Thermoresponsive xylan hydrogels via copper-catalyzed azide-alkyne cycloaddition

Nikolaos Pahimanolis, Anni Sorvari, Nguyen Dang Luong, Jukka Seppälä*

Department of Biotechnology and Chemical Technology, Aalto University School of Chemical Technology, Kemistintie 1, 02150 Espoo, Finland

ABSTRACT

In the present work, hydrogels of birch wood xylan and thermoresponsive poly(ethylene glycol)-b-poly(propylene glycol)-b-poly(ethylene glycol) (PEG-PPG-PEG) were prepared using copper catalyzed alkyne-azide cycloaddition (CuAAC) in aqueous reaction conditions. First, reactive azide groups were introduced on the backbone of xylan by etherification of 1-azido-2,3-epoxypropane in alkaline water/isopropanol-mixture at ambient temperature, providing degree of substitution (DS) values up to 0.28. On the second step, the azide groups were reacted with propargyl bifunctional PEG-PPG-PEG utilizing CuAAC, leading to formation of crosslinked hydrogels. The novel xylan derivatives were characterized with liquid and solid state nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FT-IR) and elemental analysis (EA). The temperature controlled swelling behavior of the developed hydrogels was evaluated in the range of 7–70 °C by water absorption and compressive stress–strain measurements, which showed a reduction in water content and change in stiffness with increasing temperature. The morphology of the hydrogels at different temperatures was studied by scanning electron microscopy (SEM), which showed a reduction in pore size with increasing temperature.

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1. Introduction

Hemicellulose is the most abundant polysaccharide family next to cellulose, present as a component in most plant cell walls. In hardwood species, the main hemicellulose type is xylan, which predominantly consists of D-xylopyranoside units connected by β-(1→4)-linkages along with acetyl-, glucuronic acid and species dependent side groups (Deutschmann & Dekker, 2012). Among other methods, xylan can be conveniently extracted from biomass by alkali extraction (Ebringerová & Heinze, 2000; Glasser, Kaar, Jain, & Sealey, 2000) where the acetyl groups are also hydrolyzed rendering the backbone hydroxyl groups available for derivatization reactions.

Several chemical modifications targeting the hydroxyl groups have been reported for xylan, including etherification using epoxides and alkyl halides in alkaline media (Bigard et al., 2011; Ebringerová, Hromádková, Kacuráková, & Antal, 1994; Fang, Fowler, Tomkinson, & Hill, 2002; Jain, Sjöstedt, & Glasser, 2001; Kataja-aho, Haavisto, Asikainen, Hyvärinen, & Vuori, 2008; Laine et al., 2013; Petzold, Günther, Kötteritzsch, & Heinze, 2008; Petzold, Schwikal, & Heinze, 2006; Ren, Sun, & Liu, 2007; Saghir,

Iqbal, Hussain, Koschella, & Heinze, 2008; Saghir, Iqbal, Koschella, & Heinze, 2009; Schwikal & Heinze, 2007; Schwikal, Heinze, Ebringerová, & Petzold, 2006; Vincendon, 1998) and development of bifunctional derivatives using acrylamide (Ren, Peng, & Sun, 2008). In addition, esterification reactions typically employing anhydrides or activated carboxylic acids (Buchanan et al., 2003; Daus & Heinze, 2010; Fundador, Enomoto-Rogers, Takemura, & Iwata, 2012; Hansen & Plackett, 2011; Hesse, Liebert, & Heinze, 2006; Hettrich et al., 2006; Salam, Pawlak, Venditti, & El-tahlawy, 2011) and sulfating agents (Daus et al., 2011; Hettrich et al., 2006) have been used to alter the chemical properties and introduce new functionality to xylans. In order to tailor the properties of natural-based polymers, the copper catalyzed azide-alkyne cycloaddition (CuAAc), often referred as "click"-reaction, offers a straightforward and efficient way to build new molecular complexity (Kolb, Finn, & Sharpless, 2001; Rostovtsev, Green, Fokin, & Sharpless, 2002; Tornoe, Christensen, & Meldal, 2002). Characteristics for click-type reactions is that they can be performed at mild reaction conditions and have high tolerance toward functional groups, oxygen and water, which makes the use of this chemistry tempting for the modification of natural polymers. The CuAAC offers extensive possibilities to tailor polymer properties (Binder & Sachsenhofer, 2007, 2008; Fournier, Hoogenboom, & Schubert, 2007; Meldal, 2008; Lallana, Riguera, & Fernandez-Megia, 2011; Lallana, Fernandez-Trillo, Sousa-Herves, Riguera, & Fernandez-Megia, 2012; Lallana,

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* Corresponding author. Fax: +358 9 47022622.
  E-mail addresses: jukka.seppala@aalto.fi, jukka.seppala@tkk.fi (J. Seppälä).

Sousa-Herves, Fernandez-Trillo, Riguera, & Fernandez-Megia, 2012; Kempe, Krieg, Becer, & Schubert, 2012). The utilization of CuAAC on the modification of polysaccharides has been reported in several publications (Bernard, Save, Arathoon, & Charleux, 2008; De Geest et al., 2008a, 2008b; Eissa, Khosravi, & Cimecioglu, 2012; Elchinger, Montplaisir, & Zerrouki, 2012; Hafrén, Zou, & Córdova, 2006; Hasegawa et al., 2006; Koschella, Richter, & Heinze, 2010; Liebert, Hänsch, & Heinze, 2006; Pohl, Schaller, Meister, & Heinze, 2008; Ritter, Knudsen, Mondrzik, Branscheid, & Kolb, 2012; Schatz, Louguet, Le Meins, & Lecommandoux, 2009; Tankam, Müller, Mischnick, & Hopf, 2007; Xu, Zhang, & Kadla, 2012; Zhang, Xu, Wu, Zhang, & Zhuo, 2009). However, studies on the modification of xylan using the CuAAC-reaction is limited to a recent paper, where propargyl end-functionalized polylactide was grafted on azide-containing xylan (Enomoto-Rogers & Iwata, 2012).

Hydrogels, three-dimensional networks of hydrophilic polymers capable of retaining large amount of water, have a broad field of applications, and the research on such materials is an ongoing task. Applications such as drug-delivery systems, artificial muscles and sensors are being developed (Calvert, 2008; Coviello, Matricardi, Marianecci, & Alhaiqu, 2007; Klouda & Mikos, 2008). Polysaccharide based hydrogels have some advantages over synthetic polymers, since in addition to their abundant availability, they are biologically compatible and degradable (Coviello et al., 2007). Xylans are interesting candidates for such materials also due to their bioactive properties (Cipriani et al., 2008; Ebringerová & Heinze, 2000; Ebringerová, Kardosová, Hromádková, Malovíková, & Hribalová, 2002). For example, xylan based-hydrogels have been developed from methacrylated xylan and subsequent radical polymerization of hydroxyethyl methacrylate for drug release studies (Silva, Habibi, Colodette, & Lucia, 2011). In addition, xylan-rich hemicelluloses grafted with acrylic acid in the presence of N,N-methylene-bis-acrylamide crosslinker yielded hydrogels with multistimulus response properties (Peng, Ren, Zhong, Peng, & Sun, 2011). Allylated xylan derivatives were successfully crosslinked by UV induced radical crosslinking with and without N,N'-diallylaldardiamides yielding novel bio-based hydrogels (Pohjanlehto, Setälä, Kammiovirta, & Harlin, 2011). The xylan hydrogel formation has also been reported on enzymatically aided method (Chimphango, van Zyl, & Görgens, 2012), by physical (Hettrich & Fanter, 2010) or ionic interactions (Gabrielii & Gatenholm, 1998; Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000). The utilization of the CuAAC reaction for the synthesis of polysaccharide hydrogels has been reported for azide and alkyne derivatized hyaluronan and the obtained hydrogels were demonstrated to serve as drug reservoirs and scaffolds (Crescenzi, Cornelio, Di Meo, Nardecchia, & Lamanna, 2007, Huerta-Angeles et al., 2011; Huerta-Angeles et al., 2012). In addition, networks based on cellulose (Koschella, Hartlieb, & Heinze, 2011, Pierre-Antoine, Francois, & Rachida, 2012) and thermoresponsive cellulose/poly(N-isopropylacrylamide-co-hydroxyethyl methacrylate) hydrogels have been developed (Zhang et al., 2009).

In our previous publication, we described a method for introducing azide-groups on the backbone of dextran using aqueous reaction media (Pahimanolis, Vesterinen, Rich, & Seppala, 2010). The azide functionalities provide a combinatorial approach to discover new materials, as a wide range of possible modifications via CuAAC become available. In this paper, the modification is applied to xylan. First, azide groups were introduced to the backbone of xylan using glycidyl azide under alkaline conditions. On the second step, the novel azide modified xylan was crosslinked with thermoresponsive alkyne end-functionalized polyethylene glycol/polypropylene glycol/polyethylene glycol (PEG-PPG-PEG) triblock copolymers using CuAAC, yielding temperature responsive hydrogels. Elemental analysis, NMR and FT-IR were used to confirm

the chemical structure of the synthesized products. The temperature controlled swelling behavior of the developed hydrogels was evaluated in the range of 7–70 °C, which showed a reduction in water absorption with increasing temperature. The hydrogels could have applications as drug delivery systems, or work as part of separation, fractionation or self-cleaning membranes (Klouda & Mikos, 2008; Vermonden, Censi, & Hennink, 2012; Wandera, Wickramasinghe, & Husson, 2010).

2. Experimental

2.1. Materials

Birch wood xylan (xylose content $\geq$90%, degree of acetylation less than 4% determined with ^{1}H NMR), Mn = 11700 g/mol, PDI = 2.02 determined by GPC against PEG standards) was purchased from Sisco Research Laboratories Pvt. Ltd. and used as received.

Propargyl bromide (80 wt% in toluene) and HNO_3 (65%) were obtained from Fluka Chemicals. L-Ascorbic acid (99%), $CuSO_4 \cdot 5H_2O$ (99%), epichlorohydrin (99%), ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA, 99%), 2-propanol (99.8%), NaH (95%) and $NaNO_2$ (97%) were purchased from Sigma–Aldrich. NaN_3 (99%), acetic acid (99.8%) and NaOH (99%) were from Merck. All chemicals were used as received. Anhydrous grade tetrahydrofuran (THF) was purchased from VWR and stored over molecular sieves. Poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers were from BASF (trade name Pluronic© PE6100 and PE 6400 both having a central PPO block of 1750 g/mol and 10% or 40% of PEO in molecule respectively). Poly(ethylene glycol) 2000 g/mol was from Fluka. The polymers were vacuum-dried at 40 °C for 48 h before use.

2.2. Preparation of 1-azido-3-chloro-propanol

The synthesis of 1-azido-3-chloropropanol was done starting from epichlorohydrin. The ring-opening reaction of the epoxide with azide-ion was done according to a modified method (Fringuelli, Piermatti, Pizzo, & Vaccaro, 1999; Pahimanolis et al., 2010; Yang, Shao, Li, Wang, & Zhang, 2011). Isopropanol (109.0 ml) and acetic acid (7.2 ml, 125.8 mmol) were mixed with a solution of NaN_3 (8.177 g, 125.8 mmol) in 74.0 ml of water. Epichlorohydrin (6.6 ml, 84.2 mmol) was then added under stirring and the reaction was continued at 30 °C for 24 h, until ^{1}H NMR analysis showed complete consumption of the epoxide. A water solution of $NaNO_2$ (14.4 ml, 41.6 mmol) was then added, followed by the dropwise addition of HNO_3 (5.76 ml, 83.8 mmol) to eliminate any excess azide-ions. The stirring was continued for 24 h at room temperature, by which time the formation of nitrous oxides had ceased. To concentrate the solution, 12 g of NaCl was added and the separated propanol phase was collected. The aqueous phase was extracted once with 50 ml of diethyl ether and the organic phases were combined. This resulted in additional phase separation of water, which was discarded. The organic phase was further concentrated by removing diethyl ether by rotary evaporation. The obtained propanol solution of 1-azido-3-chloropropanol (58 ml, concentration 1.34 mmol/ml, yield 92% by ^{1}H NMR analysis, acetic acid content 0.61 mmol/ml) was stored in dark at room temperature and used without further purification.

Warning! Low molecular weight organic azides are known to be potentially explosive. For this reason, handling highly concentrated solutions of these materials should be avoided.

^{1}H NMR (D$_2$O, ppm): δ = 3.36–3.54 (CH$_2$-Cl), 3.56–3.73 (CH$_2$-N$_3$).
^{13}C NMR (D$_2$O, ppm): δ = 70.50 (C-OH), 53.92 (C-N$_3$), 46.69 (C-Cl).