CORRELATION BETWEEN LATERAL LIPID PHASE SEPARATION AND IMMUNOLOGICAL RECOGNITION IN SENSITIZED LIPOSOMES

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1. Introduction

Existence of segregated lipids in the liquid crystalline state and in the gel state can modify essential functions in cell membranes. One of the most interesting questions is the extent to which such lipid phase separations is involved in cell surface recognition.

In the present paper, we report experiments that give evidence that lipid segregation modulates the immunological recognition process in a model membrane. It has become increasingly evident that recognition properties of natural cell membranes can be duplicated in model membranes [1–4] of well-defined composition [5]. Our results suggest that liposomes, which have been extensively used as model membranes and more recently as carriers of drugs [6,7] give a unique opportunity to control simultaneously the lipid phase separation and to mimic the response of cell membranes to antibody and complement [8].

We studied the Bordet-Wasserman immunochemical reaction on liposomes made up of egg lecithin (EggPC), DL-α-dipalmitoyl lecithin (DPPC), cardiolipin (Card) and cholesterol (Chol). The hapten sensitized liposomes loaded with CrO_4^{2-} ions were incubated with syphilitic serum and complement. The leakage of CrO_4^{2-} allowed us to detect the immune damage. The occurrence of a lateral phase separation in the liposomes was tested for with the spin label technique [9].

2. Materials and methods

EggPC, DPPC, Chol and Card were purchased from Sigma. Syphilitic serum was obtained from Pasteur Institute (Brabant). Guinea pig complement was supplied by DIFCO. The spin label TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) was synthetized as described by E. G. Rozantsev [10].

2.1. *Preparation of liposomes*

Liposomes were prepared from mixtures containing Card, EggPC or DPPC and Chol. A chloroform solution containing the required lipid mixtures was dried under reduced pressure. The thin film which formed on the walls of the flask was dispersed in a Tris–HCl buffer (pH 7.35, NaCl 0.9%, Ca^{2+} 0.15 M).

2.2. *Immune lysis of liposomes*

Liposomes were loaded with CrO_4^{2-} ions (0.145 M) by suspending the lipids in a chromate solution (Tris–HCl buffer, pH 7.35, NaCl 0.9%, Ca^{2+} 0.15 mM). External CrO_4^{2-} was removed by ultrafiltration using an XM-100 A membrane. The hapten sensitized liposomes loaded with CrO_4^{2-} were then incubated with syphilitic serum and guinea pig complement, at 25°C for 15 min. After incubation, the suspension was filtered and the release of CrO_4^{2-} determined by optical density measurements at 370 nm [11]. Both com-

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plement and serum were required because neither complement in the absence of antiserum, nor antiserum in the absence of complement has a significant effect on CrO_4^{2-} release. Moreover, no chromate was released by antiserum and complement from liposomes containing no cardiolipin.

2.3. *Electron spin resonance measurements*

TEMPO electron spin resonance spectra were recorded with a VARIAN E-3 spectrometer (Microwave power, 4 mW; Modulation amplitude, 0.5 G). Liposomes were introduced into 50 μl capillaries (Drummond microcaps). TEMPO concentration was 7.5×10^{-5} M.

3. Results and discussion

The paramagnetic resonance spectrum of the spin label TEMPO in aqueous lipid dispersions is shown in fig.1 (inset). The high field line has two components, h and p. Line p arises from TEMPO dissolved in water, line h arises from TEMPO dissolved in the hydrophobic environment. The ratio $f = h/(h+p)$ is a convenient parameter for measuring the label partition between the aqueous and the lipid phase. Breaks located in plots of TEMPO partition parameter against temperature allows one to define the onset and completion of the lateral phase separation. Such a technique has been applied in many systems and the coexistence of fluid and solid phases has been visualized often by freeze fracture electron microscopy [12]. As shown in fig.1a, the curve corresponding to the liposomal system DPPC-Card (in the molar ratio 8/2) reveals clearly 2 break points. They are interpreted as resulting from the beginning and the end of the lipid lateral phase separation process. Between these temperatures, two domains of different fluidity coexist. With the EggPC-Card liposomes (in the molar ratio 8/2), the

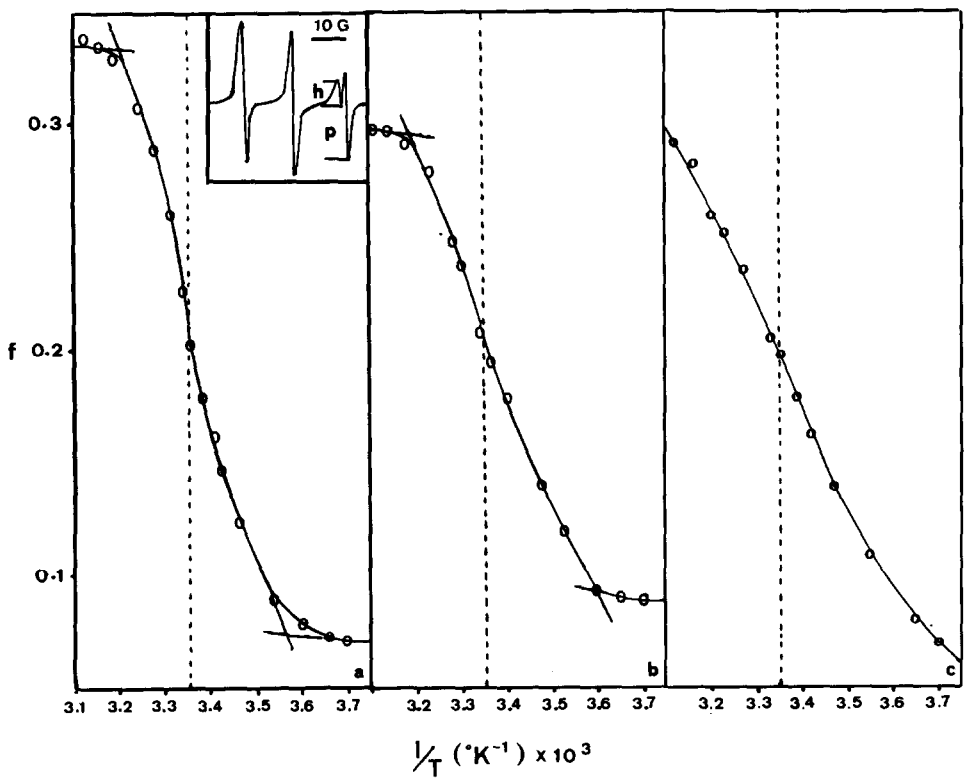

Fig.1. The TEMPO spectral parameter, $f = h/(h+p)$, as a function of the reciprocal of the absolute temperature ($1/T$) for DPPC-Card-Chol liposomes. Molar ratios: (a) 8/2/0, (b) 6.5/2/1.5, (c) 5/2/3. The dashed lines correspond to the temperature of incubation with syphilitic serum and complement.

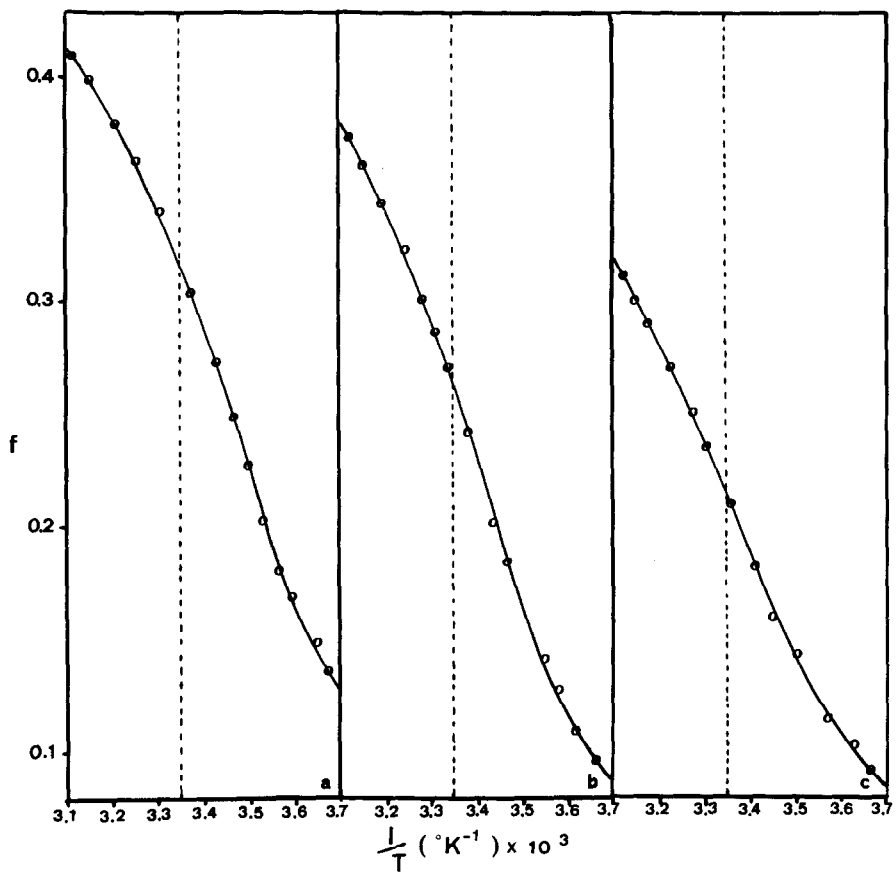

Fig.2. The TEMPO spectral parameter as a function of the reciprocal of the absolute temperature ($1/T$) for EggPC-Card-Chol liposomes. Molar ratios: (a) 8/2/0, (b) 6.5/2/1.5, (c) 5/2/3. Dashed lines as in legend to fig.1.

curve (fig.2a) exhibits no defined break points and no phase separation is detected. For these 2 liposomal systems, the results concerning the immunological action of syphilitic serum and complement on the sensitized liposomes are presented in table 1. They show clearly that significant antibody–complement damage is observed only for the liposomal systems in which a lateral phase separation is detected. Our experiments give evidence that lateral phase separation corresponding to domains of different fluidity is

Table 1
Immunological lysis of cardiolipin sensitized liposomes

	DPPC 80% Card 20%	DPPC 65% Card 20% Chol 15%	DPPC 50% Card 20% Chol 30%	EggPC 80% Card 20%	EggPC 65% Card 20% Chol 15%	EggPC 50% Card 20% Chol 30%
$A_{370}^{(1)}/A_{370}^{(2)}$	0.46	0.48	0.82	0.82	0.85	0.85

$A_{370}^{(1)}$: absorbance at 370 nm of the released CrO_4^{2-} after incubation of the liposomes at 25°C, for 15 min

$A_{370}^{(2)}$: absorbance at 370 nm of the released CrO_4^{2-} after incubation of the liposomes with syphilitic serum and complement at 25°C, for 15 min

a critical factor enhancing immune damage for high antigen concentration whereas one phase liposomal systems are inefficient for immune lysis.

Similar observations have been made for other biochemical reactions. Indeed, the sugar transport through *E. coli* membranes increases suddenly in the phase separation domain [13]. In these membranes, lateral phase separation was detected using spin labels and freeze fracture electron microscopy. An other elegant experiment was successfully conducted on the dependance of lipid hydrolysis by porcine pancreatic phospholipase A_2 [14]. The authors provided evidence that saturated lipids dispersed as liposomes can be hydrolyzed only near the transition temperature [14]. At this temperature, regions of frozen molecules coexist with lipids in the liquid crystalline phase. It has been suggested that at the border of these regions, irregularities in the packing of lipid molecules might favour insertion of enzyme [15].

In immune attack, the existence of such regions might favour the insertion of the antibody–complement system into the lipid matrix. It would be premature to exclude another possibility consistent with the idea that cardiolipin must be surrounded by a lipid phase of an intermediate fluidity, modulating the antigen compressibility and immobilizing the antigen in the most favourable orientation.

In an attempt to modulate the lipid segregation, liposomes including cholesterol were formed (fig.1b, 1c, 2b, 2c). Indeed, recent calorimetric [16] evidence suggests that cholesterol, at concentrations up to 20 mole% shows a preference, in equimolar phosphatidyl choline mixtures, for the lipid with the lower transition temperature. At higher cholesterol concentration, the heat of the upper transition is also decreased and any lipid phase separation disappears. Our TEMPO partition measurements confirm these results. Indeed, with 15% cholesterol (fig.1b), a lipid segregation still exists which disappears (fig.1c) at the higher cholesterol concentration (30%). As expected, cholesterol does not modify the phase partition in EggPC-Card liposomes (fig.2b, 2c). Again, the results presented in table 1 show evidence that coexistence of solid and fluid lipid (DPPC-Card-Chol in the molar ratio 6.5/2/1.5) enhances complement mediated immune attack. One phase liposomal systems (DPPC-Card-Chol in the molar ratio 5-2-3, EggPC-Card-Chol in the molar ratio 6.5/2/1.5, EggPC-Card-Chol in the molar ratio 5/2/3) are inefficient for immune lysis. Finally, the system we have studied demonstrates that liposomes have potential advantages in detection of antibodies against cell surface components. Indeed, if cardiolipin is substituted by another amphipathic hapten (lipid or protein), liposomes containing such components in the lipid layer should release the trapped marker when incubated with a complement source and the specific antibody. Lateral lipid phase separation is however an essential condition to initiate marker release. This could be developed into a simple procedure for screening patients with suspected allergies whose serum contains antidrug antibodies, or for the detection of antibodies against cell surface glycolipids which is of interest in cancer immunology. The potential of these sensitized liposomes in antibody detection is presently under evaluation.

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