Available online at www.sciencedirect.com
SCIENCE DIRECT®
Spectrochimica Acta Part A 61 (2005) 1153–1161
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

# Phase transition affects energy transfer efficiency in phospholipid vesicles

K.A. Kozyra[a,*], J.R. Heldt[a], M. Engelke[b], H.A. Diehl[b]

[a] *Institute of Experimental Physics, University of Gdansk, ul.Wita Stwosza 57, Gdansk, Poland*
[b] *Institute Biophysics, FB1, University of Bremen, PB 30440, D-28334 Bremen, Germany*

Received 14 April 2004; accepted 22 June 2004

This paper is dedicated to Professor Józef Heldt on the occasion of his 70th birthday

**Abstract**

The fluorescence quenching of 6-propionyl-2-dimethylaminonaphtalene (PRODAN) and 6-dodecanoyl-2-dimethylaminonaphtalene (LAURDAN) by octadecyl rhodamine B (ORB) in a model system of small unilamellar vesicles (SUV) of dipalmitoylphosphatidyl-choline (DPPC) was investigated. Non-linear Stern–Volmer behaviour was observed in both systems in the gel phase (25 °C) and in the fluid phase (50 °C), resulting from association processes and from static quenching. The relative quenching efficiencies of both dyes depend on the phase state of the bilayer and indicate a deeper incorporation of PRODAN and LAURDAN into the membrane in its fluid phase than in its gel phase.


*Keywords:* PRODAN; LAURDAN; Octadecyl rhodamine B; Fluorescence quenching; Resonance energy transfer; Phospholipid vesicles

## 1. Introduction

Fluorescence quenching has become a powerful technique to obtain topographical information about proteins, nucleic acids, and biological membrane systems [1–3]. Biological membranes are complex assemblies of lipids and proteins that allow cellular compartmentalization and act as the interface through which cells communicate with each other and with the external milieu [4].

Biological membranes are often simulated by model systems like phospholipid vesicles, which are formed from unilamellar or multilamellar bilayers dispersed in an aqueous medium. Under certain variations of temperature or pressure the liposomal membrane undergoes structural changes or phase transition. Bilayers of all lipids show a first-order transition (associated with melting of the alkyl chains) from the so-called $L_\beta$ gel phase to the fluid $L_\alpha$ phase, which profoundly affects the bilayer water content [5–8,16]. Membrane interactions and conformational changes are detectable by fluorescence quenching and various other fluorescent techniques [9–14].

Quenching of fluorescence requires a close approach of fluorophore and quencher. Quenching usually is interpreted according to theories assuming either dynamic or static quenching, or a combination of both [1]. These have been applied successfully to a wide variety of quenching studies in isotropic and low-viscosity solutions.

The Stern–Volmer theory according to which the ratio of the quantum yield of a fluorophore in absence and presence of a quencher, $\Phi_0/\Phi$ (or $I_0/I$), depends linearly on the quencher concentration, may not be applicable to fluorescence quenching in membranes because of its anisotropic character. Lipid components are constrained to two dimensions and they diffuse at much lower rates than in non-viscous solvents. A proper treatment of quenching processes in such systems requires additional considerations.

*Abbreviations:* DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; SUV, small unilamellar vesicle; MLV, multilamellar vesicle; PRODAN, 6-propionyl-2-dimethylaminonaphtalene; LAURDAN, 6-dodecanoyl-2-dimethylaminonaphtalene; ORB, octadecyl rhodamine B; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; LE, locally excited; TICT, twisted intramolecular charge transfer

* Corresponding author. Tel.: +48 58 5529542; fax: +48 58 3413175.
*E-mail address:* dokkat@univ.gda.pl (K.A. Kozyra).

1386-1425/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2004.06.034

The quenching process is determined by the partition of fluorophores between aqueous and lipid compartments. Two types of distributions have been identified, i.e., the equilibrated partitioning and the binding to a target [15]. These types of associations represent different thermodynamic processes. The various sizes, charges, and the hydrophobic character of fluorophores effect the quenching process in membranes.

In this paper, we report quenching of LAURDAN and PRODAN fluorescence by octadecyl rhodamine B (ORB) in SUV of DPPC in gel or liquid crystalline state. Because of the different lengths of PRODAN and LAURDAN alkyl residues the two probes locate differently in the bilayer depth, and their affinities for the different membrane phase domains are profoundly different [8]. Therefore, modification of the membrane structure due to phase transitions will cause the relocation of the fluorescent probes in the bilayer and will result in an efficient fluorescence quenching. It is known that the used fluorophores exhibit a dual fluorescence, i.e., emitting from the locally excited state, $S_1$(LE), and the charge transfer state, $S_1$(CT) [17–21]. In solution, the intensity ratio of the two bands depends on the microenvironment, e.g. the solvent polarity and its ability to form an intermolecular complex with the solvent molecules, and in certain cases also on the excitation wavelength [20–23]. On the other hand, a study of the photophysics of LAURDAN in organic solvents and amphiphile aggregates (mixed liposomes, mixed micelles) has shown that de-excitation arises from three different excited states governed by charge transfer and solvent relaxation processes [24,25]. During the lipid phase transition of DPPC, the freedom of the dipolar molecules increase, allowing the dielectric relaxation to occur.

In the following, we have performed intermolecular electronic energy transfer studies in DPPC liposomes, and one of the aims of those studies was to gain information about the influence of phase transition on the energy transfer mechanism, i.e., on the parameters describing this phenomenon.

## 2. Materials and methods

### 2.1. Chemicals

PRODAN, LAURDAN and ORB were purchased from Molecular Probes, Eugene, OR. High purity DPPC was purchased from Lipoid KG (Ludwigshafen, Germany) and used in experiments without further purification. Ethanol, DMF, DMSO was of spectroscopic grade and provided by Merck (Darmstadt, Germany).

### 2.2. Preparation of liposomes

DPPC dissolved in ethanol was evaporated to deposit a thin lipid film on the wall of a glass tube. The final traces of residual solvent were removed after overnight storage under vacuum (Vacutherm, Heraeus Instr., Hannover, Germany) at 42 °C. The lipid film was resuspended in an appropriate amount of Tris buffer (0.1 M, pH 7.4), and vigorously vortexed at a temperature above the phase transition, giving a lipid concentration of approximately 2 mg/ml. For the preparation of small unilamellar vesicles (SUV), the resulting multilamellar vesicle (MLV) dispersion was sonicated with a Bandelin sonoplus HD70 (Bandelin Electronics, Berlin, Germany) for 15 min at maximal power (cycle 30%) under nitrogen and transferred to a thermostatted membrane extruder system (Lipex Biomembranes Inc., Vancouver, Canada), which allowed the extrusion of unilamellar vesicles with final diameters of 25 nm. The final lipid concentration of the SUV suspension was determined for each preparation [26]. The liposomal suspension was stored under nitrogen in darkness at 4 °C to avoid lipid peroxidation. All liposomal preparations were used within 2 weeks.

### 2.3. Fluorescence measurements

Fluorescence quenching measurements of LAURDAN and PRODAN by ORB in SUV prepared from DPPC were carried out in a computer-controlled Perkin-Elmer LS-50 spectrofluorimeter equipped with a thermostatted cuvette (Julabo Labortechnik, Seelbach, Germany). PRODAN and LAURDAN were added from a 1 mM stock solution in DMF, and ORB was added from a 5 mM stock solution in DMSO. Before the measurements all samples were incubated for 1 h. The excitation wavelength was 380 nm, a 5 nm slit width was set for both excitation and emission.

The fluorescence lifetime measurements were carried out using a nanosecond single photon counting apparatus. The excitation wavelength used was 403 nm. The measurements were performed at 25 and 50 °C. In order to eliminate the influence of anisotropy on the decay curves, a polarizer set at the magic angle was inserted between the sample and the monochromator. The fluorescence lifetimes were obtained from the fit of the convolution of the excitation pulse and the double or triple exponential function to experimental decay curves using a non-linear least-squares fitting program. The average fluorescence lifetimes were obtained as $\langle\tau\rangle = \sum_i \alpha_i \tau_i^2 / \sum_i \alpha_i \tau_i$, where $\alpha_i$ are the pre-exponential factors and $\tau_i$ the $i$th lifetime components. The quality of the fit was assessed over the entire decay and tested by a plot of the weighted residuals and the reduced $\chi^2$ value.

The quantum yield, $\Phi_F$, was determined using a standard solution of β-carboline in 1 N sulphuric acid as a reference compound [1]. The fluorescence quantum yield was calculated according to the relationship [31]:

$$\Phi_F = \Phi_F^S \frac{\int_0^\infty I_F(\tilde{\nu})d\tilde{\nu}}{\int_0^\infty I_F^S(\tilde{\nu})d\tilde{\nu}} \frac{1-10^{-A_S}}{1-10^{-A}} \frac{n^2}{n_S^2} \quad (1)$$

where $\Phi_F^S = 0.6$ [1] is the quantum yield of the standard solution, the integrals $\int_0^\infty I_F(\tilde{\nu})d\tilde{\nu}$ and $\int_0^\infty I_F^S(\tilde{\nu})d\tilde{\nu}$ are the areas under the emission curves of the investigated and standard compound, respectively, $A$ and $A_S$ are the respective