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Role of the hydrophilic spacer of glucosylated amphiphiles included in liposome formulations in the recognition of Concanavalin A

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ABSTRACT

The functionalization of liposomes with glycosylated amphiphiles is an optimal strategy for targeted drug delivery, leading to enhanced efficacy as well as to reduced side effects of drugs. In fact, the presence of natural or synthetic glycolipids in vesicle formulations might increase their specificity toward lectins, a class of non-enzymatic sugar-binding proteins involved in cellular recognition and adhesion. The capability of a new glucosylated synthetic amphiphile to interact with Concanavalin A (Con A), a plant lectin used as model system, was investigated by a synergic experimental and computational approach, both as pure component and in formulation with a natural phospholipid. The comparison of the affinity with Con A of the new glucosylated amphiphile with respect to that of a previously described structural analogue demonstrates that the hydrophilic spacer length controls the exposure of the glucose residue on liposome surface, and consequently the recognition by the lectin.

1. Introduction

The highly specific interactions of carbohydrates with lectins, a class of non-enzymatic sugar-binding proteins, are involved in a wide variety of cellular recognition processes including bacterial and viral infections, immune response and tissue growth [1,2]. Since lectins locate on various cell surfaces, including microbial and viral ones, targeting devices exploiting the recognition of sugar moieties have been frequently investigated in recent years [3]. Glycosylated liposomes (glycoliposomes), obtained by functionalization of vesicles with glycosylated amphiphiles, represent a promising approach to obtain the selective targeting of drugs to diseased tissues *in vivo*, leading to reduction of drug toxicity and improved therapeutic outcomes. It was reported that galactosylated liposomes loaded with doxorubicin showed a higher specific cell uptake of the drug by human hepatocellular carcinoma HepG2 cells with respect to non-targeted liposomes both *in vitro* and *in vivo* experiments [4]. Liposomes decorated with galactose and mannose residues were used to deliver azidothimydine and stavudine in the treatment of HIV-1-infected monocytes and macrophages, attaining enhanced cellular uptake [5–12]. Furthermore, the presence of lectins on pathogen surface can be exploited by using glycoliposomes as an attractive drug delivery strategy in antibacterial therapies [13].

The distance of the carbohydrate moiety from the liposome surface is a crucial parameter for the interaction with lectins [14,15]. In fact, in the absence of a hydrophilic spacer linking the sugar residue to the lipid, the sugar moieties do not extend into the water in a manner favorable to bind lectins [16]; however, an excessive length of the spacer might hamper the interaction with lectins [17]. Hence a molecular design that guarantees the proper exposure of the saccharidic residues on the surface of the lipid nanocarrier is a delicate issue.

Herein we report on the synthesis and the physicochemical characterization of the new glucosylated amphiphile **1** (Chart 1) and of the aggregates that it forms as a pure component and in formulation with dimyristoyl-*sn*-glycero-phosphocholine (DMPC). The binding of monomeric glucosylated amphiphile (GA) with the

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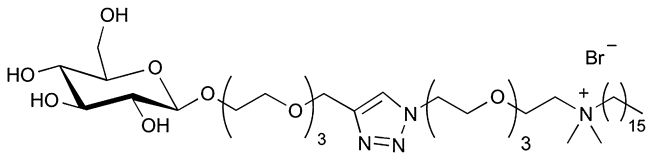

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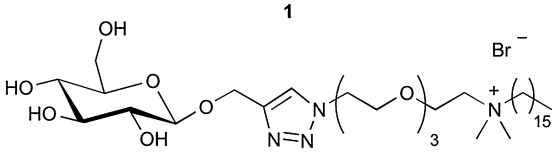

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Chart 1. Glucosylated amphiphiles **1** and **2**.

plant lectin Concanavalin A (Con A), used as model system, was investigated by fluorescence experiments on Con A fluorescently labeled with fluoresceinisothiocyanate (FITC-Con A), whereas the binding of glucosylated liposomes DMPC/**1** with the protein was investigated by agglutination monitored by optical density (OD) and dynamic laser light scattering (DLS). The affinity of GA **1**, both as a monomer and as a liposome component, with the lectin is compared with analogous experiments carried out on its analogue **2** (Chart 1) and described previously [18]. The difference between GAs **1** and **2** concerns the length of the hydrophilic spacer that connects the glucose residue to the quaternary nitrogen. The effect of the presence of GA **1** or **2** in the DMPC bilayer organization was investigated in the presence and in the absence of Con A by differential scanning calorimetry (DSC). Molecular dynamics calculation on DMPC/**1** and DMPC/**2** lipid bilayers were carried out to obtain a thorough understanding of the experimental results.

2. Experimental

2.1. Instrumentation

A Bruker 300 Avance spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C) and a Bruker 400 Avance spectrometer (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C) were used to record NMR spectra. ^{1}H resonances of deuterated solvents were used as internal standards.

A LTQ Orbitrap XL instrument was used to record HRMS-ESI spectrum. A Fluoromax-4Horiba-JobinYvon spectrofluorimeter was used to carry out steady-state fluorescence experiments.

A Cary 300 UV–vis double beam spectrophotometer (Varian Australia PTY Ltd., Mulgrave, Vic., Australia) was used to carry out OD and UV measurements.

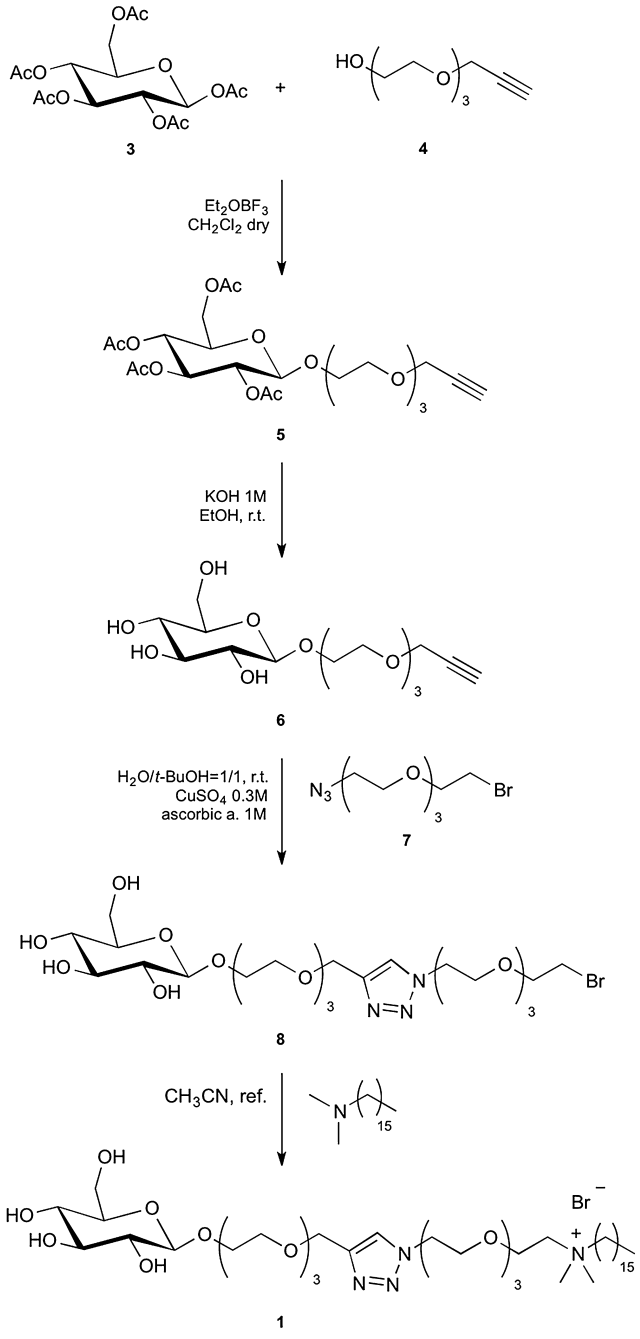
A Hanna conductimeter, HI-9932, equipped with a thermostating apparatus, was used to carry out conductivity measurements in the temperature range 4–60 °C. All experiments were performed in a jacketed cell kept at the appropriate temperature (±0.1 °C).

A Malvern Nano-ZetaSizer spectrometer, equipped with a 5 mW HeNe laser (λ_{ecc} = 632.8 nm) and a digital logarithmic correlator was used to perform DLS measurements. The normalized intensity autocorrelation functions were measured at an angle of 173 ° at 25.0 ± 0.1 °C. The autocorrelation functions were analyzed by using the cumulant fit. The first cumulant was used to obtain the apparent diffusion coefficients D of the particles, further converted into apparent hydrodynamic diameters, d_h, by using the Stokes–Einstein relationship $d_h = k_B T/3\pi\eta D$, where $k_B T$ is the thermal energy and η *is* the solvent viscosity.

A METTLER TA 3000 calorimeter provided with a TC 10 A processor by keeping the cell (DSC30) under N_2 flow was used to perform DSC measurements.

2.2. Materials

DMPC was purchased from Avanti Polar Lipids (Alabaster, AL, USA). FITC-Con A was purchased from Invitrogen. Con A, from Canavalina ensiformis (Jack bean), glycogen, from bovine liver, phosphate-buffered saline (PBS; Aldrich; 0.01 M phosphate buffer; 0.0027 M KCl; 0.137 M NaCl; pH 7.4), and all reagents employed for the synthesis of **1** were purchased from Sigma-Aldrich.



Scheme 1. Synthetic pattern to obtain glucosylated amphiphile **1**.