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PHASE TRANSITIONS IN PHOSPHOLIPID DISPERSIONS STUDIED WITH AN INTRAMOLECULAR EXCIMER FORMING FLUORESCENT PROBE, 1,3-BIS (β-NAPHTHYL)PROPANE

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SUMMARY

We introduce the use of an intramolecular excimer forming, non-conjugated bichromophoric molecule : 1,3-bis (β-naphthyl)propane as a new probe for measuring thermal phase transitions in aqueous dispersions of phospholipids.

Intermolecular excimer forming systems, such as pyrene, have been intensely studied as probes for the "microfluidity" of phospholipid dispersions.

The probe we used has the added advantage that intramolecular excimer formation follows a pseudomonomolecular mechanism. This makes observations independent of probe concentration and allows for minute concentrations of the probe to be used, lowering the risk of perturbation of the phospholipid phase.

Phase transition temperatures determined from 1,3-bis (β-naphthyl) fluorescence are in good agreement with differential scanning calorimetry and light scattering measurements.

INTRODUCTION

A great number of fluorescent probes has been used in membrane studies and among them an important place is taken by excimer forming molecules (1). Intermolecular excimer formation, e.g. with pyrene, follows a bimolecular mechanism (2) and hence is concentration dependent. Pyrene has been used widely (3,4) as a probe of the "microfluidity" (5) of the hydrocarbon interior of a variety of phospholipid dispersions. "Microfluidity" changes are measured from the change of the ratio of emission intensities of excimer and monomer (I_E/I_M). However, for intermolecular excimer forming probes the ratio I_E/I_M is concentration dependent. Thus, relatively large concentrations are required in order to obtain a sufficient intensity of excimer emission.

Abbreviations : ββDNP : 1,3-bis (β-naphthyl)propane ; DMPC : dimyristoylphosphatidylcholine ; DPPC : dipalmitoylphosphatidylcholine ; DMPG : dimyristoylphosphatidylglycerol ; T_t : phase transition temperature.

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Intramolecular excimers are formed by molecules containing two or more aromatic residues linked by flexible aliphatic carbon chains (6). In contrast with intermolecular systems, the formation of intramolecular excimers is concentration independent. The latter have already been used in the study of the "microfluidity" of micelles (5,7,8). In the present communication we describe the fluorescence behaviour of an intramolecular excimer forming probe, 1,3-bis (β-naphthyl)propane (ββ DNP) (fig. 1) which has previously been studied in organic media (9,10,11) and which is now incorporated in the hydrocarbon region of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG) and dipalmitoylphosphatidylcholine (DPPC) dispersions. We have investigated the effect of temperature and different label concentrations on the ratio I_E/I_M of lipid-embedded ββ DNP.

We will demonstrate that ββ DNP is a useful probe to determine the lipid phase transition temperature (T_t) and that the ratio I_E/I_M is concentration independent over a wide range of ββ DNP/lipid molar ratios.

MATERIALS AND METHODS

Materials

Synthetic L-α-dimyristoylphosphatidylcholine and L-α-dipalmitoylphosphatidylcholine were purchased from Sigma. Dimyristoylphosphatidylglycerol was synthetised according to Papahadjopoulos et al. (12). ββ DNP was synthetised according to Chandross and Dempster (9) and purified by high pressure liquid chromatography. 2-Ethylnaphthalene was obtained from Aldrich and purified by column chromatography on silicagel. All solvents were spectroscopic grade Uvasol from Merck.

Labeling phospholipid dispersions with ββ DNP

DMPC, DMPG and DPPC dispersions were prepared as follows : a thin lipid film was deposited on the wall of a 20 ml glass flask by evaporating a chloroform solution containing 10 mg of the lipid and an appropriate amount of ββ DNP. The ββ DNP/lipid molar ratio was 0.01 in DMPG and ranged from 0.00034 to 0.05 in DMPC and from 0.001 to 0.05 in DPPC dispersions. The evaporation was effected with a stream of purified, dry nitrogen. After the addition of 10 ml 0.05 M phosphate buffer (pH 7.4) the mixture was sonicated above its T_t with an ultrasonic desintegrator MSE (150 watt) operated at maximum power, until a clear dispersion was obtained. The sample was then centrifuged above T_t to remove titanium residues. Sepharose CL-4B chromatography and electron microscopy show that these preparations are mainly single-walled vesicles with a diameter of 250 to 400 Å. The lipid dispersions containing ββ DNP were stored above their T_t and used for spectral studies on the day of preparation. The influence of oxygen quenching on the ratio I_E/I_M was assessed to be negligible and therefore the lipid dispersions were prepared without purging with nitrogen.

In order to correct the recorded spectra for light scattering due to the lipid dispersion, a control containing the same lipid component without ββ DNP was prepared under identical conditions. In evaluating the monomer and excimer bands, which overlap, the monomer band is assumed to have the spectral characteristics of the corresponding arylalkane 2-ethylnaphthalene. An appropriate amount of 2-ethylnaphthalene was therefore incorporated into the phospholipid phase by addition of an ethylalcohol solution to the probe-free dispersions. The final alcohol concentration did not exceed 0.1 % (v/v).

Measurement of fluorescence spectra

Right angle steady state fluorescence measurements were performed using an Aminco Bowman ratio spectrofluorometer or a Spex Fluorolog spectrometer. The cell compartment of both instruments was thermostatted, and the temperature of the cell was monitored with a thermocouple. Temperature increments were 1-4° C and the sample was allowed to equilibrate for 10 min at each temperature before the spectra were measured. The fluorescence spectra are uncorrected. The lipid-embedded ββ DNP was excited at 275 nm. Emission maxima of excimer and monomer were found to be at about 402 and 338 nm respectively.

Phase transition measurements by light scattering

For each preparation used in ββ DNP fluorescence measurements, the lipid phase transition was determined by 90° light scattering (λ excitation and λ emission set at 400 nm) using the same instrumentation.

RESULTS AND DISCUSSION

Analysis of the recorded spectra

A typical emission spectrum of ββ DNP in a DMPC dispersion above its T_t is shown in fig. 1 (curve b). Curve a is the light scattering curve of the control dispersion used for correction. The monomer band was calculated from the emission spectrum of lipid-embedded 2-ethylnaphthalene (curve c) after normalisation in the 320-330 nm region. The excimer emission was obtained after subtraction of the monomer component from the total emission curve. The fluorescence intensity ratios I_E/I_M were calculated from the surfaces under the excimer and monomer bands.

Effect of ββ DNP concentration on the fluorescence ratio I_E/I_M

The ratio of the fluorescence intensities I_E/I_M as a function of different probe/lipid molar ratios is reported in table 1, for a temperature of 30° C. At this temperature DMPC dispersions are in the liquid crystalline state, while DPPC dispersions are in the gel phase. As can be seen from the data in

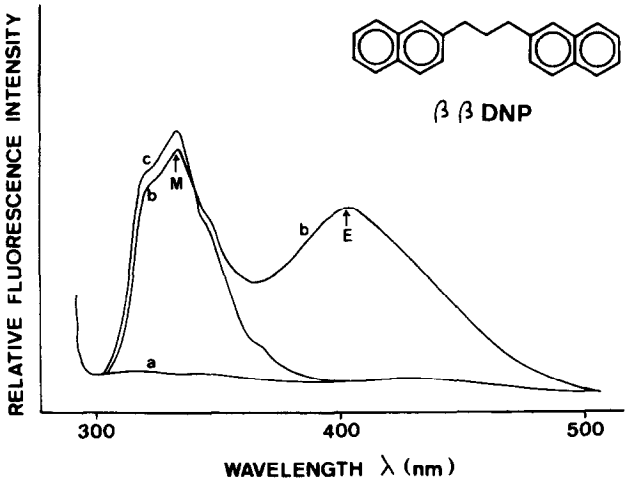

Figure 1. Analysis of the fluorescence spectrum of ββ DNP incorporated in a DMPC dispersion.
Excitation was done at 275 nm ; the temperature was 27° C ; the aqueous dispersion contained 1 mg of DMPC per ml phosphate buffer 0.05 M (pH 7.4).
a. Stray light from a control dispersion of DMPC without ββ DNP added.
b. Emission spectrum of ββ DNP embedded in the DMPC dispersion. The ββ DNP/lipid molar ratio was 0.001.
c. Emission spectrum of 2-ethylnaphthalene embedded in the DMPC dispersion.
The capitals M and E refer to the monomer resp. excimer emission bands.

Table 1

Fluorescence ratio I_E/I_M for ββ DNP in phospholipid dispersions as a function of the probe/lipid molar ratio at 30° C.

Probe/lipid molar ratio	I_E/I_M [a]	
	DMPC	DPPC
0.00034	1.77	--
0.001	1.78	1.25
0.005	--	1.27
0.01	1.78	--
0.05	1.79	1.27

[a] The ratio I_E/I_M was calculated from the surfaces under the excimer and monomer bands as described in the text.

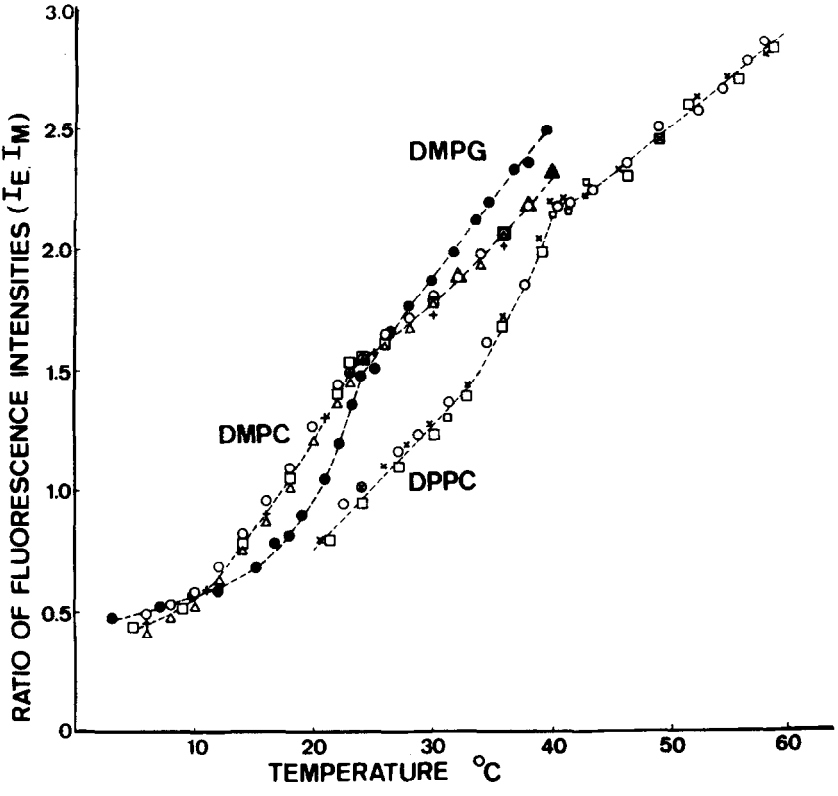

Figure 2. Effect of temperature on the ratio of the excimer to monomer fluorescence intensities (I_E/I_M) of ββDNP incorporated in DMPC, DMPG and DPPC dispersions. The ββDNP/lipid molar ratios were resp. 0.00034 (+), 0.001 (□), 0.005 (x), 0.01 (Δ, and ● in the case of DMPG) and 0.05 (o).
The lipid transition and the pretransition are characterised by well defined points of discontinuity as discussed in the text. For two phospholipids the I_E/I_M vs. T.plots coincide for all ββDNP concentrations used.

table 1, the ratio I_E/I_M is concentration independent within the range of ββDNP concentrations used, even at a probe/lipid molar ratio of 0.00034 representing an average of one probe molecule per vesicle.

Effect of temperature on the fluorescence ratio I_E/I_M.

The effect of temperature on the fluorescence intensity ratio I_E/I_M is depicted in fig. 2 for DMPC, DMPG and DPPC dispersions. For all probe concentrations a point of discontinuity in the I_E/I_M vs. T plot is observed at 23°, 24° and 40° C for DMPC,DMPG and resp. DPPC dispersions. The transition temperature T_t for each of the lipid dispersions (labeled and unlabeled) used

in our fluorescence measurements was determined by the light scattering method and found to coincide with the temperature at which the discontinuity in the I_E/I_M vs T plot occurs. These values are in good agreement with previously reported data obtained by differential scanning calorimetry (13,14). This demonstrates the feasibility of measuring transition temperatures by using ββ DNP as a fluorescent probe, even at very low probe/lipid molar ratios.

The increase of I_E/I_M with temperature is related to the increase of the "microfluidity" in the hydrocarbon interior of the phospholipid bilayer, while the point of inflexion at T_t is due to an abrupt change in the membrane fluidity. These changes in the physical state of the phospholipid bilayer are well known from spin label studies (15). The different slopes below and above T_t probably result from differences in the temperature coefficient of viscosity of the gel and liquid crystalline phases, suggesting a correlation to exist between the viscosity of phospholipid phases and the fluidity of the probe microenvironment. From the curves in fig. 2 we suggest that intramolecular excimer formation is also sensitive to the "pretransition" phenomenon (13) occuring below T_t, at about 12° and 33° C for DMPC resp. DPPC dispersions. With DMPG dispersions we observe also a change of slope at about 15° C. The sensitivity of the ββ DNP intramolecular excimer formation to the pretransition is in agreement with observations made with pyrene (4) and with nitroxide spin labels (16), indicating that this transition does not only affect the polar head group region but also the arrangement of the alkyl chains in the hydrophobic membrane region.

From our results we conclude that ββ DNP and hence non-conjugated bichromophoric systems are useful probes to determine transition temperatures of artificial phospholipid membranes, even if used at minute concentrations, minimalising the risk of perturbation of the whole phospholipid phase.

Further detailed analysis of the kinetics of excimer formation in these media might result in a better understanding of the dynamical aspects of changes occuring in phospholipid bilayers.

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