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Short communication

Hydrolysis of the terminal dimethylacetal moiety on the spacers bound to carboxy groups containing glucans

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Abstract

Series of carboxymethylglucan (CMG) and oxidized glucan (OXG) derivatives containing spacers of different length with terminal dimethylacetal groups were treated with different acid media. The kinetics of acid hydrolysis was monitored via carbonyl groups formation measurements. Pseudo-first order rate constants k_1 and second-order rate constants k_2 were calculated, $k_1 = k_2[H^+]$. Observed overall yields and reaction rates varied with different linkers and flexibility of whole linker moiety. Derivatives of OXG hydrolysed about five times faster than analogous derivatives of CMG.

Keywords: Glucan; Dimethyl acetal; Hydrolysis; Linker; Conjugate

1. Introduction

Construction of saccharide-protein conjugates is challenging. The effective biological functionality of the glycoconjugates depends on the proper chemistry of the conjugate construct. Usage of heterobifunctional spacer (linker) molecules resulted in a great improvement. Highly reactive spacers are those containing a nucleofile group (amino, hydrazine) at one end and a carbonyl group at the other. However, the carbonyls should be protected to prevent cross-reactions of the end groups. A diacetal moiety which can be simply converted to carbonyl by mild acid hydrolysis is well known for this. The conversion is quantitative as expected with small molecules (Zhang, Yergey, Kowalak, & Kováč, 1998). The situation may be different with high molecular weight polymers. Unexpectedly, we observed different performance of hydrolysis of acetals after spacers were attached to polysaccharide molecules.

Recently we have reported the preparation of two glucan derivatives with spacer-arm linkers containing dimethylacetals as latent carbonyl groups (Farkaš & Bystrický, 2007). Two polysaccharides: carboxymethylated β-(1 → 3)-D-glucan (CMG) and C6 oxidized β-(1 → 3)-D-glucan (OXG), bearing three different length spacer-arms are examined here for acid catalyzed hydrolysis of acetals (see Fig. 1).

2. Experimental

2.1. Preparation of glucan derivatives

We chose two types of model polysaccharides, for our purposes, namely carboxymethyl glucan (CMG) with degree of carboxymethylation 0.87, $M_w = 120{,}000$, and oxidized glucan (OXG), $M_w = 47{,}000$, with degree of carboxylation 0.45 prepared according to the literature (Machová, Kogan, Alföldy, Šoltés, & Šandula, 1995; Painter, Cesaro, Delben, & Paloeti, 1985). The glucan carboxy-derivatives were transformed to amides by the specific linker attachment (Farkaš & Bystrický, 2007). Carboxymethyl-glucans with different linkers were desig-

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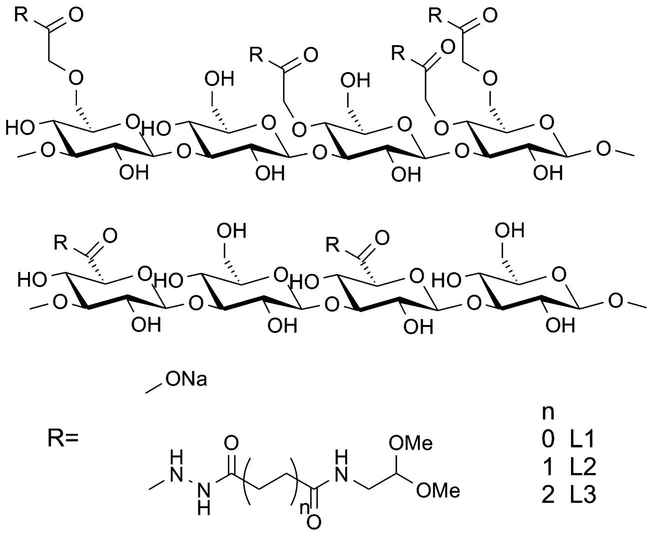

Fig. 1. Derivatives of carboxymethylated glucan (CMG) and oxidized glucan (OXG).

nated CMG-L1, CMG-L2, CMG-L3, and those prepared from oxidized glucan, were designated OXG-L1, OXG-L2, OXG-L3. For these polysaccharides the unbound linker dimethylacetal ends have latent reactive carbonyl groups.

2.2. General procedure for hydrolysis experiments

Acid hydrolysis was performed as follow: Solutions of ~7 mg of CMG-LX or OXG-LX in 10 mL water were incubated at 50 or 80 °C in an oil bath. 5 mL of the Dowex-50 W H^+ suspension or a 0.05 mol L^{-1} HCl was then added. 0.5 mL samples were taken out at defined time intervals and the reaction was quenched by the addition of 0.5 mL solution of 0.1 mol L^{-1} $NaHCO_3$. The samples were kept in the refrigerator until the analyses were carried out.

2.3. Carbonyl group determination

Samples with carbonyl groups were examined by NMR spectroscopy. ^{1}H NMR spectra were measured in D_2O at 25 on Bruker 300 MHz Avance DPX spectrometer. ^{1}H NMR spectra confirmed that aldehydes form as the products of hydrolysis. Two new signals replaced a singlet of methoxy group, and triplet of acetal hydrogen. The triplet of the aldehyde group appears at 8.49 ppm and triplet of hydrated form at 5.32 ppm ($J = 5.33$ Hz). The integral ratio of the signals is approximately 0.09:1, thus more than 90% of the aldehyde is in the hydrated form.

The content of aldehyde groups was quantitatively determined by alkaline ferricyanide assay. The determination of the content of carbonyl groups is based on the reduction of ferricyanide ions in alkaline solution (Park & Johnson, 1949). Freshly prepared solution of D-glucose was used as the standard.

2.4. High-performance size exclusion chromatography

Size exclusion chromatography (SEC) experiments were performed with a system from Laboratorní přístroje (Prague, Czech Republic) containing two columns connected in series (250 × 8 mm) packed with Biospher GM 300 and Biospher GM 1000 sorbents from Labio, a.s. (Prague, Czech Republic). Biospher GM is a co-polymer of glycidylmethacrylate and ethylenedimethacrylate accompanied by special porogens. The separation process was monitored with a differential refractometric detector. The mobile phase used was 0.1 M $NaNO_3$ solution. A set of pullulans was used for molecular weight calibration of SEC system.

3. Results and discussion

First, we tested reaction conditions known from literature on CMG derivatives. According the hyaluronic acid acetals hydrolysis authors used 0.025 mol L^{-1} HCl and 25 °C (Bulpitt & Aeschliman, 1999). The results under these conditions show only 4% hydrolysis of acetal after 1 h and only 20% hydrolysis after 24 h. Then we tested 0.05 mol L^{-1} trifluoracetic acid and 100 °C (Zhang et al., 1998). Under these conditions we obtained 99–100% conversion after half an hour with all CMG derivatives, with a small decrease in the molecular weight of the products. After 24 h the carbohydrate chains were significantly hydrolysed and oligosaccharides with M_w of 300–1200 Da were obtained. In both cases the pH was not strictly constant but varied slightly during the course of the reaction.

To simplify the manipulation and isolation of the products and to determine the rate of hydrolysis at constant pH we used Dowex H^+ resin. Despite the wide utilization of

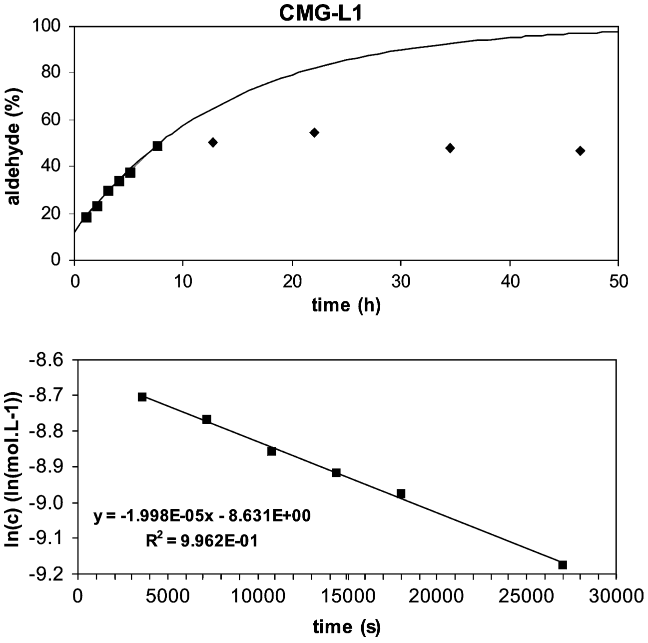

Fig. 2. Hydrolysis of CMG-L1 derivative with Dowex H^+ at 80 °C, pH 1.56.