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Binding of aromatic anion amphiphile to phospholipid vesicles: a fluorescence study

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Abstract

The fluorescence properties of an aromatic anion amphiphile, *N*-(4-decyloxy-2-hydroxy-benzylidene)glycine (C_{10}HG) have been investigated. The fluorescence spectra of C_{10}HG in lipid vesicles showed stronger intensity than that in aqueous solution, which is due to the less polarity of microenvironment in the former case. A blue-shift maximum of C_{10}HG fluorescence spectra caused by the carbonyl and phosphate groups in the membrane lipid was also observed. The fluorescence lifetimes and anisotropy data showed that the C_{10}HG molecule is immobilized in the membrane, and its motion is blocked by phospholipid. Fluorescence quenching suggested that the molecule is embedded but does not penetrate deeply in the bilayer.

Keywords: Amphiphile; Phospholipid; Fluorescence study

1. Introduction

Fluorescence technique has been attracting great attention for application of complex macromolecular system [1–3]. By the use of steady state and time resolved fluorescence technique, extensive insight has been provided into the environment within phospholipid vesicles, which have been used as membrane model system for analyzing protein–lipid or drug membrane interaction [4,5]. Similar to micellar systems, vesicles have the ability to solubilize compounds in different region of the bilayer: (a) in the hydrophobic core; (b) at the head group region; or (c) in the inner or outer aqueous phase. The site of molecule located in lipid is of great importance, since it relates to properties and functions of vesicles. Many works have been carried out to investigate the location within lipid by fluorescence technique [6,7].

In our previous work, a novel aromatic anion amphiphile, *N*-(4-decyloxy-2-hydroxy-benzylidene)glycine (abbreviated as C_{10}HG, shown in Fig. 1), was synthesized, and special surface and aggregation properties were found [8]. In the present paper, fluorescence properties of C_{10}HG are studied. The spectra are analyzed to investigate its behavior in lipid. The quenching experiments of C_{10}HG embedded in lipid by three quenchers of

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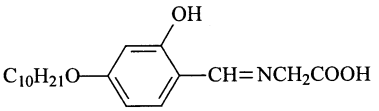


Fig. 1. The structure of *N*-(4-decyloxy-2-hydroxy-benzylidene)glycine (C_{10}HG).

different hydrophilicity are performed, and the site of location within lipid vesicles is also discussed.

2. Experimental

2.1. Material and methods

C_{10}HG was prepared by method reported earlier [8]. The structure is shown in Fig. 1. Fluorescence quenchers dibutylaniline (DBA), *p*-benzoquinone (BQ) and 1-decyl-pyridinium chloride (C_{10}PyCl), as well as dimyristoylphosphatidylcholine (DMPC) were obtained commercially.

Phospholipid vesicles were prepared by thin film method followed by ultrasonication [9]: the lipid was dissolved in chloroform, and the solvent was removed until a thin film was formed on the inner surface of the round-bottomed flask. Then a 10^{-4} mol l^{-1} solution of C_{10}HG was added, and the resulting mixture was sonicated for 20 min. Thus the prepared lipid solution was incubated in the dark for 12 h before the use.

In fluorescence measurement, solution were kept at pH 12.0 by 0.01 mol l^{-1} KOH. And temperature was controlled at 25 °C. The quenchers (DBA and BQ in ethanol and C_{10}PyCl in aqueous solution) were added directly to the solution of C_{10}HG. At no time did the amount of ethanol in the samples exceed 1 vol.%. All the solutions were deoxidated with high-pure nitrogen for 15 min before measurements. The excitation and the emission wavelength were 390 and 450 nm, respectively.

Steady-state anisotropy (r_s) values were calculated according to

$$r_s = \frac{I_{VV} - GI_{HV}}{I_{VV} + 2GI_{HV}}$$

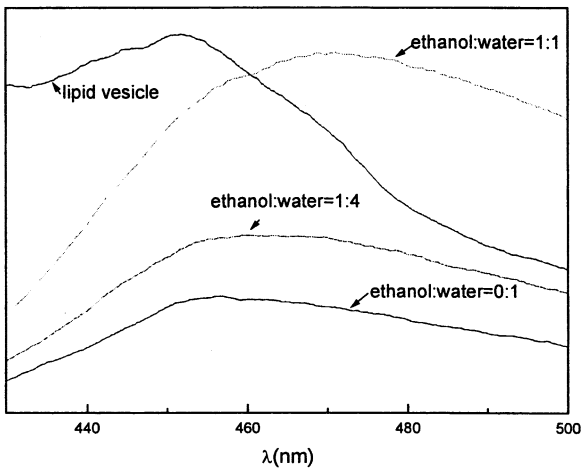


Fig. 2. Fluorescence spectra of C_{10}HG in ethanol/water solution and lipid vesicle. [C_{10}HG] = 10^{-4} mol l^{-1}, [DMPC] = 5×10^{-3} mol l^{-1}.

where I_{VV} and I_{HV} are the intensities measured in the directions parallel and perpendicular to the exciting beam and G is the grating correction factor equal to I_{HV}/I_{HH}.

2.2. Physical chemical measurements

The fluorescence emission spectra were measured using a Hitachi F-4000 fluorescence spectrometer. Time-resolved fluorescence decay of C_{10}HG was performed on a Horiba NAES 1100 ns fluorometer.

3. Results and discussion

3.1. Fluorescence spectra

The fluorescence emission spectra of C_{10}HG in aqueous solution, the mixture of ethanol/water and phospholipid vesicles were measured at 25 °C (Fig. 2). Fluorescence properties of C_{10}HG in the mixture of ethanol/water and phospholipid vesicles are summarized in Table 1.

The intensity of C_{10}HG in the mixture of ethanol/water increases with the addition of ethanol.

The fluorescence intensity in 1:1 mixture of ethanol/water is about 2.6 times stronger than that in aqueous solution. These facts can be attributed to the decrease of the polarity of environment around fluorophore [10]. It was interesting to find that the fluorescence intensity in lipid vesicles is even stronger than in that in 1:1 mixture of ethanol/water. This experimental result suggested that the fluorophore of $C_{10}HG$ molecule is incorporated into the hydrophobic region of the vesicles. The polarity of microenvironment around $C_{10}HG$ is reduced by hydrophobic chain of lipid molecules, and less than in 1:1 mixture of ethanol/water.

Usually the fluorescence maximum would be influenced by two factors [11]. One is the polarity of the environment around fluorophore. The emission maximum would shifts toward the red with the decrease of the environment's polarity. The other is the existence of quencher. The effect of fluorescence quenching would lead to the blue-shifted maximum. The fluorescence maximum of $C_{10}HG$ in aqueous solution is 456.2 nm, while in 1:1 mixture of ethanol/water it changes to be 471.0 nm. This phenomenon can be attributed to the decease of solvent's polarity. However, it is not the case in lipid vesicles. As mentioned above, the polarity in lipid vesicles is less than that in 1:1 mixture of ethanol/water, therefore it is far less than that in aqueous solution. However, the fluorescence maximum in lipid vesicles of $C_{10}HG$ shifts to 451.8 nm much smaller than that in aqueous solution (456.2 nm). Thus blue-shifted fluorescence maximum can be ascribed to the presence of carbonyl and phosphate groups acting as quenchers in the membrane lipid. Therefore, there should exist collisions between $C_{10}HG$ molecule and the head groups of lipid. In the meantime, it also suggests the fluorophore is close to the head group of lipid.

Table 1
Fluorescence maximum λ_{max} and fluorescence intensities in ethanol/water (v/v) and lipid vesicles

	0:1	1:4	1:1	In vesicle
λ_{max} (nm)	456.2	459.6	471.0	451.8
Relative intensity	0.70	0.98	1.84	1.93

3.2. Fluorescence lifetime and anisotropy

The time-resolved fluorescence of $C_{10}HG$ in aqueous solution and lipid vesicles were measured at 25 °C. The lifetime of the former is 0.327 ns, and that of the latter is 0.277 ns. The decease in lifetimes of the fluorophore in phospholipid vesicles media can also be attributed to the presence of the carbonyl and phosphate group as quenchers in the membrane [12]. According to Stern–Volmer equation, lifetime of $C_{10}HG$ will be reduced by the existence of quencher.

The motional freedom of a fluorophore is reflected in the value of steady state anisotropy values (r_s) which has been examined for $C_{10}HG$. In aqueous solution the steady state anisotropy value is found to be very low (0.05). However, the anisotropy value measured in vesicles (0.12) suggested that the $C_{10}HG$ molecule gets to some extent immobilize in the lipid bilayer. The results can be explained by Perrin relationship [11], $r_s = r_0/[1 + (\tau/\tau_R)]$, where τ is the fluorescence lifetime of the fluorophore, τ_R is the rotational correlation time of the fluorophore, and r_0 is anisotropy in highly viscous medium. From the present results, it is evident that when the fluorophore is incorporated in vesicles, the lifetime (τ) decreases and the anisotropy value (r_s) increases. This means that the motion of $C_{10}HG$ molecule immobilized in the lipid bilayer is considerably restrained by its microenvironment.

3.3. Fluorescence quenching

Fluorescence quenching may be employed to investigate the position of a fluorophore in a bilayer. The fluorescence quenching of $C_{10}HG$ was studied using different quenchers: $C_{10}PyCl$, DBA, and BQ. Solutions of $C_{10}HG$ embedded within lipids were monitored by their fluorescence emission as a function of quencher concentration. The data were processed by Stern–Volmer equation:

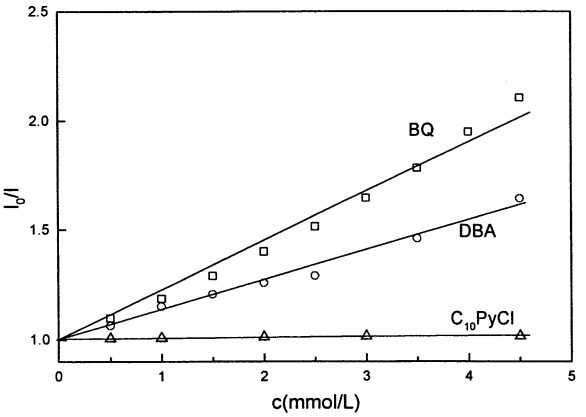


Fig. 3. Fluorescence quenching by three quenchers ($[C_{10}HG] = 10^{-4}$ mol l^{-1}, $[DMPC] = 5 \times 10^{-3}$ mol l^{-1}).

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

where I and I_0 are the fluorescence intensity in the presence and absence of quenchers, respectively. K_{SV} is the Stern–Volmer constant and $[Q]$ is the quencher concentration.

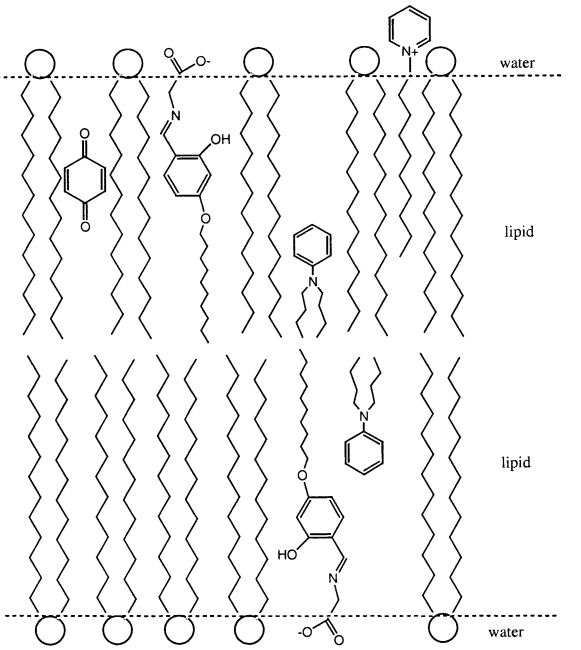
Stern–Volmer plot obtained in fluorescence quenching experiments are shown in Fig. 3. The plots are linear in the concentration range of quenchers used. The quenching constants are listed in Table 2.

According to Fig. 3 and Table 2, the value of K_{SV} decreases following the sequence BQ > DBA > C_{10}PyCl. The sequence of K_{SV} can be explained by the relative location of fluorophore and quenchers in the lipid bilayer.

The fluorophore of C_{10}HG molecule is the aromatic group, the distance between it and hydrophilic group is almost 4 Å. While the total length of a DMPC molecule is about 20 Å. Therefore, C_{10}HG molecule should be embedded in the hydrophobic region of lipid, but the location is quite shallow, very close to the interface of lipid vesicles (see Scheme 1).

Table 2
Stern–Volmer constant (K_{SV}) and the octanol–water partition coefficient (log $P_{oc/w}$)

	BQ	DBA	C_{10}PyCl
K_{SV}	0.126	0.069	0.001
log $P_{oc/w}$	0.96	3.99	<0



Scheme 1.

The depth of quencher located in lipid can be decided by the octanol–water partition coefficient log $P_{oc/w}$, which is widely used in pharmaceutical chemistry to provide a measure of the lipophilic versus hydrophilic nature of a compound. The pyridinium moiety of C_{10}PyCl, which acts as quencher, can not penetrate the hydrophobic region of the bilayer; BQ has a log $P_{oc/w}$ of 0.96 [13], which may be at a shallow location in the hydrophobic region of the bilayer; DBA can penetrate most deeply into lipid, for it has a value of log $P_{oc/w}$ as large as 3.99 [14]. The relative locations of C_{10}HG and quenchers in lipid bilayer are demonstrated in Scheme 1.

Thus the collision between the C_{10}HG molecule and BQ is very efficient, for their site of location in the bilayer is readily access to each other. On the contrary, it is difficult for C_{10}HG and the pyridinium moiety of C_{10}PyCl to meet each other, because the pyridinium can hardly penetrate into the hydrophobic bilayer. While to DBA, in most case it stays in the hydrophobic core, but it still

has chance to access $C_{10}HG$. Therefore, the efficiencies of fluorescence quenching, i.e. Stern–Volmer constants K_{SV}, decrease following the sequence BQ > DBA > C_{10}PyCl.

4. Conclusions

In this paper, the fluorescence properties of $C_{10}HG$ was investigated in aqueous solution and phospholipid vesicles. The fluorescence spectra of $C_{10}HG$ in lipid vesicles showed stronger intensity than that in aqueous solution, which is due to the less polarity of microenvironment. And it also shows a blue-shift maximum, which is attributed to the presence of carbonyl and phosphate groups acting as quenchers in the membrane lipid. The fluorescence lifetimes and anisotropy data showed that the molecule is immobilized in the membrane, and its motion is blocked by phospholipid. Fluorescence quenching suggested that the molecule is embedded in the hydrophobic region of vesicles but does not penetrate deeply in the bilayer.

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