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Synthesis and characterisation of glucose-functional glycopolymers and gold nanoparticles: study of their potential interactions with ovine red blood cells

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ABSTRACT

Carbohydrate–protein interactions can assist with the targeting of polymer- and nano-delivery systems. However, some potential protein targets are not specific to a single cell type, resulting in reductions in their efficacy due to undesirable non-specific cellular interactions. The glucose transporter 1 (GLUT-1) is expressed to different extents on most cells in the vasculature, including human red blood cells and on cancerous tissue. Glycosylated nanomaterials bearing glucose (or related) carbohydrates, therefore, could potentially undergo unwanted interactions with these transporters, which may compromise the nanomaterial function or lead to cell agglutination, for example. Here, RAFT polymerisation is employed to obtain well-defined glucose-functional glycopolymers as well as glycosylated gold nanoparticles. Agglutination and binding assays did not reveal any significant binding to ovine red blood cells, nor any haemolysis. These data suggest that gluco-functional nanomaterials are compatible with blood, and their lack of undesirable interactions highlights their potential for delivery and imaging applications.

1. Introduction

Carbohydrate–protein interactions play a fundamental role in a variety of biological recognition/communication processes. A specialised subset of carbohydrate binding proteins called lectins, which are neither antibodies nor enzymes, are responsible for this, but suffer from intrinsically low affinity for individual glycans, exhibiting dissociation constants in the mM range.[1] Nature overcomes this by presenting multiple copies of the same glycan on cellular surfaces, leading to a non-linear increase in binding affinity: the cluster glycoside effect.[2,3] Consequently, there has been much interest in the application of synthetic macromolecules (polymers, particles) with pendant carbohydrates as high-affinity lectin binders.[4,5] This effect has been exploited in anti-adhesion therapy towards cholera,[6,7] HIV,[8] Shiga toxins,[9] biosensing,[10] or in synthetic vaccines,[11] to name a few. The synthesis of glycopolymers has been facilitated by the development of controlled radical polymerisation (CRP) and improvements in post-polymerisation modification chemistry.[12] These are tolerant to most functional groups and give rise to well-defined polymers of various architectures.[13] Understandably therefore, there is significant interest in their potential for therapeutic applications.[14]

Modern CRP techniques further afford high levels of control over polymer chain-end functionality for immobilisation onto nanoparticle surfaces. The use of nanomaterials offers additional advantages such as providing a core of fixed, tuneable size which can be modified with, for instance, pre-designed polymer densities,[15] and can be combined with carbohydrates to generate glyconanoparticles.[16] For instance, Marín et al. have functionalised gold nanoparticles with a combination of polyethylene glycol (PEG) and a trivalent α-2,6-thio-linked sialic acid ligand to detect human influenza virus X31 and then discriminate between human (α2,6 binding) and avian (α2,3 binding) RG14 influenza virus.[17] Penadés and co-workers have prepared paramagnetic gold glyconanoparticles as MRI probes.[18]

One attractive (potential) application of glycosylated (nano)materials is as targeted delivery vehicles which improve the pharmacokinetic profiles of therapeutics. The most frequently employed approach is to incorporate galactose residues to target the liver via the asialoglycoprotein receptor, which normally functions to recycle degradable red blood cells, and can hence internalise macromolecules.[19,20] Cameron and co-workers demonstrated that galactose-rich glycopolymers can be used to deliver antioxidants to spermatozoa to improve their storage time.[21] Cancerous cells overexpress the transmembrane GLUT-1 transporter on their surface to enable increased glucose uptake. This overexpression has been exploited to improve the delivery of ifosfamide, a DNA alkylating agent as glufosfamide, and conjugates of paclitaxel.[22]

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Reineke et al. have explored the use of glucosamine-functional polymers as the corona-forming component of micelles for drug-delivery applications as an alternative to the commonly employed PEG (poly(ethyleneglycol)) layer.[23] Glycosylated cationic dendrimers have also been employed.[24]

If glucose-rich glycopolymers/particles are to be successfully employed in vivo, their unwanted interactions with other biological components together with issues related to the inherent promiscuity of lectin–carbohydrate interactions must be understood.[25,26] For instance, a drug delivery vehicle in the blood stream should not interact with red blood cells or any other components of the vasculature, such as endothelial cells which line the arteries, if unwanted effects are to be avoided and a high efficacy at the target site is to be maintained. The GLUT-1 transporter comprises approximately 5% of all the membrane proteins in human erythrocytes.[27–29] Whilst not a lectin, the surface exposed nature of the transporter makes it conceivable that multivalent glucose-functional materials could interact with this transporter, leading to agglutination (a serious problem in vivo) or effective depletion of the concentration of the drug delivery vehicle. Park et al. have observed that poly([3-*O*-(vinylbenzyl)-D-glucose] was capable of binding rat erythrocytes (via GLUT-4 which has high homology to GLUT-1), and that addition of the specific inhibitor phloretin prevented these interactions.[30,31] In the above case, the glucose moiety was linked at the 3-*O*-position to the styrenyl backbone, with the anomeric position free (i.e., reducing). The same authors also showed that poly[*N*-*p*-vinylbenzyl-*O*-α-D-glucopyranosyl-(1-4)-D-gluconamide] which bears a terminal 1-*O*-α-linked glucose unit, did not bind rat erythrocytes. The reason for these differences is not immediately obvious. Detailed structure–activity relationships conducted on GLUT-1/glucose interactions suggest that modifications at the glucose 1-position are allowed by GLUT-1, though data regarding the tolerance to modifications at the 3-position remain inconclusive.[22] The polymers used in these prior investigations were also obtained by free-radical polymerisation, so contain a wide range of molecular weights preventing detailed structure–activity relationships to be drawn. Additionally, it is desirable to employ non-reducing (i.e., 1-position protected) carbohydrates to prevent unwanted glycation reactions with amines in proteins or drug molecules[32] or other biomolecules which might compromise their performance. It is clear, therefore, that there is a need to evaluate glycopolymer–blood interactions to enable the successful transition of such vehicles to 'real' therapies.

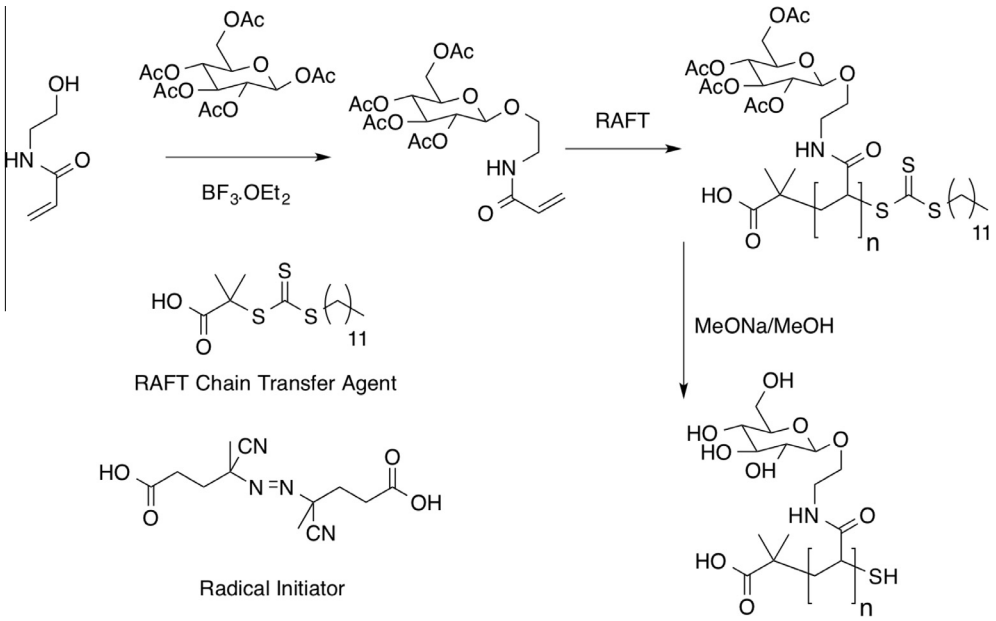
Considering the above, this work sought to synthesise a range of well-defined gluco-functional polymers and nanoparticles, and probe for interaction (binding/agglutination) with erythrocytes to assess their biocompatibility. A range of assays are employed, including haemolysis, agglutination and fluorescence microscopy.

2. Results and discussion

2.1. Polymer and nanoparticle synthesis

In order to probe for interactions blood cell–glucosylated material interactions synthetic routes towards both functional linear polymers and gold nanoparticles were developed. The glycosylated monomer tetra-*O*-acetyl-β-D-1-glucopyranosyl-hydroxylethylacrylamide (GluHEA) was prepared by the reaction of *N*-hydroxyethyl acrylamide with glucose pentaacetate using $BF_3{\cdot}OEt_2$ as the activating agent. This yielded the beta-linked monomer in 21% yield after column chromatography, Scheme 1. An acrylamide (as opposed to the more commonly employed acrylate/methacrylate) was chosen to enable direct polymerisation of the protected monomer, followed by deprotection of the macromolecule. This avoided complications associated with competitive hydrolysis of the (meth)acryloyl ester.[33] GluHEA was polymerised using 4,4′-azobis(4-cyanovaleric acid), ACVA, as the radical source and a trithiocarbonate reversible addition-fragmentation chain transfer (RAFT)[34] agent to give a small library of polymers. These were characterised by SEC (size exclusion chromatography), ^{1}H NMR and FT-IR (Table 1). The polymerisation of GluHEA proceeded slower than the non-glucose containing *N*-hydroxyethyl acrylamide (HEA—required for the assembly of glycosylated gold nanoparticles, vide infra), resulting in lower than expected conversions (~20%). SEC confirmed the well-defined nature of the polymers with dispersities <1.2, as expected from this controlled process. The molecular weights determined by SEC (Table 1) deviate from the theoretical values due to the different elution behaviour of the polymers compared to the poly(methyl methacrylate) standards used (Fig. 1).

In order to obtain the desired gluco-functional polymers, the acetate protecting groups were removed using standard sodium



Scheme 1. Synthetic scheme for the synthesis of poly(β-D-1-glucopyranosyl-hydroxylethylacrylamide).