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Influence of the insertion of a cationic peptide on the size and shape of nanoliposomes: A light scattering investigation

Olivier Wattraint[a,*], Imene Saadallah[a], Viviane Silva-Pires[b], Pascal Sonnet[b], Catherine Sarazin[a]

[a] Unité de Génie Enzymatique et Cellulaire, UMR 6022 CNRS, Université de Picardie Jules Verne, 33 rue Saint-Leu, 80039, Amiens cedex, France
[b] Laboratoire des Glucides, FRE 3517 CNRS, UFR de Pharmacie, Université de Picardie Jules Verne, 1 rue des Louvels, 80037, Amiens cedex 1, France

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ABSTRACT

Nanoliposomes are widely used for drug delivery in the human body. Cell-penetrating peptides are amphiphilic peptides inserting in the lipid bilayer of these lipid vesicles to induce the fusion with target cells. Separation by size exclusion chromatography coupled with the analysis by light scattering detectors provides both the hydrodynamic radius and the radius of gyration of all the liposomes in a sample. In this paper, the influence of the insertion of a cationic peptide, K_2LA_{12}, on the size and shape of anionic liposomes has been studied by this approach. The results obtained highlighted an increase in size and a slight deformation of the lipid vesicles depending on the concentration of peptides incorporated into the lipid bilayers.

Nanoliposomes are nanoscale lipid vesicles composed of a single phospholipid bilayer encapsulating an aqueous core. Their applications in cosmetics and in drug delivery are often reported in the literature (Gregoriadis, 1992; Lasic and Papahadjopoulos, 1995; Lasic, 1998). Cell-penetrating peptides (CPPs) are amphiphilic molecules containing a high abundance of cationic amino acids and a hydrophobic fragment allowing their insertion in the lipid bilayers of nanoliposomes. They constitute one of the most promising tools for delivering various biomolecules in cells such as DNA, peptides or proteins (Elliott and O'Hare, 1997; Frankel and Pabo, 1998; Morris et al., 2008; Lebleu et al., 2008; Belting and Wittrup, 2009). Synthetic amphiphilic peptides such as the series of $K_2(LA)_x$ (where $x = 6$, 8, 10, 12) constitute good models of CPP (Dieudonne et al., 1998). These peptides have a strong affinity for anionic phospholipid bilayers (Wadhwani et al., 2012). The present work focuses on the influence of the a $K_2(LA)_{12}$ cationic peptide on the size and shape of anionic liposomes studied by Gel Permeation Chromatography coupled to a triple detection. Photon Correlation Spectroscopy (PCS) is currently used to determine the size of nanoliposomes and to validate the concept of encapsulation of molecules. However, since liposomes are polydisperse samples, separation of lipid vesicles according to the size before size measurement by PCS provides more accurate values (Carrozzino et al., 2004). Size distribution of liposomes separated on ethylene glycol-methacrylate gel in HPLC column has been extensively studied (Lesieur et al., 1993; Lundahl et al., 1999; Grabielle-Madelmont et al., 2003). In this paper, we demonstrated that the separation of liposomes by size exclusion chromatography with the simultaneous measurement of the R_h and the radius of gyration (R_g) was a powerful approach to determine both the size and shape for all the liposomes composing a sample.

SUV composed of a mixture (65:35, mol/mol) of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Sigma-Aldrich, France) and 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG, Avanti Polar Lipids, USA) were prepared starting from a multilamellar vesicle (MLV) dispersion. The phospholipids were dissolved in a mixture of chloroform/methanol (3:1, v/v). The solvent was evaporated under nitrogen and dried in a desiccator under vacuum overnight. The obtained lipid film was hydrated with ultrapure milliQ water and freeze-dried to remove the solvent traces. MLV were then obtained by suspending the lipid-dried film at room temperature in a HEPES buffer (10 mM, pH 7.4) with NaCl (145 mM). After shaking with a vortex mixer, the samples were subjected to 5 freeze–thaw cycles around the lipid transition phase temperature (23 °C) to ensure complete equilibrium. The suspension was then sonicated using a sonicator equipped with a 13 mm titanium probe at a power level of 100 W (Fisher Bioblock Scientific, France). Several cycles of sonication with a length of

* Corresponding author. Tel.: +33 03 22 82 74 73; fax: +33 03 22 82 75 95.
E-mail address: olivier.wattraint@u-picardie.fr (O. Wattraint).

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5 minutes were used and the samples were cooled by an ice bath. The nanoliposomes are then filtered through a 0.45 μm membrane filter to remove titanium particles. The peptide $K_2(LA)_{12}$ was synthesized from commercially available Fmoc-Ala-Wang-Tentagel® (Iris Biotech GmbH, Germany) on an Applied Biosystems Model 433A peptide synthesizer, using standard automated continuous-flow solid-phase peptide synthesis methods (Pires et al., 2010). Mass spectra were recorded on a Micromass-Waters Q-TOF Ultima spectrometer equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound. HRMS calculated for $C_{120}H_{219}N_{28}O_{27}$ [MH+] 2485.66643, found 2485.66137. SUV containing the $K_2(LA)_{12}$ peptide are obtained with the same protocol by dissolving several peptide molar ratios with phospholipids in chloroform/methanol (3:1, v/v).

HP-SEC experiments were performed with an HPLC system equipped with a LC-20A HPLC pump (Shimadzu, Japan). The column used was a Shodex OHpak SB-806 HQ (Japan) and was conditioned at 25 °C. The mobile phase is a HEPES buffer (pH 7.4) with NaCl (145 mM) filtered through a 0.1 μm membrane filter. The column was previously saturated by liposomes in order to avoid their non-specific adsorption on the polymeric phase. The sample was eluted at a flow rate of 0.5 mL/min and monitored by three detectors in series composed by a refractometer (RID-10 Shimadzu, Japan), a Multi Angle Laser Light Scattering detector (Dawn-Heleos 8+, Wyatt Technology, USA) and a Photon Correlation Detector (Dynapro Nanostar, Wyatt Technology, USA). R_g, R_h and cumulative number fraction plots were processed with ASTRA 5.3.4 software (Wyatt Technology, USA) based on the Zimm formalism (Korgel et al., 1998) with a dn/dc value equal to 0.15.

Phosphatidylcholine is classically used in biomimetic membranes as this zwitterionic phospholipid is largely represented in biological membranes. The negative charge is provided by phosphatidylglycerol. In this work, liposomes composed by DMPC and DMPG (65:35, mol/mol) were used and the corresponding elution chromatogram is presented in Fig. 1. The elution of the liposomes occurs between 16.2 and 21.7 min. The R_h values decrease throughout the elution and range from 54 to 22 nm. The R_g values also decrease and range from 61 to 20 nm. It seemed that the Shodex column presents a low selectivity for radii lower than 22 nm as seen for elution times greater than 19.5 min. The distributions of the R_h and R_g values are broad and confirm that the liposome samples obtained after sonication are polydisperse.

In order to determine the number of sonication cycles required to obtain the lipid vesicles with the smaller sizes, liposomes composed of DMPC and DMPG have been prepared from 6, 9 and 12 sonication cycles. The corresponding cumulative number fraction curves (Fig. 2A and B), reflecting the effective number of vesicles

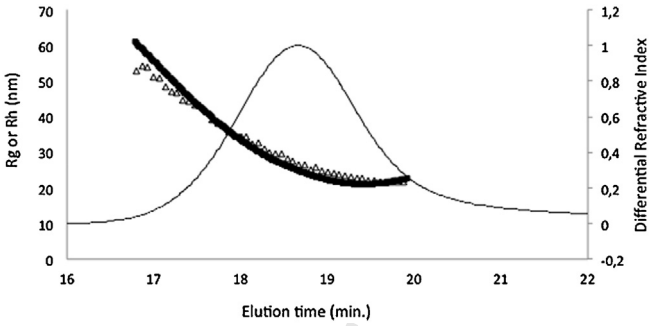

Fig. 1. Elution chromatogram obtained from SEC-MALLS of liposomes composed by DMPC and DMPG (65:35, mol:mol) and obtained after 9 sonication cycles. dRI signal (—), R_g (■) and R_h (△) are plotted as a function of the elution volume.

reveal a weak decrease in all liposome sizes as the number of the sonication cycles increase. The median R_g and R_h values, corresponding to the size of 50% of the vesicle population (Table 1) highlight that the liposome sizes are only weakly affected by the sonication cycles as more than a number of 6 cycles appears to be sufficient to obtain the smaller vesicles. The shape factor (ρ) calculated from the ratio R_g/R_h is a parameter providing significant information on the morphology of particles in suspension in a medium (Yamakawa, 1971; Shen et al., 2005). The calculated ρ values (reported in Table 1) are close to 1.0 and indicate that the sonicated vesicles are spherical and unilamellar (Fig. 3). Q2

The influence of the insertion of a hydrophobic positively charged peptide (K_2LA_{12}) in SUV composed by DMPC and DMPG has been investigated through the same method. The cumulative number fraction curves for R_g and R_h show a decrease of these values according to the number of sonication cycles. From the cumulative number fraction of R_g, the vesicles with the smaller sizes are obtained after 12 cycles of sonication. The median R_g and R_h values (Table 1) seem to be influenced by the sonication cycle since the R_g and the R_h values decreased respectively of 9 nm and 8 nm from 6 to 12 sonication cycles. Furthermore, the liposome sizes determined from the R_g values are greater than those obtained for the liposomes that do not incorporate the peptide since a difference of 13 nm is calculated for 12 cycles of sonication. The values of the shape factor (reported in Table 1) are greater than those observed for vesicles and indicate that the insertion of the peptide inside the lipid bilayers of liposomes leads to its weak deformation.

In order to highlight the influence of the peptide concentration on the size and morphology of liposomes, three lipid-to-peptide (L/P) molar ratio values ranging from 10 to 40 have been used, the lowest value (L/P = 40) corresponds to the value classically used

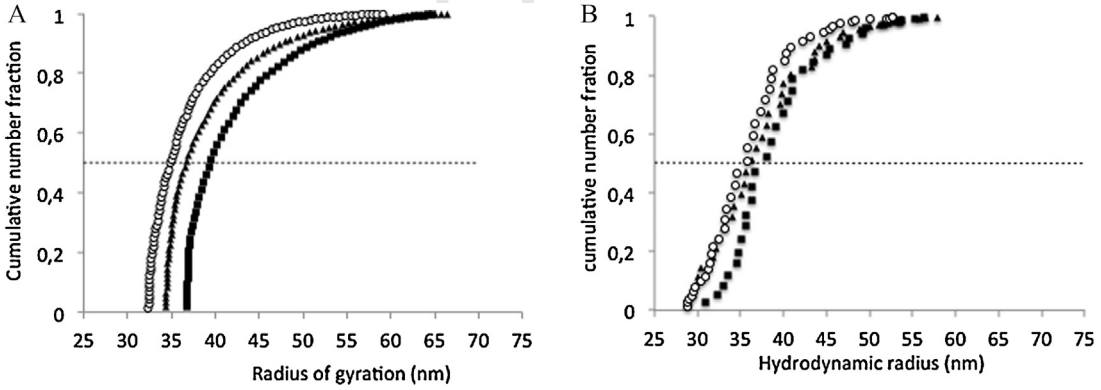

Fig. 2. Cumulative number fraction plots of liposomes composed by DMPC and DMPG (65:35, mol:mol) obtained after 6 (■), 9 (▲) and 12 (○) sonication cycle number. (A) Distribution of the R_g and (B) distribution of the R_h. Median values are represented by the dotted line.

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