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Spontaneous association of hydrophobized dextran and poly-β-cyclodextrin into nanoassemblies. Formation and interaction with a hydrophobic drug

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Abstract

New nanoassemblies were instantaneously prepared by mixing two aqueous solutions, one containing a β-cyclodextrin polymer (pβCD), and the other a hydrophobically modified by alkyl chains dextran (MD). The formation mechanism and the inner structure of these nanoassemblies were analysed using surface tension measurements and ^{1}H NMR spectroscopy. The effect of a hydrophobic guest molecule, such as benzophenone (BZ), on the formation and stability of the nanoassemblies was also evaluated. MD exhibited the typical behaviour of a soluble amphiphilic molecule and adsorbed at the air/water interface. Whereas the injection of native β-CDs in the solution beneath the adsorbed MD monolayer did not produce any change in the surface tension, that of the pβCD resulted in an increase in the surface tension, indicating the desorption of the polymer from the interface. This result accounts for a cooperative effect of β-CDs linked together in the pβCD polymer on dextran desorption. The presence of benzophenone in the system hindered the sequestration of dextran alkyl moieties by β-CD in the polymer without impeding the formation of associative nanoassemblies of 100–200 nm. ^{1}H NMR investigations demonstrated that, in the BZ-loaded nanoassemblies, the hydrophobic molecule was mainly located into the cyclodextrin cavities.

Keywords: Nanoassemblies; Self-assembly; Hydrophobically modified polymer; Cyclodextrins; Surface tension; ^{1}H NMR

1. Introduction

Over the past decades, there has been a growing interest in the development of hydrophobically modified water-soluble polymers (HMP). They consist of a hydrophilic polymer backbone, on which hydrophobic moieties are grafted [1]. One of the most interesting feature of these polymers is their unique associative behaviour in aqueous solutions due to the strong tendency of their hydrophobic groups to aggregate together in order to minimize their contact with the solvent [2]. In semi-dilute solutions, these groups can form hydrophobic microdomains [3] resulting from intra and/or intermolecular interactions. The mode of association depends on polymer concentration, and on structural parameters such as the content, length and distribution of the hydrophobic groups along the polymer backbone [3–5]. At sufficiently high polymer concentrations, intermolecular associations prevail and act as transient cross-links connecting polymer chains.

Among the associative polymers, hydrophobized polysaccharides, such as cellulose derivatives [6], dextran [7,8], chitosan [9,10] or pullulan [11,12] are particularly attractive due to their biocompatibility, biodegradability and low toxicity, which are advantageous for biological and pharmaceutical applications. For instance, cholesteryl-bearing pullulans that can self-assemble to form nanoparticles and associate with various small hydrophobic molecules or proteins [13–16] were proposed as

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potential drug carriers [17]. Many studies also reported that aggregates formed of hydrophobized polysaccharides were able to solubilize poorly water-soluble drugs entrapped into their hydrophobic microdomains [18–21]. Moreover, the physicochemical and biochemical stability of these drugs appeared significantly increased when they were associated to these hydrophobized polysaccharides [22,23].

The solubilization and protection of drugs may also be achieved by using cyclodextrins (CDs). Indeed, CDs are known to form inclusion complexes with a wide range of low molecular weight compounds such as lipophilic drugs, modifying thereby their physicochemical properties [24,25]. The inclusion of a guest drug can improve its apparent solubility, physical and chemical stabilities, dissolution and bioavailability [26], thus making CDs very attractive drug carriers. The development of supramolecular assemblies, in which CDs were associated to macromolecules attracted much attention [27–29]. Harada et al. reported on the design of supramolecular structures consisting of CDs and poly(ethylene oxide) with a relevant cross-sectional area for its inclusion into CD cavities [27–29]. Huh and co-workers described systems, in which CDs formed inclusion complexes with poly(ethylene oxide)-grafted polysaccharides [30,31]. More recently, supramolecular gel-like networks were obtained by mixing a CD-bearing host polymer and a hydrophobically modified guest polymer [32–36]. Using the same approach, studies were carried out in our laboratory to design a stable dispersion of 200 nm in size nanoassemblies, combining the properties of both polysaccharides and CDs [37]. We showed that these nanoassemblies spontaneously form by mixing two aqueous solutions of soluble polymers: a hydrophobically modified dextran obtained by grafting alkyl moieties onto the polysaccharide backbone (MD) and a β-cyclodextrin–epichlorohydrin polymer (pβCD). The present study aimed at gaining information on the mechanism of formation of the MD–pβCD nanoassemblies, which has not yet been fully elucidated, and to determine whether a hydrophobic guest drug might be solubilized into these nanoassemblies without interfering with their formation and stability. The interaction of the host and guest polymers leading to the formation of the nanoassemblies was analysed by surface tension measurements [38–44] in the absence and in the presence of a model guest hydrophobic molecule (benzophenone). ^{1}H NMR spectroscopy was used to determine the inner structure of the nanoassemblies and locate the guest molecules in the CDs inclusion complexes [45–47].

2. Materials and methods

2.1. Materials

The guest polymer, a dextran grafted with dodecyl pendant groups (MD, Mw = 40 000 g/mol, polydispersity ~1.5, degree of polymerisation ~247, Fig. 1a, was synthesized as previously described [36,48]. Briefly, 0.43 mL of lauryl chloride and 0.031 mL of pyridine were reacted for 3 h at 80 °C with 4 g of dextran solubilized in 100 mL of a lithium chloride solution (1%) in dimethyl formamide. The MD was isolated by precipitation in isopropyl alcohol. It was further solubilized in distilled

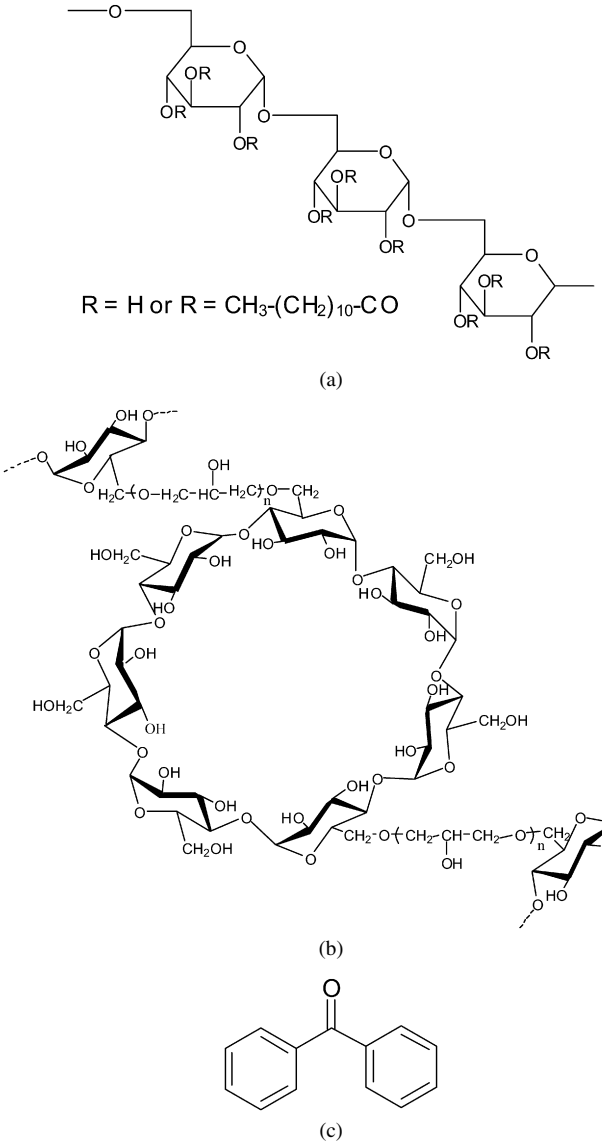

Fig. 1. Schematic representation of the chemical structures of (a) MD, (b) pβCD and (c) benzophenone.

water, purified by dialysis for 48 h using membranes with a cut-off of 6000–8000 g/mol (Spectra/Por®) and finally freeze-dried. The substitution yield of MD was 3.5 wt% of glucose units according to the ^{1}H NMR spectra, corresponding to about 8 alkyl chains per dextran macromolecule. In the MD polymer, the glucose units bearing alkyl chains are separated by spacers of 31 glucose units length. Thus, the average mass of one MD repeating unit is 5200 g/mol. As dextran Kuhn length is about 10–25 Å [49], the spacers between grafted alkyl chains can be considered as flexible.

The host β-cyclodextrin polymer (pβCD), which has a branched structure as represented in Fig. 1b, was prepared by reacting β-cyclodextrin (β-CD) with epichlorohydrin (EP) under strong alkaline conditions [50]: 100 g of anhydrous β-CD were dissolved in 160 mL NaOH 33% w/w aqueous solution and left under mechanical stirring overnight. Then, 81.52 g of EP (molar ratio β-CD/EP = 10) were rapidly added to the solution heated to 30 °C. In order to obtain a high molecular weight