Properties of Imidazolinium-containing Multiblock Amphiphile in Lipid Bilayer Membranes

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A multiblock amphiphile CBA bearing a cationic imidazolinium moiety at its center formed different types of assembly in THF and CHCl₃, which show characteristic emission bands around 300 and 465 nm upon excitation at 295 and 320 nm, respectively. These assemblies were able to be transferred into lipid bilayer membranes, keeping the similar spectral profiles with those in solutions. These results indicate a new potential of self-assembling processes for the control of supramolecular architecture hierarchically formed in lipid bilayer membranes.

Keywords: Amphiphile, Self Assembly, Lipid Bilayer Membranes.

1. Introduction

Control of self-assembled architecture formed in lipid bilayer membranes is an important issue [1–11], in particular for regulating the transportation of materials across the membrane by synthetic molecules [1–7]. Meanwhile, the domain formation due to the lateral phase separation in lipid bilayer membrane has also been drawing interest, in association with raft formation which is considered to play important roles for controlling activity of membrane proteins [12–14]. We have been involved in development of multiblock amphiphiles which form stimuli-responsive ion channels [15–21] and thermally responsive assemblies in lipid bilayer membranes [22,23]. Recently, we have developed a multiblock amphiphile bearing an imidazolinium moiety at the center of molecule (CBA), which are able to transport anions through lipid bilayer membranes as a mobile carrier [24]. In the course of investigation of the properties of CBA, we have noticed that CBA may adopt different states of assembly in solution depending on the solvent, which were also transferable into the lipid bilayer membranes. In this study, we investigated such properties of CBA in different solvents based on spectroscopic analysis.

Fig. 1. Chemical structure of CBA.

2. Experimental

2.1. Instruments and Reagents

UV-Vis absorption spectra were recorded on JASCO V-530 UV-Vis spectrophotometer. Fluorescence spectra were recorded on JASCO FP-6500 spectrometer using quartz cell of 10-mm optical path length. TEM images were obtained using a JEOL model JEM–1400 electron microscope operating at 100 kV. TEM support grids (Catalog No. U1015) were purchased from EM Japan Co., LTD. Negative staining was performed using NANO–WTM manufactured by Nanoprobes. Large unilamellar vesicles were prepared using Avanti Mini Extruder with 100 nm polycarbonate membranes. 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids. CBA was synthesized following the procedure reported in our previous paper [24].

2.2. UV-Vis absorption and emission spectroscopy in various solvents.
15 µL of CBA in CHCl₃ (1 mM) was taken into vial and dried under vacuum in dark, and 3 mL of each solvent (THF, CHCl₃, MeOH, DMSO, and H₂O) was added to the vial so as to prepare 5 µM solution. The solution was transferred into clean, dried cuvette for absorption and emission spectroscopic measurements.

2.3. Preparation of DOPC LUVs for spectroscopic analysis

CHCl₃ solution of DOPC (10 mM) was evaporated in glass tube to form a thin lipid film. The resulting film was dried for at least 1 h under vacuum, and hydrated with HEPES buffer (20 mM HEPES, 50 mM NaCl, pH 7.1, same volume as CHCl₃, 10 mM final concentration) at 37 ºC, followed by freezing and thawing (5 cycles). The resulting solution was incubated at 37 ºC for at least 1 h, and then extruded by polycarbonate membranes for 21 times at room temperature.

2.4. Spectroscopic analysis in lipid bilayer membranes.

60 µL of DOPC LUVs were added to 2.94 mL of 20 mM HEPES, 50 mM NaCl, pH 7.1 buffer solution and stirred for 30 s. THF or DMSO solution of CBA were added to DOPC LUVs and stirred for 30 s for incorporation to lipid bilayer membranes.

2.5. TEM Observation

A 1 mM stock solution of CBA in THF or CHCl₃ was prepared and then heated at 60 ºC for 30 min followed by dilution to 100 µM.

A 5 µL of sample solution ([CBA] = 100 µM in THF or CHCl₃) was deposited onto a copper TEM grid with a carbon support film (200 mesh, EM Japan Co., LTD.) and held in place with tweezers for 1 min. The sample solution was removed by capillary action using a filter paper, and the grid was dried for another 1 min. The sample was then stained with 5 µL of NANO–WTM (1wt%) for 1 min and the solution was removed by capillary action using a filter paper. The staining process was repeated once again. The grid was dried overnight before imaging.

2.6. Preparation of DOPC GUVs for microscopic observation

100 µL of 6 mM DOPC in CHCl₃ was coated on the ITO-coated side of glass plate and dried on heat block of 50 ºC for 5 min to make a thin lipid film. The lipid film was dried under vacuum for overnight. Silicon sheet with squared gap was filled with milliQ and covered with another ITO glass plate so that ITO glass plate would face each other. The plates were connected to the electrode, and hydrated at 50 ºC for 2 h to afford a DOPC GUV solution (ca. 2 mM).

100 µL of DOPC GUVs solutions prepared above were transferred to a vial. To this was added 1 µL of 1 mM CBA in THF or DMSO, and the resulting mixture was incubated at 37 ºC for at least 1 h for incorporation of CBA_T and CBA_C into the lipid bilayer membranes.

3. Results and discussion

3.1. Spectroscopic analysis of CBA in various solvents

First, a CHCl₃ stock solution of CBA was prepared which was dried under vacuum. Then, to the residue were added THF, CHCl₃, MeOH, DMSO, and H₂O, respectively, for UV-Vis absorption and emission spectral analysis.
2.6. Preparation of DOPC GUVs for microscopic observation overnight before imaging. The grid was dried using a filter paper. The staining process was repeated once again. The resulting solution was incubated at 37 ºC for at least 1 h, and then extruded by polycarbonate filters, and hydrated with HEPES buffer (20mM CHCl3, 10 mM final concentration) at 37 ºC, followed by freezing and thawing (5 cycles). The resulting mixture was incubated at 37 ºC for at least 1 h for incorporation of CBA into DOPC LUVs. The residue were added THF, CHCl 3, MeOH, DMSO and H 2O, respectively, for UV-Vis absorption and emission spectral analysis. As for the emission spectra, the maximum emission bands were observed around 465 nm for the solvents besides THF, which showed the maximum band around 366 nm, with a shoulder around 465 nm. Interestingly, after annealing at 60 ºC (Fig. 3a), both absorption and emission spectra in THF showed hypsochromic shifts while the those in the other solvents remained substantially unchanged. From these results it is likely that CBA forms two kinds of assemblies depending on solvents; one was thermally stabilized in CHCl 3, MeOH, DMSO and H2O, and gave emission bands around 465 nm, while another one was thermally stabilized in THF, and gave emission around 330 nm.

3.2. TEM observation
Based on the above-mentioned spectroscopic studies, we have carried out microscopic studies, we have carried out microscopic observations of CBA stained with NANO–WTM. Both samples showed two kinds of assemblies depending on solvents; one was thermally stabilized in CHCl 3, MeOH, DMSO and H2O, and gave emission bands around 465 nm, while another one was thermally stabilized in THF, and gave emission around 330 nm.

3.3. TEM observation

![Fig. 3. (a) UV-Vis absorption and (b) emission spectra of CBA in various organic solvents (λex = 295 nm (THF), 315 nm (MeOH and H2O) and 320 nm (CHCl3 and DMSO) after annealing at 60 ºC for 30 min. All measurements were carried out at 5 µM, 20 ºC.](image)

The absorption spectra showed a broad band around 310 nm, where the absorption maximum differed depending on solvents (Fig. 2a). As for the emission spectra, the maximum emission bands were observed around 465 nm for the solvents besides THF, which showed the maximum band around 366 nm, with a shoulder around 465 nm (Fig. 2b). Interestingly, after annealing at 60 ºC (Fig. 3a), both absorption and emission spectra in THF showed hypsochromic shifts while the those in the other solvents remained substantially unchanged. From these results it is likely that CBA forms two kinds of assemblies depending on solvents; one was thermally stabilized in CHCl 3, MeOH, DMSO and H2O, and gave emission bands around 465 nm, while another one was thermally stabilized in THF, and gave emission around 330 nm.

![Fig. 4. TEM images of CBA prepared in (a) THF (CBA T) and (b) CHCl 3 (CBA C). Both samples were prepared as [CBA/DOPC] = 1/200 in milliQ, λex = 330 nm–385 nm, λem = 420 nm. Scale bars indicate 10 µm.](image)

![Fig. 5. (a), (c), Phase contrast and (b), (d) and fluorescent microscopic images of (a), (b) CBA T and (c), (d) CBA C. All samples were prepared as [CBA/DOPC] = 1/200 in milliQ, λex = 330 nm–385 nm, λem = 420 nm. Scale bars indicate 10 µm.](image)

![Fig. 2.  (a) UV-Vis absorption and (b) emission spectra of CBA in various solvents (λex = 305 nm (THF), 320 nm (MeOH and H2O) and 310 nm (CHCl3 and DMSO) after annealing at 37 ºC, 30 min. All measurements were carried out at 5 µM, 20 ºC.](image)
observations of CBA assemblies prepared in THF (CBAT) and CHCl3 (CBAC), which showed different emissions as shown in Fig. 3. Considering the possibility that CBA could form assemblies by both kinetic and thermodynamic controls, we dissolved CBA in THF and CHCl3 and annealed the resulting solutions at 60 °C respectively for complete formation of each assembly. Importantly, TEM observation of CBAT and CBAC revealed differences in morphology (Fig. 4): CBAT showed sharp-edged and ribbon-like structures (Fig. 4a) while CBAC showed small spherical aggregates (Fig. 4b). From these observations, it is likely that the assembly formed in THF has relatively higher order compared to that prepared in CHCl3.

3.3. Incorporation of CBAT and CBAC into lipid bilayer membranes

Since preparation of two different assemblies could be controlled by proper choice of solvents, we investigated the possibility whether these two assemblies could be transferred into lipid bilayer membranes. Thus we prepared CBAT and CBAC in THF or DMSO, respectively, and added them to the vesicles. In this case, DMSO was used to prepare CBAC instead of CHCl3, due to its miscibility with water.

Incorporation of CBAT and CBAC into the lipid bilayer membranes was confirmed by microscopic observations of vesicles after addition of CBAT and CBAC. To giant unilamellar vesicles (GUVs) of DOPC, was added CBAT in THF or CBAC in DMSO, respectively. In the phase contrast and fluorescent microscopic images shown in Fig. 5, circular objects corresponding to GUVs were clearly observed, indicating that CBAT and CBAC were both successfully incorporated into the lipid bilayer membranes.

Importantly, the emission spectra of CBAT in DOPC LUVs showed little difference from that in THF (Figs. 6a, b, dashed lines). The emission maximum wavelength was 333 nm in DOPC LUVs, which was very close to that in THF (328 nm). This result strongly indicates that CBAT was maintained within the lipid bilayer membranes. Similarly, emission spectra of CBAC in DOPC LUVs was very similar to that in CHCl3 (Figs. 7a, b, dashed lines), where the emission maximum wavelength was 466 nm in both cases. Therefore,
CBAC was also preserved within the lipid bilayer membranes. From these results, it is strongly suggested that the assemblies of CBA formed in solvents could successfully be transferred to in the lipid bilayer membranes.

4. Conclusion
The self-assembling properties of multiblock amphiphile CBA is unique in that the structure formed in CHCl₃ gradually changed into other forms in THF as shown in Figs. 2 and 3. This apparently slow conversion process between two states likely allows transfer of CBAT and CBAC into lipid bilayer membranes, respectively. These findings suggest a possibility of controlling supramolecular architectures in lipid bilayer membranes through kinetically controlled self-assembling processes in solution [25].

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