DNA initiates polymorphic structural transitions in lecithin

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Received 29 April 1996; revised version received 27 May 1996

Abstract The inverted micellar phase, obtained by treating lecithin and Ca^{2+}-DNA complex with chloroform, was used as an intermediate step in the preparation of DNA-Ca^{2+}-lecithin complex. DSC analysis demonstrated the involvement of a large fraction of lipid in the interaction with DNA. Freeze-fracture electron microscopy revealed (i) rod-like structures on the hydrophobic fracture surface of membranes and (ii) regular bundles of fibrils with a repeat distance of about 6 nm, which were located free in solution. Similar regular bundles of fibrils were also revealed by staining the samples with uranyl acetate. According to the suggested model, the observed structures are hexagonally packed inverted lipid tubes, with DNA located in their central cores. The possible biological relevance of the capability of Ca^{2+}-DNA to initiate polymorphic phase transitions of lecithin is discussed.

Key words: DNA-lipid interaction; Transfection; Lipid polymorphism; Membrane ultrastructure

1. Introduction

Recently, DNA-lipid interactions have attracted much attention because of the possibility of using liposomes as carriers of genetic material to target cells [1]. Lipids could also be involved in some natural processes of DNA translocation through membranes and gene expression in cells [2–4]. They could participate in DNA replication [2,3] and in the formation of complexes of DNA with histone proteins and some enzymes responsible for DNA metabolism [5–7].

Phospholipids cannot directly interact with DNA because of the repulsive forces that exist between the negatively charged phosphates of both lipid and DNA. However, the interaction can be mediated by cationic molecules like synthetic [1] or natural [8] cationic lipids, cationic polypeptides [9] and proteins [5–7], or divalent metal cations [10–12]. The ability of DNA to influence the structure of membranes and to initiate the formation of tubular structures in bilayers was demonstrated with synthetic cationized lipids [13]. The potential ability of DNA to influence the phase behavior of natural lipids was in our view in part because of the supposed biological importance of polymorphic phase transitions of lipids in living cells [14–18].

2. Materials and methods

2.1. Preparation of complex

Calf thymus DNA was purchased from Sigma, additionally purified using phenol and chloroform (A_{260}/A_{280} value being greater than 1.9) and mildly ultrasonicated until the formation of rather homogeneous native fragments with a chain length of about 300–500 bp. The DNA fragments were mixed with a 1 mg/ml suspension of sonicated small unilamellar vesicles of egg PC or DPPC from Avanti in 0.5 mM HEPES solution (pH 7.5) and then a 100 mM stock solution of $CaCl_2$ was slowly added with rapid stirring to yield a final Ca^{2+} concentration of 20 mM. The mixture was lyophilized and treated with chloroform to disintegrate initially existing membrane bilayer structures and to favour the formation of the inverted phase in organic solvent [19]. After the removal of chloroform under vacuum, the DNA-Ca^{2+}-lipid complex was rehydrated with the initial volume of water.

2.2. Microcalorimetry

Measurements were performed using a DASM-4 (Russia) differential scanning microcalorimeter at a heating of rate of 0.25 K/min. The

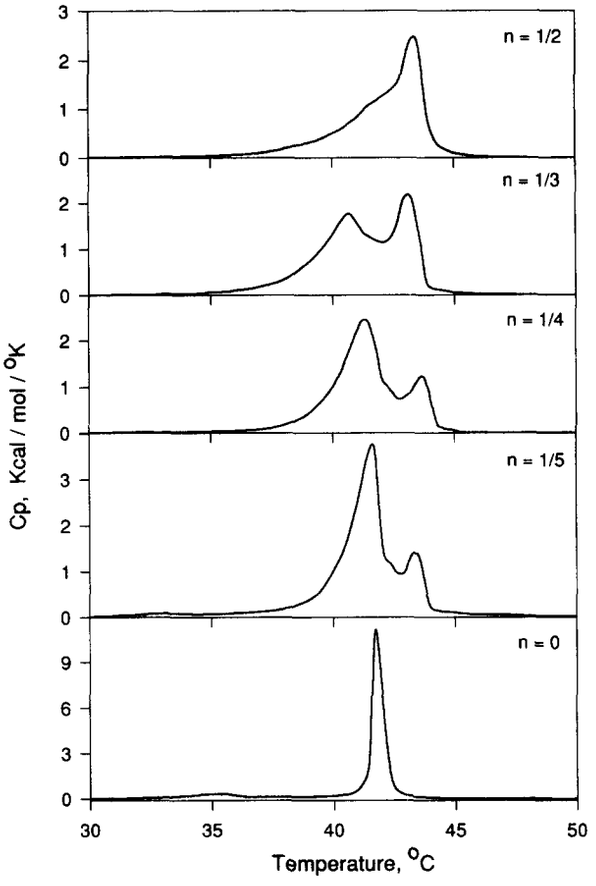

Fig. 1. DSC thermograms of DNA-Ca^{2+}-DPPC complexes. The molar proportion (n) of DNA:lipid is indicated.

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry

PII S0014-5793(96)00643-6

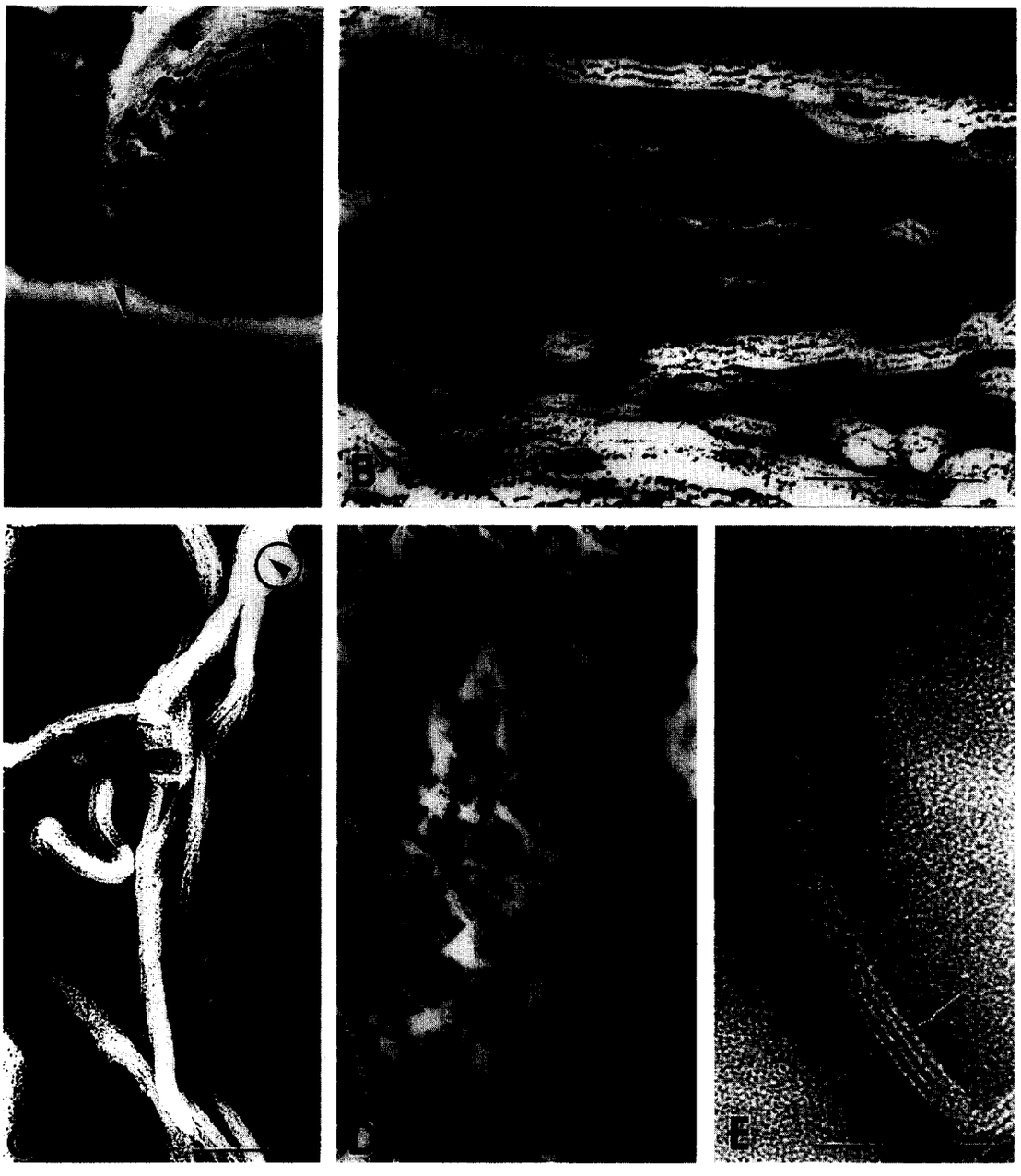

Fig. 2. Freeze-fracture (A–D) and negative contrast (E) micrographs of DNA-Ca^{2+}-egg PC complexes. Circled arrowheads in the corners of all freeze-fracture micrographs mark the direction of shadowing. Bars represent 100 nm. (A) Intramembrane particles and rods on fracture surface of membrane vesicles (asterisk) and free regular fibrils in suspension (arrowheads). (B) Fibrils from A at higher magnification. (C) Another type of fibrils where their capability to form coils and branches is demonstrated. Note that in B,C negatives images are presented. The rod-like fibrils on the hydrophobic fracture surface of membranes are presented in D. Regular bundles of fibrils similar to that demonstrated in A,B are revealed also by staining with uranyl acetate (see E).

DNA-Ca^{2+}-DPPC complexes were vortexed at 45–50°C and kept for about 30 min at 18°C before DSC measurement.

2.3. Microscopy

For freeze-fracture the samples were quenched in propane according to the sandwich technique. No cryoprotectors or chemical fixators were used. Both the fracture procedure and carbon-platinum shadowing were carried out at −150°C and under a vacuum of 1×10^{-6} Torr in a JEOL device as described [21].

For negative contrast, the samples were placed on a carbon-coated grids and then stained with 2% uranyl acetate for about 30 s.

All samples were examined in a JEM 100B (JEOL) transmission electron microscope.

3. Results and discussion

The DSC thermograms of DNA-Ca^{2+}-DPPC complex (Fig. 1) reveal the appearance of a distinct maximum at a temperature of about 43.3°C in addition to the main maximum at 41.6°C [10]. A direct relationship between the molar proportion of DNA in samples and the value of the second peak was observed, which indicates that the high temperature transition corresponds to formation of DNA-Ca^{2+}-DPPC complex [10]. The total enthalpy of both transitions in all presented scans was about 7 ± 0.6 kcal/mol. The thermograms show that, un-

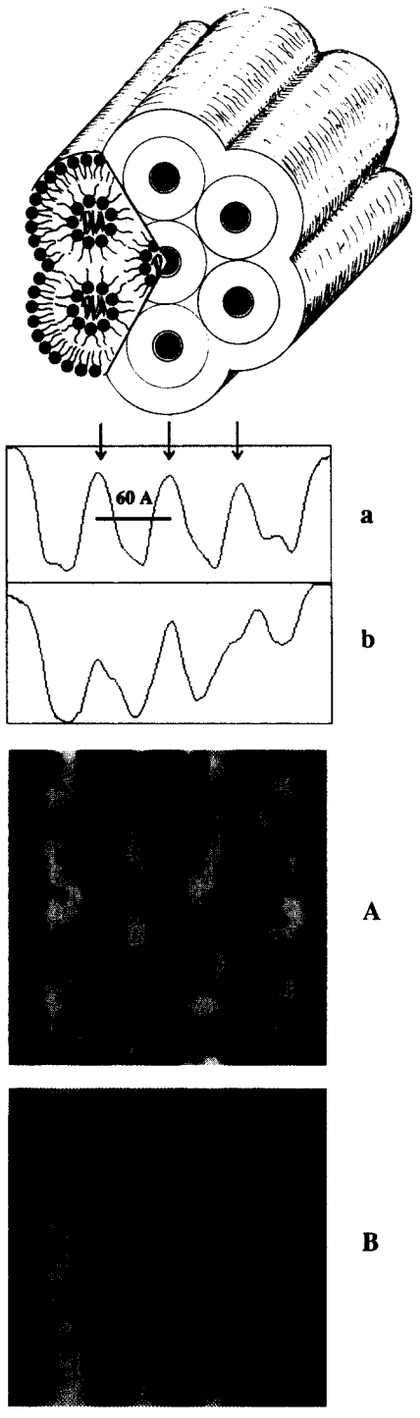

Fig. 3. A hypothetical model (top) of the structural organization of fibrillar DNA-lipid bundles based on negative staining (A) and freeze-fracture (B) images. The scanned computer images (A,B) represent selected regions from Fig. 2B,E at higher magnification. The averaged cross density profiles of the negative staining image (A) and of the freeze-fracture image (B) are presented in (a) and (b), respectively. The presented curves demonstrate a repeat distance between maximums of about 60 Å. According to the scheme presented, the regions of low electron density observed in the samples stained with uranyl acetate correspond to tubular lipid structures, whilst dark strips correspond to cores of the tubes filled by DNA.

der the conditions of our experiment, a large part of the lipid was involved in the formation of DNA-lipid complex with new thermotropic properties.

Electron microscopy reveals changes in the structural organization of both DPPC and natural egg PC lipids. Here we present the data obtained for natural lipids, where the structural changes were more obvious and could not be caused by the inherent properties of synthetic lecithins of producing regular structures such as the 'ripples' known for DPPC [20]. Our work on the structural transitions of DPPC will be presented elsewhere.

We have observed two types of structures: round, or more often, rod-like particles revealed on the hydrophobic fracture surface of some liposomes, and regular bundles of fibers, which were located free in the suspension (Fig. 2). The rod-like fibers (Fig. 2A,D) on the hydrophobic fracture surface are rather similar to the 'spaghetti' structures found in complexes of DNA with synthetic cationized lipid and are believed to represent bilayer-covered DNA tubules [13]. The results presented here clearly demonstrate that DNA, like hydrophobic proteins, can modify the structural organization of membranes and initiate the formation of structures similar to well-known intramembrane particles.

The regular bundles of fibrils (Fig. 2A-C,E) represent another kind of DNA-lipid complex. Most of the bundles were not connected to membranes and existed free in suspension. The visual appearance of the bundles revealed both by freeze-fracture (Fig. 2A–C) and negative contrast (Fig. 2E) microscopy was similar. The samples stained with uranyl acetate revealed electron-dense strips separated by unstained white strips of lipid. The affinity of DNA to uranyl acetate could be responsible for the formation of the dark strips.

We suppose that the fibrillar bundles correspond to hexagonally organized lipid tubes filled by DNA in the central core (Fig. 3). The repeat distance revealed of about 60 Å (Fig. 3a,b) is typical for hexagonal tubes of phospholipids [22]. It should be noted that we used DNA fragments of about 300–500 bp, corresponding to a DNA length of about 100–150 nm. However, the length of the observed bundles was much greater which indicates that in lipid tubes the DNA fragments might be oriented tail-to-tail.

It is well known that some phospholipids can produce a very great diversity of regular structures such as hexagonal and cubic phases [14–18,22]. In contrast, lecithins usually form stable bilayer structures and do not reveal a pronounced tendency to polymorphic behavior, except for cases where some specific biologically active modulators are present [23,24]. It seems quite realistic to suppose that complexes of DNA with polyvalent cations could occupy a prominent place among modulators of polymorphic transitions not only in lecithins, but also in some another phospholipids. The ordered polymorphic structures could be formed in complexes of phospholipids with DNA in the presence of cationic proteins, lipids or polyamines. The capability of lipids to produce the ordered structures with DNA could be used by living cells for the arrangement of the chromosomal material in the nucleus, in the processes of DNA translocation through membranes during virus infection, or in the exchange of genetic information between cells.

Acknowledgements: This work was supported by RFFI 96-04-48192 and INTAS 93-2084. We thank I. Masulis for technical support.

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