Nanoscale DNA - Zwıtterionıc Vesicle Formulation Compacted For Gene Delivery: Adiabatic Differential Scanning Calorimetric Study

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Citation

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Abstract

INTRODUCTION

Nucleic acids-membrane associations comprise the least functionally studied macromolecular assembly, yet attract the attention of researchers due to their potential in the field of gene therapy (Leckband & Israelashvili, 2001). The design of novel nucleic acid delivery formulations proceeds mainly as searches of alternatives to highly efficient but risky viral based vehicles (Templeton, 2001; Liu & Huang, 2002; Miller, 2003). The main objective is to achieve compaction of genetic material within highly restricted compartments, while decreasing its cytotoxicity. In the light of well-established potential of liposomes as gene carriers (Templeton, 2001), the current work concerns mainly the stability and physical properties of DNA within the lipid surrounding. Such particles, referred to as lipoplexes are composed of positively charged lipid species and a helper neutral lipid, used for the stabilization of the liposome complex. Despite the considerable efforts that had been made to characterize the structure of these complexes, the origin of molecular forces responsible for self-assembly formation, determination of their charge, colloidal properties, stability against dissociations, cytotoxicity issues, and unravelling characteristics related to efficient intracellular delivery and gene expression remain unclear. A possible alternative to the toxic cationic lipids is the employment of zwitterionic lipid species, which are much safer for target cells (Kharakoz, 1999). Neutral liposome-DNA self-organization is mediated by various inorganic cations, acting as condensing agents. In the light of recent strong evidence that divalent cations enhance the efficacy of plasmid DNA-cationic lipid formulations (Lam & Cullis, 2000), it is of particular interest to study the effect of different divalent cations on the transfection potency of lipid-DNA complexes. In this context, a preliminary results of promissimg ternary DNA-DPPC-Mg^{2+} complex preparation and its thermodynamic properties are presented herein.

MATERIALS AND METHODS

MATERIALS

Synthetic dipalmitoylphosphatidylcholine (DPPC) and calf thymus DNA were a kind gift of Prof. P. Balgavy (J. A. Comenius University – Bratislava, Slovakia). EDTA was purchased from Sigma Chemical Co., St. Louis, MO, USA. $MgCl_2.6H_2O$, $NaHPO_4$ and NaCl were obtained from Merck, Darmstadt, Germany. The presented nucleic acid concentrations and the molar ratios are based on on the average nucleotide molecular weight of 308 calculated from the known DNA composition employed (Uhrikova, et. al. 1998).

METHODS

PREPARATION OF VESICLES

1.2 mM lipid in standard SSC buffer, pH=7.2 was used in all experiments and was stored at 4°C. The formation of a thin layer of lipids of a 15 ml round-bottomed flask was achieved by a hand-shaking and hydration of in SSC buffer at arround 70°C. Vortexing of the lipid with the desired aqueous solution above the gel-to-liquid crystalline phase transition of the lipid (T_m) for arround 30 min resulted in multilamellar vesicles. The DNA concentration used troughout all experiments was 1.8 mM based on the abovementioned assumption. Unilamellar vesicles were obtained by extrusion of multilamellar vesicle suspension through two stacked polycarbonate filters (Nucleopore, Inc.) of 100 nm pore size at arround 60°C. Repeated extrusion (10

times) through the extruder (Lipex Biomembranes, Inc., Vancouver, B.C., Canada) creates homogeneous vesicle suspension. This allows the preparation of vesicles with a mean diameter of 90 nm and a trap volume in the range of 1.5 – 2.0 l/mole.

Preparation of liposome-nucleic acid mixtures – DPPC-DNA formulations were obtained by mixing appropriate volumes of unilamellar vesicles dispersion, calf thymus DNA solution and $MgCl_2$ solution in SSC buffer to obtain the desired molar ratios.

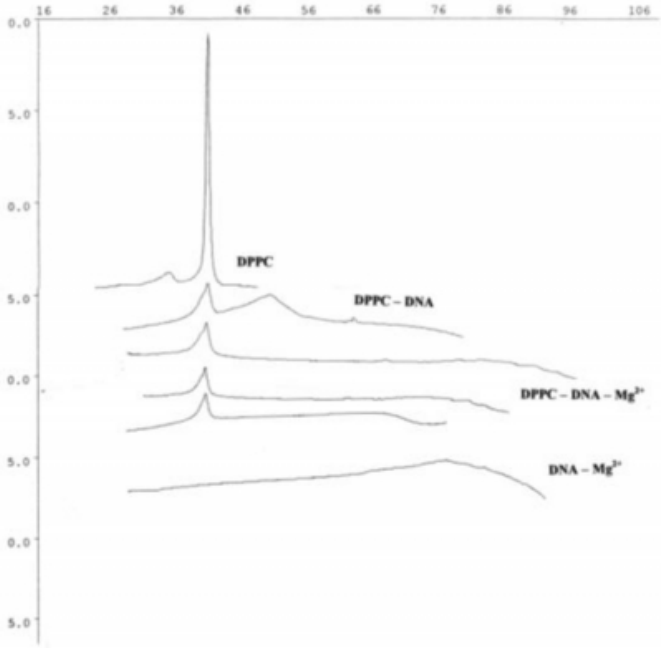
Differential Scanning Calorimetry – Differential scanning microcalorimetric measurements were performed employing Privalov type DASM-4 adiabatic differential scanning microcalorimeter (Russian Academy of Sciences-Puschino, Moscow Region). With a scan rate of 0.5 K.min $^{-1}$. Instrumental base line calibration mark was obtained by scanning at 50 μW, ΔT=4, as described (Ivanov, 1988).

RESULTS AND DISCUSSION

Although double-stranded DNA (dsDNA) has been shown to bind to zwitterionic lipids (Malghani & Yang, 1998), its thermodynamic stability features remain to be elucidated. Only results obtained with unilamellar DPPC vesicles are presented, in the light of recent evidence for their better performance in gene delivery studies with respect to internalization mechanisms (Templeton, 2001). The interaction of calf thymus DNA with phosphatidylcholine liposomes in the presence of Mg^{2+} ions was studied using the adiabatic differential scanning microcalorimetry. Fig 1. depicts representative thermograms of DPPC liposomes and their complexes with DNA and Mg^{2+} . The first curve on the top is calibration mark and shows a typical DPPC multilayer phase transitions, with pre-transition 36°C with a ΔH_{cal}=3.9 kJ/mol and the gel – liquid crystal, or main phase transition (T_m) at 41.9°C. The subsequent marked curves to the bottom represent the change of phase transitions upon interactions with various quantities of DNA and Mg^{2+} . DPPC unilamellar vesicles' thermogram peak appears broader with a decreased maximum. The pre-transition peak disappears. The curve denoted for nucleic acid - phosphatidylcholine mixture DPPC – DNA) indicates that phase separation occurs between lipids and nucleic acid, the DNA phase having its peak further at arround 51.3°C when mixed in equimolar amounts (1:1) with DPPC.

Figure 1

Figure 1: DSC heating scans of the main phase transition of DPPC multilayers and unilamellar vesicles obtained from them, upon their associations with various amounts of calf thymus DNA and 0.5 mM Mg 2+ . Each complex is denoted in the abreviated form. Data is presented as excess apparent heat capacity vs. temperature curves. Details of sample preparation and measurements are outlined in Materials and Methods.



In this type of complex formation, the measured value of T_m=41.9 and ΔH_{cal}=31.9 kJ/mol were determined. The interaction with liposomes results in the significant decrease of excess apparent specific heat capacity. The next three curves to the bottom marked as DPPC – DNA – Mg^{2+} show the phase behaviour of ternary mixtures of DPPC:DNA:Mg^{2+} in equimolar 1:1:1, 1:3:1 and 1:5:1 ratios with increased DNA amount, respectively. The equimolar peak possesses narrower signal, compared to DPPC vesicles' peak, with further DNA phase separation. The Tm value remained, however ΔH_{cal} diminished to 9.7 kJ/mol. The specific heat capacity remains upon complexation with divalent cation. The main phase transitions shifts somehow to 41.7°C. Intere stingly the DNA phase peak moves further to 89°C. At the 1:1:1 molar ratio of the tripple complexation, the self - assemlies display two peaks. The first one is at main phase transition temperature corresponding to the melting of DNA-lipid aggregate. The second one is at 86 °C and corresponds for the DPPC-DNA complex. The former peak is attributed to the stabilization of the DNA secondary structure by a tight packing of DNA molecules several unilamellar vesicles,

bridged by Mg^{2+} -ions. This is a particular case of liposome surface induced nucleic acid condensation of the "spaghetti and meatballs" structure (Templeton, 2001). The effect is driven by surface cationization of vesicles, sensed by a conformational change in the choline group of DPPC. It tilts towards the bilayer surface plane since its positively charged quaternary nitrogen is attracted by the opposite charge of the nucleic acid polyanion. The main phase peak sharpens upon increasing the DNA amount twice, as shown in the next curve beneath. Interestingly, the DNA phase peak shifts to lower values of 71°C. This trend is maintained upon increasing the DNA amount more (taernary molar ration of 1:5.1).

The tripple complex of DNA-metal ion-phosphatioline vesicle remains stable at different incubation times, which is in agreement with small- and wide-angle X-ray scattering measurements [7]. Apparently, Mg^{2+} decreases the DNA efective radii and creates groove narrowing, by ligand binding to six or eight water molecules, or alternatively to nucleic acid phosphate in the minor groove in a fully hydrated state (Hud & Polak, 2001). The last curve denoted as DNA – Mg^{2+} represents equimolar mixture of DNA and Mg^{2+}, which brings about a major signal at arround 90°C. The Mg^{2+} ions at the equimolar ratio of Mg^{2+}:DNA increase the T_m value by 33.7°C, reaching a maximum at 85°C, due to Mg^{2+} -induced phase separation with an increased gel – liquid crystal phase transition temperature, which indicates the divalent cation trigered high temperature DNA stabilization. Unilamellar vesicles treated with the same concentration of Mg^{2+} did not produce such a shift, which is normally detected spectroscopically. Obviously, here divalent metal cation does not contribute essentially in stabilizing of the zwitterionic lipid structure. Therefore, DNA contributes to stabilization of ternary complex towards higher temperatures. Apparently, Mg^{2+} – DNA creates polymorphic phase transitions in phosphatidylcholine moiety.

It is well-established that such a positively charged particle deliver DNA into cultured cells by electrostatic mechanisms of binding to their negatively charged membranes. Liposomes enter cells by various routes, such as through endocytic pathway and direct membrane fusion. Gene delivery designs involving receptor mediated transfer face problems, since endosomes fuse rapidly with lysosomes, thus degrading the nucleic acids. The ternary complex between nucleic acid, divalent inorganic cation and extruded liposome formulated from zwitterionic lipid, described in the work herein, can deliver genes into cells via direct fusion with the cell membrane. This model is is accordance with recent proposal (Templeton, 2001). The major advantage of such non-viral nanocondensate formulation is the ability to act across tight barriers in vivo.

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