

# DNA affinity to biological membranes is enhanced due to complexation with hydrophobized polycation

A.A. Yaroslavov\*, S.A. Sukhishvili, O.L. Obolsky, E.G. Yaroslavova, A.V. Kabanov\*\*, V.A. Kabanov

Department of Polymer Sciences, Faculty of Chemistry, M.V. Lomonosov Moscow State University, Leninskie Gory, Moscow 119899, Russian Federation

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**Abstract** The interaction of negatively charged liquid phosphatidylcholine/cardiophilin liposomes with water-soluble negatively charged DNA/cetylpyridinium bromide and DNA/poly(*N*-alkyl-4-vinylpyridinium bromide) complexes was studied. It is shown that the DNA/cetylpyridinium bromide complex while interacting with the liposomes is destroyed, so that the cetylpyridinium cation is incorporated into the liposomal membrane and DNA remains in the solution. The DNA/poly(*N*-ethyl-4-vinylpyridinium bromide) complex does not interact at all with the liposomes. On the contrary, the complex of DNA with the poly(vinylpyridinium) cation carrying a small amount of *N*-cetyl groups is adsorbed on the membrane as a whole. The data obtained indicate that complexation of DNA with hydrophobized polycations can be used for enhancing DNA affinity to biological membranes.

**Key words:** DNA-containing complex; Liposome; Polyelectrolyte adsorption

## 1. Introduction

The targeting of alien nucleic acids into cells is one of the goals of genetic engineering [1]. A number of approaches have been developed to introduce DNA into cells [2–13]. Recently, for this purpose, it was proposed to incorporate nucleic acids into soluble IPECs with polycations [11–16]. Incorporation of DNA into soluble IPEC with quaternized poly-4-vinylpyridine was shown to enhance significantly the efficiency of DNA penetration into *Bacillus subtilis* cells when compared to native DNA [14–16]. At the same time, it was shown that amphiphilic oligocations (such as spermine modified by hydrophobic substituents) complexed with DNA effectively stimulated gene transfer into mammalian cells [13]. However, little is known about the mechanism of interaction between the above-mentioned DNA-containing complexes and the cell membrane. Our research examined the influence of positively charged ligands on the efficiency of DNA binding with liposomes.

\*Corresponding author.

\*\*Present address: Department of Pharmaceutical Science, College of Pharmacy, University of Nebraska Medical Center, 600 South 42nd St., Box 986025, Omaha, NE 68198-6025, USA.

**Abbreviations:** PC, phosphatidylcholine; CL, cardiophilin; PEA-FITC, dipalmitoylphosphatidylethanolamine, *N*-fluorescein isothiocyanate; P<sub>2</sub>, 4-vinylpyridine/*N*-ethyl-4-vinylpyridinium bromide copolymer (7/93); P<sub>2,16</sub>, 4-vinylpyridine/*N*-ethyl-4-vinylpyridinium bromide/*N*-cetyl-4-vinylpyridinium bromide copolymer (36/60/4); D<sub>e</sub>, mean hydrodynamic diameter; EPM, electrophoretic mobility; DP, degree of polymerization; IPEC, interpolyelectrolyte complex.

somal membranes which was considered to mimic the cell surface.

## 2. Materials and methods

P<sub>2</sub> and P<sub>2,16</sub> were prepared according to [17]. Polymer compositions were determined by IR Spectroscopy. The DP of both polymers was 1070. The concentration of the polymers is given in ionogenic repeating units per l. CPB was obtained from Chemapol, Czech Republic.

DNA from chicken embryos (Reanal, Hungary) was fragmented using a 4710 ultrasonic generator (Cole-Parmer Instrument Co., USA) and then fractionated in a 5–20% sucrose gradient. DP of DNA was determined by gel electrophoresis in agarose, the 50–100-base DNA fraction was used. Modification of DNA with a fluorescent label was performed as follows. Aqueous solutions – 4 ml of 12.5 mg/ml DNA, 0.2 ml of 0.5 M ethylenediamine and 1 ml of 1 M NaOH – were mixed. The mixture was incubated at 60°C for 15 min. Then 1.5 ml of 2 M LiClO<sub>4</sub> aqueous solution and 20 ml of acetone were added to precipitate DNA. DNA was sedimented after centrifugation for 10 min at 8000 rpm using a J-21 centrifuge (Beckman, USA) and dissolved in 4 ml of 0.1 M NaClO<sub>3</sub> and 0.2 ml of 0.5 M ethylenediamine aqueous solution. 1 ml of 0.1 M aqueous *N*-bromosuccinimide was then added. The resulting mixture was incubated at 0°C for 10 min, then treated with 1.3 ml of 0.15 M aqueous solution of 1-aminohexamethylene fluorescein and heated at 50°C for 1 h. The DNA modified with a fluorescent label (DNA\*) was isolated from the reaction solution using the procedure described above, then washed with acetone and dried under vacuum.

PC, CL and PEA-FITC were obtained from Sigma, USA.

PC-CL liposomes (4:1 molar ratio) were prepared according to the following procedure. Corresponding amounts of PC and CL solutions were mixed in a flask. The solvent was evaporated under vacuum, the thin lipid layer being dispersed in 2 ml of borate buffer solution, 10<sup>−3</sup> M, pH 9.2. The mixture was sonicated in a 4710 ultrasonic generator under ice cooling. Liposomes with a fluorescent label incorporated into the bilayer (PC-CL\* liposomes) were prepared as described above, 0.5 mol% of PEA-FITC being included in the lipid mixture. The average diameter of liposomes was 40–60 nm.

D<sub>e</sub> was measured by photon correlation spectroscopy with an Autosizer 2C (Malvern, UK), EPM by laser microelectrophoresis with a Zetasizer 2C (Malvern, UK). Fluorescence analysis was performed with an F-4000 fluorescence spectrophotometer (Hitachi, Japan). pH values were measured using a PHM83 Potentiometer (Radiometer, Denmark) with a G2040C standard glass electrode. Experiments were performed in 10<sup>−3</sup> M borate buffer, pH 9.2, at 20°C. Solutions were prepared using bidistilled water, additionally purified by passage through a Milli-Q water system (Millipore, USA).

## 3. Results and discussion

The native cell surface is known to carry a net negative charge. Therefore, negatively charged mixed liquid PC-CL liposomes were used as a cell-mimetic species. Due to the negative charge of DNA chains they cannot interact with PC-CL liposomes in aqueous salt solution. To increase the DNA affinity for the liposomal membrane, the ability of

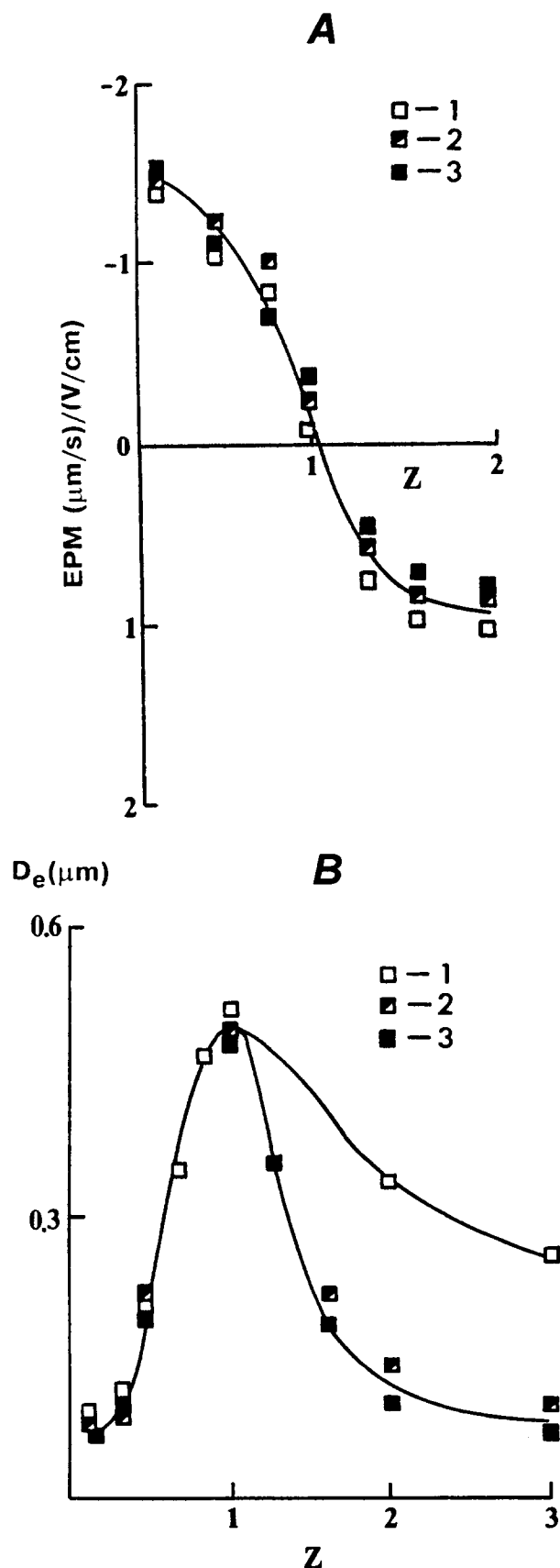


Fig. 1. Dependence of EPM (a) and  $D_e$  (b) of DNA-CPB (1), DNA- $P_2$  (2) and DNA- $P_{2,16}$  (3) complex particles on  $Z = [CR]/[DNA]$ . Measurements were performed in 10 min after mixing of constituents.  $[DNA] = 10^{-4}$  M; phosphate buffer,  $10^{-2}$  M; pH 7.

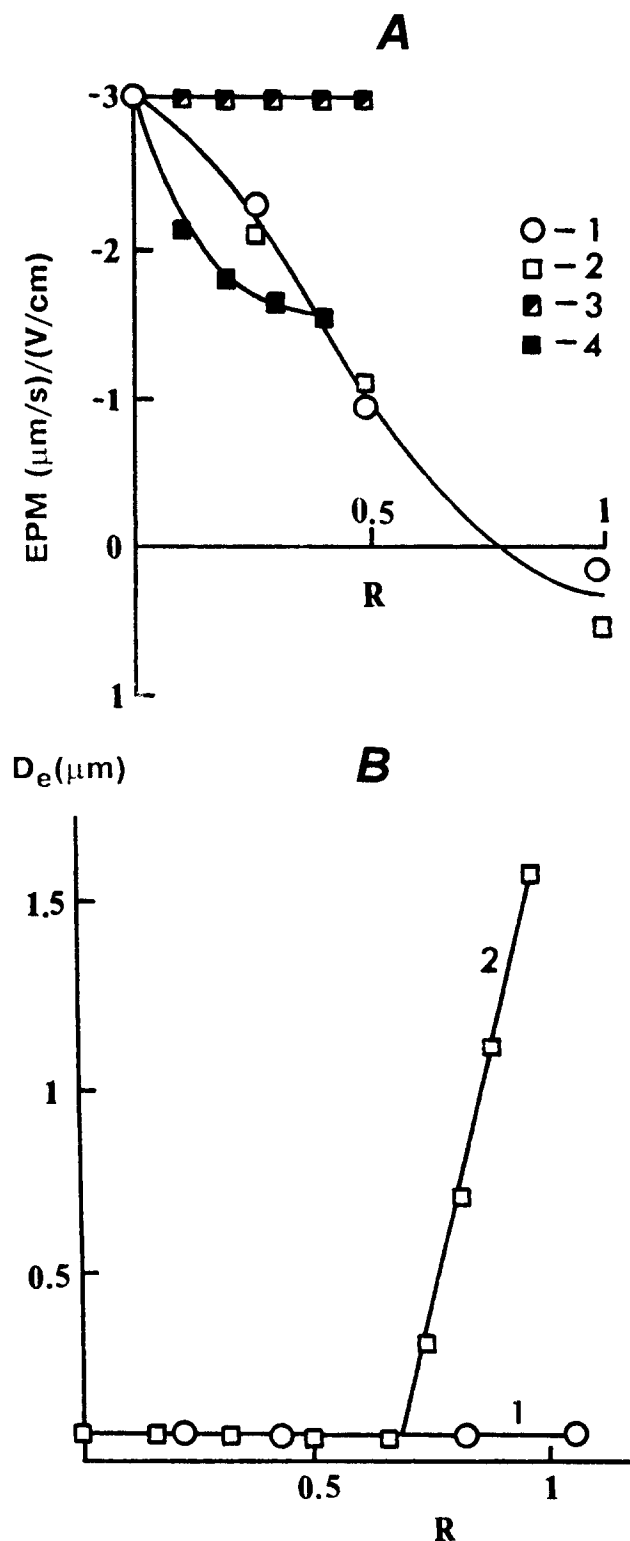


Fig. 2. (a) Dependence of EPM of PC-CL liposomes after addition of CPB (1), DNA-CPB (2), DNA- $P_2$  (3) and DNA- $P_{2,16}$  (4) complexes on  $R = [CR]/[CL]$ . (b) Dependence of  $D_e$  of PC-CL liposomes after addition of CPB (1) and DNA-CPB complex (2) on  $R = [CR]/[CL]$ . Measurements were conducted in 10 min after mixing of constituents. Liposome concentration 1 mg/ml; phosphate buffer,  $10^{-2}$  M; pH 7.

DNA to complex with polycation and cationic surfactants was used. We expected that these complexes would be able to

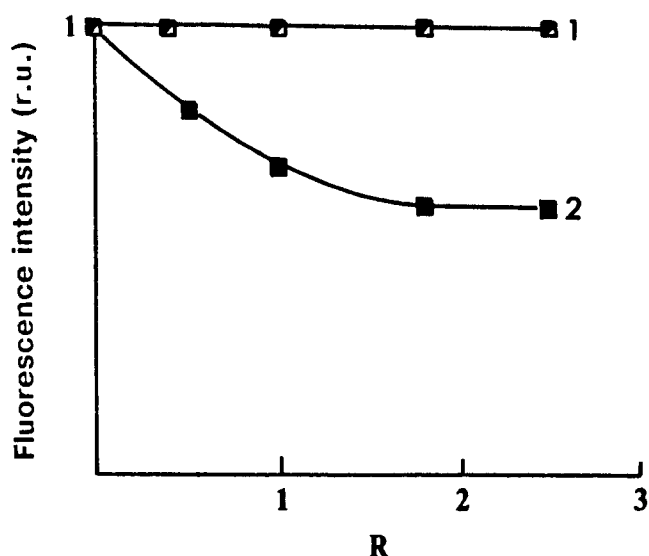


Fig. 3. Dependence of relative fluorescence intensity of PC-CL\* liposomes after addition of DNA-P<sub>2</sub> (1) and DNA-P<sub>2,16</sub> (2) complexes on  $R = [CR]/[CL]$ . Measurements were performed in 10 min after mixing of constituents. Liposome concentration 1 mg/ml; phosphate buffer,  $10^{-2}$  M; pH 7.

interact with the liposome surface due to incorporation of their hydrophobic domains into the hydrophobic part of the lipid bilayer.

To understand the mechanism of interaction of DNA-containing complexes with the negatively charged liposomal

membrane, complexes of DNA with three different types of cationic reagents (CR) were used: the complex of DNA with the low molecular weight surfactant, CPB; the complex of DNA with the linear polycation, P<sub>2</sub>; and the complex of DNA with the polycation containing side-chain cetyl groups, P<sub>2,16</sub>.

The addition of CRs solution to DNA solution resulted in the neutralization of the DNA charge (Fig. 1a) and the appearance of large DNA-CR complex particles (Fig. 1b). Zero charge and the maximum particle size were observed at stoichiometric ratio of the components in the system ( $Z = [CR]/[DNA] = 1$ ). This indicates that DNA was quantitatively bound in the strong complex with CRs. In other words, the ratio of the complexed components  $\nu = [CR]_c/[DNA]_c$  was equal to  $Z$  in the range  $0 < Z < 1$ . Increase of the  $Z$  value over 1 resulted in recharging of the complex particles and a decrease in their size. In further experiments the soluble negatively charged DNA-CR complexes with  $Z = 0.1$  were used. It was shown in [14–16] that  $\nu$  values equal to 0.1–0.2 corresponded to the largest transformation effect of DNA-containing IPECs in the experiments with *B. subtilis*.

Fig. 2a (curves 1,2) shows that the addition of CPB and DNA-CPB complex to the liposomes resulted in a decrease in EPM of the liposomes close to zero. (The parameter  $R$  is the ratio of the concentration of CPB or CPB incorporated in the original DNA-CPB complex to the concentration of CL incorporated in the liposomal membrane.) It is important to note that both dependencies were nearly the same. The CPB-liposome interaction did not affect the liposome size

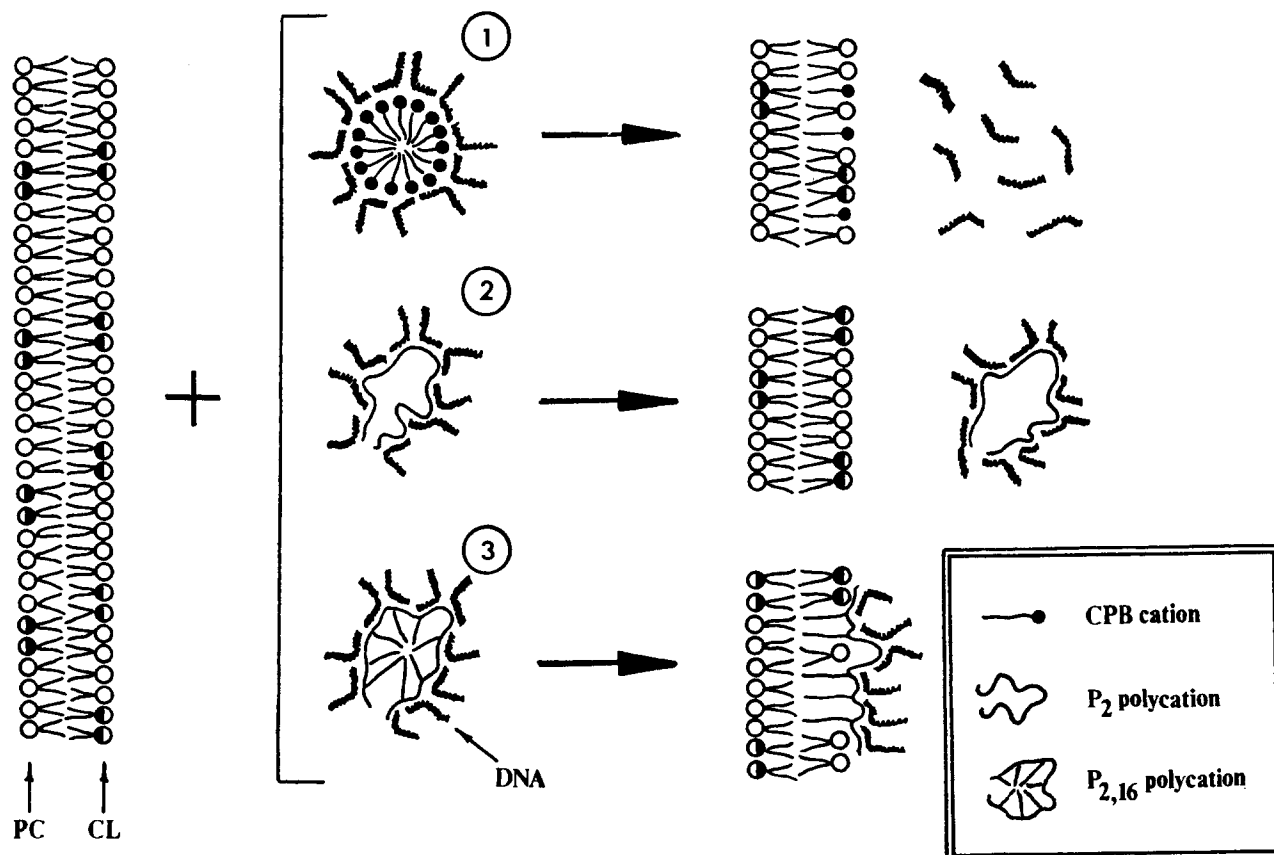


Fig. 4. DNA-CPB (1), DNA-P<sub>2</sub> (2) and DNA-P<sub>2,16</sub> (3) complexes in contact with PC-CL liposomes (schematic representation).

(Fig. 2b, curve 1). The same was true for the interaction of DNA-CPB complex with the liposomes unless the  $R$  value exceeded 0.7. At higher  $R$  values larger aggregates were formed (Fig. 2b, curve 2). Based on these data, one can conclude that the DNA-CPB complex is destroyed on contact with the liposomes so that CPB molecules likely are incorporated into the liposomal membrane, neutralizing the liposome surface charge, while DNA molecules are released in solution. At higher  $R$  values the liposome charge changes from negative to positive. Then free DNA molecules act as a classical polyelectrolyte flocculant with respect to oppositely charged colloid species causing their aggregation.

The complex of DNA with  $P_2$  had no effect on the EPM of the liposomes (Fig. 2a, curve 3), indicating that no interaction exists between this complex and the liposomal membrane. In this case, the complex most likely did not dissociate but remained in solution as a whole. To confirm this assumption, a complex of  $P_2$  with fluorescence labeled DNA\* was prepared of which the fluorescence was partially quenched by  $P_2$  units. Addition of the liposomes to the complex had no effect on the fluorescence intensity (Fig. 3, curve 1), providing evidence of the stability of the DNA- $P_2$  complex in the presence of the liposomes.

Thus, neither the DNA-CPB nor DNA- $P_2$  complex was able to enhance the DNA affinity to the liposomal membrane.

The negatively charged DNA- $P_{2,16}$  complex with  $Z=0.1$  characterized by  $EPM = -1.5$  (mm/s)/(V/cm) was added to PC-CL liposomes, resulting in a decrease in the EPM of the liposomes to the that of the original complex (Fig. 1a, curve 4). As shown in Fig. 3 (curve 2), the fluorescence intensity of PC-CL\* liposomes decreased when the DNA- $P_{2,16}$  complex was added. The quenching of the fluorescence could result from adsorption of the entire DNA- $P_{2,16}$  complex of any of its components on the liposome surface. To clarify whether or not the DNA- $P_{2,16}$  complex dissociated on contacting the liposome surface, the complex of the fluorescence labeled DNA\* with  $P_{2,16}$  was prepared and then added to the PC-CL liposomes. It was found that the DNA\* fluorescence, originally quenched in the DNA\*- $P_{2,16}$  complex, was not recovered. This is proof that in contrast to the DNA-CPB and DNA- $P_2$  complexes, the DNA- $P_{2,16}$  complex interacted with the liposomes without dissociation and adsorbed on the liposome surface as a whole, probably due to incorporation of the hydrophobic cetyl groups into the membrane. The behaviour of DNA-CPB, DNA- $P_2$  and DNA- $P_{2,16}$  complexes in PC-CL liposome solution is schematically represented in Fig. 4. In other words, modification of DNA by electrostatic coupling with the polycation carrying hydrophobic groups makes it 'sticky' with respect to a biological membrane.

At the same time, it was shown in the experiments with living cells that complexation of plasmid DNA with both  $P_2$

and  $P_{2,16}$  considerably enhanced DNA affinity to living cell membranes [14–16]. Such a difference in the behavior of DNA-containing complexes regarding liposomes and cells probably resulted from the rigidity of small liposomes, of which the membranes could be hardly curved without disruption. Therefore, they could not incorporate the polycomplexes containing rather extended hydrophobic parts formed by coupling oppositely charged DNA and  $P_2$  units. This problem might be solved by a comparative study of DNA/ $P_2$  and DNA/ $P_{2,16}$  interaction with large liposomes. Another reason for the difference might be brought about by the presence of membrane proteins incorporated into the cell membrane. However, the complexation of DNA with the polycation carrying side-chain fatty fragments was found to ensure strong binding of the whole complex to both the liposomal and cell membranes.

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