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Short communication

Characterization of DNA–lipid complexes commonly used for gene delivery

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Abstract

Cationic liposomes are used to deliver genes into cells. Here we describe some poorly understood basic features of DNA–lipid complexes (lipoplexes), especially the electrostatics, stability and DNA structure of lipoplexes, and their effects on transfection (lipofection). Use of the lipophilic, pH-sensitive fluorophore 4-heptadecyl-7-hydroxycoumarin, in combination with Gouy–Chapman calculations, showed that cationic liposomes had a large positive surface potential (180–240 mV) and a high pH (10–11.5) at the location of the probe on the liposomal surface in 20 mM Hepes buffer (pH 7.4). Other electrostatic characteristics were also found, such as the existence of protonable groups of cationic or helper lipids or salt bridges between those. Addition of DNA resulted in neutralization of cationic lipids, which was lower than expected and depending on the type of lipid and the DNA/cationic lipid ratio. The liposomes containing DOTAP (*N*-(1-(2,3-dioleoyloxy)propyl)-*N*,*N*,*N*-trimethylammonium chloride) were unstable upon dilution, probably due to the high critical micellar concentration of DOTAP, 7×10^{-5} M. Large instablility expressed as continuous size increase was demonstrated by the time-dependent static changes in light-scattering monitored following the mixing of cationic liposomes and DNA at DNA/cationic lipid molar ratios between 0.2 and 0.8. Addition of cationic liposomes composed of 100% DOTAP or DOTAP/DOPE (1/1) liposomes, induced instantaneous transition of the plasmid DNA from the B- toward a partial C-type conformation as shown by circular dichroism (CD) spectroscopy and at certain conditions Ψ^--DNA could be found as well. The Ψ^--DNA is characterized by inter-helical interaction between parallel helices. The highest

Abbreviations: DC-CHOL, 3β(N-(N′,N′-dimethylaminoethane)carbamoyl)cholesterol; DMRIE, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine; DOTAP, N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride.

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lipofection was obtained under conditions of lipoplex instability, and when the DNA was partially dehydrated and had a partial Ψ^-- structure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Fluorescence; Surface potential; Electrostatics; Cationic liposomes

1. Introduction

DNA–cationic lipid complexes (also called lipoplexes) are commonly used in gene therapy to deliver DNA into mammalian cells and are currently also under test in several clinical trials (Ledley, 1995). The lipoplexes enter cells most likely by adsorptive endocytosis. In spite of extensive efforts, many results obtained with lipoplexes are hard to explain. Fundamental knowledge is lacking and most of the achievements reached are based on trial and error. We studied some poorly understood basic features of DNA–lipid complexes (lipoplexes), especially the electrostatics, stability and DNA structure of lipoplexes and their effects on transfection (lipofection).

2. Elecrostatics

Using the lipophilic, pH-sensitive fluorophore 4-heptadecyl-7-hydroxycoumarin (HC), in combination with Gouy–Chapman calculations, allows one to characterize the electrostatic parameters of the cationic liposomes alone and the lipids complexed with DNA at the water–lipid interface (Zuidam and Barenholz, 1997, 1998). HC is a weak acid. At $pH < pK_a$ the maximal fluorescence intensity is found at an excitation wavelength of approximately 320 nm (emission at 450 nm), and at $pH > pK_a$ the excitation wavelength of the maximum is shifted to approximately 380 nm. The fluorescence intensity at excitation wavelength of 330 nm is independent of surface pH and surface potential, thus 330 nm is an isosbestic point. Therefore, the 380/330 nm ratio can be used to monitor the dissociation degree of HC in liposomes as function of pH and to estimate its apparent proton binding constant (pK_a). Fig. 1 shows such titration curves of HC in LUV composed of 100% DOTAP, DOTAP/DOPE (mole ratio 1/1), DOTAP/DOPC (mole ratio 1/1) DMRIE/DOPE (mole ratio 1/1), DC-CHOL/DOPE (mole ratio 1/1), 100% DOPC and DOPC/DOPE (mole ratio 1/1). All titration curves in Fig. 1 consist of a single sigmoid, except that of DC-CHOL/DOPE. The curve of DC-CHOL/DOPE shows that DC-CHOL in these bilayers had a pK_a of 8.0. Thus, in contrast to DOTAP and DMRIE which were 100% charged, DC-CHOL in DC-CHOL/DOPE (1/1) liposomes was only about 50% charged in 20 mM Hepes buffer (pH 7.4).

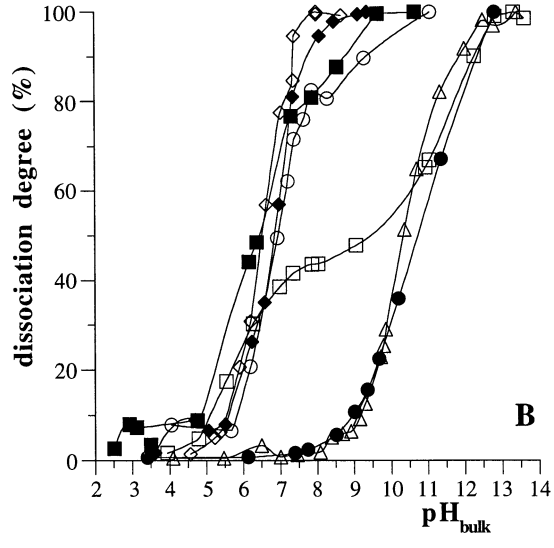

Fig. 1. The dissociation degree of HC in liposomes as monitored by the ratio of the excitation fluorescence intensities at 380 nm and at the isosbestic point 330 nm translated into percentages of the maximum value against the pH_{bulk}. The liposomes were composed of DOTAP/DOPE (1/1) (○), DOTAP/DOPC (1/1) (◆), DOTAP (◇), DMRIE/DOPE (1/1) (■), DC-CHOL/DOPE (1/1) (□), DOPC (△), and DOPC/DOPE (1/1) (●). Taken from Zuidam and Barenholz (1997).

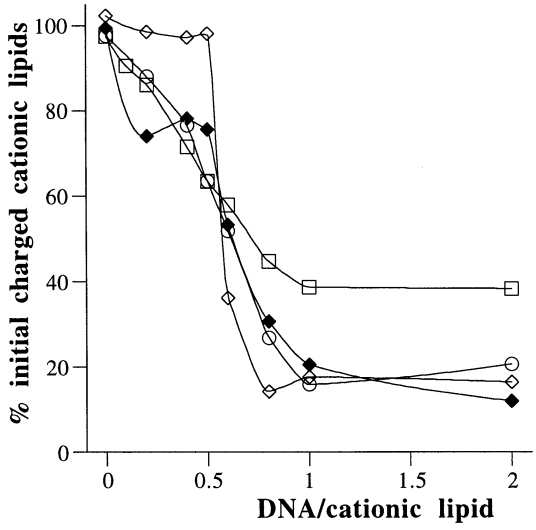

Fig. 2. Percentage of charged cationic lipids in the lipid assemblies upon addition of plasmid DNA to cationic liposomes as a function of the mole ratio of DNA/cationic lipid at 15 min ($n = 2$). The compositions of the cationic liposomes were DOTAP/DOPE 1/1 (○), DOTAP/DOPC 1/1 (◆), 100% DOTAP (◇), and DC-CHOL/DOPE 1/1 (□). Taken from Zuidam and Barenholz (1998).

The difference in pK_a of HC in neutral DOPC liposomes and in cationic liposomes can be used to estimate the surface potential (Ψ_O^{HC}) using the Boltzmann equation:

$$\Psi_0^{HC} = -\frac{\Delta pK_{el} kT}{e \ln 10} = -\frac{(pK_a^{charged} - pK_a^{neutral})kT}{e \ln 10} \quad (1)$$

where k is the Boltzmann constant (1.38×10^{-23} $J \cdot K^{-1}$), T is the absolute temperature (here 295 K), e is the electron charge (1.6×10^{-19} C) and $pK_a^{charged}$ and $pK_a^{netural}$ are the pK_a's in charged bilayers and in neutral DOPC bilayers, respectively. The pH at the surface can be estimated by comparing the dissociation degree of HC in cationic liposomes with the one in neutral DOPC-liposomes as a function of pH. The cationic liposomes shown in Fig. 1 had a large positive surface potential (180–240 mV) and a high pH (10.1–11.5) at the location of the probe in the liposomal bilayers in 20 mM Hepes buffer (pH 7.4). These experimental data were in good agreement with the Gouy–Chapman calculations. Other electrostatic characteristics shown in Fig. 1 are that the primary amine group of DOPE in cationic liposomes dissociated at high pH_{bulk} (> 7.9) and that a salt bridge was likely between the quaternary amine of DOTAP or DMRIE and the phosphate group of DOPE or DOPC (as indicated by the plateau at the dissociation degree of 8% between pH 4 and pH 5.5), but not between the tertiary amine of DC-CHOL and the phosphate group of DOPE.

A novel approach of combining the Gouy–Chapman calculations and fluorescence measurements of the pH at the surface of lipid assemblies by the fluorophore 4-heptadecyl-7-hydroxycoumarin showed that the electrostatic parameters played a key role in the instantaneous formation of the DNA–lipid complexes upon addition of different amounts of plasmid DNA to cationic liposomes in 20 mM Hepes buffer (pH 7.4) (see Zuidam and Barenholz (1998)). At low DNA/cationic lipid mole ratio, neutralization of cationic lipids was lower than expected, and the addition of large amounts of plasmid DNA led to neutralization of 60% of the protonated DC-CHOL in DC-CHOL/DOPE (1/1) assemblies and 80% of the DOTAP in lipid assemblies (see Fig. 2). The characteristics of these electrostatic parameters of the complexes suggests better surrounding of plasmid DNA by lipids when DOPE is present.

3. Stability

The liposomes containing DOTAP were unstable upon dilution, probably due to the high critical aggregation concentration of DOTAP, 7×10^{-5} M (Zuidam and Barenholz, 1997). Large instablility expressed as continuous size increase was demonstrated by the time-dependent static changes in light-scattering monitored following the mixing of cationic liposomes and DNA at DNA/cationic lipid molar ratios between 0.2 and 0.8 M (Zuidam and Barenholz, 1998).

4. DNA structure

Addition of cationic liposomes composed of 100% DOTAP or DOTAP/DOPE (1/1) liposomes

induced instantaneous transition of the plasmid DNA from the B- toward a partial C-type conformation as shown by circular dichroism (CD) spectroscopy (results to be published). Above a DOTAP/DNA charge ratio of 1.0, the DNA in the presence of DOTAP/DOPE (1/1) liposomes (but not 100% DOTAP liposomes) was in a partial tertiary structure of Ψ^--DNA as well. The Ψ^--DNA is characterized by inter-helical interaction between parallel helices.

5. Lipofection

The highest lipofection was obtained under conditions of lipoplex instability, and when the DNA was partially dehydrated and had a partial Ψ^-- structure (results to be published).

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