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Polypyrrole Oligosaccharide Array and Surface Plasmon Resonance Imaging for the Measurement of Glycosaminoglycan Binding Interactions

Emilie Mercey,[†] Rabia Sadir,[‡] Emmanuel Maillart,[§] André Roget,[†] Françoise Baleux,[||] Hugues Lortat-Jacob,[‡] and Thierry Livache*[,†]

CREAB, UMR 5819 (CEA, CNRS, UJF), INAC CEA Grenoble; 17, Rue des Martyrs 38054 Grenoble Cedex 9, France, Institut de Biologie Structurale UMR 5075 (CEA, CNRS, UJF), 41 Rue Jules Horowitz, 38027 Grenoble Cedex 1, France, Genoptics SA, Orsay, France, and Institut Pasteur, 28 Rue du Dr. Roux, 75015 Paris, France

In order to construct tools able to screen oligosaccharide−protein interactions, we have developed a polypyrrole-based oligosaccharide chip constructed via a copolymerization process of pyrrole and pyrrole-modified oligosaccharide. For our study, GAG (glycosaminoglycans) or GAG fragments, which are involved in many fundamental biological processes, were modified by the pyrrole moiety on their reducing end and then immobilized on the chip. The parallel binding events on the upperside of the surface can be simultaneously monitored and quantified in real time and without labeling by surface plasmon resonance imaging (SPRi). We show that electrocopolymerization of the oligosaccharide−pyrrole above a gold surface enables the covalent immobilization of multiple probes and the subsequent monitoring of their binding capacities using surface plasmon resonance imaging. Moreover, a biological application was made involving different GAG fragments and different proteins, including stromal cell-derived factor-1α (SDF-1α), interferon-γ (IFN-γ), and monoclonal antibody showing different affinity pattern.

In the wake of "genomics" and "proteomics", "glycomics" is now an emerging field that allows one to study oligosaccharides structures and protein−oligosaccharide interactions. Nevertheless, the structure complexity and diversity of carbohydrates make it difficult to dissect the individual roles of each oligosaccharide−protein interaction. Thus tools for studying protein−carbohydrate interactions are necessary to gain an understanding of biological functions and the roles that these interactions play in disease. The presentation of carbohydrates in the format of an array provides a means to simultaneously monitor multiple binding events between immobilized oligosaccharides and proteins in solution. Several such studies have appeared; for example, the work by Park and Shin[1] deals with the preparation of microarrays by immobilizing maleimide-linked carbohydrates on thiol-derivatized glass slides. Wang et al.[2] developed a carbohydrate-based microarray, where the polysaccharides can be immobilized on a nitrocellulose-coated glass slide without chemical conjugation. Fukui and co workers described microarrays of oligosaccharides as neoglycolipids and their display on nitrocellulose.[3] Houseman and Mrksich[4] prepared carbohydrate chips by the Diels−Alder-mediated immobilization of carbohydrate−cyclopentadiene conjugates to self-assemble monolayers that present benzoquinone and penta(ethylene glycol) groups. Smith et al.[5] fabricated microarrays on gold films by an immobilization consisting of the formation of a surface disulfide bond used to attach thiol-modified carbohydrates. More recently, Chevolot et al.[6] have used a DNA chip to array oligonucleotide−carbohydrate conjugates and de Paz et al.[7] have developed a chip-bearing heparin derivative on which protein interactions are detected by a scaffold of different antibodies. All these processes involve an end point and indirect fluorescence detection. In order to avoid the use of labeled proteins, Smith et al.[5] uses a SPRimaging approach associated with a fluidics network, which allowed the construction of a disaccharide chip. In this case, the chemical grafting steps and the reading of the chip needed to be carried out in the presence of a fluidic network confining the reactions. A classical DNA spotter cannot be used, thus limiting the potentiality of this interesting approach. Moreover, no kinetic data related to monosaccharides or disaccharides interaction with proteins were given.

Parallel with such work, we have developed a process to immobilize DNA sequences covalently on a gold surface, via an electrocopolymerization of DNA−pyrrole and pyrrole monomers.[8] This process allows the grafting of a biomolecule on the gold surface at a localized position via a polypyrrole film. Initially developed for the construction of DNA chips, the polypyrrole

* Corresponding author. E-mail: thierry.livache@cea.fr.
[†] CREAB, UMR 5819 (CEA, CNRS, UJF), INAC CEA.
[‡] Institut de Biologie Structurale UMR 5075 (CEA, CNRS, UJF).
[§] Genoptics SA.
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approach is extended in this work to oligosaccharides. This method allows for the copolymerization of pyrrole molecules with a biomolecule bearing a pyrrole group. This reaction is based on an electrochemical process allowing a very fast coupling of the biomolecule (probe) to a gold layer used as a working electrode.

In order to measure the protein−oligosaccharide interaction in real time without labeling, we used a SPR imaging process previously developed to read DNA chips[9] or protein chips.[10] Surface plasmon resonance (SPR) is a label-free, surface-sensitive spectroscopic technique that can be used to detect the binding of biomolecules onto arrays of probe biomolecules covalently attached to chemically modified gold surfaces.[11] This technique allows us to measure kinetics data in real time, showing complex association or dissociation between oligosaccharide and the injected protein.

In this paper, we show that we can combine a real chip array approach, in other words to construct an array with a *x*, *y*, *z* spotter, to store the chip for a long time, with a label free monitoring of the interactions occurring on all the oligosaccharide spots deposited on the surface of the chip in order to obtain kinetic data.

The biological application is carried out using glycosaminoglycans (GAG) as a model of oligosaccharides. Within the carbohydrate family, the sulfated GAGs play a major role in several biological phenomena. They are involved in cell-biological processes such as cytokine and growth factor action, cell adhesion, host−pathogens interaction, and regulation of enzymatic catalysis. These activities generally depend on interactions of polysaccharides with proteins, mediated by saccharide sequences, expressed at various levels of specificity, selectivity, and molecular organization. Heparin (HP) and Heparan sulfate (HS), which belong to the family of the GAG, are linear, complex, and highly sulfated polymers. Their high negative charge is prone to ionic interaction with a variety of proteins in which they recognize clusters of basic amino acids,[12] such as enzymes, enzyme inhibitors, extracellular-matrix proteins, various cytokines, and others.[13] Heparin (used as an anticoagulant drug) is isolated from animal tissues such as pig intestinal mucosa. Heparan sulfate, on the other hand, has ubiquitous distribution on cell surfaces and in the extracellular matrix. It is generally less sulfated than HP and has a more varied structure. Interactions between HP/HS and proteins generally depend on the presence of sulfate groups. Difficulties in evaluating the role of heparin and heparan sulfate in vivo may be partly ascribed to ignorance of the detailed structure and sequence of these polysaccharides. Some studies have been made by NMR,[14] mass spectroscopy, isothermal titration calorimetry (ITC),[15] or the classical SPR approach[16] to obtain information on interactions between HP/HS and proteins. For most heparin-binding proteins, however, where there is no such observable change following the binding event, it is very difficult to study the kinetics of the interaction. The development of the classical surface plasmon resonance (SPR)[17] such as the Biacore system allows one to study the kinetics of heparin−protein interactions, however.

In order to illustrate the feasibility of such an approach for biological applications, we studied in this work the interactions between GAGs, heparin/heparan sulfate, and chondroitin sulfate and proteins including stromal cell-derived factor-1α (SDF-1α), interferon-γ (IFN-γ), and monoclonal antibody. The chemokine SDF-1α is the natural ligand for CXC chemokine receptor 4 (CXCR4). Moreover, SDF-1α inhibits cells infection by human immunodeficiency virus (HIV) and interacts specifically with heparin or heparan sulfate.[17] It is generally supposed that attachment of the chemokine to cellular HS proteoglycans may contribute to optimize the anti-HIV activity by increasing the local concentration of the chemokine in the surrounding environment of CXCR4.[18] IFN-γ is a T cell secreted cytokine, centrally involved in many facets of the immune response, and its activity is regulated upon binding to GAGs.[19,20]

We report in this work (i) the chemical modification of natural oligosaccharides (molecular weight from 3 to 45 kDa), (ii) their grafting on the surface of the chip, and (iii) the interaction measurement in real time between the oligosaccharides deposited on the chip and different proteins of biological interest without labeling.

MATERIALS AND METHODS

Materials and Reagents. Prisms bearing a gold surface were provided by Genoptics (Orsay, France). Chemicals were obtained from the following sources: 2,5-dimethoxytetrahydrofuran, 11-aminoundecanoic acid from Acros; acetic acid, dioxan, anhydrous ethanol from SDS; *N*-hydroxysuccinimide (NHS) from Fluka; dimethylformamide (DMF) from Carlo Erba; dimethylsulfoxide (DMSO), dichloromethane from Prolabo; sodium cyanoborohydride from Merck; *N*,*N*′-dicyclohexylcarbodiimide (DCC), hydrazine, 4-(dimethylamino)benzaldehyde (DMAB), 6 kDa heparin (HP6), chondroitin sulfate (CS), dermatan sulfate (DS), anti-CS IgM from Sigma-Aldrich. Pyrrole was purchased from Tokyo Kasei, and stromal derived cell factor-1 and interferon-γ were prepared as previously described.[18,21]

Synthesis of 10-(Pyrrol-yl)-undecanoyl-hydrazide (Product 3). *10-(Pyrrole-yl)-undecanoic acid (Product **1**).* A mixture of 2,5-dimethoxytetrahydrofuran (490 mmol), 11-aminoundecanoic acid (430 mmol), acetic acid (430 mL), and dioxan (570 mL) was heated under reflux for 4 h and stirred at room temperature overnight.[22] The volatiles were removed under reduced pressure; the residue was dissolved in ethanol (2 × 100 mL) and concen-

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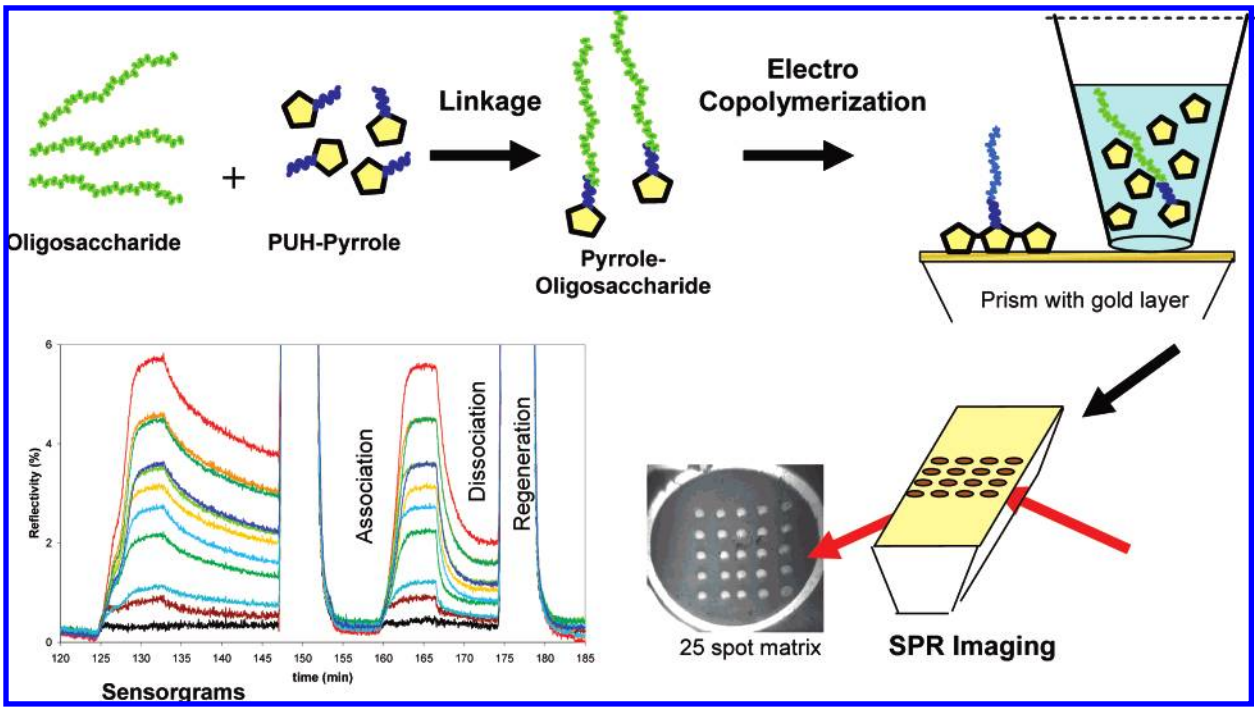

Figure 1. Principle of preparation of oligosaccharide chip. First, covalent linkage of the carbohydrate to the pyrrole group and then the oligosaccharide arrays are fabricated using an electrocopolymerization process on a prism covered with a gold layer. These arrays can be analyzed by the SPR imaging technique, allowing one to obtain sensorgrams, corresponding to the association and dissociation phases of the oligosaccharide−protein complexes.

trated. The yield of the product **1** (solid) was 65% after chromatographic purification on a silica column; CH_2Cl_2/EtOH as the eluents. MS: product **1** (m/z) = 182.1 (M^+); product **2** (m/z) = 252.1 (M^+). ^{1}H NMR product **1** (200 MHz; $CDCl_3$/TMS) δ (ppm): 1.64 (m, 16H, $-CH_2-(CH_2)_8-CH_2-$); 2.34 (t, 2H, $-CH_2-CO_2H$); 3.86 (t, 2H, $-CH_2-N$); 6.13 (dd, 2H, 3-**H** and 4-**H** pyrrole); 6.64 (dd, 2H, 2-**H** and 5-**H** pyrrole).

*N-Hydroxysuccinimidyl-10-(pyrrole-yl)-undecanoate (Product **2**)*. The product **1** (144 mmol, 26.055 g), *N*-hydroxysuccinimide (NHS) (144 mmol, 16.56 g), *N*,*N*′-dicyclohexylcarbodiimid (DCC) (159 mmol, 32.75 g), and DMF were mixed, at room temperature, overnight. Then the mixture was filtered to eliminate *N*,*N*′-dicyclohexylurea. The volatiles were removed under reduced pressure. The product **2** is used without any other purification. ^{1}H NMR product **2** (200 MHz; $CDCl_3$/TMS) δ (ppm): 1.64 (m, 16H, $-CH_2-(CH_2)_8-CH_2-$); 2.6 (t, 2H, $-CH_2-CO$); 2.89 (tt, 4H, $-CH_2-CH_2-NHS$); 3.86 (t, 2H, $-CH_2-N$); 6.13 (dd, 2H, 3-**H** and 4-**H** pyrrole); 6.65 (dd, 2H, 2-**H** and 5-**H** pyrrole).

*10-(Pyrrol-yl)-undecanoyl-hydrazide (Product **3**, PUH)*. A mixture of the product **2** (20 mmol), hydrazine 1M in THF (20 mmol), and DMF (180 mL) was stirred at room temperature overnight. Then DMF was removed under reduced pressure. The product **3**, called PUH, was obtained after chromatographic purification on a silica column; CH_2Cl_2/DMSO as the eluents. MS: T = 200 °C, solvents CH_2Cl_2/DMSO, product **3** (m/z) = 266.3 $(M + H)^+$. ^{1}H NMR product **3** (200 MHz; $CDCl_3$/TMS) δ (ppm): 1.63 (m, 16H, $-CH_2-(CH_2)_8-CH_2-$); 2.6 (t, 2H, $-CH_2-CO$); 3.86 (t, 2H, $-CH_2-N$); 6.13 (dd, 2H, 3-**H** and 4-**H** pyrrole); 6.65 (dd, 2H, 2-**H** and 5-**H** pyrrole); 8.9 (s, 1H, $-NH-NH_2$).

Preparation of Pyrrolylated Oligosaccharides. The preparation of oligosaccharide−pyrrole hydrazides was derived from the biotin-hydrazide−oligosaccharide coupling method.[23] The oligosaccharide was dissolved at 1 mM in sodium acetate buffer (20 mM, pH 4.2), and then PUH dissolved in DMSO was added (50 mM). Immediately after adding PUH, sodium cyanoborohydride (4 M in EtOH) was added at a final concentration of 150 mM. The mixture was heated at 56 °C for 2 days. The reaction products were purified by using a PD10 column (Pharmacia-Amersham). The pyrrolylated oligosaccharides were then quantified by the method described by Bitter at al.[24] allowing us to determinate oligosaccharide concentrations in the samples.

Determination of the Linkage Yield. The estimation of the pyrrole content was derived from Muhs and Weiss method.[25] An amount of 1% 4-(dimethylamino)-benzaldehyde (DMAB) (50 μL in o-phosphoric acid) was mixed with the oligosaccharide sample at concentrations of 2.5, 5, or 10 μM (dilution in H_2O) and acetic acid (550 μL). The mixture was heated at 40 °C for 2 h. The optical density was then read at λ = 559 nm. A standardization series was made with 99% purified pyrrolylated oligonucleotide.

Copolymerization on SPR Prisms. The glass prisms (n = 1.717 at λ = 633 nm) covered with 50 nm gold films were purchased from Genoptics (Orsay, France). The electrochemical copolymerization was carried out by the "electrospot" method[26] on the gold layer through the use of a 200 μL pipet tip as the electrochemical cell. Electrical contact was established inside the tip by inserting a platinum wire. The tip was filled up with 10 μL of the polymerization solution (sodium-phosphate buffer 0.1 M, pH 6.8 containing 10% glycerol and pyrrole 20 mM) and pyrrole−oligosaccharide (from 0 to 25 μM) and was then applied to a

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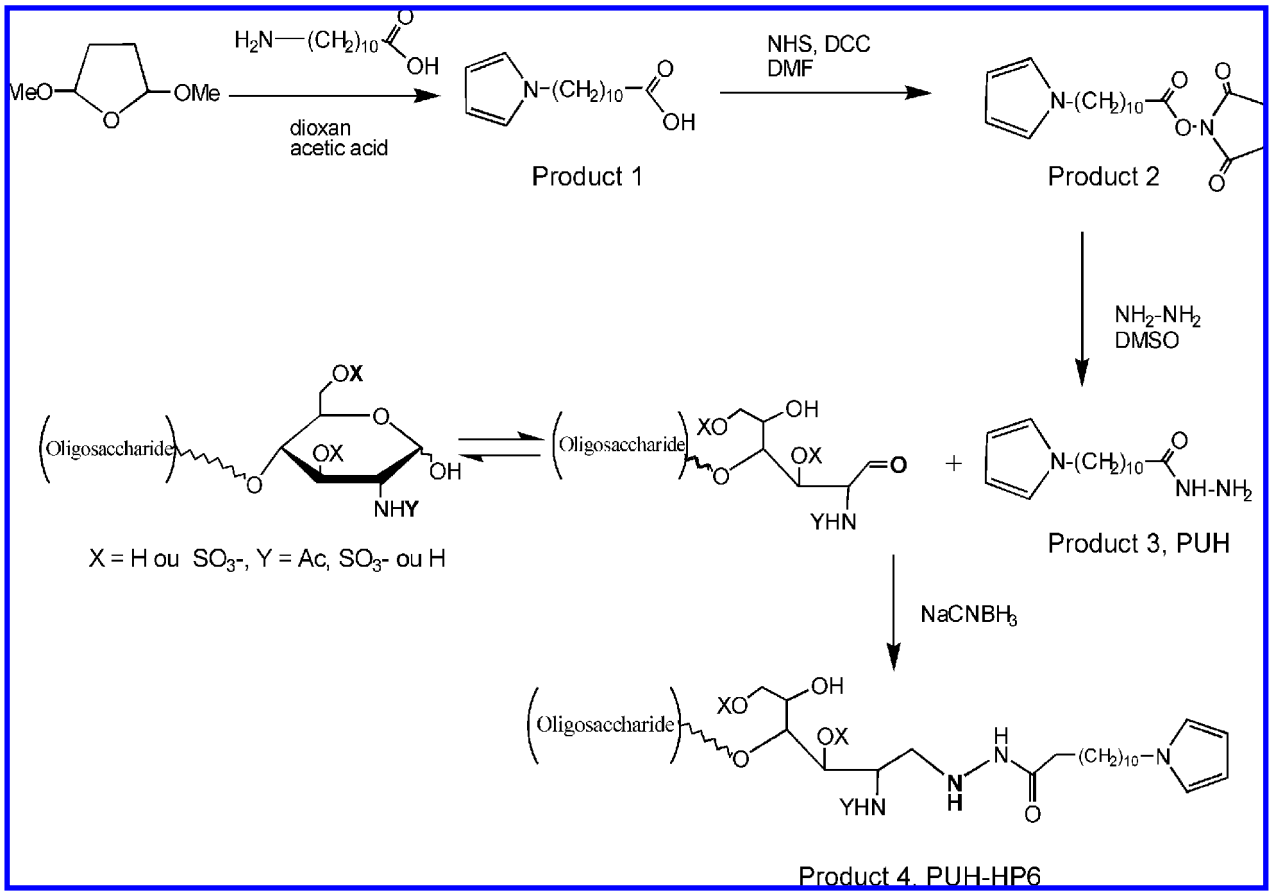
Scheme 1 Preparation of Modified Oligosaccharide Bearing a Pyrrole Group and a Chemical Linker[a]



[a] First the pyrrole group is synthesized. Second, modifications of the terminal chemical function to react with the aldehyde group of the oligosaccharide in a third step.

precise location on the gold layer used as the working electrode. This electrochemical system was connected to an EG&G Princeton Applied Research model 283 potentiostat and to an 83000 Schlumberger *X/Y* recorder. The polypyrrole film was synthesized by electrocopolymerization on the gold layer (working electrode) by a 2 V electrochemical pulse for 250 ms (the voltage was defined with respect to the platinum counter electrode). Following the electrosynthesis, the tip was emptied and rinsed with water. The successive polymerizations were carried out by the same process with the different oligosaccharides to be copolymerized on spatially defined areas of the gold slide. When all the oligosaccharides spots were synthesized, the slide was disconnected, rinsed with water, dried, and stored at 4 °C.

SPRi Interactions Monitoring. The optical setup was described elsewhere[9] and constructed by Genoptics (Orsay, France). The system was designed and implemented using the Kretchmann configuration.[27] Briefly, oligosaccharide−protein interactions produce changes in the refractive indexes near the gold surface. These results in changes of the reflectivity were recorded by a 12-bit CCD camera as gray-level contrasts. During interaction experiments, images were recorded at fixed intervals of time (0.2 s). All the images captured were analyzed on a computer with Imagin software (Genoptics). The interactions were carried out in a 10 μL Teflon cell, connected to a syringe pump, in the washing buffer (HEPES 10 mM, NaCl 150 mM, Tween 20 0.005%, pH 7.4) at room temperature. The flow rate of running solutions within the cell was 60 μL/min. The buffers used in SPRi were filtered and degassed. Following the protein injection, the sensor surface was rinsed with the washing buffer to remove unbound molecules and dissociate the complex of the oligosaccharide−protein. It was regenerated with 1 M NaCl dissolved in washing buffer for 3 min.

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RESULTS AND DISCUSSION

In this study, we developed a new oligosaccharide array to measure the carbohydrate-binding protein interactions by using SPR imaging. The oligosaccharides used are covalently linked to a pyrrole monomer via a linker at their reducing end (acyclic aldehyde form). We grafted oligosaccharide−pyrrole onto the surface using electrocopolymerization of the modified oligosaccharide and pyrrole monomers. Following the successive copolymerizations of the different oligosaccharides, the chip was used to measure oligosaccharide−protein interactions by SPR imaging (Figure 1).

Chemical Modification of Oligosaccharides. Oligosaccharide probes were grafted on the surface via a method initially developed for the construction of DNA chips: the polypyrrole approach. This process allows for direct electrochemical deposition. In order to carry out biological interaction studies, it is necessary that the oligosaccharides have the same orientation. In this way, all the modifications will be made on the reducing end. It is also important, especially for the smallest molecules, to

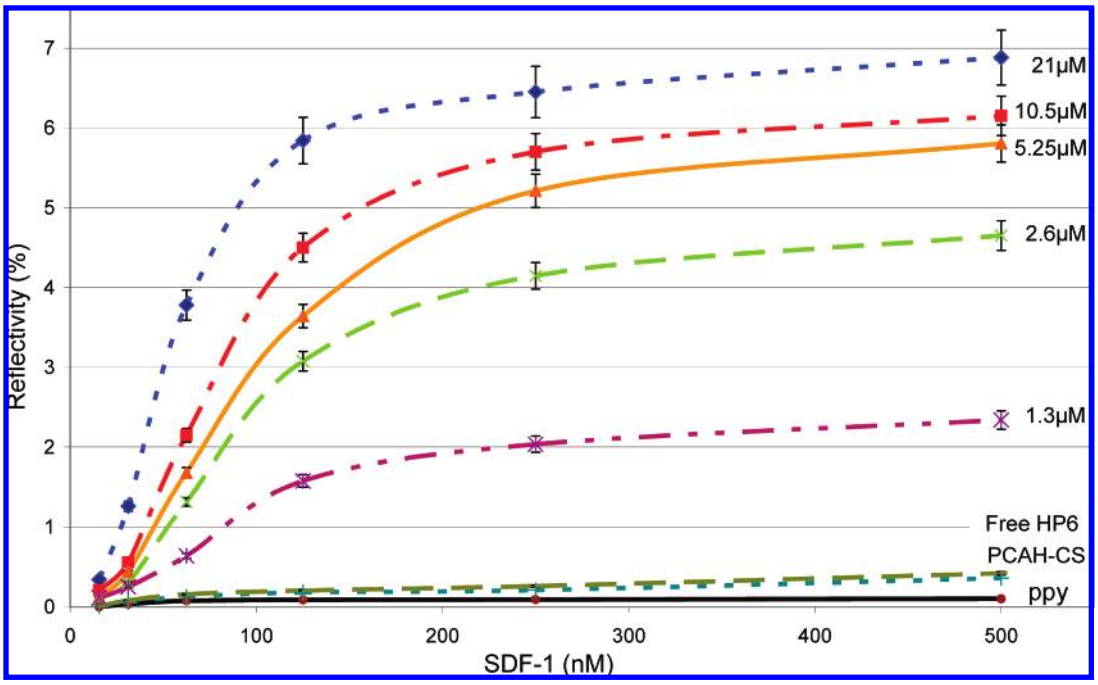

Figure 2. Reflectivity signal from bound SDF-1α at the end of the injection step versus different concentrations of SDF-1α. The different curves correspond to polypyrrole spots (ppy), pyrrolylated CS spots (PCAH-CS), nonmodified HP6−ppy spots (Free HP6), and pyrrole modified HP6 used in different concentrations (from 1.3 to 21 μM) during the copolymerization process.

incorporate a spacer between the covalently attached sugar and the chip surface, in order to guarantee accessibility of these small molecules for recognition by protein.

The pyrrole residue is coupled to the oligosaccharide at its aldehyde group using a hydrazide linkage, as it is usually used to couple biotin and oligosaccharide.[24] The linkage via this reducing end avoids the modification of the oligosaccharide structure or its functional properties. First, the "pyrrole-linker" PUH (product **3**, pyrrole−undecanoyl−hydrazide) was synthesized (Scheme 1). The second step consists in linking the oligosaccharide to the pyrrole monomer. This reaction was followed by a simple purification step on an exclusion column to give the final product **4**, PUH-HP6.

Other "pyrrole-linkers", such as 5-(pyrrol-yl)-caproyl-adipate-hydrazide (PCAH) and 10-(pyrrol-yl)-undecanoyl-adipate-hydrazide (PUAH), having a more hydrophilic or a longer chain were also synthesized and coupled to the oligosaccharide but were less efficient than the PUH. The yield of the linkage was estimed through (i) the determination of the pyrrole residue quantity (adapted from the Muhs and Weiss method[25]) and (ii) the quantification of the oligosaccharide concentration by the Bitter method.[26] Thus, the yield of the pyrrolylation of oligosaccharides was in the range of 20%. Optimization of this yield was very difficult, but the electrocopolymerization remains very efficient. We will show in this paper that this product can be used without separation of modified and nonmodified oligosaccharides because nonmodified product cannot be grafted on the support. Subsequently, we will use only the concentration of pyrrolylated oligosaccharides in discussing the results described in this article, i.e., concentration is determined by the linkage yield of pyrrole−linker−carbohydrate by the Muhs and Weiss method.

Construction of the Oligosaccharide Chip. Heparin HP6 arrays were constructed using the electrospotting method (Figure 1). This process allows us to graft the modified oligosaccharides onto gold via a polypyrrole layer. Briefly, pyrrolylated oligosaccharide conjugates (from 0 to 25 μM) were copolymerized with pyrrole through an electrochemical pulse in a modified pipet tip including a Pt wire used as a counter electrode. With the use of this setup, a reference electrode is not necessary because a very short electrocopolymerization time (250 ms) is used; in this case, the real potential of the working reference is in the range of 0.7 V vs SCE. Following spotting, it must be noted that the chips can be dried and stored at 4 °C for at least 6 months without modification of the oligosaccharide properties.

Interaction Measurements. A preliminary experiment was performed with SPRi on a chip bearing 25 spots to evaluate the efficiency and specificity of the target interaction. SPR imaging allows direct monitoring of different steps (Figure 1). Injection of the protein (SDF-1) corresponds to the association phase; washing buffer injection allows us to see the dissociation phase in the first sensorgram. The second sensorgram illustrates a dissociation phase with a protein interaction competition between the washing solution with free heparin and grafted oligosaccharides. Finally, because of the ionic interaction between oligosaccharide and SDF-1, the surface can be easily regenerated by an injection of 1 M NaCl allowing us to carry out successive analyses without loss of signal. More than 20 cycles can be carried out during 1 day on the same chip.

Figure 2 shows the SPRi intensity at the end of the injection of SDF-1: a very low signal (background) can be observed for spots of nonmodified polypyrrole and on the spot bearing chondroitin sulfate (CS), used as a negative control. Only pyrrole-functionalized HP6 spots interact with the SDF-1 protein, whereas no signal is recorded for unmodified HP6. This shows that the oligosaccharide grafting occurs by the pyrrole moiety. Different pyrrole-functionalized HP6 concentrations (from 0 to 21 μM in

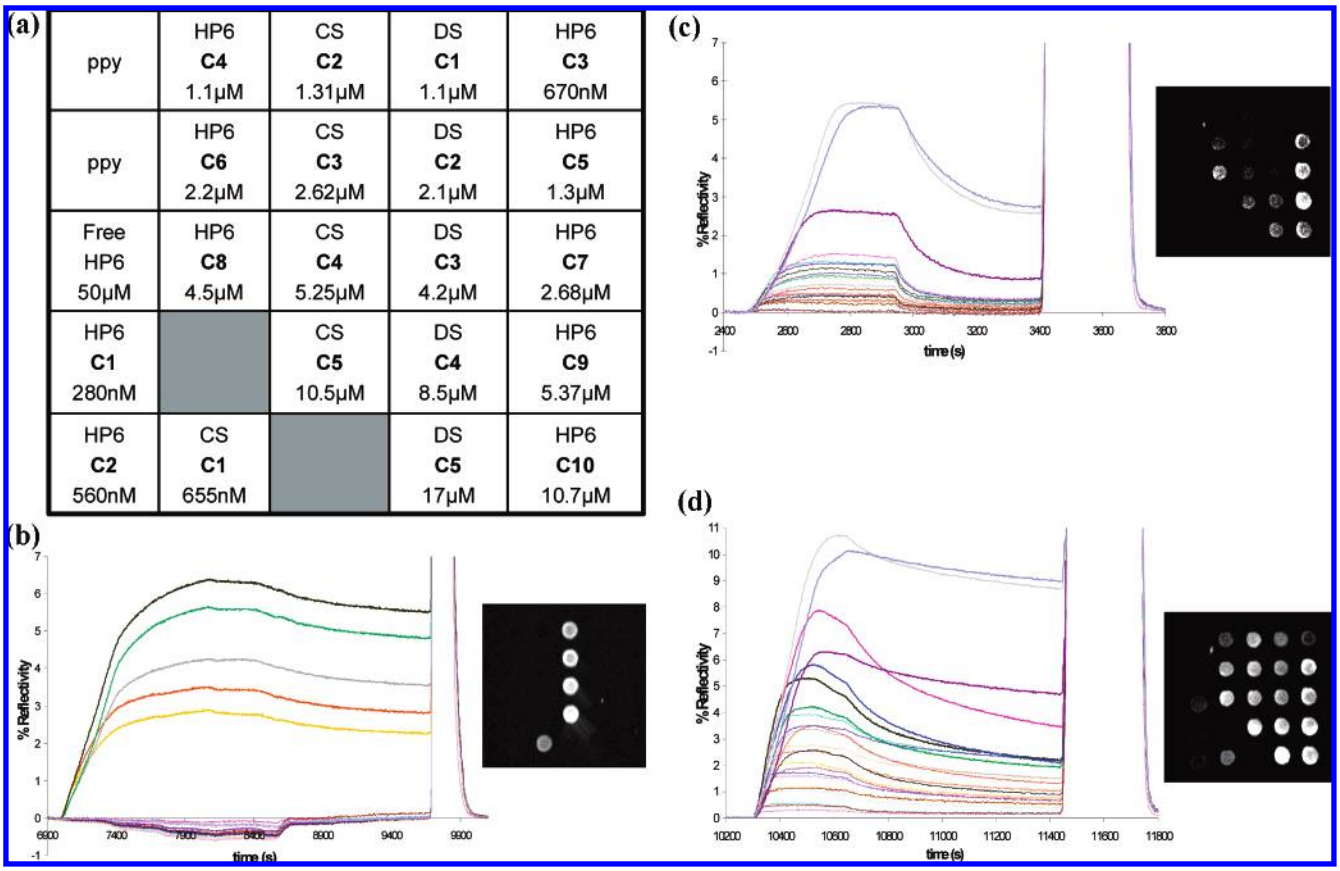

Figure 3. Real-time observation of oligosaccharides−proteins interactions. Each spot is about 700 μm large and the distance between the centers of two spots is 1.2 mm. The proteins were injected for 7 min (association phase), then buffer was injected (dissociation phase), and the chip was regenerated for 5 min. (a) Design of the chip: copolymerizations were performed with a mix of 20 mM free pyrrole and various concentrations of oligosaccharide probes C_1, C_2, C_3, etc. (b) Sensorgrams corresponding to the interactions of CS, DS, and HP6 functionalized spots with 10 nM of IgM anti-CS injected in the cell. On the right, image of the reflectivity increment caused by the biological interactions. (c) Sensorgrams corresponding to the interactions of CS, DS, and HP6 functionalized spots with 250 nM of SDF-1α injected in the cell. On the right, image of the reflectivity increment caused by the biological interactions. (d) Sensorgrams corresponding to the interactions of CS, DS, and HP6 functionalized spots with 100 nM of IFN-γ injected in the cell. On the right, image of the reflectivity increment caused by the biological interactions.

solution before polymerization) were used to synthesize the spots, leading to different oligosaccharide grafting densities.

For all grafting densities, a plateau is reached for an injection of SDF-1 concentration of 200 nM. For a SDF-1 concentration lower than 150 nM, the reflectivity is directly related to the grafting concentration. However, at higher SDF-1 concentration (>200 nM), the signal depends linearly on the HP6 concentrations used for the grafting and reaches a plateau for a concentration higher than 4 μM (5−6% reflectivity). At this saturation level, the data show that the limit is being approached by the steric hindrance of the recognition.[28] The highest sensitivity is obtained with the 21 μM oligosaccharide spot (slope at origin), allowing us to detect a concentration of 25 nM of SDF-1. For a SDF-1 concentration in the range of 50−200 nM, a grafting concentration of 10 μM is a convenient compromise. Although it is possible to use higher probe concentrations to increase the uptake of SDF-1, a low concentration is preferred to avoid steric hindrance that could occur during the interaction of protein (molecular weight of the SDF-1 is 7.8 kDa). These preliminary results allowed us to use this process in a biological application.

Biological Applications. Glycosaminoglycans (GAG) form a group of negatively charged molecules that have been shown to bind and directly regulate the bioactivity of growth factors and cytokines. In the sulfated glycosaminoglycans family, different carbohydrates have similar structures but different recognition and affinity properties. After showing that it was possible to covalently graft the natural oligosaccharide HP6, we constructed a chip bearing three oligosaccharides from the GAG family: heparin HP6, chondroitin sulfate A (CS), and dermatan sulfate (DS). Unlike HP6, CS and DS have a higher molecular weight (32−45 kDa and 37 kDa) and are known not to interact with the chemokine SDF-1. In contrast, interferon-γ IFN-γ is recognized by both HP and DS but not with the same affinity.[29] As CS cannot be recognized by these proteins, a commercial antibody was used to detect CS spots.

The pyrrole−CS and pyrrole−DS conjugates were prepared with the same procedure as HP6 with a yield of 10.5% and 17%, respectively. A chip containing DS, CS, and HP6 at different concentrations was constructed by the electrospotting method according to the pattern shown in Figure 3a.

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Table 1. Reflectivity Changes Generated by the Different Proteins Injected on the Oligosaccharide Chip

	samples		
probes	IgM anti CS 10 nM	SDF-1α 250 nM	IFN-γ 100 nM
HP6 (C9)	−0.4	5.3	10.1
HP6 (C10)	−0.4	5.4	10.75
DS (C4)	−0.4	0.9	5.8
DS (C5)	−0.4	1.5	7.9
CS (C4)	5.7	0.8	4.2
CS (C5)	6.5	1.1	5.35

The three proteins were successively injected in the cell. Each run was followed by a NaCl regeneration step. The sensorgrams (Figure 3b) show that the anti-CS IgM antibody (10 nM) binds only to the different CS spots; no cross reaction can be seen with the other compounds, and the response is roughly proportional to the grafting density. One can observe a typical Ag−Ac interaction with a very slow dissociation rate. This recognition is highly sensitive, and interaction can be observed until 250 pM (data not shown).

Injection of SDF-1α (250 nM) gives a strong signal on HP6 spots, as expected, and a small interaction signal on CS and DS spots (Figure 3c). As anticipated, grafted HP6 spots recognize the protein proportionally to their grafting concentration (Figure 3c). During the dissociation phase, only the reflectivity of HP6 spots do not decrease up to the baseline, showing that the interaction of HP6 with SDF-1 is much stronger than with CS or DS.

The last injection of 100 nM IFN-γ shows that all grafted oligosaccharides recognize this protein. The curves which have the highest reflectivity increase correspond to spots with grafted HP6 (C10), and the five reflectivity curves decreasing in the intensity correspond to HP6 (C9), DS (C5), HP6 (C8), DS (C4), and CS (C5) (Figure 3d). Differences between the affinities are observed, as expected.[20,30] Table 1 summarizes the results obtained with the three proteins. In particular, the reflectivity level obtained with CS and IgM α CS is as high as for the HP6−SDF-1 complex, only 10 nM antibody was injected. For comparison, the SDF-1 concentration must reach 250 nM to have the same signal. This difference is probably related to the difference of molecular weights of the two proteins and to the different affinities.

A last series of experiments were made to determinate the affinity constants between anti-CS IgM and CS. To our knowledge these have not been reported in the literature. An antibody concentration series was injected on the same chip (Figure 3b). Using the Scatchard plot method, we obtained values from 3.9 to 7.2 nM for each CS spot. We proposed that this affinity constant is, then, lower than 10 nM, the value generally obtained for antigen−antibody interactions. That is smaller than that of SDF−HP6 (90 nM, unpublished observation).

In conclusion, our study has shown that it is possible to graft natural oligosaccharides of a range of molecular weights on a surface compatible with a SPR imaging interaction detection process. Whereas only natural relatively long oligosaccharides are grafted in this study, synthetic oligosaccharides bearing a reducing end (or functionalized by any other methods) can also be grafted by the same approach. The chemical procedure developed here leads to a very strong and stable grafting allowing the chips to be dried, stored, and regenerated between the different biological experiments. SPR imaging is, moreover, an efficient method to study protein−oligosaccharide interactions without labeling of the probes or the targets and to monitor simultaneously a number, in this case 25, of interaction kinetics in real time. This number can be significantly extended by using miniaturized electrochemical setups, if necessary.[31] Equilibrium dissociation constant (K_d) and kinetic parameters (i.e., rate constants) can be obtained for each biological interaction process at all spots at the same time allowing the direct comparison of the behavior of a number of compounds. In the same way, injection of compounds with competiting binding interactions can also be studied. This approach opens the field of multiple and direct studies of synthetic or natural oligosaccharides properties.

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SUPPORTING INFORMATION AVAILABLE

A video from SPR imaging showing a typical interaction experiment between the protein SDF-1 and a GAG array. This material is available free of charge via the Internet at http://pubs.acs.org.

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