

## Spin Label and Microcalorimetric Studies of the Interaction of DNA with Unilamellar Phosphatidylcholine Liposomes

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**Abstract.** High-molecular DNA from chicken erythrocytes interacts with 1,2-dipalmitoylphosphatidylcholine in unilamellar liposomes, both in the presence and absence of  $Mg^{2+}$  ions. This interaction results in a phase separation in liposome membranes. The new phase induced by DNA and  $Mg^{2+}$  has a higher gel-liquid crystal phase transition temperature as measured by microcalorimetry. In the liquid crystalline state, the 16- and 5-doxyl stearic acid spin labels indicate changed local bilayer properties at the label position in the new phase.

**Key words:** DNA-membrane interaction — 1,2-dipalmitoylphosphatidylcholine — Microcalorimetry — Spin labels — Unilamellar liposomes

### Introduction

It is generally accepted that chromosomes are attached to the (cell or nuclear) membranes both in prokaryotic and eucaryotic cells. Besides the membrane proteins, also the lipids may interact with the chromosome. Lipid-nucleic acid interaction is of interest also because of the use of lipid vesicles for the nucleic acids entrapment in genetic engineering. Therefore, aggregates of lipid liposomes with nucleic acids are widely studied. It has been observed that the presence of bivalent cations (Hoffman et al. 1978; Budker et al. 1978; 1980; Gruzdev et al. 1982) or cationic surfactants (Horniak and Balgavý 1988) is necessary for polynucleotides to be able to tightly adsorb on phospholipid membranes. In multilamellar phospholipid dispersions, this adsorption induces the formation of a new phase which phase transition from the gel to the liquid crystalline state occurs at higher temperatures (Vojčíková and Balgavý 1988). Results of studies with fluorescent probes (Gruzdev et al. 1982; Grepachevsky et al. 1986) and <sup>31</sup>P-NMR (Viktorov et al. 1984) and <sup>13</sup>C-NMR (Budker

et al. 1986) studies suggested an increase of lipid immobilization in the presence of DNA and bivalent cations. We investigated the effects of DNA-phosphatidylcholine liposomes interaction using the techniques of spin label ESR spectroscopy and scanning microcalorimetry.

## Materials and Methods

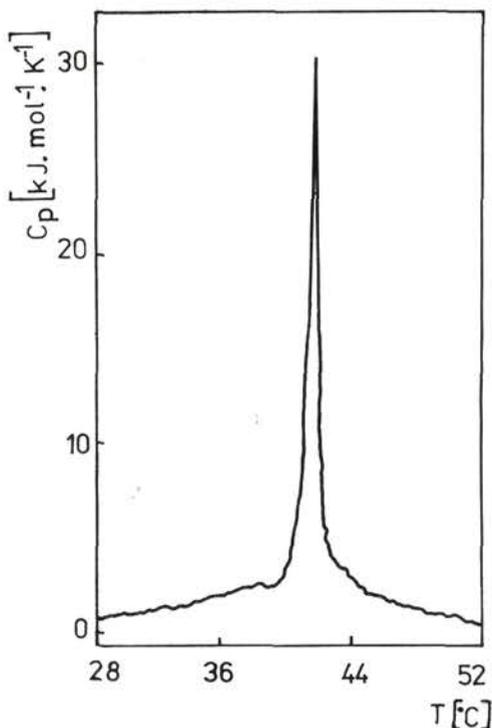
The spin labels 16-doxyloystearic acid (16-DSA) and 5-doxyloystearic acid (5-DSA) were purchased from Syva (USA). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) was from Fluka (Switzerland). High-molecular DNA from chicken erythrocytes was from Reanal (Hungary). Standard sodium citrate (SSC) buffer containing  $1.5 \cdot 10^{-4} \text{ mol} \cdot \text{l}^{-1}$  sodium citrate,  $1.5 \cdot 10^{-3} \text{ mol} \cdot \text{l}^{-1}$  NaCl, pH 6.9, was prepared with redistilled water.

For sample preparation, DPPC was dispersed in SSC buffer and hydrated at 50°C. From this multilamellar liposome dispersion, unilamellar liposomes were prepared by sonication at 65°C using a Dynatech ultrasonic desintegrator (FRG). Multilamellar liposomes and sonicator tip debris were sedimented by centrifugation at 15,000 rpm on K24 Janetzki centrifuge (GDR). Unilamellar liposomes in the supernatant were stored at room temperature for 7–10 days. Small DPPC vesicles formed by sonication are known to spontaneously fuse below the gel-liquid crystal phase transition temperature, forming stable unilamellar vesicles with diameters of about 70 nm (Schullery et al. 1980). This stable vesicle suspension was mixed with DNA and/or  $\text{MgCl}_2$  solutions in SSC buffer and allowed to equilibrate for 2–4 days at room temperature. Thereafter, a sample for ESR spectroscopy was taken and spin labeled by vortexing in a plastic vial, the bottom of which was covered with a thin layer of the spin label. The DPPC: spin label molar ratio was 100:1 or more. Finally, the sample was enclosed in a flat plastic ESR cell. Samples for calorimetric experiments were prepared essentially as described above, except for the spin labelling step.

ESR spectra were recorded by means of an ESR 230 (GDR) X-band spectrometer using the 100 kHz modulation technique. Typical instrumental settings were 5 mW or less microwave power, modulation amplitude maximally 0.2 mT, scan rate  $1.5 \text{ mT} \cdot \text{min}^{-1}$  or less, and the accuracy of temperature setting was  $\pm 0.5^\circ\text{C}$ . Differential scanning calorimetry was performed with a Pivalov DASM-4 adiabatic differential microcalorimeter (USSR) using a scan rate of  $0.5^\circ\text{C} \cdot \text{min}^{-1}$ . The calorimetric enthalpy of phase transitions,  $\Delta H$ , and the apparent specific heat,  $C_p$ , were evaluated from electrically calibrated microcalorimeter cell by planimetric integration of the signals obtained.

## Results and Discussion

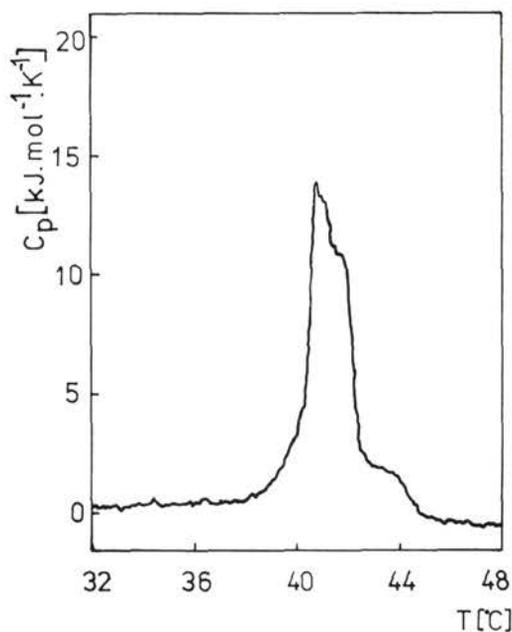
A typical curve of excess apparent specific heat vs. temperature for one preparation of unilamellar DPPC liposomes is shown in Fig. 1. The maximum of  $C_p$  is observed at  $T_c = 41.50 \pm 0.02^\circ\text{C}$  which corresponds well with the gel-liquid crystal phase (main) transition in multilamellar DPPC liposomes (Lipka et al. 1984; Vojčiková and Balgavý 1988). Noteworthy is the absence of pre-transition which usually occurs at about 35°C in multilamellar DPPC liposomes (Lipka et al. 1984). The absence of pre-transition in unilamellar liposomes was noted



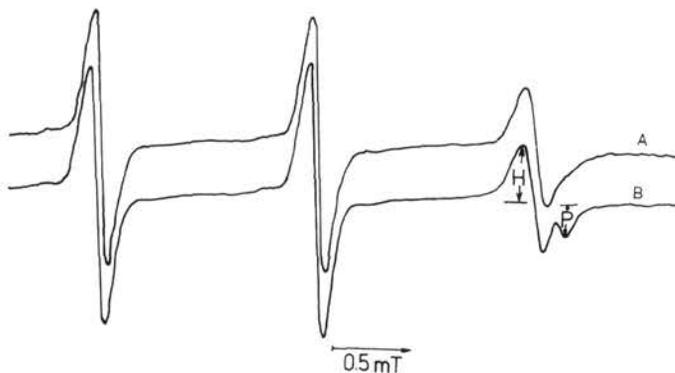
**Fig. 1.** Temperature dependence of excess apparent specific heat  $C_p$  of unilamellar DPPC ( $0.31 \text{ mmol.l}^{-1}$ ) liposomes.

and discussed also by Surkuusk et al. (1976). The origin of a rather broad background onto which the main transition endothermic peak is superposed is unknown.

The plot of  $C_p$  vs. temperature for DPPC-DNA- $\text{Mg}^{2+}$  aggregates shown in Fig. 2 clearly indicates the formation of a new phase (or phases) with increased phase transition temperatures of about  $41.7^\circ\text{C}$  and  $43.5^\circ\text{C}$ . The relative areas of the composite curves in Fig. 2 varied between different experiments. The phase transition temperature shifts observed in our work correspond well to those observed by Gruzdev et al. (1982) who worked with diphenylhexatriene fluorescent probe. In the absence of  $\text{Mg}^{2+}$  ions, the results obtained for DPPC-DNA were very sensitive to sample handling (incubation time, temperature, etc.). In some samples phase separation such as illustrated in Fig. 2 was seen, whereas only a slight broadening of the calorimetric peak occurred in

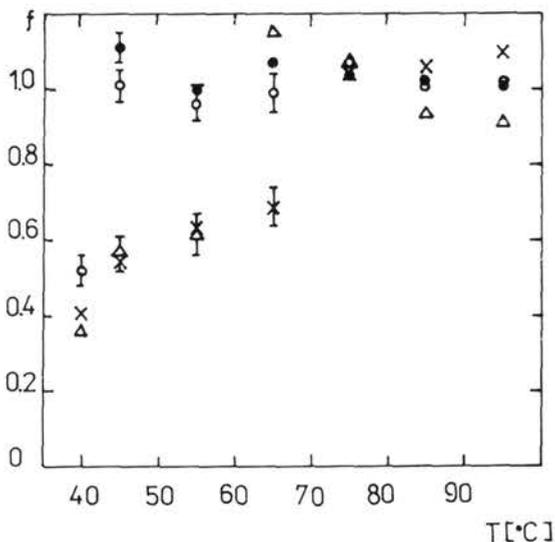


**Fig. 2.** Temperature dependence of excess apparent specific heat  $C_p$  of unilamellar DPPC ( $0.33 \text{ mmol.l}^{-1}$ ) liposomes in the presence of  $\text{MgCl}_2$  ( $0.33 \text{ mmol.l}^{-1}$ ) and DNA ( $100 \text{ mg.l}^{-1}$ ).



**Fig. 3.** ESR spectra of 16-DSA spin label located in unilamellar DPPC ( $9.5 \text{ mmol.l}^{-1}$ ) liposomes in the absence (A) and in the presence of  $\text{MgCl}_2$  ( $9.5 \text{ mmol.l}^{-1}$ ) and DNA ( $2.89 \text{ g.l}^{-1}$ ),  $T = 95^\circ\text{C}$ .

others. The calorimetric enthalpies of the observed transitions,  $\Delta H$ , obtained by the integrating the signals were  $41.5 \text{ kJ.mol}^{-1}$ ,  $31.1 \text{ kJ.mol}^{-1}$ ,  $23.9 \text{ kJ.mol}^{-1}$ , and  $28.1 \text{ kJ.mol}^{-1}$  for the DPPC, DPPC- $\text{Mg}^{2+}$ , DPPC-DNA, and DPPC-



**Fig. 4.** Temperature dependence of parameter  $f = H/(H + P)$  for the 16-DSA spin label located in unilamellar DPPC ( $9.5 \text{ mmol} \cdot \text{l}^{-1}$ ) liposomes. Concentration of DNA:  $0 \text{ g} \cdot \text{l}^{-1}$  ( $\circ \bullet$ ),  $2.98 \text{ g} \cdot \text{l}^{-1}$  ( $\Delta \times$ ); concentration of  $\text{MgCl}_2$ :  $0 \text{ mol} \cdot \text{l}^{-1}$  ( $\circ \times$ ),  $9.5 \text{ mmol} \cdot \text{l}^{-1}$  ( $\bullet \Delta$ ). Vertical bars indicate typical experimental errors.

DNA- $\text{Mg}^{2+}$  systems, respectively: the experimental error of  $\Delta H$  was maximally  $\pm 2.2 \text{ kJ} \cdot \text{mol}^{-1}$ . These data indicate that DNA interacts with DPPC liposomes also in the absence of  $\text{Mg}^{2+}$  ions.

Phase separation is seen also in the ESR spectra of the 16-DSA spin labeled DPPC-DNA- $\text{Mg}^{2+}$  aggregates (Fig. 3). In comparison with pure DPPC liposomes in the absence or presence of  $\text{Mg}^{2+}$  ions, an additional peak can be observed in the high-field region of the spectrum. To quantify the observed spectral changes induced by temperature increases above the phase transition temperature, parameter  $f = H/(H + P)$  was evaluated from the spectra, where  $H$  and  $P$  are the amplitudes of the peaks as shown in Fig. 3. The temperature dependence of parameter  $f$  is shown in Fig. 4. Not only DNA in combination with  $\text{Mg}^{2+}$  ions, but also DNA itself induces phase separation which can be detected by the 16-DSA spin label. With the increasing temperature, the amplitude of peak  $P$  decreases. These data indicate a DNA-phosphatidylcholine interaction both in the presence and absence of  $\text{Mg}^{2+}$  ions. With the increasing temperature, DNA-DPPC complexes most probably dissociate. The locations of 16-DSA spin labels which give rise to two different high-field peaks are

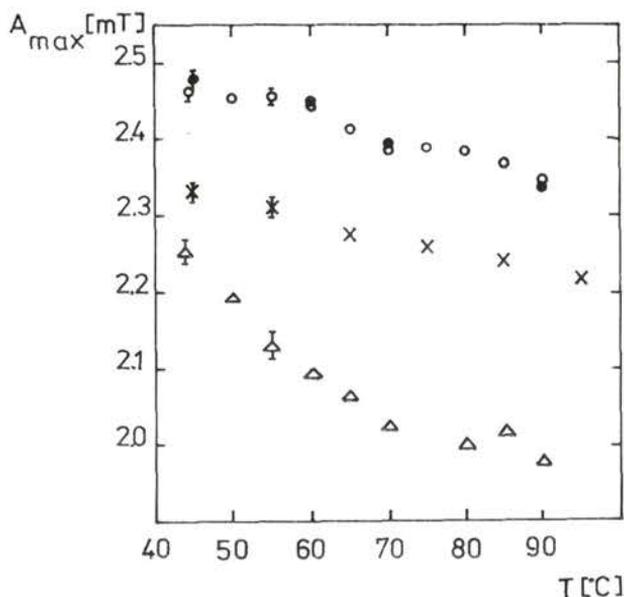


Fig. 5. Temperature dependence of hyperfine splitting constant  $A_{\max}$  of the 5-DSA spin label located in unilamellar DPPC liposomes. For concentrations and symbols see Fig. 4.

unknown; they may differ because of local polarity effects (different hyperfine splitting constants), but also because of different local membranes fluidities.

The 5-DSA spin label in DPPC liposomes display axially symmetric powder pattern spectra. In the DPPC-DNA-Mg<sup>2+</sup> aggregates, a superposition of two different axially symmetric spectra was observed (not shown). From these spectra two different hyperfine splitting  $A_{\max}$  could be evaluated as a distance of their outer extrema. The values of  $A_{\max}$  may be used as parameters characterizing the ordering of the label in a lipid bilayer (Marsh 1981). The temperature dependence of  $A_{\max}$  is shown in Fig. 5. Above the main phase transition temperature,  $A_{\max}$  decreases with the increasing temperature as expected from activation of *trans-gauche* isomerization (an increase of fluidity) in DPPC acyl chains. Noteworthy, the new signals induced in DPPC by the presence of DNA and Mg<sup>2+</sup> have significantly smaller values of  $A_{\max}$  than that observed for pure liposomes both in the presence or absence of Mg<sup>2+</sup> ions. This may indicate an increase in membrane fluidity at the 5-DSA paramagnetic fragment location. However, this does not necessarily mean that DNA in combination with Mg<sup>2+</sup>

ions fluidize the lipid bilayer, as the observed change in the hyperfine splitting of about 0.2–0.3 mT would correspond also to a shift in the spin label position to a distance of 4–5 chain carbon atoms perpendicular to the lipid bilayer (see Barratt and Laggner (1974) for data concerning  $A_{\max}$  vs. label position in phosphatidylcholine bilayer). In the DPPC-DNA system, the outer extrema were rather broad and thus could not be resolved into components. The values of  $A_{\max}$  are between those for pure DPPC and for the new phase in DPPC-DNA- $Mg^{2+}$  aggregates. It is not excluded that resolution of the extrema into components might become observable after a longer period of sample equilibration. Similar equilibration effects were observed in calorimetric experiments as noted above.

In conclusion, we could demonstrate phase separation in unilamellar DPPC liposomes due to interaction with DNA in the presence (but also in the absence) of  $Mg^{2+}$  ions using calorimetry and spin label ESR spectroscopy. The spin label method indicated changed local bilayer properties at the spin label position in the new phase/s.

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Final version accepted January 12, 1989