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Micelle-like association of polysaccharides with hydrophobic end groups

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ABSTRACT

New dextran derivatives with hydrophobic end groups were synthesized by reductive amination of dextran chain ends, followed by chemical modification of the dextran main chain by attachment of cationic groups and/or by crosslinking. Properties of the aggregates formed by hydrophobic association of the end groups were studied by fluorescence, dynamic light scattering, atomic force microscopy and transmission electron microscopy and depended on the length of the dextran chain (6, 10, 25 kDa) and the hydrophobicity of the end group (alkyl, dialkyl, bile acid). All neutral derivatives were able to form micelle-like aggregates above a critical aggregation concentration (0.008–0.159 g/dL). Polarity of the micelle hydrophobic core was close to or lower than that of neutral low molecular surfactants (polarity parameter $I_1/I_3 \approx 0.8–1.13$), aggregation number was 20–30 and hydrodynamic radius 20–30 nm. Attachment of cationic groups to the dextran main chain increased critical aggregation concentration and core polarity, but cationic polymeric surfactants with good association ability could be obtained by an appropriate choice of the content and hydrophobicity of the cationic groups. Cross-linking of the micelle shell with divinylsulfone increased micelle stability to dilution.

1. Introduction

Hydrophilic polymers with hydrophobic end groups can associate with formation of different types of interpolymeric aggregates, similar to low molecular surfactants or amphiphilic block copolymers. Double end (or α, ω−) modified polymers form flower-like micelles and are usually called associative telechelic polymers as they can associate in physical networks giving highly viscoelastic fluids at high concentrations. Therefore, these polymers attracted widespread interest due to their applications as thickeners, adhesives, adsorbants, coatings, flocculants, or surfactants. By comparison, single end modified polymers (or α− modified, semi-telechelic) which can associate into spherical (star like) micelles are less extensively studied (Rubinstein & Dobrynin, 1997) despite their potential application in biotechnology and medicine. Among applications foreseen for semi-telechelic amphiphilic polymers one can mention the steric stabilization of liposomes (Pignatello et al., 2013; Torchilin et al., 2001), nano-scaled colloidal drug carriers (Kuskov et al., 2010) or nanoreactors (Patterson, Cotanda, et al., 2013). In comparison with block-copolymers, these block-like telechelic polymers can be prepared by easier, more reproducible and less expensive methods.

Poly(ethylene glycol) was the polymer of choice for the design of both double and single end modified polymers, due to its known hydrophilicity, presence of reactive end groups, biocompatibility and stealth properties (Beaudoin, Hiorns, Borisov, & François, 2003; Chassenieux, Nicolai, & Durand, 1997; Song, Dai, Tam, Lee, & Goh, 2003; Zhou, Zhuang, Yuan, Jiang, & Zhang, 2000). Other polymers modified at one chain end with hydrophobic moieties were poly (N-isopropylacrylamide) (Ishii et al., 2010; Patterson, Kelley, et al., 2013; Winnik, Davidson, Hamer, & Kitano, 1992), poly[N^5-2-(hydroxyethyl) L-glutamine] (Inomata, Doi, Yamada, Sugimoto, & Nakanishi, 2007), poly (vinyl pyrrolidone) (Kuskov et al., 2010; Rizos, Tsikalas, Tsatsakis, & Shtilman, 2006; Torchilin et al., 2001), poly (2-alkyl-2-oxazoline) (Obeid, Maltseva, Thünemann, Tanaka, & Winnik, 2009; Volet, Chanthavong, Wintgens, & Amiel, 2005), polyvinyl alcohol (Uemura, Hirayama, Hatate, & Macdonald, 2001). Ionic polymers such as poly-(sodium 2-acrylamido-2-methylpropanesulfonate) (Mizusaki, Morishima, Raju, & Winnik, 2001; Yusa, Kamachi, & Morishima, 2000), poly(acrylic acid) (Klijn, Kevelam, & Engberts, 2000; Patterson, Cotanda, et al., 2013) or poly(2-(dimethylamino)ethyl methacrylate) (Báñez, Robinson, Vamvakaki, Lascelles, & Armes, 2000) were also used. Most of the end groups were alkyl (C8–C18) but other hydrophobes were also

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attached to polymers, for example fullerene (C60) (Song et al., 2003), cholesterol (Yusa et al., 2000), phospholipids (Auguste, Prud'homme, Ahl, Meers, & Kohn, 2003), azobenzen (Ishii et al., 2010) or pincer ligands (Patterson, Cotanda, et al., 2013; Patterson, Kelley, et al., 2013).

There are also few oligo- and polysaccharides end modified with hydrophobes: cellulose (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011), dextran with very low molar mass (about 1300) (Zhang & Marchant, 1996) or high molar mass (40 kDa) (Hirsjärvi et al., 2013; Richard, Barras, Younes, Monfilliette-Dupont, & Melnyk, 2008), highly branched oligoxyloglucan (Greffe, Bessueille, Bulone, & Brumer, 2005), O-acetyl-galactoglucomannan (Dax et al., 2013; Lindqvist, Holmback, Eosling, & Salminen, 2013). These derivatives were designed for application as surface active agents (Zhang & Marchant, 1996; Dax et al., 2013), surface coatings for lipid nanocapsules, which can increase their blood half-life (Hirsjärvi et al., 2013; Richard et al., 2008), or additives in paper making (Lindqvist et al., 2013). The hydrophobically modified polysaccharides can be perceived as polymeric analogues of commercially available saccharide surfactants, obtained either from pure saccharides (glucose, maltose or sucrose) (Garofalakis, Murray, & Sarney, 2000) or as mixtures of compounds containing alkyl chains of varying lengths and 1–5 condensed glucoside units) (so called alkyl polyglucosides (Biermann, Schmid, & Schulz, 1993; Sulek & Wasilewski, 2006). Due to their low toxicity, these surfactants are commonly chosen as additives for personal care products (von Rybinski & Hill, 1998), for biological membranes solubilization (Biermann et al., 1993), or as drug delivery systems (Abdelrahim, Simerska, & Toth, 2012; Söderlind, Wollbratt, & von Corswant, 2003).

The use of polysaccharides in the design of new semi-telechelic amphiphilic polymers takes the advantage of the presence of only one reactive end group, which makes possible a chemoselective end modification and allows the maintaining of polymer intrinsic biological properties (aqueous solubility, molecular recognition). Besides, the numerous reactive OH groups along the polymer backbone can be further chemically modified by introducing other functionalities (ionic groups, biologically active compounds) or by cross-linking.

The aim of the present work is the synthesis of a new series of block-like amphiphilic polymers by end attachment of hydrophobic moieties (alkyl, dialkyl, bile acids) to a polysaccharide, dextran of different molar masses. Chemical modifications of the polysaccharide main chain (crosslinking, cationic group introduction) were also performed in order to improve stability and applicability of aggregates. The properties of nanoaggregates formed by the modified polymers in aqueous solutions were studied as a function of the polymer chemical structure (nature of the hydrophobic end chain, molar mass of the dextran chain, presence of crosslinks and/or cationic groups). The size, shape, and polarity of aggregates were determined by a combination of different techniques: fluorescence, dynamic light scattering (DLS), atomic force microscopy (AFM), transmission electron microscopy (TEM).

2. Experimental part

2.1. Materials

Dextran samples from Leuconostoc mesenteroides with molecular weights M_r (as indicated by the supplier) 4000 (D4), 6000 (D6), 9000–11,000 (D10) and 15,000–25,000 (D25) were purchased from Sigma. The weight-average (M_w) and number-average molar masses (M_n) determined by size exclusion chromatography are presented in Table 1. (2′-Aminoethylene)-3α,12α-dihydroxy-5β-cholanoamide (NH_2-CH_2-CH_2-NH-CO-DCA) was prepared from deoxycholic acid (DCA) and ethylenediamine (EDA) using the procedure described by Liu et al. (Liu, Avoce, Song, & Zhu, 2001). All the other reagents were from Aldrich and used as received. DMSO and N-methylformamide (MeF) were dried on molecular sieves.

Table 1
Characteristics of dextran samples used for end modification.

Dextran sample	M_w	M_n	*PI*
D4	4250	2820	1.50
D6	6385	4500	1.42
D10	10,700	7955	1.34
D25	22,946	14,900	1.54

2.2. Polymer synthesis

The codes used for synthesized polymer samples and their descriptions are presented in Fig. 1.

2.2.1. Synthesis of end-modified dextran (DM-R_1)

End modified dextrans (DM-R_1) were synthesized by the reaction of reductive polysaccharide end with an amino group terminated hydrophobe. Synthesis of D10-C18 is given as an example. D10 (1 g, 0.122 mmol) was dissolved in dry DMSO (10 mL) at room temperature (r.t.), then $NaBH_3CN$ (0.1 g) was added to obtain the solution A. Another solution (B) was separately prepared from octadecylamine (0.3 g, 1.12 mmol) and dry MeF (5 mL) heated at 60 °C. Solution B was added drop-wise to solution A, under stirring and heating at 60 °C. The resulted homogeneous mixture was stirred for 48 h at 65 °C, and then poured into methanol (100 mL) for polymer precipitation. The precipitate was recovered by filtration and rinsed on the filter with methanol and chloroform. Two other precipitation/rinsing operations were performed in order to remove all unreacted amine. Drying under vacuum led to 0.8 g white powder (79% yield related to dextran). The modification degree was determined from ^{1}H NMR spectrum taken in DMSO-d_6 (Bruker Avance DRX 400 spectrometer) (Fig. 2) using formula: $(A_{dex}/DP_{dex})/(A_{CH3}/3)$, where A_{dex} and A_{CH3} are the integrals of the peaks assigned to the anomeric protons of the dextran (4.7 ppm,) and methyl protons of the alkyl chain (0.85 ppm,), respectively, and DP_{dex} is the dextran degree of polymerization. The obtained value, 1.02, corresponds to 98% end capped dextran chains. UV analysis (325–330 nm) performed on a 0.1 wt% aqueous solution indicated the complete reduction of intermediate Schiff base derivative to amine derivative.

No degradation of dextran chain occurred during reductive amination, therefore the theoretical molar mass of the end-modified derivatives was calculated as a sum of dextran and end group molecular weights (Table 2).

2.2.2. Synthesis of cationic end modified dextrans (DM-R_1-QR_2X)

Attachment of quaternary ammonium groups to the dextran main chain was realized by a procedure previously elaborated for both linear and crosslinked polysaccharides (Nichifor, Stanciu and Simionescu, 2010), based on the reaction of dextran with an equimolar mixture of a tertiary amine and epichlorohydrin (ECH) in an aqueous medium. The synthesis of D10-C18-QBz16 is given here as an example. D10-C18 (1 g) was dissolved in deionized water (10 mL), then N,N-dimethyl-N-benzylamine (2 mL) and ECH (1 mL) were sequentially added. After stirring for 6 h at 40 °C, the mixture was diluted with 5 mL methanol and precipitated in acetone. The precipitate was further purified by redissolution in a mixture water/methanol (10 mL, 2/1, v/v) and precipitation in acetone. The gel-like product was triturated with acetone until a white solid was obtained. After drying under vacuum, 0.9 g of the final product resulted. The degree of substitution ($X = 100x/(x+y)$, mol%) was