Analytica Chimica Acta 873 (2015) 71–79

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Plasmon waveguide resonance for sensing glycan–lectin interactions

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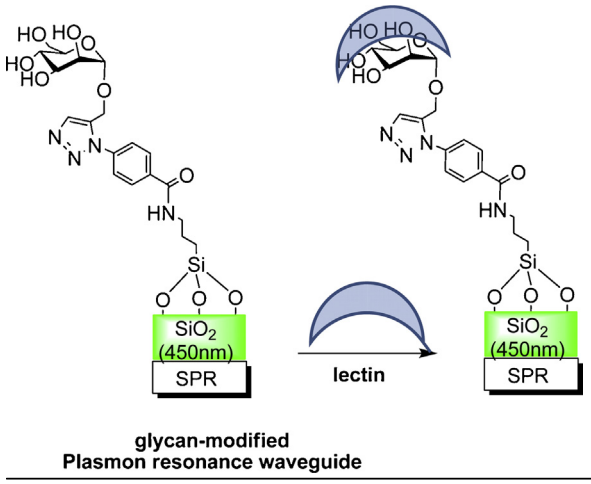
HIGHLIGHTS

- Determination of glycans/lectin interactions on planar optical waveguides.
- Development of an appropriate surface chemistry strategy.
- Subnanomolar detection range is observed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 18 February 2015
Accepted 22 February 2015
Available online 24 February 2015

Keywords:
Planar optical waveguide
"Click" chemistry
Glycan chip
Plasmon waveguide resonance

ABSTRACT

Carbohydrate-modified interfaces have been shown to be valuable tools for the study of protein–glycan recognition events. Label-free approache such as plasmonic based techniques are particularly attractive. This paper describes a new analytical platform for the sensitive and selective screening of carbohydrate–lectin interactions using plasmon waveguide resonance. Planar optical waveguides (POW), consisting of glass prisms coated with silver (50 nm) and silica (460 nm) layers were derivatized with mannose or lactose moieties. The specific association of the resulting interface with selected lectins was assessed by following the changes in its plasmonic response. The immobilization strategy investigated in this work is based on the formation of a covalent bond between propargyl-functionalized glycans and surface-linked azide groups *via* a Cu(I) "click" chemistry. Optimization of the surface architecture through the introduction of an oligo(ethylene glycol) spacer between the plasmonic surface and the glycan ligands provided an interface which allowed screening of glycan–lectin interactions in a highly selective manner.

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http://dx.doi.org/10.1016/j.aca.2015.02.060
0003-2670/

The limit of detection (LOD) of this method for this particular application was found to be in the subnanomolar range (0.5 nM), showing it to constitute a promising analytical platform for future development and use in a pharmaceutical or biomedical setting.

1. Introduction

Advances in biology continue to reveal that carbohydrate–protein interactions play an essential role in the development and maintenance of living systems as they are fundamental to many cellular processes such as cell adhesion, viral/bacterial infection, the working of the immune system and in tissue growth and engineering [1–3]. Progress in the synthesis of complex glycans [4,5] accompanied by the development of methods for engineering glycan-modified surfaces [6–14] has allowed the generation of carbohydrate-linked substrates suitable for interrogating the interactions of glycans with biomolecules. A key challenge in the fabrication of glycan interfaces is the development of reliable and reproducible chemistries for the immobilization of chemically and structurally diverse glycans onto an appropriate interface. To achieve high-affinity binding and selective recognition surface, chemical approaches and detection methods that enable multivalent interactions between the binding partners to occur are aimed at [15,16].

One of the label-free methods which allows carbohydrate–protein interactions to be effectively probed is surface plasmon resonance (SPR) [10,14,16–23]. The principal motivation behind the rapid development of SPR for the screening of glycan–ligand interactions is that it provides real-time, label-free and rapid analysis of molecular interactions occurring at the glycan-modified interface. The presentation of selected sugar moieties on an SPR interface has been achieved in the past through two main strategies: self-assembly of thiol-functionalized carbohydrates on surfaces as monolayers [16,18,20], or self-assembly of pre-functionalized thiol linkers carrying a selected terminal functional group on interface to which sugar analogs armed with complementary reactive groups have been covalently attached [12,20,24]. We have recently taken advantage of the second approach using the Cu(I) catalyzed 1,3-dipolar Huisgen cylcoaddition (CuAAC) reaction of surface-linked azide functions and alkyne-functionalized glycans for the generation of carbohydrate functionalized silica-coated SPR interfaces [10]. Indeed, while the surface chemistry developed for the generation of various gold interfaces has proven of great value [25,26], their limitations are amplified with the requirements for fabrication of increasingly complex biomimetic systems and arrays. Alternative silicon dioxide-based interfaces have also attracted interest for biosensing and benefit from a rich variety of well-developed functionalization strategies which take advantage of silane-coupling chemistry.

Surface modification strategies based on silane-coupling chemistry are also well-adapted for the modification of plasmon waveguides, which are constituted by the deposition of a dielectric layer (*e.g.*, silicon or titanium dioxide) of several hundreds of nanometers depth over a thin gold or silver film [27–29]. Plasmon waveguides allow the intrinsic limitations of traditional SPR substrates to be overcome. Particularly the generation of an optimal penetration depth (usually lower than 300 nm), while keeping the fabrication process simple [30]. In contrast to silica-based SPR interfaces [10,31–35], in plasmon waveguide resonance (PWR) sensors, the film thickness of the silica dielectric ($n = 1.48$) needs to be carefully considered. The waveguide thickness has to be greater than that of the cut-off thickness, which is around $\lambda/2$ with λ being the excitation wavelength (632.8 nm in our case). To support a propagating mode, the silica film has thus to be at least ≈320 nm. In addition, the angular position of the resonances depends on the thickness of the silica waveguide and must be above the total internal reflection values in order to be observable. Taking into account both technical parameters and the refractive index of silica, thicknesses between 430 and 610 nm are appropriate. Indeed the thickness chosen by Salamon et al. for their PWR studies is in line with the range used here [27]. In addition, adjusting the thickness allows to marginally tune the ratio of *p*- and *s*-sensitivities. For *s*-polarization, the higher the thickness, the lower the sensitivity; on the other hand, for *p*-polarization, the sensitivity reaches a maximum for a thickness of about 550 nm. As a rule of thumb, a silica thickness of 460 nm is a good compromise that slightly favors the *s*-pol sensitivity.

The role of the silica layer is linked to the generation of waveguide modes by *p*- or *s*-polarized light excitation. The choice of the materials depends on the wavelength used as well as on the sensing applications. ITO, which behaves as a dielectric material in the optical frequency, can be used as a waveguide material offering the ability to combine optical analyses with electrochemical studies [36]. Hydrogel based waveguides were proposed by Zhang et al. for the detection of 17β-estradiol [37]. A monolayer of epithelial cells was employed by Yashunsky et al. to perform infrared SPR [38]. Independent on the waveguide material used, the modes are highly sensitive to changes in the refractive index under both polarizations and can give access to mass density measurements. In addition, in contrast to SPR, PWR provides information about molecular order and conformation of oriented anisotropic materials (not exploited here as the molecules investigated are not oriented). The favorable hydrophilicity of these silica-based waveguides makes them suitable platforms for formation of planar lipid membranes and thereby facilitates investigation of membrane protein activity as well as peptide/lipid interactions [39–43]. Surprisingly, no attempt has until now been reported to chemically modify waveguides with glycans and to use the resulting interfaces for the investigation of carbohydrate–lectin interactions.

We show here that glycans can be covalently linked to silica-based waveguides in the same manner as shown for silica-coated SPR interfaces through a combination of surface silanization chemistry coupled with “click” chemistry of alkynyl-derivatized glycans and azide-functionalized waveguide surfaces (Fig. 1). The sensitivity and selectivity of the glycan-functionalized waveguide interface toward various lectins have been assessed. This study thus represents the first example where PWR is harnessed for the study of carbohydrate–protein interactions.

2. Experimental

2.1. Materials

All chemicals were of reagent grade or higher and were used as received unless otherwise specified. (3-aminopropyl) trimethoxysilane (APTMS), *N*,*N*-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)-pyridine (DMAP), copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), ascorbic acid, D(+)-mannose, O-(2-azidoethyl)-O-[2-(diglycolyl-amino)ethyl]heptaethylene glycol, sulfuric acid (H_2SO_4), phosphoric acid, phenol, dimethylformamide (DMF), dichloromethane (CH_2Cl_2), methanol (MeOH) and ethanol (EtOH) were purchased from Sigma–Aldrich and were used without further purification. 4-Azido-benzoic acid (ABA) was purchased