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A DSC investigation on the influence of gemini surfactant stereochemistry on the organization of lipoplexes and on their interaction with model membranes

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ABSTRACT

Previous investigations showed that the extent of DNA condensation and the efficiency in the transfection of liposomes formulated with 1,2-dimyristoyl-*sn*-glycero-phosphocholine and cationic stereomeric gemini surfactants depend heavily on the stereochemistry of the gemini. The influence of the stereochemistry on the interaction of lipoplexes with zwitterionic and anionic cell membrane models was investigated by differential scanning calorimetry to rationalize their different biological behavior. Further, the thermotropic behavior of the corresponding liposomes and of the spontaneous self-assemblies of gemini surfactants in the presence and in the absence of DNA was evaluated to correlate the physicochemical properties of lipoplexes and the stereochemistry of the cationic component. The obtained results show that the stereochemistry of the gemini surfactant controls lipoplexes organization and their mode and kinetic of interaction with different cell membrane models.

1. Introduction

Since Felgner pioneering investigation to exploit cationic lipids as vehicles for the transfer of DNA into eukaryotic cells (Felgner et al., 1994), cationic liposomes have been extensively studied as non viral transfection vectors in gene therapy (Ma et al., 2007). The electrostatic interactions between the cationic lipids and the negatively charged nucleic acids lead to the condensation of DNA and to the formation of compact structures (lipoplexes) that, reducing the size of DNA, enhances its cellular uptake, protects it from degradation by endogenous nucleases and promotes interaction between positively charged DNA complexes and the negatively charged cellular membrane (Plautz et al., 2011). The structure of lipoplexes and their transfection activity vary considerably with lipid composition (lipid molecular structure and molar ratio of the components, surface charge, acyl chain saturation) (Zuhorn and Hoekstra, 2002), preparation protocol (Tros de Ilarduya et al., 2002), size of liposome (Regelin et al., 2000) and charge ratio ($\rho_{+/-}$, defined as the ratio of positive charge equivalents of cationic component to negative charge equivalents of the nucleic acid) (Felgner et al., 1997). A number of physical and physico-chemical factors have been suggested as lipofection modulators, however, the specific parameters that rule the interaction of lipoplexes with cells and their efficiency of delivery are still under investigations. In this context, the influence of the molecular structure of cationic lipids on the complexation of DNA, on the organization of the lipoplexes and on their biological activity is an issue of major interest.

We previously reported a detailed investigation on the capability of liposomes formulated with 1,2-dimyristoyl-*sn*-glycero-phosphocholine, DMPC, and each one of the stereomeric cationic gemini surfactants (gemini) (*2S,3S*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N,N*-dimethylammonio)butane bromide, **1**, (*2R,3R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N,N*-dimethylammonium)butane dibromide, **2**, (*2R,3S*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N,N*-dimethylammonium)butane dibromide, **3** (Chart 1) to compact calf thymus DNA (CT DNA) and a plasmid DNA and it was shown that the stereochemistry strongly affected DNA condensation (Bombelli et al., 2005a,b) and transfection (Bombelli et al., 2005b).

Here it is reported on a DSC investigation aimed at rationalizing the biological features of the above mentioned formulations at DMPC/gemini equimolar ratio. In particular the DSC measurements

Abbreviations: $\rho_{+/-}$, ratio of positive charge equivalents of cationic component to negative charge equivalents of the nucleic acid; DMPC, 1,2-dimyristoyl-*sn*-glycero-phosphocholine; CT DNA, calf thymus DNA; DSC, differential scanning calorimetry; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt); HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-ethanesulfonic acid; Tm, main transition temperature; TEM, transmission electron microscopy.

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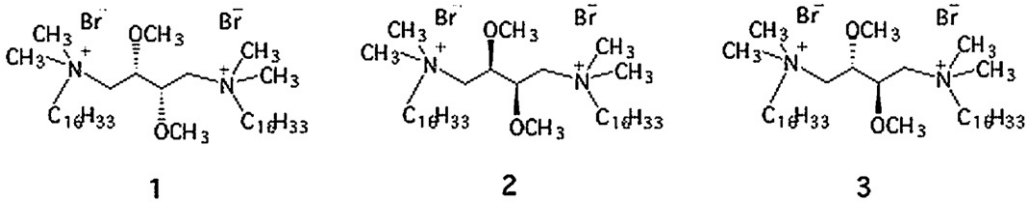

Chart 1. Structure of the stereomeric cationic gemini surfactants **1**, **2**, **3**.

were carried out to investigate the interaction of CT DNA lipoplexes with liposomes of DMPC or 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DPPG) used as zwitterionic or anionic cell membrane models, respectively. With the aim of rationalizing the influence of the stereochemistry on the complexation of DNA and on the organization of lipoplexes, DSC measurements were carried out on aggregates of gemini surfactants and on their complexes with CT DNA, on the DMPC/gemini liposomes and on their corresponding lipoplexes with CT DNA. For the sake of clarity, lipid/DNA complexes formed by the pure gemini in the absence of DMPC will be indicated as gemini/DNA complexes whereas lipid/DNA complexes containing also DMPC will be indicated as lipoplexes. Lipoplexes were formulated at DMPC/gemini equimolar ratio and at $\rho_{+/-} = 2$ because these formulations had previously shown high efficiency of DNA condensation (Bombelli et al., 2005a) and transfection (Bombelli et al., 2005b). Investigating the mode of interaction by which cationic lipoplexes interact and fuse with biological membranes is important to understand how these lipid vesicles mediate cell transfection.

2. Experimental

2.1. Materials

DMPC and DPPG (purity > 99%) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. CT DNA, *N*-(2-hydroxyethyl) piperazine-*N*'-ethanesulfonic acid (HEPES) were purchased from Sigma Aldrich. Surfactants **1**, **2** and **3** were prepared and purified as reported previously (Bello et al., 2006).

2.2. Sample preparation

2.2.1. Determination of the transition temperature

A 20 mL sample of a 60 mM (1 mg/20 μL) aqueous solution of the gemini was heated to obtain a clear solution that was kept at 4 °C for 12 h before the measurements were started. During the measurements, the solution was continuosly stirred and the temperature was raised at a rate of 0.5 °C/min till complete solubilization of the surfactant. Complete solubilization was occasionally checked also by turbidimetry.

2.2.2. Preparation of gemini self-assemblies and gemini–DNA complexes

A solution of gemini surfactant 60 mM (1 mg/20 μL) in HEPES buffer solution (5 mM HEPES and 0.1 mM EDTA at pH 7.4) was prepared on the inside wall of a round-bottom flask. Gemini–DNA complexes were prepared adding to the surfactant solution a known volume of an aqueous solution of DNA in HEPES buffer in order to obtain cationic complexes (at 1/1 gemini/DNA molar ratio, *i.e.* $\rho_{+/-} = 2$).

2.2.3. Preparation of MLVs

A lipid film was prepared on the inside wall of a round-bottom flask by evaporation of $CHCl_3$ solutions containing the proper amount of lipids to obtain either the cationic liposomes or the cell membrane models. The obtained films were stored overnight under reduced pressure (0.4 mbar), then a HEPES buffer solution was added to obtain a lipid dispersion 1 mM for preparation of LUVs and a concentration of 1 mg total lipids/20 μL (67.5 mM) for preparation of MLVs (both cationic liposomes and cell membrane models). Lipoplexes were prepared by addition of a known volume of an aqueous solution of DNA 30.7 mM in HEPES buffer to the cationic MLVs solution to obtain cationic complexes ($\rho_{+/-} = 2$) and a concentration of 1 mg total lipids/20 μL (67.5 mM). The solutions were then heated at 45 °C and vortex-mixed.

2.3. Measurements

2.3.1. DSC measurements

DSC measurements were carried out (i) on assemblies of pure gemini surfactants in the presence and in the absence of DNA, (ii) on cationic DMPC/gemini MLVs and on the corresponding lipoplexes and (iii) on mixtures, at a 1:1 ratio, of DMPC or DPPG MLVs and lipoplexes. Each sample, kept under continuous stirring, was incubated at 45 °C and 35 μL aliquots of the suspension were examined every 2 h. All calorimetric experiments were performed with an adiabatic differential scanning calorimeter Pyris1 (Perkin Elmer). The cells were pressurized with nitrogen to about 2.75 mbar to prevent bubbling when heating and the loss of solvent by evaporation. Heating scans of micelles, micelle–DNA complexes and lipoplexes and of mixture were recorded at 1 °C/min and 5 °C/min rate, respectively. In the case of pure gemini, gemini–DNA complexes and lipoplexes, a scan rate of 0.5 °C/min was used to verify if the shape of the heat capacity curves could depend on the scan rate, and based on these experiments, it was ascertained that the phase transitions under consideration were not influenced by the scan rate (Biltonen and Lichtenberg, 1993). Each sample was heated several times up to the achievement of reproducible thermograms. The experiments were all repeated and were reproducible. The given values are calculated as the mean and standard deviation of two sample for each one of three independent experiments.

3. Results

3.1. Transition temperature of gemini surfactants

Enantiomeric gemini surfactants **1** and **2** form aggregates that feature different transition temperatures and different phase behavior with respect to the aggregates of **3**. In a 60 mM solution **1** or **2** are not completely dissolved in water below 40 °C, whereas above 40 °C an opaque and dense suspension (coagel phase) is clearly observed; by increasing temperature the coagel phase begins to dissolve and at 47 °C the transition from the coagel to a completely transparent gel phase is complete. The coagel phase is constituted by densely packed surfactant lamellae separated by thin interlayers of strongly bound water molecules (mostly hydration water) with the aliphatic chains in *all-trans* conformation, whereas in the gel state the hydrophobic tails are arranged in a hexagonal lattice and the water interlayer thickness is larger with