Stoichiometry of dipalmitoylphosphatidylcholine-DNA interaction in the presence of Ca^{2+}: a temperature-scanning ultrasonic study

D.P. Kharakoz[a,*], R.S. Khusainova[a], A.V. Gorelov[a,b], K.A. Dawson[b]

[a] *Institute of Theoretical and Experimental Biophysics, 142 292 Pushchino, Russia*
[b] *Irish Centre for Colloid Science and Biomaterials, Chemistry Department, University College Dublin, Belfield, Dublin 4, Ireland*

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Abstract DNA-DPPC complexes can be prepared by means of a single step procedure of mixing DNA solution and aqueous lipid dispersion in the presence of calcium ions. Interaction between DPPC and DNA brings about a biphasic shape of melting curves corresponding to the free lipid and the strongly bound one. The amount of the strongly bound lipid is 5 molecules per nucleotide which is close to the size of the first lipid monolayer around DNA molecule.

Key words: DNA-lipid interaction; Phase transition; Transfection; Ultrasonics

1. Introduction

Interaction between DNA and lipids has become an active area of research recently due to the fundamental importance and various applications of such interaction. The ability of DNA to influence the structure of membranes and to initiate polymorphic phase transitions in bilayers might have important biological implications [1,2]. Also DNA-lipid complexes are used as a non-viral system for gene delivery to target cells [3].

Cationic lipids can easily form stable complexes with DNA owing to the attraction forces between the opposite net charges of the lipid and DNA. Unfortunately, the cationic lipids are frequently toxic for the cells. Zwitterionic lipids, which are safer for the cells, do not interact with DNA directly. However, the interaction can be mediated by Ca^{2+} and other divalent metal cations ([1,4] and the references therein). The Ca^{2+} mediated complexes were used recently for transfection [5].

The complexes of dipalmitoylphosphatidylcholine (DPPC) with DNA and Ca^{2+} had been obtained earlier with a procedure which included sonication, freeze-drying, treatment with chloroform, drying again, and redispersion of the lipid-DNA mixture [1]. This procedure was adopted to ensure the formation of multilamellar vesicles with a high DNA to lipid ratio, and allowed the observation of DNA induced structural transition in the bilayer. On the other hand, this is a many-step and laborious procedure that can increase a risk of distorting DNA and introducing harmful impurities into the complexes. In this present work, we have shown that the procedure can be simplified to a single step of mixing DNA with an aqueous lipid dispersion. Elimination of extra steps is of evident importance in view of transfection problems. The minimum amount of lipid required to obtain the stable lipid-DNA dispersion has been approximately determined. By means of ultrasonic interferometry, we have studied the melting transition in lipid bilayers, estimated the stoichiometry of DPPC-DNA complexes, and found that the amount of bound lipid is enough to cover the accessible surface of DNA.

Velocity and absorption of ultrasound in liquids are determined by their density and compressibility. Any change in the intermolecular interactions, hydration, and/or in the kinetics of fast structural processes result in a change of the compressibility of the system, and thus, is reflected in the sound velocity and absorption [6]. These parameters were successfully used to characterize the thermodynamic and dynamic properties of biomolecular systems (see [6–9] and references therein) and also to characterize lipid-biopolymer interaction [10,11].

2. Materials and methods

Calf thymus DNA was purchased from Sigma (St. Louis, MO, USA) additionally purified using phenol and chloroform (A_{260}/A_{280} value being greater than 1.9) and mildly sonicated, resulting in the formation of fragments with a length of about 300–500 base pairs, and then dialyzed against 0.5 mM HEPES solution (pH 7.5). Final concentration of the stock-solution thus obtained was 4.84 mM, as determined spectrophotometrically (hereinafter, the DNA concentration is presented in terms of nucleotides). The stock-solution was stored at 4°C. Immediately prior to use, a stock solution of $CaCl_2$ was slowly added with rapid stirring to yield a final Ca^{2+} concentration of 22 mM.

To prepare dispersions of multilamellar vesicles of DPPC (Avanti, Richmond, Canada), a portion of dry lipid powder was mixed with the aqueous solution of $CaCl_2$ (22 mM) and HEPES (0.5 mM), hydrated during 1–2 h at room temperature, and then vortexed for 20 min at 50°C (above the melting temperature of the lipid bilayer). The lipid concentration was 30 mg/ml (determined by weight). The MLV dispersion thus obtained was stored at 4°C. Unilamellar vesicles (ULV) were prepared by the repeated extrusion (11 times) of the MLV dispersion at 50°C through a pair of stacked polycarbonate filters (Millipore, Bedford, MA, USA) with pore size of 0.1 μm.

Lipid-DNA mixtures were prepared 1–2 h before measurements by rapid mixing and stirring the calcium-containing DNA solution, lipid dispersion (MLV or ULV), and solvent, to get a required lipid-DNA molar ratio at a constant final concentrations of DPPC (4.7 mM), the concentration of DNA being a variable. Prior to measurements, the DNA-lipid mixtures, as well as the pure lipid dispersions, were subjected to a heating/cooling cycle between room temperature and 50°C, in order to pass over the melting temperature of the lipid and thus to equilibrate the structure of vesicles and the DNA-lipid complexes. In control experiments, the DNA-lipid mixtures were prepared in the absence of calcium.

Sound velocity and absorption were measured with a temperature-scanning differential ultrasonic fixed-path interferometer (acoustic resonators are described earlier by Sarvazyan and Kharakoz [12], an automatic ultrasonic resonance analyzer was developed and kindly donated by Profs. L. De Maeyer and T. Funck, of the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). Basic tech-

*Corresponding author.
E-mail: kharakoz@pbc.iteb.serpukhov.su

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; MLV, multilamellar vesicle; ULV, unilamellar vesicle; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]

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nical characteristics were as follows [8,9]. The frequency of ultrasound was 6.5–8 MHz, the sample volume was 1 ml. The reproducibility at the highest scan rate was 3×10^{-4}% for sound velocity and 5% for absorption (relative to water). The accuracy and the resolution of temperature measurements were 0.1 K and 0.005 K, respectively. The dynamic hysteresis of temperature was 0.05 K at the highest scan rate. The device was calibrated as described earlier [7,8].

Base lines for sound velocity, $u_o(T)$, and absorption per wavelength, $\alpha\lambda_o(T)$, as functions of temperature were measured when the sample acoustic cell was filled in with a solvent containing all components but lipid and DNA, and the reference cell with pure water. Then a pure lipid dispersion or a DNA-lipid mixture was placed into the sample cell and measured to obtain $u(T)$ and $\alpha\lambda(T)$. The base line was subtracted to calculate the excess relative sound velocity, $[u(T)-u_o(T)]/u_o(T)$, and the excess absorption, $\alpha\lambda(T)-\alpha\lambda_o(T)$. These quantities characterize the state of lipid and DNA-lipid complexes in the system.

3. Results and discussion

All the dispersions studied contained about 4 mM lipid. The temperature dependences of excess sound velocity and absorption were measured in heating and cooling modes in the range of temperatures across the main phase transition. Typical melting curves are presented in Figs. 1 and 2.

3.1. Minimum amount of lipid required to form stable dispersions

In preliminary experiments we have found that in the presence of DNA, the dispersions were stable only if the DNA/lipid molar ratio did not exceed 1/10–1/12 nucleotide per lipid. At any higher amount of DNA, a phase discontinuity was visually observed: a portion of aggregated material appeared in the sample. Therefore, 10–12 molecules per nucleotide is the minimum amount of lipid required to form stable dispersion. In the systems studied, this condition was fulfilled.

3.2. Pure lipid vesicles

In pure lipid vesicles, the transition temperature upon heating is about 42.2°C (the half-transition point). This is about one degree higher then in the calcium-free aqueous dispersions of DPPC (41.3–41.5°C for MLV [13,14]). The whole transition is accomplished within 41.5–42.4°C interval in the case of MLV. In the case of ULV, the transition range is broader (41.0–42.6°C). A similar difference in the transition zone width is known for the calcium-free MLV and ULV lipid dispersions [13].

The transition is evidently non-equilibrium because a steady hysteresis is observed. On cooling, the transition temperature is by 0.2 K less then on heating, and this difference is practically independent of scan rate varied within 0.1–1.0 K/min in our study.

The shape of the curves outside the transition region is noticeably non-linear. This is due to the so-called heterophase fluctuations, i.e. a spontaneous process of creation/dissipation of the new phase nuclei of subcritical size [7]. When temperature approaches the melting point, the magnitude of the fluctuations sharply increases resulting in the additional decrease of sound velocity and increase of sound absorption.

3.3. DNA-lipid mixtures

The transition curves obtained in the DNA-containing dispersions are presented in Figs. 1 and 2, and, for comparison, in pure lipid dispersions at the same lipid concentration (the ordinates of the curves of sound velocity in the mixtures are shifted to and superimposed on those for pure lipid, in order to facilitate comparison). The melting curve becomes biphasic in the presence of DNA. An additional transition appears at the temperature 43.0°C, which is indicative of a modification of a lipid fraction interacting with DNA. For convenience, we call this fraction the bound lipid.

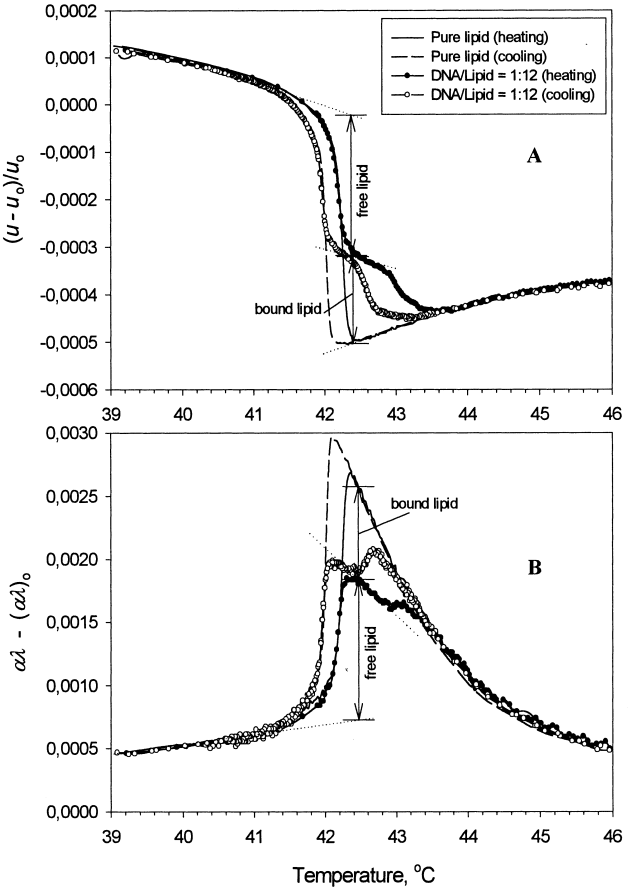

Fig. 1. Melting curves (relative sound velocity, A, and absorption, B) for MLV and their complexes with DNA. Scan rate, 0.4 K/min. The DNA/DPPC ratio is given at the upper right corner. Stoichiometry of strong binding obtained is 4.5 (by sound velocity) to 5 (by sound absorption), in terms of lipid molecules per nucleotide. The ordinates of the curves of sound velocity in the mixtures are shifted to and superimposed on those for pure lipid, in order to facilitate comparison.

As seen from Figs. 1 and 2, the magnitude of the total transition-induced changes in the sound velocity and the absorption coincide in the pure lipid and lipid-DNA dispersions. This means that the molar changes are the same in the free and bound lipid (note that the lipid concentrations were the same in both pure lipid dispersions and lipid-DNA mixtures). Only the transition temperature is changed, allowing the amount of bound lipid to be determined by means of a simple phenomenological analysis: from the ratio of magnitudes of the changes (shown by arrows in the figures). If Δx_{free} is the magnitude for free lipid fraction and Δx_{bound} is that for the bound fraction, the stoichiometry of binding, s (the number of bound lipid molecules per nucleotide), is calculated as

$$s = S\times\Delta x_{bound}/(\Delta x_{bound}+\Delta x_{free})$$

where S is the total lipid to DNA molar ratio in the mixture

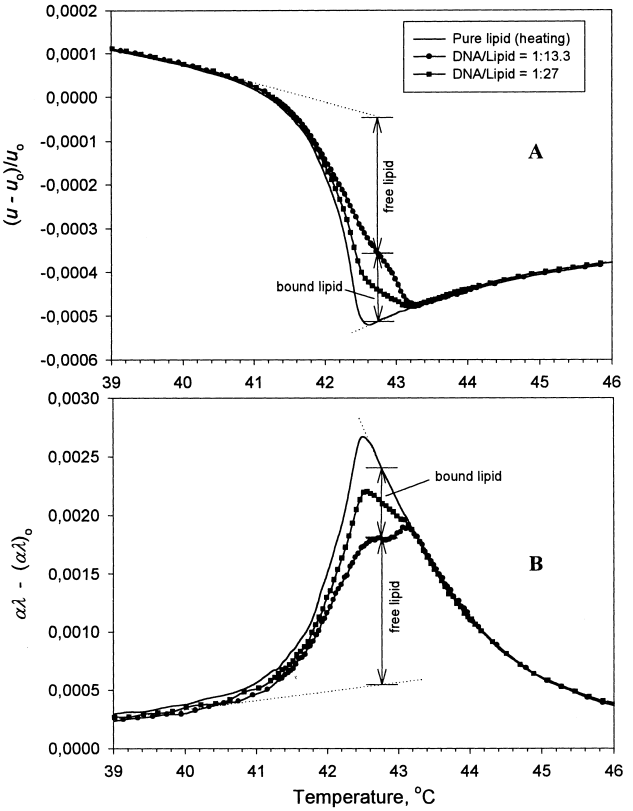

Fig. 2. Melting curves (relative sound velocity, A, and absorption, B) for ULV and their complexes with DNA. Scan rate, 0.4 K/min. The DNA/DPPC ratios are given at the upper right corner. Stoichiometry of strong binding obtained is 4.5 lipid molecules per nucleotide (from both sound velocity and absorption data). The ordinates of the curves of sound velocity in the mixtures are shifted to and superimposed on those for pure lipid, in order to facilitate comparison.

(in lipid per nucleotide). The magnitudes Δx are shown in Figs. 1 and 2 by arrows.

The stoichiometry of binding thus obtained is about $s = 4.5$–5, as obtained from both sound velocity and absorption. The result does not depend on the kind of vesicles (MLV or ULM) and the total molar lipid/DNA ratio in the mixture (varied from 12 to 27). This is close to the maximum number of lipid molecules which can be placed in the first layer around DNA, as calculated from the DNA double-helix geometry [15] and the area occupied by DPPC in the bilayer plane (taken as 0.4 nm^2, see [16]). This fact probably means that the observed amount of strongly bound lipid molecules per nucleotide belong to the first monolayer adjacent to DNA molecule.

Surprisingly the main features of transition in the bound lipid are the same as in the pure lipid vesicles. For MLV the width of transition of bound lipid is narrow which reflects high cooperativity of transition and the preservation of the multilamellar structure of vesicles (Fig. 1). For ULV the transition in bound lipid is broad (Fig. 2). It is clear that interaction of DNA with MLV not only occur on the surface of vesicles, but DNA is actually incorporated in the vesicles, probably intercalated between bilayers. The incorporation of DNA in MLV, in our opinion, is facilitated by the preparation procedure where DNA-MLV mixture is brought across transition before measurements.

The structure of the DNA-DPPC complexes has yet to be determined. Also, we do not know how calcium ion participates in the structure of the complexes. The stoichiometry of its binding has to be investigated.

4. Conclusions

It was shown that DNA-DPPC complexes can be prepared by means of a simple procedure: by just mixing the DNA solution with an aqueous lipid dispersion in the presence of calcium ions. The formation of complexes was observed for both MLV and ULV.

The stoichiometry of strong binding of lipid to DNA is about 5 molecules per nucleotide where the fraction of bound lipid is determined by shifted temperature of transition. Most probably this amount could be attributed to the first lipid monolayer around DNA molecule.

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