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New Structure–function Relationships of Carbohydrates

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Abstract

The potential of glycoarrays for the investigation of carbohydrate interactions has not been fully exploited to date. In addition to the saccharide specificity of lectins, carbohydrate recognition and carbohydrate binding most likely also comprises aspects of pattern formation, density regulation, as well as the mode of sugar presentation on a surface. When glycoarrays – which allow for systematic alteration of such parameters – become available, new structure–function relationships are likely to be discovered in the carbohydrate regime. In this account some of our work on fabrication of special glycoarrays is summarised including the 'dual click approach' to glyco-SAMs, and fabrication of photosensitive glycoarrays which allow 'switching' of carbohydrate orientation between two distinct states.

Introduction

Organismic life has been partitioned into life of cells and cell–cell interactions, and hence, cell biology has emerged as an own scientific discipline during the late 20th century. However, till this date, it has been greatly overlooked, that the glycosylated cell surface ('glycocalyx') is a key feature of all cells, and of eukaryotic cells in particular. In addition, it has been frequently underestimated, how important the carbohydrates as glycocalyx main components are for cell biology and as regulators of cell–cell interactions. Likewise, the meaning of molecular diversity of the carbohydrates remains insufficiently understood in comparison to the two other major biopolymer classes, the nucleic acids and the proteins.

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The growing understanding of DNA and protein functions has led to meaningful research fields, namely the genomics and the proteomics. These are given priority in biochemistry, whereas the 'glycomics' [1] rather receive second-rate treatment. A possible explanation for this situation lies in the overwhelming complexity of carbohydrate chemistry as well as of carbohydrate biochemistry, which is indeed difficult to manage.

Thus, a cell's glycocalyx appears like a molecular puzzle to researchers, which needs to be unravelled to understand the meaning of carbohydrates for life. Apparently, the lectins, a huge class of carbohydrate-recognizing proteins, contribute critically to glycobiology through the formation of specific carbohydrate–protein interactions. Consequently, a great deal of attention has been paid to experimental formats that allow systematic as well as versatile testing of carbohydrate–protein interactions. Glycoarrays have emerged as number one tool in this regard [2 – 5]. Glycoarrays are artificial glycosylated surfaces in which more or less complex oligosaccharides are immobilised on a suitable material of different dimension. Experiments with glycoarrays typically involve fabrication of glycoarrays on the one hand and their interrogation with lectins on the other. Thereby, much valuable information about the specificity of lectins has been generated and related to the structures of complex carbohydrates [6,7].

Pride and Prejudice of Glycoarrays

While the approach of probing sophisticated glycoarrays with proteins has clearly increased our understanding of carbohydrate–lectin interactions, a number of questions in addition to the aspects of sugar specificity of recognition, remain open as highlighted in Figure 1. Two fundamental problems of glycoarray fabrication are illustrated, namely (i) the regulation of density of carbohydrates on a surface, and (ii) the challenges connected to fabrication of mixed (thus more divers) glycoarrays, with both topics related to the possibility of patterning or raft formation, respectively. The requirement of uniform distribution of different carbohydrates on a surface to form a regularly mixed glycoarray is difficult to fulfil as well as the success of any such attempt is hard to analyse. Segregation effects occurring on a surface regardless of whether one or more types of carbohydrates were immobilised, normally remain undetected and consequently, even misinterpretation of testing results might happen.

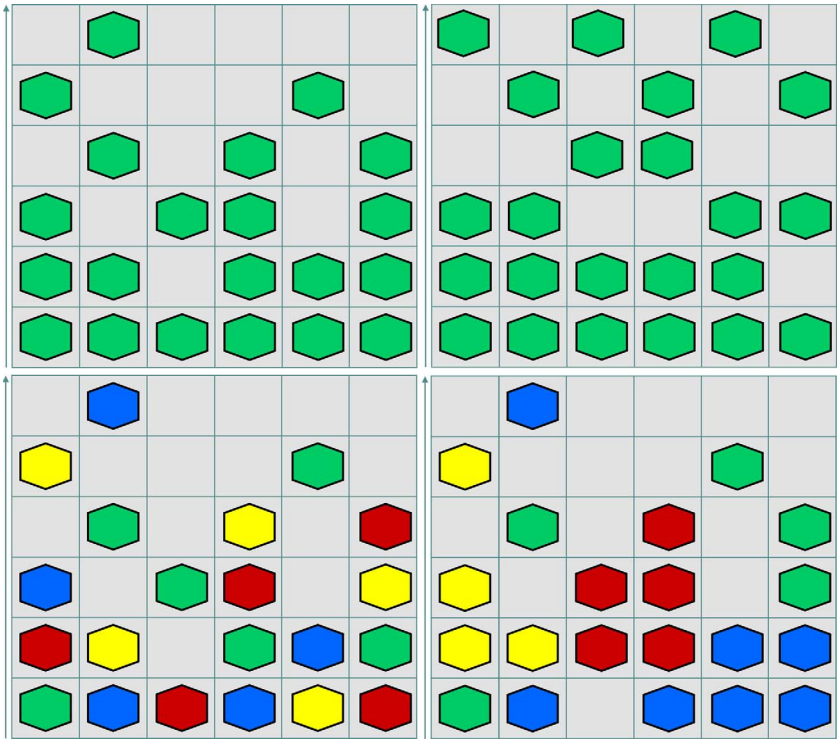

Figure 1. Density of carbohydrates on a surface (top) and regular mixing of different sugars in glycoarray formation (bottom) are difficult to control as well as to analyse. Segregation effects (on the right) might occur in all cases regardless of whether a pure or a mixed glycoarray is prepared. Hexagons represent carbohydrates (not further specified). Arrows on the left next to the depicted surface squares indicate increasing dilution of sugar solutions that are applied for glycoarray fabrication.

A lot of our own research has been dedicated to the investigation of bacterial adhesion, in particular adhesion of uropathogenic *Escherichia coli*, in short UPEC [8 – 11]. This work bears the potential for advancement of our understanding of the mechanisms of cell adhesion and, secondly, the possibility of progress towards an 'anti-adhesion therapy' against microbial infection. Adhesion of bacteria cells is mediated, inter alia, by extracellular adhesive organelles, called fimbriae that carry lectin domains to attach the bacterial cell to the glycosylated cell surface of a target cell through multiple carbohydrate-(bacterial) lectin interactions. For UPEC infections, the so-called type 1 fimbriae are of particular relevance, mediating α-D-mannoside-specific adhesion [12]. Consequently, type 1 fimbriae-mediated bacterial adhesion can be inhibited by α-D-mannosides or suitable antagonists. Based on the structure of the type 1 fimbrial lectin, the protein FimH, a large number of carbohydrate inhibitors of mannose-specific bacterial adhesion has been invented and investigated in

different assays [13, 14]. Importantly, in addition to using inhibitors of bacterial adhesion in solution, we have started testing various designer surfaces, in other words glycoarrays, to test bacterial adhesion.

Our bacterial adhesion experiments often involve the fabrication of glycoarrays using a microtiter plate format. For this, differently concentrated carbohydrate solutions are employed for different rows of wells. In this procedure, it is assumed that the carbohydrates density of surface coverage can be systematically varied across the microplate wells (Figure 1). Typically, this procedure of concentration variation reveals an optimal carbohydrate concentration for a particular cell adhesion experiment, resulting in a maximally adhesive surface, while further dilution of the carbohydrate solutions that are employed for glycoarray fabrication leads to less dense glycoarrays and consequently less adhesion of (bacterial) cells.

Density Control of Glycoarrays

Recently, we have obtained indications, that the clustering of carbohydrates, such as α-D-mannosyl moieties, in *one* molecule resulting in so-called cluster glycosides might be used as means to control the carbohydrate density of glycoarrays [15]. In this experiment a series of simple (monovalent) mannosides (Figure 2, **1**) and their di- and trivalent glycocluster analogues (**2** and **3**) were employed.

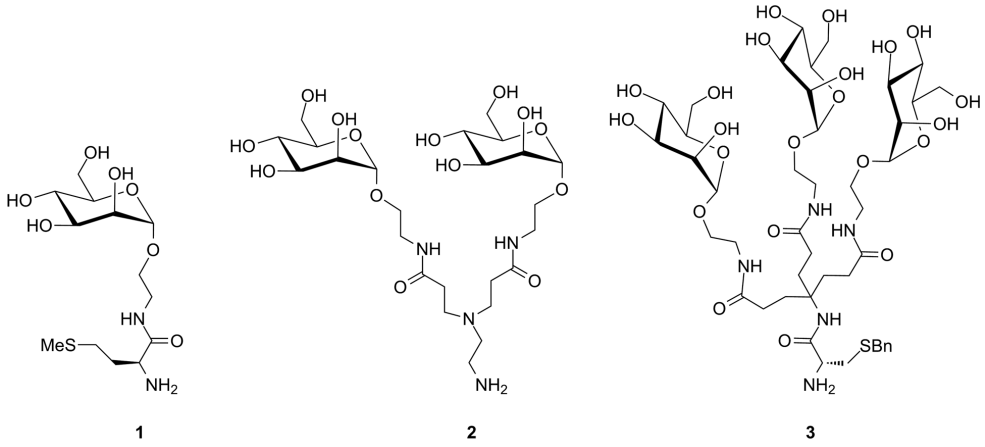

Figure 2. Mannoside **1**, and its di- and trivalent analogues **2** and **3** were prepared to test density carbohydrate control of glycoarrays [15].

Testing of bacterial adhesion using pre-functionalised microtiter plates and solutions of **1**, **2**, and **3**, respectively, to fabricate the respective glycoarrays led to a picture as reflected in Figure 3. It was shown that at higher concentrations (~15 mmol), all three types of glycoarrays are approximately equally adhesive within the experimental error. On the contrary, at

lower concentrations (~2 mmol), the glycoarray prepared from a solution of the trivalent cluster mannoside **3** became more adhesive in comparison to the respective glycoarrays made from **1** or **2** in the same concentration range.

The interpretation of the results was that each of the three types of employed glycoarrays, prepared from **1**, **2**, or **3**, respectively, shows different concentration dependencies in bacterial adhesion assays. When higher sample concentrations were used for the immobilisation, density of mannose coverage should be comparable in all three cases. On the other hand, further dilution of the carbohydrate solutions employed for surface functionalisation would affect carbohydrate density more critically in case of the glycoarrays resulting from **1**, whereas glycoarrays prepared from the trivalent cluster glycoside **3** can still provide relatively high local α-mannoside density (Figure 3, right).

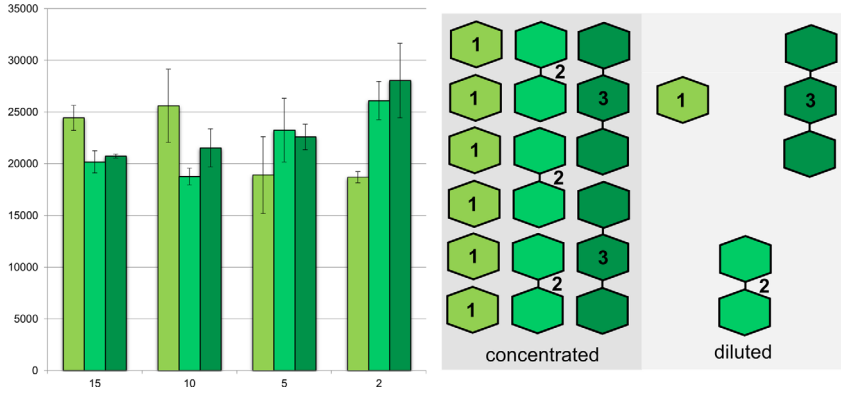

Figure 3. Three types of glycoarrays were prepared using pre-functionalised microtiter wells and solutions of 1 (Figure 2, left bars), 2 (middle bars), or 3 (right bars), respectively, at concentrations of 15, 10, 5, and 2 mmol (x axis). Concentrations are valency-corrected (that means that the specified concentrations refer to α-D-mannosyl moieties rather than to the molecular concentration of the solution that was used for glycoarray fabrication). Adhesion of fluorescent *E. coli* cells was determined in each case by fluorescence read-out (standard deviations are indicated for each bar) (y axis). For interpretation of these findings see the cartoon on the right: When higher sample concentrations were used for the immobilisation, density of mannose coverage should be comparable in all three cases. On the other hand, dilution of the carbohydrate solutions employed for surface functionalisation should be more critical regarding the resulting carbohydrate density in case of the glycoarrays resulting from 1 than those fabricated from 3.

In the near future it has to be investigated if this or other alternative concepts [16 – 17] for the control of carbohydrate density on surfaces can be advanced into a reliable methodology. In parallel, we have worked on methods for the consecutive build-up of glycoarrays to increase our options in making and manipulating glycosylated surfaces. Thus, we have recently expanded the repertoire of glycoarray fabrication by a 'dual click approach' in which glycoarrays were constructed as 'glyco-SAMs' using three simple consecutive steps.

Dual Click Approach to Glyco-SAMs

The term SAM stands for self-assembled monolayer, founded by Whitesides and co-workers in the 1980s [18]. Typical SAMs are fabricated by the reaction of an alkanethiol on a gold surface through formation of Au–S bonds, leading to rather well-organised, regular monolayers that are amenable to a number of (bio)physical experiments comprising an impressive explanatory power [19, 20]. Moreover, glyco-SAMs have been shown to be of great value in the glycosciences including the testing of bacterial adhesion. In the dual click approach to glyco-SAM fabrication we have employed long chain mercapto-alkynes for the assembly of a principal, alkyne-terminated SAM, followed by a first 'click reaction' involving Cu(I)-catalysed coupling to α-azido-ω-amino-difunctionalised oligoethylene glycol (OEG) on SAM [21]. This step provides 'biorepulsive' properties of the SAM, this is, non-specific adhesion of proteins to this surface is prohibited through the OEG unit. Next, in the second 'click reaction' the terminal amino group can be further refined on SAM by employing NCS-functionalised bio-molecules such as glycosyl isothiocyanates, or other isothiocyanato-modified glycosides or glycoclusters, respectively (Figure 4). We could show that the prepared glyco-SAMs provide suitable platforms to test bacterial adhesion.

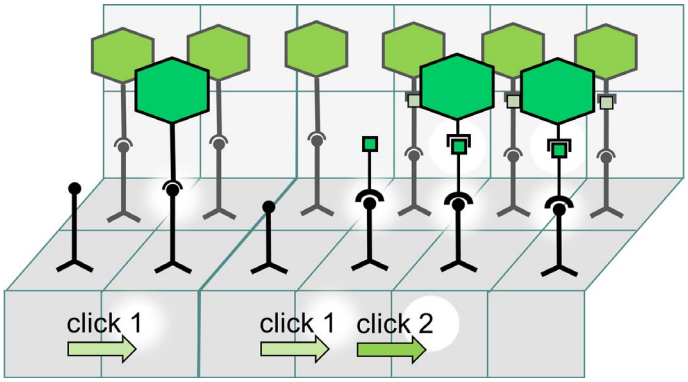

Figure 4. A 'dual click' approach allows consecutive assembly of glyco-SAMs 'on SAM' [21].

Photosensitive Glycoarrays

An addition to aspects of glycoarray fabrication with regard to the problems of density control and consecutive build-up, we have become interested in glycosylated surfaces, which allow for controlled manipulation of carbohydrate exposition; ideally with spatiotemporal resolution. This is of importance in the context of conformational control as a regulatory mechanism in glycobiology. Conformational control of biological function is well-known and highly appreciated in other areas of biochemistry, for example in structural biology. In glycobiology it has remained a neglected area to date.

Searching for glycoarrays that can be switched between to different steric states without changing other parameters of the glycosylated surface (except carbohydrate orientation), we became interested in azobenzene glycosides [22]. The photochemical *E*/*Z* isomerisation of the azobenzene N = N double bond can be easily achieved by appropriate irradiation and has been established as a biocompatible method for photocontrol in biology [23, 24]. Thus, the azobenzene N = N double bond might be utilised as a hinge region in an azobenzene glycoconjugate, permitting controlled steric manipulation of a glycoarray as outlined in Figure 5.

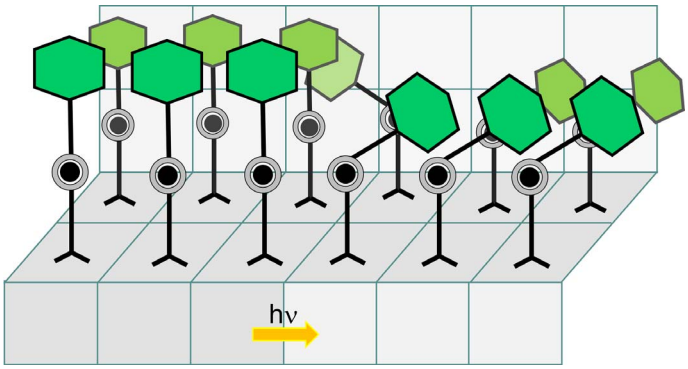

Figure 5. Azobenzene glycoarrays should in principle be isomerisable using light of appropriate wavelength. The azobenzene N = N double bond serves as a hinge region. The effected steric changes are reversible as E→Z isomerisation requires a different wavelength than Z→E back isomerisation. Such 'photo switching' on surfaces can be applied to probe conformational control of carbohydrate recognition.

We have shown that azobenzene glycosides as those depicted in Figure 6 can be readily synthesised [25 – 27], possess favourable photochromic properties and are biocompatible and non-toxic [28]. Moreover, azobenzene derivatives including azobenzene glycosides can be elaborated into molecular tools for bioorthogonal ligation chemistry [29]. Thus, azobenzene glycosides are emerging into a class of photosensitive glycoconjugates that are suited to test conformational control of carbohydrate recognition. However, the question remains if photocontrol is effective on glycosylated surfaces. Thus, we have employed IRRAS (infrared reflection absorption spectroscopy) for the characterisation of glyco-SAMs. IRRAS allows to measure vibrational changes of films or molecular monolayers on surfaces and is therefore suited to determine if photoisomerisation of chemisorbed azobenzene glycosides (Figure 7) [30].

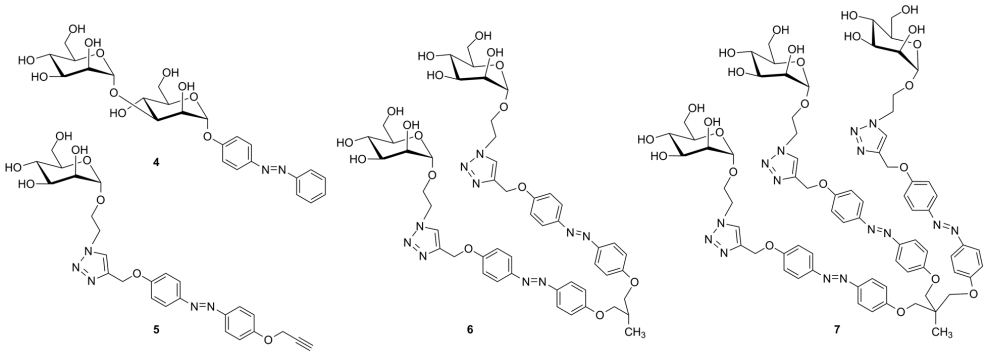

Figure 6. Examples of synthetic mono-, di- and trivalent azobenzene glycoconjugates.

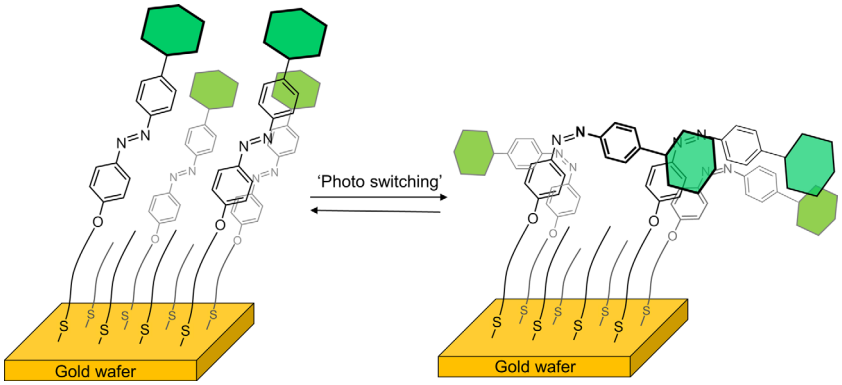

Figure 7. Glyco-SAMs were fabricated using mercapto-functionalised azobenzene glycoside derivatives. Photoswitching was proven by IRRAS [30].

To this end, it was shown that photosensitive glycoarrays can be prepared and their carbohydrate orientation can indeed be manipulated using light of appropriate wavelength (~365 nm). The next step in this project will be testing of cellular adhesion in relation to carbohydrate orientation on a surface.

Conclusion

The glycosylated surface of cells is probably one of the most exciting surfaces known. Compared to the surface of our planet it is small, but its complexity and its potential for molecular diversity might be comparable to mother earth. Glycoarrays are useful tools to investigate features and functions of glycosylated surfaces but many adventures lie ahead of researchers who try to expand the potential of glycoarrays. So far it remains difficult to control patterning of glycoarrays and density of carbohydrate coverage as well as to enable controlled manipulation of glycoarray surfaces. This account is meant to inspire further research and discoveries in this area.

Acknowledgements

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