The Fluorescent Polydiacetylene Liposome

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Development of efficient sensors utilizing conjugated polymer as sensing matrices has gained much attention among many research scientists.1-11 Especially, polydiacetylene (PDA)-based sensors for the detection of biologically important species have been intensively investigated due to the unique color changing properties upon stimulation.12-27 Closely packed and properly designed certain diacetylenes can undergo polymerization via 1,4-addition reaction to form an ene-yne alternated polymer chain upon UV irradiation with 254 nm as shown in Figure 1.28-35 The resulting polydiacetylenes, if obtained under optimized conditions, appear to be intense blue color to the naked eyes. The blue-colored polydiacetylenes can be prepared in the form of liposomes in aqueous solutions or as thin films using Langmuir-Blodgett or Langmuir-Schaefer methods. The advantage of the nanostructured polydiacetylenes as biosensors comes from the fact that visible color change from blue to red occur in response to a variety of environmental perturbations, such as temperature,36-39 pH,40 and ligand-receptor interactions.12-14,27 Many researchers have tried to understand the mechanism of the color transition. Although it is not clear, it has widely been accepted that color change is associated with a conformational change of polydiacetylene backbone. Accordingly, the polydiacetylenes in the blue form have extended conjugation of $p$-orbital in the main chain of the polymers. The conjugated $p$-orbitals undergo distortion by environmental stimuli, leading to partial twist of the $p$-orbitals. Thus, the dark blue color of the polymers gradually shifts to the red color depending on the amount of the stress.

One of the most commonly used matrix lipid monomer for polydiacetylene-based biosensor is 10,12-pentacosadiynoic acid (PCDA 1) (Figure 2). The PCDA lipid monomer can readily be assembled in aqueous media in the form of polymerized liposome vesicles after UV irradiation. When indicating the lipid concentration in a liposome solution, one calculates it based on the total lipid monomers used, assuming all of the monomers are transformed into the liposome. Since it is practically impossible to directly measure the lipid concentration of polymerized diacetylene liposomes, the nominal concentration has been widely used as a standard measure, even without rigorous proof. However, in order to use such nominal concentration for the purpose of calibration of PDA-based sensors, we will need to prove most of, if not all, the PCDA molecules assembled to bilayered liposomes in aqueous solution. This could be done by using a fluorescent lipid monomer which is newly derived from PCDA. If a liposome solution is prepared with

10,12-pentacosadiynoic acid (PCDA, 1)

Figure 1. Schematic representation of polymerization of assembled functional diacetylenes by irradiation with UV light.

Figure 2. Schematic representation of the liposome prepared with 10,12-pentacosadiynoic acid (PCDA, 1).
a mixture of a fluorescent lipid monomer and PCDA and if all of the lipid monomers are converted to liposome, we should not be able to detect fluorescence in the mother liquid after dialysis. We now report a straightforward method for the measurement of efficiency of the formation of polymerized diacetylene liposomes with a fluorescence-labeled diacetylene monomer.

Results and Discussion

The preparation of the diacetylene monomer 6 having a fluorescent head group is shown in Figure 3. Synthesis of the monomer 6 having a fluorescent head group was very straightforward. Firstly, the commercially available 10,12-pentacosadiynoic acid (PCDA, 1) was converted to the corresponding acid chloride 2 with oxalyl chloride. Secondly, tetra(ethylene glycol) (3) was coupled with dansyl chloride (4) to give a linker molecule with fluorescent chromophore 5. The tetra(ethylene glycol) unit was introduced to make the fluorescent head group hydrophilic. Final step of obtaining the diacetylene monomer 6 was accomplished by reacting the 10,12-pentacosadiynoic acid chloride (2) prepared as described above with the tetra(ethylene glycol) derivative having fluorescent chromophore 5.

Having prepared the fluorescent diacetylene monomer 6, next phase of our efforts focused on the preparation of polydiacetylene liposomes with the fluorescent diacetylene monomer 6. General procedures for the preparation of polydiacetylene liposome in aqueous solution is presented in Figure 4. In the first step, a diacetylene monomer is dissolved in chloroform in a test tube and the organic solvent is removed by purging with N₂ to give a thin film of the lipids on the glass surface. Deionized water or a proper buffer is added to yield typically a total lipid concentration of 1 mM. The sample is then heated at 80 °C for 15 min and sonicated for 15 min. The resulting solution is filtered through a 0.8 µm filter and the filtrate is cooled at 4 °C for 12 h. Polymerization is carried out at room temperature by irradiating the solution with 254 nm UV light (1 mW/cm²) to induce conjugated backbone of alternating double and triple bonds.

When the fluorescent diacetylene monomer 6 was subjected to above procedures, no polymerization was occurred. This is presumably due to the flexible tetra(ethylene glycol) units which prevent molecular assembly of the monomers. In general, monomers having headgroups which can not
form intermolecular hydrogen bonding tend not to generate stable polydiacetylene liposomes. Therefore, the fluorescent diacetylene monomer 6 was mixed with 10,12-pentacosadiynoic acid (PCDA, 1) which is widely used as matrix lipid monomer and known to form stable liposome vesicles.1 The mixture of the fluorescent monomer 6 and PCDA 1 (monomer 6:PCDA = 3:7, molar ratio) was dissolved in chloroform and followed routine protocols for polydiacetylene vesicle formation. The liposome vesicles prepared showed intensive blue color and Figure 5 presents a schematic picture of the resulting polydiacetylene vesicle.

In order to investigate how efficiently the fluorescent diacetylene lipid monomers were incorporated into the matrix lipid 1 for liposome formation, the blue-colored liposome solution was dialyzed by using a membrane (cut-off molecular weight: 10,000) against ample amount of deionized water. Since the diameters of polydiacetylene vesicles, in general, are above 100 nm, the polymerized vesicles should not be able to penetrate the membrane. If there are any fluorescent monomers which were not participated in the liposome formation, we should be able to detect them in mother liquid because they can cross the membrane freely. Accordingly, we took both visible and fluorescence spectra of the solutions inside and outside the membrane after dialysis (Figure 6). As can be seen from the inspection of the spectra, the solutions inside the membrane show a typical visible spectrum for the blue-colored polydiacetylene vesicle (Figure 6, left (a)) and a fluorescence spectrum for the dansyl group (Figure 6, right (a)). No absorbance or emission peaks were observed in the mother liquid outside the membrane. These observations demonstrate that most, if not all, of the fluorescent diacetylene monomers 6 were incorporated into the liposome vesicles.

Conclusions

We have prepared a diacetylene lipid with a strongly fluorescent head group. The fluorescent monomer was readily synthesized from the commercially available 10,12-pentacosadiynoic acid (1). The fluorescent monomer was mixed with the matrix lipid 1 and subjected to routine procedures for the formation of polydiacetylene liposomes. Irradiation of the resulting liposomes with 254 nm UV light induced intermolecular addition polymerization and generated blue-colored polydiacetylene vesicles. Dialysis of the obtained liposomes with a membrane of molecular weight cut-off of 10,000 was performed. No fluorescent monomers were detected in the mother liquid after dialysis, confirming most of the fluorescent monomers were involved in the liposome formation. This is the first experimental proof using a fluorescent lipid monomer that the 10,12-pentacosadiynoic acid and its structurally analogous derivatives can be efficiently transformed into polydiacetylene liposomes. Thus, the results described above should give useful information on the development of polydiacetylene-based sensors.

Experimental Section

General. 10,12-Pentacosadiynoic acid (PCDA) was obtained from GFS Chemicals. Dansyl chloride, triethylamine (TEA), tetra(ethylene glycol), oxalyl chloride and 4-dimethylaminopyridine were purchased from Aldrich Chemical Co. UV-vis spectra were measured using an Agilent 8453E spectrophotometer. Fluorescence spectra were obtained with a Shimadzu RF-5301 spectrofluorophotometer. 1H and 13C NMR spectra were recorded using a Varian 300 NMR spectrophotometer.

Synthesis of the fluorescent lipid monomer 6. Oxalyl chloride (0.64 g, 5.08 mmol) was added dropwise to the solution of 10,12-pentacosadiynoic acid (1.00 g, 2.54 mmol) in 20 mL of methylene chloride. After stirring the resulting solution for 1 h, a drop of DMF was added to complete the reaction. The solution was stirred for another 3 h and concentrated in vacuo to give the acid chloride 2 which was used for the next reaction without further purification.

Triethylamine (0.74 g, 7.31 mmol) and dansyl chloride (1.65 g, 6.12 mmol) were added to a solution of tetra(ethylene glycol) (4.77 g, 44.48 mmol) in 50 mL of THF. After stirring overnight, the mixture was concentrated in vacuo and the residue was subjected to silica gel column chromatography (ethyl acetate) to give 1.64 g (62%) of the
highly fluorescent intermediate 5.

The acid 2 (0.88 g, 2.23 mmol) prepared as described above and triethylamine (0.28 g, 2.79 mmol) was added to a solution of intermediate 5 (0.80 g, 1.86 mmol) in THF. After stirring overnight at room temperature, the mixture was concentrated to give a residue which was subjected to silica gel column chromatography (50% hexane-ethyl acetate to 10% chloroform-methanol) to yield 0.38 g (26%) of the desired fluorescent monomer 6. 1H NMR (300 MHz, CDCl3) δ 0.88 (t, 3H), 1.20-1.60 (32H), 2.23 (t, 4H), 2.31 (t, 3H), 2.89 (s, 6H), 3.46-3.69 (m, 12H), 4.14 (t, 2H), 4.12 (t, 2H), 7.19-8.61 (6H); 13C NMR (75 MHz, CDCl3); δ 14.1, 19.2, 22.7, 24.8, 28.3, 28.9, 29.1, 29.6, 31.9, 34.2, 45.4, 63.3, 65.2, 68.6, 69.2, 69.5, 70.4, 115.5, 119.6, 123.1, 128.6, 129.8, 129.9, 130.5, 131.3, 131.5, 173.8.

Preparation of liposome. A mixture containing the fluorescent diacetylene monomer 6 and 10,12-pentacosadiynoic acid 1 (6 : 1 = 3 : 7 molar ratio) was dissolved in chloroform in a test tube. The solvent was evaporated by a stream of N2 gas and deionized water was added to the test tube to give the desired concentration of lipid (1 mM). The resultant suspension was sonicated (Fisher Sonic dismembrator model 550) at a temperature of around 80 °C. Following sonication, the solution was filtered to remove dispersed lipid aggregates by using 0.8 μm filter and cooled at 4 °C overnight. Polymerized diacetylene liposomes were prepared by UV irradiation (1 mW/cm²) with 254 nm for 10 minutes. Dialysis of the liposome was carried out with a membrane (MW cut-off: 10,000) against deionized water.

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References