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Fluorescence quenching of fluorescein by Merocyanine 540 in liposomes

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ABSTRACT

The fluorescence quenching of fluorescein (FL) by merociyanine 540 (MC540) was examined in L-egg lecithin phosphatidycholine (PC) liposomes using spectroscopic methods. The type of quenching mechanism (dynamic or static) was evaluated using the Stern–Volmer plots. Findings were also supported by the temperature studies and florescence decay measurements. The Stern–Volmer equation was utilized to calculate bimolecular quenching constants (K_q). Furthermore, the bimolecular quenching constant of the quencher in the liposomes (K_{SV}), partition coefficient (K_p), binding constant (K), and corresponding thermodynamic parameters ΔH, ΔS, and ΔG were calculated. The quenching property was also used in determining quantitatively (K_p) the partition coefficient of Merociyanini 540 in PC liposome.The obtained data indicated that static quenching occurred in the system and the K_{SV} values decreased with increasing lipid concentration. In addition, thermodynamic analysis suggested that van der Waals interactions and hydrogen bonding were the main acting forces between fluorescein and merociyanine 540 molecules in the medium.

1. Introduction

Fluorescence quenching is widely applied within the studies in association with biological and medical phenomena [1]. Fluorescence quenching techniques provide information about the internal structure of membranes or lipid characteristics of the membrane, quencher molecules permeability between the surface molecules (lipid–water), and drug distribution in the lipid–water phase. Moreover, the extent of fluorescence quenching of the molecules in the bilayer yields information about the concentration and mobility of the quencher in the membrane [2–5]. The quencher molecules in the membrane lead to a decrease in the fluorescence intensity of the probe, which depends on the average number of quencher molecules [6]. Besides, the spectral characteristic of fluorescent probes depends on the variety of the depths that probe molecules are located at and the surrounding environment [7]. FL and MC540 molecules are dye compounds that are exploited in different fields [8–10]. For example, MC540 is used in the photodynamic treatment of various human cancer cells as photosensitizer [11–13] whereas FL is used for labeling membrane and proteins [14]. Such compounds are especially suited for studies with natural and model membranes, for instance liposomes or micelles [2]. The interactions between these dye molecules in liposomes have not been reported so far. But, the interactions between these dye compounds were characterized in micelle systems [15]. In this study, the nature of interactions between FL and MC540 dye molecules in egg PC liposomes will be determined. Therefore, we examined the fluorescence quenching of FL by MC540 in liposomes and discussed the type of quenching mechanism. The Stern–Volmer constant was used to determine quantitatively the partition coefficient of MC540 in liposomes.

2. Experimental procedures

2.1. Chemicals and solutions

Fluorescein, NaOH, L-egg lecithin phosphatidylcholine (purity 99%), and phosphate buffer saline were purchased from Sigma. Merocyanine 540 was obtained from Fluka. Chloroform was purchased from Merck. Stock solutions of the dye compounds (1.0×10^{-3} M) were prepared in ethanol. Regarding the measurements, fluorescein concentration was kept constant at 1.0×10^{-6} M for all the experiments.

2.2. Liposome preparation

The required amount of the lipid (PC) (3–55 μL/ml) was dissolved in chloroform and the solvent was evaporated under vacuum in a rotary evaporator for at least 30 min. The lipid film was then left under stream nitrogen to remove all trace amount of

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chloroform. The remnant of dried lipid film was dispersed into phosphate buffer saline (pH 7.4) and then mixed by vortex to form liposomes for 1 h at 25 °C and the resulted liposome containing solution was used directly as a solvent in the studies. After this point, the required amount of FL (1 μM) and MC540 (3–40 μM) were dissolved in the liposome solution and allowed to equilibrate for 2 h before conducting the experiments.

2.3. Spectrofluorometric measurements

The UV–visible absorption spectra were measured with a Shimadzu UV-3101PC UV–vis–NIR spectrophotometer in a 1 cm quartz cuvette. Steady-state fluorescence spectra were recorded with Shimadzu RF-5301PC. LaserStrobe Model TM-3 lifetime fluorometer from Photon Technology International was used for fluorescence decay measurements to determine fluorescence lifetime (τ_f) values. Fluorescence quantum yields (ϕ_s) were determined by comparison with a reference solution. The following equation was used to calculate the fluorescence quantum yields for this purpose, [16]:

$$\phi_s = \phi_r \left(\frac{D_s}{D_r}\right)\left(\frac{n_s}{n_r}\right)^2 \left(\frac{1-10^{OD_r}}{1-10^{OD_s}}\right) \quad (1)$$

where D_s and D_r are the integrated area under the corrected fluorescence spectra for the sample and reference, respectively, n_s and n_r are the refractive indices of the sample and reference, respectively. OD_s and OD_r are the optical densities for the sample and reference at the excitation wavelength, respectively. The reference used for this study is fluorescein of 1.0×10^{-6} M in 0.01 N NaOH. This reference has a known fluorescence quantum yield of 0.92 in this condition [17].

3. Results and discussion

3.1. Absorption and fluorescence spectra of FL

The molecular absorption and fluorescence spectra of FL in aqueous and in the increasing amounts of PC liposomes are shown in Fig. 1. At constant FL concentration (1.0 μM), addition of PC liposomes caused an increase in the fluorescence intensity of FL at 490 nm. This increase in fluorescence intensity of FL was seen in Fig. 1b. As the solubility of FL is less in aqueous solution, the fluorescence intensity is also low in the aqueous solution as seen in Fig. 1a [18]. The spectral data, quantum yield, and lifetime (τ_f) values are listed in Table 1 for FL in PC liposome.

3.2. Fluorescence quenching

The fluorescence spectra of FL in PC liposomes with varying concentration of MC540 are shown in Fig. 2. From Fig. 2, it was found that the fluorescence intensity of FL decreased gradually with the increase of MC540 concentration, indicating that interactions between MC540 and FL occurred in the system. However, the maximum of the FL emission wavelength exhibited a blue shift, indicating that the FL molecules were located in a more hydrophobic environment upon the addition of MC540. The fluorescence quenching is generally described by the Stern–Volmer equation:

$$\frac{I_o}{I} = 1 + K_{SV}[Q] \quad (2)$$

where I_0 and I are the fluorescence intensities of the probe in the absence and presence of the quencher, respectively. [Q] is the concentration of the quencher, and K_{SV} is the Stern–Volmer constant. Fluorescence quenching generally is investigated in two categories: Dynamic and Static quenching. In both there is an interaction between the fluorophore and the quencher. The S–V plot shows a linear, which indicates the dynamic quenching process mechanism in which fluorescence quenching occurs as a result of collision between fluorophores and the quencher. In some cases, the experimental results exhibit positive deviation from the linear, which indicates the static quenching, which is due to the ground-state complex formation between fluorophore and a quencher [1]. In addition, dynamic and static quenching can be differentiated regarding their different dependence on temperature. Dynamic quenching depends on the diffusion of

Table 1
Photophysical and spectral properties of FL in aqueous [18] and PC liposome.

Solutions	λabs (nm)	λ_{fl} (nm)	τ_f (ns)	Φ_s
Water	479	509	3.840	0.400
8 μM liposome	490	509	3.057	0.759
20 μM liposome	490	509	3.093	0.799
80 μM liposome	490	509	3.068	0.716
150 μM liposome	490	509	3.082	0.798

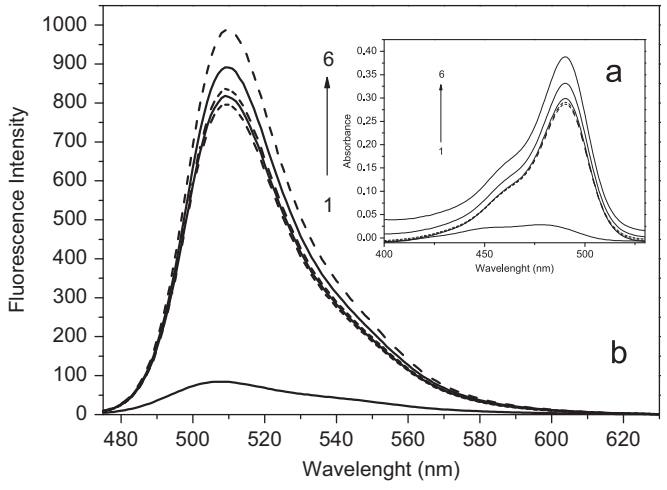

Fig. 1. (a) Absorption and (b) fluorescence spectra of FL in PC liposome. Total concentrations of PC liposome (1) 0 μM; (2) 2.5 μM; (3) 8 μM; (4) 20 μM; (5) 80 μM; and (6) 150 μM.

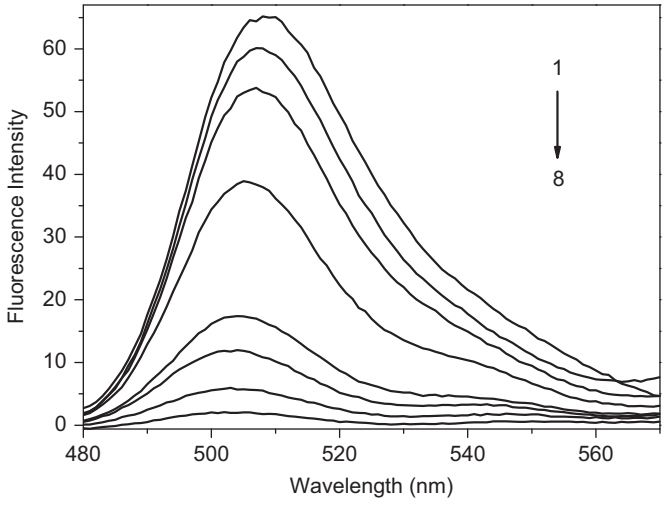

Fig. 2. Fluorescence spectra of FL in the presence of MC540. Total concentrations of MC540: (1) 0 μM; (2) 3 μM; (3) 6 μM; (4) 12 μM (5) 20 μM; (6) 25 μM; (7) 35 μM; and (8) 40 μM. The concentration of FL is 1 μM.