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Competitive binding of nile red between lipids and β-cyclodextrin

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ABSTRACT

Fluorometric measurements are exploited to explore the binding interactions of nile red (NR) with anionic lipid dimyristoyl-L-α-phosphatidylglycerol (DMPG), zwitterionic lipid dimyristoyl-L-α-phosphatidylcholine (DMPC) as well as neutral cyclic oligosaccharide β-cyclodextrin (β-CD) solutions. The binding constants are found to be quite high and comparable (within a factor of five). Series of spectral techniques like steady state fluorescence and fluorescence anisotropy study, micropolarity study, quenching study and time resolved experiments reveal that the addition of β-CD to the probe–lipid complexes results in weakening of the lipid–probe interaction and formation of probe–β-CD inclusion complexes leading to the removal of some of the probe (NR) molecules from the lipid environments. The extent of removal of NR is, however, more from DMPG than DMPC lipid. The phenomena are explained from the concept of competitive binding of the probe between the lipids and β-CD. Since lipids are the principal constituents of the cell walls, the work might make a foundation for the possible removal of excess of molecules like nile red adsorbed on the cell walls.

1. Introduction

In recent years intense attention has been given to the study of the photophysical and photochemical properties of molecules embedded in restricted geometries. Fluorescent dyes have been exploited as molecular probes to study the interaction of the dye molecules with different bio-mimicking microheterogeneous environments like micelles, cyclodextrins, lipids, DNAs, proteins, etc. The fluorescent nonionic dye from the phenoxazone family, nile red (NR) (Scheme 1) has been extensively used as a staining agent in biological tissues [1]. Its nonlinear optical properties [2], application as a laser dye [3] and as an active material for white light emitting diode (LED) [4] have enhanced the importance of the fluorophore. The photophysical properties of NR have also been the subject of many studies because of the intense solvent-dependence of its fluorescence enabling the probe to be used conveniently to study the polarity related aspects in the restricted microenvironments. The large spectral shifts of it in the absorption and emission bands in solvents of different polarities are due to the changes in the excited state dipole moment of the molecule. This corresponds to nearly complete charge separation between the diethyl amino group which acts as electron donor and the quinoid part which acts as electron acceptor. The charge separation is explained by the formation of the twisted intramolecular charge transfer state (TICT) of the molecule in polar solvents due to the rotation of the flexible diethylamino group attached to the rigid structure of the molecule [5]. Unlike anilinonaphthalene sulfonate [6], it has a very high partition coefficient from water to hydrophobic solvents such as xylene or chloroform [7]. In aqueous medium, NR has only a feeble fluorescence. However, in hydrocarbon solvents, it fluoresces strongly in the yellow gold region while in ethanol or phosphatidylcholine vesicles, the dye fluorescences in the red region [7]. Due to the remarkable sensitivity of the absorption and fluorescence properties of NR to the environment, it has been exploited in studies of local polarity in various microheterogeneous environments including zeolites, reverse micelles, micelles, cyclodextrins, etc. [8–14]. It has also been used to infer the heterogeneity of membranes [15,16] and to study the formation of dendrimer–surfactant supramolecular assemblies [17].

Lipid vesicles are closed systems consisting of one or more lamellae containing the amphiphiles. In vesicles, the hydrophobic part of the amphiphiles orients in a way to form hydrophobic interior of the bilayer and the hydrophilic portion remains in contact with the aqueous phase [18]. Liposomes serve as valuable models for biological membranes and correspond to the environment in which many drugs, proteins and enzymes display their activities. Since the structure of the biological membranes is complicated due to a wide diversity in their composition, one uses synthetic liposomes that mimic the structure and the geometry of the cell membrane [19]. Interaction of drug molecules with lipid membrane is the prime concern in membrane biochemistry. To characterize the interaction behavior, location of the probe molecules within the membrane different spectroscopic techniques have been extensively used, among which fluorescence technique is perhaps the most sensitive one [20].

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Cyclodextrins (CDs) have been recognized as important hosts for the drug molecules in aqueous solutions. The reduced polarity inside the CD cavity and the restricted space, influence the photophysical and photochemical properties of the probe [21,22]. The studies have revealed that depending on both the probe and the CD, formation of inclusion complexes of different stoichiometries like 1:1, 1:2, 2:1, etc. are possible [13,23,24]. In some cases, formation of the probe-anchored nanotubes has also been demonstrated [25,26]. Such preference in the formation of well-defined nanoconjugates in microheterogeneous environments is of much interest to the present day science. Many investigations have employed such properties of CD inclusion complexes to understand the mechanistic details of many photoprocesses like excited state proton transfer (ESPT), intramolecular charge transfer (ICT) and so forth [24,27].

In a recent work [28] using a cationic phenazinium dye, zwitterionic dimyristoyl-L-α-phosphatidylcholine (DMPC), anionic dimyristoyl-L-α-phosphatidylglycerol (DMPG) lipids (Scheme 1) and β-CD, we demonstrated two aspects. Firstly, the dye was found to bind with both the lipid environments (of course, to different extents). Then, considering this as a mimic of drug–membrane binding, our experiments revealed that the lipid-bound probe may be disrupted and hence the probe may be excreted from the lipid membrane simply by the use of health friendly β-CD. The work, thus, projected possibility of development of a simple strategy to expel the adsorbed drugs in the cell membranes. Since this was the first proposition of such a strategy, one needs to establish its applicability using more dye/drug systems. With this objective of establishing the general applicability of the proposed strategy and hence strengthen it, here we have extended the study with another dye, namely nile red, which is non-ionic in nature and has a very different geometry compared to the earlier dye used.

2. Experimental section

Nile red (NR), β-CD, DMPG and DMPC are purchased from Sigma–Aldrich (USA) and they are used as received. Triply distilled water is used for making the experimental solutions. UV spectroscopic grade (Spectrochem, India) 1,4-dioxane is used. AR grade potassium iodide and Tris–HCl buffer are purchased from SRL, India. Concentrated stock solution of NR is prepared in methanol. Small aliquots from this stock solution were added to Tris–HCl buffer solutions to give a final fluorophore concentration of ca. 2×10^{-6} mol dm^{-3}. The concentration of methanol in the final solution was <1%. All the experiments are performed with Tris–HCl buffer solution at pH 7.4.

Steady state fluorescence measurements are performed with a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. Measurements of the steady state fluorescence anisotropy are made using the same spectrofluorometer. Fluorescence anisotropy (r) is defined as:

$$r = (I_{VV} - G \cdot I_{VH})/(I_{VV} + 2G \cdot I_{VH})$$

where I_{VV} and I_{VH} are the emission intensities obtained with the excitation polarizer oriented vertically and emission polarizer oriented vertically and horizontally, respectively. The G factor is defined as [20]:

$$G = I_{HV}/I_{HH}$$

where the intensities I_{HV} and I_{HH} refer to the vertical and horizontal positions of the emission polarizer, with the excitation polarizer being horizontal. All the time resolved measurements are performed on Horiba–Jobin–Yvon FluoroCube fluorescence lifetime system using NanoLED at 490 nm (IBH UK) as the excitation source and TBX photon detection module as the detector. The decays are analyzed using IBH DAS-6 decay analysis software. The instrumental response time for our set up is ~1 ns. Goodness of fits is evaluated from χ^2 criterion and visual inspection of the residuals of the fitted function to the data. All of the experiments are performed at 25 °C temperature with air-equilibrated solutions.

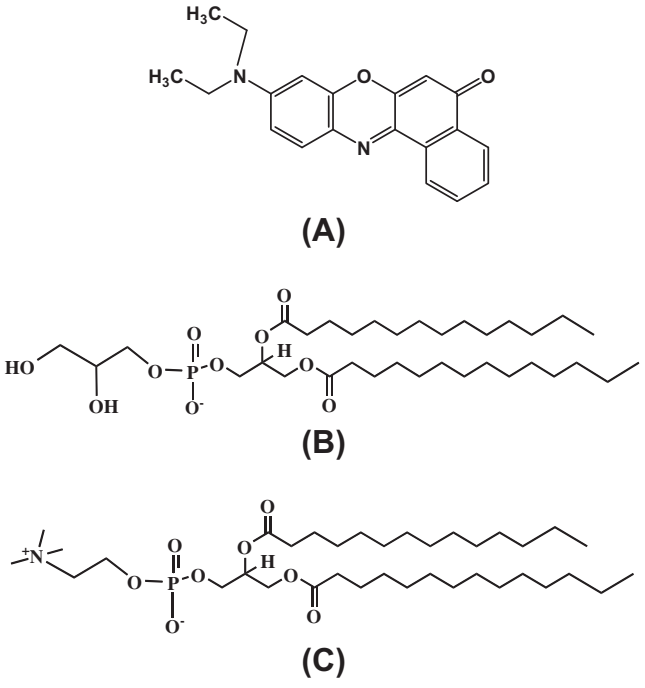
2.1. Preparation of the lipids

The lipid solutions have been prepared following the same procedure as was used and described in our previous work [28]. A defined amount of lipid in 2:1 (v/v) chloroform:methanol solution is dried under a stream of nitrogen and, subsequently, kept overnight under high vacuum. The dry film is hydrated and swelled in Tris–HCl buffer at pH 7.4 containing 20 mM NaCl and vortexed rigorously. The dispersion is then sonicated in ice water using a Vibronics Ultrasonic P1 sonicator until the solution became transparent. Foreign particles, if any, are removed by centrifugation (Spinwin, MC-02) at 6000 rpm for 10 min [29,30]. Like the previous work, both the lipids (DMPG and DMPC) are single walled (SUV).

3. Results and discussion

3.1. Steady state absorption and emission studies: binding of NR with the lipids

The absorption spectrum of the aqueous buffer solution of NR shows a broad absorption maximum at around 580 nm. Addition



Scheme 1. Skeletal structures of (A) nile red, (B) DMPG and (C) DMPC.

Table 1
Absorption maxima of NR in different aqueous environments.

Environment	λ_{abs}^{max} (nm)
Aqueous buffer	580
1.5×10^{-3} mol dm^{-3} DMPG	550
1.5×10^{-3} mol dm^{-3} DMPC	532
12×10^{-3} mol dm^{-3} β-CD	574