Glycopolymers with Branched Architectures: Sugar Balls and Sugar Sticks

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Introduction

Glycopolymers are carbohydrate-bearing polymers, in particular, synthetic polymers containing sugar moieties as pendant groups.[1] Synthetic carbohydrate polymers with biocompatible and biodegradable properties are used in tissue engineering and controlled drug release devices. They are widely used for investigating glycopolymer-protein interactions.[2] Highly branched polymers due to their polyvalency can be used as effective tools to understand and manipulate carbohydrate-protein interactions.

Controlled/"living" radical polymerization has allowed well-defined and controlled synthesis of glycopolymers by a very facile and simple approach.[3-5] Here, we report the synthesis of branched glycopolymers of different architectures by employing atom transfer radical polymerization (ATRP). Hyperbranched glyco(meth)acrylates were synthesized using the protected sugar-carrying monomers, 3-O-(meth)acryloyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (AIGlc and MAIGlc, respectively), using self-condensing vinyl copolymerization (SCVCP) via ATRP, followed by deprotection.[6,7] The effect of different catalyst systems and comonomer ratios, γ, were investigated. Glycocylindrical brushes ("sugar sticks") having poly(3-O-methacryloyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside), (PMAIGlc) side chains, using the "grafting from" approach are also reported. Well-defined glycopolymer stars ("sugar balls") using a silsesquioxane-based macroinitiator and MAIGlc monomer were also synthesized successfully.

Experimental

Materials. Bis(triphenylphosphine)nickel (II) bromide ((PPh_3)$_2$NiBr$_2$, 99 %, Aldrich) was used as received. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97 %, Aldrich) and ethyl 2-bromo-2-isobutyrate (98%, Aldrich) were distilled and degassed. MAIGlc monomer was synthesized as reported very recently.[7] Synthesis of a methacrylic AB* inimer, 2-(2-bromo-isobutyryloxy)ethyl methacrylate (BIEM) was synthesized as reported previously.[8] The synthesis of the polyinitiator, poly[2-(2-bromoisobutryloxy)ethyl methacrylate] (PBIEM), employed for the synthesis of the glycocylindrical brushes was described earlier.[9] The synthesis of functionalized silsesquioxane nanoparticles with ca. 58 hydroxyl groups was reported earlier.[10] A macroinitiator for glycostars was prepared by treating the dried nanoparticles with 2-bromo-2-methylpropionyl bromide in the presence of 4-(N,N-dimethylamino) pyridine. This was then dried *in vacuo*. The molecular weight data obtained by MALDI-TOF spectrometry were M_n = 10,200 (calcd: 12,500) and M_w /M_n 1.25. The transformation of branched poly(MAlGlc)s of different architectures into the corresponding water-soluble hydroxyl polymers, poly (3-O-methacryloyl-α,β-D-glucopyranoside)s was achieved by conventional method as reported by Fukuda et al.[3]

Characterization. The branched glycopolymers were characterized by conventional GPC and GPC/viscosity using THF as eluent at a flow rate of 1.0 ml/min at room temperature. GPC with a multi-angle light scattering detector (GPC-MALS) was used to determine the absolute molecular weights of the brushes and stars with THF as a eluent at a flow rate of 1.0 ml/min.

^{1}H NMR spectra of the polymers were obtained on a Bruker AC-250 spectrometer at room temperature. The scanning force microscopy (SFM) images were taken with a Digital Instruments Dimension 3100 microscope operated in Tapping Mode. The SFM measurements in water were performed on a Digital Instrumetns Nanoscope III Multimode AFM operated in Tapping mode equipped with a 12 μm scanner and a liquid cell. The samples for SFM measurements in air were prepared either by dip-coating from dilute solutions of brushes in THF onto to a freshly cleaved mica surface or by spin-casting onto a carbon-coated mica surface. Cryogenic transmission electron microscopy (cryo-TEM) images were measured using a Zeiss EM922 EF-TEM.

Results and Discussion

Synthesis of Hyperbranched Glycopolymers. Linear polymers of MAIGlc could be obtained by both the CuBr/HMTETA and the (PPh_3)$_2$NiBr$_2$ catalyst systems. We used (PPh_3)$_2$NiBr$_2$ in ethyl acetate at 100 °C for the synthesis of hyperbranched polymers via SCVCP. The influence of the monomer-to-inimer ratio, $\gamma = [MAIGlc]_0/[BIEM]_0$ ($1 \leq \gamma \leq 25$), was investigated, keeping the comonomer-to-catalyst ratio at a constant value of $\mu = ([MAIGlc]_0 + [BIEM]_0)/[(PPh_3)_2NiBr_2]_0 = 100$. Under that condition, almost full conversion was reached within 5 h for $\gamma > 10$. The tendency is markedly different from the case of the glycoacrylate AIGlc,[6] where ca. 120 h were required to reach full conversion at $\gamma = 10$. This indicates that the bulky isopropylidene-protected glucofuranoside side group is not a crucial factor to retard the polymerization rate. The molecular weights and molecular weight distribution of the copolymers were characterized by GPC/viscosity using universal calibration and conventional GPC in THF. The results are given in Table 1. In all samples, the molecular weights determined by GPC-viscosity are higher than the apparent ones obtained by GPC, indicating highly branched structures. The ratios of $M_{n,GPC-VISCO}$ to $M_{n,GPC-THF}$ of the copolymers are 2.3-1.9, suggesting that a suitable amount of AB* inimer, BIEM, in the feed leads to a considerably compact structure, and the difference in the amount has an influence on the molecular weights and compact structure in solution. It is known that the Mark-Houwink constant typically varies between 0.5 and 0.2 for branched polymers depending on the degree of branching. Typical Mark-Houwink plots are shown in Figure 1. ^{1}H-NMR spectra depicting the homopolymer and the copolymers of MAIGlc are shown in Figure 2. It can be seen that with the increase in the comonomer ratio, γ, the intensity of the doublet at 1.85 ppm (marked by an ellipse) decreases, which is assigned to methyl protons adjacent to a bromine atom (A* and M* in the polymer chain end and B* in the 2-bromoisobutyryloxy group), respectively.

Table 1. Self-Condensing Vinyl Copolymerization of BIEM and MAIGlc at Different Comonomer Ratios γ[a]

γ[b]	$M_{n,GPC-THF}$[c] (M_w/M_n)	$M_{n,GPC-VISCO}$[d] (M_w/M_n)	α[e]
1	9300 (1.81)	17500 (2.01)	0.20
5	10100 (1.51)	21000 (1.55)	0.30
10	11100 (1.53)	23300 (1.57)	0.34
25	15400 (1.44)	29800 (1.71)	0.28

[a] Copolymerization at 100 ºC with (PPh_3)$_2$NiBr$_2$ at a constant comonomer-to-catalyst ratio, $\mu = ([MAIGlc]_0 + [BIEM]_0)/[catalyst]_0 = 100$ in the presence of ethyl acetate (50 wt % to MAIGlc). [b] $\gamma = [MAIGlc]_0/[BIEM]_0$. [c] Determined by GPC using THF as eluent with PtBMA standards. [d] Determined by GPC/viscosity measurement. [e] Mark-Houwink exponent as determined by GPC/viscosity measurement.
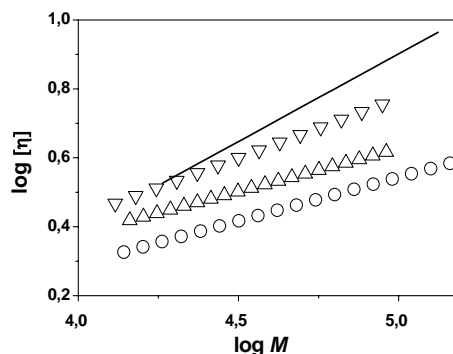

Figure 1. Mark-Houwink plots for the polymers formed by SCVCP of BIEM and MAIGlc: γ = 1.5 (○), 5.0 (△), 10 (▽). The intrinsic viscosity of a linear poly(MAIGlc) (—) is given for comparison.
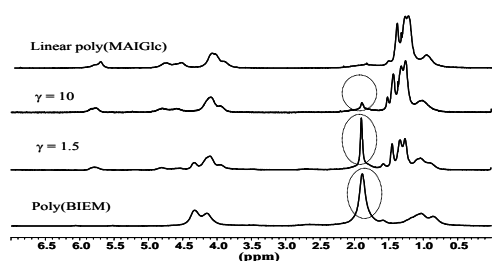
Figure 2. ^{1}H NMR spectra ($CDCl_3$) of the linear and branched poly(MAIGc)s.

Synthesis of Glycocylindrical Brushes ("Sugar Sticks"). Results of the synthesis of glycocylindrical brushes using CuBr/HMTETA as the catalyst system, MAIGlc as monomer and PBIEM as polyinitiator are reported in Table 2. The polyinitiator was synthesized from the precursor, poly(2-hydroxyethyl methacrylate), PHEMA, made by anionic polymerization.[9] The number-average degree of polymerization is 1500. Very low conversions are maintained in order to avoid inter-macromolecular coupling reactions. The molar masses obtained by light scattering are significantly higher than those obtained by GPC indicating the compact structure of the brushes. In our system, larger radii of gyration, R_g, were observed compared to brushes with PtBuA side chains at a given DP, caused by a stronger stretching of the backbone due to the bulky sugar moiety of the glycomonomer.[9] The characteristic worm-like structure of the glycocylindrical brushes was directly visualized on mica and carbon-coated mica by SFM as seen in Figure 3. The number-average length of 20 individual cylinders in Figure 3a of Brush 1 is $L_n = 110$ nm, with a polydispersity $L_w/L_n = 1.04$. The brushes are not completely stretched due to the low DP of the side chains. The deprotection of the isopropylidene groups of the PMAIGlc side chains resulted in water-soluble glycocylindrical brushes. SFM in water and cryogenic transmission electron microscopy showed a highly stretched, rod-like structure as seen in Figure 4.

Table 2. Synthesis and Characterization of Glycocylindrical Brushes[a]

brush	$[M]_0/[I]_0$	Time (min)	conv[b] (%)	10^{-4} x $M_{n,GPC}$[c]	PDI	10^{-4} x $M_{n,MALS}$	R_g (nm)
1	300	30	5	40.80	1.19	900	41.5
2	200	25	10	58.64	1.07	1120	59.5

[a] Solution polymerization in ethyl acetate (50 wt % to MAIGlc) at 60 ºC at constant $[I]_0 : [CuBr]_0 : [HMTETA]_0 = 1: 0.5 : 0.5$. [b] Determined by ^{1}H-NMR. [c] Determined by GPC using THF as eluent with PS standards.
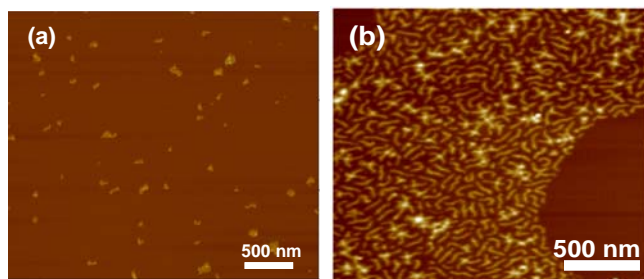

Figure 3. SFM Tapping Mode height image of (a) Brush 1, spin-coated from dilute solution on carbon-coated mica, (z-range: 20 nm) and (b) Brush 2 dip-coated from dilute solution on mica, (z-range: 15 nm).

The SFM image of the deprotected Brush 1 in water in Figure 4a appears to be more stretched than before hydrolysis owing to the hydration of sugar moieties. The insert shows a single cylinder having a length of 135 nm which is considerably larger than that of the protected polymer, but still shorter than the contour length, which could be due to the very low DP of the side chains. These results are supported by cryo-TEM measurements as shown in Figure 4b. The length of a single cylinder shown as an insert in Figure 4b is 130 nm which is comparable to the length measured by SFM.
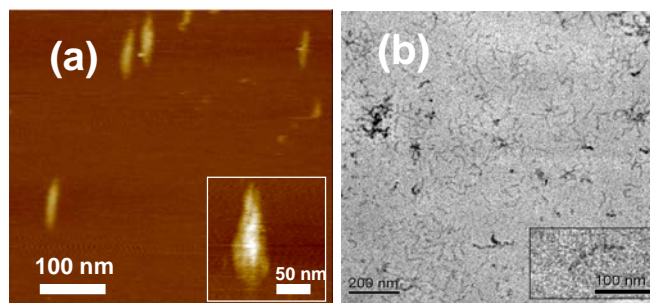

Figure 4. (a) SFM Tapping Mode height image of deprotected Brush 1 in water (z-range: 10 nm) and (b) cryo-TEM image of the deprotected Brush 1.

Synthesis of Glycopolymer stars ("Sugar balls"). A macroinitiator with ca 58 functions was prepared from functionalized silsesquioxane nanoparticles and used for the synthesis of well-defined glycopolymer stars ("sugar balls") of MAIGlc. When the solution polymerization in ethyl acetate (50 wt % to MAIGlc) was carried out at 60 ºC in the presence of CuBr/HMTETA (0.5/0.5) and $[MAIGlc]_0/[I]_0 = 100/1$, the conversion reached 16 % in 10 min. Very low conversions were maintained to avoid coupling reactions. The molecular weight data of the resulting polymer obtained by conventional GPC using PtBMA calibration are $M_n = 1.18 \times 10^5$ and $M_w/M_n = 1.09$. The data obtained by GPC/viscosity and GPC-MALS were 2.45×10^5 (1.25) and 2.43×10^5 (1.10), repectively, which are fairly in agreement with the calculated molecular weight (3.04×10^5) of the glycostar. Synthesis of glycostars of different arm lengths are under progress.

Conclusions

We have demonstrated that both the $(PPh_3)_2NiBr_2$ and the CuBr/HMTETA catalyst systems can be successfully used for the ATRP of MAIGlc. Copolymerization with BIEM resulted in randomly branched poly(MAIGlc)s with relatively high molecular weights. Glycocylindrical brushes and glycopolymer stars could be synthesized successfully. The deprotection of isopropylidene protecting groups resulted in water-soluble brushes with branched architectures. This work substantially broadens and extends the scope of facile and straightforward strategy for generating water-soluble glycopolymers and their precursors by a controlled polymerization technique. Such branched glycopolymers can be manipulated for various biological and medicinal applications.

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