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Attachment of glycosaminoglycan oligosaccharides to thiol-derivatised gold surfaces†

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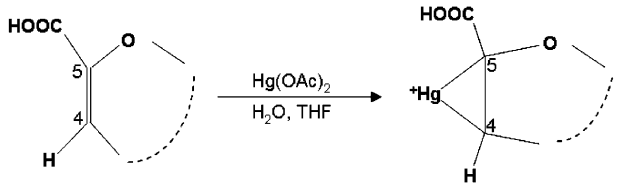
Glycosaminoglycan oligosaccharides have been attached to thiol-derivatised gold surfaces, *via* the formation of mercury–sugar adducts at the non-reducing end, representing a new method of generating versatile glycoconjugates incorporating this class of biologically and medically important carbohydrate.

The glycosaminoglycans (GAG) are a family of linear, sulfated polysaccharides which are known to mediate a large number of protein interactions and biological processes.[1] They are implicated in many clinical conditions and are involved in host cell invasion by a variety of pathogenic microorganisms.[2] The efficient coupling of GAG oligosaccharides to labels, allowing more sensitive detection and aiding sequencing, as well as their attachment to surfaces to form arrays and biosensor surfaces, is an attractive prospect. Such developments would assist the search for structure–activity relationships and the identification of binding partners within this structurally complex, but biologically important family. Currently, the condensation reaction between reducing end aldehyde groups of sugars and the amine groups of labels and surfaces to form imines is widely exploited. Examples include labelling with fluorophores and tags to improve detection in biological systems[3] or sequencing procedures[4] and as a means of attachment to derivatised glass surfaces.[5] However, this reaction can vary in efficiency depending on the structure of the reducing end sugar unit. The reaction is acid catalysed but this cannot be exploited for some GAGs, including the biologically and medically important heparan sulfate (HS), because of their sensitivity to acid.[6]

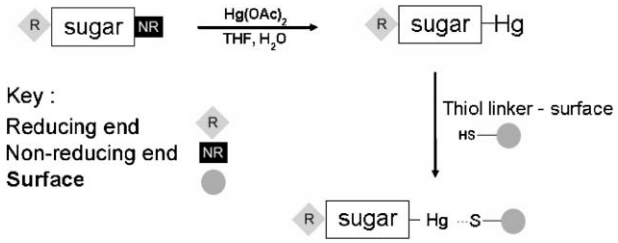
An alternative strategy for GAG-derived oligosaccharides is provided by the presence of a carbon–carbon double bond in the unsaturated uronic acid (ΔUA) residue, which is generated at the non-reducing end by lyase enzymes during cleavage from the parental polysaccharide.

Reaction of the double bond with $Hg(OAc)_2$ is a long-established reaction in which the π electrons of the double bond attack the electrophilic Hg^{2+} to displace an acetate group and form a cyclic mercurinium intermediate[7] (oxymercuration). This approach is used, following subsequent reduction, to eliminate Hg (demercuration)[8] to produce ethers or alcohols and to remove the terminal non-reducing end monosaccharide from GAGs.[9]

However, if the reduction step is omitted, alternative reactions become possible. Here, formation of mercury intermediates (Scheme 1) and their avid reaction with sulfhydryl groups is exploited to produce conjugates attached *via* the non-reducing end of the saccharide as shown in Scheme 2. This permits the generation of versatile glycoconjugates, illustrated here by the preparation of a Au–thiol surface derivatised with octadecasaccharides derived from HS. The binding of a known heparin binding



Scheme 1 Oxymercuration of the 4,5-carbon double bond at the non-reducing terminal unit of GAG saccharides forming a mercurinium intermediate.



Scheme 2 Formation of a GAG-thiol derivatised Au surface *via* the mercury adduct of the carbohydrate.

protein, fibroblast growth factor 2 (FGF-2) and inhibition of binding with HS confirmed attachment of the GAG to the surface.

A model disaccharide, ΔUA–GlcNAc (10 μmol), derived from HS by heparitinase enzyme digestion and bearing an unsaturated 4,5-uronic acid at the non-reducing end was reacted in water–THF (1 mL, 1 : 1 (v : v) 2 h, 40 °C) with $Hg(OAc)_2$ (0.1 mmol). The product was characterized by MALDI-MS, confirming the formation of a mercury adduct (*m*/*z* calc. 639.9, obs. 640 with the expected spread of 6 mass units for Hg^{198} to Hg^{204}, consistent with the production of a cyclic 4,5-mercurinium intermediate.† ^{1}H NMR‡ (Fig. 1) demonstrated opening of the double bond by $Hg(OAc)_2$ and this was accompanied by loss of absorbance by the double bond at 232 nm.

Model disaccharide compounds derived by digestion with the appropriate lyases from a range of GAGs including chondroitin sulfate (CS)† reacted in the same way. The applicability of this reaction to larger oligosaccharides was shown by reacting size-defined HS oligosaccharides including the hexadecasaccharide,

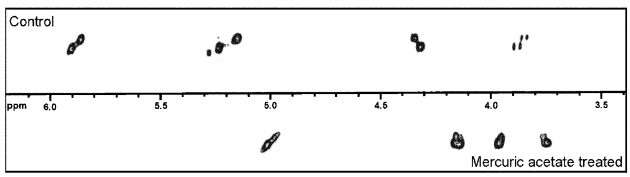


Fig. 1 Detail of TOCSY NMR spectrum of the unsaturated uronic acid residue of ΔUA–GlcNAc disaccharide (upper) and after opening of the carbon double bond with mercuric acetate (lower).

† Electronic supplementary information (ESI) available: ^{1}H NMR spectrum and COSY with MALDI-MS of Hg-derivatised UA–GlcNAc. ^{1}H NMR spectrum of Hg-derivatised UA–GalNAc,6S. See http://www.rsc.org/suppdata/cc/b4/b411726c/

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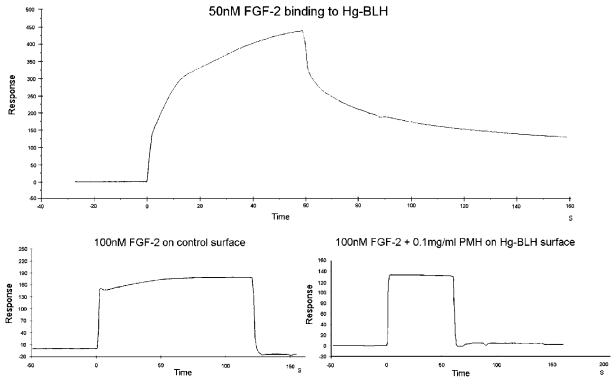

Fig. 2 Detection of protein binding (FGF-2) to HS hexadecasaccharide derivatized gold–thiol surface (upper), inhibition of binding with heparin (lower right) and background binding to surface alone (lower left).

ΔUA2S(α1-4)GlcNS,6S{(α1-4)IdoA2S(α1-4)GlcNS,6S}$_7$, with Hg(OAc)$_2$ and, following purification twice in the presence of excess EDTA, their attachment to a thiol-derivatised Au surface that had previously been derivatised with 1,8-octanedithiol (5 mM in EtOH, 24 h)[10] to generate a novel biosensor surface.

A protein known to bind heparan sulfate, fibroblast growth factor 2 (FGF-2),[11] was used to demonstrate the attachment of HS saccharides to the surface. Binding of FGF-2 (50 nM, 3 h) to this surface is shown in Fig. 2 (upper). Control experiments were conducted to eliminate the possibility of FGF-2 binding to the underivatised Au surface (100 nM FGF-2, no binding observed) or the thiol-derivatised Au surface alone (Fig. 2, lower left) and to exclude the involvement of free Hg^{2+} ions bridging from surface thiols to anionic groups on the oligosaccharide (incubated with excess Hg(OAc)$_2$ then 100 nM FGF-2, no binding observed). Furthermore, the binding of FGF-2 (100 nM) to the thiol–Au surface coated with Hg-derivatised HS oligosaccharides was prevented by the addition of heparan sulfate (0.1 mg mL^{-1}) (Fig. 2, lower right).

The reactions described here will permit improved detection of labelled GAG saccharides attached to a variety of surfaces and increase access to a range of hitherto unavailable experiments. Derivatised Au surfaces will be useful for the study of biological systems involving GAG mediated processes, which include growth factor activation, protease inhibition, neuron guidance and inhibition of the attachment of microbial pathogens especially where these depend on the presentation of multivalent oligosaccharide ligands. Mercury derivatisation of GAGs also allows dual-end labelling with widespread potential applications. This method will aid the development of biosensor surfaces suitable for the study of GAGs and their activities as well as GAG saccharide microarrays. Further derivatisation of the mercury adducts of GAGs and the preparation and application of heparan sulfate gold nanoparticle glycoconjugates in biological systems will be reported in future work.

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Notes and references

‡ Hg treated HS disaccharide; ΔUA(1-4)GlcNAc: uronic acid residue ^{1}H NMR (D$_2$O) assignments δ (ppm): H-1 5.00, H-2 3.74, H-3 3.96, H-4 4.15; glucosamine residue (principal anomer) H-1 5.26 H-2 3.95 H-3 3.76 H-4 4.76 H-5 3.78 H-6 H-6′ 3.92–3.94; (minor anomer) H-1 5.25 H-2 3.99 H-3 3.74 H-4 4.77 H-5 3.78 H-6 H–6′ 3.92–3.94. Hg treated chondroitin disaccharide; ΔUA(1-3)GalNAc,6S: ^{1}H NMR (D$_2$O) assignments of uronic acid residue δ (ppm) H-1 5.25 H-2 4.15 H-3 4.33 H-4 4.23. EDTA was added to chelate excess Hg_2^+ ions during purification and to record NMR spectra. For detection of FGF-2 binding, experiments were performed at 30 μL min^{-1} flow rate in a BIAcore 2000 in HBS-P buffer (BIAcore) at 25 °C. Surfaces were washed with EDTA (3 mM), NaCl (2 M) and HCl (20 mM) prior to injection of FGF-2 (100 nM).

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