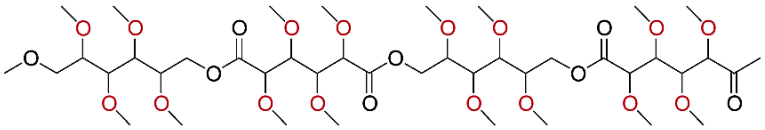
Communication

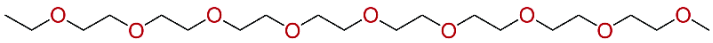
A Novel Carbohydrate-Derived Side-Chain Polyether with Excellent Protein Resistance

Mark Metzke, Jane Z. Bai, and Zhibin Guan

J. Am. Chem. Soc., **2003**, 125 (26), 7760-7761• DOI: 10.1021/ja0349507 • Publication Date (Web): 30 May 2003



1 Carbohydrate-derived polymer: side-chain polyether



2 PEG: main-chain polyether

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Published on Web 05/30/2003

A Novel Carbohydrate-Derived Side-Chain Polyether with Excellent Protein Resistance

Mark Metzke, Jane Z. Bai, and Zhibin Guan*

Department of Chemistry, 516 Rowland Hall, University of California, Irvine, California 92697-2025

Received March 2, 2003; E-mail: zguan@uci.edu

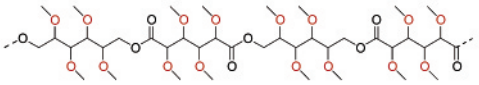
The ability to resist nonspecific protein adsorption is a major indicator of a material's biological inertness or biocompatibility.[1] Applications of protein resistant materials include prostheses, sensors, substrates for enzyme-linked immunosorbent assays (ELISAs), materials for use in contact lenses, and implanted devices.[1] More recent applications include systems for patterned cell cultures,[2] tissue regeneration,[3] microfluidic systems,[4] drug delivery,[5] and systems for high-throughput screening of proteins[6] or cells.[7] With the recent intense development of new biomedical applications, there is a great need to develop new biomaterials with new structures and properties. Herein we report a new carbohydrate-derived side-chain polyether (**1**) as a protein resistant material (Chart 1).

Poly(ethylene glycol) (PEG) is among the most commonly used biomaterials because of its exceptional biocompatibility.[8] PEG shows unsurpassed resistance to nonspecific protein binding. As a simple main-chain polyether, however, PEG has several limitations. Two of those limitations follow: PEG can only be functionalized at the chain ends, and the polymer is not biodegradable. For many biomedical applications, such as in vivo drug delivery and tissue engineering, biodegradability and flexibility to incorporate desired functionalities are critical. Phosphorylcholine (PC)-containing polymers were also shown to have good protein resistance; however, they are generally nonbiodegradable because PC groups were usually attached to side chains of vinyl polymers.[9] If a polymer can be created to be functionalizable and biodegradable while maintaining protein resistance, it would be invaluable for many biomaterial applications.

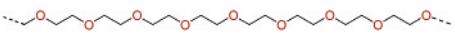
Despite extensive research, the molecular mechanism for PEG's excellent protein resistant ability remains to be fully understood.[10,11] To generate structure−property correlation and to discover new structures resistant to protein binding, Whitesides[11] and Mrksich[12] groups prepared self-assembled monolayers (SAMs) presenting substrates with various functional groups to test the protein binding of those substrates. Through their studies, Whitesides and coworkers[11] observed the following properties among protein resistant substrates: (a) hydrophilicity, (b) the ability to accept hydrogen bonding, (c) the inability to donate hydrogen bonding, and (d) a net neutral charge. Among other motifs, Whitesides[11] and Mrksich et al.[12] reported SAMs presenting carbohydrates exhibiting good protein resistance. Others have also reported that incorporation of carbohydrate moieties could enhance polymer protein resistance.[13] These studies inspired us to design a carbohydrate-derived side-chain polyether (Chart 1) that we envision to combine protein resistance, biodegradability, and functionalizability.

The design of **1** as a protein resistant biomaterial is based on the following considerations. First, structure **1** matches the four criteria outlined in Whitesides's studies. It has a polyether structure analogous to PEG; however, it is a side-chain instead of main-chain polyether. More specifically, on the basis of the structural resemblance between our monomeric units and the methylated sorbitol motif shown by Whitesides et al.,[11] we envision polymer **1** to demonstrate good protein resistance. Second, the repeating units in **1** are connected with ester linkages, rendering it biodegradable. Because ester linkages can be cleaved by hydrolysis, polymer **1** should eventually degrade into monomers in biological systems. Finally, the carbohydrate-derived monomers are naturally abundant and can be functionalized with various ligands. In addition, copolymerization with other diol or diacid monomers carrying desired functional groups should serve to introduce further functionalities to the polymer. The biodegradability and functionalizability should potentially lead to a broad range of applications for this polymer.

Chart 1. Comparison of Main-Chain and Side-Chain Polyether

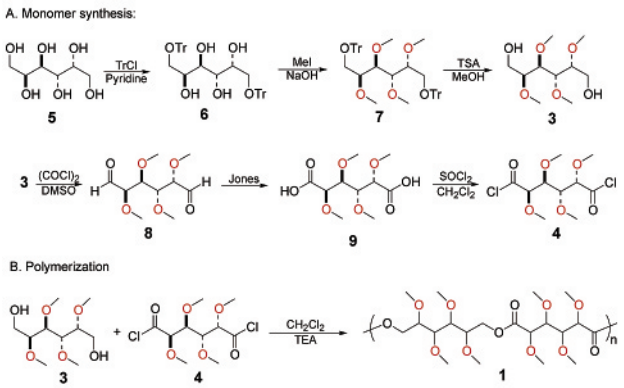


1 Carbohydrate-derived polymer: side-chain polyether



2 PEG: main-chain polyether

Scheme 1. Synthesis of Monomers and the Side-Chain Polyether **1**



The synthetic route for the monomeric building blocks and the polymer **1** is shown in Scheme 1. The diol monomer **3** was synthesized from a commercially available reduced sugar, dulcitol **5**. After selective protection of the two primary hydroxyls with a trityl group, the secondary hydroxyl groups were permethylated with MeI under basic condition. The diol monomer **3** was obtained in good overall yield following deprotection of the trityl groups.

Direct oxidation at various conditions to convert **3** into diacid **9** was complicated by side reactions.[14] To circumvent this, a two-step oxidation was applied in which the diol **3** was first oxidized to dialdehyde **8** through Swern oxidation and then further oxidized to the diacid **9** through Jones oxidation. Treatment of **9** with thionyl chloride at room temperature gave clean formation of the diacid-chloride monomer **4**. After testing different polymerization conditions, we determined that a homogeneous solution polymerization

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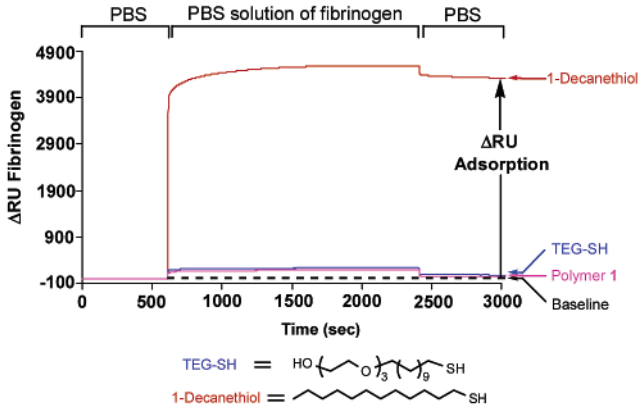

Figure 1. SPR data for the adsorption of fibrinogen onto polymer **1** and control surfaces.

of **3** and **4** in dichloromethane using triethylamine as base gave the best results, affording polymer **1** with a number-averaged molecular weight (M_n) of 29 000 g/mol and a weight-averaged molecular weight M_w of 44 000 g/mol. The monomodal GPC trace and the relatively narrow polydispersity (M_w/M_n = 1.5) indicate that the polymerization follows the standard step-growth mechanism. Polymer **1** is shown without specification of stereochemistry because there exists a combination of enantiomeric and diastereomeric repeating units in the polymer.[15]

After the successful synthesis of the side-chain polyether **1**, its protein resistant ability was measured by surface plasmon resonance (SPR) spectroscopy. Following Whitesides and Mrksich's studies,[11,12] we measured the adsorption of two proteins: fibrinogen, a large (340 kD) blood plasma protein that adsorbs strongly to hydrophobic surfaces, and lysozyme, a small protein (14 kD, PI = 12) that is positively charged under the conditions of our experiment (phosphate buffered saline, PBS, pH 7.4). Fibrinogen is used as a model for "sticky" serum proteins,[16] and lysozyme is used as a model for electrostatic adsorption of proteins to surfaces.[17] Because of the good water solubility of polymer **1**, a terminal thiol-functionalized polymer **1** was prepared and chemisorbed onto a gold slide for the SPR studies (Supporting Information). SAMs of 1-decanethiol and a tri(ethylene glycol) functionalized alkylthiol (TEG-SH) on gold were used for positive and negative control studies.

The SPR data for adsorption of fibrinogen in Figure 1 show that polymer **1** has excellent protein resistance. The amount of protein adsorbed (ΔRU = change in response units) as measured by SPR was determined by subtracting the value of RU prior to the injection of protein from the value of RU measured 10 min after the completion of the protein injection. Like the TEG model SAM, the polymer **1** surface showed minimum adsorption of fibrinogen. Within the experimental error, the amount of protein adsorbed onto the polymer **1** surface is about the same as the amount adsorbed onto the model PEG SAM, which is about 1% of the amount adsorbed onto the hydrophobic 1-decanethiol SAM. The lysozyme adsorption data also showed that polymer **1** has the same protein resistance as the model TEG SAM (Supporting Information). The mechanism for protein resistance may operate differently between the polymer **1** film and TEG SAM. While the interaction of water with the SAM surface plays a critical role in TEG SAM, for the polymer **1** film, the entropic penalty associated with displacing the polymer chains by proteins may play an important role in preventing protein adsorption.[18]

In summary, we report a carbohydrate-derived side-chain polyether as a novel biomaterial exhibiting excellent protein resistance. The polymer was synthesized through step-growth polymerization with carbohydrate-derived monomers. The ester linkage in the polymer renders it biodegradable. We are currently investigating the influences of various structural permutations on the biocompatibility and biodegradability of this family of polymers. Through the design of monomer structure and copolymerization, polymers with desired structures and properties will be constructed to meet various biomedical applications. This introduces a novel biomaterial that combines excellent protein resistance, biodegradability, and functionalizability.

Acknowledgment. We thank the Arnold and Mabel Beckman Foundation and the University of California at Irvine (UCI) for partial financial support. We thank Prof. Larry Marsh and Ms. Adeela Syed at the Department of Developmental and Cell Biology of UCI for their assistance in the SPR study. Z.G. gratefully acknowledges a Beckman Young Investigator Award, a DuPont Young Faculty Award, a NSF CAREER Award, and a 3M Non-Tenured Faculty Award.

Supporting Information Available: Experimental details for the synthesis, polymerization, characterization, and SPR studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Lactonization was observed in direct oxidation, which was presumably formed through oxidation of cyclic hemiacetal intermediate after the first oxidation of one terminal alcohol to aldehyde.
(15) Four types of stereoisomeric repeating units can be formed in the polymer: (i) SRSR/SRSR, (ii) SRSR/RSRS, (iii) RSRS/SRSR, and (iv) RSRS/RSRS, where S and R represent the stereoconfiguration of the chiral centers in both monomeric units. Among them, i, iv and ii, iii are two enantiomeric pairs, and the two pairs are diastereomers. Two sets of peaks were observed in the ^{1}H NMR spectrum of polymer **1**, corresponding to the two sets of diastereomeric repeating units. A model dimer (**13** in the Supporting Information) was prepared whose ^{1}H NMR spectrum also shows two sets of peaks for the diastereomers.
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JA0349507