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DNA-directed immobilisation of glycomimetics for glycoarrays application: Comparison with covalent immobilisation, and development of an on-chip IC_{50} measurement assay

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ABSTRACT

Glycoarrays are powerful tools for the understanding of protein/carbohydrate interactions and should find applications in the diagnosis of diseases involving these interactions. Immobilisation of the carbohydrate probe is a key issue in the elaboration of high performance devices. In the present study, we have compared the fluorescent signal intensity and determined the lower detection limit of glycoconjugates immobilised at two concentrations (0.5 and 25 μM) by DNA-directed immobilisation (DDI), to glycoconjugates covalently immobilised on the solid support (borosilicate glass slide). At 0.5 μM, DDI led to a stronger fluorescence signal (by a factor of 4.5) and to a lower detection limit (20 nM) than covalent immobilisation (higher than 200 nM). We also report the development of an IC_{50} measurement assay of DDI immobilised glycoconjugates. We found that the relative affinity per galactose residue of RCA 120 for glycoconjugates bearing one or three galactose residues was different by a factor of 23 when measured under IC_{50} conditions or by direct fluorescence reading.

1. Introduction

Oligosaccharides are involved in crucial physiological and pathological events (Varki, 1993; Sharon and Lis, 2004; Seeberger and Werz, 2007; Galonicǐ and Gin, 2007; Bishop et al., 2007). Research in this field has been slowed down by the wide structural diversity of oligosaccharides and the difficulty to synthesize them. Glycoarrays or carbohydrate microarrays have emerged as a key technology for deciphering the glycocode (Wang, 2003; Wang et al., 2002).

Grafting of the oligosaccharide probes on a surface is one of the key steps in the final performance of analytical devices. The grafting process should guarantee good availability of the probe for the target, a proper orientation, a sufficient degree of freedom, sufficient distance from the surface, and should preserve the 3D structure of both probe and target. It should also proceed with good and reproducible yields and ideally the immobilisation yield should be similar for all probes.

Oligosaccharides have been immobilised by various methods that may be classified into three main categories: physisorption, chemical ligation (covalent grafting) and specific biological-based interactions.

Physisorption of carbohydrate ligands relies on weak interactions of the molecules with the substrate. Due to their low molecular weight, oligosaccharides need to be modified with an anchoring tail such as hydrophobic chains (Fukui et al., 2002; Fazio et al., 2002; Wang et al., 2002), fluorous tags (Jaipuri et al., 2008) or macromolecules (Wang et al., 2002).

Carbohydrates have been covalently attached to a surface with unmodified saccharides (Seo et al., 2007; Smith et al., 2003; Miura et al., 2002; Larsen et al., 2006) or with functionalised glycosides. The latter strategy has been far more documented in the literature. In most cases, it requires surface functionalisation of the substrate. Examples of immobilisation through photochemistry (Angeloni et al., 2005; Chevolot et al., 1999,2001; Léonard et al., 2001; Leonard et al., 1998a; Leonard et al., 1998b), reaction of thiols with double

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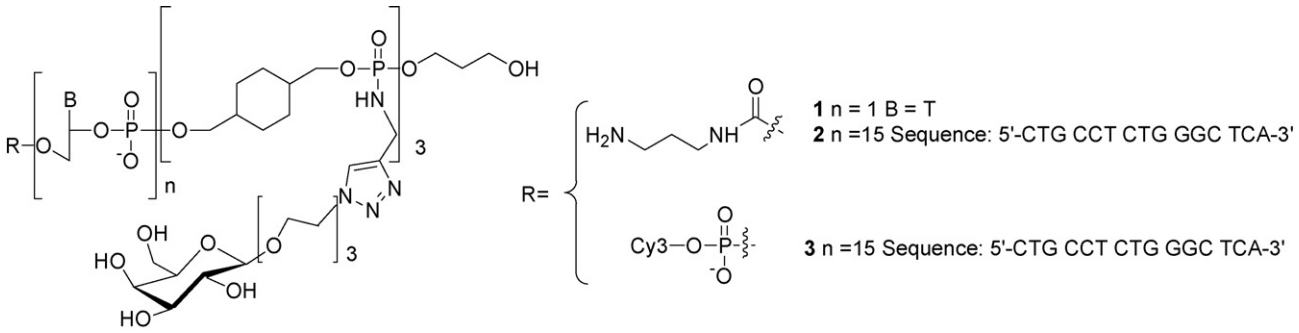

Fig. 1. Structure of 5′-amino-tris-(DMCH galactosyl) glycomimetics **1** and **2** for covalent immobilisation and **3** for DDI.

bonds (Brun et al., 2006; Park et al., 2004; Houseman et al., 2003) or thiol-derivatised surfaces (Shin, 2007), reaction of amines with activated esters (Blixt et al., 2004), aldehydes (Biskup et al., 2005) or epoxy-modified substrates (Lee and Shin, 2005), cycloaddition reaction (Houseman and Mrksich, 2002; Bryan et al., 2004; Huang et al., 2006), Staudinger ligation (Kohn et al., 2003) or reaction between a cyanuric chloride and an aminophenyl group (Schwarz et al., 2003) are reported.

Finally, immobilisation has also been achieved by means of specific biological based interactions, through biotin/streptavidin interaction (Bochner et al., 2005), or by DNA-directed immobilisation (DNA/DNA hybridisation) (Chevolot et al., 2007).

DNA-directed immobilisation (DDI) was first reported by the group of Niemeyer for proteins (Niemeyer et al., 1999; Wacker and Niemeyer, 2005; Wacker et al., 2004) and more recently for peptides (Schroeder et al., 2007). Their approach is based on the immobilisation of streptavidin by DDI and further immobilisation of the probe by biotin/streptavidin interaction. Other approaches rely on DNA-tagged proteins (Boozer et al., 2006), m-RNA PROfusion protein (Weng et al., 2002) or with the incorporation of the GAL4 DNA binding domain into a protein.

In an earlier report (Chevolot et al., 2007), it was demonstrated that glycomimetics bearing one to three monosaccharides (galactose or mannose) can be assembled on a scaffold, immobilised on glass slides by means of DDI and can then interact with the galactose binding lectin *Ricin Communis* agglutinin 120 (RCA 120). The advantages of the DDI approach are (Chevolot et al., 2007):

- A detection limit between 2 and 20 nM,
- Linearity from 0.02 to 2 μM (semi log scale),
- A reusable DNA platform,
- Quality control of immobilised glycomimetics (relative surface density) thanks to the Cy3-labelling of glycoconjugates,
- Biological lectin/oligosaccharide recognition can be performed in solution before hybridising the whole complex onto the DNA chip.

Furthermore, carbohydrate moieties are introduced into the glycomimetics in a very flexible and rapid manner thanks to powerful techniques such as automated oligonucleotide synthesis, amidative oxidation and microwave assisted 1,3-dipolar cycloaddition (Bouillon et al., 2006; Chevolot et al., 2007).

Here, more work on the DDI of glycomimetics is reported. First, we compared the influence of direct covalent immobilisation of glycomimetics onto the solid surface with DDI, with respect to the interaction with RCA 120. Three glycomimetics exhibiting the same tri-galactosyl structure were synthesised (Figs. 1 and 2). Two of them have a 5′-end amine function with either a short (deoxythymidine) **1** or a long (oligonucleotide) **2** spacer linked to the glycomimetic moiety so they can be further immobilised by reaction with ester activated modified glass slides. The third glycomimetic **3** was synthesised as described in (Chevolot et al., 2007) and was immobilised by DDI. After the interaction of RCA 120 with the immobilised glycomimetics, the fluorescence intensity signal and lower detection limit were compared. Next, the efficiency of their immobilisation on the surface was studied according

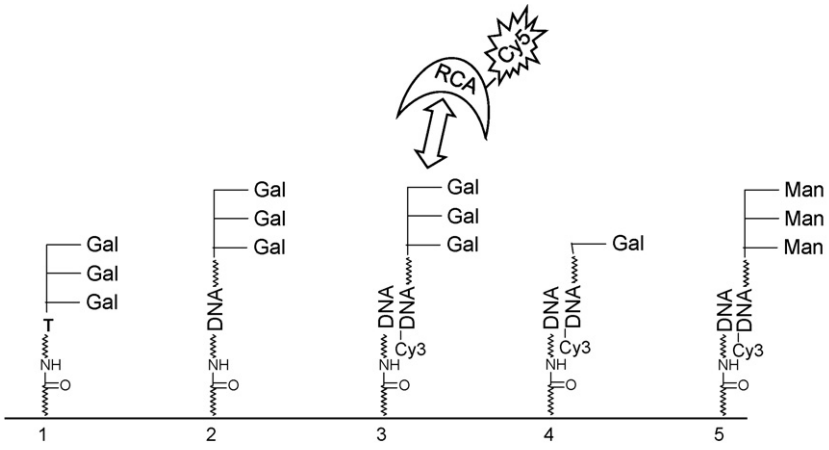

Fig. 2. Schematics of the five immobilised structures. Molecules **1** and **2** were directly immobilised on activated ester modified glass slides, while glycomimetics **3–5** were immobilised by DDI. Cy3 allows for the quality control of the DDI immobilised glycomimetics. The interaction with the Cy5 labelled lectin Ricin Agglutinin Communis was probed by fluorescence scanning. Glycomimetic **5** is a negative control with regard to RCA 120 (galactose specific lectin). The DNA sequence of molecules **2**, **3**, **4** and **5** is the same.