Aromatic Fluorination of Multiblock Amphiphile Enhances Its Incorporation into Lipid Bilayer Membranes

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We designed multiblock amphiphiles AmF and AmH, which consist of perfluorinated and non-fluorinated hydrophobic units, respectively. Absorption spectroscopy revealed that both amphiphiles are molecularly dispersed in organic solvent, while they form aggregates under aqueous conditions. Furthermore, we investigated whether AmF and AmH can be incorporated into DOPC lipid bilayer membranes, and found that the maximum concentration of AmF that can be incorporated into DOPC lipid bilayer membranes is 43 times higher than that of AmH.

Biological membranes that separate the inner and the outer environment of cells play diverse roles in controlling important cellular events.[1] Among numerous biomolecules within cell membranes, transmembrane proteins are particularly intriguing due to their sophisticated functions such as selective material transport,[2] receptor-ligand binding,[3] and signal transduction.[4] In order to mimic their structures and functions, various synthetic molecules that can be incorporated into lipid bilayer membranes have been developed.[5] In this regard, our research group have reported the synthesis of multiblock amphiphiles that are composed of hydrophilic oligo(ethylene glycol) chains and hydrophobic aromatic groups. Interestingly, these molecules self-assembled within lipid bilayer membranes and showed transmembrane ion transport properties.[6] Alongside these studies, we sought to enhance the affinity of multiblock amphiphiles for lipid bilayer membranes, which is necessary for further expansion of their functions. For this purpose, we focused on the effect of aromatic fluorination, which has been known to improve the affinity of drug molecules for the hydrophobic layer of lipid bilayer membranes.[7][8] Moreover, co-assembly of fluorinated amphiphiles with phospholipids can exert a unique influence on physical properties of membranes.[9]

Taking these into account, here we designed a multiblock amphiphile AmF, which possesses 1,4-bis(4-tetrafluorophenyl)-tetrafluorobenzene as a hydrophobic unit and tetraethylene glycol chains as hydrophilic units, and its non-fluorinated analogue AmH (Scheme 1). We expected that the effect of aromatic fluorination would enhance the incorporation of AmF into lipid bilayer membranes.

The aromatic units of AmF and AmH were synthesized by Sonogashira cross-coupling reaction, and tetraethylene glycol chains were introduced by nucleophile aromatic substitution or Williamson ether synthesis. These multiblock amphiphiles were unambiguously characterized by 1H, 13C, and 19F NMR spectroscopy, and MALDI-TOF-MS spectrometry, and their purity was confirmed by elemental analysis (see Supporting Information). First, we investigated the optical properties of AmF and AmH in solution. The absorption spectra of AmF and AmH in THF (10 μM) showed absorption maxima at λ = 333 nm and λ = 332 nm, respectively (Figure 1, blue curves). In clear contrast, in HEPES buffer ([HEPES] = 20 mM, [NaCl] = 50 mM, pH 7.5), AmF and AmH showed blue-shifted absorption peaks (AmF: λmax = 304 nm, AmH: λmax = 314 nm) (Figure 1, red curves). In addition, fluorescence spectra of AmF and AmH in HEPES buffer showed red-shifted emissions in comparison with those in THF (Figure S8). These spectroscopic changes are in agreement with the observed conformational changes in solution. Therefore, we conclude that aromatic fluorination can increase the affinity of amphiphiles for lipid bilayer membranes.

Scheme 1. Molecular structures of AmF and AmH.

Figure 1. Absorption spectra of a) AmF ([AmF] = 10 μM) and b) AmH ([AmH] = 10 μM) in THF (blue) and in HEPES buffer ([HEPES] = 20 mM, [NaCl] = 50 mM, pH 7.5, containing 1% THF) (red) at 20°C.
with our previous study that demonstrated the molecularly dispersed state of such amphiphiles in THF and an aggregated state in aqueous conditions. Moreover, dynamic light scattering (DLS) measurements of AmF and AmH (10 μM) in HEPES buffer showed the presence of aggregates with average hydrodynamic diameters of 298 nm and 866 nm, respectively (Figure S9). Therefore, we concluded that AmF and AmH hardly assemble in THF while they form aggregates under aqueous conditions.

Next, we investigated whether AmF and AmH can be incorporated into lipid bilayer membranes or not. Using an electro-formation method, we prepared giant unilamellar vesicles (GUVs) that are composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and AmF ([DOPC] = 500 μM, feed amount of AmF: [AmF] = 50 μM in aqueous 200 μM sucrose). We then tried to visualize the GUVs using phase-contrast and fluorescence microscopy (λ_ex = 330–385 nm; λ_obsd > 420 nm). Formation of micrometer-sized GUVs was confirmed by phase-contrast microscopy (Figure 2a), and the corresponding fluorescence image clearly showed an emission along the shape of GUVs (Figure 2b). These observations demonstrated the successful incorporation of AmF into lipid bilayer membranes (Figure 2c). In sharp contrast, when we added AmH to DOPC and prepared GUVs (Figure 2d), we found that a fluorescence micrograph of GUVs remained dark (Figure 2e). We also found that the fluorescence intensity of vesicles containing AmF was an order of magnitude higher than that of vesicles containing AmH (Figure S10). These results indicate the enhanced incorporation of AmF into lipid bilayer membranes in comparison with AmH (Figures 2c and 2f).

Using absorption spectroscopy, we also quantified the amount of AmF and AmH that are incorporated into lipid bilayer membranes. This time, we prepared DOPC small unilamellar vesicles (SUVs) that incorporated AmF or AmH ([DOPC] = 100 μM in HEPES buffer) using an entrainer equipped with a filter membrane with a pore diameter of 50 nm (see Supporting Information). By DLS measurements, we confirmed the formation of SUVs with a controlled diameter of ca. 50 nm (Figure 3a). Note that this process is necessary to remove aggregates formed by multiblock amphiphiles that are not incorporated into lipid bilayer membranes. We then performed absorption spectroscopy, and SUVs containing AmF (e.g. [AmF]/[DOPC] = 0.10) showed an absorption maximum at λ = 331 nm (Figure 3b, orange curve). We found that the absorption profile is similar to that of AmF in THF (λ_max = 333 nm) (Figure 1a, blue curve), in comparison with that of AmF in HEPES buffer (λ_max = 304 nm) (Figure 1a, red curve). This result indicates that AmF does not form large aggregates when it is incorporated into lipid bilayer membranes. We therefore created a standard curve based on absorption spectra of AmF in THF at several concentrations, and estimated the net concentration of AmF molecules that are actually incorporated into lipid bilayer membranes (Figures S11 and S12). As we increased the feed amount of AmF, the calculated concentration of AmF that are incorporated into lipid bilayer membranes also increased, and reached to a maximum at 5.8 μM (5.5 mol% to DOPC molecules) (Figure 3c, red line). Similarly, we measured the absorption spectra of SUVs containing AmH (Figure 3d), and calculated the concentration of AmH that was incorporated into lipid bilayer membranes (Figure 3c, blue line). Surprisingly, the maximum concentration of AmH that can be incorporated into lipid bilayer membranes was...
calculated to be 135 nM, which was 43 times lower than that of AmF. Although this multiple number may depend on the type of lipid molecules, these results clearly demonstrated that the aromatic fluorination enhanced the affinity of the amphiphile to DOPC lipid bilayer membranes. Because 2s or 2p orbitals of fluorine atom overlap with the corresponding orbitals on aromatic carbon, the C-F bond becomes highly non-polarizable and therefore, an affinity to the lipid bilayer membranes increases. This is proposed to be the main reason for the enhanced incorporation of AmF into lipid bilayer membranes.

In conclusion, we demonstrated that aromatic fluorination of a multiblock amphiphile enhanced its affinity to lipid bilayer membranes. We believe this is a powerful strategy for designing new series of functional multiblock amphiphiles. Furthermore, because fluorous compounds are rarely found in living organisms, we expect that development of fluorinated compounds that can be localized within biological membranes may exhibit unprecedented functions that are not found in nature.

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