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Improved antiviral properties of chain end lipophilic fucoidan-mimetic glycopolymers synthesized by RAFT polymerization

Mattias Tengdelius[a,*], Kwan Yee Cheung[b], May Griffith[b,c], Peter Påhlsson[b], Peter Konradsson[a]

[a] Division of Organic Chemistry, Department of Physics, Biology and Chemistry (IFM), Linköping University, SE-581 83 Linköping, Sweden
[b] Division of Cell Biology, Department of Clinical and Experimental Medicine (IKE), Linköping University, SE-581 83 Linköping, Sweden
[c] Maisonneuve-Rosemont Hospital Research Centre and Dept. of Ophthalmology, University of Montreal, Montreal, QC H1T 4B3, Canada

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ABSTRACT

Sulfated polysaccharides and synthetic glycopolymers are promising candidates as antiviral drugs but have failed in clinical trials most likely due to lack of virucidal activity. However, studies have shown that incorporation of lipophilic end groups to oligosaccharide chains is a mean to gain the desired virucidal properties. Here, we describe the introduction of lipophilic end groups to sulfated α-L-fucoside-pendant polymethacrylamides, also known as fucoidan-mimetic glycopolymers, by RAFT polymerization. RAFT agents bearing octadecyl, dioctadecyl and cholesteryl groups were used to synthesize lipoglycopolymers of different chain lengths. Short lipoglycopolymers bearing lipophilic end groups showed an improved ability to block viral entry and infection of cells compared to glycopolymers without lipophilic end groups. Short lipoglycopolymers bearing octadecyl or dioctadecyl end groups, also completely stopped the spreading of the viral infection. However, these lipoglycopolymers did not show actual virucidal properties. Nevertheless, we have described a first step towards obtaining virucidal synthetic glycopolymers for clinical use.

1. Introduction

The enveloped, neurotropic DNA virus Herpes Simplex Virus type 1 (HSV-1) is one of the most frequent pathogens found in humans. 50–90% of individuals worldwide are estimated to be seropositive for this virus. Infections by HSV-1 are caused by direct contact with an infected individual and typical clinical manifestations are blisters around the mouth and keratitis in the eyes. Around 500,000 cases of corneal HSV infections are reported in the US alone each year and the resulting stromal keratitis is the leading cause of corneal blindness due to an infectious agent in developed countries. HSV-1 infection can also be the cause of life-threatening diseases such as neurological disorders and encephalitis. The viral infection is considered incurable as the virus avoids clearance by the immune system and can enter neurons in a dormant state called latency. These neurotropic and neuroinvasive properties have also been linked to neurodegenerative disorders such as Alzheimer's disease [1].

The majority of clinically used therapies assert their antiherpetic activity by inhibiting the DNA replication of the virus. Most of these DNA replication inhibitors, e.g. acyclovir, are efficient but suffers from low bioavailability and increasing viral resistance [1]. To circumvent these shortcomings, the use of naturally occurring sulfated polysaccharides has, amongst others, emerged as an alternative therapeutic option. Sulfated polysaccharides such as heparin, chondroitin sulfate, carrageenan, ulvan, xylomannan sulfate, and fucoidan do not inhibit viral DNA replication, but they interfere with viral entry to the host cells by mimicking the heparan sulfates that serve as attachment points for the viral glycoproteins to the cell surface [2–5].

Of these polysaccharides, the heterogeneous marine α-L-fucoside-based fucoidan is perhaps the most studied one. Apart from its antiviral properties, fucoidan is also known to be anticoagulant, antithrombotic, antitumorigenic, and anti-inflammatory [6,7], and can induce platelet aggregation [6]. Despite showing antiviral properties both *in vitro* and *in vivo*, fucoidan is not virucidal [8]. The lack of virucidal activity has been suggested as a reason for the failure of sulfated polysaccharides in human clinical trials [9]. To bring fucoidan one step closer to clinical use, this would be a desirable obstacle to overcome.

In general, the anti-herpetic properties of sulfated polysaccharides have been shown to increase with increasing degree of sulfation (DS) and molecular weight [2]. Nyberg et al. demonstrated that a heparan sulfate-mimetic tetrasaccharide was the minimum fragment necessary to inhibit HSV-1 infection and reduce cell-to-cell spread by interfering

Abbreviations: CAC, critical aggregation concentration; Chol, cholesteryl; DAPI, 4′,6-diamidino-2-phenylindole; DiOct, dioctadecyl; DS, degree of sulfation; FC, flash column chromatography; HCEC, human corneal endothelial cells; HSV-1, herpes simplex virus-1; LPG, lipoglycopolymer; Oct, octadecyl

* Corresponding author.
E-mail address: matte@ifm.liu.se (M. Tengdelius).

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with viral entry to the cells [10]. The same group later showed that introduction of certain lipophilic groups, particularly cholestanyl, at the oligosaccharide chain end would also give the heparan-mimetic oligosaccharides virucidal properties. These lipo-oligosaccharides showed antiherpetic properties by both interfering with virus attachment to the cells and irreversibly inactivating the virus [9,11].

We have previously demonstrated that polymethacrylamides with pendant sulfated α-L-fucosides exhibit antiherpetic properties similar to those of fucoidan [12]. Here, we attempted to impart virucidal properties to these fucoidan-mimetic glycopolymers by introducing lipophilic groups to one of the polymer chain ends through reversible addition-fragmentation chain transfer (RAFT) polymerization using tailored RAFT chain transfer agents (CTAs). The antiherpetic and potentially virucidal properties of the resulting lipoglycopolymers were then evaluated.

2. Results and discussion

2.1. Synthesis of lipophilic RAFT CTAs

We have previously synthesized fucoidan-mimetic glycopolymers through cyanoxyl-mediated free radical [12], thiol chain transfer free radical [13] and RAFT [14] polymerization techniques. Of the three, RAFT polymerization has shown superior control over the polymer chain length in the synthesis of fucoidan-mimetic glycopolymers and was thus chosen as the polymerization technique in this work. Partially lipophilic polymers are generally synthesized by RAFT polymerization via two different approaches. The most common approach is to synthesize block-*co*-polymers where at least one block is made up of lipophilic monomers [15,16], but partially lipophilic polymers have also been synthesized by using RAFT CTAs bearing lipophilic groups, yielding chain end lipophilic polymers [17]. The lipophilic groups that are known to confer virucidal properties to sulfated oligosaccharides are comparatively small in size [9]. As the synthesis of block-*co*-polymers generally yield comparatively large lipophilic blocks, the use of lipophilic RAFT CTAs was chosen as the strategy for synthesizing partially lipophilic fucoidan-mimetic glycopolymers.

The previously synthesized RAFT-derived fucoidan-mimetic glycopolymers were polymerized using 2-Cyano-2-propyl benzodithioate (CPBDT) as the CTA [14]. In the synthesis of lipophilic CTAs a derivative of CPBDT, 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid *N*-succinimidyl ester (**1**), was used as the starting material (see Scheme 1). To avoid hydrolysis of the lipophilic end groups during the synthesis of the polymers, **1** was coupled with amines rather than alcohols to yield amides which do not undergo Zemplén deacetylation compared to esters. Coupling of **1** with commercially available octadecylamine and dioctadecylamine yielded lipophilic **Oct–CTA** and **DiOct–CTA** in 84 and 83% yield, respectively.

Reagents and conditions: (a) cystamine dihydrochloride, Et_3N, DCM, MeOH, r.t., overnight; (b) octadecylamine *or* dioctadecylamine *or* **3**, CH_2Cl_2, r.t., overnight; (c) (i) AIBN, 1,4-dioxane ([M]:[CTA] = 50:1), 90 °C, 24 h; (ii) AIBN (6.6 eq./polymer chain), 1,4-dioxane, 90 °C, overnight; (iii) NaOMe, MeOH, r.t., overnight; (d) (i) AIBN, 1,4-dioxane ([M]:[CTA] = 25:1), 90 °C, 24 h; (ii) NaOMe, MeOH, r.t., overnight; (e) (i) SO_3•Pyr, DMF, r.t., overnight; (ii) NaCl, H_2O, r.t., over two nights; (f) (i) SO_3•Pyr, DMF, r.t. overnight; (ii) $NaHCO_3$, NaCl, H_2O, r.t. overnight.

In addition to the octadecyl and dioctadecyl lipophilic groups, cholesteryl was also introduced to the glycopolymer chain as this lipophilic group has shown promising results in a previous study by Ekblad et al. [9]. Cholesteryl with an amine functionality has previously been synthesized via multiple steps [18]. As a more rapid approach, the amine functionality was introduced to the cholesteryl group via thiol-disulfide exchange. Thiocholesterol (**2**) reacted with cystamine which gave cholesteryl-functionalized amine **3** in 69% yield. Coupling of amine **3** with **1** in turn produced lipophilic **Chol–CTA** in 74 % yield.

2.2. Synthesis of chain end lipophilic fucoidan-mimetic glycopolymers

Using our previously described procedure [14], the monomer (M) 2-Methacrylamidoethyl 2,3,4-tri-*O*-Acetyl-α-L-fucopyranoside (**4**) [12] was polymerized in 1,4-dioxane using azobisisobutyronitrile (AIBN) as the initiator and Oct–CTA, DiOct–CTA or Chol–CTA as the CTA with [M]:[CTA] = 50:1 (see Scheme 1). In short, the monomer was polymerized, the benzodithioate end group removed by treatment with excess AIBN and the lipoglycopolymers furnished by Zemplén deacetylation of the acetyl protecting groups on the pendant fucosides. Polymerization with Oct–CTA, DiOct–CTA or Chol–CTA gave lipoglycopolymer Oct–LPG_{50}, DiOct–LPG_{50} or Chol–LPG_{50} in 41, 15 or 8% yield, respectively, after polymer modification and work-up (see Table 1). The drastic drop in yield is believed to be caused by unwanted dissolution of the polymers when suspended in diethyl ether during the purification steps. The increase in solubility was most likely caused by the larger lipophilic groups on the initiating chain ends of **DiOct–LPG_{50}** and **Chol–LPG_{50}** compared to **Oct–LPG_{50}**.

To study the effect of differences in chain length on the antiviral properties, lipoglycopolymers were also synthesized using the same monomer, initiator and CTAs but with a target [M]:[CTA] of 25:1. As the resulting polymers would likely be even more soluble in diethyl ether than **Oct–LPG_{50}**, **DiOct–LPG_{50}** and **Chol–LPG_{50}** due to an expected shorter chain length, all purification steps involving suspension in diethyl ether were avoided. The crude lipoglycopolymers given after the polymerization step were instead, without purification, directly subjected to Zemplén deacetylation in NaOMe/MeOH overnight and then purified, from e.g. unreacted monomers, by dialysis. Using **Oct–CTA**, **DiOct–CTA** and **Chol–CTA** as CTAs gave lipoglycopolymers **Oct–LPG_{25}**, **DiOct–LPG_{25}** and **Chol–LPG_{28}** in more evenly distributed 33, 44 and 29% yields, respectively.

^{1}H NMR spectra of the lipoglycopolymers (see Fig. 1) showed results in good agreement with our previously synthesized glycopolymers [12–14]. Peaks corresponding to the lipophilic groups on the initiating chain ends of the lipoglycopolymers were visible in all spectra.

Analysis by gel permeation chromatography (GPC) showed that all lipoglycopolymers had a bimodal distribution (see Fig. 2) with a low molecular weight major product and high molecular weight minor product, as previously observed with RAFT-derived fucoidan-mimetic glycopolymers [14]. The high molecular weight minor fractions likely stem from self-polymerization of glycomonomer **4**, independent of the RAFT mechanism. The polymers in these fractions are therefore not expected to bear lipophilic end groups. The extent of the minor peaks were 12, 9, 8, 6, 13 and 13% w/w of the total polymer compositions for **Oct–LPG_{50}**, **Oct–LPG_{25}**, **DiOct–LPG_{50}**, **DiOct–LPG_{25}**, **Chol–LPG_{50}** and **Chol–LPG_{28}**, respectively. The number average molecular weight (M_n) and dispersity $Đ$ of the minor high molecular weight polymers were for **Oct–LPG_{50}** (M_n = 107,000 g/mol, $Đ$ = 2.76), **Oct–LPG_{25}** (M_n = 52,100 g/mol, $Đ$ = 2.07), **DiOct–LPG_{50}** (M_n = 56,100 g/mol, $Đ$ = 1.55), **DiOct–LPG_{25}** (M_n = 84,900 g/mol, $Đ$ = 1.87), **Chol–LPG_{50}** (M_n = 44,100 g/mol, $Đ$ = 1.51) and **Chol–LPG_{28}** (M_n = 42,000 g/mol, $Đ$ = 1.76). The M_n of the major products of the lipoglycopolymers were significantly lower (see Table 1) and all the lipoglycopolymers showed fairly narrow dispersities ($Đ$ = 1.13–1.33). Comparing the M_n of lipoglycopolymers polymerized with the same CTA but different [M]:[CTA] ratio (M_n = 4410 and 1970 g/mol for **Oct–LPG_{50}** and **Oct–LPG_{25}**; 4650 and 2560 g/mol for glycopolymers **DiOct–LPG_{50}** and **DiOct–LPG_{25}**; and 3080 and 2140 g/mol for glycopolymers **Chol–LPG_{50}** and **Chol–LPG_{28}**, respectively) showed control over the polymer chain length by varying the [M]:[CTA] ratio. There were initial concerns the thiols on the terminating chain ends of **Oct–LPG_{25}**, **DiOct–LPG_{25}** and **Chol–LPG_{28}** would cause coupling of the polymer chains via disulfide formation. The lack of noticeable shoulders on the major peaks in the GPC elugrams of these lipoglycopolymers indicates no significant amount of such disulfide formation occurred.

The chain end lipophilic glycopolymers were then furnished by