Preparation, Characterization, and Sensing Behavior of Polydiacetylene Liposomes Embedded in Alginate Fibers

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ABSTRACT Polydiacetylene (PDA)-doped calcium alginate fibers were created by the solution blending of polymerized 10,12-pentacosadiynoic acid liposomes with sodium alginate in water prior to extrusion. The liposomes maintained their blue color during wet spinning and drying of the fibers but changed to red with exposure to specific external stimuli (heat, solvent, and chemical). In the latter case, the color change only occurred when the fibers were sufficiently permeable for the reacting species to reach the interior. A parameter termed the "Raman response" (RR) has been developed to quantify the amount of PDA liposomes in each of two critical conformations within the fibers. The RR attributes a quantitative measure of PDA response to individual stimuli. This method provides advantages over the commonly used "colorimetric response" in systems where sample limitations and chromophore activity make UV−vis spectroscopic measurements difficult or inaccurate. PDA liposomes are shown to effectively add a versatile sensing component to alginate fibers.

KEYWORDS: polydiacetylene • alginate • liposome • sensor • Raman spectroscopy

INTRODUCTION

Polydiacetylenes (PDAs) are highly conjugated materials that are typically formed through UV or γ irradiation of crystalline solids of substituted 1,3-butadiynes (diacetylenes, DAs). The resulting polymers undergo dramatic chromatic transitions that have generated interest in using them for sensing and reporting on changes in their local environment (1). Most PDAs appear blue as polymerized but change to red as they are exposed to stimuli such as heat, solvent, chemical, or mechanical stress.

PDA single crystals and crystalline fibers have been mixed into resins and laminates for the purpose of mapping strain in the host (2), while PDA microcrystals have been generated in situ by photolysis of phase-separated DA-containing copolymers (3, 4). In these studies, the strain in the host system was monitored by Raman spectroscopy, where small changes in the vibrational frequencies of the polymer backbone stretching modes were found to be linearly related to applied stress. Recently, work by Chae et al. has shown that DA monomers can be self-assembled during electrospinning of a host polymer (5) and then polymerized in situ.

Other self-assembled DA structures undergo topotactic polymerization as well, including Langmuir−Blodgett (LB) films, self-assembled monolayers or multilayers, and vesicles. The use of such structures has been of particular interest for the development of biosensors, as illustrated by recent review articles (6, 7). Liposomes, in particular, have been shown to be highly versatile, with the DA polymerization proceeding despite the incorporation of significant fractions of non-PDA lipids. This allows for additional functionality to be added to the system in order to modify the sensing behavior and physical and optical properties. Aqueous suspensions of liposomes tend to aggregate in the presence of bacteria or divalent cations. Therefore, efforts have been made to immobilize the vesicles in various ways. Approaches to this include immobilization on various surfaces or encapsulation in sol−gel matrixes (8) or agar (9). In some cases, the immobilization process itself triggers the blue−red color change, while in others, the sensing behavior of the liposomes can be preserved. The development of products made from polymer composites that include PDA sensors requires the development of procedures for preparing and processing the composites without triggering the color change.

Here, we demonstrate that PDA liposomes can be dispersed in alginate solutions and extruded as fibers without triggering the blue-to-red color change. Unlike the electrospinning work by Chae et al. in which the DAs are assembled during the spinning process and are polymerized in the resulting fibers (5), we are able to extrude the fully organized and polymerized liposomes. The liposomes retain their sensing ability and are able to detect chemical agents if the fibers are sufficiently porous for these species to reach the liposomes.

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Received for review March 13, 2009 and accepted May 14, 2009
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DOI: 10.1021/am900167r
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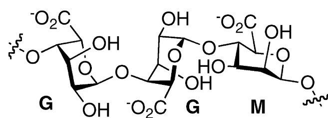

FIGURE 1. Alginic acid structure containing M and G segments.

Alginate, a general term used to describe alginic acid and its related salts, is a polymer extracted from the cell walls of brown algae. Alginic acid is a linear copolysaccharide containing β-D-mannuronate (M) and α-L-guluronate (G) segments (Figure 1) (10). The wet spinning of alginate fibers is based heavily upon its unique gelation properties with respect to mono- and divalent cations. When alginate chains are exposed to calcium, junction zones appear where G segments are dimerized by the divalent cations. This is known as the egg-box model (11). Fibers are created through extrusion of the spinning "dope" (degassed sodium alginate dissolved in water) through a spinneret into a calcium chloride coagulation bath (12, 13).

Sodium salts of alginates are widely used by the food and pharmaceutical industries as thickening agents. The insoluble calcium salts form gels, films, and fibers that are in products such as fireproofing agents, dental molds, textiles, and paper (14). The biocompatibility of alginates has also made them useful for biotechnology applications, such as in the area of tissue engineering (15).

MATERIALS AND METHODS

Materials. The DA 10,12-pentacosadiynoic (PCDA) was purchased from GFS Chemicals. The monomer was isolated from polymer impurities by acetone extraction before use. Medium-viscosity sodium alginate, calcium chloride, and α-cyclodextrin (α-CD) were purchased from Sigma Aldrich. Other reagents were purchased from commercial houses and used as received.

PCDA Liposome Synthesis. PCDA liposome dispersions (1 mM) were prepared by dissolving PCDA (3.75 mg, 0.01 mmol) into 5 mL of tetrahydrofuran (THF) in a 5 dram vial. THF was removed by rotary evaporation to leave a thin film of PCDA on the inside of the vial. Deionized water (10 mL) was added, and the vial was heated to 75 °C. After 10 min, a Branson Sonifier 450 sonication probe (10-mm-diameter tip) was lowered into the vial and the sample was sonicated at output control 5 (35%) for 15 min. Using a syringe, the sample was filtered through a 0.45 μm Whatman PES with a GMF disk filter. The filtrate was cooled at 3 °C for 12 h and then polymerized for 5 min using the 254 nm UV light from above (3 cm). For some experiments, 2 mM solutions were prepared by the same procedure, doubling the amount of PCDA.

Wet Spinning of PDA Liposome in Alginate: Small Scale. Small-scale spinning solutions were prepared by dissolving 2 g of sodium alginate in 100 g of deionized water. The viscous solution was stirred using a VWR Power Max Elite motorized stirring rod at 900 rpm. For the control solution, 2 mL of deionized water was added to 9 mL of a 2% sodium alginate solution. The PCDA–alginate blend was prepared by adding 2 mL of 2 mM PCDA liposome to 9 mL of a 2% sodium alginate solution. The solutions were mixed for 1 h with a magnetic stir bar and loaded into a syringe. Fibers were produced by extruding the alginate syringe contents through a 16-gauge needle into a 400 mL 15% calcium chloride ($CaCl_2$) coagulation bath at a rate of 1 mL/min. Once extruded, the calcium alginate fiber was moved into a 400 mL 3% $CaCl_2$ equilibration bath for 12 h. The fiber was cut into 12-in. sections and moved to a room temperature drying box for a minimum of 72 h.

Wet Spinning of PDA Liposome in Alginate: Large Scale. A large-scale control dope was prepared by dissolving 15 g of sodium alginate in 300 g of deionized water. Spinning dope containing PDA was prepared by dissolving sodium alginate (15 g) in 225 g of deionized water, to which 75 g of a prepared 2 mM PCDA liposome solution was added. The viscous solutions were stirred using a VWR Power Max Elite motorized stirring rod at 900 rpm. The alginate solutions were allowed to rest for 12 h to degas.

The control dope was placed in a stainless-steel vessel, pressurized with nitrogen gas at 25 psi, and allowed to equilibrate for 4 h. The solution was pumped into a 15% $CaCl_2$ coagulation bath through a 100-hole spinneret, hole diameter ca. 300 μm, at a rate of 1.65 cm^3/min. The resulting calcium alginate fibers were wound with a take-up speed of 31.4 cm/min. Samples were obtained by cutting yarn bundles from the roll at 30 s intervals and placing them into $CaCl_2$ equilibration baths of varying percentages (below). The procedure was then repeated for the spinning dope-containing PDA liposomes.

Equilibration baths, 1.5 L each, were prepared at $CaCl_2$ concentrations of 0%, 1%, 3%, 5%, and 10% by weight in deionized water. A minimum of two control and two doped bundles were immersed in the equilibration baths for 24 h and then dried under tension at room temperature. All experiments used dried fibers obtained from the 0% $CaCl_2$ equilibration bath unless otherwise specified.

Fiber Thermal Response. The temperature response of the PDA liposome within the alginate fiber was examined via thermal treatment. Fibers were cut into 30 mm sections (five doped and five control) and heated to 60 °C. The time was recorded at the first sign of red and when the fiber appeared to have completely changed in color. Treated fibers were inspected visually, and Raman spectra were collected.

Fiber α-CD Response. Sensitivity of the PCDA liposome to α-CD in solution has been documented (16). It is also known that introducing sodium ions to a calcium alginate fiber increases its water solubility (5). Therefore, 20 mm samples of doped calcium alginate fibers (and controls) were exposed to varying levels of NaCl combined with several different α-CD molarities. To separate vials, 0 mg, 24.9 mg (10 mM), 124.5 mg (50 mM), 249 mg (100 mM), 1.25 g (500 mM), or 2.49 g (1 M) of α-CD was added to 10 g of deionized water. To the same vials, 0%, 1% (100 mg), 2% (200 mg), or 5% (500 mg) of sodium chloride was added, by weight, to the water.

Raman Spectroscopy. Raman spectra were collected on a Renishaw Raman Fiber Optic Probe system using a 745 nm high-powered diode laser. Data were collected by 180° backscattering on a ~25 μm sample size. Scans were set to 100% laser power for a minimum of 30 s (60 s when a higher S/N ratio was needed) within the wavenumber range of 700–2500 cm^{-1}. Computer analysis was performed using *GRAMS/32* software, and peak positions were calculated by combined Gaussian/Lorentz statistical methods. Statistical sample analysis revealed deviations of less than 0.5 cm^{-1} per peak center position.

Liposome Size Analysis. Particle size analysis was carried out on PDA liposomes in deionized water by dynamic light scattering using a PCS submicrometer particle size analyzer with a polystyrene standard. Particle imaging utilized a 4800 scanning electron microscope with a transmission electron microscopy (TEM) attachment. Samples were placed on a TEM mesh grid and examined up to 80 000× magnification.

RESULTS AND DISCUSSION

Liposome Preparation. DA liposomes were prepared as previously described (17). Dynamic light scattering indicated a bimodal particle distribution. The largest particles (69.7%) were 144 ± 22 nm in diameter and are consistent with a liposome (dual-layer micelle) assembly of PCDA. The

secondary particle size (21%) was 46 ± 6 nm in diameter and most likely represents a micelle of PCDA. Both sizes of particles were found to be spherical by TEM. Polymerization of the DA dispersion with UV light led to formation of the characteristic deep-blue PDA dispersion. Previous reports demonstrate that the polymerization reaction does not significantly affect the liposome diameter (18).

Quantification of Sensor Response: Raman Response (RR) Equation. The response of PDA liposomes to stimuli has previously been quantified by measuring the ratio of the electronic absorbance of the blue form to that of the red form (19). This "colorimetric ratio" (CR) has made the usefulness of the PDA sensor response semiquantitative; however, there are limitations to the technique. The electronic absorbencies are quite broad and are not completely resolvable. Scattering at shorter wavelengths (especially problematic when the liposomes begin to aggregate) distorts the baseline and the CR. The measurement is also susceptible to interferences by other species that might be present in the testing solution or within the liposome itself. For example, we recently described a PDA liposome pH sensor that was reversible in both its absorbance response and the response of a fluorophore embedded in the hydrophobic lipid layer (20). The absorbance of the fluorophore interfered with the calculation of the CR.

In the current system, we found that scattering from the alginate host matrix made collection of the UV−vis spectrum problematic. However, there is an alternative to the CR that takes advantage of the Raman scattering of the PDA backbone. The polymer undergoes dramatic resonance-enhanced scattering from the conjugated backbone vibrational modes. The alkyne stretching vibration, in particular, is an efficient scatterer and comes in a region of the vibrational spectrum that has little interference from other functional groups. The alkyne stretching vibration of the blue, planar form of PCDA was observed at 2085 cm^{-1}, while that of the red form was observed at 2111 cm^{-1} (21). Thus, the changes in PDA-embedded alginate fibers were quantified through the development of a RR equation (eq 1). RR is defined as the intensity (I) of the red form alkyne stretching peak divided by the sum of the intensities of the red and blue carbon triple-bond peaks. Advantages of RR as compared to CR include a larger variety of sample materials, much narrower line widths, and relative immunity to many of the matrix interferences that plague CR analysis. As-formed PDA liposomes should have a RR of approximately 0%, while liposomes that have completely converted to the red form should have a RR of 100%.

$$\mathrm{RR} = \frac{I_{2111\ \mathrm{cm}^{-1}}}{I_{2111\ \mathrm{cm}^{-1}} + I_{2085\ \mathrm{cm}^{-1}}} \times 100 \qquad (1)$$

PDA/Alginate Fiber Preparation and Characterization. Extrusion of a monovalent cation alginate solution (sodium alginate) into a divalent cation coagulation bath (calcium chloride) results in an ion exchange and alginate fiber formation (11). In wet spinning of alginate, the calcium acts as a cross-linker between alginate polymer chains, creating a water-insoluble fiber. In this study, PDA liposomes were blended into a sodium alginate solution prior to extrusion. Control and PDA-doped fibers were produced on a small scale by extrusion through a syringe needle into a coagulation bath. A loading percentage of 0.5% of 2 mM PCDA to alginate by weight produced brilliant blue fibers upon drying.

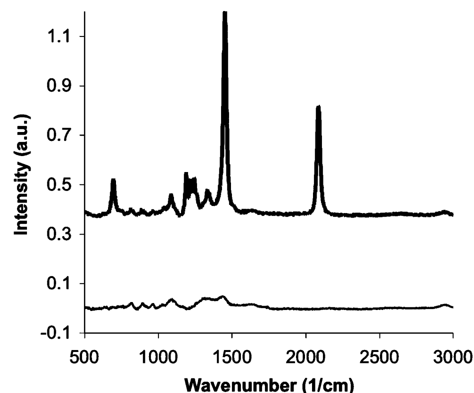

FIGURE 2. Raman spectra of the alginate fiber control (bottom) and with PCDA liposome (top).

Fibers were also produced on a large scale using typical wet-spinning apparatuses. In agreement with the small-scale wet-spinning experiment, the control alginate fibers were white and the fibers with embedded PCDA liposome were blue. The filament was generated from a 100-hole spinneret and samples were collected in bundles of 10 filaments. Separation of the bundles was not possible because of cross-linking of the fibers that took place in the equilibration bath, though single filaments could have been isolated by placing them into individual equilibration baths. Filaments were dried for 1 week under slight tension to produce fibers without bends or kinks.

The Raman spectrum of alginate microspheres has a rich fingerprint region (Figure 2) (22). The Raman spectrum of the alginate control fiber contained peaks assigned to the following groups: C═O (1670−1640 cm^{-1}), ethers (1300−1000 cm^{-1}), $-CH_2$ bend (1465 cm^{-1}), and C−H out-of-plane bend (1000−650 cm^{-1}). However, these peaks were difficult to resolve in the spectrum of the PCDA embedded alginate. This is a result of the peaks corresponding to the PDA, which even at low loading greatly overshadow those from the alginate. The most prominent peaks within the PCDA spectrum are the carbon triple-bond stretch at 2085 cm^{-1} and the carbon double-bond stretch at 1450 cm^{-1}. Spectral peaks within these regions have been correlated to the blue, planar state of the PDA backbone (23).

Sensing Performance. Alginate fibers are typically spun into a coagulation bath and then placed in an equilibrium bath for several hours. While the fiber spinning and coagulation process did not affect the deep-blue color of the encapsulated liposomes, it was seen that samples submerged in calcium chloride equilibration baths for 12 h did result in the partial conversion of the polymer to the red form. Increasing the submersion time over 12 h did not yield a larger colorimetric transition; however, the color change

FIGURE 3. Photographs of alginate bundles (1 in. long) with embedded PCDA liposome dried after $CaCl_2$ baths. From left: 0%, 1%, 3%, 5%, and 10%.

Table 1. RR of Alginate Fibers with PCDA Liposomes Dried after Submersion in Various Concentrations of $CaCl_2$

$CaCl_2$ bath (%)	color	RR (%)
0	blue	0.0
1	purple	7.7
3	purple	14.0
5	red	15.4
10	red	20.8

was directly related to the concentration of calcium chloride (0%, 1%, 3%, 5%, and 10% $CaCl_2$; Fig. 3). The color shift to red was quantified using the RR equation, and the results are tabulated in Table 1.

During the initial extrusion of the fibers into the $CaCl_2$ bath, large changes occur in the molecular structure of the polymer and in the physical properties of the fiber. However, these changes did not trigger the red-to-blue color change, nor were small shifts in the backbone vibrational modes observed, as seen with PDA crystals under mechanical stress (2−4). This suggests that the liposomes are not interacting strongly with the host polymer and that the color change caused by the equilibration bath was due to a secondary interaction between the liposomes and the calcium ions. This is not unexpected because immobilized PDA vesicles have been found to be sensitive to physical, chemical, and biological stimuli (15, 24). Fibers that were taken from the coagulation bath and allowed to equilibrate in pure water have physical properties that are similar to those that equilibrated in the $CaCl_2$ baths, yet the fibers remain blue.

Calcium and cadmium chloride have previously been used with PCDA LB films as a stabilization agent for the carboxylic acid head group during monolayer deposition. The divalent cations impact the DA organization through chelation and inhibited polymerization (25). In these cases, the DA's inability to polymerize indicated a perturbed stacking arrangement by the divalent cations. In our alginate composite, chelation of the calcium was thought to alter the organization of the carboxylic acid-containing side chain and trigger the blue−red transition.

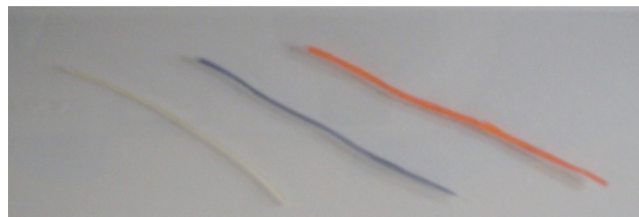

FIGURE 4. From left: Photograph of the control alginate fiber (white), as-spun alginate fiber with PCDA liposome (blue), and alginate fiber with PCDA liposome after exposure to 60 °C for 45 s (red).

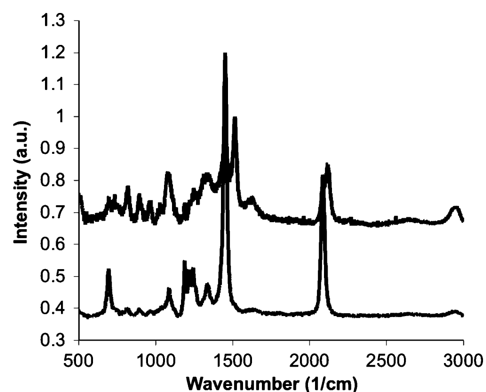

FIGURE 5. Raman spectra of alginate fibers with PCDA liposome as-spun (bottom) and after exposure to 60 °C for 45 s (top).

To test this idea, sodium chloride was added to an aqueous PDA liposome dispersion. No change in the RR (or CR) was observed. However, the addition of calcium chloride to the PDA liposome dispersion yielded an immediate color change from blue to red, indicating disruption to the planarity of the backbone. The alginate fibers mimicked the colorimetric transitions observed in the liposome dispersions with respect to the sodium and calcium ions. This experiment suggests that alginate fibers containing PCDA liposomes may also be useful in the detection of divalent cations.

The doped fibers, like the liposome dispersions, displayed a rapid response when heated to temperatures greater than or equal to 60 °C. Raman spectra were taken of alginate fibers with embedded PCDA liposomes after thermal exposure and compared to the spectra obtained from the as-spun fibers (Figures 4 and 5). The as-spun fiber contained a carbon triple-bond peak at 2087 cm^{-1}; when exposed to heat, a portion of that peak shifted to 2119 cm^{-1}, corresponding to the red portion of PCDA. The RR calculated for the thermally treated fibers was 78.9%. The visual color of the fiber as well as the RR did not change upon cooling.

The ability of α-CD to penetrate into a film or fiber and interact with the PDA molecule is a good preliminary indicator of the system's ability to act as a chemical or biological detector (25−27). The calcium alginate fibers showed no sensitivity to α-CD when combined in deionized water because of the inability of water to penetrate into the fiber. However, the addition of sodium chloride to the solution enabled some conversion to sodium alginate, thus reducing the cross-linking effect of calcium to enable a degree of water solubility; as a result, the solution could penetrate the fiber. In solutions containing NaCl and α-CD, a bundle of alginate containing PCDA liposomes showed a visual color change

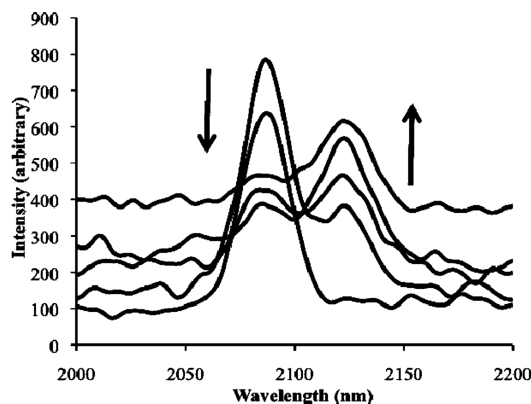

FIGURE 6. Raman spectra collected for alginate fibers with PCDA liposomes after exposure to (bottom to top) 10, 50, 100, 500, and 1000 mM α-CD and 1% NaCl.

Table 2. RR Percentages (%) of Alginate Fibers with PCDA Liposomes Exposed to α-CD and NaCl

α-CD (mM)	0% NaCl	1% NaCl	2% NaCl	5% NaCl
0	0	0	0	0
10	0	0	10.1	11.8
50	0	24.6	12.9	11.3
100	0	54.7	50.1	52.6
500	0	72.0	71.5	82.8
1000	0	75.6	76.0	76.6

to red and a RR up to 75% (Table 2 and Figure 6). Higher concentrations of α-CD dissolved in the solutions caused an increase in the RR; however, adding more than 1% NaCl by weight did not have an effect on the color or the RR.

CONCLUSION

Polymerized PCDA liposomes were solution-blended with sodium alginate and wet-spun into calcium alginate fibers. Using deionized water as an equilibration bath instead of the common 3% $CaCl_2$ allowed the embedded PCDA liposomes to retain their blue color within the alginate fiber. A color response was observed in fibers that were exposed to calcium ions, heat, various solvents, and α-CD (with 1% NaCl). In all experiments, the RR equation provided quantitative values for the percentage of PCDA chains in the red and blue forms. This method provides significant advantages over the commonly employed CR in systems where sample limitations and chromophoric activity make analysis by UV—vis spectroscopy difficult. The alginate fiber acts as a stable host for the PCDA liposome with only minor limitation to its functionality. The packaging of PDAs into alginate fibers allows for application into various types of thermal and chemical sensing.

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