A fluorescence probe study of the mixed surfactant vesicles

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Vesicle can be prepared from aqueous mixtures of simple commercially available, single-tailed cationic and anionic surfactants. In this work, the I_3/I_1 value, I_e/I_m value, and fluorescence lifetime of pyrene in different systems (see the preparation of samples) were determined. The essential affecting factors in the formation of vesicle can be deduced from the obtained results. It showed that large vesicle must form naturally before sonication in 0.082 M octyltrimethylammonium bromide and sodium laurate pH = 9.2 aqueous solution. While after sonication, only small vesicle exists, which can be proved further through electron microscope.

Keywords Vesicle, fluorescence probe, pyrene

Introduction

Although a variety of sophisticated physical methods have been used to study the properties of vesicles, such as differential scanning calorimetry,[1] NMR,[2] ESR probe,[3,4] fluorescence probe analysis is still an important area in biophysical studies of multimolecular aggregates as vesicles.[5-8] Pyrene has several interesting photophysical properties, the long fluorescence lifetime of pyrene monomer and efficient formation of excimer, which make it suitable for use as an effective probe. The intensities of the various vibronic bonds were found to show a strong dependence on the solvent environment.[9]

The strong electrostatic interactions between oppositely charged head groups in surfactant aggregates when oppositely charged surfactants are mixed in aqueous solution make vesicles form spontaneously or under sonication. The effective "neutralization" in the head group plane reduces the repulsion between head groups, and as a result, the effective surfactant packing parameter depends on composition.[6] The unilamellar structure of a vesicle mimics the cell membrane, consequently, vesicles have been widely used as model systems for *in vitro* investigations such as the study of membrane proteins.

In this paper, we designed five different systems and employed pyrene as a fluorescence probe to study carefully the influencing factors in the formation of vesicle prepared by available single-tailed surfactants with oppositely charged head group octyltrimethylammonium bromide (OTAB) and sodium laurate (SL).[10,11] The solubilized site of fluorescence probe molecules in vesicle and the size of vesicle were discussed simultaneously.

Experimental

Material

Surfactants octyltrimethylammonium bromide and sodium laurate were Aldrich products, and were recrystallized twice from ether-ethanol before use. Doubly distilled water was used throughout this work. Pyrene was recrystallized in ethanol three times before use.

Instruments

Steady-state and time-resolved fluorescence measurements were performed on a Hitach MPF-4 spectrofluorometer and a Horiba NAES-1100 single-photon-counting instrument, respectively. The vesicle samples were negatively stained with a 2% (*W/W*) uranyl acetate solution, and electron microscopy was examined on a Hi-

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tach H-600 electron microscope.

Preparation of samples

Pyrene was used as probe in the following five systems: I) 0.082 M OTAB pH = 9.2 aqueous solution, II) 0.082 M SL pH = 9.2 aqueous solution, III) 0.082 M OTAB and SL pH = 9.2 aqueous solution before sonication, IV) 0.082 M mixed OTAB and SL pH = 9.2 aqueous solution after 0.5—1 h sonication at 50℃, V) pH = 9.2 aqueous solution. The fluorescence spectra and fluorescence lifetime of pyrene at various concentrations were obtained.

Results and discussion

Pyrene monomer fluorescence in five systems

Pyrene is one of the few condensed aromatic hydrocarbon which shows significant fine structure (vibronic bands) in its monomer fluorescence spectra in solution phase. The ratio I_3/I_1 of the intensities of the third over the first vibronic peak can be used as an index of environmental polarity. When pyrene besets in the microenvironment of different polarity in micelle or vesicle, its fluorescence spectra suffer large change, and I_3/I_1 can be used to discuss the environmental effects on pyrene monomer fluorescence.

While the concentration of pyrene is very low ($C = 1.0 \times 10^{-7}$ M), only monomer exists no matter in homogeneous or in heterogeneous solutions. The value of I_3/I_1 determined is listed in Table 1. System I has almost the same I_3/I_1 value as system V, which indicates that no surfactant molecule aggregates while only 0.082 M OTAB exists in pH = 9.2 aqueous solution. The small values of I_3/I_1 in systems I and V suggest that the polarity of microenvironment in which pyrene resets is higher than that in other systems. The values obtained for the I_3/I_1 ratio in systems II, III and IV are different from those in systems I and V. The I_3/I_1 ratio is 0.92 in system IV, and they are similar to the reported values of I_3/I_1 ratio in the small vesicle.[12] The high value of I_3/I_1 in system IV shows that after sonication, only small vesicle (see electron microscope graph 1) is formed and the polarity of microenvironment in which pyrene resets is the smallest. Thus, we can conclude that the probe molecule penetrates deep into the lipidic zone of vesicle in system IV. The value of I_3/I_1 ratio in system III is 0.78, and it is similar to the reported value of I_3/I_1 ratio in the large vesicle,[12] which indicates that large vesicle must be present in system III. It is interesting to note that the value of I_3/I_1 ratio in system III is smaller than that measured in system II (it is well understood that only micelle exists in system II), which results from much more open structure of large vesicle. It also shows that the ordering of the chains in the large vesicle efficiently excludes pyren emolecules from the lipidic region, and pushes it toward the interface.

Table 1 I_3/I_1 values in five systems

System	I	II	III	IV	V
I_3/I_1	0.55	0.85	0.78	0.92	0.54

The fluorescence lifetime of pyrene in these five systems is also determined and listed in Tables 2 and 3. For the high concentration of pyrene ($C = 1.0 \times 10^{-5}$ M), the fluorescence lifetime shows different exponential decay in different microenviroments. In systems I and V, fluorescence lifetime of pyrene shows two exponential decay, while in systems II, III, IV, it shows mono exponential decay. This is readily understood from the property of pyrene. As well known, the excimer is readily formed for pyrene at a high concentration ($C = 1.0 \times 10^{-5}$ M) in aqueous solution V, so the long fluorescence lifetime is ascribed to monomer of pyrene, and the short fluorescence lifetime comes from excimer of pyrene. While there are the surfactant molecules in systems II, III, and IV, and their concentrations are much higher than those of pyrene, so the surfactant molecule aggregates can prevent pyrene from forming excimer efficiently because of their isolate effects. Although there is OTAB molecules in system I, the concentration of OTAB is lower than that of CMC, the micelle can not be formed, thus the excimer is formed in system I.

As compared with Table 2, although the long fluorescence lifetime of pyrene in low concentration ($C = 1.0 \times 10^{-7}$ M) is similar to that in high concentration, the short fluorescence lifetime is very different from that in high concentration (Table 3). And the fluorescence lifetime only shows a monoexponential decay in systems I and V. According to the above discussion, for the low

Table 2 Fluorescence lifetimes of pyrene (1.0×10^{-5} M) in five systems

Systems	A_1	τ_1 (ns)	Q_1 (%)	A_2	τ_2 (ns)	Q_2 (%)
I	0.37	46.3	15.2	0.52	185	84.8
II	—	—	—	0.9	391	100
III	—	—	—	0.975	283	100
IV	—	—	—	0.84	305	100
V	0.74	44.2	39	0.35	205	61

Table 3 Fluorescence lifetimes of pyrene (1.0×10^{-7} M) in five systems

Systems	A_1	τ_1 (ns)	Q_1 (%)	A_2	τ_2 (ns)	Q_2 (%)
I	—	—	—	0.31	172	100
II	0.60	4.70	10.5	0.07	277	89.5
III	1.45	1.13	15.7	0.03	287	84.3
IV	0.70	2.87	18	0.03	300	82.0
V	—	—	—	0.612	200	100

concentration of pyrene ($C = 1.0 \times 10^{-7}$ M), excimer can not be formed. Moreover, the short fluorescence lifetime in low concentration ($C = 1.0 \times 10^{-7}$ M) is only one tenth of that in high concentration ($C = 1.0 \times 10^{-5}$ M). This indicates the short fluorescence lifetime in low concentration ($C = 1.0 \times 10^{-7}$ M) can not be ascribed to excimer of pyrene. It can be well understood that long fluorescence lifetime in low concentration ($C = 1.0 \times 10^{-7}$ M) is from monomer of pyrene and the fluorescence lifetime shows a monoexponential decay in systems **I** and **V**. While, in systems **II**, **III**, and **IV**, where does the short fluorescence lifetime originate from? We propose that this short fluorescence lifetime can be ascribed to the pyrene that is closed to polar head and interface. The polar head and counter ion can decrease efficiently the fluorescence lifetime of pyrene by quenching.[12] Moreover, it is deduced easily that the pyrene, which shows long fluorescence lifetime, resides inner site of molecular aggregates in systems **II**, **III**, **IV**. The membrane of small vesicle is tightly packed and reduces collisional interactions of pyrene molecules in low concentration with dissolved oxygen in the bulk solution, so the fluorescence lifetime of pyrene in the small vesicle is longest (Table 3).

I_e/I_m values of pyrene in different concentrations in systems **II**, **III** and **IV**

With the increase of pyrene concentration, an excited pyrene molecule can react with an identical pyrene molecule in the ground state to form an excimer. The excimer spectrum appears as a structureless band peaking around 480 nm. The ratio of the maximum of the monomer spectra (I_e/I_m) can be used to judge the efficiency of excimer formation. While the concentration of pyrene remains the same, in various microenvironments the efficiencies of excimer formation are different. Here, I_e/I_m values of pyrene change, and these changes in I_e/I_m values with [pyrene] for systems **II**, **III** and **IV** are shown in Fig. 1. As shown in Fig. 1, for systems **II**, **III** and **IV**, in low concentration of pyrene (more than 4.0×10^{-5} M) no excimer is formed because of the isolate effect. This conclusion has already been drawn from the above determination of the lifetime of pyrene (Table 2). While, with the increase of concentration of pyrene, excimer can be formed, although isolate effect exists. In the same concentration of surfactants, the concentration of vesicle is much lower than that of micelle. Therefore, the value of I_e/I_m in system **II** is much smaller than that in system **IV**, and the value of I_e/I_m in system **III** is between the above two systems as indicated from Fig. 1. It means that before and after sonication, surfactant molecules aggregate in different forms. In system **III**, there must coexist micelle and vesicle, while in system **IV**, after sonication, the surfactant molecules rearrange and small vesicle is formed.

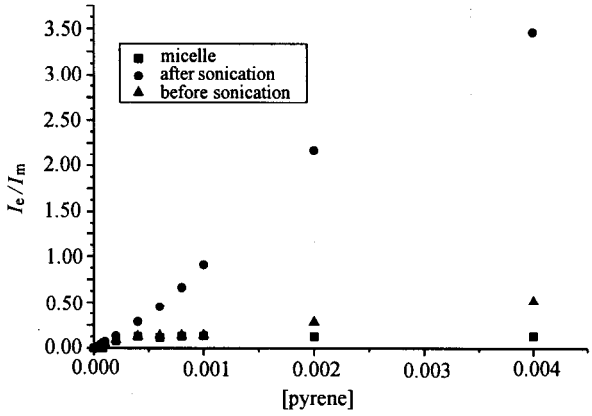
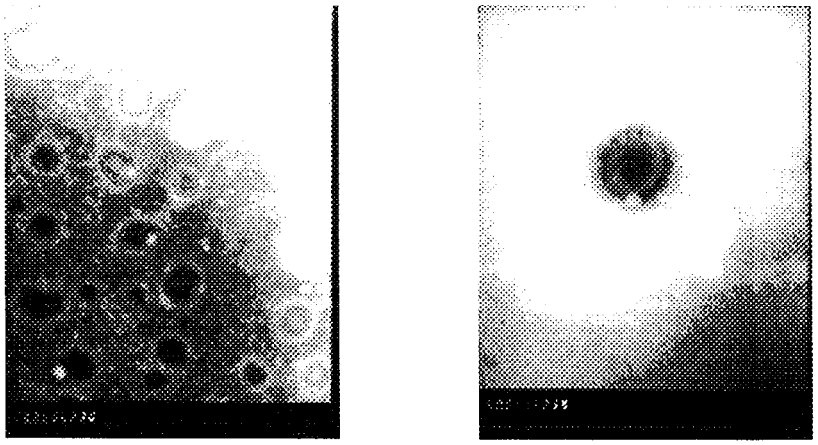


Fig. 1 I_e/I_m value of pyrene-[pyrene] in three systems.

At pH = 9.2, some lauric acid molecules exist, and they are favor for the formation of vesicle.[14] Equal molar octyltrimethylammonium bromide is the essential factor in forming vesicle. Sonication accelerates the rearrangement of the surfactant molecules and small vesicle is formed quickly.

In a word, formation of vesicles from the mixture of these cationic and anionic surfactants arises from the strong electrostatic interaction between the oppositely charged headgroup of the components. As a result, the mean effective headgroup area decreases considerably, while the mean hydrophobic volume of the tails remains the same. Thus, this dynamic ion pairing yields a pseudo-double-tailed zwitterionic surfactant, which is known to have the preferred geometry of a vesicle-forming surfactant. We also demonstrated the vesicle formation for system **IV** by transmission electron microscopy with negative technique (Graph 1). The thickness of the unilamellar layer of the vesicle is ca. 4—5 nm. The vesicles are polydisperse with radii less than 100 nm. Vesicles formed in this way are stable, and the situation is optically clear.[13]



20,000 × magnification 50,000 × magnification

Graph 1 TEM image of vesicle formed from 1:1 OTMAB-SL mixture.

Conclusion

The photophysics properties of pyrene in five different systems are investigated. The obtained results can be used to deduce the essential affecting factor in forming vesicle. It is confirmed that large vesicles must be present in system **IV**, while after sonication, the surfactant molecules will rearrange and small vesicle is formed. The results that fluorescence lifetime of pyrene show different exponential decay can be explained from properties of pyrene and microenvironment where it residues.

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