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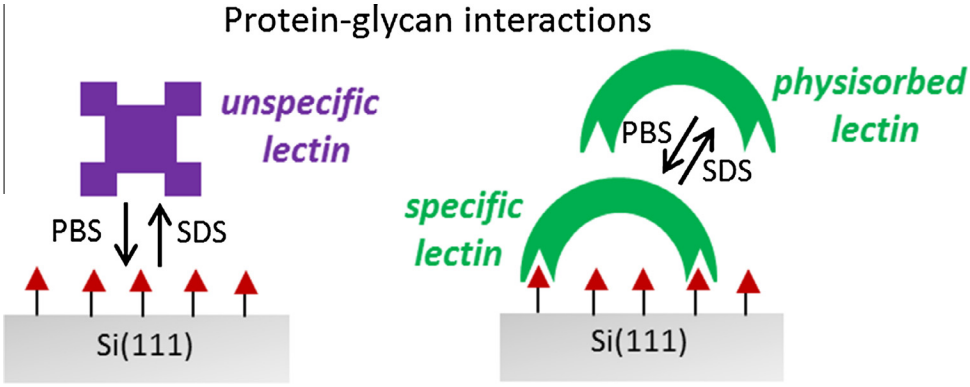
A quantitative method to discriminate between non-specific and specific lectin–glycan interactions on silicon-modified surfaces

Jie Yang [a,*], Aloysius Siriwardena [b], Rabah Boukherroub [c], François Ozanam [a], Sabine Szunerits [c], Anne Chantal Gouget-Laemmel [a,*]

[a] Physique de la Matière Condensée, Ecole Polytechnique-CNRS, France
[b] Laboratoire de Glycochimie des Antimicrobiens et des Agroressources (LG2A), (FRE 3517-CNRS), Université de Picardie Jules Verne, 33 Rue St Leu, 80039 Amiens, France
[c] Institut d'Electronique, de Microélectronique et de Nanotechnologie (IEMN, UMR CNRS 8520), Université Lille1, Avenue Poincaré-B.P. 60069, 59652 Villeneuve d'Ascq, France

GRAPHICAL ABSTRACT



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ABSTRACT

Essential to the success of any surface-based carbohydrate biochip technology is that interactions of the particular interface with the target protein be reliable and reproducible and not susceptible to unwanted nonspecific adsorption events. This condition is particularly important when the technology is intended for the evaluation of low-affinity interactions such as those typically encountered between lectins and their monomeric glycan ligands. In this paper, we describe the fabrication of glycan (mannoside and lactoside) monolayers immobilized on hydrogenated crystalline silicon (111) surfaces. An efficient conjugation protocol featuring a key "click"-based coupling step has been developed which ensures the obtention of interfaces with controlled glycan density. The adsorption behavior of these newly developed interfaces with the lectins, *Lens culinaris* and *Peanut agglutinin*, has been probed using quantitative IR-ATR and the data interpreted using various isothermal models. The analysis reveals that protein physisorption to the interface is more prevalent than specific chemisorption for the majority of washing protocols investigated. Physisorption can be greatly suppressed through application of a strong surfactinated rinse. The coexistence of chemisorption and physisorption processes is further demonstrated by quantification of the amounts of adsorbed proteins distributed on the surface, in correlation with the results obtained by atomic force microscopy (AFM). Taken together, the data demonstrates that the nonspecific adsorption

* Corresponding authors.
E-mail addresses: jie.yang@polytechnique.edu (J. Yang), anne-chantal.gouget@polytechnique.fr (A.C. Gouget-Laemmel).

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of proteins to these glycan-terminated surfaces can be effectively eliminated through the proper control of the chemical structure of the surface monolayer combined with the implementation of an appropriate surface-rinse protocol.

1. Introduction

Key players in the development and maintenance of living systems are cell-surface glycans (encompassing glycoproteins, glycolipids, proteoglycans, etc.). Specific interactions between cell-surface glycans and their protein receptors (for example, lectins) have also been implicated in numerous disease processes including bacterial and viral infections [1–3]. However, the wide-spread impact of lectin–glycan recognition events in nature is enigmatic in that they are often characterized by intrinsically weak monovalent affinity constants ($K_a \approx 10^2$–10^3 M^{-1}) [4,5]. In biological settings this low binding has been shown to be augmented by exploiting multivalency. This latter phenomenon usually kicks in when multiple copies of a glycan are presented on a cell surface and interact simultaneously with a target protein partner featuring multiple glycan recognition sites. The operation of multivalent effects has been observed to lead to affinities in the $K_a \approx 10^5$–10^8 M^{-1} range [6].

It is no surprise, given their importance, that the development of appropriate tools for studying glycan–lectin interactions such as microarrays has been intensively researched [7–9]. This technology requires only small amounts of glycan and in addition, offers the possibility that the glycan density on the array surface be modulated so as to favor the establishment of multivalent interactions with the correct lectin partners [10–14]. Nevertheless, fabrication of glycan microarrays continues to present a challenge. The need for efficient strategies for the immobilization of glycans onto selected interface material is primordial and must necessarily allow control of glycan presentation in terms both, of their density and orientation. An absolute prerequisite is that any non-specific binding of lectins with the glycan array be minimal so as to avoid analysis artefacts and to maximize the sensitivity of the interface. Proteins tend to spontaneously adsorb to most materials having potential for array development resulting in their denaturation with a corresponding loss of their biological function and/or substrate specificity [15,16]. Physisorption remains a general concern in the study of proteins with high-affinity interactions (ie. antibody-antigen or aptamer/proteins) and even more so with low-affinity ones such as those encountered between lectins and their monomeric glycan ligands. Interfering physisorption leads to an overestimation of the "actual" protein amount bound to the surface ligand. It can therefore result in errors in the determination of binding affinity K_a of specific glycan–lectin interactions [17–20]. The incorporation of hydrophilic poly(ethylene glycol) (PEG) molecules on interfaces is one of the most widely accepted strategies to minimize non-specific binding of proteins and its effectiveness is found to depend on both the length and density of the PEG units employed [21–24]. An alternative strategy relies on the implementation of appropriate buffer recipes or surface cleaning protocols that efficiently disrupt weakly physisorbed proteins and facilitate their selective removal [25–27]. The surfactant sodium dodecyl sulfate (SDS) has been successfully employed to remove proteins such as Bovin Serum Albumin (BSA) physisorbed on oligo(ethylene glycol) alkyl chains grafted to silicon surfaces [28]. In present bioassays, the actual success of limiting the non-specific binding is usually difficult to evaluate so that a set of surface structures or rinse protocols has to be tested in order to obtain an optimal assay condition. Independent of the strategy employed for minimizing non-specific binding, quantitative information on the amount of surface-adsorbed proteins and their distributions would undoubtely help in optimizing glycan arrays.

This work sets out to interrogate the adsorption behavior of lectins on glycan-modified crystalline (1 1 1) silicon interfaces. The interest in using hydrogenated crystalline (1 1 1) Si is two-fold: angstrom-level flatness can easily be obtained and chemical modification of the surface in a controllable way *via* Si—C formation is possible [29–31]. In the present study quantitative IR spectroscopy in ATR geometry (IR-ATR) is exploited to assess the characteristics of the surface at a molecular level and contact-mode atomic force spectroscopy (CM-AFM) allowing detailed information to be obtained about the morphological changes that occur after a specific lectin–glycan interaction has been established. We have recently developed an efficient surface-functionalization strategy for the covalent attachment of simple glycans to crystalline silicon and shown that the glycan-terminated surface is effective in the detection of selective lectin interactions [32]. As an extension of this work, we interrogate here the performance of mannoside- and lactoside-modified Si(1 1 1) surfaces in evaluation of the interaction of the lectins *Lens culinaris* (LENS), specific for the mannosyl moieties, and *Peanut agglutinin* (PNA), specific for the lactosyl moieties. The effects of the oligo(ethylene glycol) OEG chain length on the effectiveness of the surface and the post-treatment with SDS rinses after interaction will be discussed on the basis of IR-ATR and CM-AFM data.

2. Experimental section

2.1. Materials

All chemicals were of reagent grade or higher and were used as received without further purification. All cleaning reagents (H_2O_2, 30%; H_2SO_4, 96%), and etching (HF, 50%; NH_4F, 40%) reagents were supplied from Carlo Erba. Undecylenic acid (99%) was purchased from Acros Organics. All other chemicals and lectins (*Lens culinaris*, *Peanut agglutinin,* lyophilized powders free of salt highly purified by affinity chromatography) were purchased from Sigma–Aldrich and were used as received. Ultrapure water (Milli-Q, 18 MΩ cm) was used for the preparation of the solutions and for all rinses. The synthesis of α-propargyl mannoside was described in Ref. [32]. The synthesis of β-propargyl lactoside was described in Ref. [10].

2.2. Preparation of glycan-derivatized surfaces

2.2.1. Safety considerations

The H_2SO_4/H_2O_2 (piranha) solution is a strong oxidant which reacts violently with organic materials. Hydrogen fluoride (HF) is a hazardous acid, which can result in serious tissue damage if burns are not appropriately treated. They must be handled with extreme care in a well-ventilated fume hood, while wearing appropriate chemical safety protection.

2.2.2. Carboxydecyl-terminated surface

The silicon platelet was cleaned in a 1/3 H_2O_2/H_2SO_4 piranha solution at 100 °C and rinsed with Milli-Q water. It was either etched in a 50% HF solution for 5 s or in an oxygen-free 40%