PHASE TRANSITIONS IN PHOSPHOLIPID MONOLAYERS AT THE AIR–WATER INTERFACE: A FLUORESCENCE STUDY

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1. Introduction

In the last few years, fluorescent probes have proved to be powerful tools in elucidating several aspects of the dynamics of phospholipids in biomembranes. The main body of investigation has been carried out either on biomembranes [1,2] or on membrane models such as phospholipid dispersions in water (liposomes) [3–5]. In these materials, it is extremely difficult to have any control on the molecular packing of the phospholipids. Instead, monomolecular films of lipids spread at the air–water interface are interesting membrane models from this point of view since molecular packing is easily controlled in addition to temperature, subphase pH and ionic strength and composition. The aim of this paper is to report on fluorescence experiments on lipid monolayers at the air–water interface using an apparatus specially designed in our laboratory. Transitions from liquid–crystal to gel phase of dipalmitoylphosphatidylcholine have been revealed by using the fluorescent probes DPE, DPH and AS*. The phase-dependent fluorescence properties observed can be accounted for by environment modifications and/or reorientations of the probes and are discussed with regard to data already inferred from experiments on phospholipid dispersions in water.

**Abbreviations used*: DPE, dansylphosphatidylethanolamine; DPH, diphenyl hexatriene; AS, 12(9-anthroyl)stearic acid; DPC, dipalmitoylphosphatidylcholine.

2. Materials and methods

2.1. *Chemicals*

DPC and AS were purchased from Sigma (USA) and DPH from Fluka (Switzerland). DPE was a generous gift from Dr Lussan (Bordeaux, France). All these compounds were pure as considered by thin layer chromatography and were used without further purification. Salts were of analytical grade. Ultra pure water for semiconductor industry (Motorola, Toulouse, France) was used throughout the experimentation. Solvents were spectroscopically pure.

2.2. *Monolayer and fluorescence experimentation*

The experimental procedure for surface pressure measurements, including the Langmuir moving-barrier automated apparatus of our own design, will be described in Chem. Phys. Lipids [6]. Pure DPC or DPC–probe mixtures in the molar ratio 99:1 were dissolved in an hexane–ethanol solvent mixture (9:1 v/v). For fluorescence experimentation, the film pressure was raised stepwise, at constant area, by consecutive additions of the lipids using a cylindrical trough (ϕ = 5 cm; h = 2 cm). Fluorescence was measured with an apparatus specially devised by one of us (J. Teissie) which will be described elsewhere in which the relative angle between the excitation and observation light beams is 45°, the axis of observation being perpendicular to the film surface (fig.1). For each measurement of fluorescence intensity, a 30 s illumination period was used after which time the film was allowed to rest in the dark in order to avoid any photo-degradation. Signal-to-noise ratio was 50 typically and reproducibility within 5%. In all experiments temperature was 20.0 ± 0.5°C.

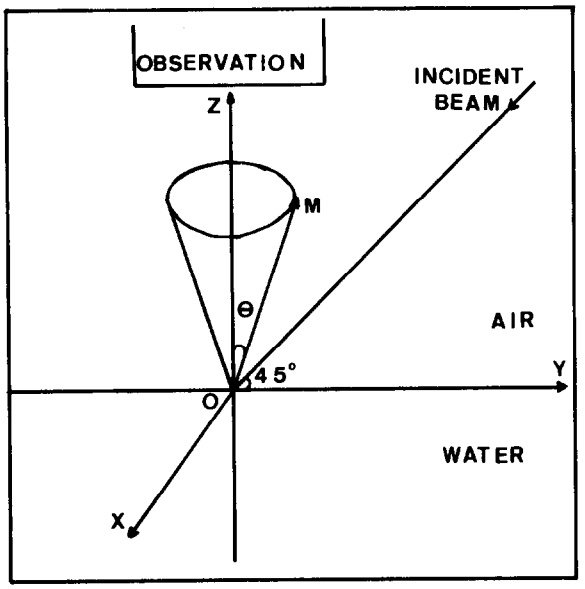

Fig.1. Fluorescence measurements on monolayers: schematic drawing of the geometrical disposition.

3. Results

3.1. *Compression isotherms*

A typical compression isotherm of DPC recorded through continuous film compression is shown in fig.2A. Along the curve, nearly identical to those already described [7], the gas–liquid ($\pi < 0.2$ dyne/cm), liquid–crystal ($0.2 < \pi < 6$ dyne/cm; molecular area 75 Å^2 at $\pi = 4$ dyne/cm) and gel ($\pi > 8$ dyne/cm; molecular area 46 Å^2 at $\pi = 20$ dyne/cm) phases of the film are clearly observable, the intermediate plateau region being specific of the liquid–crystal to gel phase transition.

The experimental points observed during step-wise compression with the fluorescence trough (triangles in fig.2A) fit satisfactorily the preceding continuous curve, particularly the different states of the film are still recognizable.

The compression isotherm of DPC was not perturbed at all when any of the three probes used was present in the film (molar ratio 99:1).

3.2. *Fluorescence experiments*

No fluorescence signal could be detected with films of pure DPC whatever their compression state.

It is currently accepted that due to its chemical structure DPE inserts between the phospholipid molecules and probes for the polar head region [8]. As observed in fig.2B, the reduced fluorescence intensity (fluorescence signal divided by the number of probe molecules; doing so it is assumed that the probe is distributed at random in the film) increases sharply (by 60%) at the beginning of the liquid–crystal to gel phase transition. Reorientation of the optical transition moments and changes in the environment of the probe can occur concurrently and be responsible for the

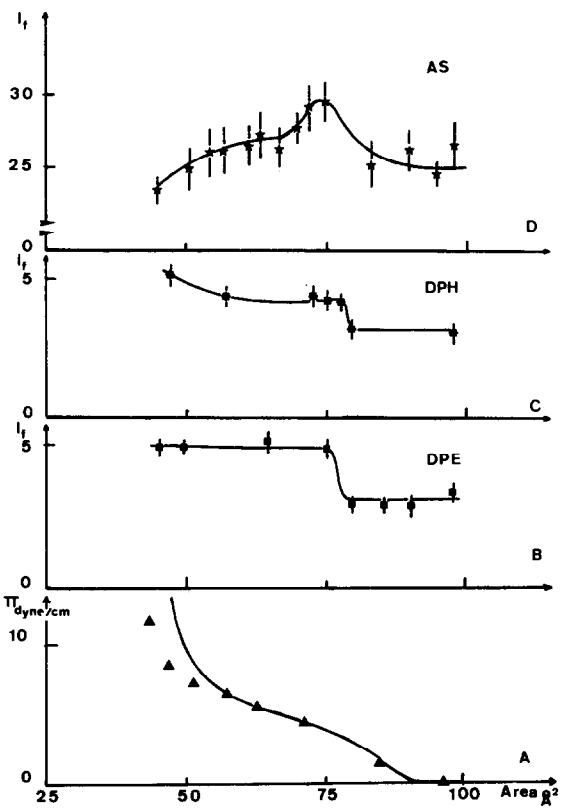

Fig.2. Phase transitions of DPC monolayers. Liquid subphase is 0.1 M NaCl, pH 6.0. (a) Surface pressure measurements with continuous compression (full line) and stepwise compression (triangles); (B – D) changes of the reduced fluorescence intensity (arbitrary units) during stepwise compression of DPE, DPH and AS respectively. Excitation wavelengths are 347 nm ($\Delta\lambda$ = 20 nm) for DPE and DPH and 360 nm ($\Delta\lambda$ = 60 nm) for AS. Observation wavelengths are 498 nm ($\Delta\lambda$ = 10 nm) for DPE and 476 nm ($\Delta\lambda$ = 10 nm) for DPH and AS. Abscissa scale is area per molecule. Error bars show the standard deviation observed with a minimum of six determinations.

Table 1
Spectral distribution of the emission of DPE embedded in a DPC monolayer in expanded state (π = 3 dyne/cm) and in condensed state (π = 10 dyne/cm)

λ (nm)	476	498	519	544
I_3	4	10	8.5	4
I_{10}	12.2	20	10	5
Ratio I_{10}/I_3	3.05	2.0	1.2	1.25

Subphase is 0.1 M NaCl, pH 6.0. I_3 and I_{10} (arbitrary units) are reduced fluorescence intensities observed at surface pressure* π = 3 dyne/cm and π = 10 dyne/cm respectively. I values were not corrected for wavelength dependence of the apparatus sensibility. Spectra bandwidth of the filters used is $\Delta\lambda$ = 10 nm. Standard error is 0.2. Excitation wavelength is 347 nm ($\Delta\lambda$ = 20 nm).

observed behavior. To decide on the relative weight of these factors, a spectral analysis of the emitted signal was carried out by comparing the fluorescence properties of the film in the expanded state (π = 3 dyne/cm) and in the condensed state (π = 10 dyne/cm). From the changes of the fluorescence signal ratio I_{10}/I_3 as a function of emission wavelength (table 1), it can be inferred that a blue shift is brought about on condensing the lipid film. This suggests that part of the enhancement of DPE fluorescence is due to environmental changes along the phase transition. Orientation factors might be quantitated directly in the near future with equipment of our fluorometer for anisotropy measurements.

Due to its hydrophobic character, DPH is considered as probing for the lipid hydrocarbon chains [3]. Its behavior is very similar to that of DPE as the liquid–crystal to gel phase transition is triggered (fig.2C) though the fluorescence increase in somewhat smaller (30%).

The fluorescent moiety of AS is also embedded within the acyl chains of the lipid but now at a fixed distance from the interface since its hydrophilic carboxyl group is supposed to anchor at the polar head–water interface [8]. As shown in fig.2D, the behavior of the fluorescence signal of AS upon film compression is strongly different from that observed with both DPE and DPH. The fluorescence of the probe is enhanced once more in the region of the liquid–crystal to gel phase transition, but to a lesser extent (15%), then progressively decreases towards its original value (in the limit of compressions allowed). This peculiar biphasic profile of the fluorescence response of AS was substantiated by numerous measurements, the subphase ionic environment being either 10 mM phosphate buffer, pH 7.2, or 20 mM NaCl, pH 3.0.

4. Discussion

Although DPE, DPH and AS probe for different regions in the phospholipid monolayer, all display an increase of their fluorescence signal as the DPC film starts to condense from the liquid–crystal to the gel phase. This phenomenon can be accounted for either by environment modifications and/or reorientation of the probe. The influence of environment follows the Franck–Condon principle; in other words any modification of the physical state of the lipid in the monolayer, in modifying the solvent cage around the probe and then the associated relaxation processes, can bring about a change in its quantum yield and a shift of its maximum emission wavelength. Changes in the environment can be regarded as depending upon a change in the solvation of the lipid polar heads at the interface [9] or as a modification of the fluidity of the aliphatic chains within the lipid matrix. Recent developments in the field of membrane structure clearly suggest, however, that the two concepts are closely intertwined [10]. The blue shift observed in the emission spectrum of DPE as DPC condenses is characteristic of a modification of the environment of the probe.

Furthermore, the geometric relationship between the orientation of the molecules in the monolayer and that of the axes of the optical system in the apparatus (fig.1) allows for detection of probe reorientation. Indeed, let us assume that the absorption and emission moments lie along the main axis OM of the probe. The axial symmetry of the monolayer system allows the probe molecule to be regarded as distributed on a cone centered on the Oz axis, the angle θ being specific of a given molecular orientation. With this model, it is easily demonstrated that the observed fluorescence intensity depends on this angle according to the equation:

$$I = K\left(1 + \frac{\sin^2\theta}{2}\right)\sin^2\theta \qquad (1)$$

In thermal transitions of DPC dispersions in water, changes in fluorescence intensity of the probes have been reported to be 20% and 10% with DPE and DPH respectively [3,5] and are attributable to environment changes only since the distribution of axes in microvesicles is at random. The higher values observed with monolayers, 60% and 30% respectively, make it reasonable to propose (through equation (1)) that probe reorientation does occur and is responsible for the difference observed in the fluorescence enhancement factor. Evidence for molecular reorientations correlated with phase transition have been reported for DPC multilayers either by fluorescence techniques [3] or by X-ray [11] and electron [12] diffraction studies.

As far as AS is concerned, an interpretation of the complex fluorescence transition observed is premature; however, it is worth noting that a similar behavior has been very recently reported for AS in microvesicles of DPC [4]. The fluorescence decrease observed in the high pressure region of the compression isotherm might correlate with AS segregation in microdomains; such a phase separation of AS has been already suggested [13] and is currently under investigation.

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