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Synthesis and lectin recognition of glycosylated amphiphilic nanoparticles

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ABSTRACT

Carbohydrate–protein interactions play an important role in many biomolecular recognition events and thus artificial glycopolymers and glycoparticles are actively developed. Nanoprecipitation and aerosol flow reactor techniques were used to prepare glucose-decorated nanoparticles capable of binding to lectin Concanavalin A (ConA). The nanoparticles were based on a polystyrene-block-polypentafluorostyrene (PS-b-PFS) block copolymer that was post-functionalised with glucose units via thiol *para*-fluorine click reaction to yield PS-b-PFS-GlcOH. Thus it was of interest to see how different methods of fabrication affect the particle size and, more importantly, the localization of the glucose units on the particle surfaces. Nanoprecipitation of the PS-b-PFS-GlcOH gave monodisperse particles with a mean diameter of 97 nm, while the particles made with aerosol flow reactor had mean diameter of 357 nm. For comparison, aerosol particles with mean diameter of 194 nm were made from the starting polymer PS-b-PFS and these nanoparticles were further surface modified with glucose. With light scattering and fluorescence spectroscopy it was shown that all the prepared particles bind fluorescently labeled lectin Con A via multivalent binding between the glucose units of nanoparticles and the lectin. Fluorescence resonance energy transfer (FRET) was used to study the interaction of fluorescent moieties on sub-nanometer scale and used to quantify the efficiency of binding. The studies showed that all the glycosylated nanoparticles gave FRET effect, and, when compared to the glucose concentration, the surface modified nanoparticles gave the highest response.

1. Introduction

Glycopolymers, synthetic macromolecules bearing sugar functionalities are increasingly studied due to their potential for applications in biomedicine as well as in understanding biological interactions of natural carbohydrates [1–3]. The pendant carbohydrate units can act as ligands for a broad spectrum of protein receptors and, therefore, play a significant role in a variety of biological processes. These include immunological recognition, interaction between bacteria or viruses with cells, tissue growth and repair. The interactions between carbohydrates and proteins are usually very weak but they can be multiplied if the polymer carries several carbohydrate pendants. This is the so called glycoside cluster effect.

Glycopolymers can be synthesised for example by direct polymerization of sugar-containing monomers. Well-defined polymers may be obtained by use of a variety of controlled or living polymerization methods [4,5]. However, synthesis conditions need to be carefully adjusted in order to solubilize both the glycomonomer

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and glycopolymer. When preparing block copolymers this becomes a major hurdle if the solubilities of the blocks are significantly different. On the other hand, during the last decade the so called "click" reactions have offered a straightforward route to tailor the polymer structures after polymerization. Click reactions combined with ionic or controlled radical polymerizations have become an indispensable tool for polymer chemists to prepare polymers with both controlled chain lengths and with novel functionalities. Thiol–para fluorine click chemistry is one of the click reactions that can be conveniently utilized in the case of poly(pentafluorostyrene) polymers [6–10], especially as the synthesis of well-defined poly(pentafluorostyrene) copolymers is possible with many routes [11–19]. The reaction can be easily monitored via ^{19}F NMR [2,6] and the functionalization can be done with a multitude of commercially available thiol reagents.

While glycopolymers often show good bioactivity and display the multivalent effect efficiently, their use as such for drug delivery or biosensing might be limited [1]. More complex polymeric structures such as micelles or particles are sometimes necessary to be able to encapsulate drugs or to prepare nanomaterials for applications such as sensing. Ways to achieve such structures is to make use of self-assembling of copolymers having a non-glycosylated hydrophobic block and a glycopolymer block via nanoprecipitation [20–23], via polymerization of hydrophilic macromonomers and hydrophobic comonomers in a polar solvent [24] or by grafting polymer particles [25,26]. In addition to these techniques it is also possible to prepare nanoparticles of amphiphilic polymers by aerosol flow reactor technique [27,28]. In the aerosol preparation a polymer solution is atomized as nanosized droplets into gaseous phase and allowed to dry in a laminar flow in the aerosol reactor. During the solidification the particles self-assemble and by varying the preparation conditions the size of the particles can be easily tuned. Importantly, preparation of such dry nanoparticles allows their post-modification. This enables their dispersion in the preferred solvent medium with possible diagnostic or sensing properties and the method could also allow addition of for example markers or excipients inside the particles during the solidification that may be difficult to achieve via self-assembly of particles from a solvent.

In this paper we describe the preparation of glucose-decorated nanoparticles via three different methods – nanoprecipitation from solution to form micellar structures, aerosol preparation of a glycosylated copolymer and thirdly post-glycosylation of naked aerosol nanoparticles. The aim is to study the possibility to make use of aerosol technique in preparation of glycosylated nanoparticles, either by directly preparing glycosylated particles or by post-modification of nanoparticles by click-reactions and compare them to self-assembled particles. The effect of nanoparticle size and their surface glucose content on the lectin recognition efficiency is investigated. Here, the surfaces of the different nanoparticles are further labeled with a Rhodamine B isothiocyanate and their interaction with a glucose-binding fluorescent lectin is studied. For these studies we make use of light scattering and fluorescence resonance energy transfer (FRET) to determine and quantify the lectin binding with nanoparticles.

2. Experimental

2.1. Materials

The synthesis of the semifluorinated polystyrene-block-polypentafluorostyrene copolymer, PS-b-PFS, via Atom Transfer Radical Polymerization (ATRP) has been published earlier [29]. The polymer used, PS_{74}-b-PFS_{74} was characterized by SEC and NMR. The reagents for PS-b-PFS modification, 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose, SH-GlcAc4 (GLYCON Biochemicals GmBH), triethylamine (Fluka), anthrone (Merck) (as well as the analysis grade solvents and reagents (THF, chloroform, DMF, methanol, sulfuric acid) were used as received unless otherwise stated. The fluorescent markers, lectin–fluorescein isothiocyanate conjugate from Canavalia ensiformis (ConA-FITC) (Sigma–Aldrich) and Rhodamine B isothiocyanate (RITC) (Sigma–Aldrich) were used as received.

2.2. Modification of PS-b-PFS

The glucopyranose modification [6,30] of PS-b-PFS was done by dissolving PS-b-PFS (80 mg, 0.27 mmol repeating units of PFS) and SH-GlcAc4 (112.5 mg, 0.309 mmol) in 10 mL dry DMF followed by addition of triethyl amine (78 mg, 0.77 mmol) to the solution. After stirring for 4 h at room temperature, the reaction mixture was concentrated and precipitated into cold methanol. The white precipitate was filtered, washed twice with methanol, and dried in a vacuum oven to afford 158 mg of a white powder, PS-b-PFS-GlcAc4, (yield 90%). The acetonide protected glycopolymer, PS-b-PFS-GlcAc4, was deprotected by adding sodium methanolate in methanol dropwise into PS-b-PFS-GlcAc4 (100 mg) dissolved in 10 ml of DMF and stirred for 1 h in room temperature. After stirring reaction mixture was concentrated and PS-b-PFS-GlcOH precipitated into cold methanol. After drying in vacuum a yield of 63% was achieved.

2.3. Nanoparticle preparation

2.3.1. Nanoprecipitation

2.5 ml PS-b-PFS-GlcOH in DMF (2 mg/ml polymer concentration) was titrated with 2.5 ml of distilled water and the solution turned turbid. The solution was placed in dialysis tube (Orange Scientific Cellu Sep T1 MW cutoff 3500) and dialysed against distilled water for several days yielding glycomicelles, Glu-Mic. Gravimetric analysis of the nanoparticle solution gave a polymer concentration of 1.0 mg/ml. For the light scattering and lectin binding studies the solution concentration was adjusted to 0.1 mg/ml.

2.3.2. Aerosol method

PS-b-PFS and PS-b-PFS-GlcOH copolymer precursor solutions of 1 w-% in DMF were used to prepare solid nanoparticles in the aerosol flow reactor [27,28]. A