

MICROSTRUCTURE OF DNA - LIPOSOME AGGREGATES IN PRESENCE OF METAL CATIONS

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In presence of metal cations or cationic amphiphiles, DNA aggregates with electrostatically neutral phospholipids dispersed in aqueous phase. These aggregates can be used as nonviral vectors for transfer and expression of DNA in cells. The ability of phospholipids to aggregate with DNA in presence of metal ions is also important for the interactions of DNA with biomembranes. The interaction of DNA with liposomes charged positively by cationic amphiphiles results in three different types of microscopic structures: (i) spaghetti-like structure, in which DNA is covered by a cylindrical lipid bilayer, (ii) sandwich-like structure with DNA molecules intercalated between lipid bilayers (condensed lamellar L^c phase), and (iii) honeycomb-like condensed columnar inverted hexagonal phase H_{II}^c with DNA molecules surrounded by lipid monolayers forming inverted cylindrical micelles arranged in a hexagonal lattice. Even if some of these structures formed in presence of cationic amphiphiles have been studied in great detail using various experimental and theoretical methods, the information about structures formed in presence of metal cations is scarce.

In the present contribution we summarize results of our recent studies of aggregates resulting from DNA interaction with liposomes prepared from saturated dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), monounsaturated dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) in presence of 1-77 mM solutions of divalent Ca^{2+} and Mg^{2+} cations using synchrotron X-ray diffraction on the beamline A2 of the storage ring DORIS at HASYLAB, DESY Hamburg.

Aggregates prepared from DPPC or DMPC liposomes (lipid:DNA=3:1 mol/base) in 5-50 mM metal cation solutions show a well organized structure of L^c phase with 2-3 Bragg reflections of periodicity $d \sim 8$ nm in the gel L_{β}^c (at 20°C) and $d \sim 7.2$ nm in the liquid-crystalline phase L_{α}^c (at 60°C), resulting from the lipid bilayer stacking. One broad reflection with lower intensity is identified as a peak from DNA-DNA organization. The interhelical DNA-DNA distance is $d_{DNA} = 4.6-5$ nm in the gel phase, depending on used phospholipid and cation concentration. With increasing temperature, we observe an increase of the width and a decrease of the intensity of DNA peak. We find the distance $d_{DNA} = 4.3-4.8$ nm in the liquid-crystalline phase at 60°C. The peak relative to DNA-DNA organization merges in the background

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when the samples are heated up to 70°C and above. Applying a repeated heating-cooling process in the range 10-80°C, the original diffraction patterns reappear or we can observe two lamellar phases with repeat periods $d \sim 7.4$ nm and $d \sim 5.8$ nm at 20°C and there is no reflection from DNA organization.

When aggregates are prepared from DOPC liposomes at DOPC:DNA= 1:1 mol/base and within the cation²⁺ concentration range 0-77 mM, the diffractograms show the coexistence of two lamellar phases: L phase with the spacing $d_L \sim 8.3$ -7.4 nm identified as a phase with the DNA strands intercalated in water layers between adjacent charged lipid bilayers, and L_{DOPC} phase with the spacing $d_{DOPC} \sim 6.5$ -5.7 nm identified as a phase of partially dehydrated DOPC bilayers without any divalent cations and DNA strands. If the amount of lipid increases, the fraction of partially dehydrated L_{DOPC} phase is limited and depends on the portion of DNA in the sample and also on the length of DNA fragments. The coexistence of two phases in one aggregate can be explained by a lateral segregation of DNA and metal cations to minimize electrostatic energy of the whole system, as suggested Mitrakos P. and MacDonald P. M. (Biochemistry 35 (1996) 16714) for DNA-lipid-cationic surfactant.

DOPE in excess of water forms columnar inverted hexagonal phase. At 20°C we have observed five reflections proportional to 1, $\sqrt{3}$, 2, $\sqrt{7}$, and 3 times the spacing $a = 7.75 \pm 0.01$ nm, according to the relation $s_{hk} = 2(h^2 + hk + k^2)^{1/2} / \sqrt{3}a$, where h and k are Miller indices. With increasing temperature (20 - 80°C), a decrease of the unit cell dimension with the transversal thermal expansivity $\alpha = -(26.3 \pm 0.2) 10^{-4} \text{ K}^{-1}$ is observed. The aggregate formed due to DNA interaction with DOPE in presence of 30 mM Mg^{2+} shows a condensed columnar inverted hexagonal phase H_{II}^c with unit cell spacing $a = 7.12 \pm 0.01$ nm (at 20°C) and transversal thermal expansivity $\alpha = -(9.7 \pm 0.1) 10^{-4} \text{ K}^{-1}$.

In this way, it is demonstrated that neutral phospholipid bilayer and divalent metal cations can compact DNA into structures with morphologies similar to those observed for DNA-lipid-cationic surfactant aggregates.

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