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FLUORESCENCE TEMPERATURE JUMP RELAXATIONS OF DANSYL-PHOSPHATIDYLETHANOLAMINE IN AQUEOUS DISPERSIONS OF DIPALMITOYLPHOSPHATIDYLCHOLINE DURING THE GEL TO LIQUID—CRYSTAL TRANSITION

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Summary

The change of fluorescence of the probe dansylphosphatidylethanolamine embedded in multilamellar liposomes of dipalmitoylphosphatidylcholine was used to investigate the dynamics of the thermotropic gel to liquid-crystalline phase transition by use of the temperature-jump technique. The results are discussed and compared to published observations on the same system in which the phenomenon was reported by turbidity changes.

The thermotropic phase transitions of phospholipid assemblies (multilamellar liposomes and closed monobilayered microvesicles) have been investigated by use of various techniques including ESR, NMR, fluorescence spectroscopy, ultrasonic absorption, light scattering and calorimetry [1—5]. Numerous data have been collected on the equilibrium properties of these transitions but very few have concerned their dynamic behaviour [6]. Several distinct classes of structural change are expected to take place when the so-called pretransition and main melting processes are triggered, which we hoped could be revealed by fast kinetic investigation using different observation signals. Here we report observations by temperature-jump experimentation in the gel to liquid-crystal transition region of dipalmitoylphosphatidylcholine. The relaxation process was followed by the change of the fluorescence of the probe dansylphosphatidylethanolamine embedded in the lipid phase, a probe known to be located at the glycerol level [14]. We expected that it could reveal transients occurring at the glycerol level which could be compared with results obtained by measurements of turbidity which report overall properties of the membrane.

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In the experiments 0.25 to 1.5 mg/ml lipid suspensions in 0.1 M NaCl pH 5.5 were used. Weighed amounts of dipalmitoylphosphatidylcholine (Sigma, U.S.A. P6138) and dansylphosphatidylethanolamine (a gift of Professor C. Lussan, Bordeaux, France) were first dissolved in chloroform. The required volume of organic solution was evaporated to dryness with nitrogen, then the aqueous milieu was added after being deozygenated by bubbling nitrogen. Homogenization was achieved at 50° C with repeated Vortex mixing, above the transition temperature [4]. The probe: lipid molar ratio was 1:250 and the dispersion was used within a few hours after preparation.

Fluorescence spectra were recorded with the FICA 55 spectrofluorimeter (ARL, France) working at a 7.5 nm resolution, and the temperature of the observation cell was kept constant to within ± 0.1° C. To minimize possible artifacts due to sedimentation, the following time sequence of operations was used: temperature equilibration, manual mixing of the cell content, the spectrum was always recorded starting 5 min after mixing (a second spectrum run immediately was almost identical to the original one).

Temperature-jump experiments were conducted using home-built apparatus based on the description given by Rigler et al. [7] with modifications concerning mainly the optics and resulting in increased sensitivity (Teissie, J., unpublished). An *F*/1 collecting optic gives a high incident light flux resulting in a good signal to noise ratio. Observation cells and heating device were from Messanlagen (F.R.G.). The light source used was an XBO lamp from Osram (F.R.G.) and the regulated power supply was from Spotlight (France). Transients were observed on the oscilloscope and recorded simultaneously in a Datalab DL 905 recorder (U.K.) coupled either to a charge potentiometric recorder or to a Texas ASR tape recorder (U.S.A.). The size of the jump was calculated to be 0.1° C as described by Tsong, using an effective volume of 5 ml [6]. Fluorescence was excited at 340 nm ($\Delta\lambda$ = 6.6 nm), and emitted light was selected with use of a Kodak 4 long wavelength pass filter (U.S.A.) ($\lambda > 450$ nm).

Static observations of the fluorescence emitted by dansylphosphatidylethanolamine showed that increasing the temperature results in both a reduced fluorescence intensity and a red shift of the spectra (Fig. 1). From 33° C and above, the intensities of fluorescence of the probe (observed at 520 nm) showed a distinctive pattern (Fig. 1A). A general decrease of fluorescence with increasing temperature is contrasted by a plateau region and subsequent steeper profile in the temperature range from 37 to 41° C where the main melting transition of the host lipid is expected to occur. These properties are in qualitatively good agreement with the observations reported earlier by Faucon and Lussan [4] for the change of anisotropy of the same probe in the same environment. These changes were interpreted by the authors as indicative of the transition between P_{β}' and L_{α} states in Luzzati's nomenclature [8]. This transition will induce a change in viscosity or in hydrophobicity in the neighbourhood of the fluorescent moiety, resulting in the observed red shift of the emission.

As already discussed by Tsong [6], the joule heating temperature-jump method [9] can be used with lipid suspension. The associated electric field effect is expected to be very small for the small size of the temperature jump we have used.

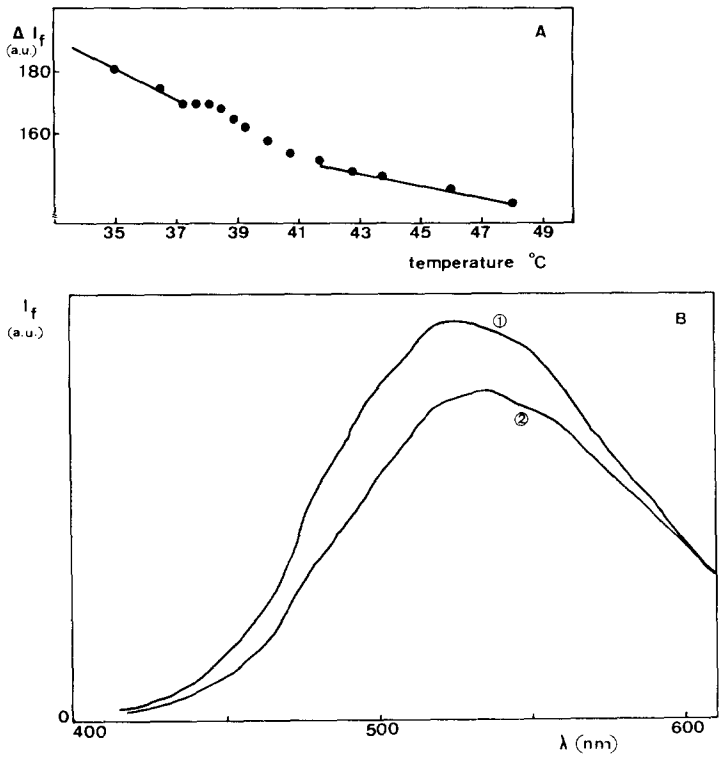

Fig. 1. Fluorescence changes of dansylphosphatidylethanolamine during dipalmitoylphosphatidylcholine phase transition. (A) Equilibrium melting curves. Heating and cooling curves were identical. Fluorescence changes observed at 520 nm emission wavelength with excitation at 340 nm. The time elapsed between two consecutive recordings of spectra was 15 min. (B) Temperature induced changes of emission spectra. The upper curve was recorded at 21.2°C, the lower at 44.2°C. Excitation wavelength was 340 nm. Conditions: 0.7 mg/ml lipids dispersed in 0.1 M NaCl at pH 5.5. The probe: lipid molar ratio was 1:250.

At all temperatures used, a signal was present in the 1—10 μs time range that was too fast to be studied further. Its amplitude could not be accurately determined owing to the time constant of the electronics. Its apparent value was a fraction of one percent of the total intensity. When the temperature was set to within the transition region, in addition to this fast transient, a single major exponential relaxation process was observable in the 0.01-s time range (Fig. 2). The relaxation time of this process showed a sharp maximum culminating at 38.4°C and was practically unmeasurable either at temperature below 37.9°C or higher than 40°C, its amplitude vanishing outside this temperature range (Fig. 3). In addition, a slower relaxation process was detected in the 10-s time range, with a decay being clearly nonexponential. However, due to temperature drifts following the jump and to associated convection in the solution, investigation of this slow reaction was not conducted further. In fact this time range is known to be outside the usable time scale in classical temperature-jump experimentation.

The very fast relaxation could be due either to a direct physical perturbation of the fluorescence of the probe or most probably to a transport of solvent molecules through the lipid leaflets as reported by other groups using

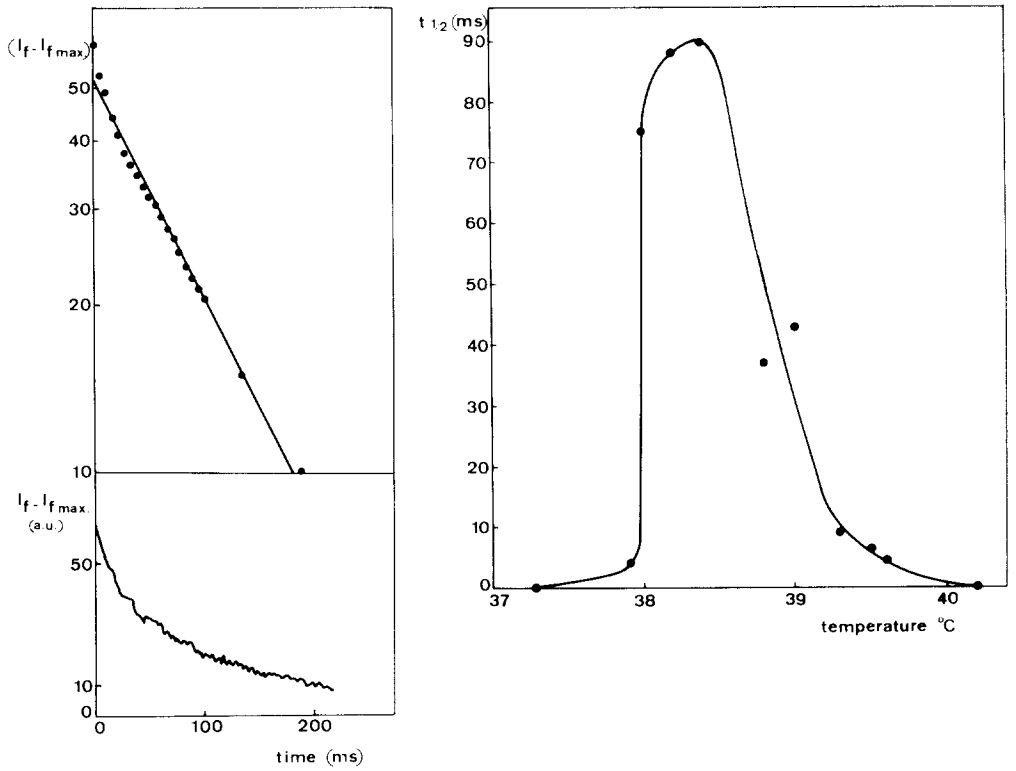

Fig. 2. The kinetics of the crystalline-liquid crystalline phase transition of dipalmitoylphosphatidylcholine dispersions. The lipid concentration was 1 mg/ml in 0.1 M NaCl, pH 5.5; temperature jump from 38.2 to 38.3°C; fluorescence of dansylphosphatidylethanolamine was used to monitor the reaction. Electronic time constant was 0.5 ms. The probe: lipid molar ratio was 1:250. $I_{f,\,max}$ is the steady intensity recorded 1 s after the jump.

Fig. 3. Temperature dependence of the relaxation times for the fluorescence change of dansylphosphatidylethanolamine correlated with the crystalline to liquid-crystalline phase transition of dipalmitoylphosphatidylcholine. A maximum is observed at 38.4°C. Conditions were as described in Fig. 2. $t_{1/2}$ is the time required to relax to $I_{fmax} - I_f(0)/2$ following a temperature jump.

temperature-jump experimentation with light scattering detection [6, 10, 11].

The major fluorescence relaxation observed in the millisecond time range is strikingly similar to the relaxation process with time constant τ_2 described by Tsong and Kanehisa [6]. The similarity concerns not only the absolute value of the relaxation time but extends to the shape of the very sharp spike profile of the temperature dependance in the phase transition region. We note, however, that the position of τ_{max} is lower by about 1.5°C as compared to the position observed by Tsong and Kanehisa using turbidimetry measurements. Moreover, no trace has been found of a fluorescence relaxation in the second time range for which turbidity revealed a slow process with time constant τ_1 so that we can rule out the possibility that fluorescence changes be simple feed back effects of the turbidity changes. The kinetic scheme proposed by Tsong and Kanehisa [6] indicates that maximum values of relaxation times occur at T_m, the transition temperature. Their hypothesis is based on a cluster model of lipid phase-transition. Phase-transition is linked to the growing

of clusters of lipids in the liquid state among the lipids in the gel state.

Dansylphosphatidylethanolamine is known to probe the glycerol moiety [14], whose fluidity is the lowest in the lipid assembly [2]. the apparent shift to lower temperature of T_m observed by fluorescence as compared to turbidity is unexpected. Though any impurity present in the lipid can lower the T_m [12, 13], we do not favour such an explanation for the probe is used at a very low concentration (1:250 molar ratio). We rather suggest that dansylphosphatidylethanolamine reveals early kinetic events in the transition. Following this view, the glycerol region should be the locus of the very trigger of the phase transition. In conclusion, the transition to the liquid-crystalline state, which is associated to a modification of the conformation of the hydrocarbon chains, would, indeed, be controlled at the glycerol level. This triggering process is associated with a change in hydrophobicity or in mobility at the glycerol level as reported by our probe.

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