Cu-I-mediated degradation of polysaccharides leads to fragments with narrow polydispersities

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Cu(I) mediated degradation of polysaccharides leads to fragments with narrow polydispersities

Jani Rahkila,[a] Filip S. Ekholm,*[b] and Reko Leino*[a]

Abstract: Biomass derived oligo- and polysaccharides are important compounds for various applications, including biomedicine and material science. Their use, however, is often limited by inherent structural inhomogeneity of the starting materials. Here, a method for depolymerization of naturally occurring polysaccharides, including dextran, starch, xylan and galactoglucomannan, into well-defined fragments of narrow polydispersities is described, based on the use of in situ generated Cu(I) species under the commonly employed CuAAC reaction conditions. The main strength of the reported method is its high versatility, both in terms of substrate scope and operational simplicity.

Introduction

Oligo- and polysaccharides belong to the most abundant classes of biomolecules and find numerous applications in material and biological sciences.[1–5] They also play a central role in understanding of biological recognition events[6] and in contemporary processing and refining of biomass.[7] The nearly infinite ways in which the different monosaccharide residues can be linked together and the commonly observed broad polydispersities of many naturally occurring polysaccharides lead, however, to various challenges associated with preparation of well-defined saccharide fractions, their isolation and structural characterization.

Oligosaccharides and small polysaccharides can be constructed by fragmentation of larger polysaccharides, or by chemically combining mono- and oligosaccharides into larger ones. Direct conversion of monosaccharides into oligosaccharides remains an expensive, time-consuming and tedious process despite the various automated synthetic protocols developed during the past decades.[8–10] On the other hand, the generally applied methods for fragmentation of polysaccharides are often either substrate specific (in the case of enzymatic protocols[11]) or difficult to control, particularly when strong mineral acid or alkaline hydrolysis[12] or oxidative depolymerization conditions[13] are applied. Therefore, a need exists for new reaction technologies that would enable the preparation and isolation of well-defined oligo- and polysaccharide fractions. In cases where larger polysaccharides are easily obtainable from abundant natural sources, a controlled fragmentation process represents the best synthetic strategy and shortest possible route for production of smaller polysaccharide fractions. In addition, controlled fragmentation reactions should be useful for future biomass feedstock valorization applications.

Here, we describe the development of a general fragmentation methodology for bioprocessing of polysaccharides. As such, the degradation of polysaccharides in the presence of Cu(I) has been reported in the literature already decades ago.[14] While this issue should always be considered and potentially avoided in copper(I) catalyzed azide-alkyne cycloaddition (CuAAC) reactions applied to polysaccharide materials, it is occasionally neglected or not clearly addressed even in more recent studies.[15–20]

From the biomass valorization perspective, a controlled fragmentation methodology based on Cu(I) with inexpensive reagents would be desirable. In this work, we have specifically explored the use of Cu(I) as an opportunity for *controlled fragmentation* of different polysaccharides. We demonstrate that the commonly utilized copper(I) catalyzed azide-alkyne cycloaddition (CuAAC) reaction protocol can also be applied for precisely controlled degradation of polysaccharide feedstocks commonly used in biorefineries, as exemplified by the degradation of 70 kDa dextran, 36 kDa birch wood xylan, 70 kDa starch and a 12 kDa galactoglucomannan (GGM) (structures displayed in Figure 1).

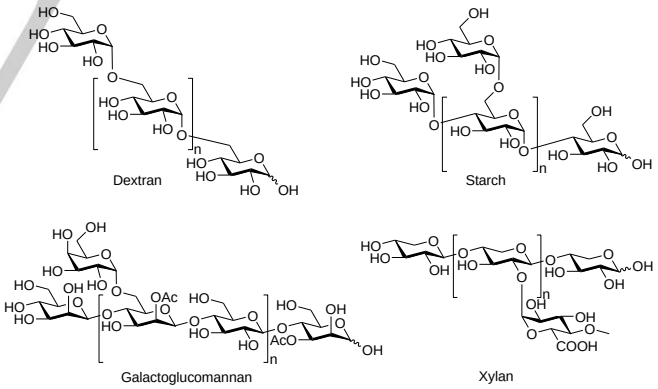

Figure 1. Chemical structures of dextran, starch, GGM and starch

Results and Discussion

Dextran as the model compound

The aim of this work was to investigate the potential use of Cu(I) for controlled degradation of polysaccharides. In order to study the participating chemical species and their influence on the overall degradation reaction, a suitable model compound, allowing for simple monitoring of both the progress of the reaction and the product distribution, was required. For this purpose, dextran, a polysaccharide consisting of α-(1→6)-D-

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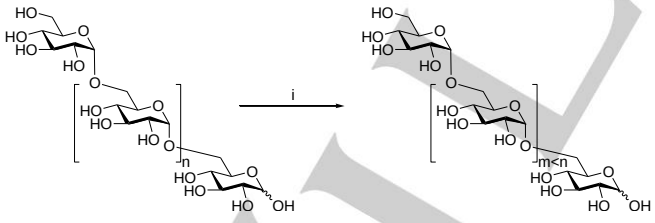
glucopyranosyl units, was selected due to the high homogeneity of both the starting material and the expected degradation products. Furthermore, analytical dextran standards are commercially available, which proved beneficial for analysis of the product distribution. The results are summarized in Table 1.

First, a negative control experiment (Entry 1, Table 1) was performed with 70 kDa dextran in water at 55 °C in order to rule out the possibility of degradation taking place in the presence of only the solvent at the targeted reaction temperature. As expected, spontaneous degradation of dextran was not observed. Next, the influence of the different chemical species involved in the standard CuAAC-reaction [Cu(I), Cu(II) and Na-ascorbate] was investigated.[21] Cu(I)-ions were generated from CuCl, Cu(II)-ions from $CuSO_4$ and Na-ascorbate was used as such. Neither Cu(II) nor Na-ascorbate (Entries 6 and 7) induced any degradation of dextran. The Cu(I)-ions, on the other hand (Entry 8), degraded the 70 kDa dextran into smaller polysaccharides with a molecular weight of approximately 17-18 kDa, as determined by both high pressure size exclusion chromatography (HPSEC) and multiangle laser light scattering (MALLS).[22–24] The synergistic effect of $CuSO_4$ and Na-ascorbate was investigated by varying the amounts of these reagents (Entries 2-5). Under these conditions, smaller dextran oligomers with molecular weights between 7-10 kDa were obtained. These findings suggest that for degradation of dextran, the $CuSO_4$/Na-ascorbate-reagent system is more efficient than CuCl alone. Mechanism of the degradation is believed to be a Fenton reaction where the depolymerization is mediated by a hydroxyl radical, which can be formed by the reaction of Cu(I) species with hydrogen peroxide.[25,26] The peroxide itself can be formed either by reduction of O_2 by Na-ascorbate catalyzed by Cu(II) species, or by direct reduction of O_2 by Cu(I). Our findings support this theory, as consumption of Cu(I) species during the production of H_2O_2 would reduce the amount of Cu(I) available for producing hydroxyl radicals required for the degradation. This also explains the difference in reactivity of Cu(I) species alone vs. a reagent system consisting of $CuSO_4$/Na-ascorbate.[25,27,28]



Scheme 1. Reagents and conditions: i) CuCl or $CuSO_4$ and Na-ascorbate, H_2O, 55 °C, 18 h.

Table 1. Degradation of dextran under different reaction conditions.

Entry	$CuSO_4$ (equiv.)	Na-asc (equiv.)	t (h)	M_{HPSEC} (kDa)	M_{MALLS} (kDa)	M_W/M_n	~DP
1	0	0	0	70	72	1.20	430
2	0.1	0.2	20	12.8	15.9	1.43	100
3	0.3	0.6	20	10.3	11.1	1.34	70
4	0.5	1.0	20	9.1	10.2	1.22	65
5	0.5	1.0	48	7.1	10.9	1.55	65
6	0.5	0	20	67	71	1.21	430
7	0	1.0	20	68	71	1.21	430
8	0.5 (CuCl)	0	20	18	17	1.67	105

All equivalents are given as equivalents per monosaccharide unit and molecular weights are given as M_w.

Having studied the effects of the different chemical species on the degradation reaction, the combined $CuSO_4$/Na-ascorbate reagent system was selected for further experiments. The reaction was next reproduced on 200 mg scale using 0.05 equivalents of $CuSO_4$ and 0.1 equivalents of Na-ascorbate. The reaction progress was monitored for five days (Figure 2). In all of the reactions, a specific point was observed after which the reaction rate decreased significantly. This can be interpreted as a terminal oligomer size, after which the reaction does not proceed. In order to verify this hypothesis, we studied the behavior of maltoheptose, one of the larger commercially available oligosaccharides in this series, under the employed reaction conditions. Maltoheptose remained intact as verified by NMR-spectroscopy (data not shown), supporting the hypothesis on terminal oligomer size. For dextran the terminal size was found to reside between 7-10 kDa, corresponding to a degree of polymerization (DP) of 40-60 (the DP of 70 kDa dextran is approximately 430). It should be noted that this terminal oligomer size is unaffected by concentrations of the reagents and the reaction time, which suggests that the degradation mechanism is also linked to the structural features of the polysaccharide itself, not only to the Fenton reaction as such. Furthermore, polysaccharides in this size range are difficult to produce by other existing protocols and the narrow polydispersity witnessed in the product should make this protocol appealing from the biomass valorization and material science perspective since it leads to end products with a more predictable behavior. In addition to the narrow polydispersities observed in the degradation reaction, the molecular weight distribution of the product follows a logarithmic dependency on the reaction time ($r^2 = 0.998$). In principle, this logarithmic decay enables the calculation and determination of fixed reaction conditions for preparation and isolation of oligomers of a desired terminal molecular size (above the DP-limit of 40-60).

While the results on dextran depolymerization proved promising, it would, however, be important to investigate whether the same conditions could also be applied to other than linear, homogeneous polysaccharides. Consequently, we continued the

work by studying the applicability of this new Cu(I)-based degradation protocol on other biomass derived polysaccharides in order to gain more information on the substrate scope and the link between the polysaccharide structure and terminal oligomer size.

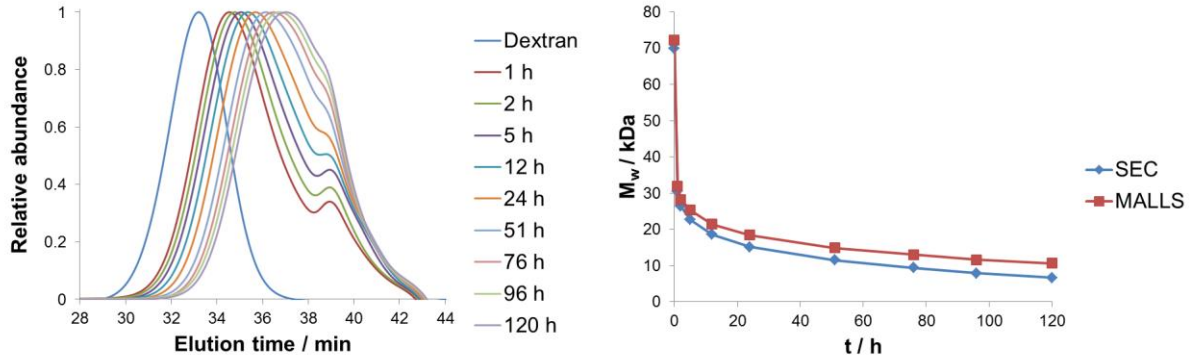


Figure 2. Degradation of 70 kDa dextran over 5 days. Normalized elution profiles (left). Molecular weights are calculated from elution time and MALLS (right). The peak at 39 min in the chromatograms is a contamination in the solvent used for dissolving the samples, not an actual product of the reaction.

Degradation of galactoglucomannan, xylan and starch

Next, the degradation of polysaccharides representing other biomass constituents was investigated. As model substrates, 36 kDa birch wood xylan, 70 kDa starch and 12 kDa spruce wood GGM were selected. The degradation experiments were conducted under three different reaction conditions with the results summarized in Table 2. Initially, the poor solubilities of starch and xylan at room temperature resulted in some analytical challenges, especially in the case of starch which did not become appreciably soluble even after the degradation reaction. In order to avoid precipitation of the products in the SEC column, the size distribution of the challenging samples was determined by the NMR-spectroscopic technique Diffusion Ordered SpectroscopY (DOSY). In general, DOSY provides information similar to SEC, distinguishing molecules based on their physical size and has therefore become a solid technique in polymer studies.[29,30] Regardless of the analytical techniques used here, or in cases where all of the techniques could be applied, the results displayed a similar trend for all three polysaccharides studied. Starch and xylan were successfully depolymerized into smaller polysaccharides with molecular weights in the 5-12 kDa range corresponding to DPs of 30-75, which are similar to the DPs obtained in the model reactions with dextran. The GGM polysaccharide, on the other hand, was degraded into even smaller fragments of ca. 2-2.5 kDa size, corresponding to DPs of 10-15. In all these cases, the terminal oligomer size was unaffected by the reaction time or the amount of reagents used. Moreover, the results obtained indicate that both linear and branched homo- and heteropolysaccharides degrade in a selective manner in the presence of Cu(I)-ions. This raises some concerns about the integrity of recent scientific reports on the structural modification of polysaccharides by CuAAc reactions, where the potential occurrence of degradation of the polysaccharide backbone has not been considered.[16,18-22] On a general level, it should be noted that in all of the examples reported here, the end products represent small polysaccharides of molecular weights which are difficult to produce by any existing reaction technology (including both synthetic transformations and earlier reported degradation protocols). While the Fenton reaction is believed to be the main cause of the degradation, it has been suggested, in previous studies using copper, that the complexation between the metal species and the polysaccharide plays a crucial role during the generation of the reactive oxygen species which possibly causes the degradation phenomenon.[31] These assumptions would partially explain the significant decrease in the reaction rate once a specific oligomer size has been reached. Our study also demonstrates that the final oligomer size depends on the structure of the polysaccharide itself. Especially the stereochemistry displayed in close proximity to the glycosidic linkage appears to significantly influence the terminal oligomer size. Here, the polysaccharides containing “gluco”-stereochemistry, *i.e.* dextran, starch and xylan, resulted in terminal DP-values of 30-75. For GGM, where mannopyranosyl units dominate, DP-values of 10-15 were obtained. In order to gain more detailed insights into the actual reaction mechanism and its correlation with the terminal oligomer size, further experiments with a wider series of substrates will be needed. It can also be noted that the method described here could be considered as a chemical equivalent of some natural, enzymatic depolymerizations induced by lytic polysaccharide monooxygenases, responsible for degrading, for example, cellulose and chitin.[32]

Table 2. Degradation reactions of xylan, starch and GGM.

Entry	Polysaccharide	$CuSO_4$ (equiv.)	Na-asc (equiv.)	t (h)	M_{HPSEC} (kDa) M_{MALLS} (kDa) M_{DOSY} (kDa)	M_w/M_n	~DP
1 a	Xylan	0	0	0	- - 36		- - 200
1 b	Xylan	0.1	0.2	18	5.5 12[a] 6.4	1.29	35 75 40
1 c	Xylan	0.5	1.0	18	5.0 11[a] 6.4	1.32	30 70 40
2 a	Starch	0	0	0	- - 70		- - 430
2 b	Starch	0.1	0.2	19	- - 5.5		- - 35
2 c	Starch	0.5	1.0	19	- - 5.5		- - 35
3 a	GGM	0	0	0	12 11 -	1.38	75 70 -
3 b	GGM	0.1	0.5	18	4.2 3.9 -	2.01	25 24 -
3 c	GGM	0.5	1.0	18	2.0 2.3 -	1.38	12 14

[a]The observed large deviation is likely caused by the different nature of the analytical methods and the use of dextran standards for SEC and DOSY. Reaction conditions: starting material, H_2O, 55 °C, reagents. Molecular weights are given as M_w.

Conclusions

We have developed a new aqueous bioprocessing method for degradation of polysaccharides into oligomeric fractions of controlled size, based on the widely used CuAAC “click”-reaction protocol. Size distributions of the oligomeric products were found to depend on the starting polysaccharide used. Notably, a terminal oligomer size was observed for all of the polysaccharides studied here, after which the degradation rate decreased significantly. For dextran, GGM, xylan and starch this terminal oligomer size was in the range of 2-10 kDa. Oligo- or polysaccharides in this molecular weight range are typically challenging to produce by other existing reaction technologies. Consequently, the method developed has significant potential for processing of polysaccharide streams from, for example, wood and paper industries, as showcased here by the degradation of the naturally occurring polysaccharides xylan, GGM and starch. In addition, the developed method should also be useful to various scientific disciplines, especially in material and biosciences, since it enables the production of polysaccharide fragments with well-defined and controlled molecular weight distributions. For material purposes, such narrow polydispersities may result in consistent and predictable molecular behavior. In biological recognition studies, polysaccharides with narrow polydispersities can be expected to enhance the reproducibility of the obtained research results.

Experimental Section

General information

All reagents were purchased from Sigma-Aldrich and used as such without further purification. GGM was obtained from the Laboratory of Wood and Paper chemistry at Åbo Akademi University. NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 500.20 MHz (^{1}H) and 125.78 MHz (^{13}C) equipped with a Prodigy BBO CryoProbe or a Bruker AVANCE III spectrometer operating at 600.20 MHz (^{1}H) and 150.92 MHz (^{13}C) equipped with a Prodigy TCI inverted CryoProbe optimized for proton detection. DOSY spectra were recorded at a concentration of approximately 2 mg/mL and HSQC at a concentration of 10 mg/mL. The starch samples and the unreacted xylan sample had to be heated to approximately 80 °C to get everything to dissolve. Upon cooling some precipitate was observed which could mean that the lower molecular-weights might be slightly overrepresented in these samples. The HPSEC/MALLS analysis was performed on an Agilent 1260 series (G1311B) instrument equipped with a refractive index detector (Shimadzu RID-10A) and MALLS detector (Wyatt Technology miniDAWN Tristar). The setup consisted of a guard column (Waters, Ultrahydrogel 6 mm × 40 mm) and two columns (2 × Ultrahydrogel linear 7.8 mm × 300 mm) connected in series. Eluent: 100 mM $NaNO_3$; flow rate: 0.5 mL/min; injection volume: 200 µL. The dn/dC values used for MALLS were 0.146 mL/g (dextran, xylan) and 0.150 mL/g (GGM).[33,34] All chromatogram traces are drawn using data from the RI detector.

Degradation experiments

The polysaccharides were dissolved in H_2O (dextran: 10 mg/mL, xylan and starch: 5 mg/mL). The xylan and starch samples needed to be heated to 80 °C to make everything dissolve. Once everything had dissolved $CuSO_4$ and Na-ascorbate were added and an intense yellow color was observed. The reaction mixture was heated to 55 °C and stirred at that temperature for approximately 20 h during which the reaction mixture took on a green color. The reaction mixture was then cooled down to room temperature and the copper was removed by adding a generous amount of QuadraPure® TU thiourea-based metal ion chelator. The reaction mixture was then concentrated to dryness.

Analysis of degradation products

The degree of degradation was determined by High Pressure Size Exclusion Chromatography (HPSEC) combined with MALLS to obtain both the molecular weights and polydispersities.[22–24] Calculation of the molecular mass from HPSEC chromatograms was performed by using dextran standards ranging from 50 kDa to 270 kDa. As additional support, the degradation was also studied by NMR spectroscopic techniques DOSY and Heteronuclear Single Quantum Coherence (HSQC). The DOSY data was found to be in good agreement with the HPSEC/MALLS results, especially for the dextran samples where the same molecular weight standards as used for the HPSEC analysis could be employed. The poor solubility of starch and xylan at room temperature resulted in some analytical problems, especially in the case of starch which did not become appreciably soluble even after the degradation reaction. In order to avoid possible precipitation of the compounds in the SEC column, these samples were mainly analyzed by NMR methods. HSQC and DOSY experiments turned out to be invaluable for visualization of the degradation of these compounds. While the HSQC spectra may become visually complex, they clearly show the increased number of oligomer end groups after the copper induced degradation reaction. It should be noted, however, that due to the poor solubility of some samples the smaller oligomers might be somewhat overrepresented in the NMR data.

Due to the small size, resulting in rather well-resolved signals the maltoheptaose was analyzed by ^{1}H NMR before and after the reaction and no increase in end-groups could be observed.

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Keywords: Polysaccharides • controlled depolymerization • biomass refining • renewable resources

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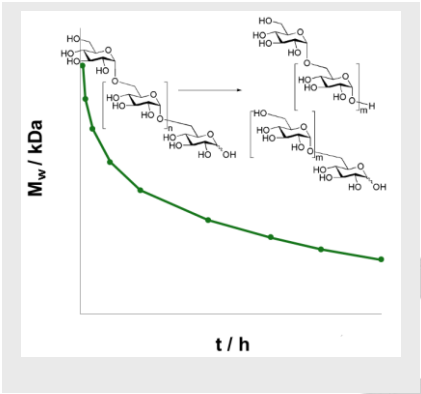
Entry for the Table of Contents

FULL PAPER

Degradation of natural polysaccharides using reaction conditions commonly applied in click chemistry. Readily available chemicals are used for obtaining polysaccharide fragments with narrow polydispersities by depolymerizing polysaccharides from natural sources



Polysaccharide fragmentation

Jani Rahkila,Filip S. Ekholm, Reko Leino**

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Cu(I) mediated degradation of polysaccharides leads to fragments with narrow polydispersities