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Fluorescence resonance energy transfer mediated by vesicles containing naphthalene moiety

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Abstract

A single chain synthetic amphiphile, 4-(2-hexadecyloxynaphthalene-6-oxy)butyl trimethylammonium bromide (C_{16}Naph(2,6)C_4N^+), can self-assemble into vesicles in the dilute solution. The fluorescence resonance energy transfer has been studied in aqueous vesicle solution of C_{12}Naph(1,5)C_4N^+ and hydrophilic dye fluorescein sodium salt (FS). The donor, naphthalene group, was embedded in the interior of the vesicles. The acceptor, FS with two negatively charged polar groups, was bound electrostatically to the positively charged surface of (C_{16}Naph(2,6)-C_4N^+) vesicles. The FS quenched the fluorescence intensity of the naphthalene, which fitted a Stern–Volmer plot, and the Stern–Volmer quenching-constant K_{sv} is $5.65 \times 10^5\ \mathrm{mol}^{-1}$. The energy transfer efficiency increased with the increasing concentration of FS acceptor in the range of $0–4.4 \times 10^{-6}\ \mathrm{dm}^3\ \mathrm{mol}^{-1}$ up to ca. 71% which could be ascribed to the ordered arrangement of chromophores inside the vesicles.

Keywords: Fluorescence resonance energy transfer; Dyes; Vesicles; Amphiphiles; Self-assembly; Quenched fluorescence

1. Introduction

In natural photosynthetic systems, arrangement of porphyrin derivatives with regulated distances and geometry through noncovalent interaction provides highly efficient photo-induced energy transfer which has been recognized as the primary step in vivo photosynthesis [1,2]. The arrangement of functional chromophores would provide a good model for artificial photosynthetic systems [3]. Also, energy transfer processes have also wide applications in energy conversion processes, dye lasers, solar cells and photochemical synthesis [4]. The rate and efficiency of the non-radiative photo-induced energy transfer from the excited state of a donor (D) to an acceptor (A) depend on several factors such as the extent of overlap of the emission and absorption spectra of the donor and acceptor, relative orientation of the chromophores, and distance between these molecules [5]. Thus, the energy transfer processes will depend not only on the characteristics of the donor–acceptor pair but also on the arrangement of donor and acceptor in their immediate environment. In order to mimic this process operating in nature, several approaches have been exploited in decades such as Langmuir–Blodgett films, micelles and vesicles [6,7]. Artificial amphiphiles can self-assemble into vesicles in the dilute solution and organize functional molecules or ions in their microenvironments [8]. The constrained and organized systems have proved to be useful media for the processes occurring in photosynthesis, such as photoionization [9], electron transfer [10], efficient charge separation [11] and photoexcitation energy transfer [12,20]. These artificial mimetic media can support a higher local concentration and thus improve energy transfer efficiency [13].

From the viewpoint of molecular architecture, a specific chromophore introduced into the single chain amphiphiles not only increases intermolecular interaction in the aggregates [14], but also provides energy donor, which can be excited to perform energy transfer to appropriate closing acceptor in the vesicles [15]. Vesicles as simplified models of cell membranes have been used as model systems for in vitro investigations

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[6]. Therefore, studies of the physical and chemical processes in highly organized vesicles are the basis of comprehending complicated mimetic membranes. We are interested in the photochemical process of synthetic bilayer membranes containing chromophores and have observed efficient energy transfer in the vesicles [16].

In this work, we synthesized an amphiphile, 4-(2-hexadecyloxynaphthalene-6-oxy)butyl trimethylammonium bromide ($C_{16}Naph(2,6)C_4N^+$), which can form vesicles in dilute solution. The rigid naphthalene group embedded in the vesicles as energy donor, and the hydrophilic dye fluorescein sodium salt (FS) with negatively charged head anchored onto the surface of the vesicles as energy acceptor. The fluorescence resonance energy transfer was further studied in the aqueous vesicles' solution.

2. Experimental

Synthesis, purification and characterization of $C_{16}Naph(2,6)C_4N^+$ amphiphile have been described previously [17]. The $C_{16}Naph(2,6)C_4N^+$ vesicles (2×10^{-4} mol dm^{-3}) were prepared by sonication (Branson, B-19) for ca. 4 h to give an aqueous transparent solution. Globular vesicles with diameters ranging from 50 to 200 nm were observed under an electron microscope (JEM-2000 FM). The vesicles were stable in the pH range of 4–10 for weeks. The phase transition from crystal to liquid crystal (T_c) determined by differential scanning calorimetry (ca. wt. 3%, Perkin–Elmer, DSC-2C) is located at 46 °C. The sample of FS (Beijing Chemical Factory, A.R. grade) was used without further purification. Water was doubly distilled after passing through an ion-exchange resin column. Ethanol (Beijing Chemicals Factory) was redistilled before use. The structure of $C_{16}Naph(2,6)C_4N^+$ and FS is shown in Fig. 1.

Steady-state fluorescence spectra were recorded on an RF-5301PC spectrophotometer. UV spectra were measured with a Shimadzu UV-3100 spectrophotometer. Since N_2-bubbled and air-saturated solutions gave the same results, the data presented here are all for air-saturated solutions.

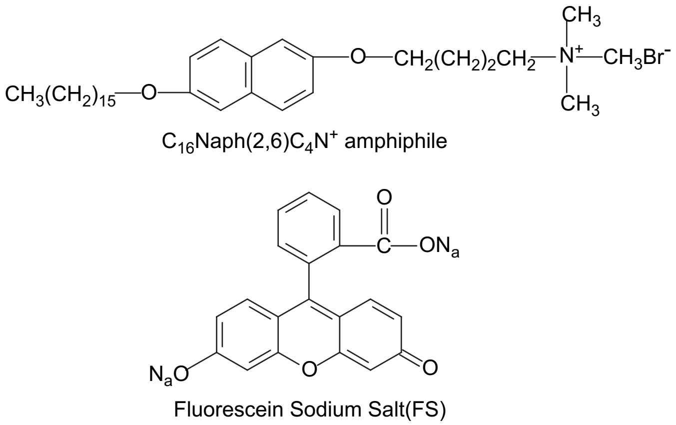

Fig. 1. Structural formulae of $C_{16}Naph(2,6)C_4N^+$ amphiphile and fluorescein sodium salt (FS).

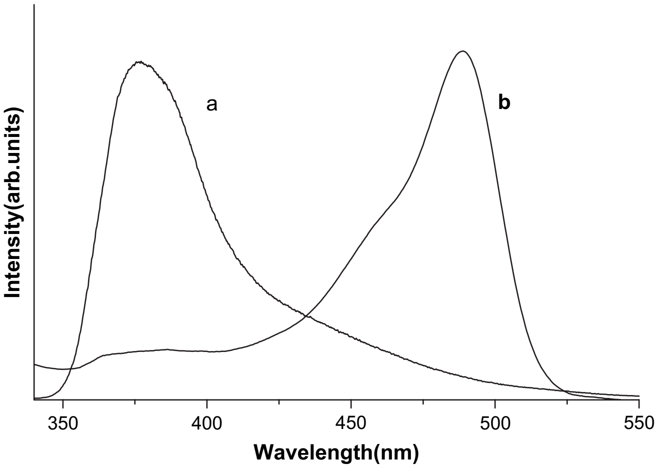

Fig. 2. Emission spectrum of $C_{16}Naph(2,6)C_4N^+$ aqueous vesicles (2×10^{-4} mol dm^{-3}) excited at 320 nm and absorption spectrum of FS aqueous solution (2×10^{-6} mol dm^{-3}) (b).

3. Results and discussion

3.1. Fluorescence resonance energy transfer from naphthalene to the membrane-bound FS anions in the vesicles

Fig. 2 shows the emission spectra of aqueous $C_{16}Naph(2,6)C_4N^+$ vesicles (a, 2×10^{-4} mol dm^{-3}) excited at 320 nm and absorption spectra of aqueous FS solution (b, 2×10^{-6} mol dm^{-3}), respectively. Partial overlap of emission peak centred at 378 nm of naphthalene (a) with the absorption peak centred at 489 nm of FS (b) suggests possible Förster-type intermolecular energy transfer from the naphthalene rings to the membrane-bound FS anions in the vesicles. Mention should be made here concerning the fluorescence of FS by direct excitation at the same wavelength of 320 nm. To check this, we measured the fluorescence spectra for the $C_{16}Naph(1,5)C_4N^+$ vesicles and aqueous FS solution with

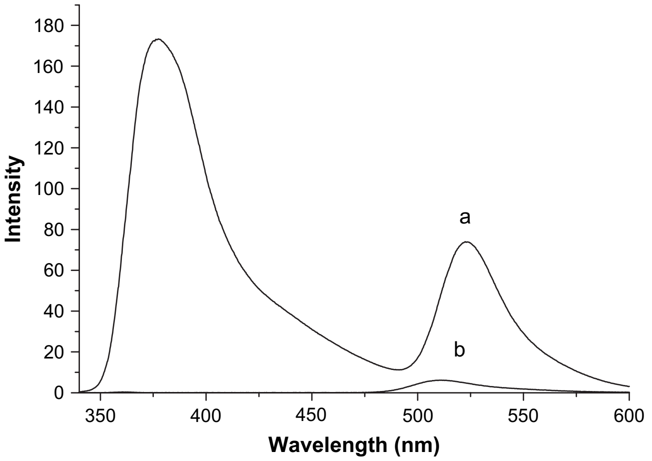

Fig. 3. Fluorescence emission spectra of $C_{16}Naph(2,6)C_4N^+$ (2×10^{-4} mol dm^{-3}) and FS aqueous vesicles (4×10^{-7} mol dm^{-3}) (a) and FS aqueous solution (4×10^{-7} mol dm^{-3}) (b), excited at 320 nm.

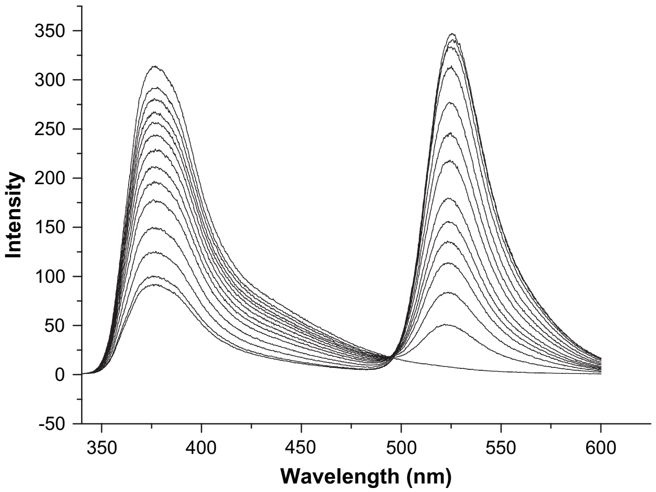

Fig. 4. Fluorescence spectra of C_{16}Naph(2,6)C_4N^+ vesicles when FS was added gradually into the aqueous vesicle solution, excited at 320 nm, [C_{16}Naph(2,6)C_4N^+]: 2.0×10^{-4} mol dm^{-3}; [FS]: 0, 1×10^{-7}, 2×10^{-7}, 3×10^{-7}, 4×10^{-7}, 5×10^{-7}, 6×10^{-7}, 8×10^{-7}, 1×10^{-6}, 1.2×10^{-6}, 1.4×10^{-6}, 1.6×10^{-6}, 2×10^{-6}, 2.4×10^{-6}, 2.8×10^{-6}, 3.2×10^{-6}, 3.6×10^{-6}, 4×10^{-6}, 4.4×10^{-6} mol dm^{-3}, respectively.

excitation at 320 nm, respectively, as shown in Fig. 3. In this case, the FS fluorescence (Fig. 3(b)) is emitted very weakly and the contributions through direct excitation other than the excitation transfer are negligible.

When C_{16}Naph(2,6)C_4N^+ and FS aqueous vesicle solution were excited at 320 nm, the energy transfer virtually occurred as shown in Fig. 3(a). Fig. 4 shows the changes of the fluorescence spectra of aqueous C_{16}Naph(2,6)C_4N^+ vesicles with the increasing concentration of FS at room temperature, which is below the phase-transition temperature of the vesicles. The fluorescence intensity of the naphthalene emission of C_{16}Naph(2,6)C_4N^+ vesicles which gives a peak centred at 378 nm, gradually decreases with the increasing concentration of FS in the aqueous C_{16}Naph(2,6)C_4N^+ vesicle solution. The fluorescence intensity of the emission peak of the FS centred at ca. 526 nm increases with the increasing concentration of FS up to a stoichiometric concentration of 4.4×10^{-6} mol dm^{-3}. These results imply that the FS anions were anchored by electrostatic interaction on the surface of the vesicles, which resulted in the energy transfer from naphthalene to FS. It is obvious that the organization of C_{16}Naph(2,6)C_4N^+ amphiphilic molecules in aqueous solution plays a decisive role in the energy transfer from naphthalene to FS.

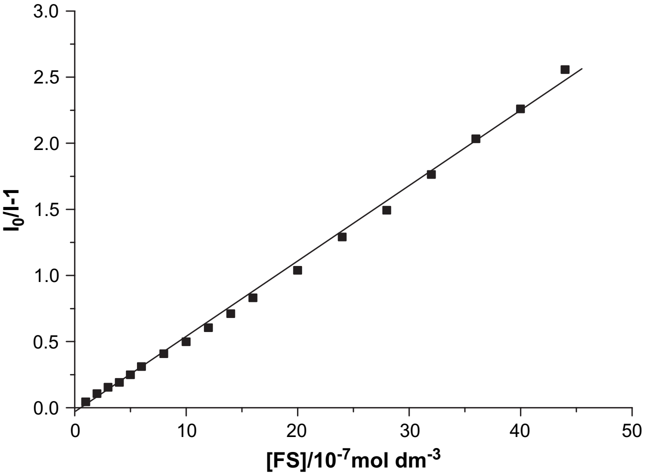

Fig. 5. Stern–Volmer plot of C_{16}Naph(2,6)C_4N^+ and FS vesicles.

The FS molecules anchored onto the surface of C_{16}Naph(2,6)C_4N^+ vesicles quench the emission intensity of naphthalene chromophore in C_{16}Naph(2,6)C_4N^+ amphiphiles. The concentration quench obeyed the Stern–Volmer relationship [18], Eq. (1), over the concentration range studied (0–4.4×10^{-6} mol dm^{-3}), as shown in Fig. 5.

$$I_0/I - 1 = K_{sv}[\mathrm{FS}] \tag{1}$$

where I_0 and I are the emission intensities of C_{16}Naph(2,6)C_4N^+ vesicle in the absence and presence of fluorescein sodium, respectively, and K_{sv} is the Stern–Volmer quenching-constant. Quenching data are plotted in Fig. 5 according to Eq. (1). The slope of the Stern–Volmer plot gives $K_{sv} = 5.56 \times 10^5$ mol^{-1}.

3.2. Efficiency of the energy transfer from naphthalene to FS in the aqueous vesicle solution

The efficiencies of energy transfer from the naphthalene of the C_{16}Naph(2,6)C_4N^+ amphiphiles in the vesicles to the membrane-bound FS, Φ_T values, were evaluated from the absorption of FS and the relative fluorescence intensity according to the following equation [19]:

Table 1
The energy transfer efficiency from naphthalene to FS at various concentrations of FS

FS (10^{-7} mol dm^{-3}):	1	3	5	8	12	16	24	32	40
$\frac{A(\lambda_D)}{A_D(\lambda_D)}$	0.9974	0.9898	0.9869	0.9809	0.9756	0.9743	0.9663	0.9544	0.9486
$\frac{I_D(\lambda_D\lambda_D^{em})}{I_D^0(\lambda_D\lambda_D^{em})}$	0.9577	0.8667	0.8008	0.7104	0.6232	0.5462	0.4367	0.3620	0.3068
φ_T	4.479	14.217	20.964	30.316	39.195	46.785	57.807	65.451	70.896

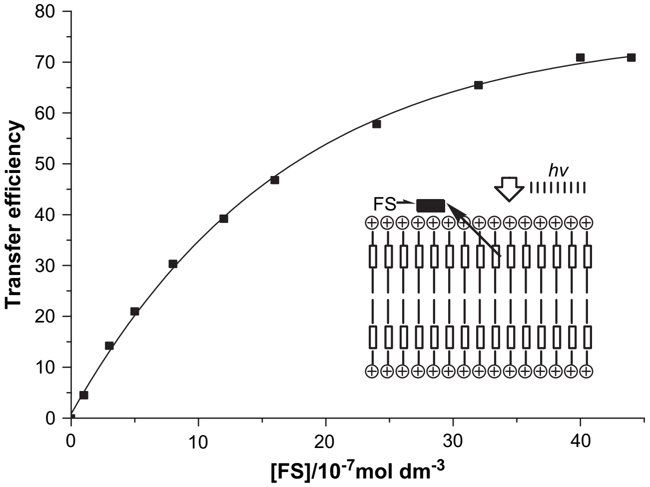

Fig. 6. Plot of energy transfer efficiency from naphthalene to membrane-bound FS against the relative concentration of FS. Inset: schematic drawing of molecular arrangement and energy transfer from naphthalene to FS in C_{16}Naph(2,6)C_4N^+/FS vesicles.

$$\phi_T = 1 - \frac{\phi_D}{\phi_D^0} = 1 - \frac{A(\lambda_D)}{A_D(\lambda_D)} \frac{I_D(\lambda_D, \lambda_D^{em})}{I_D^0(\lambda_D, \lambda_D^{em})}$$

where Φ_D and Φ_D^0 are the quantum yields of C_{16}Naph(2,6)C_4N^+ in the presence (Φ_D) of FS and absence (Φ_D^0) of FS. $A(\lambda_D)$ and $A_D(\lambda_D)$ are the absorption intensities of the C_{16}Naph(2,6)C_4N^+ donor in the presence ($A(\lambda_D)$) and absence ($A_D(\lambda_D)$) of FS, respectively. $I_D(\lambda_D, \lambda_D^{em})$ and $I_D^0(\lambda_D, \lambda_D^{em})$ are the fluorescence intensities of C_{16}Naph(2,6)C_4N^+ donor in the presence ($I_D(\lambda_D, \lambda_D^{em})$) and absence ($I_D^0(\lambda_D, \lambda_D^{em})$) of FS, respectively. The efficiency of the energy transfer in the C_{16}Naph(2,6)C_4N^+ and FS vesicle system is given in Table 1. The efficiencies are plotted as a function of the relative content of FS as shown in Fig. 6. The chromophores were arranged in vesicles by self-assembly as shown in the inset of Fig. 6. The energy transfer efficiency increases with the increasing concentration of FS, and levels off at higher acceptor contents (4.4×10^{-6} mol dm^{-3}), reaching ca. 71% of the 2D surface as the energy-trapping area. The high efficiency of energy transfer might be ascribed to the higher local concentration in the vesicles which was formed by self-organization from C_{16}Naph(2,6)C_4N^+ amphiphile and the interaction between C_{16}Naph(2,6)C_4N^+ and FS molecules. These results reveal that the amphiphile molecules have first self-assembled into vesicles with molecular order structure, and closed distance between the chromophores to satisfy the long distance resonance energy transfer. The constrained, order and organized system might provide a useful media for the processes occurring in photosynthesis.

4. Conclusions

Completely synthetic single chain amphiphile, C_{16}Naph(2,6)C_4N^+, can form vesicles in the dilute solution. The efficient energy transfer was performed through the naphthalene chromophore introduced into the amphiphilic molecules of the vesicles and FS anchored onto the surface of the vesicles. The synthetic vesicles exhibited some functionalizations similar to the natural system but readily manufactured. Thus, specific chromophore-containing vesicles will have many applications in relative fields, although the functionalization of aqueous vesicles for practical use is still an exploring subject.

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