TRANSITIONS IN ALIPHATIC AND POLAR HEAD REGIONS OF PHOSPHATIDYL CHOLINE VESICLES DETERMINED WITH FLUORESCENT PROBES

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1. Introduction

Phospholipid dispersions are largely used as models of biological membranes. The vesicles which result from prolongated sonication of these dispersions have an aqueous compartment and a bilayer membrane and furthermore can be carefully calibrated [1]. Transport of ions and molecules through phospholipid models, or even membranes, must require motions or structural changes of the constituents. Fluorescence polarization appears promising in this regard and the principal aim of this work is to calibrate fluorescent probes with model systems of well-defined structure and phase transition. The dispersions of egg phosphatidyl choline (EPC) does not show transition in the 10 to 55° temperature range, but the dipalmitoyl phosphatidyl choline (DPC) exhibits a large endothermic order–disorder transition of the aliphatic chains at 41° [2]. We report here on the polarization of fluorescence of three probes specifically located into the two characteristic regions of the bilayer: the phosphatidyl choline polar heads at the membrane–water interface and the aliphatic chains into the membrane matrix. The 1-anilino-8-naphtalene sulfonate (ANS) does not detect any transition on both systems. But the dansyl phosphatidyl ethanolamine (DPE) and the 9-methyl anthracene (9MA) are reliable and sensitive indicators of structural changes within DPC vesicles.

2. Materials and methods

Egg phosphatidyl choline was extracted from chicken egg yolk according to the method of Singleton et al. [3] and checked for purity by thin layer chromatography (TLC). The purified phospholipid was dissolved in benzene, then lyophilized and stored until use under vacuum at −20°.

Synthetic dipalmitoyl phosphatidyl choline was purchased from N.B.C., dipalmitoyl ethanolamine from Fluka, dansyl chloride from Baker, ANS and 9-methyl anthracene from K and K.

Synthesis of DPE [4]: Lα-dipalmitoyl phosphatidyl ethanolamine, dansyl chloride and dry triethylamine were shaken 7 hr at 50° in dry benzene. After washing with dilute bicarbonate and HCl, the solution was dried and benzene removed. The pale yellow residue was dissolved in ether, precipitated by acetone and centrifuged at −15°. The powder then obtained was purified by preparative TLC.

Different methods of labeling were used. Generally the fluorescent probe and phosphatidyl choline were mixed with chloroform which was then removed under reduced pressure. This process was repeated several times before the addition of water. For 9MA we followed the procedure adopted by Shinitzky et al. for labeling of micelles with 2-methyl anthracene [5].

The phosphatidyl cholines were suspended in water at a concentration of 15 mg/ml. The suspensions were ultrasonically irradiated 15 min with an Annemasse F 50 sonifier at 0° for EPC, and 50° for DPC and then chromatographed on a Sepharose 4B column [1]. The concentration of solutions containing vesicles was about 1 mg/ml.

Fluorescence measurements were performed with an apparatus built up in the laboratory. The wavelength of excitation was 366 nm and the fluorescence observed above 450 nm. Exciting light was polarized vertically. In both instances, excitation and emission, proper filters were used. The solutions were generally

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clear, but sometimes showed a very slight turbidity. In that case a possible source of error is due to scattering of fluorescence light which reduces the degree of polarization $P = (I// - I\perp)/(I// + I\perp)$ [6]. With our apparatus, this effect is barely detectable, being less than 1%.

3. Results and discussion

Depolarization of fluorescence from labeled small vesicles (diameter 250 Å) results from two superimposed contributions: the intrinsic rotational motion of the chromophore within the bilayer and the rotational diffusion of the whole vesicle. We have recently shown that this later contribution is negligible [7] at least, when the lifetime of the excited state is around 15 nsec.

3.1. *Vesicles labeled with ANS*

The 1-anilino naphtalene sulfonate (ANS) is localized at or near the lipid–water interface [8]. In fig. 1 the degree of polarization P of ANS bound to EPC and DPL vesicles, plotted as a function of temperature, decreases progressively when the temperature increases from 10 up to 55° and P is always slightly higher for DPC than for EPC. Both curves do not show any discontinuity, therefore with the accuracy of our measurements we cannot detect phase transition of phospholipids. This result is consistent with the localization of ANS assumed above, because if the chromophore was within the aliphatic chains of DPC, it would be sensitive to their order–disorder transition at 41°. Here however we need to note a study in which ANS indicates structural reorganization of membrane fragments [9]. But it does not seem possible to take into account these differences, because natural membranes contain proteins which may also be labeled with ANS [10].

3.2. *Vesicles labeled with DPE*

In contrast with the ANS experiments the two systems differ remarkably from each other (fig. 2). At low temperature P is significantly greater for DPC vesicles, a break in the temperature curve occurs near 40° and afterwards both curves are closely identical. These results indicate that the fluidity of the chromophore environment is quite different at low temperature but almost similar above 40°. According to Waggoner and Stryer [11], the DPE probes the glycerol region of the bilayer, therefore our findings strongly suggest that a phase transition occurs at 40°. It is likely that the glycerol moiety, very close to the phospholipid chains, melts with the hydrocarbon matrix.

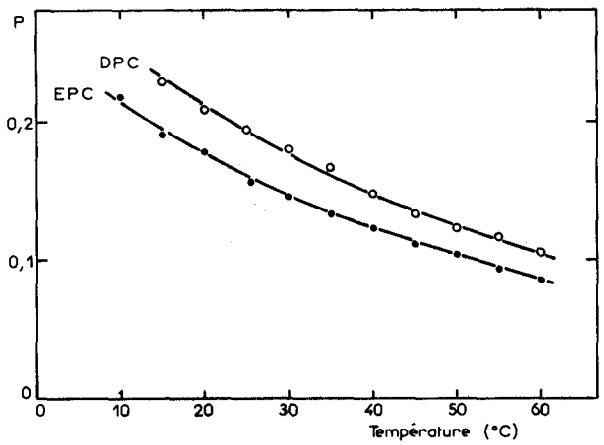

Fig. 1. Effect of temperature on the degree of polarization P of 1-anilino-8-naphtalene sulfonate (ANS) inserted into egg phosphatidyl choline (EPC) and dipalmitoyl phosphatidyl choline (DPC) vesicles.

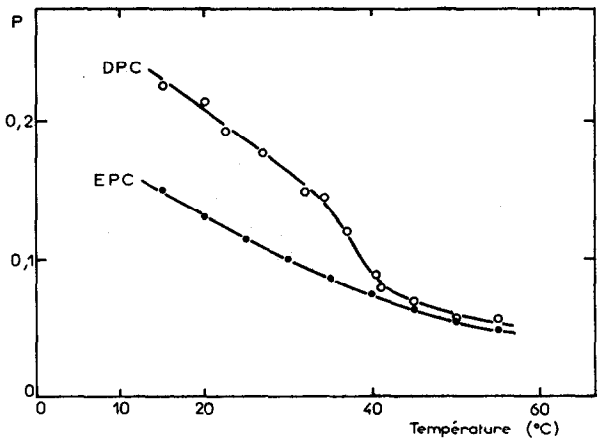

Fig. 2. Effect of temperature on the degree of polarization P of dansyl phosphatidyl ethanolamine (DPE) inserted into egg phosphatidyl choline (EPC) and dipalmitoyl phosphatidyl choline (DPC) vesicles.

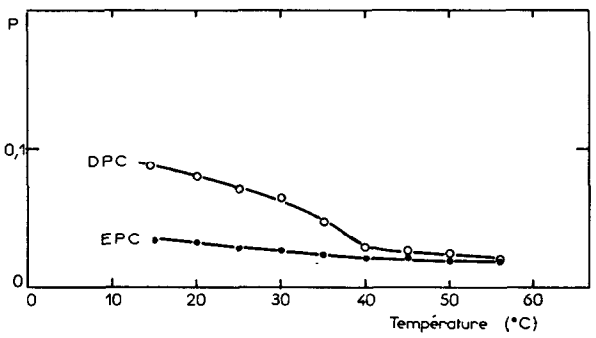

Fig. 3. Effect of temperature on the degree of polarization P of 9-methyl anthracene (9MA) inserted into egg phosphatidyl choline (EPC) and dipalmitoyl phosphatidyl choline (DPC) vesicles.

3.3. *Vesicles labeled with 9MA*

The 2-methyl anthracene was found to have good probe properties for the hydrocarbon region of spherical micelles [5] and we assume that 9-methyl anthracene labels this region in the bilayer. As we see (fig. 3) the degree of polarization of 9MA in EPC vesicles is very small and remains roughly constant over the temperature range 10 to 55°. On the other hand, the chain transition of DPC is clearly detected around 40°. We also notice that the viscosities of the hydrocarbon region are identical for both systems above 40°. Although slightly smaller, the values of P then obtained are comparable with those of Shinitzky et al. [5] on alkyl trimethyl ammonium bromide micelles labeled with 2MA. Our results suggest that above the melting temperature of the aliphatic chains the phospholipid bilayer interior is similar in nature to the hydrocarbon region of these micelles.

In summary, DPE and MA are sensitive indicators of structural transition within the bilayer. But it is relevant to point out in this context that we cannot yet ascribe the rapid decrease of P around the melting temperature merely to the expected local change of viscosity, since $P = f(\eta/T\tau)$. Preliminary study on DPC vesicles labeled with DPE indicates a diminution of 35% of the excited-state lifetime τ when the temperature is raised from 20 up to 55°. Since η and τ have opposite effects, this result indicates that the decrease of τ reduces somewhat the influence of the viscosity change on the degree of polarization. A more detailed analysis is now in progress to clarify this matter.

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