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Homogeneous tosylation of agarose as an approach toward novel functional polysaccharide materials

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ABSTRACT

The homogeneous tosylation of agarose was studied with respect to the effects of reaction parameters, such as reaction medium, time, and molar ratio, on the reaction course, the degree of substitution (DS) with tosyl/chloro deoxy groups, and the molecular structure. Tosyl agaroses (TOSA) with $DS_{tosyl} \leq 1.81$ could be obtained in completely homogeneous reactions by using *N*,*N*-dimethylacetamide (DMA)/LiCl or 1,3-dimethyl-2-imidazolidinone (DMI) as solvents. The products were characterized by FT-IR and NMR spectroscopy and it was demonstrated that two types of substitution pattern can be achieved: (i) non-preferential substitution at position 6 of the 1 → 3-linked β-D-galactose unit (G-6) and position 2 of the 1 → 4-linked 3,6-anyhdro-α-L-galactose unit (LA-2) and (ii) regioselective tosylation at G-6, depending on whether the reaction is performed with or without LiCl. Finally, the nucleophilic displacement reaction of TOSA was studied using azide and ethylenediamine as representative nucleophiles. Novel deoxy-agarose derivatives were obtained that showed an interesting solubility behavior and will be used for creating functional polysaccharide materials.

1. Introduction

With respect to the limitations of conventional, oil-based materials, the exploration of 'novel' bioresources as well as the innovative use of already exploited ones is a topic of increasing importance (Zhang, 2008). With an annual production of nearly 90 kt in 2009, equivalent to a sales volume of about 10^9 US$, polysaccharides from seaweeds (in particular agar, alginate, carrageenans) are interesting alternatives to their wood and crop derived counterparts (e.g., cellulose, starch, hemicelluloses) (Bixler & Porse, 2011). Renowned for their ability to form gels, these hydrocolloids have been used 'traditionally' for centuries in food applications but found increasing interest also in biomedical and pharmaceutical industry (Armisén & Galatas, 2009; De Maria, Rincon, Duarte, Vozzi, & Boland, 2013; Rinaudo, 2008). In particular agarose, which is commercially extracted from red algae (*Rhodophyta*) as a subfraction of agar, is an ideal starting material for the preparation of functional polysaccharide materials. It is characterized as a heteropolysaccharide with a disaccharide repeating unit of alternating 1 → 3-linked β-D-galactose (G) and 1 → 4-linked 3,6-anhydro-α-L-galactose (LA; see Fig. 1) (Meena et al., 2007). Agarose forms thermoreversible, physical gels in water that melt above 80–90 °C and reform upon cooling below 35–40 °C (Fernández et al., 2008; Millán, Moreno, & Nieto, 2002; Yokokawa & Nishiyama, 2005).

Agarose is widely used in biotechnological routine applications in the form of beads for affinity chromatography (protein/antibody purification) and gels for electrophoresis (DNA/RNA purification) (Cuatrecasas, 1970; Koontz, 2013; Serwer, 1983). Moreover, agarose hydrogels are of considerable interest for biomedical applications as 3D scaffolds for tissue engineering (Thiele, Ma, Bruekers, Ma, & Huck, 2014; Zhao, Jin, Cong, Liu, & Fu, 2013). The gels are non-toxic, stable at room- and body temperature, not pH-sensitive, relatively inexpensive, and their elastic moduli can be tuned to meet the stiffness of natural tissue. However, agarose is bio-inert and lacks active signals to stimulate important cell processes like adhesion, migration, and proliferation. Thus, functionalization of the native polysaccharide, either by blending or chemical modification, is indispensable (Ulrich, Jain, Tanner, MacKay, & Kumar, 2010; Yamada et al., 2012).

It has been demonstrated for several polysaccharides, including cellulose, starch, dextran, and chitosan, that polymer

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analogs chemical derivatization reactions provide access to highly engineered compounds with tailored properties for specific applications (Cumpstey, 2013; Heinze & Liebert, 2012; Heinze, Liebert, & Koschella, 2006). Of particular interest are homogeneous conversions that enable efficient control over the degree of substitution (DS), and yield uniform reaction products with respect to the distribution of substituents between the individual polymer chains. Agarose is an interesting bioresource and starting material for the chemical derivatization because it provides a large density of hydroxyl groups and is non-ionic. In contrast, the two other main types of seaweed derived polysaccharides of commercial importance, alginates and carrageenans, are highly negatively charged. Surprisingly few reports on the homogeneous derivatization of agarose can be found in the scientific literature in comparison to other non-ionic polysaccharides such as cellulose, dextran, and starch. In a homogeneous reaction with *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine as activators, several agarose amino acid esters have been prepared that could be cross-linked subsequently into hydrogels (Kondaveeti, Prasad, & Siddhanta, 2013; Mehta, Kondaveeti, & Siddhanta, 2011). Also the homogeneous preparation of amino and iodo deoxy-agarose derivatives in dipolar aprotic solvents has been reported (Chhatbar, Godiya, & Siddhanta, 2012; Kondaveeti, Mehta, & Siddhanta, 2014). Water has been used as homogeneous reaction medium for the preparation of cationic and anionic derivatives by etherification and for selective, TEMPO-mediated oxidation of the primary hydroxyl group (Prado, Matulewicz, Bonelli, & Cukierman, 2011; Yixue et al., 2013).

In an ongoing process to develop novel, highly functional polysaccharide materials, the homogeneous tosylation of agarose was studied in the present work. Tosylated polysaccharides are key intermediates that can be converted with a broad variety of nucleophilic compounds to yield deoxy-polysaccharide derivatives with specific properties (Petzold-Welcke, Michaelis, & Heinze, 2009). By this approach, polysaccharides with azido moieties have been prepared that could be further functionalized to highly engineered derivatives via copper catalyzed 1,3-dipolar cycloaddition with alkynes e.g., in order to introduce chemical functionalities, graft co-polymers onto the polysaccharide or to obtain cross-linked polysaccharide hydrogels (Hasegawa et al., 2006; Koschella, Hartlieb, & Heinze, 2011; Koschella, Richter, & Heinze, 2010; Liebert, Hänsch, & Heinze, 2006; Pahimanolis, Sorvari, Luong, & Seppälä, 2014; Pahimanolis, Vesterinen, Rich, & Seppälä, 2010). Moreover, the azido moiety can be reduced to an amine group yielding amino deoxy-polysaccharide derivatives (Matsui, Ishikawa, Kamitakahara, Takano, & Nakatsubo, 2005). The conversion of tosyl cellulose with di- and triamines provides access to amino group containing polysaccharides with a unique supramolecular self-assembling behavior (Heinze et al., 2011; Nikolajski et al., 2014). These 'aminocelluloses' also form monolayers on various types of materials, which have been exploited for the bio-functionalization with enzymes and antibodies (Berlin et al., 2003; Berlin, Klemm, Tiller, & Rieseler, 2000). It is of great interest to introduce these type of substituents (highly reactive and prone to induce supramolecular interactions) into the polymer backbone of agarose (bioresources with self-assembling behavior) to create innovative polysaccharide based materials.

The aim of the present work was to evaluate different reaction media for the homogeneous tosylation of agarose and to study the effect of the different reaction conditions. Moreover, the subsequent nucleophilic displacement of tosyl moieties with azido- and amino groups was studied as a next step toward novel functional agarose based materials. Special emphasis was placed on comprehensive structural characterization of the products obtained in order to gain deeper understanding of the regioselectivity of chemical reactions at the agarose backbone. This issue has not been previously studied but is of outmost importance for developing correlations between molecular structure and specific properties of agarose and its derivatives.

2. Materials and methods

2.1. Materials

Dimethylsulfoxide (DMSO), *N*,*N*-dimethylacetamide (DMA), *N*,*N*-dimethylformamide (DMF), 1,3-dimethyl-2-imidazolidinone (DMI), pyridine, and triethylamine, purchased from Acros Organics, were of anhydrous grade and stored in sealed vessels containing molecular sieves as received by the supplier. Agarose (type I, low EEO: 0.09–0.13, sulfate content ≤ 0.15%, Lot.: SLBD2493V) was obtained from Sigma–Aldrich. 1-Butyl-3-methylimidazolium chloride (BMIMCl, >99%, Lot.: I00427.1.1) was purchased from IoLiTec GmbH, Germany. All other chemicals were obtained from Sigma–Aldrich and used as received. Prior to use, agarose and LiCl were dried for 3 h in vacuum at 60 °C and 130 °C respectively.

2.2. Measurements

All measurements were performed in-house according to standardized procedures. NMR spectra of agarose derivatives (100 mg/ml) were recorded at 70 °C in deuterated dimethylsulfoxide (DMSO-d_6) with a Brucker Avance 400 MHz spectrometer (^{1}H NMR: 16 scans, ^{13}C NMR: >10,000 scans). FT-IR spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer using translucent KBr tablets containing the solid polysaccharide samples. A CHNS 932 analyzer (Leco) was used for elemental analyses. The chlorine content was determined by combustion of the organic samples and potentiometric titration with $AgNO_3$ using a chloride sensitive electrode. The individual DS values were calculated from the elemental composition according to the formulas provided in the supplementary content. Based on the DS values found, theoretical elemental composition was calculated. Size exclusion chromatography (SEC) was performed on a JASCO system (isocratic pump PU-980, RI-930 refractive index detector) with a Novema 3000 and a Novema 300 column in series. DMSO with 0.5 wt% LiBr was used as eluent (65 °C, flow rate: 0.5 ml/min) and pullulan as calibration standard.

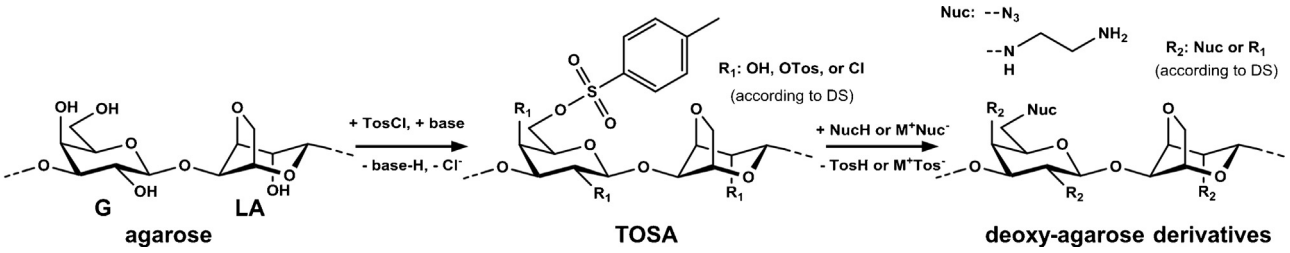

Fig. 1. Scheme for the reaction of agarose with tosyl chloride (TosCl) and the subsequent conversion of tosylated agarose with a nucleophile (NucH/M⁺Nuc⁻).