SPHERICAL GLYCOPOLYMER BRUSHES

André Pfaff,[1] Andreas Walther,[1] Yan Lu,[2] Alexander Wittemann,[2] Matthias Ballauff[2] and Axel H. E. Müller[1]

[1] Makromolekulare Chemie II and [2] Physikalische Chemie I
Universität Bayreuth
95440 Bayreuth, Germany

Introduction

Carbohydrates are involved in a multitude of biological functions in living systems. Glycopolymers, synthetic polymers carrying carbo-hydrate moieties, have been widely investigated for medical and pharmaceutical applications, like drug delivery[1,2], cell culture substrates[3,4], macromolecular drugs[5,6] and surface modifiers[7,8]. The multitude of potential applications has prompted researchers to develop various synthetic strategies for the preparation of a variety of sugar-containing polymers with controlled functionalities and architecture[9-11].

Earlier we reported on the synthesis of glycopolymers of different topologies based on glucose-containing (meth)acrylates, in particular linear, hyperbranched, star-shaped polymers as well as cylindrical and planar brushes and their use in binding proteins and cells.[12-15]

Here we report the synthesis and characterization of a spherical glycopolymer brush via ATRP of the functional monomer methacryloyl-2,3:5,6-di-*O*-isopropylidene-D-mannofuranose (MAIMan). Using the "grafting from" technique these PMAIMan chains were grown from a crosslinked polystyrene core covered with a thin layer of an ATRP initiator (Figure 1). The PS cores were prepared by a 2-step emulsion polymerization.[16,17] After the polymerization of styrene with 5% divinylbenzene to form the crosslinked core, addition of the initiator-monomer BIEM implemented initiator functions for the following Atom Transfer Radical Polymerization (ATRP). After polymerization of the arms, the deprotection of the sugar units, under mild acidic conditions, led to spherical brushes consisting of 1-*O*-methacryloyl-α-D-mannose (MAMan) chains. The final product is a potentially biocompatible carrier for nanoparticles, proteins or pharmaceutics.

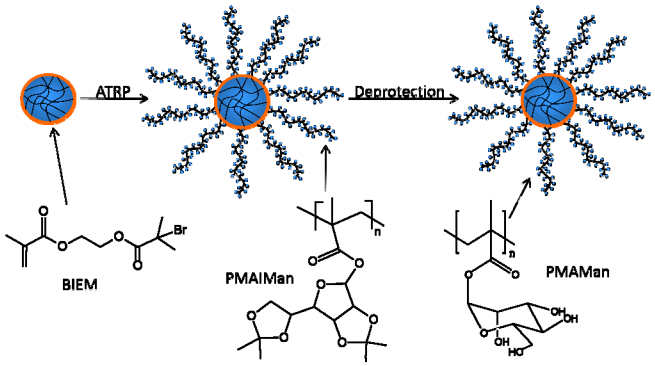

Figure 1. Synthesis of the sugar containing polymer brush.

One motivation for the preparation of the spherical glycopolymer brushes, was to investigate whether these brushes could act as carriers for catalytically active gold nanoparticles, formed in situ. Gold nanoparticles are of special interest because of their potential applications in electronic, optical and biomedical materials.[18-23]

Apart from these applications, glycopolymers are in general of great interest in medical and pharmaceutical applications. As a basic principle the adsorption of proteins from solution to solid substrates is driven by hydrophobic, electrostatic interactions or hydrogen bonding. Due to the high concentration of hydroxyl functions on the sugar-containing polymer brush, we also investigated the capability of these brushes to adsorb a commonly used model protein bovine serum albumin (BSA).

Experimental

ATRP towards linear PMAIMan. Polymerizations were carried out in a round-bottom flask sealed with a plastic cap. A representative example is as follows: A mixture of CuCl (1.51 mg, 0.0152 mmol), ethyl 2-bromoisobutyrate (2.96 mg, 0.0152 mmol) and MAIMan (0.5 g, 1.52 mmol) in ethyl acetate (1.0 g) were degassed for several minutes. After addition of PMDETA (2.63 mg, 0.0152 mmol) the color of the solution turned into green, indicating the dissolution of CuCl. The flask was placed in an oil bath at 60°C for 150 minutes. The conversion of the double bonds was 85%, as detected by ^{1}H-NMR. The solution was passed through a silica column and subsequently the polymer was precipitated from THF into MeOH.

ATRP towards spherical glycopolymer brushes. 50 mg of cross-linked PS-Inimer cores and 1.31 g of MAIMan (4 mmol) were added to a round bottom flask containing 2.6 g ethyl acetate and placed in a sonic bath for several minutes to create a homogenous dispersion. After addition of 2 mg CuCl (0.02 mmol) the mixture was degassed for 15 minutes, followed by the addition of PMDETA (3.47 mg, 0.02 mmol). The flask was placed in an oil bath at 60 °C for 420 minutes. The resulting polymer was precipitated from THF in MeOH, filtrated and finally freeze-dried.

Deprotection. The deprotection of PMAIMan and the spherical brushes was achieved under mild acidic conditions according to the method reported by Muthukrishnan et al.[12]

Formation of Au-nanoparticles. Au nanocomposite brushes were prepared by heating a mixture of $HAuCl_4$ and the polymer brush in water, without any additional reducing agent. For a typical experiment, 0.05 ml $HAuCl_4$ (0.09 M) solution was added to sugar-containing polymer aqueous solution (24 mg polymer dissolved in 10 g water) and stirred for 30 minutes under N_2. The mixture was heated to 98° C for 45 minutes and subsequent cleaned by dialysis against purified water (membrane: regenerated cellulose with MWCO 12.000 supplied by ZelluTrans ROTH).

Protein adsorption. The adsorption experiments were carried out as described by Wittemann et al.[24] In a typical run 100 mg of polymer were dissolved in 5 ml MES-buffer followed by the addition of 80 mg BSA in 5 ml MES-buffer. After stirring for 24 h at 4°C to equilibrate the system, the mixture was entered in a serum-replacement cell and flushed eight times with MES-buffer to remove non-adsorbed protein. The amount of non-adsorbed protein was determined by the extinction of the eluate at a wavelength of 278 nm by UV/VIS spectroscopy.

Results and Discussion

ATRP of MAIMan. In order to find a suitable system for the ATRP of the protected glycopolymer brushes we first investigated the effect of different catalysts and ligands on the ATRP of linear poly(MAIMAn). $(PPh_3)_2NiBr_2$ works as a single component-system, i.e. there is no ligand needed to dissolve the catalyst. Polymerizing MAIMan by the use of $(PPh_3)_2NiBr_2$ in ethyl acetate at 60°C led to low conversion due to a low polymerization rate. An increase of the reaction temperature from 60°C to 100°C results in a polymer with a number-average molecular weight of 30,500 and a narrow molecular weight distribution (PDI = 1.19) as determined by GPC in THF calibrated with PtBMA standards.

Other well-known catalysts in ATRP of (meth)acrylates are copper-(I)-halogenides in the presence of ligands. The synthesis of poly(MAIMan) by the use of CuBr/HMTETA in ethylacetate at 60 °C led to a polymer with a relatively broad molecular weight distribution of 1.48 and $M_{n,\,GPC}$ = 36,200. Also the change of the catalyst system to the less reactive CuCl led to no significant decrease of the PDI (1.45). As the activity of N-based ligands increases with the number of coordinating sites, the use of PMDETA should lead to a lower concentration of active species and therefore less termination reactions and a narrower PDI.[25]

The catalyst system CuBr/PMDETA led to well-defined PMAIMan after 150 minutes with a unimodal molecular weight distribution even at high conversion (95%) and $M_{n,GPC}$ = 40,300. An additional amount of 15% $CuBr_2$ further narrowed the molecular weight distribution from 1.17 to 1.09 at almost full conversion (98%) with an increase of the reaction time from 2.5 to 3.5 hours. Usage of CuCl/PMDETA led to well defined PMAIMan (PDI = 1.09) without any additional amount of Cu(II). The molecular weight (M_n = 39,000)

and molecular weight distribution (1.10) determined by MALDI-TOF MS are in agreement with those obtained by THF-GPC.

Synthesis of spherical glycopolymer brushes. As shown above, CuCl/PMDETA is an excellent system for the ATRP of linear PMAIMan. The use of these compounds for the preparation of spherical glycopolymer brushes led to core-shell-corona particles with well-defined arms (M_n = 78,000; PDI = 1.05), determined by MALDI-TOF MS after arm cleavage.

To obtain water soluble polymer brushes the protected sugar units were deprotected under mild acidic conditions.

Spherical brushes as carriers for catalytically active gold nanoparticles. The addition of $HAuCl_4$ to an aqueous solution of sugar containing polymer brushes and subsequent heating for 45 min led to gold nanoparticles with an average diameter of 4.3 nm. The change of the color from yellowish to violet indicates the reduction of the $AuCl_4^-$ ions. To investigate the catalytic activity of the gold nanoparticles we chose the reduction of *p*-nitrophenol by excess sodium borohydride. This reaction can be easily monitored by UV/VIS-spectroscopy. As can be seen in Figure 2 the characteristic absorption peak of *p*-nitrophenol at 400 nm disappeared whereas a new peak at 290 nm (due to *p*-aminophenol) appeared, confirming the capability of the sugar-containing polymer brushes to act as a carrier for catalytically active gold nanoparticles.

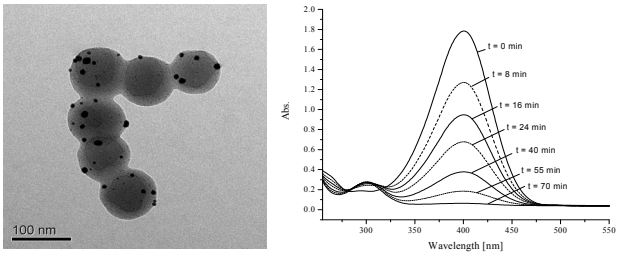

Figure 2. (left) TEM image of gold nanocomposite particles. (right) Catalytic reduction of *p*-nitrophenol in the presence of Au nano-composite particles.

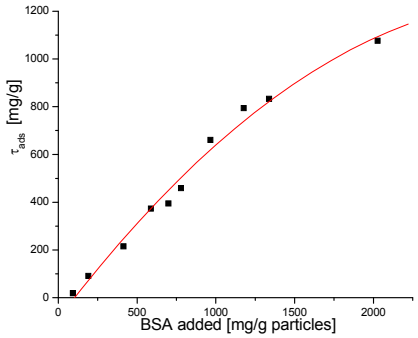

Figure 3. Amount of adsorbed BSA τ_{ads} per unit mass of latex particles plotted against the amount of added BSA per unit mass of latex particles.

Protein adsorption. In order to investigate the adsorption of proteins on the spherical brushes, we carried out several experiments with different concentrations of BSA. In Figure 3 the amount of adsorbed BSA τ_{ads} per unit mass of latex particles is plotted against the amount of added BSA per unit mass of latex particles. This indicates a strong adsorption of BSA to the polymer brushes. Even at the highest amount of added BSA (2g per g particles) the uptake of protein is higher than 50%, which could be attributed to the high concentration of hydroxyl groups in the polymer brush side chains.

Conclusions

Well-defined linear polymer and spherical brushes of mannose-containing monomers were obtained. The prepared brushes posses a high capability to adsorb bovine serum albumin. Furthermore the mannose units act as reducing agent for the formation of gold nanoparticles. Finally we demonstrated the catalytic activity of the nanocomposite particles.

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