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Host–guest interaction and structural ordering in polymeric nanoassemblies: Influence of molecular design

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ABSTRACT

Host–guest nanoassemblies made from spontaneous self-association of host and guest polymers in aqueous solutions have been studied. The specific motivation behind this work was to clarify the impact of the molecular design of the polymers on the interactions between them and on the inner structure of the resulting nanoassemblies. The polymers were composed of a dextran backbone, functionalized with either pendant β–cyclodextrin (CD) or adamantyl (Ada). Those groups were connected to the backbone either directly or with hydrophilic polyethylene glycol (PEG) spacers. To study the impact of those spacers we have proposed a synthetic pathway to new guest polymers. The latter relied on the use of thiol-substituted dextrans as a scaffold, which is subsequently transformed into PEG-Ada grafted guest polymers *via* nucleophile-mediated thiol-click reaction. Surface plasmon resonance (SPR) studies evidenced strong mutual affinities between the host and guest polymers and showed that the stoichiometry was close to the ideal one (CD/Ada = 1/1) when PEG spacers were introduced. The structure of the nanoassemblies was studied by a combination of dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS). The nature of the individual host or guest polymers has a strong impact on the size and internal structure of the resulting nanoassemblies. The presence of PEG spacers in the polymers led to smaller and less compact nanoassemblies, as evidenced by their large correlation length values (4–20 nm compared to 2 nm without PEG spacers). At the same time, all types of nanoassemblies appear to have radial density distribution with denser cores and pending polymer chains at the periphery. This study, centered on the influence of the molecular design on the host–guest interactions and structural ordering in polymeric nanoassemblies, will help to tailor host–guest nanoassemblies with attractive drug delivery profiles.

1. Introduction

Complex, often hierarchically structured, polymeric nanoassemblies are gaining increasing importance in drug delivery and gene transfection applications (Angelova et al., 2011; Gref et al., 1994; Park et al., 2006; Xu et al., 2012). The main types of weak interactions exploited to construct such systems include, but are not limited to: electrostatic assembly (Faul, 2014), hydrogen bonding, van der Waals forces and host–guest interactions (Antoniuk and Amiel, 2016). Host–guest or inclusion complexation occurs as a result of "molecular recognition", i.e. synergy between van der Waals forces, hydrogen bonding and precise matching in size between a guest molecule and the hydrophobic cavity of a macrocyclic host. It results in higher specificity and manipulability of host–guest interactions compared to their simpler non-covalent counterparts (Harada et al., 2014). A wide range of macrocyclic "hosts" were rendered into polymeric form and used in supramolecular nanoassembly. Yet, to date cyclodextrins (CDs) remain undoubtedly the most profoundly studied and widely exploited (Duchêne and Bochot, 2016). CDs are naturally occurring cyclic oligosaccharides commonly composed of six, seven, or eight (α, β and γ-cyclodextrins, respectively) D-glucose units connected by α-1,4-glucosidic linkages (Khan et al., 1998; Rekharsky and Inoue, 1998; Tan et al., 2014). In addition to their rich host–guest chemistry cyclodextrins (Messner et al., 2010) also demonstrate high biocompatibility and are the only class of macrocycles

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approved by the Food and Drug Administration (FDA) for usage in food, personal care products and drug formulations (El Fagui and Amiel, 2012; Krauland and Alonso, 2007; Moya-Ortega et al., 2012; Qi and Schalley, 2014). Some notable examples of their applications include the first siRNA delivery vehicles studied in humans (Davis, 2009; Godinho et al., 2014). All these features make CDs and CD-containing polymers indispensable building blocks for the construction of hierarchically structured systems with potential biomedical applications.

Within this paradigm, size-controlled host–guest nanoassemblies based on cyclodextrin-modified polymers were previously described by our group (Daoud-Mahammed et al., 2009; Daoud-Mahammed et al., 2007; Wintgens et al., 2011). Those can be formed in pure water by self-assembly between hydrophobically substituted (alkyl, adamantyl (Ada)) dextrans and either βCD-epichlorohydrin branched copolymers (Daoud-Mahammed et al., 2007, 2009; Gref et al., 2006) or βCD-substituted linear dextrans with short spacers between the backbone and grafted βCD-groups (Wintgens et al., 2011). Attractive host–guest interactions between such polymers might lead to associative phase separation (Wintgens et al., 2008). At low weight concentrations this phase separation occurs on the nanoscale, leading to the formation of nanoassemblies, instead of a macroscopic phase separation. The cohesion of the polymers happens in a “zip fastener” manner through establishment of numerous “lock and key” physical cross-links. Furthermore, it was shown that by controlling the density of such cross-links one might tailor the compacity and colloidal stability of the nanoassemblies (Wintgens et al., 2011). It can be simply achieved either by changing the molar ratio between the host (βCD) and guest (Ada, alkyl) moieties in the mixture or by varying the degree of substitution (DS), or equivalently the grafting ratio, of the respective polymers. The closer to stoichiometrical host/guest molar ratios and higher DS typically yield more stable nanoassemblies with higher compactness.

Regarding their excellent biocompatibility profile together with their safe preparation stage done in aqueous solution, avoiding the use of organic solvents and surfactants, promising drug delivery applications of the host–guest nanoassemblies (HG nanoassemblies) were considered. However, so far little is known about the organization of the polymers and internal density profile in such nanoassemblies; nor is there clear understanding of how the polymer architecture influences their overall properties. In an attempt to answer these questions, we have introduced a flexible hydrophilic poly(ethylene glycol) (PEG) spacer between the dextran backbone and βCD (DT40-g-PEG-βCD) or adamantyl (DT40-g-PEG-Ada) groups. Several studies in the literature report the use of PEG spacers in the host or guest copolymers (Bellocq et al., 2004; Harada et al., 2014; Tan et al., 2014; van de Manakker et al., 2009) but none of them was centered on the inner structure of the self- assemblies. The idea was to see how the properties of the nanoassemblies may be tuned varying the properties of their component polymers (i.e. adding or removing the PEG spacers). Within this concept, we have recently reported the synthesis of DT40-g-PEG-βCD host polymers prepared using a combination of thiol-Michael addition and azide–alkyne cycloaddition click reactions (Antoniuk et al., 2014).

Here, we report the synthesis of adamantane-bearing guest polymer counterparts of DT40-g-PEG-βCD. We describe the impact of hydrophilic PEG spacer on the binding properties of the guest polymers in solution by isothermal titration microcalorimetry (ITC). Affinities of the guest toward host polymers are studied by SPR. The formation of host–guest nanoparticles upon mixing the host and guest counterparts’ solutions in water is further investigated by DLS and SAXS. The impact of the presence of the hydrophilic PEG spacer between the dextran backbone and the host/guest functions is estimated by comparing their binding properties with those of previously described dextran-based host–guest polymers with short hydrophobic spacers (Wintgens et al., 2011).

2. Experimental

2.1. Materials and reagents

Pyridine (99.9%, anhydrous), 4-nitrophenyl chloroformate (4-NC, 96%), cysteamine (98+%), *N*,*N*′-dimethylformamide (DMF, 99.8%, anhydrous), trimethylamine (≥99%) and dithioerythritol (99+%) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) and used as received. Hexylamine (99%) and sodium hydroxide (98+%, anhydrous pellets) were purchased from Acros Organics and used as received. 4-(Dimethylamino)pyridine (DMAP, 98+%) were purchased from Fluka and used as received.

Dextran (DT40, M_w 4.3×10^4 g mol^{-1}, PDI = 1.5, Amersham Pharmacia Sweden) and lithium chloride (Alfa Aesar) were dried overnight in vacuum at 95 °C prior to use. Poly(ethylene glycol) acrylate (M_n 375 g mol^{-1}, Sigma Aldrich) was dried overnight in vacuum at room temperature before use. 2-(1-Adamantyl) ethyl trimethyl ammonium bromide (AdaTMA) (Burckbuchler et al., 2006), thiolated dextrans (DT40-SH, DS_{SH} = 6.5 and 12.5 mol%) (Hiemstra et al., 2007), host polymer with short spacer (DT70βCD, $DS_{\beta CD}$ = 16.4 mol%) (Nielsen et al., 2010), guest polymers with short spacers (DT40Ada, DS_{Ada} = 6.6 and 8.8 mol%) (Layre et al., 2009) and epichlorohydrin-βCD branched copolymers (pβCD (Renard et al., 1997) and pβCDN+ (Blomberg et al., 2004)) were prepared according to previously described procedures. The molecular weight M_w of pβCD (used as a parent for the preparation of pβCDN+) is 2.1×10^5 g mol^{-1} and βCD content of 60% (w/w) as determined by ^{1}H NMR.

2.2. Synthetic procedures

PEG-acrylate-Ada: PEG-acrylate with $M_n \sim 375$ (0.27 mL, 0.8 mmol) was dissolved in 12 mL of $CHCl_3$ in a round-bottom flask equipped with a water-cooled condenser. A catalytic amount of DMAP (20 mg, 0.16 mmol) and NEt_3 (0.22 mL, 1.6 mmol) were added and the solution was cooled to 2–3 °C on ice bath followed by addition of 1-adamantanecarbonyl chloride (0.286 g, 1.44 mmol) as solid. The reaction mixture was then left under stirring at 2-3 °C and 500 rpm for 2 h, allowed to warm to room temperature and subsequently refluxed for 3 h. After evaporation of the solvent under reduced pressure the product was dissolved in 25 mL of 1:1 water–acetone mixture and stirred for 3 h at 45 °C and 400 rpm to destroy the excess of 1-adamantanecarbonyl chloride. The solvents were removed by evaporation under reduced pressure and drying in vacuum at 50 °C. The resulting PEG-acrylate-Ada was isolated as white solid and used in the next step without purification.

DT40-g-PEG-Ada: Thiolated DT40-SH with DS–=–12.5 mol% (0.38 g, 0.28 mmol of –SH groups) and lithium chloride (0.30 g, 7.1 mmol) were dissolved in 15 mL of DMF at 50 °C in a round-bottom flask while bubbling the solution with argon. The temperature was lowered to 35 °C and PEG–acrylate-Ada (0.430 g, 0.8 mmol) solution in 15 mL of DMF was added to the reaction mixture with a Pasteur pipette followed by hexylamine (152 μL, 1.02 mmol). After stirring at 65 °C and 500 rpm under argon for 16 h, the reaction mixture was precipitated in 420 mL of Et_2O in an Erlenmeyer flask, washed twice with Et_2O on a sintered-glass funnel with a porosity P4 (pore size 10–16 μm), filtered, redissolved in water and dialyzed against pure water for 5 days. The adamantyl-grafted guest copolymer (here DT40-g-PEG-Ada2) was isolated by freeze drying (m = 0.42 g). DS (from ^{1}H NMR = 10.5 mol%).