Time Resolved Fluorescence in Unimellar Vesicles

Luis F. Aguilar and Carlos P. Sotomayor
Department of Chemistry
Pontificia Universidad Católica de Valparaíso
Valparaíso, Chile

Introduction

The lipidic matrixes of biological membranes are lyotropic liquid crystals in lamellar phase. As highly structured supramolecular phases, their dynamics and structural properties, that not necessarily correlate, are all important to characterize unambiguously a membrane and/or its changes. Many studies concerning membrane's properties and processes make use of the fluidity concept that has become an ill-defined term since it groups together different parameters, related with diverse structural and dynamics properties.

Here we present the characterization of dynamic and structural properties of large unilamellar vesicles (LUV) of the artificial lipid dipalmitoylphosphatidylcholine (DPPC) using time resolved fluorescence techniques, including fluorescence intensity decay and fluorescence anisotropy decay. As fluorescent probes DPH, and its derivatives TMA-DPH and TMAP-DPH were used.

Materials and Methods
Sample Preparation

Large unilamellar vesicles(LUV) suspension were formed using an aqueous solution of the non ionic detergent octaethylene glycol dodecyl ether $(C_{12}E_8)$, to solubilize DPPC. Vesicles spontaneously formed when the detergent was subsequently removed by addition of hydrophobic Bio-Beads, and incubated at $4°C$ during 12 h.

Equipment

Time resolved fluorescence measurements were done using a K2 multifrequency phase and modulation spectrofluorometer from ISS, Inc. The instrument was equipped with Glan-Thompson polarizers. For all probes, the exciting light was from a modulable ISS 375 nm LED laser. The emission was measured through Schott KV-399 and WG-420 long band-pass filters. Intensity decay measurements were done with the exciting light polarized parallel to the vertical laboratory axis, and the emission was viewed through a polarizer oriented at $55°$ ("magic angle" condition) and the phase and modulation values were obtained at ten modulation frequencies in intervals according to the fluorophore lifetime.

For anisotropy decay determination, differential phase angles and modulation ratios were obtained from parallel and perpendicular oriented sinusoidal polarized emission at twelve modulation frequencies between 2 and 200 MHz. Dimethyl-POPOP (1,4-bis[2]4-Methyl-5-phenyloxazoly benzene) in ethanol (t =1.45 ns) was used as a reference of intensity decay. 5 mm path-length square quartz cuvettes were used . Sample temperature was controlled by an external bath circulator.

Data Analysis

Time-resolved fluorescence data were analyzed using the ISS Vinci analysis software package. The fitting function for the lifetime measurements was the sum of a continuously distributed Lorentzian component and a discrete component, fixed at 0.01 ns to account for scattered light.

Anisotropy decay data were fitted to a hindered rotator model of anisotropy decay, which is based on the "wobble-in-cone" model, including a hindered rotation component (i.e. two rotational correlation times, where the second rotational correlation time, θ_2 is fixed at a large value,1 ms, relative to the lifetime.

$$r(t) = (r_0 - r_\infty) \exp(-t/\theta_1) + r_\infty \qquad (1)$$

where r_0 is the amplitude of the anisotropy decay at time 0, θ_1 is the fast rotational correlation time of the anisotropy decay, and r_∞ is the residual anisotropy at infinite time. θ_1 with the fluorophore rotational rate R_1 by $R_1 = 1/6\theta_1$, r_∞ is related to S, the mean second rank-order parameter of the fluorescent probe in the bilayer $S = (r_\infty/r_0)^{1/2}$

Figure 1. Lifetime and Anisotropy Decays parameters in DPPC:CHO LUV of A) DPH; B) TMA-DPH; C) TMAP-DPH

Results

In this lamellar system our results show that as temperature increase, the fluorescence lifetimes and rotational correlation time of the DPH derivatives probes decrease. In this way, the fluorescence and rotational dynamics has a proportional behavior with the temperature (Figs. 1 A to C).

Analysis of the data at one temperature through the different curves indicate that the increase of cholesterol in the bilayer, elicit significant changes in water penetration into the lamella. Dynamic quenching of probe fluorescence by probe-water interactions has been proposed to reduce fluorescence lifetimes.

The migration of gauche-trans-gauche conformation along phospholipid acyl chains and dynamic phospholipids head group fluctuations may permit the transport of water into the bilayer. Indeed cholesterol has long been recognized to decrease water penetration in lipids lamellas. In DPPC vesicles, at temperatures corresponding to the liquid crystalline state (50°C), lifetime trends of all probes (Figs. 1 A to C, lifetime) indicate that cholesterol incorporation elicits an overall hydration decrease at both lamellar depths.

However, we have found that in the gel state (25°C), cholesterol incorporation induces an overall hydration increase as sensed by the two deeper probes.

From anisotropy decay analysis we recovered: θ_1 is the fast rotational correlation time of the anisotropy decay (inversely related with the fluorophore rotational rate R_1), and r_∞ the residual anisotropy at infinite time, a dynamics and structural property respectively (Figs. 1 A to C, θ_1 and $r_=$).
Considering that the probe rotational rate reflects

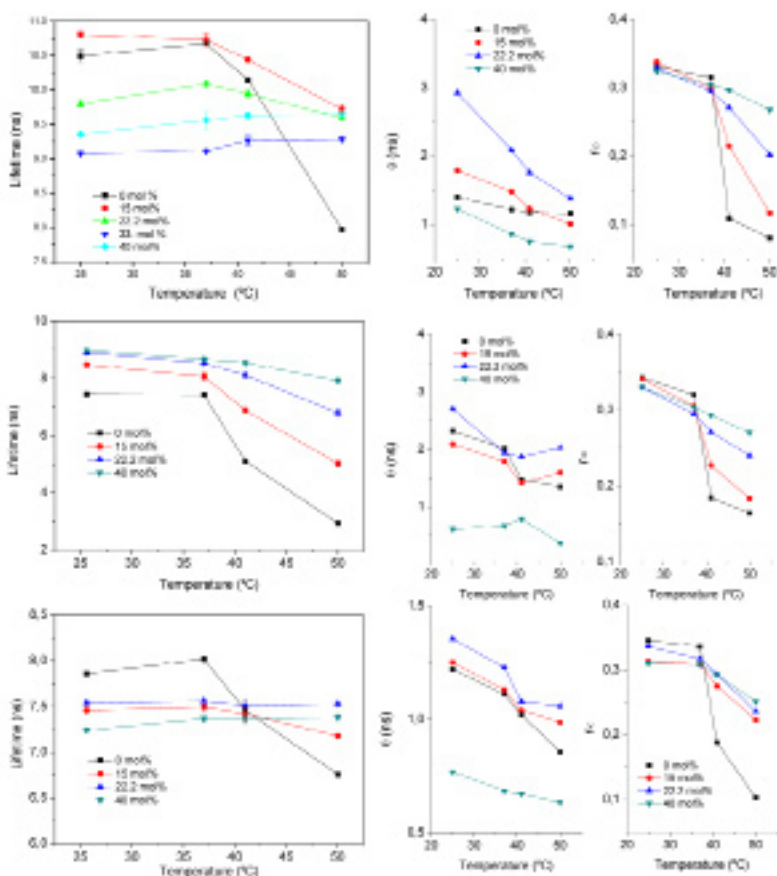the orientational dynamics of the surrounding phospholipids molecules, we suggest that cholesterol incorporation elicits non monotonic changes in the orientational dynamics of the phospholipids, with an initial decrease in the rotational rate at lower cholesterol content and a subsequent increase at 40 mol %. These variations are larger in the gel state (25ºC) and at the acyl chains deep region as sensed by DPH.

The changes in the residual anisotropy at infinite time r_∞ related to the lipid packing order, indicate a monotonous order increase with cholesterol incorporation in the liquid crystalline state (50ºC), similar at all the bilayer depths. However, at the gel state (25ºC), the results indicate slight monotonous decreases at the two shallower depths and practically no effect at the deep hydrophobic region.