Carbohydrate Polymers 95 (2013) 569–577

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Aminoalkyl functionalization of dextran for coupling of bioactive molecules and nanostructure formation

Kathrin Fiege[a], Heinrich Lünsdorf[b], Petra Mischnick[a,*]

[a] Institute for Food Chemistry, Technische Universität Braunschweig, Schleinitzstraße 20, D-38106 Braunschweig, Germany
[b] Department of Vaccinology and Applied Microbiology, EM-Unit, Helmholtz Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany

ARTICLE INFO

Article history:
Received 17 November 2012
Received in revised form 15 February 2013
Accepted 3 March 2013
Available online xxx

Keywords:
Glucan functionalization
Amino alkyl dextran
Immobilization of active molecules
Nanostructure formation
Magnetic nanoparticle

ABSTRACT

Aminopropyl dextrans and mixed aminopropyl cyanoethyl dextrans were prepared from cyanoethyl precursors by full or partial reduction with $CoCl_2/NaBH_4$. Coupling of various aldehydes to the glucan backbone by reductive amination was accomplished with 4-hydroxy-3-methoxybenzaldehyde (vanillin), 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde (BHT-CHO), maltose and maltotriose, and picoline borane as reducing agent. Successful coupling of these representatives for aroma compounds, antioxidants and sugar side-chains were verified by ESI-MS after hydrolysis and by 1D and 2D NMR spectroscopy. Degree of conversion (molar ratio of coupled aldehydes) was estimated from ^{1}H NMR spectra. Formation of secondary and tertiary amines was detectd by ESI-MS. Applying a solvent exchange process, new nanoparticles based on these modified dextrans were prepared with and without addition of iron oxide nanoparticles.

1. Introduction

Amino groups are versatile functions for immobilization of bioactive or other molecules by coupling them to surfaces or polymer backbones. Using various amino functions in molecules and macromolecules, Michael addition, addition to oxiranes or thiocyanates, amide formation and reaction with aldehydes with or without subsequent reduction are widely applied (Hermanson, 2008). In the field of polysaccharides, chitosan obtained from chitin by *N*-deacetylation has been used for such *N*-selective coupling reactions. However, the lack of a spacer between the 2-amino-2-deoxy-glucosyl units of the β-1,4-linked glycan chain restricts applicability. Periodate oxidation of dextran followed by reductive amination has been used by Piehler et al. for the functionalization of silica surfaces for direct immunoprobes (Piehler, Brecht, Geckeler, & Gauglitz, 1996).

The objective of our work with dextran, an α-1,6-linked, branched glucan, is to perform aminofunctionalization while preserving the polysaccharide backbone and maintaining the intrinsic properties of the polymer. To introduce amino functions in a polysaccharide by a polymer analog modification, different approaches have been applied. Fujita et al. described the synthesis of aminoalkyl pullulans by reaction with aminoepoxyalkyl derivatives, e.g. 3-amino-1,2-epoxypropane. Product characterization was only performed by elemental analysis (Fujita, Fukami, & Fujimoto, 1979). However, direct introduction of unprotected amino groups is affected by the higher nucleophilicity of amino groups compared to hydroxyl groups, thus favoring tandem reactions and complex products. This can be avoided by using *N*-protected reagents or by introducing nitrogen in a higher oxidation state. Gonera et al. reported on the Williamson-type *O*-alkylation with phthalimido-protected bromopropylamine and subsequent mild deprotection by reductive hydrolysis (Gonera, Goclik, Baum, & Mischnick, 2002). Cyanoethylation with subsequent reduction to aminopropyl ethers has been widely used and for example applied to inulin (Verraest, da Silva, Peters, & van Bekkum, 1996; Verraest, Zitha-Bovens, Peters, & van Bekkum, 1998) as well as to cellulose and starch (Volkert, Wagenknecht, & Mai, 2010; Gonera, 2004; Gonera et al., 2002). Daly and Munir applied borane-dimethylsulfide in THF for the reduction of cyanoethyl cellulose and proposed an application as ion-exchange resin (Daly & Munir, 1984). Amino functions have also been introduced by click reaction of aminoalkyl azides to pentynyl dextrans, allowing variation of spacer length (Tahir, Lämmerhardt, & Mischnick, 2012). By nucleophilic substitution of 6-*O*-tosyl cellulose with an excess of diamino compounds, *N*-aminoalkyl-6-amino-6-deoxy celluloses have been obtained and used for enzyme immobilization and coating of silica surfaces (Berlin, Klemm, Tiller, & Rieseler, 2000).

Aminopolysaccharides can be used as adsorbent of heavy metal ions (Nakamura, Amano, Saegusa, & Sato, 1992). *O*-Aminoethyl inulin was prepared from inulin with ethylene imine resulting in a maximum DS of 0.76, and the antioxidant effect of

* Corresponding author. Tel.: +49 5313917201.
E-mail address: p.mischnick@tu-braunschweig.de (P. Mischnick).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.03.006

inulin and its derivatives was tested. Modified inulin showed a moderate hydroxyl radical scavenging ability and considerable superoxide-radical scavenging activity (Ren, Liu, Dong, & Guo, 2011). Furthermore, aminoethyl glucans can be applied as components in glycan arrays to analyze carbohydrate–protein interactions. (2-Aminoethyl)-aniline has been coupled to the reducing end, and the so modified carbohydrate directly been fixed by amide formation on a *N*-hydroxy-succinimide (NHS)-activated carboxyl groups bearing glass surface (Seo, Kim, Hwang, & Cha, 2010). By such coupling reactions on surfaces, aminoethyl glucans act as useful interfaces for glycoconjugation (Sardzik et al., 2010). Aminopropyl amylose coatings on PVDF surfaces have been shown to be inert against unspecific protein absorption (Ademovic, Gonera, Mischnick, & Klee, 2006). They have also been successfully applied for the stabilization of horseradish peroxidase (Gonera, Mischnick, & Ukeda, 2004). Polymers with pending amino groups like polyethylene imine have been decorated with maltooligosaccharides by reductive amination to investigate their ability as DNA/siRNA transporter (Höbel et al., 2011). In the following we present the preparation of aminopropyl dextrans (APD) from cyanoethyl dextrans in a wide range of DS. Self-assembly of fully and partially reduced cyanoethyl dextrans and coupling of some model aldehyde compounds as vanillin (Van), 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde (BHT-CHO), maltose (G_2), and maltotriose (G_3) was achieved by reductive amination.

2. Experimental

2.1. Materials

Dextran from *Leuconostoc ssp.* (M_w 6 kDa, puriss) was purchased from Fluka. Cobalt(II)-chloride (p.a.) was received from Across Organics. 2-Picoline borane complex (95%), vanillin (99%) and 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde hemihydrate (99%) were purchased from Aldrich. Maltose monohydrate and $NaBH_4$ were obtained from Merck, maltotriose from TCI Europe N. V. Solvents were of p.a. quality and distilled water was used. Dialysis tubes were obtained from Spectra/Por®, Spectrum Laboratories Inc. (MWCO: 3.5 kDa).

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Bruker AMX 300 spectrometer or a Bruker AMX 400 MHz Avance spectrometer at room temperature (around 5–10 mg sample in MeOH, D_2O or DMSO-d_6). Chemical shifts are given in ppm referred to residual solvent signals. 2D experiments were performed on a Bruker Avance DMX 600 MHz spectrometer (atom number assignment according to Fig. 1).

An Esquire HCT Ultra ETD II (Bruker Daltonics, Bremen, Germany) equipped with an ion trap was used to record ESI-MS spectra (positive mode). Sample solutions (20 μg/mL) were infused directly by a syringe pump with a flow rate of 200 μL/h. Nitrogen was used as drying gas (300 °C, 6 L/min) and as nebulizer gas (10 psi). The following voltages were applied: capillary ± 4500 V, end plate offset ± 500 V, capillary exit ± 100 V, trap drive 45 or 55, smart target 100,000. The maximum accumulation time was adjusted with 200 ms and 50 scans.

The hydrodynamic particle size was analyzed using a Zetasizer Nano ZS (Malvern Instruments Ltd.). DLS measurements were directly performed after dialysis. Nanoparticle dispersions were diluted with distilled water to a final concentration of 1.33 mg PS/mL.

Transmission electron micrographs were obtained using an EF-TEM Libra 120 plus Zeiss microscope operated at 120 kV. Samples were adsorbed to a hydrophilized carbon film, which was supported by a Cu grid (carbon only, copper 300 square mesh) and dried at room temperature. All images were recorded with a 2 × 2k SharpEye cooled CCD camera (Tröndle, Moorenweiss, Germany) in the magnification range from 4000× to 25,000× in the elastic bright-field mode, with the energy slit set to 10 eV.

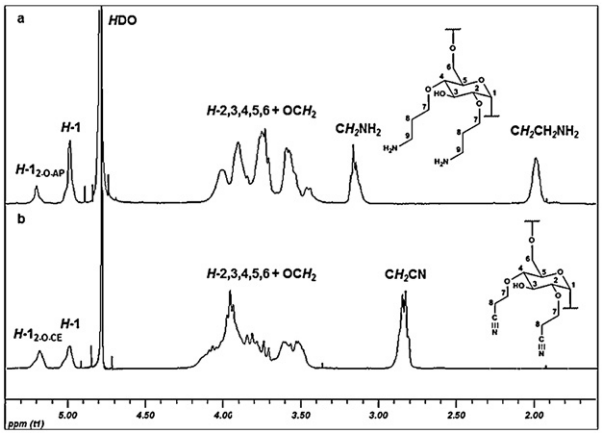

Fig. 1. ^{1}H NMR-spectra (D_2O, 400 MHz) of (a) APD (DS_{AP} = 0.64, D_{red} = 100%) and (b) CED (DS_{CE} = 1.01). The inserted structures show one example of possible substitution patterns.

2.3. Polysaccharide modification

2.3.1. Reduction of cyanoethyldextrans

Cyanoethyl dextrans were prepared by Michael addition using acrylonitrile as has been described (Fiege, Lünsdorf, Atarijabarzadeh, & Mischnick, 2012). Reduction of cyanoethyl dextrans to aminopropyl derivatives was performed according to procedures reported earlier (Gonera, 2004; Gonera et al., 2002; Verraest, da Silva et al., 1996; Verraest, Zitha-Bovens et al., 1998). The cyanoethyl dextrans were mixed with $CoCl_2$ in a big flask and dissolved in water under stirring. Highly substituted cyanoethyl dextrans (DS_{CE} > 2) were first dissolved in 1 mL DMSO and diluted with aqueous $CoCl_2$-solution. Aqueous $NaBH_4$ was added dropwise through a septum to the reaction mixture. Evolving hydrogen was effused through a needle. After 1 h stirring at room temperature, diluted HCl (0.1–1.0 M) was added to dissolve the black precipitate (cobalt boride). Products were isolated by a dialysis against distilled water. Detailed reaction parameters are given in Table 1.

IR (diamant-ATR): $\tilde{\nu}(cm^{-1}) = 3490 - 3234$ (OH + NH), 2919, 2882 (CH, CH_2, aliph.), 1640 (OH), 1550 (NH), 1159, 1099, 1001 (s, C-O).

^{1}H NMR (D_2O, 300 MHz) δ (ppm): 5.21 (1 H, *H*-1, substituted in position 2), 4.97 (1 H, *H*-1, unsubstituted), 4.25 – 3.35 (6 H + DS * 2 H, *H*-2,3,4,5,6a,b,7), 3.15 (2 H, *H*-9), 2.00 (2 H, *H*-8).

2.3.2. Reductive amination

Coupling of aldehydes to the amino functionalized dextran by reductive amination was performed according to literature (Jagadish, Divyashree, Viswanath, Srinivas, & Raj, 2012; Sato, Sato, Okubo, & Yamazaki, 1998; Unterieser & Mischnick, 2011). Detailed reaction parameters are listed in Table 2. Aminopropyldextrans were dissolved or dispersed in methanol under stirring and the aldehyde (4-hydroxy-3-methoxybenzaldehyde, 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde hemihydrate, maltose or maltotriose, 0.8 – 12 eq/NH_2, see Table 2) was added. The pH was adjusted by addition of HCl or NaOH (0.1–1 M). Reaction with aromatic aldehydes was performed at pH 4 (exception: Van-7 at pH 8). Reactions with maltose were carried out at pH 3, 7 and 9, maltotriose was applied