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Structural characterization of lyotropic liquid crystals containing a dendrimer for solubilization and release of gallic acid☆

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ABSTRACT

The role of 2nd generation polypropyleneimine (PPIG2) dendrimer in controlling the release of gallic acid (GA) as a model drug from lyotropic liquid crystal was explored. GA (0.2 wt%) was solubilized in three types of mesophases: lamellar (L_α), cubic (space group of Ia3d, Q^G), and reverse hexagonal (H_{II}), composed of GMO and water (and D-α-tocopherol, or tricaprylin in the case of H_{II} mesophases).

Small angle X-ray scattering (SAXS) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) along with UV spectrophotometry were utilized to elucidate the structure modifications and release resulting from the cosolubilization of GA and PPIG2.

Solubilization of PPIG2 into L_α and Q^G phases caused transformation of both structures to H_{II}. The diffusion of GA out of the mesophases was found to be dependent on water content and PPIG2 concentration. Rapid release from L_α + PPIG2 and Q^G + PPIG2 mesophases was recorded. The release from both H_{II} mixtures (with D-α-tocopherol and tricaprylin) was shown to be dependent on the type of oil.

Release studies conducted for 72 h showed that GA release can be modulated and sustained by the presence of PPIG2, supposedly due to the electrostatic interactions between the dendrimer and the drug molecule.

1. Introduction

Macromolecular carriers such as dendrimers have generated considerable interest as one of the most promising nano-scale delivery devices for anti-cancer and HIV therapies [1–9].

The particular properties of nanometric and monodispersed dendrimers make these molecules appealing for biomedical applications [2,10–13]. The utility of the internal void volume of dendrimers to encapsulate hydrophobic guest molecules and drugs has been demonstrated by several research groups [14–18]. In general, dendrimers are used as well-defined scaffolding or nano-containers to conjugate, complex, or encapsulate therapeutic drugs or imaging moieties [1,19]. They are hyperbranched three-dimensional molecules that consist of a central core, branching units, and terminal functional groups [20,21]. The terminal groups play an important role in controlling the properties of therapeutic moieties that are encapsulated or complexed with it [1].

Gallic acid (GA) is a polyhydroxyphenolic compound that can be found in various natural products, such as green tea, gallnuts, oak bark, and apple peels [1,22,23]. The main interest in GA is related to its anti-tumor activity. Indeed, anti-cancer activity of GA has been reported in various cancer cells, such as leukemia and prostate, cervical, and esophageal cancers [24–26]. GA and its derivatives show selective cytotoxicity against a variety of tumor cells [27–29]. The natural antioxidant GA has also demonstrated significant inhibition of cell proliferation and induction of apoptosis in a series of cancer cell lines [30].

There are several studies on the interaction between GA and dendrimers. Klajnert et al. reported on interaction of GA and different types of polyamidoamine (PAMAM) dendrimers with modified surfaces such as amino groups and hydroxyl-terminated [31]. It was found that the extent of GA incorporation into dendrimers depends on the type of dendrimer. Another study by Sharma et al. showed that GA-PAMAM dendrimer conjugates might be a promising nano-device for cancer diagnosis and targeting [1].

Nevertheless, the release of GA remains barely understood. Moreover, designing an efficient system to serve as a carrier for oral or transdermal applications and drug release in the presence of dendrimers from such carriers, and understanding the structural properties of the delivery system, are essential demanding tasks.

We designed a biological carrier in which the co-encapsulation and the delivery of GA and dendrimer can be efficiently controlled. We designed three lipid-based mesophases: lamellar (L_α), cubic (space group of Ia3d, Q^G), and reverse columnar hexagonal (H_{II})

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lyotropic liquid crystal (LLCs), in which PPIG2 dendrimer was entrapped, as described in our recent studies [32,33]. These lipid-based systems should interact with the guest molecules and influence the release profile while maintaining the PPIG2 and GA concentrations.

In the present work, GA has been chosen as a model anionic drug with strong electrostatic possibility to interact with the cationic dendrimer and to investigate its solubilization and release within several types of LLC vehicles. We prepared L_α and Q^G mesophases based on glycerol monooleate (GMO) and water (with and without PPIG2), while the H_{II} system is constructed from GMO and D-α-tocopherol (VE) [34,35] or tricaprylin (TAG) [36] and mixed with GMO and water (with or without PPIG2). The impact of the cosolubilized and entrapped PPIG2 and GA molecules on the L_α, Q^G, and H_{II} structures was investigated by several advanced analytical techniques.

2. Experimental

2.1. Materials

Monoolein, GMO, and distilled glycerol monooleate consisting of 97.1 wt% monoglycerides, 2.5 wt% diglycerides, and 0.4 wt% free glycerol (acid value 1.2, iodine value 68.0, melting point 37.5 °C) were purchased from Riken (Tokyo, Japan). D-α-Tocopherol, vitamin E 5–96 (containing 1430 international units of vitamin E) was obtained from ADM (Decatur, IL, USA). Tricaprylin (triacylglycerol, TAG; assay 97–98%) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid (GA) (97%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Polypropyleneimine (second generation, PPI-G2; >95% purity) was obtained from SyMO–Chem (Eindhoven, The Netherlands). Hydrochloric acid 37% was purchased from Sigma–Aldrich (St. Louis, MO, USA). Water was double distilled. Phosphate buffered saline (PBS, catalog number P3688) was purchased Sigma Chemical Co. (St. Louis, MO, USA). The contents of one pouch, when dissolved in one liter of distilled or deionized water, will yield 0.01 M phosphate buffered saline (NaCl 0.138 M; KCl 0.0027 M); pH 7.4, at 25 °C. All ingredients were used without further purification.

2.2. Sample preparation

The starting composition of 25 wt% PPIG2 at pH = 8.6–8.7 was chosen for the solubilization experiment in comparison with pure water [32,33]. Each LLC system (empty and loaded with PPIG2 and GA) was formed by mixing the entire component as follows: in the case of LLC loaded with PPIG2 and GA, 25 wt% PPIG2 was dispersed in the water prior to its incorporation into the LLC mesophases. The L_α LLC system was composed of 85 wt% GMO, 15 wt% aqueous phase, and 0.2 wt% GA. The Q^G mesophase was composed of 65 wt% GMO, 35 wt% aqueous phase, and 0.2 wt% GA. The H_{II} mesophase consisted of 72 wt% GMO, 8 wt% VE or TAG (GMO/(VE or TAG) 9/1 ratio), 20 wt% water solution, and 0.2 wt% GA. All these systems were also formed with pure water (without PPIG2) for comparison and with 0.2 wt% GA. After adding the water solution to GA, GMO, and the GMO/(VE or TAG) mixture, the sample was heated to ~70 °C in sealed tubes under nitrogen (to avoid oxidation of the GMO) for ca. 15 min. The samples were stirred and cooled to 25 °C. The solution of PPI-G2 in distilled water was formed at pH = ~8.6–8.7 by HCl (37% concentration) titration. It should be noted that as a result of PPIG2 solubilization, the concentrations of the water were decreased, keeping the weight ratio of GMO/(VE or TAG) (9:1) and GMO with aqueous phase constant.

2.3. Small-angle X-ray scattering (SAXS)

SAXS measurements were used to identify the structure of the LLC containing GA and PPIG2 before and during the release. The scattering experiments were performed using Ni-filtered Cu K_α radiation (0.154 nm) from an Elliott rotating anode X-ray generator that operated at a power rating of 1.2 kW. The X-ray radiation was further monochromated and collimated by a single Franks mirror and a series of slits and height limiters, and measured by a linear position-sensitive detector. The samples were held in 1.5 mm quartz X-ray capillaries inserted into a copper block sample holder. The temperature was maintained at 25 ± 1 °C with exposure time of ca. 20–60 min. The camera constants were calibrated using anhydrous cholesterol. The scattering patterns were desmeared using the Lake procedure implemented in home-written software [37].

2.4. Attenuated total reflectance Fourier transform infrared (ATR-FTIR)

An Alpha P model spectrophotometer, equipped with a single reflection diamond ATR sampling module, manufactured by Bruker Optik GmbH (Ettlingen, Germany), was used to record the FTIR spectra (GMO/water and GMO/(VE or TAG)/water with GA and PPIG2 at different aqueous concentrations). The spectra were recorded in 50 scans, with spectral resolution of 2 cm^{-1}, at room temperature. The absorbance intensities reported here were reproducible to ±0.005.

2.4.1. ATR-FTIR data analysis

Multi-Gaussian fitting was utilized to resolve individual bands in the spectra. The peaks were analyzed in terms of peak frequencies, widths at half-height, and areas.

2.5. Release experiments

To study the release from the LLC systems, solutions of ~400 mg of LLC containing 0.2 wt% GA with 25 wt% PPIG2 were prepared, diluted with ~15 ml of PBS buffer (pH = 7.4), and kept at 25 °C. Each system was compared to its reference (without PPIG2 and GA) to exclude contribution to the release of other components, e.g. VE and TAG. Samples of 4 ml of the PBS buffer were collected at constant times, and the GA and PPIG2 concentrations of each sample were determined by a Cary 100 Bio UV–vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). The GA and PPIG2 concentrations were calculated according to the absorption at 259 nm. Each sampling was followed by insertion of 4 ml of fresh PBS. Throughout the experiment, each vial containing the LLC floating in excess of PBS was gently shaken before sampling 4 ml for UV measurements.

3. Results and discussion

3.1. Solubilization of GA within the LLC, unloaded and loaded PPIG2

3.1.1. SAXS experiments

SAXS measurements were conducted to elucidate the effect of GA solubilization on the lattice parameter of the mesophases composed of GMO + aqueous phase (15 and 35 wt%, i.e. L_α and Q^G) and GMO/VE or TAG (90/10 weight ratio) + 20 wt% aqueous phase. The aqueous phase contained 25 wt% PPIG2 (Fig. 1, Supplementary data).

Supplementary data related to this article found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2013.06.051.

SAXS results indicated a noticeable increase in the lattice parameter of the L_α mesophase (no PPIG2), changing from 42 to 47.5 Å