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Self-assembled carbohydrate-based vesicles for lectin targeting

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ABSTRACT

This study examined the physicochemical interactions between vesicles formed by phosphatidylcholine (PC) and glycosylated polymeric amphiphile *N*-acetyl-*β*-D-glucosaminyl-PEG_{900}-docosanate ($C_{22}PEG_{900}GlcNAc$) conjugated with Bauhinia variegata lectin (BVL). Lectins are proteins or glycoproteins capable of binding glycosylated membrane components. Accordingly, the surface functionalization by such entities is considered a potential strategy for targeted drug delivery. We observed increased hydrodynamic radii (R_H) of PC + $C_{22}PEG_{900}GlcNAc$ vesicles in the presence of lectins, suggesting that this aggregation was due to the interaction between lectins and the vesicular glycosylated surfaces. Furthermore, changes in the zeta potential of the vesicles with increasing lectin concentrations implied that the vesicular glycosylated surfaces were recognized by the investigated lectin. The presence of carbohydrate residues on vesicle surfaces and the ability of the vesicles to establish specific interactions with BVL were further explored using atomic force microscopy (AFM) and small-angle X-ray scattering (SAXS) analysis. The results indicated that the thickness of the hydrophilic layer was to some extent influenced by the presence of lectins. The presence of lectins required a higher degree of polydispersity as indicated by the width parameter of the log-normal distribution of size, which also suggested more irregular structures. Reflectance Fourier transform infrared (HATR-FTIR), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) and ultraviolet-visible (UV–vis.) analyses revealed that the studied lectin preferentially interacted with the choline and carbonyl groups of the lipid, thereby changing the choline orientation and intermolecular interactions. The protein also discretely reduced the intermolecular communication of the hydrophobic acyl chains, resulting in a disordered state.

1. Introduction

The surface functionalization of nanoparticles and liposomes has become of increasing importance since Ehrlich publicized his vision of the "Magic Bullet" [1]. Vesicles and liposomes (phospholipid-based vesicles) have been extensively studied due to their widespread application as controlled drug delivery vehicles in the pharmaceutical industry and as biomembranes [2–4]. Because of their lipid composition, vesicles have lower toxicity than other vehicles, making them promising systems for the delivery of a wide range of drugs requiring specific treatments, controlled circulation times, reduced side effects and optimum drug action [5–11]. The possibility of directing a drug toward targeted tissues without changing its structure and hence, its biological activity, is fundamental for therapeutic applications [12]. Thus, efforts have been directed toward improving the vectorization of drug delivery systems to specific target tissues. In this context, the surface functionalization of vesicles with natural or synthetic glycolipids has been proposed to enhance the specificity of vesicles for lectins [12,13]. Lectins are non-immunological proteins or glycoproteins that specifically recognize sugar molecules and are capable of binding glycosylated membrane components. They are widely used to characterize carbohydrates on cell surfaces [14]. The *Bauhinia variegata* lectin (BVL) is particularly found in Caesalpinoideae plants. Its subunit has molecular weight of ~33 kDa and diffraction pat-

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ter similar to related lectins such as *Bauhinia purpurea agglutinin* (BPA) [15]. The carbohydrate-lectin binding typically involves two or three terminal sugar residues of mammalian glycans, including galactose, mannose, *N*-acetyl-neuraminic acid, fucose, and *N*-acetyl-glucosamine [16,17].

High levels of lectins, such as galectin-3, have been detected in various cancers [12]. The specificity of the carbohydrate-lectin interaction has been exploited to convey glycosylated liposomes to tumor cells [12,18–20]. Therefore, the development of nanoparticles with outer shells decorated with glycoconjugates for lectin targeting is considered a promising means to improve the delivery and internalization of antitumor drugs [16,21]. Previously, our research group described the physicochemical interactions between vesicles composed of phosphatidylcholine-purified soybean lecithin and the glycosylated polymeric amphiphile *N*-acetyl-β-D-glucosaminyl-PEG$_{900}$-docosanate conjugate ($C_{22}PEG_{900}GlcNAc$). Structurally, the ~100 nm composite vesicles self-assemble *via* attractive force between the lipidic region and the nitrogen groups of $C_{22}PEG_{900}GlcNAc$. The results also suggested discrete interaction among the hydroxyl and carbonyl regions of $C_{22}PEG_{900}GlcNAc$ [22]. Amphiphiles containing PEG chains have become a topic of interest due to their biocompatibility and anomalous behavior in water [23]. Through the PEGylation of vesicles, nanoparticles, and proteins, the residence times of these carriers can be significantly extended and diminish their uptake by the organs (e.g., liver and spleen) of the reticulo endothelial system (RES) [24,25].

This present work reports on the interaction between self-assembled vesicles (composed of phosphatidylcholine-purified soybean lecithin and the glycosylated polymeric amphiphile, $C_{22}PEG_{900}GlcNAc$) and BVL. The physicochemical properties of the phosphatidylcholine-based vesicle system containing $C_{22}PEG_{900}GlcNAc$ and lectin were investigated by several techniques, including zeta potential, dynamic light scattering (DLS), atomic force microscopy (AFM), small-angle X-ray scattering (SAXS), horizontal attenuated total reflectance Fourier transform infrared (HATR-FTIR), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) and ultraviolet-visible (UV-vis.) spectroscopy measurements. The physicochemical properties of these self-assembled vesicles presented herein could contribute to improved vectorizations of drug delivery systems in cancer therapy.

2. Materials and methods

2.1. Materials

Phosphatidylcholine (PC) from soybean lecithin (95% phosphatidylcholine, 5% lysophosphatidylcholine and phosphatidic acid) was a gift from Solae do Brasil S.A. The molecular composition of the soybean PC was approximately 75% distearoylphosphatidylcholine (DSPC, 18:0), 12% dioleoylphosphatidylcholine (DOPC, 18:2) and 8% dipalmitoylphosphatidylcholine (DPPC, 16:0) [26]. All reagents were of commercial grade and were used as received unless otherwise noted. The native *Bauhinia variegata* (BVL) lectin was extracted from seeds, according to the protocol described by Pinto et al. [15].

2.2. Synthesis of $C_{22}PEG_{900}GlcNAc$ and soybean lecithin purification

$C_{22}PEG_{900}GlcNAc$ was synthesized according to a previously reported method [27] and phosphatidylcholine (PC) was obtained through the purification of soybean lecithin according to previously reported methods [22,26,28].

2.3. Vesicles preparation

Vesicles were prepared by a reverse-phase evaporation method [26,29]. The preparation of the vesicles without the lectin was previously presented (reference [20]). Herein the preparation has been performed in phosphate buffered saline (PBS, 10 mM, pH 7.2) instead of water. The types of vesicle were prepared comprising self-assembled structures between PC and $C_{22}PEG_{900}GlcNAc$ (PC + $C_{22}PEG_{900}GlcNAc$) in the absence (pure) and in the presence of *Bauhinia variegata* lectin at a PC:$C_{22}PEG_{900}GlcNAc$:lectin ratio of 1:1:1 (w/w). Lectin was previously solubilized in 10 mM PBS and added in the organogel hydration step. The solution was then filtered using 0.45-μm pore-size nylon-membrane filters to remove dust and large aggregates. The final vesicle concentration was 15 mg/mL.

2.4. Electrophoretic light scattering

The zeta potential measurements were performed on a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments) with a coherent He-Ne 632.8 nm laser. Vesicle suspensions (15 mg/mL) in phosphate buffered saline (PBS, 10 mM, pH 7.2) were diluted to 1 mg/mL at 20 ± 1 °C for the zeta potential measurements. Ten measurements were performed in triplicate for each sample.

2.5. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) experiments were performed on a BI-200 goniometer with a BI-9000 AT digital correlator (Brookhaven Instruments) with a He-Ne laser (λ = 632.8 nm) as a light source. For the DLS experiments, the scattering volume was minimized using a 0.4 mm aperture and an interference filter before detecting the signal on the photomultiplier at a 90° angle. The autocorrelation functions were obtained in a multi-τ mode using 224 channels. The samples were filtered through membrane filters with a pore size of 0.45 μm directly into the sample cell and placed in the index matching liquid decahydronaphthalene (Aldrich). The autocorrelation functions were analyzed by using the nonlinear inverse Laplace transformation algorithm REPES [30] resulting the distributions of size. The temperature used for the analyses was 20 ± 1 °C.

The relaxation frequency, $\Gamma = 1/\tau$, is a function of the scattering angle [31]. The apparent diffusion coefficient D_{app} of the nanoparticles was calculated from Eq. (1):

$$q \to 0 \left| \frac{\Gamma}{q^2} = D_{app} \right. \quad (1)$$

where q is the scattering vector:

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (2)$$

λ is the wavelength of the incident laser beam (632.8 nm), n is the refractive index of the sample, and θ is the scattering angle. The hydrodynamic radius R_H (or diameter, $2R_H$) was calculated using the Stokes-Einstein relation given in Eq. (3):

$$R_H = \frac{K_b T}{6\pi\eta D_{app}} \quad (3)$$

where k_B is the Boltzmann constant, T is the temperature of the sample and η is the viscosity of the solvent (water).

2.6. Small angle X-ray scattering (SAXS)

Small angle X-ray scattering (SAXS) measurements were performed at the SAXS1 beamline of the Brazilian Synchrotron Light Laboratory (LNLS – Campinas, SP, Brazil) operating at wavelength