimer) was required for optimal transferase activities. The glycochip was also used to monitor and estimate xyloglucan fucosyltransferase activity in detergent-solubilized crude extracts from pea microsomes that are known to contain this enzyme activity. Our data indicate that the methodology can be used for efficient and rapid monitoring of glycosyltransferase activities involved in plant wall polysaccharides biosynthesis.

Keywords Cell wall · Glycosyltransferases · Glycochip · Xyloglucan · Microarray

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Abbreviations

AtFUT1	arabidopsis fucosyltransferase 1
DP	degree of polymerization
HPLC	high performance liquid chromatography
MALDI-TOF	matrix-assisted laser-desorption/ionization time-of-flight
PDL	poly-D-lysine
Sf21	*Spodoptera frugiperda 21*
TXyG	tamarind xyloglucan
XyG-FUT	xyloglucan-fucosyltransferase
XyG-XT	xyloglucan-xylosyltransferase

Introduction

The plant cell wall is a well-organized network of polysaccharides with varying degree of complexity [5, 13].

Considering the specificity of glycosyltransferases and the diversity in linkage types connecting all monosaccharides within these polymers, plant wall biosynthesis would need a diversified set of glycosyltransferases to catalyze the formation of these glycosidic linkages. Development of methodologies that allow the screening for these glycosyltransferase activities would help understand their role in building cell walls. Therefore, the current challenge in plant cell wall polysaccharides biosynthesis is to develop enzymatic assays to identify the biochemical function of any putative glycosyltransferase involved in the process. Limitations to achieve this goal include the availability of substrate acceptors and donors, and the lack of a robust high-throughput method of screening. One possible technology to reach this goal would be to generate a glyco-array that contains virtually all possible cell wall polymers and oligomers as a biochemical analytical platform. The goal of this study is to optimize the microarray methodology for an easy and efficient use in investigating glycosyltransferase activities (separately or simultaneously) in a high-throughput manner.

Nucleotide microarray technology offers the possibility for gene expression and discovery of glycosyltransferase genes; however, no such advanced methodology is currently available to study a set of glycosyltranferase activities at the same time or simply directly detect these activities. Because of carbohydrate structural complexity, microarray-based technology has been difficult to use in glycobiology and efforts to adapt this tool were not undertaken till recently [6, 20]. Several recent investigations reported the use of a glyco-array to investigate protein–carbohydrate interactions including carbohydrate recognition by lectins or antibodies [1, 2, 12, 15, 19, 20, 22]. However, among these studies, only a limited number is dealing with glycosyltransferases involved in glycoprotein N-glycan synthesis [3, 14, 17]. The only example where a solid-phase platform has been applied to plant cell wall polysaccharide glycosyltransferases was carried out to investigate pectin biosynthesis [11]. In this study, the authors reported the use of anchored pectin oligosaccharides (DP11-13) on a Sepharose gel functionalized with a disulfide-cleavable linker, to detect α(1,4)galactorunosyltransferase activity. Reaction products were released under reducing conditions and analyzed by MALDI-TOF[1] mass spectroscopy [11].

In modern biology, carbohydrate arrays represent a real opportunity for high-throughput screening of polysaccharide biosynthetic enzymes, but the methodology is still not well adapted for routine use in any research lab. Thus, it is necessary to adapt microarray methodology for plant cell wall biology. To be advantageous, a plant cell wall glyco-array platform should possess the following characteristics: (1) Use small quantities of acceptor polysaccharides and oligosaccharides. In contrast to N-glycans of glycoproteins, the structure of plant wall polysaccharides are much more complex and not easy to prepare in pure form and in large quantities, which will require adjustments of the glyco-array methodology (scaling down reaction conditions). (2) The method of detection should be sensitive enough to demonstrate the formation of glycosyltransferase products. Standard biochemical assays to monitor glycosyltransferase activities use radioactive NDP-sugars. Radiolabeling methods are very sensitive and NDP-sugar precursors are commercially available. Therefore, glyco-array platforms need adaptation for the use of such precursors. (3) The platform should be simple and easy to implement in any laboratory. Currently, the existing glyco-array platforms require sophisticated tools such as MALDI-TOF, HPLC, Dionex, etc., or well developed specific antibodies, which are not available for all cell wall polysaccharides [22, 23]. For platforms used in investigating N-glycans biosynthesis, the detection of reaction products (added sugar to the array), is achieved through glycan binding proteins that would recognize specifically that sugar, or through labeled secondary antibodies against sugar epitopes containing that sugar. Such tools are not well established for plant wall polysaccharides.

In this work we have used a well-characterized glycosyltransferase involved in xyloglucan (XyG) biosynthesis, namely XyG-fucosyltransferase AtFUT1 [9, 18] to optimize [^{14}C]Fuc incorporation onto sugar acceptors immobilized on a chip platform. Several types of acceptors, including oligosaccharides and polysaccharides, were tested at various concentrations. The [^{14}C]-radiolabel incorporation could be detected and quantified using a simple high-resolution phosphoimager. We were able to detect as little as 45 cpm [^{14}C]-radiolabel incorporation on the slides on 1 mm^2 spots. Our data suggest that glycochip technology can be adapted to glycosyltransferase assays and represent a promising methodology for high-throughput screening of new glycosyltransferase activities.

Material and methods

Reagents and enzymes

Tamarind xyloglucan (TXyG) was purchased from Megazyme (Bray, Ireland). Cello-oligosaccharides were from the Institute of Chemistry, Slovak Academy of Sciences (Bratislava Slovakia, http://www.chem.sk/products/). All chemicals were from Sigma unless stated specifically. GDP-[^{14}C]Fuc (10.1 GBq mmol^{-1}) was from NEN (Dupont). Spectra/Por Membrane MWCO: 12–14000 from Spectrum laboratories. Dowex 1-X8 Cl$^-$ ion-exchange matrix (200–400 mesh) was from Sigma. PhotoChips

(standard size glass slides, thin-film coated with the photolinker polymer OptoDex) were obtained from Arrayon Biotechnology (Neuchatel, Switzerland). Complete mini protease inhibitor tablets EDTA-free were from Roche Applied Science. AtFUT1 expressed in Bac-to-Bac baculovirus expression system, *Spodoptera frugiperda 21 (Sf21)* cells, was a gift from Dr. Kenneth Keegstra (Michigan State University-Plant Research Laboratory, East Lansing).

Preparation and purification of xyloglucan oligosaccharides

One gram of TXyG (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) dissolved in 100 ml water was digested by 100 mg *Trichoderma* cellulase (2,500 U/mg, Sigma) for 16 h at room temperature. The reaction was terminated by 5 min heating at 100°C and the undigested XyG was precipitated by two volumes of ethanol and removed by centrifugation. The supernatant was concentrated to a small volume and lyophilized. For preparation of higher TXyG oligomers (dimers, trimers), 5× scaled-up incubation mixture was incubated in an Amicon DC2 dialyzer/concentrator in dialysis mode using a 10,000 MWCO dialysis cartridge at room temperature for 4 h. The outer circuit was circulated with distilled water continuously replaced in batches of 500 ml every 30–40 min. The dialysate was then concentrated in a rotary evaporator to a small volume. Further separation of oligomers was performed on a column of Bio-gel P10 (2.4×110 cm) eluted with water with a flow rate of 0.4 ml min^{-1}. Typically, from 5 g of TXyG, 3.9 g of dialyzable sugars were obtained and the relative abundance of individual fractions were 28% of monomers (DP 7–9), 17% dimers, 18% trimers and 23% of higher oligmers. The composition of the oligomer fractions was analyzed by TLC on Silicagel M60 using the solvent system *n*-Butanol-Ethanol-water (10:10:8, by vol.), run three times with intermittent drying. Each oligomer cluster consisted of several oligosaccharide species reflecting the combination of individual monomers. Their composition was further confirmed by MALDI-TOF analysis performed on Kratos Kompact MALDI 3 using 2,5-dihydroxybenzoic acid as a matrix, which indicated that the oligomers were essentially pure. The individual fractions were concentrated by evaporation and lyophilized.

Preparation of microsomes from pea seedlings

All steps were carried out at 4°C. Microsomal membranes were prepared from the growing region of 7-day-old etiolated pea seedlings as described earlier [10] with modifications. Briefly, the tissue was homogenized with a blender in extraction buffer. After filtration, the homogenate was first centrifuged at 5,000×*g* for 15 min at 4°C, and the supernatant layered over 6 ml of 1.8 M sucrose cushion and then subjected to ultracentrifugation at 100,000×*g* for 60 min at 4°C (Sorvall OTD-65B). The membranes at the top of 1.8 M sucrose cushion were collected and adjusted to protein concentration of 70–100 mg/ml before storage at −80°C until later use.

Enzyme assays

Solution assays

The enzymatic assays were conducted as described earlier [10], namely, final reaction volume was 50 µl containing GDP-[^{14}C]Fuc (~65,000 cpm), substrate acceptors (20–5,000 ng), and 40 µl of AtFUT1 that was expressed in *Spodoptera frugiperda 21* cells (250 ng/µl). Reactions were incubated for 15 min at Room temperature and stopped by adding 0.2 ml of water and then 0.3 ml of 50% (v/v) Dowex-1×8 resin to remove excess of radiolabeled GDP-Fuc before centrifugation for 1 min at 12,000×*g* in spin columns. The flow-through was collected and radioactivity (cpm) was counted (Beckman LS 6500 scintillation counter) after adding ~3 ml scintillation fluid.

Glycochip assays

In a 1.5 ml centrifuge tube, 60 µl of recombinant AtFUT1 (250 ng/µl) or 200 µl of Triton X 100-solubilized pea microsomal proteins (6 µg/µl), 20 µl GDP-[^{14}C]Fuc (130,000 cpm), and 120 µl extration buffer were mixed. The mixture was then overlaid evenly over the glycochip and incubated for 2 h at room temperature. The reactions were stopped by washing the glycochip several times in distilled H_2O and then air-dried. Once dry, the chip was wrapped in cellophane and exposed to a phosphor screen (Kodak) for various timings (up to 48 h). The [^{14}C]-radiolabel incorporation was revealed by simple scanning the phosphor screen, at 50 µm resolution, using a Personal Molecular Imager FX phosphoimager scanner (Bio-Rad).

Glycochip preparation

Reductive amination and coupling of oligosaccharides to Poly-D-lysine (PDL)

Reductive amination reactions were carried out mostly as described earlier [16, 21] with some modifications. Briefly, 2 mg PDL, 2.5 mg $NaCNBH_3$, and 4 mg oligosaccharides dissolved in 250 µl 0.1 M borate buffer pH 8.4. The reactions were incubated at 45°C for 7 days, under periodic agitation, and then dialyzed against water using Spectra/Por membrane (MWCO: 12–14,000) at 4°C for 2 days with exchanging the water every 8–16 h. The retained PDL–

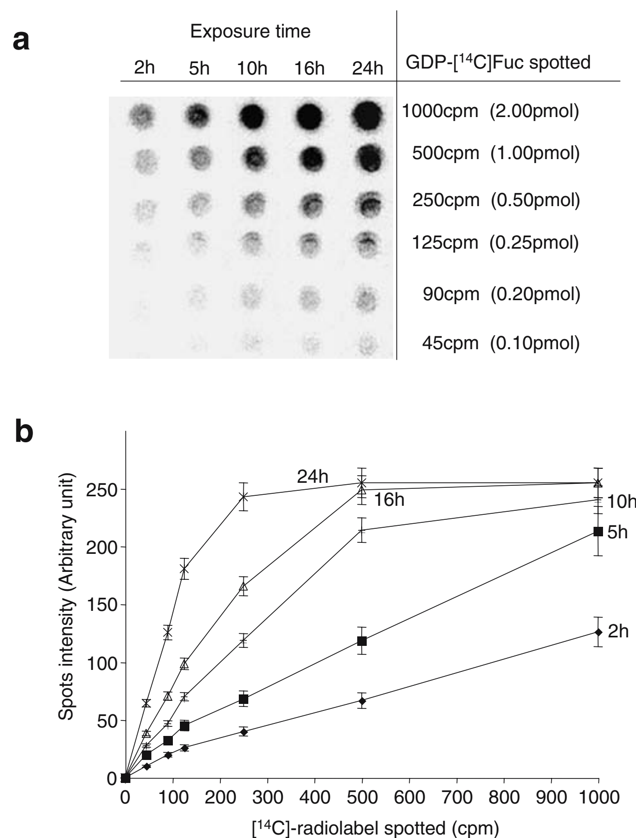

Fig. 1 Determination of lower limit detection of [^{14}C]-radiolabel spots by phosphoimager. **a** Several amounts of GDP-[^{14}C]Fuc were spotted (2 mm diameter) on a standard glass slide, dried, and then exposed to a phosphor screen for various times before visualization using Personal Molecular Imager FX (Bio-Rad). **b** The intensity of the spots was estimated using the ImageJ software and the arbitrary units were plotted as standard curves

oligosaccharide complex was then freeze-dried and weighted and carbohydrate content measured using the phenol sulfuric assay [7]. The degree of PDL substitution was then calculated by relating the moles carbohydrate to the amount of PDL (Table 2). A mean MW of PDL (~22,500) was used to calculate its degree of substitution, assuming that one PDL polymer contains 174 lysine residues.

Biomolecule printing and immobilization

Biomolecules and glyco conjugates were arrayed on PhotoChips as described earlier [1], using the NP2 Nanoplotter (GeSim GmbH, Grosserkmannsdorf, Germany). Printed features of 1 mm^2 surface area were homogeneously impregnated by multiple pL droplet deposition. Upon drying, the printed PhotoChips were illuminated for 4 min with a high-power ultraviolet lamp (1,000 W) at 365 nm (11 mW/cm^2) [4], rinsed with buffer, vacuum-sealed and stored in dry state until use. All procedures related to PhotoChip handling, printing, photoimmobilization and packaging were carried out in a dedicated clean room.

Image analysis using ImageJ software

ImageJ software is freely available at http://rsb.info.nih.gov/ij/. All glycochip images were first resized to 50% their original size using Adobe Photoshop. Measurement settings in ImageJ were set to mean grey value and the images were opened in ImageJ and inverted. Inversion of the pictures was necessary because ImageJ records 100% black as zero and 100% white as 255. A square section was drawn around a spot to be measured using selection tool. The size of the square drawn was maintained and moved over each 1×1 mm^2 spot, and the mean gray value was recorded. The background measurement was taken for the same square size from a non-printed region of the glycolchip.

Results

During the design of the glycochip we sought to develop a tool that can be easily implemented in any research laboratory working on glycosyltransferases. The most common assays for glycosyltransferases use radioactive nucleotide sugars precursors, thus, our first focus was to determine the lower detection limits of [^{14}C]-radiolabel using standard phosphor screen and phosphoimager scanner. A series of dilutions using GDP-[^{14}C]Fuc were spotted (2 mm diameter) on a standard glass slide. After drying, the slide was exposed to the phosphor screen for various exposure times (2–24 h) and then the screen was scanned with the scanner. As indicated in Fig. 1, we could detect as low as 45 cpm per spot after 10 h exposure (Fig. 1a). For 2 h exposure, the limit of detection was 125 cpm per spot.

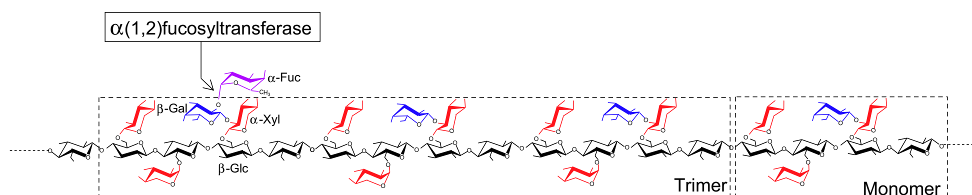

Fig. 2 Presentation of basic tamarind xyloglucan (TXyG) structure along with the linkages catalyzed by α(1,2)fucosyltransferase (FUT) activity used in this study. TXyG is formed of a repeating unit (*monomer*). Three monomers linked together form a trimer. TXyG does not contain fucose residues, thus, both the polymer and trimer are good acceptor substrates for FUT activity [9]

Table 1 Determination of lower limit detection of recombinant Arabidopsis XyG-α(1,2)fucosyltransferase activity (AtFUT1) in tube assays

Amount of acceptor (ng)	[^{14}C]Fuc incorporated (cpm/reaction)	
	Onto TXyG polymer	Onto TXyG trimer
20	727±150	0
50	Not tested	840±100
100	2,330±200	1,400±300
500	Not tested	4,484±600
5,000	Not tested	2,234±400
20,000	41,526±1,500	46,772±1,000

The activity was determined using both TXyG polymer and trimer oligomers (see Fig. 2 for structure) as acceptors in presence of GDP-[^{14}C]Fuc.

From these data we generated standard curves where the intensities of the spots for various amounts of [^{14}C]-radiolabel (cpm) are plotted for each exposure time (Fig. 1b). These standard curves can be used to estimate the incorporation of [^{14}C]Fuc onto the acceptors during transfer reactions. The data showed that 5–10 h exposure times are best for spots having less than 200 cpm, and shorter exposure times are required for spots with strong signals (>250 cpm). The linear increase of the standard curves suggests that the method can be used for quantification purposes (Fig. 1b).

Next, we focused on identifying the minimum amounts of substrate acceptor that a glycosyltransferase would require to incorporate as little as 125 cpm of [^{14}C]-radiolabel. To this end, we used a well-characterized glycosyltransferase involved in xyloglucan (XyG) biosynthesis, namely Arabidopsis XyG-fucosyltransferase, AtFUT1 [8, 10, 18]. A simplified structure of XyG and the linkage catalyzed by this enzyme are illustrated in Fig. 2. AtFUT1 was tested on TXyG polymer and trimer (three repeating monomer subunits as indicated in Fig. 2). The enzyme was expressed in *Spodoptera frugiperda 21 (Sf21)* cells and detergent-solubilized before use in standard biochemical assays [9, 10]. The data indicated that AtFUT1 could incorporate [^{14}C]Fuc onto as little as 20 ng TXyG polymer (727 cpm) and 50 ng trimers (840 cpm; Table 1). These data demonstrate that glycosyltransferases involved in plant wall polysaccharides biosynthesis are active on small acceptor quantities, and therefore, the assays can be scaled down and adapted to glycochip procedures.

Next step was to find an easy, quick, and efficient way to attach covalently these substrate acceptors on a standard glass slide and generate the glycochip. Recently Arrayon Biotechnology (Neuchâtel, Switzerland) made available standard glass slides coated with a thin-film of OptoDex, a polysaccharide-based polymer carrying several photo-activable chemical groups (aryldiazirines), which can be used to directly and randomly immobilize biomolecules through the action of photo-generated carbenes [1, 4]. The process does not require prior functionalization of either biomolecules or the material surface. In our preliminary tests we printed *via* Arrayon Biotechnology, a glycochip where the size of spots are 1 mm^2 separated by ~0.4 mm. The design of the chip is illustrated in Fig. 3.

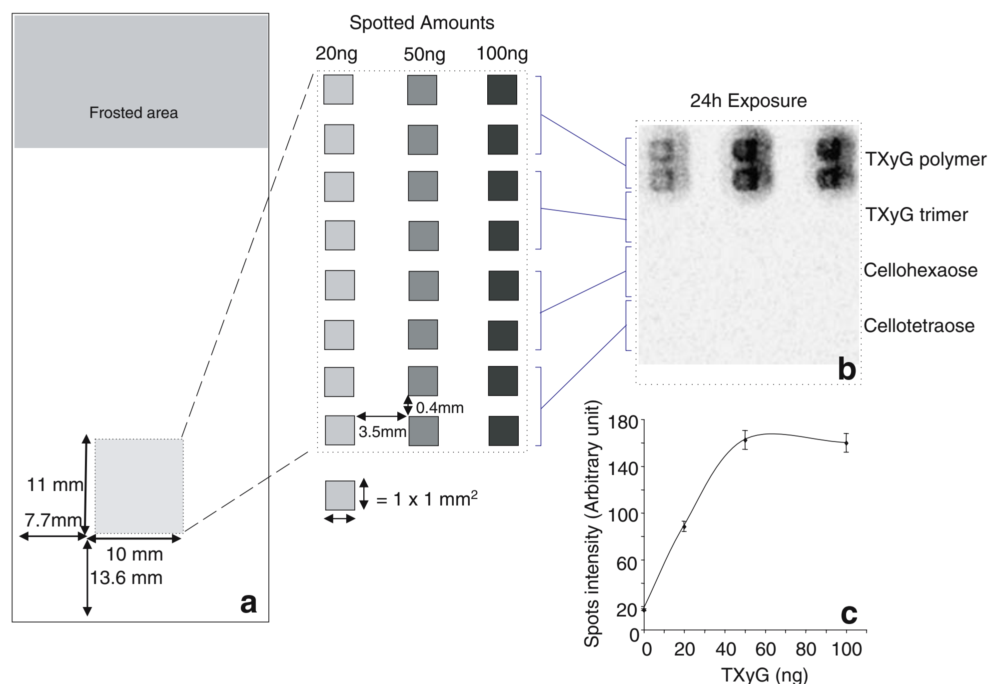

Fig. 3 The design of the glycochip. **a** TXyG polymer and trimers (acceptors for AtFUT1 activity), and cellohexaose and cellotetraose were printed at 20, 50, and 100 ng in duplicate spots of 1 mm^2. **b** Detection of [^{14}C]Fuc incorporation onto TXyG polymer using a Personal Molecular Imager FX (Bio-Rad) at 50 µm resolution. The glycochip was incubated with AtFUT1 expressed in *Sf21* cells, washed and exposed for 24 h to a standard phosphor screen and used for detection. **c** The intensity of the detected spots in **b** was estimated as indicated in Fig. 1 and the values plotted as graph

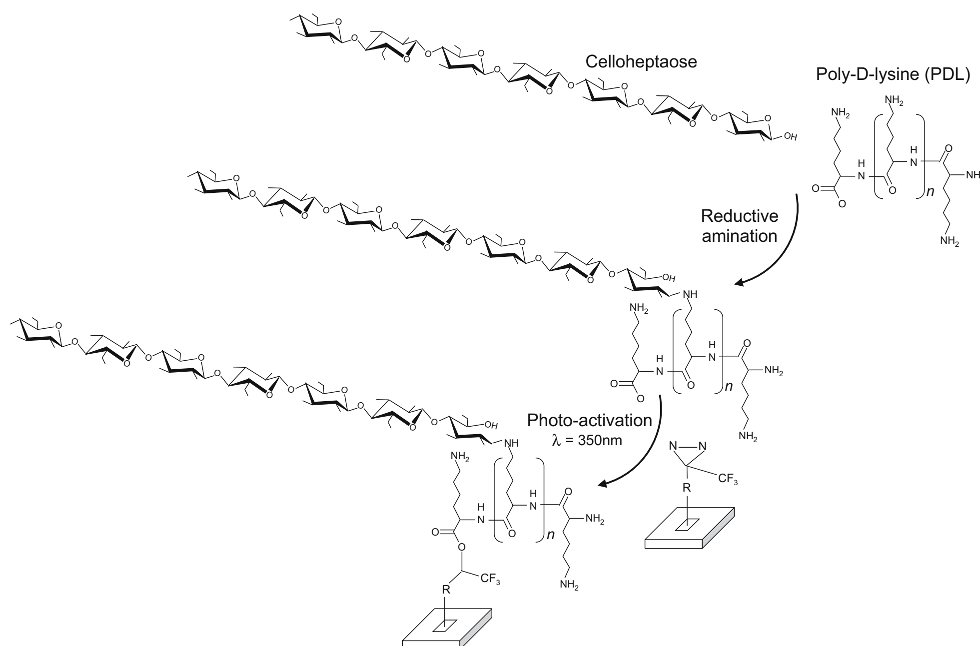

Fig. 4 Illustration of poly-D-lysine (PDL)-oligosaccharide conjugates synthesis by reductive amination and their anchoring to the glycochip by photo-activation as described in "Material and methods." Celloheptaose oligosaccharide is depicted as example. Conjugate anchoring efficiencies are listed in Table 2

The first attempts were to test TXyG polymer, TXyG trimers, celloheptaose, and cellohexaose that were directly and randomly immobilized on the glycochip at three different amounts (20, 50, and 100 ng; Fig. 3, see Fig. 2 for sugar structures). When the glycochip was incubated with AtFUT1 in presence of GDP-[^{14}C]Fuc and exposed to the phosphor screen for 24 h, the data in Fig. 3 indicate that AtFUT1 incorporated [^{14}C]Fuc onto immobilized TXyG polymer but not onto the trimer nor onto cello-oligosaccharides (Fig. 3c). In addition, the intensity of the spots was proportionally increasing as function of the amounts of TXyG polymer spotted (Fig. 3c). Using the standard curve for 24 h exposure time (Fig. 1) and the intensity of the spots in Fig. 3, we could estimate the total [^{14}C]Fuc incorporation to 0.14 pmol Fucose (24 pg), which is equivalent to 1.2 pg Fuc residues incorporated per 1 ng TXyG polymer.

Several possibilities may explain the lack of fucosylation of the trimers by the enzyme. The first possibility could be that the oligosaccharides did not attach or did attach but at lower amounts to the chip surface. This possibility is less likely to occur because the method proved to be efficient in anchoring different biomolecules at 45–80% efficiency (data not shown). The second possibility is that the sites of fucosylation on the trimers are engaged in linking them randomly to the glycochip surface, thus reducing the number of available sites for transfer reactions. To verify this possibility, we decided to use oriented covalent immobilization of TXyG trimers and cello-oligosaccharides, which would require the anchoring *via* their reducing ends. Several methods have been developed to anchor a carbohydrate to a linker in an oriented linkage *via* the reducing end of the carbohydrate [15]. Our goal was to use a simple method that can be applied to a range of carbohydrates without additional chemical functionalization and can be easily carried out in a lab. The base polymer for synthetic glyco-conjugates used in this study was a poly-D-lysine polymer (PDL), which is commercially available and can attach *via* reductive amination reaction [16, 21] several oligosaccharides on a single PDL molecule. The PDL–oligosaccharide conjugate can then be photo-linked to the glycochip *via* carbene-mediated insertion. Reductive amination forms an imine between the aldehyde of reducing

Table 2 Determination of anchoring efficiency of different substrate acceptors on PDL

Acceptor	Estimated MW	Total carbohydrate content (mg)	Substitution of PDL (mol/mol)	Degree of PDL substitution (%)
TXyG Trimers	~5,000	1.2	0.8	0.5
Celloheptaose	1,152	0.74	6.5	3.7
Cellohexaose	990	0.92	1.8	1
Cellotriose	666	1.2	41	23
Xylohexaose	810	0.7	66	38
Xylopentaose	678	0.7	60	34

The substitution (mol carbohydrate/mol PDL) was calculated by relating the moles carbohydrate to the amount of PDL. The mean MW of PDL (~22,500) is used to calculate its degree of substitution, assuming that one PDL polymer contains 174 lysine residues.

Table 3 Efficiency of untreated trimer versus PDL-trimer conjugate to act as acceptors for XyG-fucosyltransferase activity (AtFUT1 expressed in *Spodoptera frugiperda 21* cells)

Amount used (ng carbohydrates)	[14C]Fuc incorporation (cpm/reaction)	
	Untreated trimers	PDL-trimer conjugate
50	998±300	2,798±200
200	1,657±500	10,238±1,000
500	1,500±600	37,809±1,500

PDL (50 μg) was used as control in the assay, and [14C]Fuc incorporation obtained was about 400 cpm. The assays were performed as described in "Material and methods."

end and amino groups of PDL. The reduction of the imine produces a stable amine (Fig. 4). Although the method is simple and can be easily carried out in any lab, it will not prevent completely the loss of some fucosylation sites *via* random photo-linkage to the surface, but conditions can be optimized to increase the anchoring of "PDL–oligosaccharide" conjugates through the carboxyl or amine groups of the lysine residues of the PDL. We first optimized the conditions of reductive amination reaction for each oligosaccharide and found that the attachment efficiency of the oligosaccharides to PDL depends on the size and the solubility of the oligosaccharides (Table 2). For example, TXyG trimers are soluble in water but are four times larger than celloheptaose, therefore are less efficiently attached to PDL (only 0.5%). Celloheptaose and cellohexaose are non-branched oligosaccharides with limited solubility (higher DPs are not soluble), thus, have low attachment efficiency, 3.5 and 1%, respectively (Table 2).

To verify that reductive amination did not affect the capacity of the oligosaccharides to act as acceptors, we tested the PDL–trimer conjugates along with untreated trimers in our standard AtFUT1 assay. The data indicate that neither reductive amination nor PDL affected the ability of these oligosaccharides to act as good AtFUT1 acceptors. In contrast, the PDL seems to improve the trimer's ability to act as a better acceptor for FUT activity compared to free trimer (Table 3).

Three different amounts (1, 5, and 12.5 pmol per spot) of these PDL–oligosaccharide conjugates were then printed, in duplicate, on the glycochip at Arrayon Biotechnology facility. In addition, PDL and TXyG polymers were included as controls at 4, 20, and 50 ng. The design of this new chip is illustrated in Fig. 5. When the glycochip

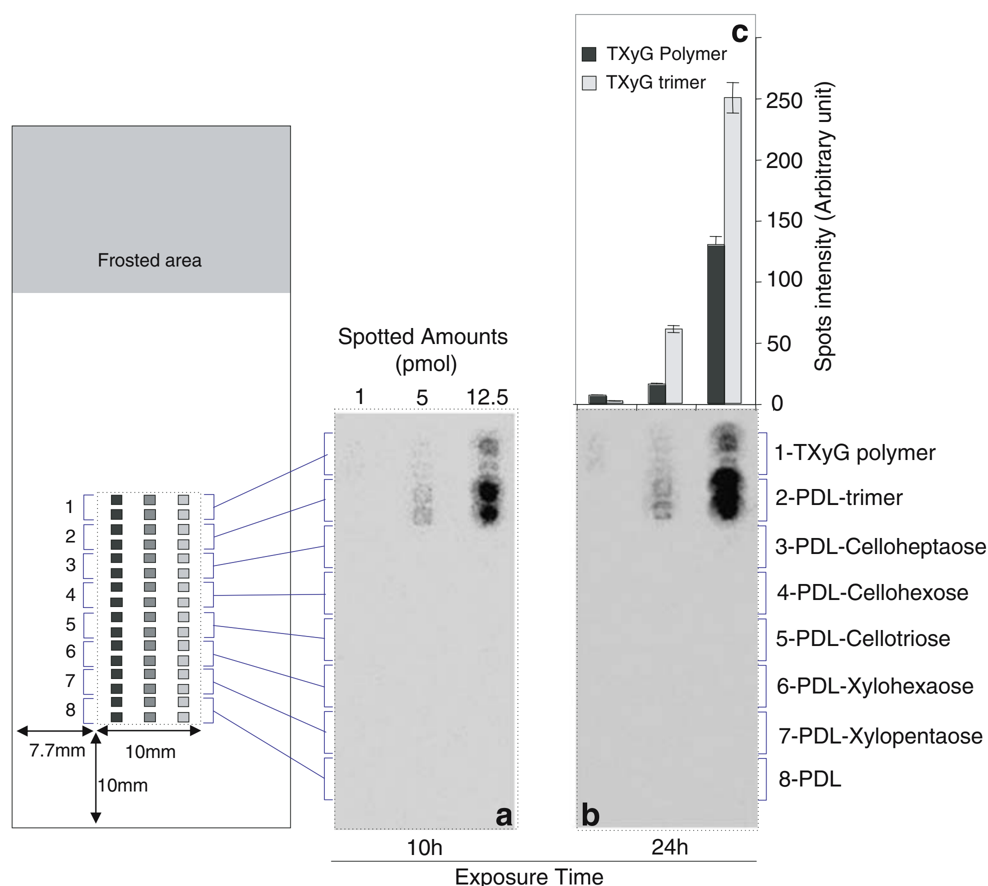

Fig. 5 Detection of [14C]Fuc incorporation onto TXyG polymer and poly-D-lysine (PDL)-trimer conjugates attached on the glycochip. All the PDL-oligosaccharides were printed in duplicate spots of 1, 5, and 12.5 pmol. TXyG polymer and PDL were printed at 4, 20, and 50 ng per spot. The glycochip was incubated with AtFUT1 expressed in *Sf21* cells and GDP-[14C]Fuc for 2 h, washed and then exposed for 10 (**a**) and 24 h (**b**) to the phosphor screen before detection with the phosphoimager. The intensity of the spots in **b** was estimated using ImageJ software and the values plotted as graph (**c**)

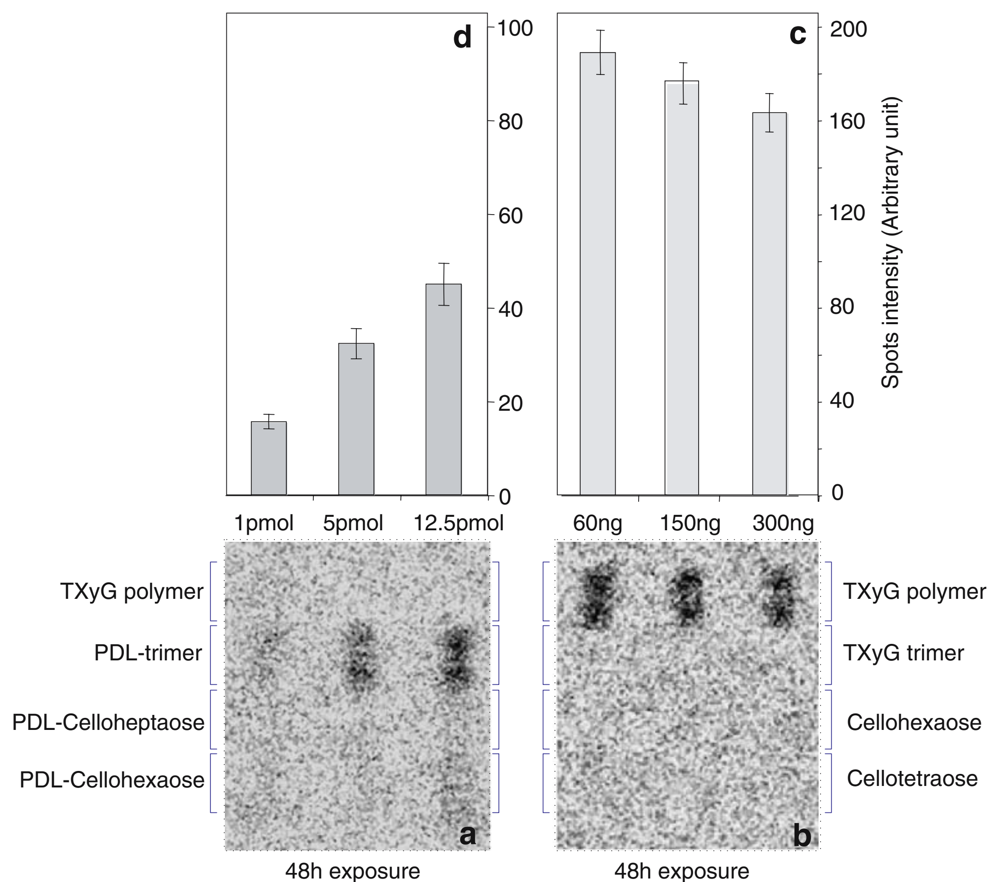

Fig. 6 Detection of [^{14}C]Fuc incorporation by triton X-100-solubilized crude extracts from pea microsomes. In **a**, the glycochip used has the same characteristics as in Fig. 5 (PDL-oligosaccharides spotted at 1, 5, and 12.5 pmol per spot; TXyG polymer printed at 4, 20, and 50 ng per spot). In **b**, the glycochip used has the same characteristics as in Fig. 3, however in this glycochip, TXyG polymer and the other acceptors were photo-anchored (random fashion), in duplicate spots containing 60, 150, and 300 ng. Exposure times are indicated, and the intensity of the detected spots in **a** and **b** was estimated as in Fig. 1 and the values plotted as histograms (**c** and **d**)

was incubated with AtFUT1 and GDP-[^{14}C]Fuc and then washed and exposed to the phosphor screen for 24 h, [^{14}C]-radiolabel incorporation was detected on both TXyG polymer and trimer (Fig. 5a, b). This result strongly suggests that oriented anchoring of the oligosaccharides was required for optimal AtFUT1 activity. Using the standard curves in Fig. 1 and the intensity of the spots in Fig. 5, we estimated [^{14}C]Fuc incorporation to 0.66 pg per 1 ng of both trimer and TXyG polymer. However, this incorporation seems to be somehow lower than that obtained with the first chip, 1.2 pg Fuc per 1 ng TXyG (Fig. 3), which may suggest that the efficiency of printing for this chip was about 55%. Also, no radioactivity was incorporated onto the PDL or cello-oligosaccharides indicating no interference with FUT activity.

In another set of experiments, we wanted to test whether the glycochip is suitable for direct monitoring of glycosyltransferase activities in a mixture of proteins from microsomal fractions. Microsomes from elongating regions of pea seedlings are known to contain XyG-fucosyltransferase activity [8, 9, 10]. Thus, Golgi-enriched microsomes were prepared from etiolated pea seedlings and tested first in our standard transferase assays to confirm the presence of FUT activity (data not shown). In the first tests we used the glycochip printed with 4, 20, and 50 ng TXyG polymer and PDL-trimer at 1, 5, and 12.5 pmol per spot to detect the presence of XyG-FUT activity in Triton X-100-solubilized proteins from microsomes. These tests indicated no incorporation of [^{14}C]Fuc onto TXyG polymer, but low signals were obtained for 5 and 12.5 pmol spots of PDL–trimer conjugate after 48 h exposure of the glycochip (Fig. 6a). The low levels of XyG-FUT activity in microsomes may explain the lack of [^{14}C]Fuc incorporation onto TXyG. To compensate for this lower activity, we increased the amounts of TXyG polymer spotted on the chip (60, 150, and 300 ng). Figure 6b indicates that at these TXyG concentrations, an exposure time of 48 h gave a stronger signal that allowed the estimation of [^{14}C]Fuc incorporation to 0.1 pg per 1 ng TXyG polymer (Fig. 6b, c). We noticed also that the spots with 300 ng TXyG polymer gave similar intensity as the spots with 60 ng, indicating that the enzyme activity in the microsomal fraction, and/or the acceptor surface density, limit [^{14}C]Fuc incorporation (Fig. 6c).

Discussion

Plant cell wall is made of complex polysaccharidic network that form up to 90% of its mass. The chemical structure of these polysaccharides is well studied but the mechanism of their biosynthesis is still not completely understood. Recent

progress in genomics and bioinformatics tremendously facilitate the discovery of putative glycosyltransferase genes that might be involved in the biosynthesis of these polysaccharides (see Carbohydrate Active enZYme database, CAZY at http://www.cazy.org/). The challenge that plant cell wall community is currently facing concerns the assignment of biochemical functions to each of these putative glycosyltransferase genes. Furthermore, in modern biology, it is also becoming increasingly important to develop tools that allow the investigation of wall glycosyltransferase activities in the context of system biology, and allow monitoring of the variations in enzymatic activities simultaneously in relation to each other at any given time or during any given developmental process. We anticipated that a glycochip containing structurally defined oligosaccharide and polysaccharide substrates for virtually all plant wall biosynthetic/modifying enzyme activities would be an effective and faster way to evaluate all the activities in a single assay for a single tissue or growth conditions. Such glycochips could be used also as a high-throughput monitoring method along with a high-throughput expression system where any putative glycosyltransferase gene can be expressed and tested in an efficient way. To be efficient the method will need to be (1) sensitive enough to detect low enzyme activities. The current microarray technology is sensitive enough to detect strong DNA–DNA/RNA and protein–protein interactions but not for sugars incorporation/modification, (2) does not require large quantities of substrate acceptors, as these substrates are difficult to prepare in large quantities, (3) easy to use in any lab with available scanners, and (4) easy and cost effective in making the glycochip.

In this report we present initial work on the adaptation of glyco-array technology for monitoring plant cell wall glycosyltransferase activities. We used XyG-α-(1,2)fucosyltransferase, which is well characterized in terms of specificity of both donor and acceptor substrates [8, 9, 18]. This enzyme could efficiently transfer [^{14}C]Fuc from GDP-[^{14}C]Fuc onto immobilized TXyG polymer, and the radiolabel could be detected using the highest resolution of a standard scanner. Under our conditions, the signal to background ratio was sufficient to detect the incorporation of as low as 0.66 pg Fuc per 1 ng TXyG. This result suggests that a glycochip containing other high MW cell wall polysaccharides can be easily made available for the detection and identification of other putative glycosyltransferase activities. The procedure does not require any derivatization of the polymers for efficient photo anchoring on the glycochip surface. However, because the anchoring on the glycochip is random, attachment conditions should be optimized for each polymer, which will depend on their size and solubility. For example, under our random anchoring conditions, TXyG trimer (DP20-25) did not give any [^{14}C]Fuc incorporation. However, oriented anchoring did overcome this problem and restored (and even improved) the trimer's ability to act as acceptors for FUT activity. Oriented anchoring was carried out by attaching the trimers onto PDL polymers, *via* reductive amination, before photo anchoring on the glycochip.

One of our goals was to optimize the glycochip for direct monitoring of glycosyltransferase activities in microsomal fractions. Thus, we used microsomes from growing regions of etiolated pea seedlings that are known to contain XyG-FUT activity. Like most of wall polysaccharide glycosyltransferase activities, XyG-FUT activity is present in low levels in microsomes, which makes its detection difficult. Our results showed that by increasing the amount per spots (up to 300 ng), and by increasing exposure time (up to 48 h), we could detect enzyme activity. However, conditions and procedures used here may request further adaptation, in particular for the detection of very low level enzyme activities such as XyG-xylosyltransferase (XyG-XT). The reason for this low activity is that XyG synthesis requires cooperative action between XyG-XT and the XyG-glucan synthase [10].

In summary, our work describes the successful use of the glyco-array technology in monitoring the transfer of [^{14}C]-radiolabel sugars from NDP-[^{14}C]sugars onto immobilized acceptors. This report delineates routes to high-throughput screening of new glycosyltransferases (recombinant enzymes), and rapid functional analysis. Most importantly, the technology is highly sensitive, it can be adapted to small-scale reactions, and is easy to implement in any research lab. Therefore, we anticipate its broad and promising applications, in particular, in the field of plant cell wall polysaccharide biosynthesis.

Acknowledgement We would like to thank Dr. Kenneth Keegstra for providing AtFUT1 protein expressed in *Spodoptera frugiperda 21 (Sf21)* cells. Our thanks go also to all of the members of the Faik laboratory and cell wall group at Ohio University, in particular to Dr. Showalter and Mr Wei Zeng, for helpful discussion and technical support. This work was supported in part by grant no. II/2/2005 to center of excellence GLYCOBIOS from the Slovak Academy of Sciences and grant and no. 2/6133/06 from Grant Agency VEGA to V.F.

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