Application of red-edge effect on the mobility of membrane lipid polar head groups

Antonio Raudino, Franco Guerrera[+], Antonio Asero° and Victor Rizza*

*Departments of Chemistry, [+]Pharmaceutical Chemistry, °Pharmacology and *Biochemistry, University of Catania, Medical School, 95100 Catania, Italy*

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The dynamic properties of membrane lipid polar head groups have been studied using the spectroscopic phenomenon known as the red-edge effect (REE). REE was measured in liposomes prepared with different composition of phospholipids and doped with a merocyanine dye. There was a gradual loss of REE with phosphatidylcholine (PC) liposomes as the temperature increased from 4–25°C. Addition of 10^{-4} M Ca^{2+} increased the REE at all temperatures measured. Liposomes prepared with phosphatidyl serine (PS) or GM_1 gangliosides showed a slight REE only at low temperatures (0–4°C). Cholesterol, Na^+, K^+ or variations of pH had no influence on the REE. The data obtained indicate that increased molecular freedom or fluidity of lipid polar head groups can be correlated to a decrease in REE.

Red-edge effect *Liposome* *Merocyanine dye* *Phospholipid* *GM_1 gangliosides* *Fluidity*

1. INTRODUCTION

Spectroscopic methods supported by mathematical models have been employed for measuring the viscosity of cellular membranes. Mobility of molecules in lipid membranes has been semiquantitatively evaluated using fluorescence depolarization techniques either statically or dynamically [1,2].

In [3], fluorescence spectra of merocyanine dye of the stibazolium betaine type exhibited a red shift when excited at the long wavelength edge of the first absorption band. This phenomenon, termed the red-edge effect (REE), has been observed primarily in cooled solvents [4,5] or very viscous media where microheterogeneities in the solvation shells are most likely due to the unrelaxed distribution of solvent molecules around the polar solute.

We have evaluated the mobility of lipid polar head groups by measuring the REE in liposomes doped with hexadecyl merocyanine (HDM). A hydrophobic chain was attached to the polar merocyanine dye (fig.1) to aid its association with the lipid matrix of the liposome.

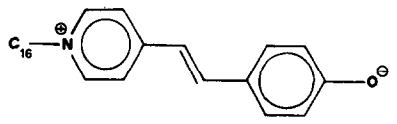

Fig.1. Molecular structure of hexadecyl merocyanine dye (HDM).

2. MATERIALS AND METHODS

2.1. *Reagents*

1-Iodohexadecane (Merck), γ-picoline (Fluka) and 4-hydroxybenzaldehyde (Aldrich) were analytical grade reagents and used as purchased.

Phosphatidylcholine (PC), phosphatidylserine (PS), cholesterol and GM_1 gangliosides were kindly provided by Fidia Res. Labs. (Abano Terme).

* To whom correspondence should be addressed

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2.2. *Synthesis of 1-hexadecyl-4-(4-hydroxystyryl) pyridinium betaine (hexadecyl merocyanine) (HDM)*

The procedure in [6] was used for preparing HDM. The condensation of γ-picoline hexadecaiodide (0.02 M) with 4-hydroxybenzaldehyde (0.04 M) was carried out in 40 ml methanol containing 1 ml piperidine. The solution was refluxed for 4 h on a steam bath. The iodide derivative was collected, washed and recrystallized from methanol twice. The final yield was 85% and the product had a melting point of 195 ± 2°C as measured on a Buchi 510. The betaine was prepared by treating the hexadecylstilbazole iodide with 2 N NaOH. The melting point of HDM showed the first phase transition at 140 ± 1°C. Elemental analysis performed on a Carlo Erba 1100 were satisfactory as compared to the theoretical values (C ± 0.30%; H ± 0.20%; N ± 0.20%).

2.3. *Preparation of liposomes*

Small closed spherical lipid bilayers (liposomes) of about 500 Å diam. were prepared as in [7]. The liposomes were doped with a small quantity of the fluorescent probe (HDM); the molar ratio of probe to total lipid was made about 1:500 and the mixture prepared in 0.25 M Tris–HCl buffer (pH 7.8). All spectroscopic measurements were carried out on a Perkin Elmer 605 spectrophotofluorimeter. The temperature of the sample was controlled with a thermoregulated circulating water bath.

3. RESULTS AND DISCUSSION

Preliminary measurements of the fluorescent dye HDM (10^{-5} M) dissolved in glycerol showed no significant REE over 4–25°C. The emission maximum consistently occurred at 507 nm and was independent on the frequency of the excitation wavelength. In contrast, when the dye was incorporated in PC liposomes a very marked REE was observed at 4°C and the emission wavelength shifted toward the red end of the spectrum with increasing excitation wavelength. This effect was gradually lost as the temperature of the sample was increased to 25°C (fig.2).

Addition of 10^{-4} M Ca^{2+} to PC liposomes showed a significant increase of the REE as compared to liposomes incubated in absence of Ca^{2+} (fig.3). Liposomes prepared with equimolar mixtures of PC and PS or with low concentration (10^{-4} M) of GM_1 gangliosides showed a slight REE only at the low temperatures (0–4°C) (fig.4).

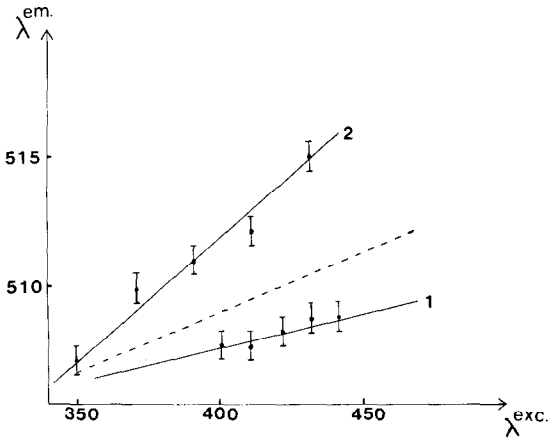

Fig.2. Excitation *vs* emission wavelength at two different temperatures for hexadecyl merocyanine dye adsorbed on PC liposomes: (1) measurements at 25°C; (2) REE at 4°C; (···) results obtained in glycerol at 4°C.

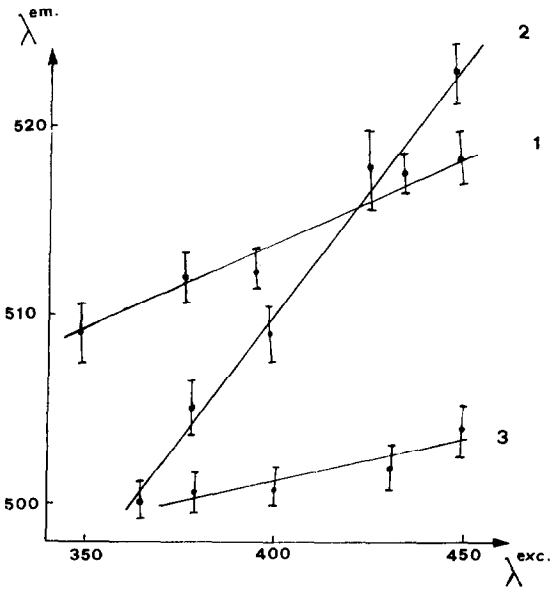

Fig.3. Excitation *vs* emission wavelength for hexadecyl merocyanine dye adsorbed on PC liposomes: PC at 4°C (1); PC plus 10^{-4} M Ca^{2+} at 4°C (2); PC plus 10^{-4} M Ca^{2+} at 25°C (3).

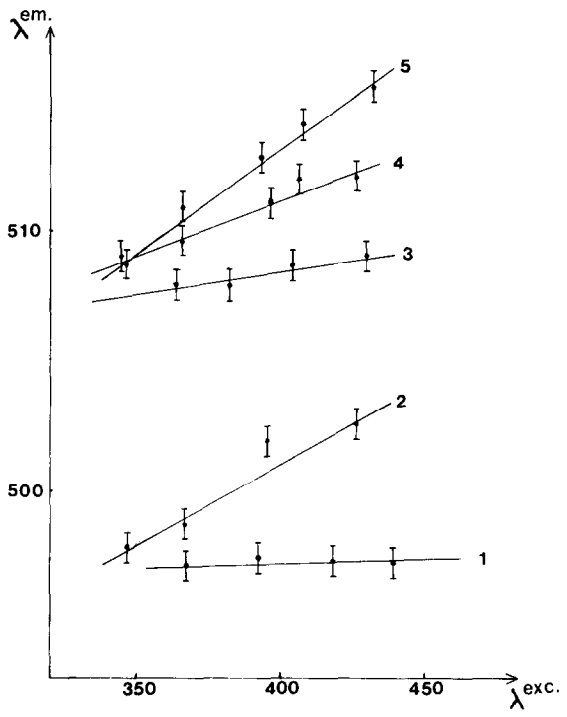

Fig.4. Excitation *vs* emission wavelength. Lower curves represent the dye adsorbed on equimolar PS + PC liposomes at 25°C (1); 4°C (2). Upper curves illustrate the effects of GM_1 gangliosides on REE; PC and PC plus GM_1 gangliosides at 25°C (3); PC plus GM_1 gangliosides at 4°C (4); PC at 4°C (5).

Addition of cholesterol, Na^+, K^+ or variations of pH had no influence on the REE at all temperatures measured. However, they did cause variations in the fluorescence intensity of the merocyanine dye.

These observations can be rationalized on the basis of the Franck–Condon and equilibrium excited state distributions using Azumi's approximation for REE [8]:

$$\nu^{max}(\nu_{exc},T) = \frac{\tau_R(T)/\tau_F(T)}{1+\tau_R(T)/\tau_F(\tau)}\,\nu_{Fc}^{max}(\nu_{exc}) + \frac{1}{1+\tau_R(T)/\tau_F(T)}\,\nu_{EQ}^{max}$$

where:

$\nu^{max}(\nu_{exc},T)$ = the energy of the observed fluorescence maximum as a function of the excitatory energy (ν_{exc}) and temperature (T);

$\tau_R(T)$ and $\tau_F(T)$ = the relaxation lifetime of solvent and fluorescence as a function of temperature, respectively;

ν_{FC}^{max} and ν_{EQ}^{max} = the steady state fluorescence spectra originating from the Franck–Condon (FC) and equilibrium (EQ) excited states.

According to Debye–Stokes theory of dielectric relaxation [9], in the usual solvents, $\tau_R(T)/\tau_F(T) \ll 1$ at room temperature. Under these conditions, eq.(1) predicts that the first term on the right-hand side is very small and the fluorescence spectra originating from the relaxed excited states show a negligible dependence on the excitation wavelength. However, when the solvent is cooled, the ratio $\tau_R(T)/\tau_F(T)$ is an appreciable quantity because of the increase in the $\tau_R(T)$ value. A decrease in temperature thus leads to a significant contribution originating from the Franck–Condon excited states and consequently a dependence of the fluorescence spectra on the excitation wavelength. Considering the surface of the liposome as a two-dimensional polar fluid on which the ionic groups of the phospholipids are free to rotate around the negatively charged phosphate, it can be expected that the rotation of the ionic heads increases with increasing temperature. This condition would lead to a decrease in the relaxation time and hence to a decrease in REE.

The decrease in REE observed with PS and GM_1 gangliosides suggests the appearance of more fluid domains on the surface of the liposomes, presumably due to the hydrophilic character of the anionic PS or the sugar moieties on the GM_1 gangliosides. These compounds would favor a decrease in the electrostatic interactions between the phosphate and polar groups resulting in a greater rotational freedom. Conversely, the addition of Ca^{2+} to PC liposomes suggests an increase in the electrostatic interactions of the polar head groups which lead to a restriction in the mobility resulting in an increased REE.

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