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Polydiacetylene Liposome Arrays for Selective Potassium Detection

Jiseok Lee,[§] Hyong-Jun Kim,[†] and Jinsang Kim*[,†,‡,§,¶]

Departments of Materials Science and Engineering, Chemical Engineering, Macromolecular Science and Engineering, and Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109

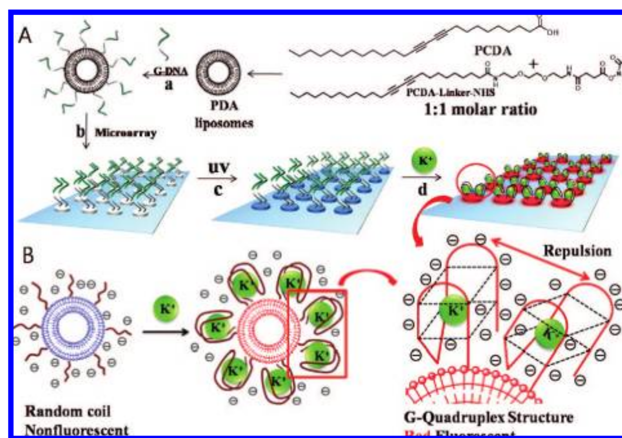
Received November 11, 2007; E-mail: jinsang@umich.edu

Conjugated polymer-based biosensor systems have gained much interest from both academia and industry.[1] This is because an environmental change at a single site along the conjugated polymer chain can affect the properties of the collective system, producing large signal amplification.[2–5] If biological materials are uniquely combined as a receptor with synthetic conjugated polymers having the tunable signal amplification property, the specificity of the biosystems will provide the ultimate selectivity in a sensor design. In this respect, developing biological and synthetic conjugated polymer hybrid biosensors through rational design, organic and bioconjugated synthesis, and molecular assembly is a promising direction to realize high sensitivity and high selectivity. Polydiacetylene-based sensor systems are unique in terms of the sensitive colorimetric/fluorescence dual detection capability and the convenient preparation method through self-assembly and subsequent photopolymerization.[6,7] It has been well-known that polydiacetylene in the blue phase is not emissive but produces red fluorescence when it transforms to the red phase by external stimuli including temperature,[8] pH,[9] ions,[10] solvent,[11] stress,[12] or ligand interactions.[13]

In this contribution, we describe the development of practical polydiacetylene (PDA) liposome-based microarrays to selectively detect potassium even in the presence of sodium. Potassium is an important cation in biology and quantitative detection of the extracellular potassium level in the blood stream is also important.[14–16] The typical physiological concentration of potassium and sodium in the blood stream is 3.5–5.3 and 135–145 mM, respectively.[17] However, selective detection of physiological potassium is a challenging task due to the presence of sodium in a much higher concentration. Our PDA liposome sensor has ssDNAs having a guanine-rich sequence (5′-GGTTGGTGTGGTTGG-3′) as a selective probe for potassium detection. We utilize the fact that the G-rich ssDNA can fold into a G-quadruplex via intramolecular hydrogen bonding by wrapping around a potassium ion exclusively.[18,19] We rationally design the PDA liposome in such a way that the G-rich ssDNA probes are presented densely at the liposome surface, and upon binding with K^+, the resulting bulky quadruplexes repulse each other as illustrated in Scheme 1. The steric repulsion of the quadruplexes will induce the perturbation of the ene-yne backbone of PDA liposomes and produce the color change from blue to red and red fluorescence, as well.

We synthesized the NHS-activated carboxylic-acid-containing diacetylene in Scheme 1A (PCDA−linker−NHS). The self-assembled diacetylene liposomes were prepared by using a 1:1 mixture of the PCDA−linker−NHS and 10,12-pentacosadiynoic acid (PCDA). The G-rich ssDNA was covalently linked to the liposome surface by means of conventional amide chemistry between the amine-modified G-rich ssDNA and the PCDA−linker−NHS. The self-assembled and functionalized liposomes were then printed onto an amine-modified glass substrate by using a manual microarrayer in a humidity chamber and photopolymerized (detailed information is in the Supporting Information).

Scheme 1 [a]



[a] A. The chemical structure of the diacetylene monomers investigated and a schematic representation of the PDA liposome-based microarray for potassium detection. (a) Surface modification of the diacetylene liposome with the amine-functionalized G-rich ssDNA. (b) Microarray of G-rich ssDNA-tethered PDA liposomes onto an amine glass. (c) Photopolymerization of the G-rich ssDNA-tethered PDA liposomes using a 254nm UV lamp. (d) Recognition of target potassium ions via the quadruplex formation results in red fluorescent emission. B. Schematic representation of the G-quadruplex formation and the resulting steric repulsion.

Scheme 1B schematically illustrates the steric repulsion and the resulting conformational change of the PDA liposome, which is induced by G-quadruplex formation with K^+ selectively. We confirmed the selective G-quadruplex formation of the PDA liposomes with K^+ by using CD analysis as shown in Figure 1a. The characteristic absorption bands at 270 and 290 nm of the quadruplex were observed only when K^+ was added to the solution of the PDA liposomes.[20] The CD analysis also shows that the presence of Na^+ does not interfere with the selective K^+ recognition. Even more than 30 times more concentrated Na^+ (100 mM) essentially did not hinder the quadruplex formation of the G-rich ssDNA probe with K^+ (3 mM). Figure 1 panels b and c show the UV–vis absorption and PL emission spectra of the G-rich ssDNA-tethered PDA liposome solution upon addition of KCl solution in various concentrations. The solution was incubated at 25 °C for 3 h. As the concentration of K^+ increases, the absorption band at 650 nm (blue phase)

[†] Department of Materials Science and Engineering.
[‡] Department of Chemical Engineering.
[§] Department of Macromolecular Science and Engineering.
[¶] Department of Biomedical Engineering.

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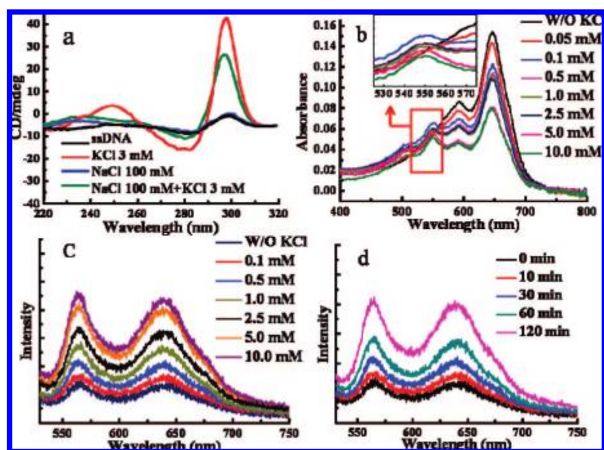

Figure 1. (a) CD spectra of the G-quadruplex at 6 °C before and after adding K^+ and Na^+. (b) UV–vis spectrum and (c) PL spectrum change of G-rich ssDNA-tethered PDA liposome solution (1 mM) upon addition of KCl. The concentration of KCl ranges from 0 to 10.0 mM. (d) PL spectrum change of the G-rich ssDNA-modified PDA liposome solution (1 mM) upon addition of 1 mM KCl (excitation at 503 nm).

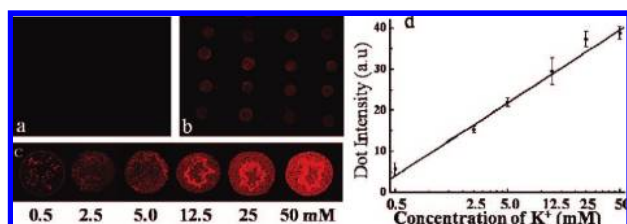

Figure 2. Fluorescent microscope images of the microarrayed PDA liposomes (excitation at 600 nm and a long-pass emission filter with 550 nm cutoff were used) (a) after adding NaCl (5 mM) and before KCl (5 mM) addition, and (b) after adding the KCl solution and 30 min of incubation at room temperature. (c) Fluorescent images of the PDA liposome arrays with KCl solutions at various concentrations (20 mL each). (d) Correlation curve between the fluorescence intensity and the amount of the K^+.

decreases and the new absorption band at 550 nm (red phase) increases. The detection limit shown in Figure 1b is 0.1 mM, and that is suitable for the detection of the physiological potassium level (3.50–5.30 mM). The same liposome solution did not show any change upon addition of even 100 times higher concentration of Na^+, providing excellent selectivity (Supporting Information). The fluorescence intensity of the liposome also increased gradually with the increasing concentration of K^+ (Figure 1c). As the incubation time at 25 °C increases, the PL intensity also increases (Figure 1d).

We further developed practically useful solid-state liposome arrays for selective potassium detection. The G-rich ssDNA-modified liposome solution was spotted onto amine-modified glass substrates using a manual microarrayer followed by incubation at 30 °C for 3 h under 90% humidity condition to prevent the liposomes from drying out and subsequently photopolymerized with a 1 mW/cm^2 254 nm UV lamp for 2 min. Twenty milliliters of 5 mM NaCl solution and 20 mL of 5 mM KCl solution were then added onto the glass substrate, respectively. As shown in Figure 2a, no fluorescence was observed after adding the NaCl solution and before adding the KCl, while the red fluorescence emission was turned on as an indication of the K^+ detection after adding the KCl solution followed by 30 min of incubation at room temperature (Figure 2b). The results demonstrate that the immobilized liposomes having G-rich ssDNA selectively detect K^+ even in the presence of Na^+ because the presence of Na^+ does not interfere with the selective K^+ detection.

We also conducted the detection limit study in the solid state. Figure 2c shows the fluorescence microscope images of the PDA microarrays after incubation with K^+ solution in various concentrations at room temperature for 30 min. The detection limit for the 30 min of incubation results in the microscope images is 0.5 mM. The correlation curve between the fluorescence intensity and the amount of K^+ is shown in Figure 2d, and therefore, a quantitative analysis of an unknown K^+ concentration is also achievable.

In summary, we have developed a highly selective PDA liposome-based sensory system to detect K^+ even in the presence of Na^+. The tethered G-rich ssDNAs at the liposome surface provide selectivity by wrapping around K^+ selectively to form quadruplexes. The bulky quadruplexes repulse each other due to the steric hindrance and induce the ene-yne backbone perturbation of the PDA liposomes. This selective event triggers the color change from the blue phase to the red phase of the PDA liposome and also produces red fluorescence emission. We demonstrated quantitative analysis of the K^+ concentration by using the PDA liposome microarray. The presented design principle can be readily applicable to many other chemical and biosensor developments and allows highly selective and sensitive quantitative analysis.

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Supporting Information Available: The detailed synthetic procedures and characterization of all chemicals. This material is available free of charge via the Internet at http://pubs.acs.org.

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