Available online at www.sciencedirect.com
ScienceDirect
Chemical Physics Letters 433 (2006) 170–174
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

Fluorescence resonance energy transfer between DPH and Nile Red in a lipid bilayer

B. Jain, K. Das *

Laser Bio-medical Applications and Instrumentation Division, Raja Ramanna Center For Advanced Technology, Indore 452 013, M.P., India

Received 14 September 2006; in final form 8 November 2006
Available online 17 November 2006

Abstract

FRET between the well-known membrane binding probes DPH (donor) and Nile Red (acceptor) is investigated in a phosphatidylserine lipid bilayer. A ~80% FRET efficiency was observed from the steady state experiments for a DPH:Nile Red:lipid molar ratio of 1:2:190, suggesting an efficient FRET process. Time resolved experiments however, provided a lower (~50%) FRET efficiency. The emission of Nile Red was observed to rise with a time constant of 0.8–0.4 ns only when DPH is selectively excited. This time constant is ascribed due to FRET from which an average donor–acceptor distance of 27 Å is obtained for a DPH:Nile RED:lipid molar ratio of 1:1:190.

1. Introduction

Fluorescence resonance energy transfer (FRET) is a process by which excitation energy is transferred from an excited donor to an acceptor (which happens due to long-range dipole–dipole interactions between the donor and acceptor) and takes place without appearance of a photon [1]. The rate of energy transfer k_T from a donor to an acceptor separated by a distance r is given by

$$k_T = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \quad (1)$$

where τ_D is the excited state lifetime of the donor in the absence of the acceptor. R_0 is the distance (in Å) between the donor and acceptor at which the transfer efficiency is 50% and given by

$$R_0 = 0.211[\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6} \quad (2)$$

where Q_D is the quantum yield of the donor in the absence of the acceptor; n is the refractive index of the medium; κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor and $J(\lambda)$ is called the overlap integral which is given (in nanometers) by

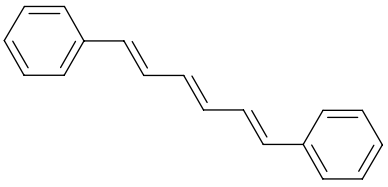
$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (3)$$

Here $F_D(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$, with the total intensity (area under the curve) normalized to unity; $\varepsilon_a(\lambda)$ is the extinction coefficient of the acceptor at λ (in units of $M^{-1}\,cm^{-1}$). Equation (1) tells that the rate of energy transfer depends critically upon 'r', the separation between the donor and acceptor molecule. This property has been utilized to measure the distances of the order of ~100 Å in biological systems utilizing different 'FRET pairs' whose R_0 values range from 20 to 100 Å [1]. FRET has been extensively used to investigate biological macromolecules like proteins and nucleic acids [2–6], in biological membranes to study bilayer organization [7–11], and in systems like micelles, reverse micelles and polymers [12–14]. In biological membranes FRET has been used as a noninvasive approach to study lipid 'rafts': small nanometer sized microdomains in a bilayer responsible for several important biological processes [7,8].

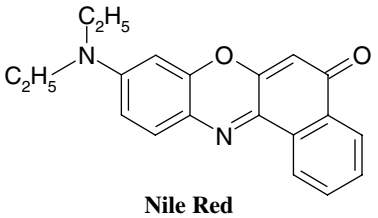
* Corresponding author. Fax: +91 731 2488430.
E-mail addresses: kaustuv@cat.ernet.in, das_kaustuv@yahoo.co.in (K. Das).

0009-2614/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2006.11.050

When using FRET to probe membrane organization, it is desirable to have both the donor and acceptor to be completely partitioned to the bilayer phase so that fluorescence contributions coming from the aqueous phase are eliminated. Diphenylhexatriene (DPH) and Nile Red (Scheme 1) are well-known hydrophobic membrane probes, and their photophysics in lipid bilayers have been well studied [1,15,16]. In a lipid bilayer, the emission of DPH overlaps substantially with the absorption of Nile Red (see, for example Fig.1) and hence there is a possibility of FRET between these two. However, to the best of our knowledge there has been no report so far, of FRET, between these two in a lipid bilayer. Here we present our preliminary investigations about FRET between DPH (donor) and Nile Red (acceptor) in a phosphatidyl serine (PS) lipid bilayer.



DPH



Nile Red

Scheme 1. Chemical structures of the probes, DPH and Nile Red.

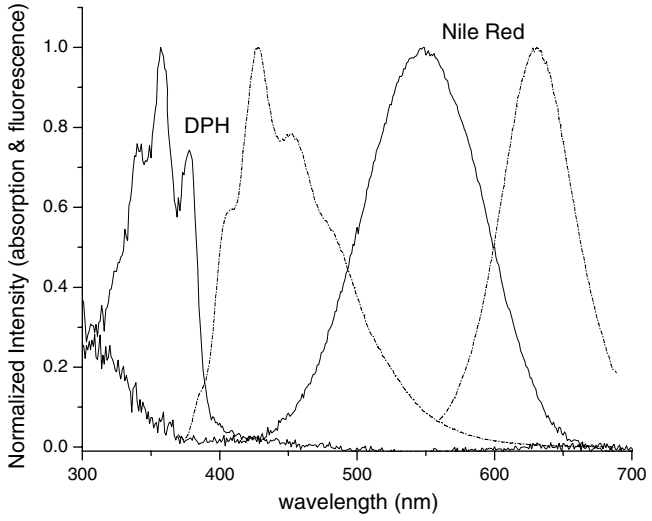


Fig. 1. Peak normalized absorption (solid line) and emission (dashed line) of DPH and Nile Red in PS liposomes with a dye:lipid molar ratio of 1:190 ($\lambda_{ex} = 350$ nm for DPH and 520 nm for Nile Red).

2. Experimental

DPH and phosphatidyl-L-serine (PS) from bovine brain were obtained from Sigma and used as received. Nile Red (Sigma) was purified by TLC as described earlier [16]. The lifetime of Nile Red in methanol was found to be single exponential (2.88 ns, excited at 450 nm) over the entire emission spectrum, assuring its purity. PS liposomes in pH 7.4 phosphate buffer were made by the process described in reference 17 yielding a final lipid concentration of 285 mM. Concentrated stock solutions of DPH and Nile Red were added in microliter quantities to the liposomal solution and allowed to equilibrate for 2 h before conducting experiments. Steady state absorption and fluorescence spectra were recorded with resolutions of 1 and 4 nm respectively. The excitation source for time resolved measurements were the second harmonic light of the pulse picked output of a tunable (720–980 nm) Ti-Sapphire femtosecond laser (Coherent Mira) having a repetition rate of 3.8 MHz. The time resolved fluorescence experiments were done in a time correlated single photon counting system (TCSPC) obtained from Edinburgh Instruments (LifeSpec Red) having an instrument response function of ~180 ps. The fluorescence decays were analyzed by the software provided by the supplier.

3. Results

Fig. 1 shows the normalized absorption and fluorescence spectra of the donor, DPH and the acceptor Nile Red in the PS liposomal solution. The absorption and emission spectra of DPH shows a small overlap, and that for Nile Red shows a substantial overlap in the liposomal solution indicating the possibility of intramolecular FRET among the dye molecules. From Fig. 1 it can be seen that the acceptor Nile Red has least absorption around 375 nm where the donor DPH absorbs strongly. In order to minimize the contribution of the acceptor fluorescence coming from direct excitation of the acceptor, the FRET measurements (steady state and time resolved) were performed by exciting the donor at 375 nm. For the lifetime and quantum yield measurements of DPH and Nile Red in PS bilayer, we have maintained a dye:lipid mole ratio of 1:190 (dye = 1.5 μM) so as to minimize any self quenching of fluorescence due to intramolecular FRET. DPH fluoresces strongly in the PS lipid bilayer ($\lambda_{ex} = 375$ nm) with a quantum yield of 0.72 and its lifetime can be best fitted by a sum of two exponentials, the major part (97%) of which is a 7.7 ns component and the minor part (3%) being 1.1 ns. We note that the presence of RF noise (see Fig. 2b) in our time resolved data prevents us to get a good fit and χ^2 (1.2 in this case). The acceptor Nile Red has a quantum yield of 0.30 and a single exponential lifetime of 3.5 ns ($\lambda_{ex} = 450$ nm) in the PS lipid bilayer (see also Table 1). In order to calculate R_0 we have assumed the following:

$n = 1.44$ and $\kappa^2 = 2/3$