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PRESSURE EFFECTS ON THE APPARENT VISCOSITY OF ARTIFICIAL DIPALMITOYLPHOSPHATIDYLCHOLINE AND DIMYRISTOYLPHOSPHATIDYLCHOLINE MEMBRANES USING INTRAMOLECULAR EXCIMER PROBE

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Emission spectroscopy of intramolecular excimer probes allows the determination of 'equivalent viscosity' of membranes. While increasing the pressure on artificial membrane suspensions, variations in viscosity – essentially related to an increase in the order parameter in the membranes – are observed. In the case of mixed phospholipids, the effect of pressure is amplified, probably due to the existence of holes on the molecular scale between the two lipidic layers.

Introduction

The lipid components of biological membranes are described as a bilayer, the fluidity of which is of great importance in determining the permeability characteristics of the membrane and also in creating the appropriate environment for the function of membrane proteins [1].

With a view to a better understanding of the nature of molecular motions in natural membranes, we have developped three different spectrofluorimetric techniques, which have been previously proposed to determine the so-called microviscosity [2] and the order parameter [3] of membranes: (i) fluorescence polarization [4,5]; (ii) intermolecular quenching [6,7] and (iii) intramolecular excimer formation [8–11].

Also, it is well known that pressure responses of membrane phenomena are complex; Heremans [12] has recently reviewed the effects of high pressure on proteins and other biomolecules.

In the present paper, we were interested in investigating the influence of the mixing of two phospholipids on their equivalent viscosity, using the high-pressure technique. But pressure is responsible for scrambling effects on windows [13] and can lead to erroneous measurement with fluorescence probes. Pressure can also effect the concentration of fluorescent molecules embedded in membranes, and it would then be better to use a probe molecule which is unaffected by concentration and polarization effects; that is the case for intramolecular excimer formation of dipyrenylpropane. The increasing pressure would force the two lipidic layers and then the phospholipids to interact, raising the order parameter at the same time. This mixture may represent a very simplified model of the membrane; in fact, biological membranes contain a large number of different lipids with chains of variable length.

Materials and Methods

1,3-Di-1-pyrenylpropane ($Py(CH_2)_3Py$) was synthesized using methods described before [9,14],

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine.

and was strictly purified. DPPC and DMPC were purchased from Sigma Chemical Co. and tested for purity, prior to the experiment, using TLC. Solvents used were spectral grade. Steady-state fluorescence measurements (uncorrected) were performed on a Jobin Yvon JY 3D spectrofluorimeter equipped with a thermostatically controlled cell compartment.

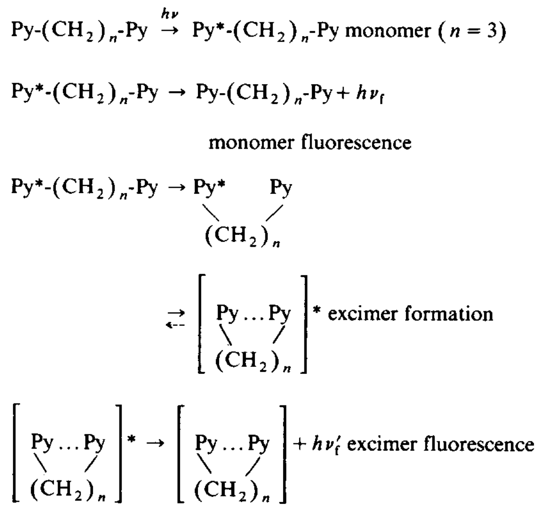
Emission spectra were recorded using excitation at 340 nm, and excimer to monomer intensity ratio (I_E/I_M) was calculated by comparing the fluorescence intensity at 480 nm to that at 376 nm.

Multilayer dispersions of phospholipids ($5 \cdot 10^{-4}$ M) were prepared in a buffer solution (1 M HCl/1 M Tris) (pH 7) with 10^{-6}M dipyrenylpropane as a fluorescent probe. To avoid the incubation of the fluorescent probe, phospholipids and dipyrenylpropane were previously dissolved in chloroform and the solution was evaporated under vacuum, then the deposit was suspended in buffer solution. According to a concentration of $1 \cdot 10^{-6}$ M for the probe and $5 \cdot 10^{-4}$ M for the lipid, the average distance between two dipyrenylpropane molecules is about 200 Å; then with a translational diffusion coefficient of about 10^{-7} cm^2/s, the probability of intermolecular reaction between excited and non-excited dipyrenylpropane is smaller than a few percent. Effectively as proved by experimental results: by changing lipidic concentration from $5 \cdot 10^{-3}$ M to $5 \cdot 10^{-4}$ M and by keeping the probe concentration constant at 10^{-6}M, the ratio I_E/I_M is only slightly affected; on the other hand, changes in the lipidic concentration from $5 \cdot 10^{-4}$ M to $5 \cdot 10^{-5}$ M for a 10^{-6} M concentration of the probe increase greatly the ratio I_E/I_M. Sonicated vesicles were obtained by sonication at 50° C for 30 min, with a home-made ultrasonic source (27 kHz).

High-pressure experiments were performed at 20°C in a NOVA-SWISS high-pressure apparatus. A stainless steel optical cell fitted with two sapphire ultraviolet quality windows was used for the measurements of fluorescence spectra. Excitation and analysis wavelengths were selected by two H 20 Jobin-Yvon monochromators. Spectroscopic conditions were as described above.

Results and Discussion

In homogeneous solution, by ultraviolet light excitation, an electronically excited monomer of dipyrenylpropane (M*) can be formed. Internal rotations, the functioning of bond torsion potentials, and the non-bonded atom interactions of molecular brownian motion and of medium viscosity, allow the formation of an intramolecular excimer (E*), the mechanism being as follows:



No satisfactory theoretical model exists describing this reaction; however, it is possible to establish a relationship between the excimer-to-monomer fluorescence intensity ratio, I_E/I_M, of dipyrenylpropane and the viscosity of homogeneous media. Then, theoretically, a comparison of this ratio for organized assemblies might give information about the cohesion which would correspond to the viscosity of a homogeneous medium and be called equivalent viscosity [2].

Fig. 1 shows the value of the ratio of fluorescence quantum yields of E* and M* (peak heights), for dipyrenylpropane, as a function of the viscosity of ethanol/glycerol mixtures.

Pressure effect in ethanol solution

In ethanol solution, the formation of an intramolecular excimer of dipyrenylpropane is affected by pressure as shown in Fig. 2 (curve a); a result which is in agreement with data already published on similar systems [15]. From the viscosity-pressure relationship [16] and from results con-

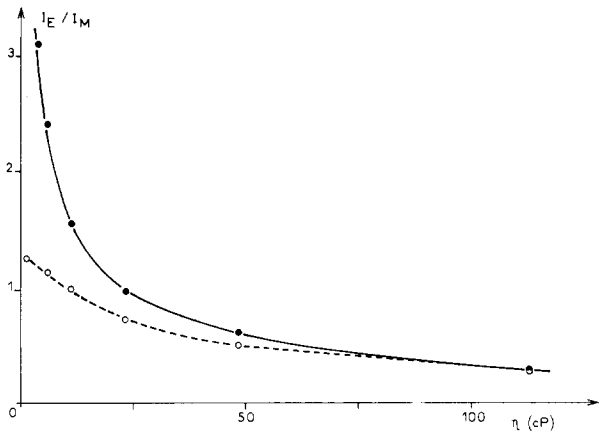
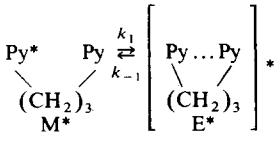


Fig. 1. Variation of I_E/I_M vs. η for dipyrenylpropane in ethanol/glycerol mixtures: ○, aerated solution; ●, degassed solution

cerning the effect of viscosity on the intramolecular excimer formation of dipyrenylpropane (cf. Fig. 1), it is possible to calculate the variation of log (I_E/I_M) relative only to the increase of viscosity due to the pressure (Fig. 2, curves b and c in degassed and aerated conditions respectively). Comparison of curves a and b of Fig. 2 shows an important additional pressure effect. This effect can be qualitatively interpreted assuming an equilibrium between the two excited species as described below:



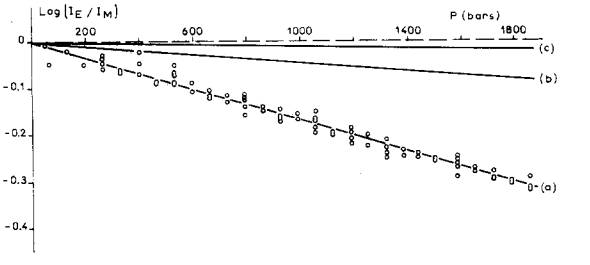


Fig. 2. Relative variation of log (I_E/I_M) vs. pressure, for dipyrenylpropane in degassed ethanol (a). Compared with the effect of the calculated effect of viscosity change ((b) degassed solution; (c) aerated solution).

If $K = k_1/k_{-1}$, the variation of log K with the pressure, P, must follow a classical law with the following form:

$$\frac{\partial \log K}{\partial P} = -\frac{\Delta V^*}{RT}$$

where ΔV^* is the variation of molar volume during the reaction, R is the gases constant and T is the absolute temperature. If the relation is true, log K must vary linearly with P. This is well verified as shown on Fig. 2 (curve a).

The calculated value of ΔV^* is of the order of 7–8 $cm^3 \cdot mol^{-1}$. This value is of the same order of magnitude as that found in formation of intermolecular excimers [17].

Pressure effect in phospholipids

At atmospheric pressure, equivalent microviscosities of artificial membranes can be determined by using intramolecular excimer formation of dipyrenylpropane [10,11]. Fig. 3 presents plots of I_E/I_M vs. temperature (range 17–50°C) of dipyrenylpropane embedded in DPPC, DMPC and

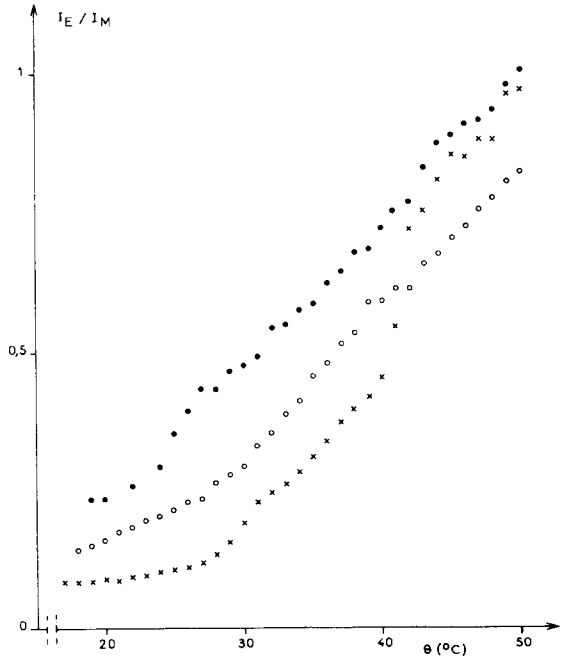


Fig. 3. Variation of I_E/I_M vs. temperature for dipyrenylpropane in undegassed solution: x, DPPC; ●, DMPC; ○, DMPC-DPPC equimolar.

an equimolar mixture of DPPC/DMPC. Note the absence of transition of the case of phospholipids mixtures. (Mixtures of DPPC and DMPC are accepted as systems which exhibit ideal mixing [18]). The estimated equivalent viscosities at 20°C are reported in Table I.

The behaviour of multilamellar vesicles in degassed medium, under pressure (up to 2000 bars) and using intramolecular excimer formation of dipyrenylpropane as a probe can be visualized in Fig. 4. The measurements have been performed at 20°C, which correspond to the solid phase of the lipid. Moreover, the application of high pressure to phospholipid will raise the phase transition temperature [12,19,20].

A similar pressure effect has been obtained in the case of small unilamellar vesicles. A typical effect of pressure on the fluorescence emission spectra of dipyrenylpropane embedded in multilayers of DPPC is represented in Fig. 5.

These results are consistent with the two following comments. (i) The phospholipid behaviour, under pressure, is different from that observed in a homogeneous medium such as ethanol. A significant decrease in I_E/I_M is observed at low pressures, until a smaller slope decrease is obtained. This result can be interpreted by an important modification of the order degree of the membrane due to pressure, and also by modifications of the equilibrium constant between the most favourable populations of conformers of unexcited probe.

Taking into account the complexity of molecular motions in membrane, on one hand, and the interpretation of variations of I_E/I_M due to those motions, on the other hand, it seems most audacious to go further with this analysis for the moment. (ii) DMPC and DPPC multilamellar vesicle behaviours are very similar. On the contrary, for an equimolar mixture of DMPC and DPPC, a more important effect of the pressure has been

TABLE I

EQUIVALENT MICROVISCOSITY OF PHOSPHOLIPID BILAYERS AT 20°C

Equivalent microviscosity was determined using the relation between I_E/I_M and viscosity of ethanol/glycerol mixtures.

Phospholipid	I_E/I_M	Equivalent microviscosity in cP
DPPC	0.08	> 500
DMPC	0.24	160
Equimolar DMPC-DPPC	0.16	250

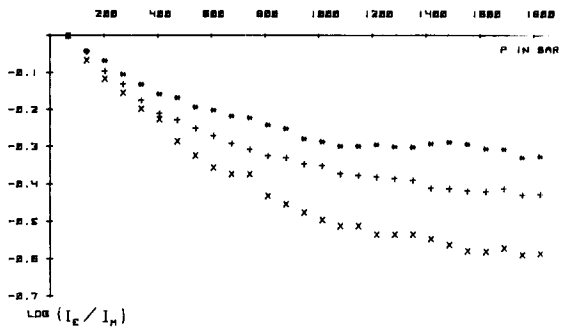


Fig. 4. Relative variation of log (I_E/I_M) vs. pressure at 20°C for dipyrenylpropane in multilamellar vesicles of: *, DPPC; +, DMPC; and x, DMPC-DPPC equimolar. values were normalized at $P = 68$ bars.

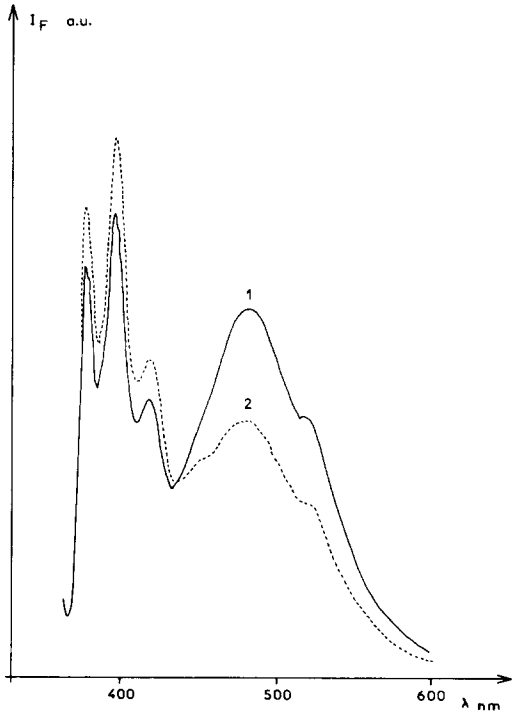


Fig. 5. Fluorescence emission spectra of dipyrenylpropane, embedded in multilayer vesicles of DPPC, at 20°C. (1) 70 bars; and (2) 1800 bars.

observed. Now the mean length of molecules of DMPC is different from that of DPPC molecules (two methylene units), which factor could be responsible for the existence of holes on a molecular scale in mixed membranes, as has already been proposed [21,22]. The effect of pressure would, as a consequence, be a decrease in the size of the holes which can allow interpenetration of the layer. Such an effect would not exist for pure phospholipids.

Conclusion

Our experiments are thus in good agreement with the assumption of the presence of holes in the proximity of interlayer zone having an influence on the apparent reactivity, bound to the environment, of an excited pyrenyl probe with an unexcited pyrenyl group. Moreover, this result implies that dipyrenylpropane must be localized in those regions in the vicinity of hydrophobic tails, and not to that of hydrophilic heads.

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