FLUORESCENCE POLARIZATION STUDY OF THE INTERACTION OF BIOPOLYMERS WITH LIPOSOMES

A. D. GRUZDEV, V. V. KHRAMTSOV+, L. M. WEINER+,†, and V. G. BUDKER*

*Institute of Cytology and Genetics, +Institute of Chemical Kinetics and Combustion, *Institute of Organic Chemistry, Siberian Branch of the USSR Academy of Sciences, Novosibirsk 630090, USSR*

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1. Introduction

Nucleic acid–protein interactions are usually studied in complexes of nucleic acids with natural membranes [1]. However, polynucleotides proved to interact also with the lipids of membranes [2,3]. Bivalent cations (Ca^{2+}, Mg^{2+}) are necessary for the formation of the complex, while univalent ions (Na^{+}) dissociate it. It is proposed, therefore, that bivalent cations are involved in binding phosphates of polynucleotides to negatively charged phosphate groups of the membrane. Changes in the secondary structure of nucleic acids during the complexing resemble those observed on thermal denaturation [3]. However, no data on membrane structure and fluidity changes have been published.

We have studied the effect of some polynucleotides and polyanions on melting of the membrane lipid phase. For that purpose the steady-state fluorescence method was used which is sensitive to a change of the structure and fluidity of the membrane during the phase transition [4,5].

2. Materials and methods

Synthetic β,γ-dipalmitoylphosphatidylcholine (DPPC) (Calbiochem) and egg phosphatidylcholine (PC), isolated and purified as in [6], were used. 1,6-Diphenylhexatriene (DPH) was chosen as a fluorescent probe which dissolved in the hydrophobic region of the membrane [7]. Liposomes were prepared according to [8], 10 μl DPH in tetrahydrofurane (8×10^{-5} M) was added to 4 ml liposome water solution to 0.2 mg lipid/ml. Final [DPH] was $\sim 2 \times 10^{-7}$ M and [DPH]/[lipid] = 1.2×10^{-3}.

To form polyanion–liposome complexes, water solutions of $MgCl_2$ were added to obtain equivalent concentrations (some 2.5×10^{-4} M) of lipid, polyanion (in monomers), and Mg^{2+}. The polyanions used were: DNA from salmon sperm, poly(A) and poly(U) (Reanal), heparin (SPOFA), dextrane–sulfate (DS) (Serva), and poly(dA) (SKTB BAC, Novosibirsk).

Polyacrilic acid (polymerized from monomers and purified on a Sephadex G-50) was used as a polyanion lacking hydrophobic groups.

To remove the bases, poly(dA) was incubated for 4 h at 37°C (pH 2). The polymer fraction was separated by gel-filtration on a Sephadex G-25. The concentration of the bases in poly(dA) after deprivation was estimated by the absorption at 260 nm; phosphorus was measured as in [9].

The steady-state fluorescence anisotropy was measured with a self-constructed instrument. The excitation light was provided by a mercury lamp equipped with a cut-off filter (cut-off 365 nm) and a polarizer. Emission anisotropy was observed through a sheet polarizer (with 2 cross-polarized positions) after passing through a filter (cut-off 436 nm).

Fluorescence intensities $I_{\parallel}$ and $I_{\perp}$ (subscripts denote the direction of polarization with respect to the polarization of the exciting light beam) were measured by using the photomultiplier tube by digital voltmeter. From these measurements anisotropy r was calculated according to the formula:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \qquad (1)$$

The temperature of the liposome solution was mea-

† To whom correspondence should be addressed

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sured with a copper–constantane thermocouple accurate to 0.3°C; light intensity to 1%.

The fluorescent probe lifetime was measured by an Ortec ns fluorometer. Experimental data were treated according to a special program.

3. Results and discussion

The temperature dependences of the fluorescence anisotropy r for lipid–polyanion complexes were obtained mostly for synthetic DPPC with a more pronounced phase transition at $T = (41 \pm 0.3)$°C (fig.1) compared to the complexes with PC at $T = (24 \pm 2)$°C. Most of the complexes with polyanions had the phase transition temperature higher than that of pure lipids, some of them showing 2 phase transitions (table 1).

According to [4,5] the measured anisotropy r in the steady-state method depends upon both the fluidity parameter τ_c (proportional to microviscosity) and the structure parameter r_∞:

$$r = \frac{r_0 - r_\infty}{1 + \tau/\tau_c} + r_\infty \qquad (2)$$

where r_0 and r_∞ are the initial and limiting values of time-resolved anisotropy; τ_c, correlation time; τ, lifetime of fluorescent probe. According to [4], the structure order parameter S is determined by the value of limiting anisotropy:

$$S^2 = r_\infty / r_0 \qquad (3)$$

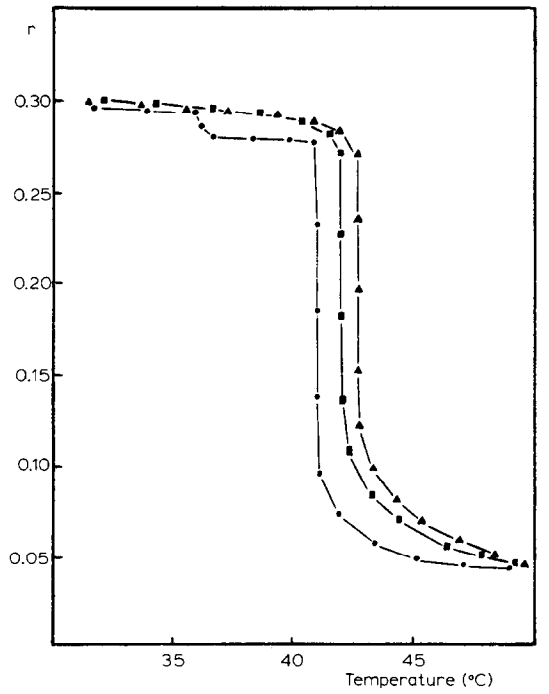

Fig.1. The temperature dependences $r(T)$ for DPH in DPPC (●), in complex DPPC with poly(U) (■), in complex DPPC with poly(A) (▲) ([DPPC] = [poly(A)] = [poly(U)] = [$MgCl_2$] = 2.5×10^{-4} M, [DPH] = 2×10^{-7} M).

In some cases the steady-state method can provide the order parameter in good agreement with experimental data. For instance, in [5] using an average value of $\tau/\tau_c = 8$ and $r_0 = 0.4$ (according to [10] for DPH in DPPC τ is ~10 ns and $\tau_c \sim 1$ ns; $r_0 = 0.395 \pm 0.01$) the following equation was derived from eq. (2):

Table 1
The phase transition temperature shifts ΔT_t in the formation of lipid–polyanion complexes compared to T_t of pure lipid

Polyanion	ΔT_t for complex with DPPC (±0.3°C)		ΔT_t for complex with PC (±2°C)
1. Mg^{2+}	+0.5		+2
2. Poly(A)	+1.5		+7
3. Poly(dA)	+1.4		–
4. Poly(U)	+0.9		+5
5. Poly(dA) with only 30% of bases	0	+1	–
6. DNA	+0.5	+2.1	+6
7. Polyacrilic acid	0		–
8. Heparin	+1.3	+2.2	–
9. Dextran–sulfate	−0.8	+3.7	–

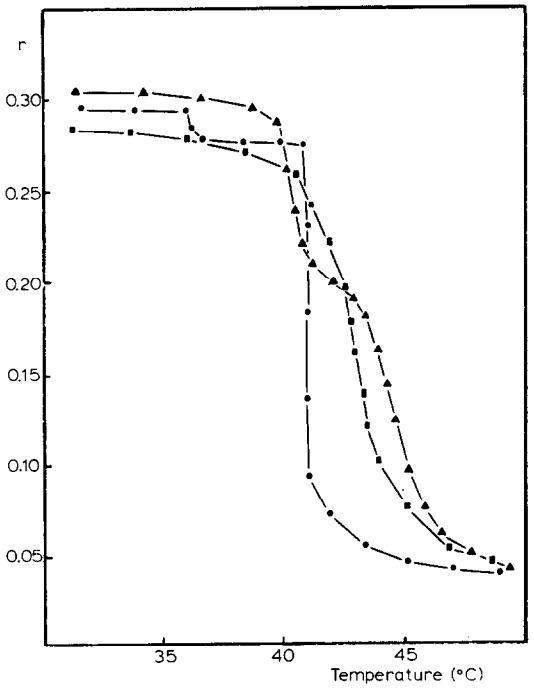

Fig.2. The temperature dependences $r(T)$ for DPH in DPPC (•), in complex DPPC with DNA (■), in complex DPPC with dextran–sulfate (▲) ([DPPC] = [DNA] = [DS] = [$MgCl_2$] = 2.5×10^{-4} M, [DPH] = 2×10^{-7} M). To analyse the temperature profile $r(T)$ and existence of the 2 phases we have considered the experimental data as a function $\frac{d\ln r}{d1/T}(T)$ [7].

$$r_\infty = 9/8\, r - 1/20 \qquad (4)$$

This formula seems to be a good approximation below the phase transition where r is large in comparison with the term $(r_0 - r_\infty)/(1 + \tau/\tau_c)$ in eq. (2). The application of this formula gives quite a good agreement with experimental data for DPH in DPPC in this temperature region involving the phase transition point [5].

To determine the order parameter, we also used the same approximation for DPH in DPPC and PC liposomes. This approximation seems to be valid since the measured temperature dependences for DPH in DPPC, PC and in complex DPPC with poly(A) gave the same order of values (some 10 ns as in [10]; see fig.3).

By means of eq. (3) and eq. (4) we calculate temperature dependences $S(T)$ for DPH in DPPC and in complex DPPC with poly(A) (fig.4). According to eq. (3) and (4) the temperature profile of the structure order parameter (fig.4) is a qualitative analogue of the

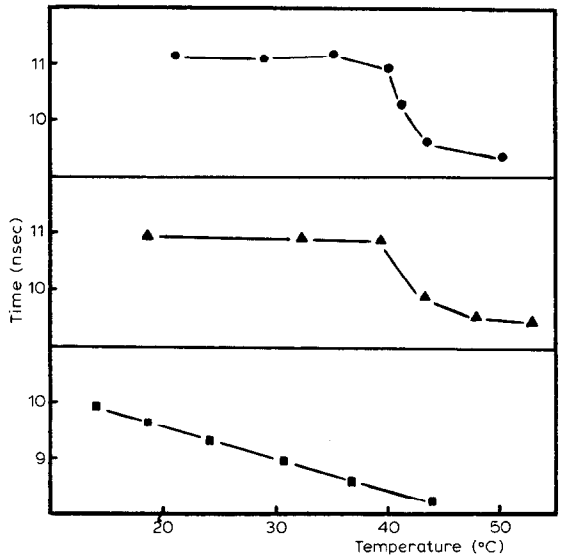

Fig.3. The temperature dependences $\tau(T)$ for DPH in DPPC (•), in complex DPPC with poly(A) (▲), in PC (■) ([DPPC] = [PC] = [poly(A)] = [$MgCl_2$] = 2.5×10^{-4} M, [DPH] = 2×10^{7} M).

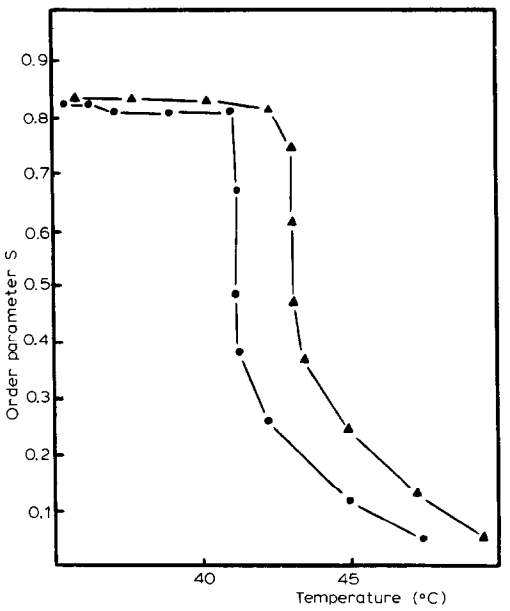

Fig.4. The temperature dependences $S(T)$ for DPH in DPPC (•), in complex DPPC with poly(A) (▲).

anisotropy profile (fig.1). Moreover, the phase transition points coincide for both profiles.

The data listed in table 1 show that polyanions with side-chain residues increase the phase transition temperature of lipid, T_t, while polyacrilic acid, which has no side-chain residues, does not shift T_t although it certainly forms a complex with lipid.

Note that purine-containing polynucleotide (poly(A)) shifts T_t stronger than pyrimidine-containing polynucleotide (poly(U)). At the same time, poly(A) and poly(dA) induce equal shifts of lipid T_t which means that the hydroxyl group does not affect the properties of the complex. A partial deprivation of the bases (~70%) from the ribophosphate chain of poly(dA) diminishes the effect of the polymer on lipid T_t.

The above features of complexes permit one to assume that a rise in T_t of the membrane after complexing results from the interaction of the bases with the interior of the membrane; on the contrary, the fixation of the lipid polar heads on the membrane surface by polyanions does not affect the phase transition temperature.

A strong DNA effect does not contradict the above assumption. It is possible to suppose that in line with [3], DNA fixation on the membrane surface results in its partial denaturation making possible interactions of the bases with the hydrophobic part of the membrane.

A strong effect of polysaccharides (heparin and dextran-sulfate) on lipid T_t, inducing 2 phase transitions (fig.2), seems to contradict the above assumption. It is known, however, that polysaccharides form complexes with the membrane in the presence of bivalent cations [11]. Moreover, even neutral polysaccharides interact with phospholipid membrane [12]. It is probable that hydrogen bonds arise between the hydroxyl groups of polysaccharides and the carbonyl groups of phospholipids. The phase transition temperature and microviscosity of such complexes can differ from those of pure membranes.

References

[1] Dworsky, P. and Schaechter, M. (1974) in: Mechanism and Regulation DNA Replication (Kolber, A. R. and Kohiyama, N. eds) pp. 63–70, Plenum, London, New York.
[2] Budker, V. G., Kazachkov, J. A. and Naumova, L. P. (1978) FEBS Lett. 95, 143–145.
[3] Budker, V. G., Godovikov, A. A., Naumova, L. P. and Slepneva, I. A. (1980) Nucleic Acids Res. 8, 2499–2515.
[4] Heyn, M. P. (1979) FEBS Lett. 108, 359–364.
[5] Jähnig, F. (1979) Proc. Natl. Acad. Sci. USA 76, 6361–6365.
[6] Wells, M. A. and Hanahau, D. I. (1969) Biochemistry 8, 414–424.
[7] Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394.
[8] Kamp, H. and Wirtz, K. (1974) Methods Enzymol. 32, 140–146.
[9] Batlett, G. R. (1959) J. Biol. Chem. 234, 446–475.
[10] Kawato, S., Kinosita, K. and Ikegami, A. (1977) Biochemistry 16, 2319–2324.
[11] Kim, J. and Nishid (1977) J. Biol. Chem. 252, 1243–1249.
[12] Minettli, M., Aucci, P. and Vivi, V. (1979) Ital. J. Biochem. 28, 119–122.