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Glycopolydiacetylene nanoparticles as a chromatic biosensor to detect Shiga-like toxin producing *Escherichia coli* O157:H7

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Abstract—Shiga toxin-producing *Escherichia coli* organisms (STEC) were detected by Gal-α1,4-Gal glycopolydiacetylene (GPDA) nanoparticles through the selective binding between Shiga toxin and GPDA nanoparticles. The binding produced a colorimetric change in the absorption wavelength of the GPDA nanoparticles. This method provides a highly selective, rapid, sensitive, and quantitative approach for the detection of STEC.
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Shiga toxin-producing *Escherichia coli* organisms (STEC) are pathogens capable of producing sporadic and epidemic diarrhea, hemorrhagic colitis, and potentially lifethreatening hemolytic-uremic syndrome (HUS).[1–6] *Escherichia coli* O157:H7, the most common identified STEC serotype, alone causes an estimated 60 deaths and 73,000 illnesses annually in the United States.[1] Most cases are associated with food or waterborne infection. Shiga toxins (Stx) are cytotoxins and major virulence factors of STEC. There are two principal serologically distinguishable types of Stx: Stx1 and Stx2. They are both composed of one enzymatically active 'A' subunit and five identical copies of a 'B' subunit. The pentamer B can bind to the terminal Gal-α1,4-Gal disaccharides on the surface of host cells.[6]

Therefore, this carbohydrate–toxin interaction can be applied to detect STEC. It has been well-known that glycopolymers, glycodendrimers, and glycol conjugated nanoparticles have been widely used as anti-adhesion molecules for toxins[7–11] and bioprobes to monitor carbohydrate–protein interactions.[12–14] Of all these different types of sugar-containing materials, glyconanoparticles show perhaps the greatest potential for the application of carbohydrate–protein interactions. The diameter of the nanoparticles is comparable with a biomolecule. In addition, they have well-defined and easily manipulated chemical structures. All these make glyconanoparticles suitable mimics of glycoproteins. 1,3-diacetylenic acid derivatives[15] were polymerized to give Glycopolydiacetylene (GPDA)[16] nanoparticles (see Scheme 1). The unique 'blue-to-red' colorimetric transition of GPDA nanoparticles bound with macromolecules has been utilized to monitor the ligand-receptor binding events for viruses,[17–19] toxins,[20,21] bacteria,[22,23] and antibody-receptor interactions[24,25].

Recently, nanoparticles were also applied for measuring biomolecules related to NIH biodefense program.[26] Therefore, it is of great interest to apply the GPDA nanoparticles to detect Shiga toxin or related toxins in the NIH biodefense program. Herein, the GPDA nanoparticles with Gal-α1,4-Gal disaccharides on the surface were used to detect the Shiga toxins from *E. coli* O157 on 96-well plates. The success of this method suggested that the GPDA nanoparticles could be applied to environmental samples as a highly selective, sensitive, and fast responsive colorimetric biosensor to monitor the STEC.

In order to select glyconanoparticles with a narrow size distribution for the quality control, the size and size distribution of GPDA nanoparticles synthesized with

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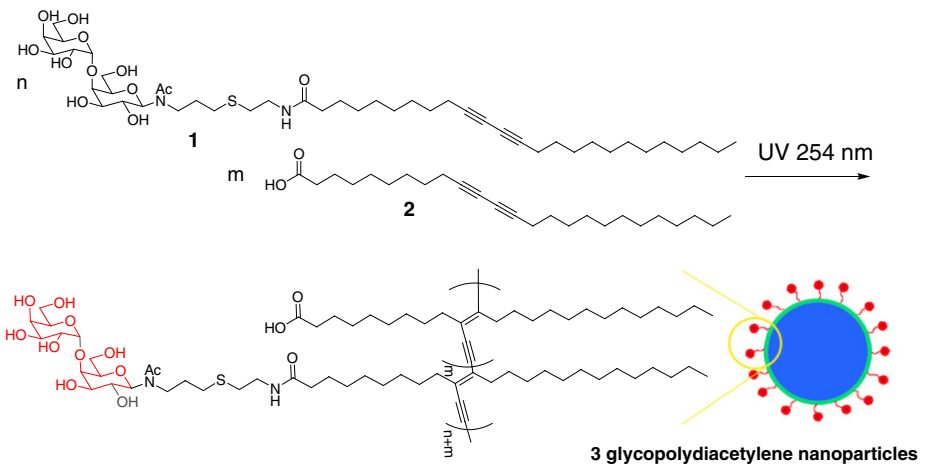

Scheme 1. Synthesis of glycopolydiacetylene nanoparticles.

different exposure times under UV light were measured by dynamic light scattering (DLS) at 30 °C by a BI-DNDC differential refractometer (Brookhaven Instruments) with a 10 mW He–Ne laser beam of 633 nm. A scattering angle was held constant at 90°. The sample solution was filtered with 0.1 μm filter from Whatman. In Figure 1, it was found that longer exposure to the UV gave a narrower distribution of nanoparticles (more than 90% particles with a diameter of 120 nm, PDI of 0.18), while the shorter exposure gave a mixture of nanoparticles (~45% of nanoparticles has a diameter of 56 nm and ~45% of that has a diameter of 100 nm). Thus, the nanoparticles synthesized with longer exposure in UV were selected to test the detection of Shiga toxins. Two experiments were carried out to detect STEC. The first experiment investigated the response selectivity of the glyconanoparticles with STEC versus non-toxin producing *E. coli*; the second one was to determine the minimum concentration of the STEC and the quantitative relationship between the concentration of STEC and the colorimetric response.

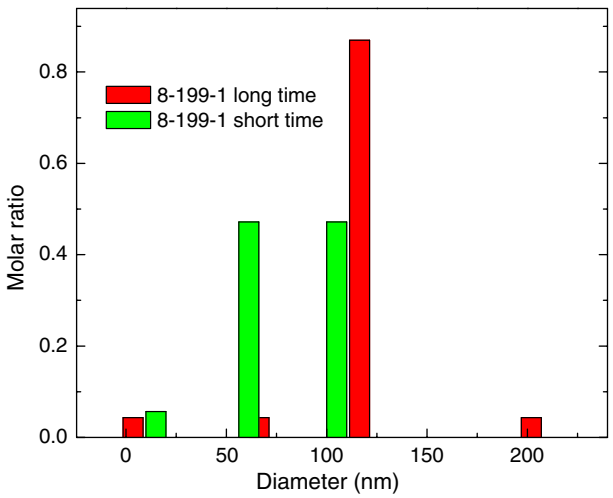

Figure 1. Measurement of the particle size of polydiacetylene liposomes by dynamic light scattering (25 °C, 633 nm).

First, non-Shiga toxin producing *E. coli* (B-5), Shiga toxin producing *E. coli* O157 (C-5, #15, Stx1; D-5, #17, Stx2; and E-5, #58, Stx1/Stx2) were mixed with GPDA nanoparticle solution (50 μL, 0.6 mg/mL) on 96-well plates and incubated for 10 min, with glyconanoparticle solution as a control (F-5) (Fig. 2). It showed that the well with *E. coli* O157 secreting Stx1 or Stx2 changed the color from purple to brown in 5 min. While non-Shiga toxin producing *E. coli* (B-5) solution retained the purple color with the added GPDA glyconanoparticle. This result demonstrated the high selectivity of this method to monitor the Shiga toxin-producing *E. coli* compared with non-toxin-producing *E. coli*. From Figure 3, the visible absorbance intensity change between 625 and 535 nm explains the visible color change of the samples. For the Shiga toxins producing *E. coli* O157 samples, the absorbance intensity increased at 625 nm and decreased at 535 nm, indicating the binding of Shiga toxin with GPDA nanoparticles and resulting a brown color. For the *E. coli* without Shiga toxins (sample G in Fig. 3a), the absorption had a similar trend as that of GPDA nanoparticle solution (sample H) to give a purple color. This indicated that there was no unselective binding between *E. coli*. and GPDA nanoparticles.

A series of *E. coli* (#17, Stx2) were mixed with GPDA nanoparticle solution (20 μL, 0.6 mg/mL) and the visible absorption was measured by a plate reader (Flexstation 3, Molecular devices). *E. coli* (#17, Stx2) was chosen because Stx2 is the major virulence factor for hemolytic uremic syndrome (HUS).[7] The experiment investigated

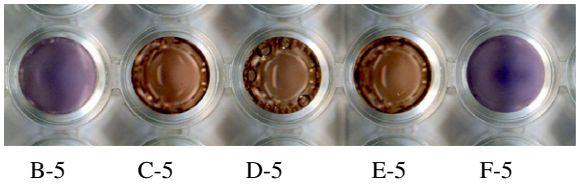

Figure 2. Colorimetric detection of Shiga toxin from *E. coli* O157 in JN#8-199-1 (long) solution. B-5: *E. coli* (non 157); C-5: *E. coli* (#15); D-5: *E. coli* (#17); E-5: *E. coli* (#58); F-5: JN#8-199-1.

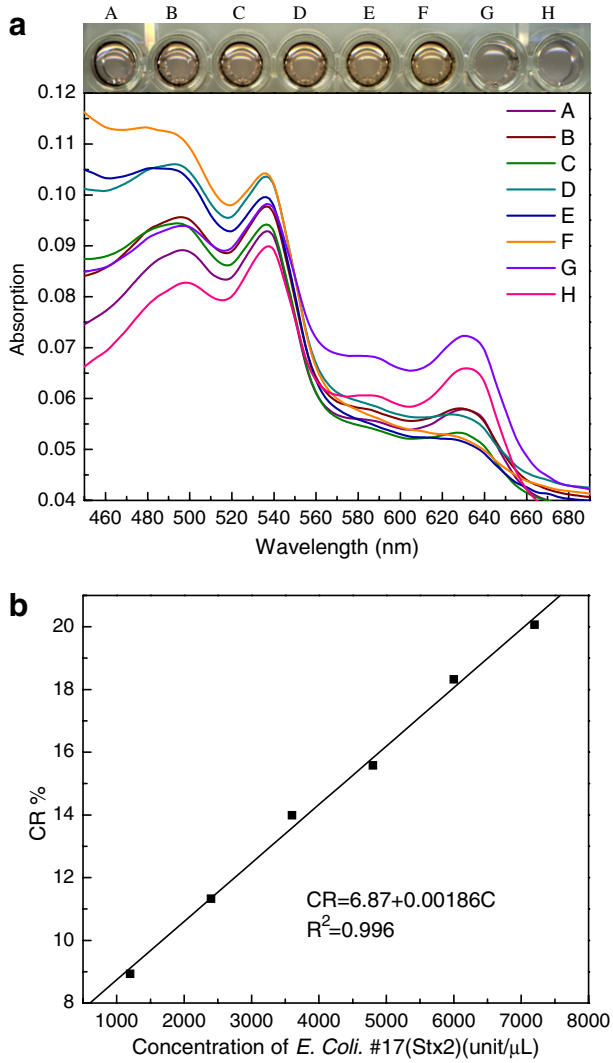

Figure 3. Colorimetric detection of Shiga toxin from *E. coli* (#17, Stx2) by PDA glyconanoparticles. (a) Visible spectra of glyconanoparticles (20 μL) with different concentrations of *E. coli* #17 (Stx2): A 1200, B 2400, C 3600, D 4800, E 6000, F 7200, G 1020 U/μL (non-Shiga toxin secreting *E. coli* O157) H PDA glyconanoparticle solution. (b) Plot of the colorimetric response of the glyconanoparticles as a function of *E. coli* concentration in U/μL.

the colorimetric relationship between visible absorption and the concentration of the samples to give the detection limitation concentration as well as the linear range. The colorimetric response (CR) is defined as the following equations according to the literature[27];

$$B = I_{625}/(I_{625} + I_{535})$$
$$\mathrm{CR} = [(B_0 - B_t)/B_0] \times 100\%$$

where B_0 refers to the ratio of absorption at 625 nm and the total absorption maxima at 625 nm and 535 nm of the GPDA nanoparticle solution; B_t refers to that of the GPDA nanoparticles mixed with *E. coli* (#17, Stx2) solution; and the percentage ratio of the variance B value due to forming complex over the original B value will be referred as CR. It was found that a CR value of 10–15% was readily discernible by the naked eye in order to verify the existence of STEC. Therefore, the CR value as well as visual difference will be compared to determine the minimum concentration of STEC that can be detected. In Figure 3a, it is shown that there was still visual difference between the lowest dose of *E. coli* (#17, Stx2) (sample A) and pure glyconanoparticle solution H. In Figure 3b, the CR value for sample A was around 9%. The color difference between sample A and sample G could be still discernible. This indicated that the limits of detectable concentration for *E. coli* (#17, Stx2) would be 1200 U/μL. Compared with some bacterial biosensors,[28] the limitation of which ranged from 10 to 6000 U/μL according to different methods, this method had a relative high limitation. However, this method does not need a sophisticated instrument and can be observed by the naked eye. Besides this, in Figure 3b, it is found that there is good linear relationship between the CR value and the concentration of *E. coli* (#17, Stx2) from 1200 to 7200 U/μL.

In summary, the successful detection of STEC by the Gal-α1,4-Gal disaccharide glyconanoparticles in solution indicates that this method is highly selective, rapid, sensitive, and quantitative. The minimum detectable concentration of *E. coli* was 1200 U/μL. It was also found that the colorimetric response (CR) from the STEC and GPDA nanoparticle mixture showed linear relationship with the concentration of STEC from 1200 to 7200 U/μL. This method can be applied to routine checking in monitoring for STEC in food and water supplies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.055.

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15. *Description of the synthesis of Gal-*α1,4-Gal *lipid 1. NMR spectra and Mass Spectra data are given in* supplementary material section. Gal-α1,4-Gal (83 mg, 0.24 mmol) was dissolved in 15 mL of freshly distilled allylamine (Aldrich) and stirred for 72 h at ambient temperature under nitrogen.[29] Excess allylamine was removed by vacuum and the crude solid evaporated several times from toluene. The white solid was cooled to 5 °C, a solution of 2 mL acetic anhydride and 2.5 mL pyridine was added, and the reaction mixture was allowed to warm to ambient temperature and stirred for 12 h under nitrogen. The resulting crude peracetylated *N*-allylglycosylamide was evaporated to viscous yellow oil and evaporated several times from toluene. The product 3 was purified by flash chromatography over silica with ethyl acetate/hexane (solvent ratio 1:1 gradient to 2:1) and obtained as colorless oil (175 mg, 99%). The peracetylated carbohydrate was deacetylated[29] by stirring in dry methanol with a catalytic amount of dry sodium methoxide for 2.5 h. DOWEX 50 WX8-400 ion exchange resin was added until the pH became neutral and the resin was removed by filtration. The deacetylated *N*-allylglycosylamide[4] was evaporated in vaccuo to give the clear oil (86 mg, 91%) and used without further purification. The *N*-allylglycosylamide product 4 (35 mg, 0.08 mmol) was dissolved in degassed water (0.5 mL) containing cystamine hydrochloride (9 mg, 0.08 mmol), sealed from oxygen, and irradiated by a UV pencil lamp for 3 h, under argon. The resulting thioether was neutralized by addition of sodium bicarbonate and evaporated to a crude solid. The product 5 was purified by flash chromatography over silica with ethyl acetate/isopropanol/water (solvent ratio 1:2:1 gradient to 0.5:1.5:2) and obtained as colorless oil (36 mg, 90%). The amino-thioether-*N*-allylglycosylamide 5 (24 mg, 0.048 mmol) was dissolved in dry DMF (0.5 ml) containing triethylamine (0.1 ml). *N*-Succinimidyl-10,12-pentacosadiynate[18] (23 mg, 0.048 mmol) was dissolved in 0.5 ml chloroform, added and the reaction stirred for 12 h. The crude product was added to NaCl and $NaHCO_3$ saturated water and extensively extracted out of the water by 25% isopropanol/chloroform. The organic layer was dried ($MgSO_4$), filtered, and evaporated to a crude product. The Gal-α1,4-Gal-(linked)-10,12-pentacosadiynate[1] product was purified by flash chromatography over silica with chloroform/methanol (solvent ratio 4:1) and obtained as a colorless oil (17 mg, 42%). The structure was confirmed by high field NMR and mass spec (MH^+ = 857.9).
16. *Description of the synthesis of GPDA nanoparticles.*[30] Briefly, Gal-α1,4-Gal-10,12-pentacosadiynate **1** (0.3 mg, 0.3 μmol) and 10,12-pentacosadiynoic acid **2** (GFS Chemicals) (0.3 mg, 0.7 μmol) were mixed and evaporated to a film. Deionized water (1 mL) was added to the films so as to give a 1 mM (total lipid) suspension. The suspension was heated to between 70 and 80 °C and probe sonicated for 10 min. The resulting clear solution was then cooled to 5 °C for 20 min, warmed to ambient temperature, and polymerized by UV light irradiation (254 nm) for 5 min (short exposure) or 15 min (long exposure). The deeply blue colored solutions were syringe filtered through 0.2 μm cellulose acetate filters in order to remove trace insoluble aggregates. Essentially all of the lipid material (>98%) is incorporated into the soluble liposomes GPDA nanoparticles as determined by the weight of material recovered after filtration.
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