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COMMUNICATIONS

Published on Web 02/08/2006

Microarrays of Synthetic Heparin Oligosaccharides

Jose L. de Paz, Christian Noti, and Peter H. Seeberger*

Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zurich, Wolfgang-Pauli-Str. 10, HCI F315, 8093 Zurich, Switzerland

Received November 7, 2005; E-mail: seeberger@org.chem.ethz.ch

Carbohydrate microarrays[1] are a powerful platform to screen interactions involving these molecules in a high-throughput manner.[2] The chip-based format offers important advantages over classical methods, such as the ability to screen several thousand binding events on a single glass slide and the miniscule amounts of both analyte and ligand required for one experiment.

One of the most complex classes of carbohydrates, heparin-like glycosaminoglycans (HLGAGs), plays a key role in regulating biological processes, including growth factor interactions, virus entry, and angiogenesis by binding to a host of proteins.[3] However, the structure−function relationships of HLGAGs are very poorly understood due to the chemical complexity and heterogeneity of this type of biopolymer. The use of microarrays of synthetic heparin oligosaccharides can substantially improve the understanding of heparin−protein interactions, opening an opportunity for the discovery of novel therapeutic interventions for a variety of disease states.

Herein, we report the creation and use of microarrays containing synthetic heparin oligosaccharides. A novel linker chemistry is compatible with the protecting-group manipulations required for the synthesis of HLGAGs following solution phase or solid phase assembly. The covalently attached, defined oligosaccharides can be used for the rapid analysis of HLGAG−protein interactions as exemplified by incubation with acidic and basic fibroblast growth factors (FGF-1 and FGF-2), two important heparin-binding proteins.[4]

Initially, we designed a suitable linker strategy to attach synthetic heparin molecules to a chip surface. Synthetic oligosaccharides obtained by automated solid phase synthesis are released as terminal pentenyl glycosides[5] (Figure 1) via olefin cross-metathesis. We elected an amine-terminated thiol to elongate the *n*-pentenyl glycosides before sulfated oligosaccharides are generated and printed onto glass slides.

Two monosaccharides **1** and **5** (Scheme 1) served as models to develop and optimize the linker chemistry. First, radical extension of the pentenyl moiety using 2-(benzyloxycarbonylamino)-1-ethanethiol was worked out. Thermal activation[6] at 75 °C in the presence of a catalytic amount of AIBN gave the best yields among a host of reaction conditions that were tested. Treatment of amine-terminated **2** and **6** with lithium hydroperoxide and then KOH hydrolyzed the acyl and methoxycarbonyl groups. Simultaneous oxidation of the sulfide afforded **3** and **7**[7] in good yield. Introduction of the sulfate groups that are frequent in heparin oligosaccharides and are placed following the assembly process was achieved by treatment with the SO_3·Py complex. Global deprotection was executed by hydrogenolysis to give the sulfated monosaccharide **4**. To demonstrate that the reduction of azide protecting groups and simultaneous O- and N-sulfation followed by hydrogenolysis can be carried out, glucosamine **7** was transformed via Staudinger reduction to afford **9** in high yield. Thereby, a method to equip

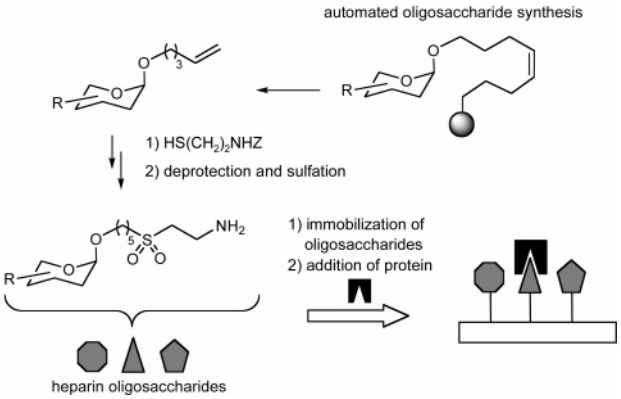
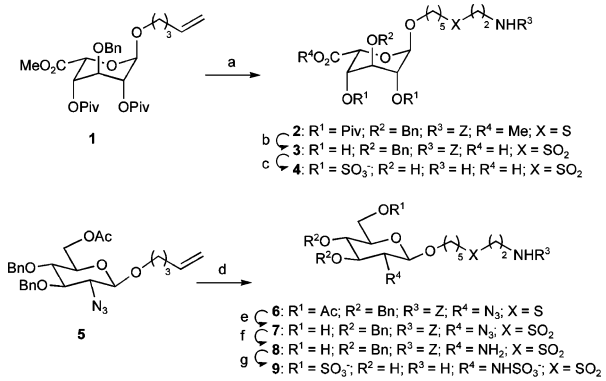


Figure 1. General strategy for the preparation of microarrays containing synthetic heparin oligosaccharides.

Scheme 1. Preparation of Sulfated Monosaccharides **4** and **9**[a]



[a] Reagents and conditions: (a) $HS(CH_2)_2NHZ$, AIBN, THF, 75 °C, 82%; (b) LiOH, H_2O_2, then KOH, 88%; (c) SO_3·Py, Py, 85%; H_2, Pd/C, 95%; (d) $HS(CH_2)_2NHZ$, AIBN, THF, 75 °C, 95%; (e) LiOH, H_2O_2; KOH; $KHSO_5$, MeOH, 88%; (f) PMe_3, THF, NaOH, 96%; (g) SO_3·Py, Py, 84%; H_2, Pd/C, 99%.

synthetic heparin oligosaccharides for covalent immobilization onto chip surfaces was established.

A series of fully protected di-, tetra-, and hexasaccharides containing the $GlcNSO_3$(6-OSO_3)−IdoA(2-OSO_3) repeating unit of the major sequence of heparin was derived as part of our ongoing program concerned with the automated solid phase synthesis of heparin oligosaccharides.[8] Application of the new linker strategy furnished the sulfated oligosaccharides **10**, **11**, and **12** (Figure 2).

With a straightforward route to procure amine-functionalized sugars at hand, the challenge to covalently attach these molecules on a suitable glass surface was addressed. Amine-reactive CodeLink slides that are coated with a hydrophilic polymer containing *N*-hydroxysuccinimide esters performed best due to the three-dimensional nature of the polymer. The immobilization chemistry was initially tested using manual spotting of 0.5 μL of heparin oligosaccharides **10**, **11**, and **12** at concentrations ranging from 5

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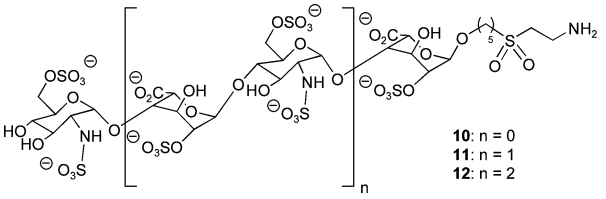

Figure 2. Heparin oligosaccharides **10**, **11**, and **12** ready for immobilization on a chip surface.

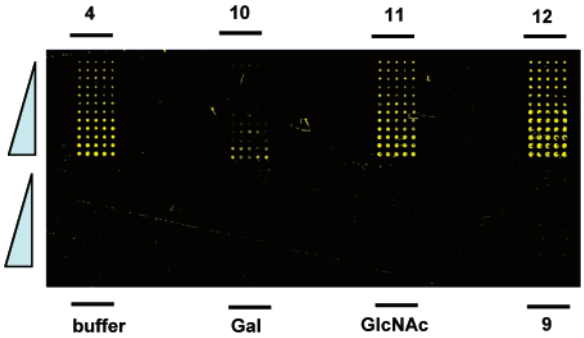

Figure 3. Top: Microarray after incubation with FGF-1. Bottom: Fluorescence signal observed for each arrayed carbohydrate binding to FGF-1 at 500 μM. Sodium phosphate buffer served as a negative control.

mM to 50 μM. After incubation and quenching of the remaining activated esters, the slides were ready for screening experiments using pure growth factor FGF-1 or FGF-2. These important members of a family of heparin-binding proteins are involved in developmental and physiological processes, including cell proliferation, differentiation, morphogenesis, and angiogenesis.[9] After removing unbound protein by washing, slides were subsequently incubated with anti-human FGF polyclonal antibodies. Finally, the human FGF captured by the heparin microarray was visualized by using a secondary antibody labeled with an Alexa 546 dye. Strongly fluorescent signals at tetra- and hexasaccharide positions were in agreement with the minimal structural requirements to bind both FGF-1 and FGF-2.[10]

Miniaturization of the spotting process using an arraying robot to construct heparin microarrays for high-throughput experiments was now simple. Oligosaccharides **4**, **9**, **10**, **11**, and **12** along with 2′-aminoethyl-2-acetamido-α-D-glucopyranoside (GlcNAc) and 2′-aminoethyl-β-D-galactopyranoside (Gal)[11] were used in concentrations ranging from 2 mM to 250 μM (Figure 3). The robot delivered 1 nL of carbohydrate-containing solutions to create spots with a diameter of ~200 μm. All samples were printed in 15 replicates to generate an array of 480 spots (Figure 3).

Following incubation with FGF, primary and secondary antibodies were utilized to detect any bound protein on the slides using a standard fluorescent slide scanner. FGF-2 bound best to **11** and **12** based on the highest fluorescence intensities, while monosaccharides were not bound (see Supporting Information). Similar results were obtained for FGF-1, but interestingly, monosaccharide **4** exhibited a spot intensity comparable to that of longer oligosaccharides (Figure 3). The presence of a 2,4-O-sulfation pattern, not found in nature, may be responsible for this result. Also, this result illustrates the possibility to employ the heparin microarrays to discover inhibitors for heparin−protein interactions.

In conclusion, we have developed a new method for the preparation of microarrays displaying synthetic heparin oligosaccharides derived by solution and solid phase assembly methods. Strategic placement of an orthogonally protected amine linker was key to the success of the array construction. The potential of the new methodology was demonstrated by probing the carbohydrate affinity of two heparin-binding proteins, FGF-1 and FGF-2, that are implicated in the development and differentiation of several tumors. On the basis of the method disclosed here, the construction of diverse heparin microarrays that can be used to rapidly screen heparin−protein interactions has become possible. Heparin arrays are expected to fundamentally impact the establishment of structure−activity relationships for heparin sequences.

Acknowledgment. This research was supported by ETH Zurich and the European Commission (Marie Curie Fellowship for J.L.P.). We thank Dr. Jens Sobek (Functional Genomics Center, Zurich) for assistance in the construction of microarrays.

Supporting Information Available: Experimental procedures for the production of the heparin microarrays, synthesis of compounds **4** and **9**, and the complete ref 2d. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For reviews, see: (a) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3583−3586. (b) Feizi, T.; Fazio, F.; Chai, W.; Wong, C.-H. *Curr. Opin. Struct. Biol.* **2003**, *13*, 637−645. (c) Shin, I.; Park, S.; Lee, M. *Chem.−Eur. J.* **2005**, *11*, 2894−2901.
(2) For examples, see: (a) Wang, D.; Liu, S.; Trummer, B. J.; Deng, C.; Wang, A. *Nat. Biotechnol.* **2002**, *20*, 275−281. (b) Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nat. Biotechnol.* **2002**, *20*, 1011−1017. (c) Disney, M. D.; Seeberger, P. H. *Chem. Biol.* **2004**, *11*, 1701−1707. (d) Blixt, O.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17033−17038. (e) Ratner, D. M.; Adams, E. W.; Su, J.; O'Keefe, B. R.; Mrksich, M.; Seeberger, P. H. *ChemBioChem* **2004**, *5*, 379−383. (f) Disney, M. D.; Seeberger, P. H. *Chem.−Eur. J.* **2004**, *10*, 3308−3314. (g) Lee, M.; Shin, I. *Org. Lett.* **2005**, *7*, 4269−4272.
(3) (a) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 390−412. (b) Noti, C.; Seeberger, P. H. *Chem. Biol.* **2005**, *12*, 731−756.
(4) Conrad, H. E. *Heparin-Binding Proteins*; Academic Press: San Diego, CA, 1998.
(5) Plante, O. J.; Palmaci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523−1527.
(6) Buskas, T.; Soderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958−963.
(7) In the case of **6**, additional treatment with oxone was necessary for the complete conversion of sulfide into sulfone **7**.
(8) (a) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem.−Eur. J.* **2003**, *9*, 140−169. (b) Noti, C.; De Paz, J. L.; Seeberger, P. H. Unpublished results.
(9) Pellegrini, L. *Curr. Opin. Struct. Biol.* **2001**, *11*, 629−634.
(10) Raman, R.; Sasisekharan, V.; Sasisekharan, R. *Chem. Biol.* **2005**, *12*, 267−277.
(11) Monosaccharide **4** without sulfate groups was used as an additional negative control (see Supporting Information).

JA057584V