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Electrochemical and Raman Studies of the Biointeraction between *Escherichia coli* and Mannose in Polydiacetylene Derivative Supported on the Self-Assembled Monolayers of Octadecanethiol on a Gold Electrode

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Here, we describe a new method to study the biointeraction between *Escherichia coli* and mannose by using supramolecular assemblies composed of polydiacetylene supported on the self-assembled monolayer of octadecanethiol on a gold electrode. These prepared bilayer materials simply are an excellent protosystem to study a range of important sensor-related issues. The experimental results from UV−vis spectroscopy, resonance Raman spectroscopy, and electrochemistry confirm that the specific interactions between *E. coli* and mannose can cause conformational changes of the polydiacetylene backbone rather than simple nonspecific adsorption. Moreover, the direct electrochemical detection by polydiacetylene supramolecular assemblies not only opens a new path for the use of these membranes in the area of biosensor development but also offers new possibilities for diagnostic applications and screening for binding ligands.

Interactions between biomolecules on the cell surface play major roles in numerous biochemistry processes. For example, the immune system can recognize an invading pathogen (disease-producing bacteria, fungi, protozoa, and viruses) through the interactions among distinct components (and, hence, treated as foreign) and then the triggering of pathways that leads to the destruction of the invader.

Self-assembled monolayers (SAMs) of alkanethiol on gold are well-suited for studying biomolecular recognition at surfaces, because they form well-defined structures and are amenable to detail characterization at a molecular level and addressable by a variety of bioanalytical techniques (e.g., optical[1] and electrochemical[2,3]). Moreover, they also provide one template for the attachment of additional molecules or molecular layers to form a bilayer, which can mimic the structural and functional roles of biological membranes.[4−8] These hybrid surfaces have been developed to overcome the fragility of conventional mimic membranes while preserving aspects of lateral fluidity observed in natural biological membranes. Therefore, the development of a bio-bilayer on a gold surface has attracted considerable attention.

Apparently, studies on the interactions between the biomolecules are necessary to understand various biological phenomena. Recent studies on the interactions have focused primarily on supramolecular assemblies coupled with the receptors of specific cell.[9−12] It has been reported that when the amphiphilic diacetylenic lipid was assembled into ordered arrays, it could be polymerized by UV irradiation into a blue polydiacetylene polymer.[13] The incorporated receptors in the polydiacetylene matrix bound to the certain target molecules, triggering a chromatic phase transition in the membranes[14−18] and, thus, provided a method for colorimetric detection of the target molecules. Different from previous approaches, this method allows molecular recognition and optical reporting to occur within one single macromolecular assembly. Most of bacteria and toxins use specific biomolecular recognition and binding at the cell surface as the first step toward invasion.[19] On the basis of these phenomena,

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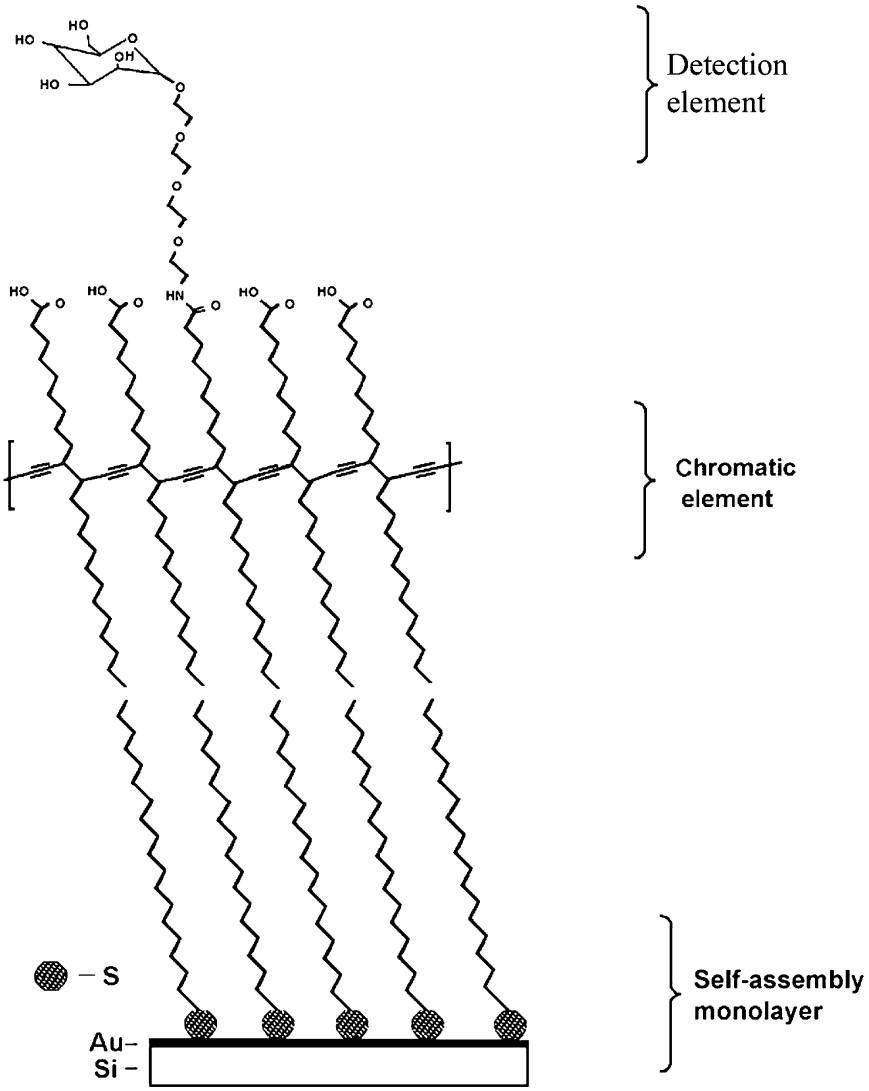

Figure 1. Schematic illustration of the bilayer structure composed of polydiacetylene derivative supported on the SAMs of octadecanethiol on a gold electrode. Mannose is the ligand-binder receptor on the polydiacetylene surface.

one expects to find a simple method to study the specific biomolecular recognition on supramolecular assembly.

In this paper, we report a simple method to study the biointeraction between mannose and *Escherichia coli*, which is the leading cause of urinary tract infection,[20] through a polydiacetylene derivative on the SAMs of a octadecanethiol-modified gold electrode by Raman and electrochemical methods. Mannose binds specifically to FimH adhesin of bacterial type 1 pili in *E. coli*.[21] Type 1 pili are filamentous proteinaceous appendages that extend from the surface of many Gram-negative organisms and are composed of FimA, FimF, FimG (structural pilus subunits), and FimH proteins (mannose-binding adhesion). [22] FimA accounts for >98% of the pilus protein, and FimH is uniquely responsible for the binding to D-mannose.[23,24] The FimH adhesin is also a two-domain protein with a carboxy-terminal pilin domain, which is used for incorporation into the pilus and an amino-terminal mannose-binding lectin domain.[25] The biointeraction between mannose and *E. coli* has been studied by using colorimetric detection,[15] fluorescent images,[26] magnetic resonance technology,[27] Raman spectroscopy, transmission electron microscopy,[21] etc.

Figure 1 shows the schematic diagram of the bilayer membrane structure with a mannose group as the ligand-binding receptor. RRS and electrochemical results showed that only the specific biomolecular interactions on the surface of the polydiacetylene could dramatically change the important interfacial characteristic, such as defect density and alkyl chain crystallinity. The direct electrochemical detection demonstrates the great potential of the bilayer as a sensitive coating for biological sensing applications.

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EXPERIMENTAL SECTION

Chemicals. 10,12-Pentacosadiyonic acid (PDA) was purchased from Farchan Laboratories (U.S.A.) and was recrystallized from petroleum ether before use. Dichlordimethylsilane was purchased from Germany, and octadecanethiol was from Aldrich. *p*-10,12-Pentacosadiyne-1-*N*-(3.6.9-trioxaundecylamide)-α-D-mannopranoside (MPDA) was provided by Dr. Peng Wang (Department of Chemistry, University of Miami), and *E. coli K12* (44106-2)[28,29] used in this study originally was a kind gift from Prof. Fangyu Hou (School of Basic Medicine, Jilin University, China). All other reagents were of analytical grade and were used without further purification.

Electrode Preparation. The gold substrates were fabricated by evaporation of an adhesion layer of titanium (10 nm), followed by a layer of gold (100 nm) in a diffusion-pumped chamber onto a pretreated silicon wafer. First, the wafer was sonicated in acetone and ethanol for 15 min, respectively, then it was placed in sulfate solution and heated for 5 min. The fresh evaporated gold film was immediately cleaned with freshly prepared piranha solution (H_2SO_4/30% H_2O_2, 3:1 (V/V)) for 5 min followed by rinsing with Milli-Q water (18 MΩ cm) and ethanol and drying thoroughly in a stream of nitrogen.

Bilayer-Modified Electrode Preparation. The self-assembled monolayer modified electrode was prepared by immersing evaporated the gold film into an ethanol solution of octadecanethiol (1 mM) at room temperature for 10 h. Solutions were then removed, and the gold films were rinsed with ethanol three times and dried under a stream of nitrogen.

PDA/MPDA (1.0 mM; mole ratio, 20:1) dissolved in a mixed solvent of chloroform and methanol (volume ratio, 5:1) was spread onto the surface of a KSV-5000 twin-compartment Langmuir trough. The film was compressed to a constant pressure of 20 mN/m at a speed of 4 mm/min, room temperature, and was allowed to equilibrate for 20 min. Subsequently, it was irradiated with 254-nm light from an ultraviolet lamp for 10 s, after which it was transferred to the gold electrode surface covered with the SAMs of octadancethiol by horizontally lifting the modified gold electrode from the water surface.

Bacterial Solution Preparation. *E. coli K12* (44106-2) was cultured aerobically at 37 °C for 18 h on solid medium, pH 7.4, in a 1-L beaker containing 10% peptone, 5% NaCl, and 20% agar. The solid medium was warmed and solved.[21] The autoclave was operated at a pressure of ~15 lb/in.^{2} (at 121 °C) for 30 min. The bacteria on the medium were washed with normal saline three times and centrifuged at 1700*g* for 10 min, and, finally, diluted to a concentration of 9×10^8 cells/mL with physiological saline, which was determined by turbidimetry.

UV−Vis Spectroscopy. UV−vis spectra were measured by using a UV-360 spectrometer (made in Japan, Shimadzu).

Resonance Raman Spectroscopy (RRS). Resonance Raman Spectra were measured by using a Raman Infinity Spectrophotometer (made in France) at a resolution of 4 cm^{-1}. The 488-nm line with a power of 50 mW from an argon ion laser was used as the excitation source. A polarizing beam splitter composed of two half-wave plates and a polarizing cube was used to control continuously the power of the exciting radiation reaching the samples. The polarization of the 488-nm beam was oriented perpendicular to the entrance slit of the spectrometer in order to generate RR scattering. The surface of the PDA/MPDA modified substrate was oriented at 90° with respect to the laser beam with a spot of 30 μm. To avoid the influence of the photoinduced thermochromism, the sample was held at lower temperature.

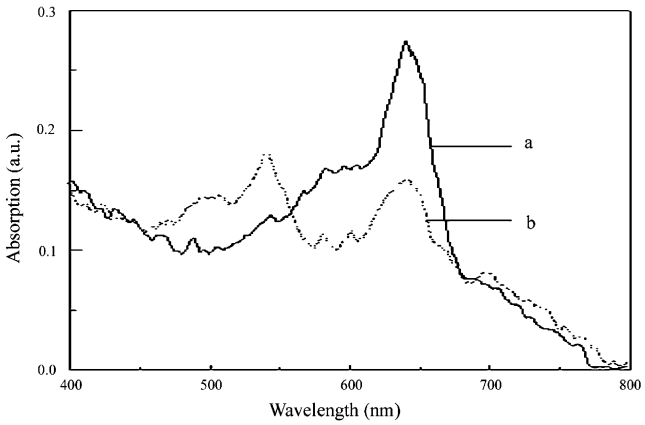

Figure 2. UV−vis spectra of the PDA/MPDA monolayer supported on the SAMs of a octadecanethiol-modified gold electrode: (a) blue polydiacetylene monolayer prepared with UV irradiation for 10 s and (b) red polydiacetylene monolayer prepared by incubating the blue monolayer with *E. coli K12* solution (9×10^8 cells/mL) for 5 min.

Electrochemical Measurements. Cyclic voltammetric measurements were carried out with a CHI 832 electrochemical system (CHI Inc.). A gold electrode or modified gold electrode was used as the working electrode; a Ag/AgCl electrode, as the reference electrode; and a platinum foil, as the counter electrode. Voltammetric measurements were performed in an aqueous solution of 0.1 M KCl, 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$. The working electrode was scanned in the potential range of −0.4 to 0.9 V at 200 mV/s, and the measured peak current of 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in solution was taken as I_0. The modified electrode was then transferred into the sample solution to bind with the bacteria for a given time. After the binding, the bacteria-bound electrode was rinsed with doubly distilled deionized water to remove those physically adsorbed bacteria. Then the electrode was returned to the electrolyte solution for voltammetric measurements. The current response from $K_3Fe(CN)_6/K_4Fe(CN)_6$ was measured as I_x; the normalized single response was calculated as I_x/I_0.

RESULTS AND DISCUSSION

Clearly, the PDA/MPDA monolayer supported on the SAMs of octadecanethiol on a gold electrode was blue. After the electrode was incubated in the solution containing 9×10^8 cells/mL *E. coli K12* for 5 min, the color of monolayer gradually changed from blue to red, which could be seen with the naked eye. Its optical feature was characterized by UV−vis spectra and shown in Figure 2. As shown in Figure 2a, before incubation with *E. coli K12*, the monolayer's main excitation peak occurred at 640 nm with a small absorption peak at 540 nm, the so-called blue monolayer. After incubation with *E. coli K12*, the monolayer's visible spectra in Figure 2b showed a gradual decrease at the 640 nm and a corresponding increase at 540 nm, the so-called red monolayer. Day and Ringsdorf[30] reported that the color shift from blue to red was attributed to a reorientation of the carbon backbone of

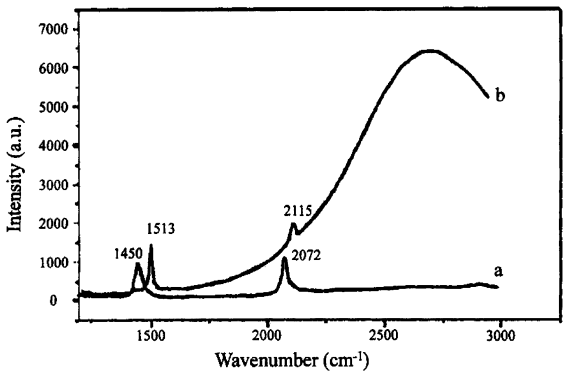

Figure 3. RRS spectra of a PDA/MPDA monolayer supported on the SAMs of an octadecanethiol-modified gold electrode in the regions of the double and triple bonds: (a) blue polydiacetylene monolayer prepared with UV irradiation for 10 s and (b) red polydiacetylene monolayer prepared by incubating the blue film with *E. coli K12* solution (9×10^8 cells/mL) for 5 min.

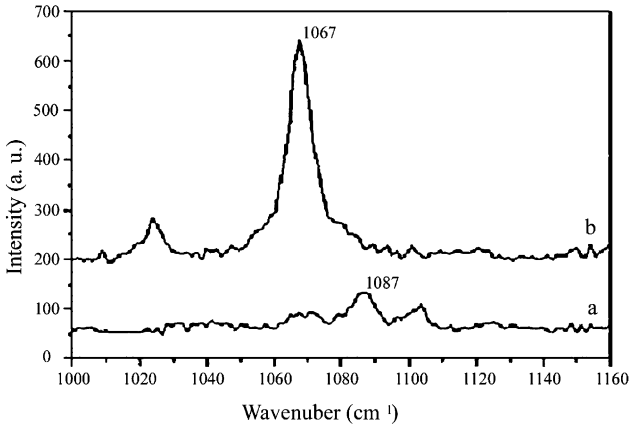

Figure 4. RRS spectra of PDA/MPDA monolayer supported on the SAMs of an octadecanethiol-modified gold electrode in the region of the single bond: (a) blue polydiacetylene monolayer prepared with UV irradiation for 10 s and (b) red polydiacetylene monolayer prepared by incubating the blue film with *E. coli K12* solution (9×10^8 cells/mL) for 5 min.

the blue polymer, which effectively reduced the conjugation length and increased the energy of the optical transition.

Figure 3 shows the RR spectra of a UV-polymerized LB monolayer of PDA/MPDA in the double- and triple-bond regions. The normal models of PDAs have been analyzed on the basis of bulk Raman spectra using a simplified model containing five independent force constants.[31,32] According to the models, the peaks around 1450 and 2072 cm^{-1} can be assigned to double- and triple-bond stretching vibrations, respectively. Because of the extensive electronic delocalization in the PDA backbone, these frequencies are lower than those for isolated double and triple bonds (1620 and 2260 cm^{-1}, respectively).[33,34] The peak positions of the double- and triple-bond stretching vibrations are in agreement with those observed in previous studies.[35−38] For example, Batchelder et al. detected two bands at 1455 and 2074 cm^{-1} for a polymerized diacetylene on smooth gold. [32]

After the PDA/MPDA monolayers were incubated with *E. coli K12* for 5 min, the color of the PDA/MPDA monolayers changed from blue to red. The RR spectra of the resulting red-colored monolayers contained two major bands at 1513 and 2115 cm^{-1} (as shown in Figure 3b), which can be assigned, respectively, to double- and triple-bond stretching modes. The results demonstrate that the double and triple bonds simultaneously shifted to higher wavenumber when *E. coli K12* interacted with mannose group on the surface of the polydiacetylene. The shift of RR bands can be due to different nonbonding interactions of the polymer backbone induced by the change in the side-chain packing.

Figure 4 shows the RRS of the blue and red monolayers in the C−C region. There was one prominent band observed at 1087 cm^{-1} in the blue monolayer in Figure 4a that is assigned to gauche defects within the monolayer. There was one prominent band at 1067 cm^{-1} in the red monolayer, as shown in Figure 4b, that is assigned to the ν_a (C−C) for trans C−C bonds. No gauche conformer band was observed at 1087 cm^{-1} for the red monolayer in Figure 4b, implying greater order in the red monolayer than in the blue monolayer. In the meantime, it also suggests the defect density gradually decreased within the monolayer when its color changed from blue to red. The molecule mechanism corresponding to the color change is believed to be an irreversible structural transition undergone by the conjugated backbone of the polymer, which is the formation of an alternating triple-bond/double-backbone structure. Such a transition is most likely associated with perturbations to the pendant side chains of the PDA following molecular processes affecting the PDA interface.[39,40] In this case, the perturbation was from the biointeraction between the mannose group in the PDA derivative and *E. coli*.

We exposed the bilayer composed of PDA/MPDA and the SAMs of octadecanethiol on the gold electrode to a 9×10^8 cells/mL *E. coli K12* solution for various times and measured the resulting current response of 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl solution from cyclic voltammograms in the potential range between −0.4 and 0.9 V at 200 mV/s. Figure 5 shows the evolution of cyclic voltammograms before or after incubation with *E. coli K12*. Before the electrode was incubated with *E. coli K12*, a very broad current peak appeared in the cyclic voltammogram (shown as Figure 5a). There was no redox substance in the bilayer, so the broad current peak resulted from the defects within the bilayer

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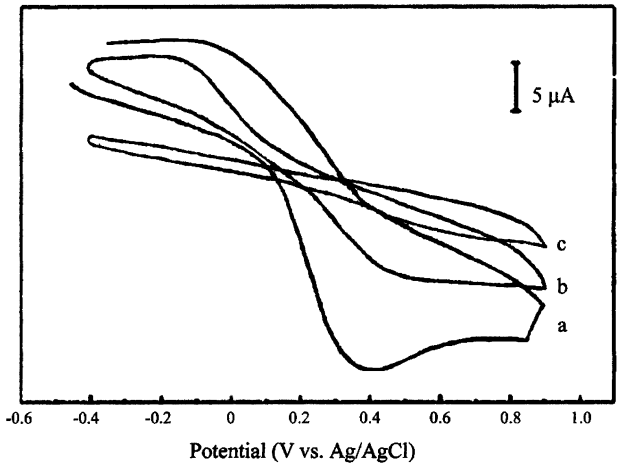

Figure 5. Cyclic voltammograms for 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl solution on the bilayer consisting of PDA/MPDA and the SAMs of the octadecanethiol modified gold electrode. Scan rate, 200 mV/s. Concentration of *E. coli K12* in solution, 9×10^8 cells/mL. (a) Before incubation with *E. coli K12* and (b, c) after incubation with *E. coli K12* for 3 and 5 min, respectively.

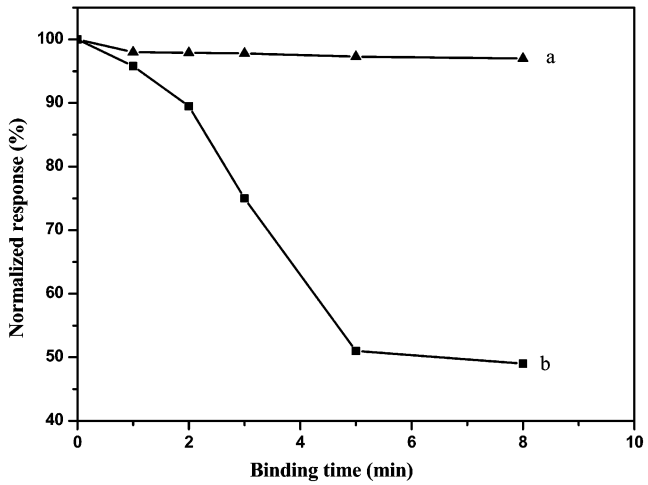

Figure 6. Effect of the binding time on voltammetric responses of 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl solution on the bilayer consisting of PDA/MPDA and the SAMs of the octadecanethiol-modified gold electrode: (a) physiological saline and (b) physiological saline containing *E. coli K12* (9×10^8 cells/mL).

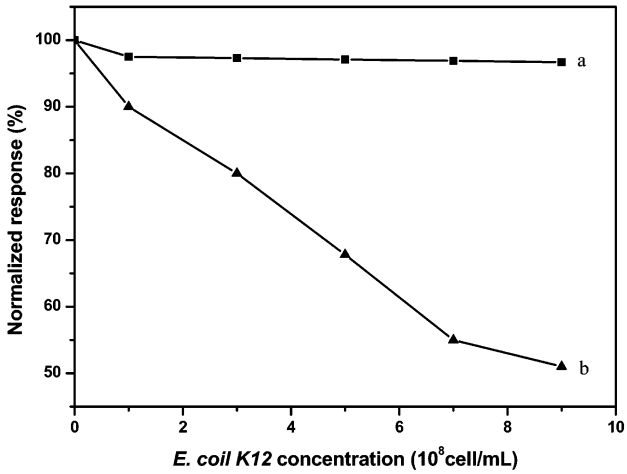

Figure 7. Effect of the concentration of *E. coli K12* on voltammetric responses of 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl solution on the bilayer consisting of PDA/MPDA and the SAMs of the octadecanethiol modified gold electrode. Binding time, 5 min for all cases. (a) Without the receptor (or PDA monolayer) and (b) with the receptor (or PDA/MPDA monolayer).

consisting of PDA/MPDA and the SAMs of octadecanethiol on the gold electrode that permitted the probe molecules ($K_3Fe(CN)_6/K_4Fe(CN)_6$) in solution to exchange electrons with the underlying gold electrode surface.[41,42] After the electrode was incubated with *E. coli K12* (9×10^8 cells/mL) for 3 min, the peak current gradually decreased and appeared plateau-shaped at potential past the redox potential of probe molecules (as shown in Figure 5b). This can be explained by a low density of micro- or nanometer-scale defects within the bilayer, and each defect could behave as a single ultramicroelectrode.[43,44] After the electrode was incubated with *E. coli K12* for 5 min, the current decreased exponentially, as shown in Figure 5c. Chidesy et al. reported that the change was attributed to the decrease of defect density within the bilayer.[41] In other words, there were no defect sites through which probe molecules can completely or partially penetrate.[45]

Figure 6 shows the effect of the binding time on the amperometric response in bacterial solution (Figure 6a) and background solution (only physiological saline, Figure 6b). Voltammetric measurements of the bilayer-modified electrode were performed in an aqueous solution of 0.1 M KCl and 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$, and the corresponding measured peak current of $K_3Fe(CN)_6/K_4Fe(CN)_6$ was taken as I_0. The bilayer-modified electrode was then transferred into the background solution or bacterial solution for a given time. After removal, it was rinsed with doubly distilled deionized water to remove those physically adsorbed bacteria and then was returned to the electrolyte solution for voltammetric measurements. The corresponding current response from $K_3Fe(CN)_6/K_4Fe(CN)_6$ was measured as I_x; the normalized signal response was calculated as I_x/I_0.

As can be seen in Figure 6a, there was <5% change of current response after the electrode was held in the background solution for 5 min, which may originate from heat influence. However, the amperometric response decreased noticeably (>40%) when the electrode was placed in a physiological saline solution containing 9×10^8 cells/mL *E. coli K12* for 5 min (see Figure 6b). The amperometric signal gradually leveled off after 8 min. A 49% decrease of current was observed after 8 min. The results clearly demonstrate that the bacterium cells in saline solution diffused onto the electrode surface and bound with the receptor (mannose) on the bilayer. The binding appeared to change the bilayer structure and gradually diminish the current response.

To further examine the specificity of the interaction between bacterium and mannose, two kinds of Langmuir−Blodgett monolayer (with and without mannose in the polydiacetylene monolayer) were prepared and inserted into the different concentrations of the bacterial solution (Figure 7). Only <5% current change was found on the electrode without the receptor mannose (see Figure 7a), demonstrating the important role of mannose in defining the sensor specificity. As for receptor-modified electrodes (PDA/

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MPDA modified electrode), the amperometric signal decreased quickly with increasing concentration of the bacterium, and the dependence of the peak current on the concentration of *E. coli K12* was a linear relationship within the range of 1×10^8 to 7×10^8 cells/mL. It is important that a negligible loss in the amperometric response was observed over multiple potential scans at the same electrode surface. This suggests that retention of the specific binding capability has been effective, and the changes in the monolayer at the surface are minimal during scans. Therefore, the bilayer composed of polydiacetylene derivative with mannose and the SAMs of octadecanethiol is similar to the biological membrane, and the direct electrochemical detection by polydiacetylene supramolecular assemblies might not only open a new path for the use of these membranes in the area of biosensor development but also offer new possibilities for diagnostic applications and screening for binding ligands.

CONCLUSIONS

In conclusion, the strong and selective binding of mannose to *E. coli*, an open platform to host biosensory elements allowing fast recognition and binding with the target molecules, presents a novel method of labeling specific protein on the cell surface using carbohydrate conjugated assemblies, which can provide a relatively easy and direct method to visualize the target receptors on the cell surface through electrochemical technology. Electrochemical and optical experiments show that the specific interactions between *E. coli* and mannose can cause the conformational changes of the polydiacetylene backbone rather than simple nonspecific adsorption. Polydiacetylene-based supramolecular assemblies are robust and can be readily applied to diagnosis of physiological molecules and for rapid screening of chemical and biological libraries.

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