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Synthesis of amphiphilic 6-carboxypullulan ethers

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ABSTRACT

Hydrophobically modified polysaccharides that contain carboxyl groups possess exceptional features for drug delivery and other applications. Carboxyl groups were introduced at C-6 in the pullulan backbone by applying the well-established oxidation with TEMPO and NaOCl/NaBr. The oxidized product, 6-carboxypullulan, is even more water-soluble than pullulan. Consequently, further chemical modifications have been mainly restricted to reactions that can be performed in water or under heterogeneous conditions. We find that the TBA salt of 6-carboxypullulan is soluble in a range of organic solvents and can be reacted homogeneously with various alkyl halides in DMSO and sodium hydroxide at 40 °C to yield 6-carboxypullulan ethers. Complete substitution (DS 7 per trisaccharide repeat unit) was achieved upon reaction with iodoethane, while products from reaction with longer chain alkyl halides (propyl and butyl derivatives) achieved DS up to about 3. The amphiphilic products have impressive surfactant properties.

1. Introduction

Pullulan is a non-ionic water-soluble polysaccharide which is produced from starch by the yeast-like fungus *Aureobasidium pullulans* (Bauer, 1938; Bernier, 1958). It consists predominantly of maltotriose units, i.e. units of three 1,4-linked α-D-glucose molecules, which are polymerized in a linear fashion via 1,6-linkages, as shown in Fig. 1 (Bender & Wallenfels, 1959). Pullulan has low toxicity and has been used for more than 20 years as an additive in the food industry (Leathers, 2003). It biodegrades in the body and does not evoke an immune response. It has also been shown to be non-toxic when administered intravenously (Yamaoka, Yasuhiko, & Ikada, 1993). In view of the attractive characteristics of pullulan and the possibility of chemical modification to suit the desired application, there have been many reports of the synthesis of new pullulan derivatives with application in drug delivery (Rekha, 2007; Shingel, 2004). Pullulan is concentrated disproportionately in the liver after intravenous administration, and so has been studied as a promising polymeric carrier for liver-related diseases (Hosseinkhani, Aoyama, Ogawa, & Tabata, 2002; Xi et al., 1996).

Most pullulan modifications are intended to reduce its water solubility or to introduce charged or reactive groups for functionality (Akiyoshi, Yamaguchi, & Sunamoto, 1991; Hirakura, Nomura, Aoyama, & Akiyoshi, 2004; Jung, Jeong, & Kim, 2003). For drug delivery applications, the ability of the drug carrier to swell or disperse in water is often more desirable than water solubility (Kost & Langer, 2001). Highly water soluble polymer carriers tend to release drugs quickly, while polymers that only swell or disperse in water have the ability to provide slow drug release (Edgar, 2007).

Polysaccharides that have been hydrophobically modified and contain carboxyl groups are commonly used in drug delivery systems because of their ability to provide pH-controlled drug release (Dulong, Le Cerf, Picton, & Muller, 2006; George & Abraham, 2006; Lu et al., 2009; Posey-Dowty et al., 2007). For example, hydrophobic drugs often are released from carboxyl-containing polysaccharide matrices only at the neutral pH of the small intestine and colon, when the carboxyl group becomes ionized and the polymer swells, thus limiting exposure of the stomach to the drug. Moreover, polymer–drug interactions also play an important role in drug delivery systems. An important example is the strong interaction of carboxyl groups with amines (many drugs contain amine functional groups) by hydrogen bonding. The presence of hydrophobic groups is also important; hydrophobicity will enhance miscibility with hydrophobic drugs, and slow their release. Due in part to these valuable features, polysaccharide derivatives, especially

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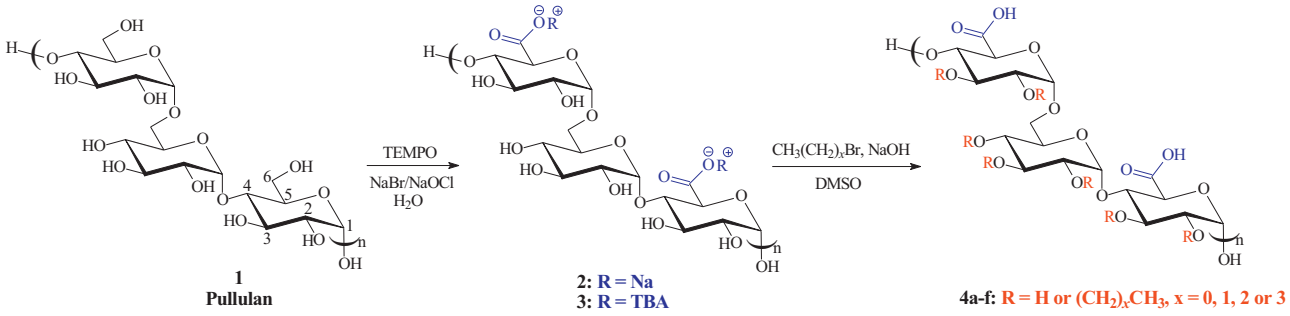

Fig. 1. Pullulan oxidation and synthesis of 6-carboxypullulan ethers.

cellulose derivatives containing pendant carboxyl groups, have been recently explored with success for amorphous solid dispersion (ASD) of drugs. These drug–polysaccharide matrix dispersions have shown the ability to significantly enhance solution concentrations and stability of many otherwise poorly soluble drugs by forming a matrix of drug, in a metastable amorphous form, entrapped within the polymer (Konno, Handa, Alonzo, & Taylor, 2008). Hydroxypropylmethylcellulose acetate succinate (HPMCAS) (Friesen et al., 2008) and cellulose acetate adipate propionate (CAAdP) (Ilevbare, Liu, Edgar, & Taylor, 2012; Kar, Liu, & Edgar, 2011) are examples of carboxylated cellulose derivatives that combine several of those attractive drug delivery functions and are promising polymers for drug delivery formulations.

Introduction of carboxyl groups to the non-ionic pullulan backbone should give an anionic derivative with interesting properties for drug delivery applications. The most widely investigated pullulan derivative containing a carboxylic acid group is carboxymethylpullulan (CMP) (Dulong et al., 2006). CMP is a promising polymeric carrier for many drugs since its high proportion of negative charges results in prolonged retention of the polymer within the organism (Yamaoka et al., 1993). CMP has been hydrophobically modified by esterification of the carboxyl groups with long alkyl chains (Henni-Silhadi et al., 2007). These derivatives self-assemble in aqueous media and efficiently solubilize hydrophobic drugs.

In the early 1990, Denooy, Besemer, and van Bekkum (1995) described the TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical)-mediated selective oxidation of various water-soluble polysaccharides with hypochlorite/bromide as the regenerating oxidant. The reaction is performed under homogeneous conditions in water and primary alcohol groups are selectively oxidized to yield carboxylates. The regioselectivity of the reaction is essentially complete, and chemoselective, favoring oxidation of the primary OH to a carboxylic acid; partial oxidation to aldehyde, or oxidation at the secondary OH groups to ketones, is minimal. Such high chemo- and regioselectivity is useful for potential use of oxidized pullulan derivatives in formulations that might reach the circulation, since full characterization and structural control on such polymers is important for regulatory approval. Oxidation of pullulan by this methodology has been reported, but modified 6-carboxypullulan compounds have not been studied much for their biomedical applications, although they may have great potential for use in drug delivery systems (Paris & Stuart, 1999; Yang, Du, Huang, Wan, & Wen, 2005). One possible reason for this is the fact that 6-carboxypullulan is even more water soluble than pullulan, therefore further chemical modification is mainly restricted to reactions which can be performed in water or under heterogeneous conditions. Additionally, highly water-soluble polysaccharide derivatives may not be highly miscible with hydrophobic drugs, and may give faster than desired release profiles (and/or with inadequate pH responsiveness) for particular drugs.

In this work we introduced carboxyl groups to the pullulan backbone by applying the selective TEMPO oxidation. We then explored methods for conversion of the oxidized pullulan product, 6-carboxypullulan, to its tetrabutylammonium (TBA) salt, seeking enhanced organic solubility that would permit more facile reactions of the remaining pullulan OH groups with electrophiles. We pursued this strategy by attempting homogeneous reaction of 6-carboxypullulan salts with various alkyl halides in DMSO, employing sodium hydroxide as base, as a route to the potentially useful 6-carboxypullulan ethers.

2. Experimental

2.1. Materials and methods

Pullulan (Mw = 450 kDa, Mn = 200 kDa) was from the Hayashibara Company (Okayama, Japan) and was dried under vacuum at 120 °C overnight prior to use. Water was deionized. TEMPO (99%, Aldrich), sodium hypochlorite (NaOCl, 14.5% chlorine, Alfa Aesar), NaBr (99%, Alfa Aesar), ethyl acetate (HPLC grade, Fisher), tetrabutylammonium fluoride trihydrate (99%, TBAF), pyridine (Py), tetrabutylammonium hydroxide (TBAOH, 1.0 M in water, Fluka Analytical), ethylene glycol (EG, laboratory grade, Fisher), and lithium chloride (99%, LiCl) were used as supplied. Dimethylsulfoxide (DMSO, HPLC grade, Acros) was dried using 4 Å molecular sieves. Dimethylacetamide (DMAc, HPLC grade, Fisher) and dimethylformamide (DMF, Fisher) were used as supplied. Bromoethane (98%, Alfa Aesar), bromopropane (98%, Aldrich), bromobutane (99%, Aldrich), iodomethane (99%, Aldrich), iodoethane (98%, stabilized with silver, Acros Organics) and iodobutane (98%, stabilized, Acros Organics) were used as supplied. Proton exchange resin was DOWEX 50WX8 100–200 (H) from Alfa Aesar. Deuterium oxide (99.9 atom % D; D_2O) containing 0.75% 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt and d_6-DMSO for NMR were acquired from Sigma–Aldrich. Trifluoroacetic acid (99%) used for ^{1}H NMR was from Acros and KBr used for FTIR analysis was obtained from International Crystal Laboratories.

For NMR analysis, samples were prepared by dissolving 8–10 mg (for ^{1}H) or 50–80 mg (for ^{13}C) of polymer in 0.7 mL of D_2O or d_6-DMSO. The solution was filtered through a pipette containing glass wool into a standard 5 mm NMR tube. ^{1}H and ^{13}C NMR spectra were acquired on Varian INOVA or Varian UNITY 400 MHz spectrometers with 32–128 scans for ^{1}H and minimum of 10,000 scans for ^{13}C. Chemical shifts are reported relative to the solvents, except for ^{13}C spectra acquired in D_2O, when TMS is used as the reference.

Degree of substitution (DS) values of the 6-carboxypullulan ethers are described as per trisaccharide repeat unit, with a